LEVERAGING PLEIOTROPY WITH BIPOLAR AND ASSOCIATED DISORDERS TO IMPROVE DISCOVERY OF GENETIC ASSOCIATIONS IN ADHD

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Background
Attention Deficit/hyperactivity disorder (ADHD) is a condition characterized by problems with inattention, hyperactivity and/or impulsivity affecting between 6-12% of children worldwide. These symptoms are often disruptive, impairing performance in broad aspects of one's life including academic, intellectual, and social settings, as well as while driving. The etiology of the disorder is accepted as multifactorial. Environmental factors, such as low socioeconomic status and familial instability, constitutional factors, such as gender and age, as well as a strong genetic component (heritability = 0.75) confer risk for ADHD. Some of these risk factors may be shared as substantial psychiatric and behavioral comorbidities have been reported. Notable are reports of Bipolar Disorder comorbid and co-segregating/aggregating within families of ADHD probands, the exact cause of which is unresolved. Despite the high heritability, genome-wide linkage and association studies have revealed few consistent genetic signals. While a number of candidate genes have shown consistent associations with ADHD, their effects sizes are modest and in aggregate they explain little of the heritability. It is likely that the genetic architecture of ADHD is highly polygenic, composed of many gene factors, each conferring a small amount of risk. Recent GWAS did not produce any associations at genome-wide significance, although suggestive evidence persisted at previous candidate loci.

Methods
We obtained the data from the Psychiatric GWAS Consortium: ADHD Subgroup GWAS of ADHD and summary statistics from a number of other traits with potential genetic overlap, including Bipolar Disorder, other psychiatric conditions and biological traits. We used a recently developed extension to the empirical Bayes local false discovery rate to compute, for each SNP in the original ADHD GWAS, a covariate modulated local false discovery rate. This cmlocFDR reflects the probability that a given SNP is null or non-null based on both the distribution of p-values in the ADHD GWAS and a set of auxiliary covariates that included pleiotropic information with related traits and disorders and functional genome annotations for each SNP.

Results
We demonstrate significant shared genetic associations between ADHD and Bipolar Disorder and use this, among other information, to improve association studies for ADHD. Using recently developed methods, we report a number of novel candidate variants for ADHD garnered from the improved power of the pleiotropy and annotation informed genetic analyses and discuss their implications in the etiology of ADHD.

Discussion
We have applied a recently developed statistical framework for genetic association studies to uncover novel candidate associations for ADHD. This framework suggests the co-occurrence of ADHD and Bipolar disorder may, at least in part, be due to shared genetic factors. Further, we have demonstrated how, in complex, highly polygenic traits, functional annotations of SNPs and pleiotropy among disorders can be used to uncover novel associations in traits with previously low yielding GWAS. This approach has the potential to aid in the quest for the missing heritability and bring together the enormous and diverse resources of GWAS data that have been created over the last decade.

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**GENOME-WIDE META-ANALYSIS OF ASSOCIATION STUDIES OF AN ADHD TRAIT IN ADULTS: GENOME-WIDE SIGNIFICANT HITS FROM THE SAGA CONSORTIUM (STUDY OF ADHD TRAIT GENETICS IN ADULTS)**

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**Background**
Attention Deficit Hyperactivity Disorder (ADHD) is characterized by symptoms of persistent inattention and/or impulsivity and hyperactivity and is thought to be the extreme of a continuum of behavior in the population. In addition to its high prevalence in children, ADHD affects 1–4% of adults and, although its symptoms have been suggested to be less heritable than in children (h² ~30-40% based on self-report questionnaires compared to 70-80% based on rater-based/diagnostic assessments in children), adult ADHD has a number of features that make it an appealing phenotype for gene-finding studies. The adult phenotype shows no or only small sex differences and a limited association with age. Across the adult life-span the same genes seem to influence the phenotype.

**Methods**
Here we present a genetic study of adult self-rated ADHD symptoms in seven Dutch population-based samples (total N = 12,894). Symptom-scores were based either on the Conners Adult ADHD Rating Scales (CAARS) or on the ADHD-DSM-IV Self Rating Scale. Firstly, all samples were imputed against the 1000 Genomes reference panel, secondly, genome-wide association analysis (GWA) of ADHD symptom counts were performed at each site, independently applying quality controls measures for minor allele frequency (≥1%) and imputation quality (rsqr ≥ 0.3). Thirdly, GWA results were uploaded to a central server, where we performed a genome-wide meta-analysis using a fixed effects model.
Results
Preliminary results for the total score of ADHD symptoms (available N = 11,651 from six samples) show a genome-wide significant SNP on chromosome 4 (p-value 4.58E-08). We also detected novel associations with one SNP on chromosome 5 (p-value 8.32E-08) and two SNPs on chromosome 2 (p-values 1.58E-08 and 1.25E-07).

Discussion
This genome-wide meta-analysis of adult ADHD symptoms will provide novel insights into the genetic underpinnings of ADHD traits in the population and potentially for the clinical disorder.

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IMAGING GENETICS OF DSM-V PROPOSED ATTENTION DEFICIT HYPERACTIVITY DISORDER SUBTYPES
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Background
Despite a voluminous literature, ADHD pathophysiology remains incompletely understood. Suggestions for maximizing future progress include:
1-isolating effects and interactions due to comorbid disorders/medication history
2-interfacing genetic-brain imaging-neuropsychologic studies
3-direct group comparisons between different ADHD subtypes
4-combining different imaging modalities.

It was aimed to investigate the association of DRD4, DAT1, BDNF, SYN III, SNAP-25 genes, which take place in neurotransmission and neuronal plasticity, with neuroimaging of attention deficit hyperactivity disorder (ADHD) subtypes.

Methods
201 ADHD children (101 ADHD-Combined type-ADHD-C, 50 DSM-V proposed ADHD Predominantly inattentive type-ADHD-PIA, 50 DSM-V proposed ADHD Inattentive (Restrictive) type-ADHD-IA) and 100 controls were included into the study. “A best estimate procedure” was used to determine the final diagnoses. “Best estimate procedure” is defined here as determining the diagnosis after reviewing all teacher and parent’s scales, K-SADS-present and lifetime version, and WISC-R results. All cases were assessed on computerized neuropsychologic test battery, consisting of continuous performance test, verbal and visual memory, finger tapping, symbol digit coding, the Stroop test, and Shift Attention Test. DNA samples were extracted from saliva specimen and samples of each subject were genotyped after all patients had finished the protocol by a researcher who was blind to diagnosis. Twenty four cases from each group were asked to take part in the multimodal neuroimaging study, consisting of functional MRI, DTI, and Arterial Spin Labeling.
Results
There was no significant difference between ADHD and control group for DAT1, SYN III, SNAP-25 genes. However, when we compared the control and ADHD groups in terms of DRD4 and BDNF;

i) at least one DRD4 7-repeat (7R) allele was significantly more frequent in ADHD-IA (restrictive) group

ii) the DRD4 4-repeat allele homozygote genotype (4R/4R) was significantly more frequent in ADHD-C group

iii) BDNF G/G genotype of the Val66Met SNP was significantly more frequent in the study group than control group. ADHD-IA cases performed worse than ADHD-PI and ADHD-C subtypes on psychomotor speed and reaction time subtests of neurocognitive test battery. Occipital activation of ADHD-IA group significantly differed from ADHD-PI group on fMRI, and from ADHD-C group on ASL.

Discussion
To the best of our knowledge, this is the first study that evaluates;

i) DSM-V proposed ADHD-IA (restrictive) subtype and compares it with the other subtypes of ADHD

ii) subtypes of ADHD has been separately evaluated, and compared in terms of neuropsychological, genetic and brain imaging aspects in a relatively large sample

iii) the same ADHD sample using three different brain-imaging modalities

These results could be explained as, occipital cortex interacts with the dorsal attention network to maintain attention and suppress attention to irrelevant stimuli. Therefore, we may speculate that, isolated attention problems, which are represented as ADHD-IA subtype in DSM-V, are related to occipital differences that lead to psychomotor speed and reaction time problems.

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GENE-BASED ANALYSES OF ATTENTION DEFICIT HYPERACTIVITY DISORDER (ADHD) GWAS DATA
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Background
Attention Deficit Hyperactivity disorder (ADHD) is a highly heritable disorder, which has led researchers to search for underlying genetic risk factors via both candidate gene studies and, more recently, large-scale Genome Wide Association Scans (GWAS’s). Many extant candidate gene studies have yielded mixed results, while recent GWAS’s have yielded some suggestive findings but no genome-wide significant results. Two possible reasons for this are the relatively small sample sizes of the first generation of ADHD GWAS's and the small effect sizes of individual SNPs that show suggestive association. These small effects are consistent with those for other psychiatric disorders and medical conditions, and have led geneticists to utilize several alternative analytic methods, such as analyses of haplotypes and gene pathway or network analyses. Alternative analytic techniques that are relatively underutilized are omnibus gene-
based analyses that simultaneously evaluate the statistical significance of the association between a trait or disorder and multiple SNPs within particular genes and their flanking regions. These methods have the advantage that they greatly reduce the number of statistical tests performed and hence involve a gene-based rather than SNP-based threshold for genome-wide significance.

**Methods**

In this study, we apply such gene-based analyses of association to the second generation of ADHD GWAS data. Specifically, we included data from 6669 parent-offspring trios and 3744 cases and 11552 controls from 9 ADHD GWAS samples and performed gene-based analyses of ADHD diagnoses using the program KGG, which combines SNP-based test results with information on LD among the SNPs within each gene and its flanking regions to obtain an association p-value for each gene.

**Results**

We used KGG to conduct gene-based tests (GATES and HYST) of the SNP-based GWAS results from both HapMap and 1000 genomes imputed dosage data from all 9 ADHD GWAS samples integrated with the HapMap and 1000 genomes LD information. We examined gene-based test results for 5' and 3' flanking region lengths of 5, 10, 20, 50 and 100 kb. While no genes consistently surpassed a genome-wide significance threshold of $2 \times 10^{-6}$, several genes had p-values in the $2 \times 10^{-5}$ to $2 \times 10^{-6}$ range, stronger than the results for their constituent SNPs.

**Discussion**

We demonstrate that the gene-based analytic methods (GATES and HYST) implemented in KGG yield evidence for association that is at least as strong as conventional SNP-based methods and in many cases is much stronger. Such procedures should evince even greater yields as the number of ADHD GWAS samples and studies increase in the near future.

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**GENETIC EVIDENCE FOR SHARED ETIOLOGY BETWEEN SMOKING BEHAVIOUR AND ADHD**

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**Background**

ADHD and cigarette smoking are two highly comorbid phenotypes. Convergent data suggests that these phenotypes may share underlying neurobiological mechanisms.

**Methods**

To test the hypothesis that there is shared genetic etiology, family-based association tests (FBAT) were conducted in children with ADHD with tag SNPs previously shown to be highly associated with different dimensions of smoking behaviour. FBAT was carried out with a clinical sample of children with ADHD and their families collected at the Douglas Mental Health University Institute, Montreal, Canada (n= 377 nuclear families). The top five single nucleotide
polymorphisms (SNPs), based on meta-analytic review of genome-wide association studies of smoking behaviour, were tested for association with ADHD. Tag SNPs at CHRNA3, BDNF, DBH and LOC100188947 (rs1051730, rs1028936, rs1329650, rs3025343, and rs6265) were selected. Main outcome measures were clinical diagnosis of ADHD, and a number of behavioural and neurocognitive phenotypes relevant to the disorder. In addition, FBAT analysis was conducted in the total sample, while stratifying based on maternal smoking during pregnancy (MSDP).

Results
One SNP (rs1329650) at the locus for non-coding RNA (LOC100188947) was significantly associated with overall ADHD diagnosis with the C* risk allele being over-transmitted from parents to children with ADHD (p=0.02). It was also over-transmitted to children with higher scores on Conners’ Parents (p=0.01) and Conners’ Teacher (p=0.002) index scores, and Child Behaviour Checklist (CBCL) withdrawn (p=0.001) and aggressive (p=0.007) behaviours. Children with poorer performance on executive and attention tasks were more likely to inherit the risk allele. These data suggest that the C* allele of rs1329650 may be increasing the risk for ADHD and smoking behaviour through a common mechanism, possibly externalising behaviours and specific cognitive deficits that manifest as ADHD in childhood and are the gateway to smoking behaviour later in life. Further evidence for shared genetic etiology was obtained when FBAT analysis was conducted in the total sample, while stratifying based on MSDP. Here, highly significant association was observed with specific SNPs within SLC6A2 (encoding the norepinephrine transporter) - rs36021 and linked SNPs, in the MSDP group. Association was noted with categorical ADHD diagnosis (Z=3.74, p=0.0002), assessments of behaviour by parents (CBCL, p=0.00008), as well as restless-impulsive subscale scores on Conners’-Teacher and Conners’-Parents (p=0.006, both measures). Significant association was also observed with deficits in cognitive function highly relevant for ADHD, specifically sustained attention, spatial working memory, planning, and response inhibition. In the group where mothers did not smoke during pregnancy, the results were in stark contrast. Here, a complete lack of association between rs36021 and related SNPs was noted.

Discussion
Taken together, these data provide supportive evidence that the association between MSDP and childhood ADHD is due to genetic factors.

CODING VARIATIONS AND THE RISK OF ADULT ADHD. AN EXOME-CHIP ASSOCIATION STUDY IN ADULT ADHD FROM THE IMPACT.
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Background
Attention deficit hyperactivity disorder (ADHD) is a highly heritable childhood onset neuropsychiatric condition that often persists into adulthood. Still, the genetic architecture of ADHD, and particularly ADHD in adults is largely unknown. The main purpose of this study was to perform a genome-wide scan of adult ADHD using the newly available Illumina HumanExome12v1 chip, and to evaluate the performance of this technology for genotyping common and rare variants.
Methods
The analyses were carried out using DNA samples isolated from saliva or blood collected by the International Multicenter persistent ADHD Consortium (IMpACT). The evaluation of HumanExome12v1 chip was implemented on 2215 individuals, 25 of which were also whole exome sequenced using Roche-NimbleGen Sequence Capture EZ Exome v2 kit and paired-end 100nt sequencing on the Illumina HiSeq. All participants were genotyped on Illumina HumanExome12v1 chip. Genotypes were called in Illumina GenomeStudio V2011.1 software, with additional genotype assignments implemented in zCall software. Performance of HumanExome12v1 chip as well as GenomeStudio V2011.1 software was assessed by direct comparison of data obtained from different DNA sources (blood and saliva), duplicate samples and sequenced genotypes. Mendelian consistency testing was also utilized. Sanger sequencing was used to validate rare variants with minor allele frequency <1%. Association testing was carried out using single marker logistic regression correcting for population substructure and gender using PLINK software.

Results
We developed a quality control analysis pipeline for the variants of HumanExome12v1 chip using series of steps implemented in PLINK, GenomeStudio V2011.1 and zCall softwares. Overall, the performance of HumanExome12v1 chip was comparable to that of next generation sequencing. However, it is worth mentioning that mismatch rates were notably higher for rare variants with minor allele frequency < 1% compared to the rest of the chip; the exact specifics will be discussed in details. Association testing in a total of 2600 adult ADHD cases and 7000 controls are currently being performed and will be presented.

Discussion
This study provides insight into the performance of the newly available Illumina HumanExome12v1 chip, serving practical guidance to its quality control with special emphasis on rare variants. Being the largest systematic adult ADHD study to date, this study expected to shed new light on our understanding of adult ADHD genetics.

ASSOCIATION BETWEEN MAOA AND AGGRESSIVE BEHAVIOR IN ADOLESCENTS RECEIVING THE PHARMACEUTICAL TREATMENT LISDEXAMFETAMINE DIMESYLATE FOR ADHD SYMPTOMS
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Background
Attention deficit hyperactivity disorder (ADHD) is one of the most common childhood neuropsychiatric disorders, affecting 5.29% of children worldwide. ADHD symptoms manifest as severely disruptive behaviors of inattention, hyperactivity, and/or impulsiveness. The pharmaceutical lisdexamfetamine dimesylate (LDX [Vyvanse®]), a prodrug stimulant prescribed for ADHD, is thought to alleviate symptoms by inhibiting the dopamine and norepinephrine
Monoamine oxidase A (MAOA) is an X-chromosome linked gene that catalyzes the degradation of dopamine and norepinephrine. The number of 30-bp repeats located in the polymorphic MAOA promoter region has been shown to alter the enzymatic degradation of dopamine and norepinephrine. Wild type alleles consist of 3.5 or 4 repeats (high enzymatic activity alleles). The genotypes of 2, 3, or 5 repeats (low enzymatic activity alleles) have been associated with increased aggression in males. This study looked at adolescents being treated for ADHD symptoms with LDX. Nearly half of the individuals discontinued the medication due to aggressive behavior. Here, we hypothesized that individuals with the low enzymatic activity MAOA alleles would be the individuals more likely to discontinue the drug due to aggressive behavior than individuals with the higher MAOA enzymatic activity genotypes.

Methods
The study sample included 73 adolescents ranging in ages between 6 and 18, averaging 12 years in age. The final study sample was 85% male and 90% Caucasian. All individuals were being treated within a behavioral health outpatient facility by licensed child psychiatrists for ADHD symptoms. After the ADHD diagnosis, each individual at one time during their treatment, received LDX medication. Each child’s buccal cell DNA was used to perform the genotyping of the MAOA 30-bp polymorphism. Odds ratios were calculated with 95% confidence intervals and significance was evaluated using a Chi-square test.

Results
We found that individuals with high MAOA enzymatic activity alleles were significantly more likely to discontinue LDX due to aggression than individuals with the low enzymatic activity MAOA alleles (OR=0.3083 [95% CI: 0.11, 0.86] p=0.02).

Discussion
Stimulant medications are the first-line pharmacological treatment for children with ADHD. Although these treatments are very effective for the majority of its users, there are patients who can experience considerable adverse side effects. This study has shown a significant association between the discontinuation of LDX due to aggressive behaviors in adolescents with the high MAOA enzymatic activity alleles (3.5 and 4).

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GENOME-WIDE ANALYSIS OF RAPID CYCLING BIPOLAR DISORDER IN THE PSYCHIATRIC GENOMICS CONSORTIUM BIPOLAR DATA
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Background
According to DSM-IV an individual suffering from bipolar disorder will be described as having rapid cycling if they have had four or more discrete mood episodes (mania, hypomania, depression, or mixed) over the course of a year. The study of rapid cycling bipolar disorder is clinically relevant due to its high prevalence, extended duration of illness and greater illness severity.

Methods
The analysis reported here used the PGC bipolar disorder sample comprising 5842 cases with
rapid cycling information (49% of the total case sample) and 15223 control samples. Amongst the bipolar subjects there were 1991 individuals (34%) with rapid cycling and 3851 individuals known not to have experienced rapid cycling. Two separate GWAS analyses were performed: a case versus case comparison of rapid cycling bipolar subjects against those known not to have experienced rapid cycling; and a rapid cycling bipolar case comparison against control individuals. These analyses were performed separately in each of the 11 sub samples and the results combined using meta-analysis.

Results
The rapid cycling case versus case analysis (\(\lambda_{gc} = 1.005\)) detected one signal that exceeds genome wide significance on 6q with rs138360464 (\(p=4.06\times 10^{-08}\), \(OR=1.44\), MAF 0.14) in an intron of the WDR27 gene; the association signal extends to nearby genes. The rapid cycling case versus control analysis (\(\lambda_{gc} = 1.09\)) detects signals on chromosome 17p13.2 rs146946310 (\(p=2.10\times 10^{-08}\), OR=1.46, MAF 0.065). rs146946310, is located in the CYB5D2 gene and the association signal extends to neighbouring genes. The case versus control analysis also detected a second signal on chromosome 16p12.2 with rs117583922 (\(p=4.45\times 10^{-08}\), OR=13.97, MAF 0.006). This finding is driven by only two of the sub samples and should therefore be treated with caution. rs117583922 is located in PRKCB which is a calcium dependent protein kinase C.

Discussion
The findings presented here represent the largest comprehensive analysis of genetic susceptibility to rapid cycling bipolar disorder. A greater insight into rapid cycling may help inform treatment for this clinically severe form of the disorder and may in turn also help to identify susceptibility variants for bipolar disorder in general.

THE KMO ALLELE ENCODING ARG452 IS ASSOCIATED WITH PSYCHOTIC FEATURES IN BIPOLAR DISORDER TYPE 1, AND WITH INCREASED CSF KYNA LEVEL AND REDUCED KMO EXPRESSION

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Background
The kynurenine pathway metabolite kynurenic acid (KYNA), modulating glutamatergic and cholinergic neurotransmission, is increased in cerebrospinal fluid (CSF) of patients with schizophrenia or bipolar disorder type 1 with psychotic features. KYNA production is critically dependent on kynurenine 3-monoxygenase (KMO). KMO mRNA levels and activity in prefrontal cortex (PFC) are reduced in schizophrenia. We hypothesized that KMO expression in PFC would be reduced in bipolar disorder with psychotic features and that a functional genetic variant of KMO would associate with this disease, CSF KYNA level and KMO expression.

Methods
Level of KMO RNA in postmortem brains was studied using data on 105 cases obtained from the
SMRI On-Line Database (www.stanleygenomics.org). *KMO* genetic association to psychotic features in bipolar disorder type 1 was studied in 493 patients and 1044 anonymous blood donors from Sweden by genotyping SNPs covering 79% of the variation in *KMO*. *KMO* Arg<sup>452</sup> was studied for association to CSF KYNA levels in an independent sample of 55 Swedish bipolar disorder type 1 patients, and to *KMO* expression in 717 lymphoblastoid cell lines and 138 German hippocampal biopsies.

**Results**

*KMO* mRNA levels were reduced in PFC of bipolar disorder patients with lifetime psychotic features (p=0.005, n=19) or schizophrenia (p=0.02, n=36) compared to non-psychotic patients and controls. The *KMO* Arg<sup>452</sup> allele was associated with bipolar disorder type 1 with psychotic features during manic episodes (p=0.003) compared to bipolar disorder type 1 patients without psychotic features and compared to blood donors. *KMO* Arg<sup>452</sup> associated with increased levels of CSF KYNA (p=0.03) and reduced lymphoblastoid and hippocampal *KMO* expression (p≤0.05).

**Discussion**

Using the five independent cohorts *KMO* expression data from SMRI, *KMO* eQTL data from HapMap3 using Genevar, and from German hippocampal biopsies, as well as *KMO* and KYNA data from two independent Swedish samples, we show findings on gene expression, gene sequence and metabolite level that collectively suggest that functional genetic variation in *KMO* influences the risk for psychotic features in mania of bipolar disorder patients. This provides a possible mechanism for the previous findings of elevated CSF KYNA levels in those bipolar patients with life-time psychotic features and positive association between KYNA levels and number of manic episodes. *Published in Molecular Psychiatry 2013 Mar 5.*

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**WHOLE GENOME DNA SEQUENCING IDENTIFIES VARIANTS SHOWING ALLELIC ASSOCIATION WITH BIPOLAR AFFECTIVE DISORDER**

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**Background**

A number of different loci and chromosomal regions have been reported to be implicated in bipolar disorder (BD) through GWAS. These studies have succeeded in identifying specific regions which could be implicated in disease aetiology. It is therefore probable that rare aetiological variants exist in these regions. Variants increasing genetic susceptibility to bipolar disorder may be found in non-protein coding control regions of genes as well as in exons. We have sought to detect these variants using whole genome sequencing data from 99 BD subjects. Two of the best implicated BD susceptibility genes are CACNA1C (L-type voltage gated calcium channel alpha-subunit) and ANK3 (ankyrin 3). The region of CACNA1C that shows the strongest linkage disequilibrium signal from markers showing allelic association with BD is entirely within intron 3. For ANK3 there are two regions that show the strongest evidence for association and these are in intron 2 and intron 26 (isoform NM_001204403).
Methods
We have selected variants in coding and noncoding cDNA regions, splicing regions, promoter regions and in potentially functional regions of CACNA1C intron 3. Variants with markedly different allele frequencies in the bipolar samples compared to European samples from the 1000 Genome Project were then genotyped in 1,510 bipolar subjects and 1,095 controls.

Results
We identified an A to G base pair change in ANK3 (rs139972937) at position 8266 resulting in a missense amino acid change from an asparagine to a serine at protein position 2643. The G allele encoding for serine was associated with BD at p=0.042. A base pair change (rs79398153) in the 3rd intron of CACNA1C likely to affect an ENCODE-defined control region also showed allelic association with BD (p=0.015).

Discussion
SNPs at the ANK3 and CACNA1C loci previously associated with BD in previous GWAS were not found to be in linkage disequilibrium with the two new possible aetiological base pair changes that we have found.

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BIPOLAR POLYGENIC LOADING AND BIPOLAR SPECTRUM FEATURES IN MAJOR DEPRESSIVE DISORDER
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Background
Understanding the relationship between bipolar disorder (BPD) and major depressive disorder remains a nosologic challenge with substantial clinical implications. Indeed, Kraepelin considered bipolar disorder and major depressive disorder (MDD) to be aspects of a common underlying illness. More recently, a bipolar spectrum disorder has been proposed to capture those individuals with depression who have risk factors and symptomatic presentation similar to bipolar depression. Features of depression that may be associated with bipolarity include family history of bipolar disorder, an earlier age of onset of depression, suicidality, more severe episodes occurring with greater frequency, atypical features, and subclinical manic and psychotic symptoms. Family and genetic studies indicate overlapping liability for MDD and BPD. Whether this shared genetic liability influences clinical presentation is not known.

Methods
Polygenic risk score for bipolar disorder, derived from a large genome-wide association meta-analysis, was generated for each subject of European-American ancestry (n=1274) in the STAR*D outpatient MDD cohort. Scores were generated using all SNPs from the meta-analysis that met 8 p-value thresholds. SNPs contribution to the score was weighted by log of the OR in the meta-analysis.
A multivariate model of features of depression associated with bipolar disorder in the literature was created. All features assessed in STAR*D were included in a correlation analysis. Features with correlation to at least one other feature were included, and the model consisted of history of suicide attempt, onset at or before age 18, manic symptoms, psychotic symptoms, atypical depression, 3 or more depressive episodes and severity as measured by the Hamilton Rating Scale for Depression. Generalized linear mixed models were employed to estimate strength of association between bipolar polygenic loading and these features as a group. Replication analyses were performed in the Netherlands Study of Depression and Anxiety and the Mannheim depression cohort. Not all clinical features were available in all cohorts, so a second model with 5 features consistent across cohorts was generated and tested in all 3 cohorts. Subclinical mania was assessed separately because of the significant difference in assessment strategies.

Results
The full multivariate model was significantly associated with bipolar polygenic risk score (F=2.07, df=7, p=0.04 at \( P_T = 0.01 \)) in STAR*D. Results were similar across all p-value thresholds though there was less evidence for association at the highest and lowest thresholds. Post-hoc univariate analyses of individual clinical features demonstrated that the major contributors to this omnibus association were onset of illness at age 18 or younger (OR=1.2, p=0.003), history of suicide attempt (OR=1.21, p=0.03), and presence of at least one manic symptom during depressive episode (OR=1.16, p=0.02). The maximal variance in these traits explained by polygenic score ranged from 0.8-1.1%. While the full 7-feature model could not be examined in the replication cohorts, a 5-feature model tested in all 3 cohorts was significant in STAR*D but was not significant in the 2 replication cohorts.

Discussion
This analysis used a multivariate approach to assess association with a set of categorical variables for which the a priori hypothesis is for association to the set rather than necessarily to individual items. It is both a more appropriate test for this hypothesis and also reduces concerns about type 1 error since only 1 omnibus test is performed. The findings suggest that bipolar genetic loading is associated with features of bipolar spectrum illness in individuals with MDD in STAR*D, but these findings were not supported in additional cohorts. Replication in studies like this is challenging. There are few cohorts with as detailed assessments as these, and ascertainment and assessment differences may still influence the results. The methodological approach developed here may prove useful in applying genetic data to clarify psychiatric nosology in future studies.

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SEQUENCE ANALYSIS OF CANDIDATE GENES IN SUICIDE SEVERITY
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Background
A number of novel candidate genes have been identified in recent genome-wide association studies of suicide attempt in bipolar disorder (Willour et al, 2011; Perlis et al, 2010). However, the historical candidate genes did not appear to be significant in these studies. Perhaps a combination of rare and common variants may contribute to the predisposition to suicidal
behavior.

Methods
We performed high-throughput DNA sequencing of 202 genes (Nelson et al, Science 2012) in our bipolar disorder cases of self-reported and genetically ascertained European ancestry. We analyzed the phenotype of suicide attempt as well as suicide severity score (from the Schedule for Clinical Assessment in Neuropsychiatry SCAN: 0=non-suicidal; 1=suicide plan/ideation; 2=suicide attempt without serious harm; 3=suicide attempt with serious harm; 4=suicide attempt designed to end life; N=227). We conducted preliminary analysis using PLINK, with history of alcohol use disorder as well as sex and age were included as covariates.

Results
We detected 3199 DNA variants across the 202 gene regions in our bipolar disorder sample. Among the findings, we found a number of DNA variants in NTRK2 and HTR1A to be nominally associated with suicide severity scores. These region is also one of the top findings for the analysis of lifetime history of suicide attempt.

Discussion
We conducted a high-throughput targeted sequence analysis of suicide severity in bipolar disorder and found a number of gene regions to be possibly associated with suicidality. We will be employing additional methods, including other analysis modalities and Sanger sequencing to further characterize and validate these findings, and will attempt to replicate the interesting results in other bipolar disorder samples.

ADVANCED PATERNAL AGE IS ASSOCIATED WITH EARLIER AGE AT ONSET IN BIPOLAR DISORDER
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Background
Advanced paternal age (APA) is a risk factor for de novo mutations that may contribute to autism and schizophrenia. APA has been reported to be associated with earlier age at onset of bipolar disorder (BD) in a population study using the Swedish national register (Frans et al. 2008). Previously we reported that de novo copy number variants were also associated with earlier age at onset in BD (Malhotra et al. 2011). Here we used independent clinically-ascertained samples to test the hypothesis that advanced paternal age is associated with earlier age at onset of BD and explore confounding variables.

Methods
A total of 427 subjects with bipolar I disorder or schizoaffective bipolar disorder and known
paternal age at birth were identified. This consisted of a discovery sample of 190 subjects and a replication sample of 237 subjects. Information on several variables was available including age-at-first-treatment (AAFT), age at onset first mania or major depression (AFMD), birth order, number of siblings, and paternal education level. Linear regression analysis was performed with XLSTAT v2011.2.01.

**Results**
We observed a significant (p=0.01) association between paternal age and earlier AAFT in both the discovery and replication samples. In both samples combined, AAFT was reduced by 1 year for every 5 years of advancing paternal age (p<0.0001; adjusted R²=0.029). A similar but less significant association was observed for AFMD (p<0.03). The observed associations between APA and AAFT were independent of proband’s sex, birth order, number of siblings, and paternal education level.

**Discussion**
APA is associated with earlier age at onset of BD in clinical samples. These results support the earlier findings and suggest that de novo mutations could play a role in BD. Further studies are warranted.

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**GENETIC AND ENVIRONMENTAL DETERMINANTS OF DEPRESSION: A GENOME-WIDE ASSOCIATION STUDY (GWAS) AND GENOME-WIDE GENOTYPE-ENVIRONMENT INTERACTION (GWGEIS) OF LATE-LIFE DEPRESSION IN THE WOMENS HEALTH INITIATIVE**

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**Background**
Depression is a common mental health problem in the general population, but especially among women; one in five people will experience a depressive disorder in their lifetime, with females being twice as likely as males to experience the disorder. Epidemiologic studies have long established that both genetic and environmental factors are important in the etiology of depression. Although the role of specific environmental pathogens (e.g. childhood adversities, stressful life events) and protective factors (e.g. social support) is well established, for both the etiology and course of depression, little progress has been made in identifying specific genes associated with depression. Genome wide association studies (GWAS) are promising in this regard. However, as of this writing, no robust and consistent genetic variants for depression have been identified through GWAS. Some have interpreted this lack of success to mean that “SNPs with substantial odds ratios are unlikely to exist” for depression. An alternative explanation is that prior GWAS are limited by their exclusive focus on diagnoses, rather than quantitative traits (e.g. depressive symptoms), and by their failure to consider the role of environmental factors in modifying relationships between genetic variants and psychiatric disorders. Our study will address these research gaps by conducting two sets of parallel analyses, both focusing on late-life depression: (1) a genome-wide association study (GWAS); and (2) a genome-wide gene-
Methods
Data come from the Women’s Health Initiative (WHI), a large population-based, longitudinal study of the determinants of cardiovascular disease, osteoporosis, and cancer among post-menopausal women in the United States. Women were between ages 50-79 at the time of enrollment. We focused specifically on African American (n=8,565) and Hispanic (n=3,709) women, making this the first large-scale GWAS of depression in these population groups.

Genetic Variation. All participants were genotyped using the Affymetrix 6.0 chip. A total of 934,940 SNPs were genotyped. Additional SNPs were imputed using the HapMap reference panel by the WHI Analytical Committee using data from the 1000genomes project.

Environmental Exposures. We examined the joint effect of common genetic variants with four environmental exposures, all of which have been associated with depression: (1) stressful life events, (2) social support, (3) smoking status, and (4) hormone therapy. Late-Life Depression. Depression was assessed using two measures. Depressive symptoms were assessed using six items from the Center for Epidemiological Studies of Depression Scale (CES-D), capturing the following symptoms: feeling sad or depressed; restless sleep, anhedonia, crying spells, and feeling people disliked you. Probable clinical depression was based on an algorithm developed by Burnam that combines the CES-D items with two items from the Diagnostic Interview Schedule (DIS). In this analysis, controls were defined as anyone with a score of 0.009 or below on the Burnam measure and who was not taking an antidepressant medication. Cases were defined according to two criteria: (1) scoring at or above 0.06 on the Burnam measure; (2) satisfying criterion 1 or taking an antidepressant medication. We performed GWAS analyses for the two depression phenotypes in African American women and Hispanic women. We used linear regression for the CES-D score analysis and logistic regression for the binary (case-control) study. Independent loci represented by a single SNP were then obtained by LD clumping using PLINK.

Discussion
Thus far, GWAS have exclusively tested genetic main effects. Moreover, studies of genotype-environment interaction (GxE) have been limited by a candidate gene approach and exclusive focus on single gene-phenotype relationships. We think results of the current study will allow us to identify, using an unbiased approach, novel loci associated with depression and depressive symptoms.

ASSOCIATION STUDY OF 240,000 RARE CODING VARIANTS IN BIPOLAR PATIENTS AND CONTROLS FROM GERMANY AND NORWAY
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Background

Genome-wide association studies (GWAS) of bipolar disorder (BD), a highly heritable disorder of mood with a lifetime prevalence of approximately 0.5-1% in all populations world-wide, have identified several common genetic risk factors. It is currently unclear, to what extent low-frequency and rare variants contribute to disease development. A valid hypothesis seems to be that low-frequency and rare variants in coding gene regions have a higher probability to have a functional (deleterious) effect. This subset of human genetic variation might therefore be enriched for disease-relevant variants. The Illumina HumanExome arrays make particularly this window of genetic variation accessible for association studies.

Methods

In the present study, we used that chip to test 895 bipolar patients and 2,366 population-based controls from Germany as well as 419 bipolar patients and 339 controls from Norway. Variants were assessed using the Illumina HumanExome v1 bead chip which contains 240,000 rare coding variants derived from whole exome sequencing. Genotypes were called in a single GenomeStudio project to minimize problems due to clustering biases. Clusters were checked and corrected by zCall. Statistical analysis was performed using a Cochrane-Mantel-Hansel test statistics with two groups. Clusters for all associated variants were re-checked manually.

Results

The two variants with the strongest association to bipolar disorder were found in the gene SYNE2. Interestingly, common variants in another member of the same gene family (SYNE1) had shown genome-wide significant association in the discovery step of the first mega-analysis of bipolar disorder performed by the international Psychiatric Genomics Consortium (PGC; Sklar et al. 2011).

Discussion

We are currently aiming to follow-up this and other strong findings of our analysis in independent samples of bipolar disorder genotyped on the HumanExome array.

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A GENOME WIDE ASSOCIATION BETWEEN MIGRAINE IN BIPOLAR DISORDER AND NEUROBEACHIN

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Background

Migraine is a common headache disorder, with a prevalence of approximately 12%. It is characterized by unilateral attacks, and in some patients accompanied by visual aura symptoms (1). Bipolar disorder is a mood disorder ranging from severe depression to mania, with migraine
as a common comorbidity seen in 25-45% (2,3). Bipolar disorder patients with migraine are more likely to have a worse outcome than those without migraine(4).

Methods
We performed a genome wide association analysis on 460 bipolar patients with self-reported migraine and 914 bipolar patients without migraine. The individuals are from the TGEN sample, a part of the Bipolar Genetics Study (BiGS). Replication was attempted in the GAIN sample, a separate part of BiGS.

Results
We found a genome wide significant association between migraine in bipolar disorder and rs1160720, an intronic single nucleotide polymorphism (SNP) in NBEA (P-value 2.97x10^{-8}, OR: 1.82, 95% CI: 1.47-2.25). The SNP resides in a linkage disequilibrium block with several other associated SNPs, spanning several exons. We were not able to replicate our finding in the GAIN sample.

Discussion
NBEA encodes the protein neurobeachin, which is involved in the transport of neurotransmitter receptors, among them glutamatergic receptors (5). This receptor system is implicated in both bipolar disorder and migraine (6). Our study provides putatively new clues to the cause of migraine in bipolar disorder, though further studies are needed to verify this association.

References:

PERCEIVED STRESS IS NOT JUST NEUROTICISM AND DEPRESSION: A MULTIVARIATE TWIN ANALYSIS
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Background
Chronic stress is a well known risk factor for psychiatric disorders such as major depression, schizophrenia and anxiety disorders. Neuroticism, is a personality trait which is defined by the inclination to worrying, being insecure, self conscious and temperamental. It is highly associated with perceived psychological stress and increases the risk for developing depression. Research has shown strong correlations between neuroticism, symptoms of depression and perceived psychological stress as well as shared genetic effects for neuroticism and symptoms of depression. Nevertheless it is still unclear whether shared genetic or environmental effects account for the association between perceived psychological stress and these two factors. The aim of the present study was to analyse the extent to which the genetic determinants of neuroticism and depressive symptoms are different from those underlying perceived psychological stress.

Methods
Multivariate structural equation models, including age and sex as modifiers, were fitted to the total sample of 798 (female=459) adolescents (mean age 15.5 years) including 139 monozygotic (MZ) and 241 dizygotic (DZ) twin pairs. Stress was measured using Item Response Theory (IRT) scores of the Perceived Stress Scale (PSS) and/or the Daily Life Stressors Scale (DLSS). Neuroticism was measured by the Neo Five Factor Inventory (Neo-FFI) or the Junior Eysenck Personality Questionnaire (JEPQ), depending on the age of the participants. Depressive symptoms were assessed by the IRT-scores of the Somatic and Psychological Health Report (SPHERE).

Results
Significant influences of additive genetic effects and unshared environmental effects were found for neuroticism, depressive symptoms and perceived psychological stress. Our data did not support a role of shared environmental effects for any of these factors. A Cholesky decomposition of genetic covariance found that the first factor, loading primarily on neuroticism and accounting for 50% of its variance, also accounted for 28% of depression and 21% of stress variance. A second factor loading primarily on depression (24%) also accounted for 14% of stress variance. This left a specific genetic contribution to stress of 18%, which was significant.

Discussion
Our results suggest that the genetic effects underlying neuroticism are largely shared with those that influence liability to depressive symptoms and perceived psychological stress: however, there are also separate genetic effects for depressive symptoms and perceived psychological stress that are not shared with neuroticism. The source of these trait specific effects needs further investigation and we are currently measuring hair cortisol in these twins towards this end.

PHYSIOLOGICAL AND GENETIC MECHANISMS UNDERLYING BROODING RUMINATION IN WOMEN AT RISK FOR DEPRESSION
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**Background**

There is growing evidence that rumination, perhaps specifically brooding rumination, is a core feature of depression and that it contributes to the development and maintenance of the disorder (Nolen-Hoeksema, 2008). However, little is known about physiological mechanisms that may underlie rumination. Building from models suggesting that rumination and low heart rate variability (HRV), one key mechanism implicated in depression risk, are driven by alterations in the same neural circuit (heightened amygdala reactivity combined with decreased prefrontal control), we predicted that lower levels of HRV would be associated with higher levels of brooding rumination, that women at high risk for future depression (i.e., those with a history of past major depressive disorder [MDD]) would exhibit lower levels of HRV and higher levels of brooding, and that HRV would mediate the link between women’s history of MDD and current levels of brooding. Second, we examined genetic influences on the variables in this model. We predicted that COMT Val158Met genotype, which has been linked to deficits in prefrontal functioning (Mier, Kirsch, & Meyer-Lindenberg, 2010), would be associated with HRV and brooding rumination, particularly among women with a history of MDD.

**Methods**

Participants in this study were 97 women recruited from the community. The average age of women in our sample was 40 years and 90% were Caucasian. Women in the depression group (n = 47) were required to have a lifetime history of MDD, but to currently be in full remission from the disorder. Women in the control group (n = 50) were required to have no lifetime diagnosis of any DSM-IV mood disorder and no current Axis I diagnosis. Electrocardiogram data was collected during a five-minute rest period and used to calculate the power density in the high frequency band of HRV. DNA was isolated from buccal cells and used to genotype COMT Val158Met. Within our sample, 28 women were met/met carriers, 50 were met/val, and 19 were val/val. Genotype frequencies did not vary significantly from Hardy Weinberg Equilibrium (χ² = .15, p = 0.69).

**Results**

As predicted, higher levels of HRV were significantly correlated with lower levels of brooding, r = -.24, p = .02. Next, the link between depression history and low HRV was significant among women homozygous for the COMT met allele, F(1, 25) = 4.67, p = .045, η² = .16., but not among val/met heterozygotes, F(1, 47) = .45, p = .53, η² = .01 or val homozygotes, F(1, 16) = 3.85, p = .09, η² = .19. In addition, history of major depression was significantly related to brooding rumination, F(1, 92) = 20.25, p < .001, η² = .18, though this link was not moderated by the COMT genotype, F(1, 92) = .10, p = .80, η² < .001. Finally, supporting the full moderated mediation model, we found that the indirect pathway from depression history to brooding rumination through HRV was significant among women homozygous for the COMT met allele, beta = .08, 95% CI = .003, .21, p < .05, but not among carriers of val/met genotype, beta = .02, 95% CI = -.01, .08, ns, or val homozygotes, beta = -.05, 95% CI = -.16, .004, ns.

**Discussion**

The results largely supported our hypotheses, suggesting that HRV may be a physiological mechanism underlying brooding for genetically at-risk individuals. High HRV is thought to reflect better inhibition of cardioaccelatory circuits and amygdala reactivity through PFC vagal pathways. Therefore, when at-risk individuals experience negative affect, those who have higher
HRV may display more efficient regulation of their emotions, leading to less brooding rumination. In summary, the current results provide important information about individual variability in the degree women at-risk for future depression (i.e., those with a prior history of major depressive disorder) exhibit brooding rumination.

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THE RELATIONSHIP BETWEEN TRAUMATIC BRAIN INJURY AND GENETIC RISK FOR BIPOLAR DISORDER

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Background
It is widely believed that traumatic brain injury (TBI) may contribute to the onset or development of bipolar disorder (BD). While those with a history of TBI are more likely to develop BD, individuals with this psychiatric disorder are also more likely to experience a head injury (HI). Comparisons of polygenic risk allele burden in BD with and without TBI is one way to parse cause from effect. We hypothesized that those with a history of TBI prior to BD would have a significantly lower polygenic allele burden, reflecting the contributory role for TBI.

Methods
Samples comprised the previously collected and genotyped sample of bipolar I cases (n=2,836) and controls (n=2,744) known as GAIN and TGEN1. To create the weights that would be used for scoring our target sample, we calculated the logarithms of odds ratios in a genotyped sample of bipolar cases and controls from the Psychiatric GWAS Consortium (PGC), for which there were 1,897,207 single nucleotide polymorphisms (SNPs) after frequency and genotype pruning. To generate the profile of polygenic scores in from the weights established in the PGC training sample, we ran a profile analysis in PLINK (v 1.07), with 108,834 SNP and allele predictors found in our pruned and imputed target sample. We analyzed the data with logistic regression models: the polygenic scores (independent variable) were analyzed across various TBI phenotypes. After excluding those in the genotype file that had missing identifiers/phenotypes, we compared the scores of BD cases (n=2,191) to controls (n=1,496). In the GAIN sample (n=1,000 BD cases), we compared scores for three case definitions: cases with and without HI, cases with HI with and without loss of consciousness (LOC), and cases with and without HI before onset of BD.

Results
Logistic regression analyses showed that the polygenic scores distinguished the cases from controls (p<0.0001) in the GAIN and TGEN samples (n=3,687), as expected given the sample overlap with the PGC. For the TBI case definitions examined in the GAIN cases sample, there was a significant difference (p<0.009) between allele burden scores of those with HI prior to onset of BD (n=147) and those with HI after onset of BD (n=159).

Discussion
BD cases with a history of TBI before BD onset have a lower polygenic allele burden. These results support a causal relationship between TBI and BD, at least in individuals at genetic risk for BD. The types of head injuries most likely to contribute to BD, and the mechanism by which
this occurs, need to be further examined. Clinically, it may important to probe for past TBI to better understand etiology and treatment for BD. These results also underscore the importance of head protection in children, particularly those with a family history of mood disorders.

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GENOME-WIDE ASSOCIATION STUDIES AND POLYGENIC SCORE ANALYSES OF SUICIDAL IDEATION AND ATTEMPT IN MOOD DISORDERS.

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Background
Suicide is a global public health problem and the 10th leading cause of death. Over 90% of suicide attempters or victims have a psychiatric diagnosis, most commonly mood disorders. While suicide is a comorbidity which has been associated with all psychiatric disorders, twin, family and adoption studies have recognised a genetic diathesis for suicidal behaviour which is independent of other psychopathologies. Candidate genetic association studies on suicide have produced many discrepant results, while genome-wide association studies (GWAS) have been more successful, with some plausible genes reaching suggestive significance in several studies. To date, molecular genetic studies have tested one gene at a time. However, suicidal behaviour is a complex trait resulting from the additive and multiplicative interaction of many risk variants with small effect sizes. Here we conduct further genome-wide investigation and, for the first time, polygenic score analysis, in cohorts of patients with mood disorders, a high risk group for suicidality.

Methods
RADIANT (n=2023) and GSK-Munich (n=807) samples of patients with recurrent unipolar depression and the Bipolar Affective Disorder Case Control (BACCs) (n=470) sample were used for these analyses (Schosser et al., 2011, Gaysina et al., 2009, Muglia et al., 2010). Participants were diagnosed according to DSM-IV criteria and information was recorded on suicidal behaviour during their worst and second worst episodes of depression, using the Schedules for Clinical Assessment in Neuropsychiatry Interview (SCAN). Attempter status (case) was defined as self-injury designed to result in death, with remaining non-attempters classified as controls. In the Genome Based Therapeutic Drugs for Depression Study (GENDEP), 747 depressed patients were randomised to a 12 week treatment with either escitalopram (a selective serotonin reuptake inhibitor) or nortriptyline (a tricyclic antidepressant) (Perroud et al., 2012). Treatment emergent suicidal ideation (TESI) or treatment worsening suicidal ideation (TWOSI) were assessed weekly using items from the Hamilton, Beck and Montgomery-Ashberg Depression Rating Scales, combined into a composite score. Individuals with either TESI or TWOSI were regarded as cases and those with no suicidal ideation or baseline ideation which did not worsen during treatment were used as controls. Genotyping was carried out on Illumina BeadChip microarrays. Following quality control procedures, GWAS were performed in each dataset using PLINK software and a meta-analysis of variants associated with suicide attempt was carried out between the RADIANT, GSK-Munich and BACCs results.

A candidate study on genes previously associated with suicide attempt, related endophenotypes
or suicidal ideation during treatment will also be carried out. Polygenic scores of GWAS data from each of these datasets will be tested for ability to predict the suicide phenotype in the other samples as well as investigating the predictive ability of the polygenic scores for major depressive disorder reported by the Psychiatric Genomics Consortium (Ripke et al., 2013).

Results
In the GWAS of individual phenotypes, no significant evidence for association was detected at any SNP, in either individual studies or in meta-analysis. Polygenic score analysis between these study cohorts, and from results of the Psychiatric Genomics Consortium studies into these cohorts is in progress.

Discussion
The low power to detect association at individual SNPs was expected given the results of other genome-wide studies. Polygenic effects across mood disorders and suicide attempts may have greater success, in particular in elucidating how the genetic susceptibility to mood disorders overlaps with the genetic susceptibility for suicide attempt. The genetic architecture of mood disorders, suicide attempt and suicidal ideation are complex, and detailed analyses within and between data sets may increase our understanding of the interrelationship between the psychiatric co-morbidities.

WHOLE EXOME SEQUENCING OF TRIO FAMILIES OF BIPOLAR DISORDER.
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Background
Bipolar disorder is one of the two major mental disorder and the heritability is around 85%(Cardno et al, Arch Gen Psychiatry 1999, McGuffin et al, Arch Gen Psychiatry 2003). Genome-Wide Association Studies (GWAS) successfully identified a number of common SNPs that are associated with bipolar disorder with genome-wide significance but the contribution is weak. Recently, Next-Generation Sequencing (NGS) technology enables us to detect rare variants and especially as mendelian disease, many causative mutations have been found. To identify molecular pathways related to bipolar disorder, we compared the deleterious mutations transmitted to, or un-transmitted to the proband using whole exome sequencing of trio families of bipolar disorder.

Methods
Fifty probands with bipolar disorder and their parents were enrolled. Each individual was interviewed by trained psychiatrists using MINI. Target regions were enriched from whole-blood DNA or saliva using the Human SureSelectXT V4. Whole-Exome sequencing for individual samples was performed using Hiseq2000(Illumina). Reads were mapped to hg19 with BWA and PCR duplicates were removed with Picards. Local re-alignment and variant calling was carried out using GATK. All variants were annotated with ANNOVAR. We only focused on the target sites that were covered with at least 20 unique reads. The criteria for candidate variants were non-synonymous single nucleotide variants, not in dbSNP137(excluding MAF < 0.01 or reported
as disease related), predicted “disease causing” by SIFT and “possibly damaging or probably damaging” by PolyPhen2. All variants observed in each family were divided into two groups, transmitted (found in a Proband and the parent) or un-transmitted (found in a parent only) variants. Finally we performed a GO analysis to assess what pathways are enriched among the transmitted or un-transmitted variants.

Results
More than 99% of targeted regions were covered by 1 read and mean target coverage was 87.8% at 20x reads. A preliminary analysis of 7 families showed transmitted mutations in 280 genes and un-transmitted mutations in 310 genes. Among these mutations, 11 transmitted and 21 un-transmitted mutations were found in two or more families. Through our approach, we found that two families have a mutation in SYNE1.

Discussion
A SNP of SYNE1 was found to be associated with bipolar disorder at the genome wide significance level (Green et al, Mol Psychiatry 2013). SYNE1 mutations were transmitted from an affected parent in both of the two families. Confirmation of these mutations by Sanger sequencing is ongoing.

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LEUKOCYTE TELOMERASE ACTIVITY IN BIPOLAR DISORDER DEPRESSION TREATED WITH LITHIUM CARBONATE
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Background
Background: telomeres are dna-protein complexes that cap linear dna Strands, protecting dna from damage. The enzyme telomerase is the main Mechanism that regulates the length of telomeres thus promoting Cellular viability. In normal human cells, telomerase levels are Insufficient to maintain telomere length resulting in progressive Attrition with each cell division; thus, telomere length can estimates of the replicative history of cells. Recently, shorter telomeres Length have been reported in bipolar disorder (bd), but it is not Clear how telomeres participate on the pathophysiology of bd. To investigate this matter we started studying the effects of lithium, a First line agent to treat bd, on telomerase activity.

Methods
Methods: Peripheral blood mononuclear cell (pbmc) telomerase activity was Assessed in 28 medication-free bd depressed individuals and 23 healthy Controls. In total, 28 bipolar depressed individuals were then treated with lithium therapy for 6 weeks, and pbmc telomerase activity was reassessed in 21 of these individuals after treatment. Pre- and Post-treatment symptom severity was rated with the Hamilton depression Rating scale (hdrs).

Results
Results: in the bipolar group, individuals with Greater decreases in hdrs during lithium treatment were associated with lower telomerase activity at endpoint. There was no difference Between pre
treatment telomerase activity and controls. There was no correlation between hdrs scores and telomerase activity. Conclusion: This is the first study describing telomerase activity in subjects with bipolar disorder treated with lithium.

Discussion
The association between pbmc telomerase activity and depressive symptomatology in lithium-treated BD patients might reflect a novel aspect of BD pathophysiology and could be a biomarker of antidepressant response. Further investigation: the study of leukocyte telomere length in the subjects described above is underway and the results will be also presented at the congress.

IDENTIFICATION OF A RARE MISSENSE VARIANT IN THE NEURONAL GLUTAMATE TRANSPORTER SLC1A1 ASSOCIATED WITH OBSESSIVE-COMPULSIVE DISORDER
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Background
Obsessive-compulsive disorder (OCD) is an etiologically heterogeneous neuropsychiatric disorder characterized by unwanted and repeated thoughts (obsessions) and repetitive behaviors (compulsions). Twin studies reveal heritability estimates for OCD symptoms in children ranging from 45-65% compared with 27-48% in adult-onset cases suggesting a strong role for genetic determinants, particularly in childhood onset cases. While many approaches have been attempted to identify genetic variants associated with OCD, the discovery of causal variants remains elusive. A limitation of earlier studies is that they have almost exclusively focused on common variants, even though there is growing evidence that rare variants play a major role in complex neuropsychiatric disorders. These rare variants are often found in the exome, the coding region of the genome in which variants are arguably most likely to have a functional effect. However, no whole exome or genome studies have been conducted in OCD. Examining the exome of pedigrees with multiple affected individuals is particularly useful. Using this method, one can prioritize variants shared by affected family members, particularly distantly related individuals that are more likely to share rare variants because of a shared disease phenotype rather than because of a shared ancestry. Our objective is to use a multi-phasic approach including CNV testing, linkage analysis and next generation sequencing on members of extended pedigree families in order to identify rare variants associated with OCD.

Methods
Written informed consent was provided by all participants, or by the parents of child subjects. DNA samples were derived from blood and extracted using standard methods. We prioritized families for the study based on: the presence of probands with an early onset of OC symptoms (<12yrs), families with greater than 2 affected individuals, and phenotypic similarity among affected individuals. CNV analysis was performed on most of the family members of 1 OCD family at The Center for Applied Genomics, SickKids (TCAG) using the Affymetrix Cytoscan HD platform. Whole exome sequencing was performed on DNA from the proband and the most
distant affected relative (2nd or 3rd degree) in the same OCD family at TCAG using the ABI SoLID platform. Genetic variants shared by both affected individuals sequenced were filtered based on location within haplotype blocks shared by all affected individuals, and frequency in the general population. In silico functional analysis (SIFT, PolyPhen2, Provean) was also used in order to identify variants that may have a detrimental impact on protein function. Rare or novel variants of high interest were validated by Sanger sequencing in order to confirm segregation with disease in the family.

**Results**

Our preliminary results have identified a rare missense variant in the *SLC1A1* gene. *SLC1A1* is a glutamate transporter expressed in neurons of brain regions implicated in OCD, and is the only gene shown to be consistently associated with OCD in multiple candidate gene studies. This is the second missense variant within this gene and to be identified and associated with OCD in a family.

**Discussion**

Our preliminary findings, which have identified a variant within a known gene associated with OCD, demonstrate the viability of our methodology to identify rare variants in common complex diseases. In addition to functionally validating these variants, we will also work to identify genetic variants in more families that will give us further clues to the biological pathways associated with OCD. This will not only be an important contribution to the current body of knowledge in OCD and genetics, but also of significance for the families affected by these genetic variants.

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THE ROLE OF POLYMORPHISMS IN ONE GENE OF THE GLUTAMATERGIC SYSTEM IN THE DEVELOPMENT OF THE OBSSESSIVE-COMPULSIVE DISORDER

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**Background**

Obsessive-compulsive disorder (OCD) is characterized by recurrent and persistent thoughts, urges, or images that are experienced as intrusive and unwanted and that usually cause marked anxiety or distress (obsessions) and/or repetitive behaviors (e.g., hand washing, ordering, checking) or mental acts (e.g., praying, counting, repeating words silently) that the person feels driven to perform in response to an obsession, or according to certain rules (compulsions). The lifetime prevalence of OCD is estimated to be 0.3–3.1%, based on population-based surveys conducted in many communities nationally and internationally. The etiology of OCD is largely unknown, but family and twin studies suggest that genes play a significant role on its underlying pathophysiological mechanisms. The objective of this study was to analyze four single nucleotide polymorphisms (SNPs) in the gene *SLC1A1* (*rs12682807, rs301443, rs301430, rs301434*) based on several lines of evidence that suggest that OCD may be due to hyperactivity of glutamatergic system. Accordingly, linkage and candidate gene association studies support the involvement of *SLC1A1*, a gene that encodes the carrier neuronal glutamate in the development of OCD. This gene is the region most closely linked to disease (9p24) in accordance with linkage studies. A number of studies found positive associations between OCD and several SNPs or
haplotypes in the gene encoding the glutamate transporter (SLC1A1). Our study aimed at determining the frequency of these five SLC1 polymorphisms in OCD patients and controls living in the Brazilian Rio de Janeiro state.

Methods
To this end, we genotyped 190 subjects with OCD and 190 healthy controls, assessed by means of the Mini International Neuropsychiatric Interview (MINI). Symptoms profiles were evaluated using the Florida Obsessive-Compulsive Inventory (FOCI) and the Obsessive-Compulsive Inventory-Revised (OCI-R). The SLC1 polymorphisms were analyzed by amplification of the fragment of interest by using Taqman (Life Technologies do Brasil) assays specific for the Polymerase Chain Reaction (PCR) in real time.

Results
The genotype distributions were in Hardy-Weinberg equilibrium. We were unable to find significant differences between patients and controls in terms of the frequency of these two SNPs (rs12682807, rs301443, rs301430 and rs301434 (P=0.731, P=0.098, P=0.685, P=0.506, respectively). No significant differences between the groups was also found when we analyzed the by the SNP allele. We did not observed significant associations between the polymorphisms rs12682807, rs301443, rs301434, rs301430 and dimensions of OCD.

Discussion
In conclusion, these preliminary results showed that SLC1A1 gene polymorphisms rs12682807, rs301430, rs301443 and rs301434 may not contribute to the etiology of OCD. Perhaps additional studies to assess the importance of these polymorphisms of the gene SLC1A1 in OCD are needed.

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SEROTONIN TRANSPORTER METHYLATION AND RESPONSE TO COGNITIVE BEHAVIOUR THERAPY IN CHILD ANXIETY DISORDERS
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Background
Anxiety disorders are the most common psychiatric disorders in childhood and are associated with a wide range of social and educational impairments. Childhood anxiety disorders have the poorest prognosis when they begin early, and often persist into adulthood. Cognitive Behaviour Therapy (CBT) is effective for the majority of childhood anxiety cases, although up to 40% of children retain significant impairments after CBT (James et al., 2005). Very few studies have investigated the source of this individual variation in treatment response. However, recent research in the newly emerging field of “therapygenetics” (Lester & Eley, 2013) indicates that individual differences in treatment response may have a genetic basis. Of particular interest is the Serotonin Transporter gene (SERT), which has been associated with disorders such as anxiety and depression. Furthermore, the short (S) allele of the serotonin promoter polymorphism (5HTTLPR) has been associated with poorer outcomes in high-stress environments but also better outcomes in positive environments. Recent research from our group demonstrated that anxious children with the SS genotype showed an increased response to CBT (Eley et al. 2012).
This finding suggests an interaction between genes and environmental influences. Epigenetic mechanisms that influence transcriptional regulation (such as DNA methylation) have been shown to be susceptible to the environment, making them plausible candidates for the biological embedding of experience.

Methods
We tested whether DNA methylation of CpG sites upstream of the SERT promoter were associated with response to CBT in a subsample of 168 children with a primary anxiety disorder. Participants were recruited from two sites (Sydney, Australia and Reading, UK), and ranged from 6-13 years of age. DNA was collected using buccal swabs at pre- and post- treatment. Extracted DNA was treated using sodium bisulfite, and SERT methylation determined using the Sequenom EpiTyper. SERT methylation was defined as a percentage at each time point (for each CpG site), and change in methylation was calculated from pre- to post-treatment time points. Only CpG probes detecting an average methylation (across all samples) of >5% were included. An average percentage of methylation at each time point and an average change score was also calculated for each sample, using only samples with data present for at least 5 of the 6 CpG sites. Treatment response was defined as the presence or absence of all anxiety disorder diagnoses, with “responders” defined as those with a clinical severity rating of less than 4 (on a scale of 0 to 8) by follow-up.

Results
We detected a significant 3-way interaction between clinical response, percentage methylation at pre- and post- treatment and CpG site ($F(5,77) = 2.39, p = 0.046$). A significant difference between treatment responders and non-responders was found in percentage methylation change (averaged across all sites) across treatment time ($t(115) = 3.20, p = 0.002$), with this effect being particularly strong for CpG site 4 in particular ($t(92) = 3.43, p = 0.001$). At this site, non responders displayed a decrease in methylation from pre to post treatment time points at CpG site 4 (mean change = -6.3%), while treatment responders showed an increase in methylation (mean change = 3.5%).

Discussion
The findings of this study imply that responders and non-responders may show differences in DNA methylation change across time (from pre to post treatment), most prominently at a CpG site upstream of the serotonin transporter gene. Results from further analyses investigating SERT methylation, 5-HTTLPR genotype and treatment response will also be presented. The possible relevance and implications of these findings within biological and clinical frameworks will be discussed.
Background
Individuals with panic disorder (PD) exhibit a hypersensitivity to inhaled carbon dioxide (CO₂), possibly reflecting a lowered threshold for sensing signals of suffocation. Animal studies have shown that CO₂-mediated fear behavior depends on chemosensing of acidosis in the amygdala via the acid sensing ion channel ASIC1a. We examined whether the human ortholog of the ASIC1a gene, ACCN2, is associated with the presence of PD and amygdala structure and function.

Methods
We conducted a case-control analysis (N = 414 PD cases, 846 healthy controls) of ACCN2 single nucleotide polymorphisms (SNPs) and PD. We then tested whether variants showing significant association with PD are also associated with amygdala volume (N = 1,048) and/or task-evoked reactivity to emotional stimuli (N = 103) in healthy individuals.

Results
Two SNPs at the ACCN2 locus showed evidence of association with PD: rs685012 (OR=1.315, gene-wise corrected p=0.011) and rs10875995 (OR=1.262, gene-wise corrected p=0.045). The association was stronger when only early-onset (age ≤ 20) PD cases were compared to controls. The PD risk allele at rs10875995 was associated with increased amygdala volume (p = 0.035), as well as task-evoked amygdala reactivity to fearful and angry faces (p = 0.0048).

Discussion
Genetic variation at ACCN2 appears to be associated with PD and with amygdala phenotypes that have been linked to anxiety proneness. These results support the possibility that modulation of acid-sensing ion channels may have therapeutic potential for PD.

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GENETIC ASSOCIATION OF THE TRANSCRIPTION OF NEUROPLASTICITY-RELATED GENES AND VARIATION IN STRESS COPING STYLE
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Background
Gene×environment (G×E) interactions are recognized to predict vulnerability to disorders such as depression and anxiety. Brain Derived Neurotrophic Factor (BDNF) has an important role in neural plasticity throughout life. It’s distinguished that BDNF almost certainly function through its high-affinity receptor, neurotrophic tyrosine kinase receptor 2 (NTRK2) (Squinto et al., 1991). BDNF and NTRK2 have also been found to be associated with major depression and antidepressant-like effects. Stress coping is defined as the cognitive and behavioral efforts made to conquer, endure, or decrease external and internal demands and the conflicts between them.
(Folkman et al., 1985). Such coping styles serve two main functions (coping strategies): the control or modification of the person–environment relationship that is the basis of stress (problem-focused coping) and regulation of stressful feelings (emotion-focused coping). The objective of this study is to investigate the genetic association of stress coping strategies with SNPs (single nucleotide polymorphisms) involved in neural plasticity, anxiety and depression (BDNF and NTRK2).

Methods
We measured and estimated stress coping using a Lazarus-type stress coping inventory (SCI), ego aptitude scale (EAS) and social adaptation self-evaluation scale (SASS). We examined 394 university students (162 females and 232 males) using SCI and EAS. Five SNPs containing BDNF (rs6265) and NTRK2 (rs11140800, rs1187286, rs1867283, rs1147198, rs10868235) and others were selected, according to their minor allele frequency (MAF >0.05). Logistic regression models were performed to test for association of the mean SCI and EAS scores with each allele (major-allele homozygote, heterozygote, and minor-allele homozygote). The significant α-value was set at α < 0.0025 (0.05/25). Statistical analysis of single-SNP or multiple-SNP was conducted using the SNPStats (Solé et al., 2006).

Results
We found the associations between SASS and confrontive-coping (Con), positive reappraisal (Pos), planful problem solving (Pla), distancing (Dis) seeking social support (See). Significant associations of BDNF with seeking social support (See), self controlling (Sel) and distancing (Dis) and that of NTRK2 with confrontive-coping (Con), positive reappraisal (Pos), planful problem solving (Pla), distancing (Dis) seeking social support (See) and were found.

Discussion
These results indicate that a common, functionally significant polymorphism in BDNF and NTRK2 differently modulates stress coping strategies including seeking social support (See), self controlling (Sel) and distancing (Dis) confrontive-coping (Con), positive reappraisal (Pos), planful problem solving (Pla). Social adaptation might be associated with stress coping style. These findings may provide insights to reactivity and vulnerability to social adaptation and stress coping strategies.

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MACHINE LEARNING METHODS TO REDUCE AND MOBILIZE THE DETECTION AND DIAGNOSIS OF AUTISM
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Background
The incidence of autism has increased dramatically over recent years, making this mental disorder one of the greatest public health challenges of our time. The standard practice of diagnosis is based on behavioral characteristics, as the genome has largely proved intractable for diagnostic purposes. Yet, the most commonly used behavioral instruments take up to 3 hours to administer by a trained specialist, contributing to the substantial delays in diagnosis experienced by many children, who may go undiagnosed and untreated until ages beyond when behavioral
therapy would have had more fundamental positive impacts. In an effort to mitigate these challenges, we have developed a machine-learning based system for accurate classification of autism that requires minutes to administer and that can be delivered via mobile technologies.

**Methods**

We use machine learning techniques to analyze a large collection of archived score sheets from two of the most commonly used behavioral instruments, the Autism Diagnostic Interview-Revised (ADI-R) and the Autism Diagnostic Observation Schedule (ADOS), in an effort to identify a small subset of behavioral categories that have most value in detection of children with autism. We then applied the resulting behavioral classifiers to over 5000 independent score sheets and several hundred home videos from children both with and without clinical diagnoses of autism to measure the sensitivity and specificity of the classification system overall. Next we administered and tested this system prospectively in a clinical sample of over 100 children to clinically validate the utility of the classification tool and its potential value for patient triage.

**Results**

Our classification approach matched the outcomes of the standard instruments in 97% of all autism and 92% of all non-autism cases, including a set of cases with learning delay and clinically challenging symptom presentation. Our results confirm that rapid analysis of home videos strengthens the confidence in classification, and that the method of video scoring can scale to match the size of the risk population. Finally our results demonstrate that pre-clinical screening through a mobilized system could have significant positive impact on the practice of screening and prioritization of the full risk population.

**Discussion**

Approaches that enable families to bridge the gap between initial warning signs of developmental delay and clinical diagnosis of autism quickly and effectively are critically needed for the field. Our goal in this study was to develop a machine-learning based system that can address this need and to test the evaluate the tool in three specific ways: (1) To prospectively validate the sensitivity and specificity of a rapid and mobilized method for detection of the core features of autism that combines home video with a parent-assessment report. (2) To assess the feasibility of obtaining relatively brief home videos of quality and content sufficient to detect behaviors consistent with an ASD diagnosis. And, (3) to detect the value of rapid, pre-clinical assessment of ASD for improving patient management at clinical sites. In both archival and clinical samples, our tool shows greater than 95% accuracy, demonstrates the feasibility of mobile, pre-clinical assessments and highlights the possibility of using mobile techniques to reduce bottlenecks and enable reach to a significantly greater percentage of the population in need.

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**FUNCTIONAL CHARACTERIZATION OF AUTISM SPECTRUM DISORDER-RELATED CNTNAP2 PROMOTER VARIANTS**

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Background

Autism Spectrum Disorders (ASD) are highly genetic pervasive disorders with early onset in childhood and encompass early childhood autism, Asperger syndrome and developmental disorders-not otherwise specified. At the genetic level, a large number of susceptibility genes were reported, which are involved in synaptic processing and neuronal development. One of the best genes investigated is the contactin-associated protein-like 2 gene (CNTNAP2). Several variants of CNTNAP2 are associated with ASD and language development. Recent exome sequencing studies report a higher frequency of missense mutations in this gene in patients compared to controls. In mouse models, knock-out of CNTNAP2 induced ASD-like behavior and a deregulation of neuronal synchronization and migration. mRNA expression studies showed a brain area-specific regulation of CNTNAP2, especially during early stages of neuronal development. It can be assumed that this regulation is mediated by the respective promoter sequence. Despite the obvious impact of genetic variants within the promoter of CNTNAP2, so far no study focused on its genetic and functional characterization in ASD. Here, we aimed at testing genetic variants of the CNTNAP2 promoter for association in ASD and at investigating for their effect on transcription factor binding sites in silico and on transcriptional efficiency during neuronal differentiation of the SH-SY5Y cell line in vitro.

Methods

The most common genetic variants localized in the CNTNAP2 promoter, rs150447075T>G, rs34712024A>G, and rs71781329GCG[6]GCG[7, 8] were tested for association with ASD and language development in a German ASD cohort of 592 families using single marker association tests and non-parametric regression models, respectively. To define the critical time-points for CNTNAP2 regulation during in vitro differentiation in the SH-SY5Y cell line, we measured mRNA expression over a time period of 2 weeks using relative quantitative RT real-time PCR. The effect of the individual variants on transcriptional efficiency was further investigated by luciferase assay in mitotically active HEK293T cells and undifferentiated SH-SY5Y cells, as well as in SH-SY5Y cells 72h after induction of differentiation (AID), i.e. when CNTNAP2 was strongly down-regulated, and 216h AID, i.e. when neuronal differentiation was completed and CNTNAP2 levels restored.

Results

At the genetic level, we observed bidirectional effects of rs34712024 in that the nominally associated minor allele G is protective for ASD, but carriers of this allele showed a suggestive delay in language acquisition based on parental reports. For the short tandem repeat rs71781329, we observed a differential role of the alleles in language development: Carriers of 7 GCG (N=2) but not of 8 GCG (N=3) repeats showed a later onset of speech compared to homozygous carriers of the major allele 6 GCG. However, the number of carriers of the minor alleles (GCG 7,
8) was limited and thus results have to be interpreted with caution. For rs150447075, we did not observe any association. The functional characterization of CNTNAP2 transcriptional regulation showed that mRNA levels are down-regulated 72h AID followed by an up-regulation 216h AID. The transcriptional efficiency of the promoter is comparable between undifferentiated cells and cells at time point 72h AID. A significant increase was observed at time point 216h AID, which is in line with the reported up-regulation at mRNA levels 216h AID. Interestingly, all three variants showed cell line- and differentiation stage-specific effects on the efficiency of the promoter. The associated variants showed significant effects on translational efficiency in the mitotic and early differentiation phase but not during the final stage of differentiation. Finally, only variants that altered neuronally active transcription factors in silico were also associated with ASD risk and/or language development.

Discussion

Our findings suggest that CNTNAP2 is tightly regulated during the neuronal differentiation in an in vitro model, and variants that are putatively associated with ASD and language development may modulate the phenotypes during early stages of differentiation. This would suggest that the functional role of genetic variants can be pleotropic and multidirectional, depending on the cellular context.

ANALYSIS OF COGNITIVE PERFORMANCE, SOCIAL FUNCTIONING, AND BODY MASS INDEX AS QUANTITATIVE RATHER THAN RICHTOMOUS TRAITS IN INDIVIDUALS WITH DELETION 16P11.2

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Background

The recurrent deletion 16p11.2 is the second most common pathogenic copy number variant identified among individuals with neurodevelopmental disorders. It was initially identified in subjects with autism and/or intellectual disability (ID) and subsequently associated with macrocephaly, obesity, seizures, speech and motor delays, congenital anomalies, and paroxysmal kinesigenic dyskinesia. Deletion carriers show substantial clinical heterogeneity, including apparently normal individuals, an observation often interpreted as evidence of incomplete penetrance.

Methods

We studied 30 individuals with de novo del 16p11.2 from the Simons Variation in Individuals Project and their non-carrier parents (n=58) and siblings (n=19). We examined parent reports from the Social Responsiveness Scale (SRS), a quantitative scale that evaluates social awareness, reciprocal social communication, social information processing, and social anxiety, resulting in a
T-score ranging from 30 (highly sociable) to 90 (severe social impairment) with a mean of 50 and a standard deviation (SD) of 10. SRS scores are highly heritable, commonly unrelated to intelligence quotient (IQ), and continuously distributed in the general population. We also evaluated full-scale IQ (FSIQ), a quantitative measure that provides an estimate of an individual's overall cognitive ability with a mean of 100 and a SD of 15, and body mass index (BMI), a proxy for human body fat based on an individual’s weight and height.

Results
Within this group, only 32% of probands met full criteria for autism using categorical diagnostic tools (ADI-R and ADOS). However, for the SRS, the mean T-score for probands was 71.9 compared to 46.8 in parents, and 44.7 in siblings. Therefore, this quantitative trait revealed a 2.6 SD shift of mean SRS scores of probands relative to unaffected intrafamilial controls ($p=3.28 \times 10^{-19}$). These results are very similar to those obtained using FSIQ to assess cognitive functioning in this subset of cases: only 16.6% met a categorical diagnostic criteria for ID (FSIQ ≤70); however, if viewed as a quantitative trait, FSIQ was 1.8 SD lower in probands compared to their parents ($p=1.72 \times 10^{-17}$). Finally, proband BMI z-scores were found to be 0.7 SD higher than parental scores ($p=0.016$).

Discussion
By using continuous, quantitative traits such as FSIQ, SRS, and BMI scores to compare probands with their unaffected, non-carrier relatives, rather than using categorical variables such as DSM diagnoses or qualitative, dichotomous traits (i.e., normal vs. abnormal), we showed that parent-reported social behavior, cognitive function, and BMI are significantly impacted in a deleterious fashion among children with deletion 16p11.2 when compared to non-carrier relatives. These data may be more consistent with phenotypic heterogeneity related to genetic/family background rather than evidence of incomplete penetrance.

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GENETIC VARIATION IN THE OXYTOCIN RECEPTOR GENE IS ASSOCIATED WITH A SOCIAL PHENOTYPE IN AUTISM SPECTRUM DISORDERS
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Background
Oxytocin has recognized associations with social cognitive behavior in animal models. As a result, translational research has begun to investigate the relation between oxytocin and social functioning particularly among disorders characterized by social impairment, such as autism spectrum disorders (ASDs). Preliminary research supports a relation between genetic variation in the oxytocin receptor gene (OXTR) and ASD. Given that Oxytocin is a neuropeptide hypothesized to influence social interaction, rather than investigating a heterogeneous disorder like ASD, research examining the association between OXTR and a specific social phenotype may provide insight into how OXTR conveys risk for social impairment.

Methods
We investigate specific social phenotypes associated with OXTR single nucleotide polymorphisms (SNPs). The current study investigated the relation between 10 SNPs in the OXTR gene that have been previously shown to be associated with autism. The current study
tests the association of these SNPs with a social phenotype that includes behaviors known to be impaired in ASD (e.g., joint attention). This is one of the largest OXTR genotype-phenotype studies to date involving greater than 900 affected participants.

**Results**
The current study found that rs7632287 was significantly correlated specifically with measures of joint attention. There is also novel evidence to support an association between social impairment across domains and rs237884. Linkage disequilibrium (LD) mapping suggests that these two SNPs are in LD.

**Discussion**
Results of this study indicate that social impairment in ASD are associated with a specific region of the OXTR gene. This may elucidate the molecular mechanism by which oxytocin contributes to social impairment in ASD. A better understanding of this mechanism will help to identify individuals that would most benefit from biological oxytocin-based treatments.

**ZINC FINGER PROTEIN 804A (ZNF804A) AND VERBAL DEFICITS IN AUTISM**

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**Background**
Autism is a complex neurodevelopmental disorder characterized by deficiencies in social interaction and communication, and by repetitive and stereotyped behaviors. The abnormalities are usually identified in the early years of childhood. It is one of the most heritable neurodevelopmental disorders. According to a recent report, the prevalence of this pervasive developmental disorder has risen to 1 in 88. In a genome-wide association study of autism, Zinc finger protein 804A (ZNF804A) SNPs were found to be nominally associated in verbally-deficient individuals with autism. ZNF804A copy number variations (CNV) have also been observed in autism. Owing to the presence of a zinc finger domain at its N-terminal end, ZNF804A has been deemed to be involved in DNA binding and transcriptional regulation. It has been found to effect neural activation during theory-of-mind (ToM, also called mentalising) tasks. ToM is a higher-order form of social cognition representing the ability to infer the mental state of others. ToM deficits are responsible for the communication and social challenges faced by autistic individuals. Given its role in ToM that in turn relates to social cognition and verbal skills, we hypothesize that ZNF804A could play a role in predisposing individuals to autism. We evaluated our hypothesis by, (i) genetic association study of ZNF804A with autism, (ii) copy
number variation (CNV) analysis at ZNF804A locus, (iii) comparison of the expression of ZNF804A in the postmortem brains of autistic individuals and healthy controls, and (iv) assessment of the effect of ZNF804A silencing on the expression of genes known to be involved in verbal efficiency and social cognition.

Methods
DNA samples from 841 families obtained from Autism Genetic Resource Exchange (AGRE) were used in genetic association study and CNV analysis. Considering the ADI-R score on overall level of language (scores: 0-2) as an indicator of verbal abilities, autistic individuals were grouped into low verbal (Lvrb; score: 0 and 1) and normal (Hvrb; score: 2) categories. Verbal deficits were found in autistic individuals belonging to 761 families (Lvrb category). 16 SNPs were examined for association with autism by family-based association test. Further, the gene expression ZNF804A was compared between the postmortem brains of autistic individuals (n=8) and healthy controls (n=13). Subsequently, the effect of ZNF804A silencing on the expression of genes involved in verbal and cognitive functions was assessed in SH-SY5Y human neuroblastoma cells.

Results
rs7603001 was found to be nominally associated with autism (p=0.018). The association was stronger (p=0.008) in the families with verbally-deficient autistic individuals. Haplotype association was also observed in these families. ZNF804A CNVs were observed in eight verbally-deficient male autistic individuals. In ZNF804A-knocked-down SH-SY5Y cells, the expression of synaptosomal-associated protein, 25kDa(SNAP25), a gene known to be associated with linguistic/verbal abilities, was found to be reduced compared to the controls (p=0.009). Furthermore, the expression of ZNF804A (p=0.009) and SNAP25 (p=0.009) were reduced in the anterior cingulate gyrus (ACG) of autistic individuals. There was a strong positive correlation between the expression of ZNF804A and SNAP25 in the ACG (p<0.0001).

Discussion
ZNF804A could be a potential candidate gene for autism. It might have a role in mediating the intermediate phenotypes associated with verbal traits in autism. Further studies on a possible interaction between ZNF804A and SNAP25 in the pathogenesis of autism are warranted.

### MONOAMINE OXIDASE DEFICIENCY: THE CLINICAL RELEVANCE OF PERSONAL GENOMICS IN A NEW DEVELOPMENTAL BRAIN DYSFUNCTION DISORDER

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Background
Now considered the standard of care for developmental disabilities, chromosomal microarray testing (CMA) has opened the door for detailed phenotypic delineation of new neurodevelopmental syndromes, defined by specific molecular criteria. Here we describe the
phenotypic, familial, and genetic characterization of a male patient with a deletion involving the X-linked MAOA and MAOB genes, critical for the metabolism of catecholamines and indoleamines, and essential for amine-neurotransmitter homeostasis. We discuss the impact of these results on clinical management.

Methods
The male proband presented at the age of 27 for psychiatric care, with prior diagnoses of autism and intellectual disability (ID). Facial dysmorphology, the combination of autistic features and ID, plus a family history remarkable for four maternal uncles with ID and premature deaths due to stroke, prompted evaluation by array comparative genomic hybridization (aCGH). We used the International Collaboration for Clinical Genomics (ICCG, formerly ISCA) consensus microarray design, containing 180K oligonucleotide probes across the whole genome. To fine-map initial results, we used a custom-designed high-density exon targeted microarray based on the same platform.

Results
The male proband presented at the age of 27 for psychiatric care, with prior diagnoses of autism and intellectual disability (ID). Facial dysmorphology, the combination of autistic features and ID, plus a family history remarkable for four maternal uncles with ID and premature deaths due to stroke, prompted evaluation by array comparative genomic hybridization (aCGH). We used the International Collaboration for Clinical Genomics (ICCG, formerly ISCA) consensus microarray design, containing 180K oligonucleotide probes across the whole genome. To fine-map initial results, we used a custom-designed high-density exon targeted microarray based on the same platform.

Discussion
The clear genotype-phenotype correlation in this individual, and the dramatic family history of premature death, is similar to the presentations of other males with deletions disrupting both MAO-encoding genes. Our observations and prior reports establish MAO deficiency as a new developmental brain dysfunction disorder with explicit clinical implications, namely the need for a low tyramine diet and clear contraindication of sympathomimetic agents. Knowledge of the genetic etiology of the phenotype in these patients allows for a tailored clinical management targeting directly the deficient pathway and serves as an example of personalized medicine.

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GENETIC STUDIES OF AUTISTIC TRAITS IN THE GENERAL POPULATION
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Background
Autism spectrum disorders (ASDs) are highly heritable, and six genome-wide association studies (GWASs) of ASDs have been published to date. There is increasing evidence that ASDs represent the extreme end of a normal distribution of autistic traits - assessed through self-report questionnaires - within the general population, which implies that general population studies of autistic traits could yield additional, novel ASD genes and loci. This could provide a cheap
phenotyping solution for the field of ASD research.

**Methods**

We developed a customized self-report questionnaire of autistic traits containing six items from the DSM-IV section about ASDs and twelve items from the Autism-Spectrum Quotient (AQ), a self-report questionnaire developed to quantify ASD-related traits in individuals with normal intelligence and validated in the Dutch general population and in people with ASDs. We validated the new questionnaire in a clinical sample of 25 adults with a confirmed ASD diagnosis as well as a Dutch general population sample of 50 healthy adults. The questionnaire was then administered to participants from the Nijmegen Biomedical Study (NBS), a large repository of biomaterials, phenotypic and genotypic data from a Dutch adult population sample. For each item, the participants responded on a 4-point scale, with the possible answer categories ‘1 = definitely agree’; ‘2 = partially agree’; ‘3 = partially disagree’ and ‘4 = definitely disagree’. The scoring was reversed for items in which an ‘agree’ response is characteristic for ASDs, and the item scores were then summed, resulting in a minimum score of 18 (no autistic traits) and a maximum score of 72 (full endorsement of all autistic traits). Subsequently, we conducted a principal component/factor analysis of the scores on the 18 individual items from the questionnaire, in order to find out which combination of factors (each consisting of the added scores from two or more individual items) would explain the largest proportion of the observed variance in the total score for ‘autistic traits in the general population’. To investigate whether the population ASD-traits show overlap with clinical ASD phenotypes, we performed association studies for seven SNPs that have yielded genome-wide significant association findings ($P < 5.00E-08$) for ASDs in the six published GWASs of ASDs and that are located within (the vicinity of) ARHGAP36, GRAMD1B, LHFPL1, MSN, MSNP1, NQO2 and PRSSL1. Additional gene-wide and genome-wide association studies of the total score for autistic traits are currently underway.

**Results**

Following the successful validation of our novel questionnaire as a useful tool for assessing autistic traits in the general population, we collected data from 5066 adults from the NBS sample. The total score for autistic traits followed a normal distribution, with a mean total score of 36 (SD=6.5). The principal component analysis of the 18 individual items from the questionnaire revealed that the combination of five factors/subscores - i.e. ‘childhood behaviour’, ‘rigidity’, ‘social skills’, ‘attention to detail’ and ‘imagination’ - constituted the best fitting model to explain the observed variance in the total score. Five of the seven genome-wide significant SNPs from the published ASD GWASs and hence the genes implicated through these SNPs were associated at nominally significant levels ($P < 0.05$) with the total score for autistic traits and/or one of the factors/subscores, i.e., ARHGAP36 was associated with ‘imagination’, LHFPL1 was associated with ‘attention to detail’, MSN was associated with the total score and ‘imagination’, MSNP1 was associated with ‘attention to detail’, and NQO2 with ‘social skills’.

**Discussion**

Our preliminary genetic analyses point towards shared genetic susceptibility factors for ASDs and autistic traits in the general population. This suggests that additional, novel ASD-linked genetic variants can be identified by performing GWASs of autistic traits assessed through self-
report questionnaires in the general population. This possibility of ‘cheap phenotyping’ could provide a strong boost to our insight into the genetic mechanisms underlying ASDs.

A NOVEL COLLAPSING METHOD FOR RARE COPY NUMBER VARIANTS (CNVS)
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Background
CNVs play an important role in the etiology of multiple psychiatric disorders. Multiple recent studies found that the genome-wide burden of rare CNVs is greater in cases with schizophrenia, autism, or bipolar disorder than in controls. Several specific and likely the most pathogenic CNVs have been discovered; however, the individual effects of most CNVs on disease risk remain unknown due to modest marginal effect size or rarity of the CNV. Collapsing approaches could be important to evaluate multigenic models to study how CNVs impact risk for psychiatric disorders. In contrast to sequence variants that affect single nucleotides, CNVs vary in size, type, dosage, and sequence level details of gene disruption. Because of its multi-faceted nature, CNV analysis is more challenging and the most important consideration is allelic heterogeneity. For example, heterogeneous clusters of rare CNVs are often observed where CNVs with variable breakpoints and sizes disrupt genes in different ways. CNVs can also have heterogeneous effects by being a mixture of neutral, risk, and protective variants. Consequently, collapsing methods developed for rare sequence variants cannot be generalized directly to rare CNVs. Existing burden tests also do not fully explore CNV-specific challenges. Typically, rare CNVs are aggregated into discreet categories, such as deletion, duplication, exon, gene, or genome; and within each category the rates of events were compared between cases and controls, treating all events equivalently. This approach falls short in two counts: ignoring heterogeneity entails loss of power and testing one-category-at-a-time has suboptimal efficiency.

Methods
We propose a novel collapsing method for CNVs that is robust to multiple types of heterogeneity. Our method is based on SimReg (Tzeng et al., 2011, PMID 21835306), a similarity collapsing approach, to collectively examine the effects of multiple CNV features (e.g. size, type, dosage, mixture effects) and weight CNVs by their frequencies and details of gene disruption. Multiple confounders can be simultaneously corrected.

Results
We conducted simulation to evaluate type I error and power in various scenarios and compared our method to the existing CNV burden test as implemented in PLINK. We then applied our method to multiple well-powered psychiatric GWAS studies where each included more than 10K subjects.

Discussion
Full results will be reported at the conference, but preliminary results suggest manifest improvement in performance.
MULTIPHEN: JOINT MODEL OF MULTIPLE PHENOTYPES CAN INCREASE DISCOVERY IN GWAS
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Background
Despite clinical overlap and statistical correlation between many phenotypes, especially psychiatric traits, GWAS are generally performed on phenotypes separately.

Methods
We introduce a new method and software, MultiPhen, that models multiple phenotypes simultaneously in a fast and interpretable way. By performing ordinal regression, MultiPhen tests the linear combination of phenotypes most associated with the genotypes at each SNP, and can thus capture effects hidden to single phenotype GWAS.

Results
We demonstrate via simulation that MultiPhen provides a dramatic increase in power over the univariate approach in many scenarios. While some multivariate methods have similar power gains, they can be prone to highly inflated type 1 error or be extremely computationally intensive. When applied to real data from the Northern Finland Birth Cohort 1966 (NFBC1966), MultiPhen discovers 21% more independent SNPs with known associations than the standard univariate GWAS approach, while applying MultiPhen in addition to the standard approach provides 37% increased discovery.

Discussion
MultiPhen can be applied to both continuous and case-control data, and is therefore well suited to application to psychiatric traits, which may benefit from large increases in power through the simultaneous modeling of both endophenotypes and clinical endpoints. We also explore how the insight into the genetic relationships between the phenotypes provided by the results of MultiPhen could be useful in refining the definition of existing phenotypes or uncovering novel heritable phenotypes.

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SEASONAL CHANGES IN GENE EXPRESSION REPRESENT CELL TYPE COMPOSITION IN WHOLE BLOOD
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Background
Seasonal patterns in human behavior are widespread and influence mood, sleeping and eating patterns in the general population. In addition, season-dependent differences have been observed in the incidence and course of vascular and psychiatric diseases. This has been linked to seasonal variation in hematological parameters and the serotonergic system.
Methods
To address seasonality in gene expression systematically, we investigated circannual patterns of gene expression in whole blood of 233 healthy controls using weighted gene co-expression analysis. We subsequently examined circannual variation in blood cell composition using a clinical database with 51,142 observations of blood cell counts over three years.

Results
We identified three gene co-expression modules that exhibited a clear circannual pattern. Enrichment analysis of the genes in these modules suggested that this signal stems primarily from red blood cells and blood platelets. Indeed, in the clinical database we confirmed seasonal patterns consistent with previous gene expression findings in red blood cells, reticulocytes and blood platelets.

Discussion
We demonstrate for the first time that seasonal patterns in gene expression of whole blood do exist and are correlated with blood cell composition. It is likely that these seasonal changes in cell counts and gene expression profiles in whole blood represent biological and clinical relevant phenomena. Moreover, our findings highlight possible confounding factors relevant to the study of whole blood gene expression profiles in subjects collected at geographical locations with disparaging seasonality patterns.

MULTIVARIATE GENE-BASED GENOME-WIDE ASSOCIATION ANALYSIS
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Background
In the search for genes underlying variation in behaviour, researchers usually study the association between one phenotype and one SNP in a genome-wide setting. This standard analysis is limited for two reasons. First, as genes, and not SNPs, are the functional units in the genome, gene-based analyses are preferred over SNP-based analyses. Second, as traits are often multivariate (or multidimensional) in nature, analyses on univariate phenotypic composite scores (such as sum scores calculated across a set of questions, either or not subsequently dichotomized into a case-control status variable) can result in a serious loss of statistical power. Various methods allow univariate gene-based tests (e.g., GATES, VEGAS, JAG), and various methods allow multivariate SNP-based tests (e.g., TATES, MultiPhen), but as yet, there is no package that integrates both, i.e., allows multivariate gene-based genome-wide association analysis.

Methods
We present an integration of the packages GATES (Li et al., 2011), geared to acquire gene-based p-values from multiple SNP tests, and TATES (Van der Sluis et al., 2013), geared to acquire trait-based p-values from multiple phenotypic measures (e.g., singular items or symptoms). Like both singular packages, the integrated method, which we refer to as GATES-TATES, is based on combining p-value information obtained in standard univariate genome-wide association analyses to eventually acquire one gene-based trait-based p-value $P_{GT}$. This combined package thus allows researchers to study the association between multiple phenotypes on the one hand and entire genes on the other.
Results
Using extensive simulation, we studied the solidity of this integrated method under various combinations of genetic and phenotypic models. These simulations show that the Type I error rate of GATES-TATES is correct. We then compared the statistical power to detect causal genes between gene-based analyses in which phenotypic composite scores (e.g. sum score) were used (GATES, multiple regression), and the new multivariate GATES-TATES method in which the individual phenotypic variables are treated separately. Depending on the exact phenotypic model, the statistical power of the TATES-GATES procedure to detect genes in which one or more SNPs together explain 1% of the variance in either one or multiple of the phenotypic variables (e.g., items, symptoms), was generally more than 8 times higher.

Discussion
Usually, the causal disease- or trait-generating genotype–phenotype model is unknown, and probably phenotypically and genetically complex. The use of a multivariate, gene-based test is therefore recommended, especially since GATES-TATES not only detects gene effects that are common to multiple phenotypes, but also genes that affect only one of multiple phenotypes in a set. GATES-TATES thus constitutes a powerful new multivariate gene-based strategy that allows researchers to identify novel genes, while the complexity of traits is no longer a limiting factor.

INTERACTIONS AMONG CANDIDATE SNPS FOR ADHD AND SCHIZOPHREния: COMPARISON OF FAMILY-BASED AND CASE-CONTROL METHODS
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Background
Most genome-wide association scans of neuropsychiatric disorders reported to date have emphasized the role of individual single nucleotide polymorphisms (SNPs) or of large numbers of such variants in combination (e.g., via their summation into a polygenic risk score). Relatively few studies have sought to identify discrete numbers of SNP-SNP interactions within such GWAS datasets. Our objective was to employ methods for detecting SNP-SNP interactions within two GWAS datasets (one case-control sample and one family-based sample) to determine the feasibility, flexibility, and efficiency of such analyses while identifying candidate interactions to be followed up in additional independent samples.

Methods
Once case-control and one family-based GWAS dataset were downloaded from the database of Genotypes and Phenotypes (dbGaP). The family-based ADHD dataset was from the International Multi-Center ADHD Genetics (IMAGE) project (dbGaP study accession: phs000016.v2.p2) and consisted of 721 complete trios. The case-control schizophrenia dataset was from the European-American subsample of the GAIN GWAS of Schizophrenia (dbGaP study accession: phs000021.v3.p2). A set of approximately 50 candidate genes for ADHD (IMAGE genes) was identified a priori based on prior evidence and expert consensus, and the most strongly ADHD-associated SNP in each of these genes was identified in the IMAGE sample by transmission disequilibrium tests of all genotyped SNPs in each gene. Interaction analyses among these top
candidate SNPs were then evaluated in the R package trio. A set of approximately 50 schizophrenia candidate SNPs was identified as those polymorphisms having evidence of association with schizophrenia surpassing a p-value threshold of $10^{-6}$ in at least one published GWAS of the disorder as determined from the genome.gov/gwastudies database. Interaction analyses among these top candidate SNPs were then evaluated in PLINK.

**Results**

Various theoretical thresholds for declaring genome-wide significance were considered, but none were surpassed due to the extremely large number of possible SNP-SNP interactions that could be tested in a typical GWAS platform. Numerous interactions did attain nominal significance and some attained a level of significance that withstood Bonferroni correction for the number of candidate interactions tested. In the family-based IMAGE ADHD dataset, the most significant interaction involved rs2311120, which maps upstream of a gene (RAB27B) encoding a member of the RAS oncogene family that regulates vesicular trafficking, and rs12596741 in the xylosyltransferase I gene (XYLT1) which is necessary for biosynthesis of glycosaminoglycan chains. In the case-control GAIN schizophrenia GWAS dataset, the most significant interaction was detected between rs10489202 in the mitochondrial pyruvate carrier 2 gene (MPC2) and rs7233060 upstream of an as-yet-uncharacterized protein (FLJ25715).

**Discussion**

Methods for detecting SNP-SNP interactions in GWAS datasets are freely available and highly efficient, but are not widely employed in the analysis of neuropsychiatric disorders. Our results show that the prudent use of such strategies can identify candidate interactions potentially worthy of direct evaluation in adequately powered replication samples. Our selection strategy was limited to SNPs known or thought to individually exert main effects on risk for the evaluated disorders; however, we acknowledge and expect that biologically important interactions need not be so constrained. Further work to refine methods for SNP-SNP interaction analyses, including the simultaneous consideration of distinct modes of inheritance at each locus and the consideration of multiple loci, will be essential to allow the missing heritability of neuropsychiatric disorder to be accounted for properly.

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**TELOMERE LENGTH AS RELATIVE T/S RATIOS IN PSYCHIATRIC AND DEGENERATIVE DISORDERS**

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**Background**

Telomere attrition is non-specific and suggests shared molecular events in degenerative conditions. Studies have shown evidence of convergence of molecular pathways in neuropsychiatric disorders. Induced pluripotent stem cell derived neurons of Parkinson’s disease from carriers of single gene mutation (LRRK2), as well as those of possible multi-factorial etiology, showed similar cellular features of auto-phagosome turnover. This suggests that in clinical syndromes arising out of differing aetiologies, the final cellular pathology may be shared¹. We measured telomere length as relative T/S ratio in psychiatric and degenerative illness.
Methods
Patients suffering from psychiatric and degenerative illness (n=185), including ataxia telangiectasia (n=9); Huntington’s disease (n=35); schizophrenia (n=71); elderly individuals with diagnosis of dementia (n=70); were recruited, and telomere length was assessed as relative T/S ratio using quantitative real time polymerase chain reaction. This was compared with relative T/S ratio in age group matched normal individuals without neuropsychiatric illness (n= 178).

Results
The relative T/S ratios were significantly lower in subjects with ataxia telangiectasia (p< 0.0001); Huntington’s disease (p=0.005); unremitted schizophrenia (n=36; p < 0.0001) and dementia (p < 0.0001) compared to their corresponding control groups. The relative T/S ratios in remitted schizophrenia subjects (n=35; p=0.441) did not differ significantly from age group matched controls.

Discussion
The lower relative T/S ratios in ataxia telangiectasia, Huntington’s disease, unremitted schizophrenia and dementia subjects suggestive of lower relative telomere length may be indicative of shared biological pathways between these disorders leading to increased cellular senescence. These results suggest that both monogenic and polygenic disorders may lead to telomere attrition through converging cellular pathways.

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CHALLENGES OF LIVING WITH FRONTOTEMPORAL DEMENTIA: THE PERSPECTIVE OF THE AFFECTED INDIVIDUAL APPRAISED THROUGH BLOG CONTENT ANALYSIS
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Background
Frontotemporal dementia (FTD) is the second most prevalent cause of early-onset dementia and, frequently, there is a genetic etiology [1]. Many studies have examined coping and adaptation among FTD caregivers [2, 3], whereas the impact of an FTD diagnosis on the psychological well-being of the affected individual is generally not considered. The goal of this study was to explore the awareness and impact of FTD on individuals affected with FTD and caregivers of persons with FTD. [1] Piguet, O., Hornberger, M., Mioshi, E., & Hodges, J. R. (2011). Behavioural-variant frontotemporal dementia: diagnosis, clinical staging, and management. The Lancet Neurology, 10(2), 162-172.
Methods
We identified weblogs (“blogs”) written by individuals affected with FTD (n=8) and those written by spouse or child FTD caregivers (n=8). A qualitative content analysis was conducted, targeted to text addressing awareness of disease, the personal impact of FTD, and coping strategies.

Results
Our data show that affected individuals are able to articulate awareness of cognitive, behavioral, and language symptoms of FTD. Bloggers with FTD described using awareness to engage in self-monitoring and in some cases behavioral control. Threat to personhood emerged as a major theme for both affected individual and caregiver bloggers; this threat was managed through both emotion-focused coping strategies such as talking to others and task-focused coping strategies such as establishing routines and self-education.

Discussion
This study is among the first attempts to understand the awareness and impact of FTD on the affected individual. It is also, to our knowledge, the first to examine public writings of individuals with FTD. Our findings support and enrich the literature describing dementia as a threat to personhood. However, our data are counter to many clinical reports that persons with FTD have poor insight and limited awareness of their cognitive decline. Finally, these FTD bloggers described a feeling of purpose for blogging and forming a supportive online community, indicating that blogs and other forms of journaling may be valuable for observing and managing the negative consequences of FTD. Genetic counselors and other healthcare professionals working with FTD patients and their families may facilitate adaptation to dementia by identifying appropriate psychosocial coping resources or initiating support groups for patients with mild to moderate FTD.

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CHRONIC VARIABLE STRESS RESULTS IN COGNITIVE IMPAIRMENT AND ALZHEIMER’S DISEASE-RELATED GENE EXPRESSION CHANGES AMONG WILD TYPE MICE

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Background
Exposure to stress and hyperactivity of the Hypothalamic-Pituitary-Adrenal (HPA) axis have long been established as risk factors for the development of neurodegenerative and psychiatric disorders [McEwen et al, 1995]. Although the underlying mechanisms remain poorly understood, chronic HPA axis activation has been consistently associated with neuronal loss, decreased neurogenesis, and altered connectivity [Montaron et al, 2006]. Further, it is thought that critical developmental periods exist during which individuals are most vulnerable to these detrimental effects and the aging brain appears to be particularly sensitive [Sapolsky, 1999]. In this study, our objectives were to (1.) determine the behavioral effects of Chronic Variable Stress (CVS) on young adult and aging wild type mice, (2.) determine the effects of CVS on expression of Alzheimer’s disease-related genes in different brain regions of wild type mice, and (3.) understand how stress-induced changes in gene expression may be epigenetically mediated.
Methods
28 wild type male CD-1 mice were exposed to 14 days of CVS or control conditions at either 6 months (‘young adult’) or 12 months of age (‘aging’). The CVS schedule involved one stressor each day that was introduced at varying times and for varying durations. Stressors included restraint, swim, cold, a moving platform, social crowding, white noise, and light. On day 1 and day 14, plasma corticosterone levels were measured. Following exposure to 14 days of CVS or control conditions, all animals underwent behavioral testing using the open field test, novel object recognition test, and Barnes maze. After behavioral testing, the animals were killed by decapitation. The adrenal glands were weighed. The brain was removed, immediately frozen, and later dissected. Tissue from the left hemisphere was used for gene expression analysis and tissue from the right hemisphere was used for DNA methylation analysis. mRNA was extracted from left-sided brain punches and qRT-PCR was used to determine relative expression levels of several genes that have been previously associated with Alzheimer’s disease, including App, Bace1, Bdnf, and Gsk3b. Genomic DNA was extracted from the right-sided brain punches and bisulfite pyrosequencing was performed to assess for DNA methylation (DNAm) of specific CpGs in the promoter regions of the genes of interest.

Results
Our findings suggest that CVS resulted in similarly elevated plasma corticosterone and adrenal hypertrophy in both young adult and aging mice. In the open field test, there were no differences between groups indicating that exposure to CVS did not result in an anxious or depressive phenotype in either stressed group. In the novel object recognition test, CVS exposure resulted in cognitive deficits in aging mice, but not young adult mice. In the Barnes maze, CVS resulted in a mild deficit among young adult mice and profound deficits among aging mice. Gene expression data suggests that CVS results in a stress-dependent increase in Bace1 expression in the prefrontal cortex and hypothalamus of young adult mice and in the prefrontal cortex, hypothalamus, and amygdala of aging mice. We also found a stress-dependent increase in Gsk3b in the prefrontal cortex of aging mice. Further, we found an age but not stress related decrease in Bdnf expression in the prefrontal cortex and hypothalamus. We also found an age and stress related decrease in Bdnf expression in the amygdala and hippocampus. Finally, we found no changes in the expression of App. Epigenetic analysis indicates that the stress-related increase in Bace1 expression may be due to decreased promoter region DNAm. Additional epigenetic studies assessing DNAm of Gsk3b and Bdnf promoter regions are ongoing. Behavioral data from the Barnes maze and representative gene expression, and DNA methylation data from the prefrontal cortex are shown in the attached figure.

Discussion
Previously published data suggest a central role for stress in the pathogenesis of neurodegenerative and psychiatric disorders. However, little is known about the precise effects of stress on cognition, gene expression, and DNAm at different points across the lifespan. Taken together, our data suggest that aging mice are especially susceptible to chronic stress and display persistent impairments in learning and memory. Further, we have found that chronic stress exposure is associated with brain region-specific changes in the expression of several genes that are important for the pathogenesis of Alzheimer’s disease, including Bace1, Gsk3b, and Bdnf. Finally, preliminary data suggest that the stress-dependent increase in Bace1 expression may be epigenetically mediated.
MACROCEPHALY AS AN ENDOPHENOTYPE IN SCHIZOPHRENIA: OVERREPRESENTATION IN PROBANDS AND THEIR CLINICALLY UNAFFECTED FIRST-DEGREE RELATIVES

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Background
We report on a large "deep-phenotyping" genetic study of psychosis that includes quantitative dysmorphology measures based on objective, reliable methods. We focus in this presentation on dysmorphic features relating to cranial size and shape, which are highly correlated with brain dimensions. One feature, that of macrocephaly, is a topic of current interest in neurodevelopmental disorders, most notably autism. There are comparatively few studies of cranial dimensions in the psychoses. We present findings on probands with schizophrenia and their unaffected first-degree relatives, and present evidence for a marked over-representation of macrocephaly in both groups.

Methods
All subjects in this study (N = 842) were seen at McLean Hospital (Belmont, MA) and examined blind to diagnosis and family membership. We utilized standardized measurements based on the methods of Farkas and Deutsch, and conditioned on demographics using a large normative database. Head circumference was measured through the glabella (g) and opisthocranion (op), head width measured laterally between the left and right eurions (eu), and anterior-posterior length between the glabella and the opisthocranion. The cephalic index, a classical anthropometric measurement relating head width to length, was computed, permitting assessment of brachycephaly (wide relative to length) and dolichocephaly (scaphocephaly; narrow relative to length), commonly used metrics in clinical and medical genetics. Macrocephaly and disproportion are recurrent features that we have noted among patients with identified genetic rare copy number variants.

Results
The most conspicuous findings were an over-representation of macrocephaly (increased head circumference, defined here as within the upper 3rd percentile) in both probands with schizophrenia and schizoaffective disorder (N = 210) and their clinically unaffected first-degree relatives (N = 335). The rate of macrocephaly among the probands was 17%, and among their relatives 10%. These figures exceed the population baserate (p = 0.0002 and 0.036, respectively) using two-tailed exact probabilities. Remarkably, these first-degree relatives had a rate of macrocephaly three-fold greater than the baserate, despite the fact they carried no psychiatric diagnosis. A contrast group of probands with bipolar disorder (N = 70) also had a high rate of macrocephaly (at 16%; contrast against baserate p = 0.0041); however, their relatives did not exhibit this over-representation. Also observed among schizophrenia probands and their relatives was cephalic disproportion, most notably dolichocephaly. Our earlier studies of multiplex autistic disorders have revealed a substantial rate of brachycephaly but not dolichocephaly -- the converse of the findings in schizophrenia reported here.
**Discussion**
These findings on macrocephaly corroborate earlier studies of schizophrenia, and are extended here to clinically unaffected first-degree relatives to reveal for the first time a statistical over-representation of this feature. If replicated, this cranial anomaly may provide (1) an endophenotype with a substantial effect size that (2) is highly correlated with whole brain volume by several laboratories, including ours, and that (3) shares a phenotypic spectrum with other neurodevelopmental disorders. We will also discuss (4) the role of covariates as sources of potential statistical bias, and methods to control them, (5) instances of cephalic disproportion in these groups, and (6) exemplars of patients with identified rare copy number variants exhibiting these cranial anomalies.

**FUNCTIONAL POLYMORPHISMS IN BDNF AND COMT GENES ARE ASSOCIATED WITH DIFFERENCES IN MATHEMATICAL FUNCTIONING IN A SAMPLE OF YOUNG SUBJECTS**

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**Background**
Understanding the molecular genetics of complex human behaviors and functions remains a big challenge for neurosciences. Previous studies have showed that there is a genetic basis for individual differences in mathematical functioning; however, the specific genes remain to be completely identified. In the present study, we explored the possibility that two functional polymorphisms in candidate genes could be associated with differences in arithmetical performance.

**Methods**
A sample of 173 healthy young Colombian subjects (university students living in the capital city, Bogotá. Mean age: 21.2 years) were included. A computerized test to analyze performance in basic arithmetical calculations (additions and subtractions) was applied, using the PEBL battery. DNA samples from these subjects were genotyped for 2 functional SNPs in candidate genes: BDNF-Val66Met and COMT-Val158Met (rs6265 and rs4680) and statistical analysis were carried out with SNPStats Program.

**Results**
We found significant differences for arithmetical processing between genotypes for both functional SNPs: For BDNF, Met/Met subjects had a worse performance (p value: 0.02, in comparison to Val/Val and Val/Met carriers) and for COMT, Met/Met carriers had a better performance (p value: 0.01, in comparison to Val/Val and Val/Met carriers).

**Discussion**
To our knowledge, this is the first study finding associations of functional polymorphisms in BDNF and COMT genes with quantitative measures of mathematical functioning in healthy young subjects.
GENOME WIDE SIGNIFICANT ASSOCIATIONS BETWEEN VARIANTS IN AURORA KINASE A GENE AND REGULATOR OF CALCINEURIN 3 GENE AND MONOAMINE METABOLITE CEREBROSPINAL FLUID CONCENTRATIONS IN A MIXED GROUP OF PSYCHIATRIC PATIENTS AND CONTROLS

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Background
We have conducted a genome wide association study searching for associations between gene variants and the concentrations of homovanillic acid (HVA), 5-hydroxyindoleacetic acid (5-HIAA), 3-methoxy-4-hydroxyphenylglycol (MHPG) and kynurenic acid (KYNA) in the cerebrospinal fluid (CSF) of psychiatric patients, mainly with psychotic disorders, and healthy controls. The monoamine metabolites HVA, 5-HIAA and MHPG are the major degradation products of the monoamines dopamine, serotonin and noradrenaline respectively, and their concentrations are considered to reflect the monoamines’ turnover rates in the central nervous system (CNS). KYNA, an end-metabolite in the kynurenine pathway of tryptophan degradation, is synthesized in astrocytes and may have neuroactive characteristics. Altered CSF levels of monoamine metabolite concentrations and KYNA have been associated with schizophrenia and other psychiatric disorders.

Methods
The CSF HVA, 5-HIAA, MHPG metabolites were measured from 218 subjects (111 controls, 66 patients with schizophrenia spectrum disorders, 11 patients with other psychotic disorders and 30 patients with other psychiatric diagnoses), whereas the CSF KYNA levels were measured in 69 individuals (27 controls, 32 patients with schizophrenia spectrum disorders, 2 patients with other psychotic disorders and 8 patients with other psychiatric diagnoses). CSF samples (12.5 ml) were drawn by lumbar puncture from all individuals. Genomic DNA was extracted from whole blood and 900,000 single nucleotide polymorphisms (SNPs) were genotyped. Allele association between SNPs and CSF monoamine metabolite concentrations and KYNA were tested with multiple linear regression (PLINK), where the concentrations were modeled as a linear function of the allele count (of each SNP separately) and one or more covariates. Back-length, gender, age at lumbar puncture and diagnostic information were selected as covariates. We have conducted the analyses in the combined sample of patients and controls as well as separately in a) patients with schizophrenia spectrum disorders, b) patients with schizophrenia spectrum disorders and other psychotic disorders and c) subjects with other psychiatric diagnoses and healthy controls.

Results
Four SNPs, i.e. rs2236207, rs11801547, rs10489444 and rs7540973, achieved genome-wide
significance ($p < 5 \times 10^{-8}$) in association with MHPG concentrations in the combined sample. These significant associations were not observed when we conducted the analyses for each group separately. No SNPs reached genome wide significance in association with HVA, 5-HIAA, MHPG or KYNA in any group analyzed.

**Discussion**

Rs2236207 is intragenic, located in aurora kinase A gene (*AURKA*). *AURKA* is located on chromosome 20 (20q13) and encodes a 403 amino acid protein. This protein is involved in microtubule formation and stabilization at the spindle pole during chromosome segregation. Overexpression of aurora kinase A is common in many types of solid tumors and it is believed that it may play a role in tumor development and progression. Rs11801547 is also intragenic, located in the regulator of calcineurin 3 gene (*RCAN3*). *RCAN3* is located on chromosome 1 (1p35.3-p33) and encodes for a 241 amino acid protein, expressed in many tissues including the CNS. *RCAN3* belongs to the regulator of calcineurin gene family. All the three members of this gene family interact with each other and inhibit calcineurin. Calcineurin is involved in many physiological processes including neurotransmission and synaptic plasticity.

In conclusion, *AURKA* and *RCAN3* SNPs were significantly associated with MHPG CSF concentrations in a combined population of psychiatric patients and controls. If replicated, this indicates an effect of these genes on noradrenergic processes in the CNS.

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**A LONGLITUDINAL INVESTIGATION OF BINOCULAR RIVALRY RATE (BRR) IN MAJOR PSYCHIATRIC DISORDERS**

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**Background**

Many psychiatric disorders are the result of complex interactions between environment and multiple genes so various avenues, especially genetic and multidisciplinary approaches, are currently being pursued to shed light on disorders such as bipolar disorder, schizophrenia and depression. It is now commonly accepted that genomic approaches focusing on categorical diagnostic entities need to be complemented by studying novel subphenotypes and endophenotypes. Slow binocular rivalry rate (BRR) has recently been suggested as a potential endophenotype for bipolar disorder (Ngo, Mitchell, Martin, & Miller, 2011). Binocular rivalry refers to the alternating perceptions that occur when two dissimilar images are presented, one to each eye. Previous studies have reported slow BRR for bipolar disorder but not for schizophrenia or major depression (Miller et al., 2003; Ngo et al., 2011; Pettigrew & Miller, 1998), with state and medication appearing not to affect BRR. Most recently, the finding of slow BRR was independently replicated in a study of nearly 100 subjects with bipolar disorder, in which depressive state, medication and level of cognitive function did not appear to affect the trait (Vierck et al., 2013). It is also known from a large twin dataset, that a healthy control’s rivalry
rate is reliable over time but varies widely between individuals, and that additive genetic factors account for around 50% of this individual variation (Miller et al., 2010). There remains insufficient data on the extent to which BRR remains stable over time in clinical subjects, or is influenced by current clinical state, specific symptomatology or medication (there has however, been recent demonstration of a slowing effect of a benzodiazepine on BRR; (van Loon et al., 2013). Furthermore, existing BRR data for subjects with schizophrenia are limited to N=18, so it is not yet clear if slow BRR is specific to bipolar disorder. The aim of this study is to investigate BRR longitudinally in patients with schizophrenia and bipolar disorder and to compare clinical data with those of healthy controls.

**Methods**
As all participants are already part of an ongoing large-scale longitudinal study in Göttingen (KFO 241, http://www.pzng.de), detailed demographic, clinical and neuropsychological data, as well as DNA samples, will be available at various assessment points.

**Results**
We will present BRR data on 30 bipolar patients, 30 schizophrenia patients and 30 controls seen twice (baseline and after 3 months). Although some data on effects of clinical state exist, this is the first study to systematically investigate BRR at multiple points over the course of psychiatric illness. Data will also be available on to what extent clinical variables such as symptomatology or medication have an impact on BRR.

**Discussion**
The data will contribute to further characterisation of slow BRR as a potential endophenotype for bipolar disorder and will clarify whether this trait is specific to bipolar disorder or may rather be an endophenotype for genes that are shared between bipolar disorder and schizophrenia.


48 TEMPERAMENT AND PSYCHOSES PRONENESS AS INTERMEDIATE PHENOTYPES IN BIPOLAR DISORDER: A DISCORDANT SIBLING-PAIR DESIGN
Background
Bipolar disorder (BPD) is highly heritable and shares significant comorbidity within families. However, the clinical phenotype of BPD is quite heterogeneous and the genetic architecture of the disorder is complex and not well understood. Given these complications, it is possible that the identification of intermediate phenotypes (“endophenotypes”) will be useful in elucidating the complex genetic mechanisms that result in the disorder. The examination of unaffected relatives is critical in determining whether a particular trait is genetically-relevant to BPD. However, few dimensional traits related to BPD have been assessed in unaffected relatives of patients and the potential impact of psychosis on these traits has not been well-established. Schizotypy is a candidate endophenotype for BPD and describes a personality type marked by odd, irritable, socially isolated, and hypersensitive behaviors. Limited data suggest that BPD patients score higher than healthy controls on measures of schizotypy. To date, there have been few studies of this trait in family members of BPD. Temperament is another potential endophenotype that has been understudied in unaffected relatives. The present study sought to assess schizotypy and affective temperament in patients, their unaffected siblings, and healthy controls to determine the endophenotypic status of these dimensional traits in BPD, as well as to examine the relationships between these two measures of personality. Furthermore, we assessed whether psychosis status influenced results.

Methods
We assessed schizotypy and affective temperament in discordant sibling pairs and healthy controls. All patients had a confirmed DSM-IV diagnosis of BPD and were euthymic at the time of assessment. Siblings of bipolar patients were unaffected with any Axis I disorder, age>25 years, and had never taken psychotropic medications. We used the Schizotypal Personality Questionnaire (SPQ) to assess schizotypy and focused on the three validated subscales: Cognitive-Perceptual; Interpersonal; and Disorganized. We used the Temperament Evaluation of Memphis, Pisa, Paris, and San Diego, Auto-questionnaire version (TEMPS-A) to assess affective temperament and analyzed the five main affective temperaments: Anxious, Irritable, Hyperthymic, Dysthymic, and Cyclothymic. We compared performance on these measures across patients (n = 37), unaffected siblings (n = 46), and healthy controls (n = 87). We also performed a correlational analysis to explore the relationships between these measures within and across the groups. Finally, we assessed whether siblings of probands with psychosis differed in schizotypy or affective temperament compared to siblings of probands without psychosis.

Results
BPD patients scored significantly higher than healthy controls on all subscales of the SPQ and on all but one subscale (Hyperthymic) of the TEMPS-A (all p <0.01). Similarly, unaffected siblings demonstrated elevated scores compared to healthy controls on all subscales other than the Hyperthymic subscale of the TEMPS-A. Unaffected siblings demonstrated scores that were intermediate to patients and controls on all subscales of the SPQ and the TEMPS-A except for the Hyperthymic subscale. Within and across all three participant groups there were strong positive correlations between all of the subscales of the SPQ and the TEMPS-A other than the Hyperthymic subscale. Siblings of probands with psychosis demonstrated significantly higher...
scores on the Disorganized subscale of the SPQ compared to siblings of probands without psychosis.

**Discussion**
The present results support the concept of a dimensional phenotype in which individuals at high genetic risk for BPD exhibit elevated levels of traits associated with the full clinical manifestation of the disorder. We also demonstrate a strong association between affective temperament and psychosis proneness across patients, unaffected siblings, and control participants, suggesting that these two traits are perhaps features of a more general vulnerability to psychopathology. Finally, the elevation in SPQ Disorganized scores among siblings of probands with compared to without psychosis is consistent with the notion that psychosis is a genetically-mediated feature of BPD.

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**DNA METHYLATION ANALYSIS OF BDNF GENE PROMOTERS IN PERIPHERAL BLOOD CELLS OF SCHIZOPHRENIA PATIENTS**

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**Background**
Brain derived neurotrophic factor (BDNF) is a neurotrophic factor, which is important for neuronal survival, development and synaptic plasticity. Accumulating evidence suggests that epigenetic alterations in BDNF promoters are associated with the pathophysiology of psychiatric disorders. Patients with psychiatric disorders generally show decreased BDNF levels, which are often associated with increased DNA methylation at the specific BDNF promoters. DNA methylation changes in BDNF were reported not only in brain tissues but also in other tissues, including peripheral blood cells (PBC) and saliva. Here we examined DNA methylation levels of BDNF promoters I and IV using genomic DNA derived from PBC of healthy controls (n = 100) and patients with schizophrenia (n = 100).

**Methods**
Genomic DNA was extracted from PBC samples using a Wizard Genomic DNA purification kit (Promega). All subjects were from the Japanese population. One microgram of genomic DNA was treated with sodium bisulfite modification using an EpiTect 96 Bisulfite Kit (Qiagen), according to the manufacturer’s instructions. A region-specific PCR with a biotinylated primer was performed for BDNF promoters I and IV. The examined CpG sites were chosen based on the previous epigenetic studies that reported altered DNA methylation. DNA methylation level of each bisulfite-PCR product was measured with the PSQ 96MA instrument (Qiagen) according to the manufacturer’s protocol.

**Results**
We obtained successful signals to analyze at promoter I from 95 controls and 90 patients with
schizophrenia, and found a significantly higher level of methylation at one of four validated CpG sites in patients with schizophrenia compared to controls (< 1.2 %, p = 0.033). Subsequent analysis revealed that in controls, the methylation level of BDNF promoters was associated with sex, and the methylation difference observed in promoter I was more prominent in male patients with schizophrenia. On the other hand, we successfully obtained signals at promoter IV from 99 controls and 96 patients with schizophrenia. However, no CpG sites in promoter IV showed a significant difference between two groups.

**Discussion**
Hypermethylation of BDNF promoter I in schizophrenia is consistent with most of the previous studies on mood disorders and other psychiatric disorders. Considering that the detected methylation difference was relatively small, further studies to validate this difference in the independent cohorts and to assess its functional significance will be needed.

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**MICRORNA SIGNATURES IN THE MOUSE HIPPOCAMPUS AFTER CHRONIC METHAMPHETAMINE ADMINISTRATION AND REPEATED ELECTROCONVULSIVE SHOCK**
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**Background**
A mouse behavioral model of the development and recovery of schizophrenic symptoms using chronic methamphetamine (MAP) administration and repeated electroconvulsive shock (ECS), respectively, was established in our previous studies. To uncover the roles of microRNAs (miRNAs), a key regulator of gene expression at the post-translational level, in underlying molecular mechanisms of schizophrenia, the expression profiles of mouse hippocampus after the exposure to chronic MAP or repeated ECS were identified. The predicted target genes of the differentially expressed miRNAs were subjected to pathway analysis to explore possible biological implications.

**Methods**
For assessing the effects of MAP and ECS on miRNA expression, male ICR mice were randomly assigned to chronic MAP administration, repeated ECS or their respective controls (total 4 groups, N=8 each group). Real-time quantitative PCR with a genome-wide miRNA PCR array was used to measure the expression levels of miRNAs in hippocampus of the experimental animals. MiRNAs that were differentially expressed in MAP and ECS pretreated mice were subjected to in silico computational target prediction with TargetScan 6.2. Ingenuity pathway analysis applying the predicted targets of the differentially expressed microRNAs to the DAVID Bioinformatics Resources v6.7 was used to identify the convergent genetic pathway among related behavioral changes.

**Results**
The results identified that miR-138, miR-328, miR-339-5p and miR-652 were up-regulated by chronic use of MAP and down-regulated after ECS, while the changes of direction of miR-126-5p and miR-203 were the opposite. These six mouse miRNAs (mmu-miRs) significantly
correlated with the changes of animal behavior during interventions. Furthermore, the expression profiling of miRNAs derived from the PCR array can accurately predict the group classification of results from animal behavioral tests. Using the in silico prediction of the target genes and analysis of the associated biological pathways of these predicted targets of the six differentially expressed miRNAs, we’ve found that pathways involved with neuronal synapse and axonal guidance were significantly enriched.

Discussion
Since MAP and ECS induced the development and recovery of psychotic symptoms respectively in the established animal model of schizophrenia, miRNAs that are both differentially expressed under the influence of MAP and ECS, but with the opposite direction of regulation, would be the most relevant candidates for the exploration of underlying molecular mechanisms. Therefore, we hypothesized that these differentially expressed miRNAs play an important role in the molecular mechanism of the progression and recovery of psychotic symptoms. Functional tests via knock-in and knock-down of these miRNAs would shed some light on the molecular mechanisms of miRNA mediated pathophysiology.

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DISTRIBUTION OF EPISNPS IN SUB-THRESHOLD VARIANTS FROM GENOME-WIDE ASSOCIATION STUDIES FOR PSYCHIATRIC DISORDERS
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Background
Purcell et al. (2009) showed that SNPs from genome-wide association studies (GWAS) with nominal but sub-threshold p-values account for a considerable proportion of the variance in independent psychiatric samples, suggesting they are enriched for causal SNPs or their proxies. Additional data, such as allele-specific methylation, may help determine which sub-threshold SNPs are causal. SNPs displaying allele-specific methylation will be referred to as epiSNPs from herein. We set out to develop a method that determines whether epiSNPs are upweighted. We hypothesized that GWAS SNPs with nominal but sub-threshold p-values are enriched for epiSNPs.

Methods
For methods development, preliminary epiSNP lists were created using DNA from prefrontal cortex tissues from control (N=76), bipolar disorder (BD) (N=67) and schizophrenia (SZ) (N=65) individuals. These samples were genotyped on Affymetrix SNP 6.0 (Affy 6) microarrays. EpiSNPs were identified from combinations of four cohorts: BD, SZ, case (ie. BD+SZ), and control at different q levels (0.05 and 0.01). This resulted in 20 BD and SZ lists. P-values for the SNPs were acquired from publically available BD GWAS and SZ GWAS from the Psychiatric Genomics Consortium (PGC). The full SNP list comprised of all the SNPs successfully genotyped on the Affy 6 array that also had a p-value from the PGC (N= 567 270 for BD and N= 574 907 for SZ). To ensure both GWAS hits and epiSNPs were independent, we pruned the lists by performing pairwise-LD pruning in PLINK
After pruning at $r^2=0.25$ there were 103,607 independent SNPs with BD GWAS p-values and 102,887 with SZ GWAS p-values. Over-enrichment of epiSNPs that are nominally significant in GWAS was examined by comparing the p-value distribution for the pruned full Affy 6 list to the p-value distribution of the various pruned epiSNP lists that were derived from BD samples. The secondary analysis compared the epiSNP lists that were derived from SZ samples to SZ GWAS data. Three methods were used. (a) The hypergeometric distribution was applied. There are white balls (epiSNPs) and black balls (non-epiSNPs on the Affy 6 array) in an urn. At various GWAS p-value thresholds, we tested to see if the number of white balls drawn without replacement is more significant than expected by chance. (b) Quantile-quantile (QQ) plots compared the distribution of ordered p-values for the epiSNPs to that of all the SNPs on the array for the various epiSNP lists. (c) Histograms of the proportion of SNPs at various p level thresholds were inspected.

Results
(a) Comparing epiSNP lists to the Affy 6 list show significant ($p<0.05$) over-enrichment of epiSNPs for 11 of the 28 comparisons performed at the $p<0.1$ threshold. However, due to the correlation structure among the lists, the true significance of these findings is hard to interpret. (b) For all of the epiSNP lists, the p-value distributions tightly followed the null distribution. This result also applied to the epiSNP list that showed the most significant over-enrichment below the $p<0.1$ threshold according to the hypergeometric distribution ($p=0.0007$). (c) We expect that the proportion of epiSNPs will taper down with each successive p-value quantile. The null distribution of the proportion of the SNPs on the array should remain roughly uniform. However, there were no obvious trends.

Discussion
The hypergeometric alone is not sufficient to assess over-enrichment of epiSNPs in GWAS data. Rather, results should be verified using the visualization techniques of QQ plots and histograms. This methodology will be applied to finalized epiSNP lists.

DIFFERENCES IN THE EXPRESSION AND DNA METHYLATION OF GENES RELATED TO BH4 PATHWAY IN DRUG-NAÏVE FIRST-EPIODE PSYCHOSIS PATIENTS
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Background
Schizophrenia is a severe mental health disorder with a high heritability. The study of gene expression levels in blood of patients in the early stages of the disease, such as first-episode of psychosis (FEP) may be useful to detect changes in gene expression despite treatment effects. In this study we aimed to analyze gene expression in whole blood, comparing: a) drug-naïve FEP patients and healthy subjects; b) drug-naïve FEP patients before and after treatment with risperidone; and c) responders and non-responders. Also, we investigated if those differentially expressed genes were regulated by DNA methylation.
Methods
All the patients (n = 38) were evaluated with standardized scales for diagnostic and symptoms assessments by a trained psychiatrist (at admission and 8 weeks after antipsychotic treatment. The healthy controls (n = 38) were also evaluated to exclude any psychiatric disorder. Response was defined by a reduction of 40% in the total PANSS after eight weeks of risperidone treatment. Whole blood was collected from each participant during clinical assessments, and DNA and RNA were extracted. For gene expression analyses, we have used RT² Profiler™ PCR Array, which is based on SYBR Green detection of cDNA amplification. First we verified the expression of 84 genes with Human Neurotransmitter Receptors and Regulators RT² Profiler™ PCR Array System in a sample of 10 FEP patients, 10 FEP patients after treatment and 9 controls, to exclude those genes that had undetectable levels of expression in blood. Then, we selected 37 genes from the pilot study and included genes related to neurodevelopment (BDNF, NRG1 and TH) to follow-up in the total sample using a customized RT² Profiler™ PCR Array. For methylation analysis, bisulfite sequencing was performed. For data analysis, we compared 2ΔCt or DNA methylation percentage values using t-test (FEP patients x healthy controls), paired t-test (before x after treatment) and general linear model with repeated measures (responders x non-responders).

Results
Significant downregulation of GCH1 gene was observed (Fold regulation (FR)= -1.34, p=0.007) comparing FEP and controls. Downregulation of GABRR2 (FR = -1.33, p=0.01) and upregulation of GCHFR (FR = 1.18, p=0.008) were found in FEP after treatment. Moreover, we verified that the differences in GABRR2 and GCHFR expression levels were not due to response to risperidone treatment, though it seemed that the responders had a more similar GCHFR expression to controls after treatment than the non-responders Also, a significant hypermethylation in GCH1 was detected in FEP comparing to healthy controls (p=0.034).

Discussion
GCH1 codes for GTP cyclohydrolase I, an enzyme involved in the synthesis of BH4, which is an essential cofactor for tyrosine, serotonin and L-Dopa. Moreover, its expression is regulated by GCHFR, which seemed to be upregulated after treatment with antipsychotic drugs. In addition, GABRR2, which is a GABA receptor, seemed to be dysregulated after treatment Therefore, these genes may play an important role in the genesis of psychosis and its treatment, independent whether being a risperidone responder or not, and can lead towards a better understanding of the illness. Funding for this study was provided by FAPESP 2010/19176-3, 2010/08968-6 and 2011/50740-5.

ANTIDEPRESSANT EFFECT OF SODIUM BUTYRATE IS ASSOCIATED WITH TET1-MEDIATED 5-HYDROXYMETHYLATION OF BDNF GENE
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Background

Changes of brain-derived neurotrophic factor (BDNF) expression in prefrontal cortex (PFC) is associated with pathophysiology and pharmacotherapy of depression. Sodium butyrate (NaB) is previously known to inhibit histone deacetylase activity and to have antidepressant effects. 5-hydroxymethylcytosine (5hmC) in genomic DNA was recently identified, shown to exist in neurons, and proposed to mark gene activation through hydroxylation of 5-methylcytosine (5mC), catalyzed by the Tet1–3 protein family. We investigated the antidepressant effect of NaB and its potential role in DNA demethylation in a genetic rat model of depressive-like behavior, the Flinders Sensitive Line (FSL) and its controls, the Flinders Resistant Line (FRL).

Methods

Rats were treated with NaB chronically for 23 days followed by forced swim test (FST). Expression of distinct BDNF transcripts and Tet1 was measured in PFC by quantitative real-time PCR (qRT-PCR). Protein expression of Tet1 was measured by Western blotting. Regional specific DNA methylation (5mC) and 5-hydroxymethylation (5hmC) in BDNF promoters were assessed by Methylated CpG Island Recovery Assay and β-glucosyltransferase modified 5hmC enrichment assay, respectively.

Results

Naive FSL rats displayed a higher degree of immobility in the FST, lower expression of specific BDNF exons and Tet1, as well as increased 5mC and decreased 5hmC levels in corresponding exons in PFC, compared to naive FRL rats. NaB treatment normalized these dysregulations in the FSL PFC. Thus, NaB decreased the immobility of FSL rat in FST indicating an antidepressant effect of NaB. NaB induced an exon-specific BDNF expression upregulation in FSL PFC. This BDNF upregulation was associated with Tet1-mediated DNA demethylation as shown by increased Tet1 and 5hmC level and decreased 5mC level in the corresponding BDNF exons in FSL PFC.

Discussion

Reduced BDNF level in depression was previously confirmed in human postmortem studies, supporting our finding of decreased BDNF transcript in PFC region of naïve FSL. Many studies have shown that changes in exon-specific BDNF expression are dependent on epigenetic modifications. Consequently, it is relevant to study antidepressant treatments that directly target epigenetic markers at BDNF promoters. NaB was previously found to influence BDNF expression by increasing acetylation of histones H3 and H4. However, no studies have investigated its effect on DNA demethylation in mental disorders and nothing is, to our knowledge, published about the role of 5hmC and Tet1 in the regulation of BDNF expression. Tet proteins also have an essential role in postnatal neurodevelopment, but few studies have investigated Tet function with regard to pathophysiology of depression. In this study, we found that Tet1 expression was reduced in the FSL PFC, and significantly upregulated after NaB treatment. This NaB-induced Tet1 upregulation occurred in parallel with a normalization of behavior and a normalization of levels of 5mC, 5hmC and BDNF transcript for specific BDNF exons. To conclude, our result implies that an antidepressant effect of NaB involves DNA demethylation mechanisms.
THE PROTEIN AND CHROMATIN INTERACTOMES OF BRD1 ARE ENRICHED WITH PSYCHIATRIC DISORDER RISK GENES

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Background

The bromodomain containing 1 (BRD1) gene has been implicated with susceptibility to schizophrenia (SZ) and bipolar disorder (BD)1,2. Recently, the BRD1 rs138880 promoter SNP was found to be the highest ranking in a large SZ GWAS meta-analysis and family-based replication study which further supports BRD1 as a SZ risk gene3. The BRD1 gene encodes a transcription factor which is important for histone H3K14 acetylation and plays an important role in mouse embryonic development.

Methods

To clarify the role of BRD1 in psychiatric disorders we used high throughput approaches to investigate the protein and chromatin interactions of the BRD1 isoforms BRD1-S and BRD1-L in HEK293T cells. A combined immunoprecipitation and proteomics analysis of epitope tagged BRD1-S and BRD1-L confirmed interaction with the histone acetyltransferase complex proteins ING4, ING5, EAF6 and HBO1. Additionally, we discovered several new protein interactions of BRD1 including interaction with the SZ risk factors poly-bromo 1 (PBRM1) and 14-3-3 tyrosine monoxygenase epsilon (YWHAE). Chromatin immunoprecipitation followed by next generation sequencing (ChIP-seq) of epitope tagged BRD1-S and BRD1-L identified major binding to promoter regions of 1054 and 563 genes, respectively. BRD1 over-expression and knockdown studies combined with expression arrays identified a subset of differentially regulated BRD1-S and BRD1-L target genes.

Results

Analyzing available GWAS data sets we provide evidence that the protein and chromatin interactomes of the BRD1 isoforms are enriched with SZ and BD risk variants indicating a key role of BRD1 in the etiology of SZ and BD.

Discussion

The functional consequences of the SZ and BD risk gene enriched interactome of BRD1 will be the subject for further studies.


THE ROLE OF NDE1 IN MAJOR MENTAL ILLNESS

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Background
Previous studies in a Finnish family cohort for schizophrenia have observed significant association with haplotypes and SNPs from five genes within the DISC1 pathway: DISC1, NDE1, NDE1, PDE4B and PDE4D. In order to elucidate the underlying biological implications of carrying these variations, we have studied their effect on genome wide gene expression levels. Using publicly available general population data we demonstrated that variation in the DISC1 pathway genes modulated expression of genes involved in neurodevelopment, synaptogenic and sensory perception genes. Through the utilisation of data from a sub-cohort of the Finnish families ascertained for schizophrenia, here we further investigate the effects of DISC1 pathway variations on gene expression with respect to patients.

Methods
Altogether, 63 individuals from 18 families provided blood samples for RNA analysis. Gene expression levels were determined genome wide using the Illumina HumanHT-12 v4 Expression BeadChip. The effect of five variants were tested, the DISC1 HEP3 haplotype and four SNPs (one from DISC1 and three from NDE1), all of which had been genotyped in this sub-cohort as part of our previous studies.

Results
After accounting for gender, affection status, family effects, and for false discovery (at the 5% level) we observed that the polymorphism rs2242549, in the NDE1 gene, was significantly associated with wide ranging effects, being associated to changes in 926 Illumina probes, representing 853 genes. Of these genes, with altered expression levels, 478 are predicted targets for the micro RNA hsa-mir-484 which is coded in a 5’ non-coding exon of the NDE1 gene.

Discussion
These findings suggest that mutations at the NDE1 locus, here tagged by rs2242549, alter risk to major mental illness through the functional modification of hsa-mir-484, leading to a cascade of changes that could potentially yield novel insight into the pathophysiology of psychiatric disorders.

References
COMPARATIVE FUNCTIONAL EFFECTS OF POLYMORPHISMS AFFECTING PSYCHIATRIC DISEASE AND TREATMENT ACROSS SPECIES

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Background
Development of high quality, translational animal models of human neuropsychiatric disorders requires an improved understanding of the genetics underlying both the disorder in humans as well as the model organism. Our focus has been on G-protein coupled receptors, key players in many psychiatric diseases as well as the targets for pharmaceutical treatments. One GPCR, the mu-opioid receptor, has seen mixed associations with various diseases, notably including addiction and anxiety, as well as their treatment. Two common and mutually exclusive polymorphisms have been identified in humans, A118G (N40D), found in non-African populations, and C17T (V6A), found in African populations. While A118G has been studied extensively, C17T is much less well understood. A parallel polymorphism C77G (P26R) has been identified in rhesus macaques (Macaca mulatta).

Methods
We resequenced five additional primate species: cynomolgus macaques (M. fascicularis), vervet monkeys (Chlorocebus aethiops sabeus), marmosets (Callithrix jacchus), cotton-top tamarins (Saguinus oedipus), and squirrel monkeys (Saimiri boliviensis). We also tested downstream cAMP signaling using an inducible CRE-responsive firefly luciferase reporter in a cell culture system. Cell lines were developed harboring each of the alleles and were treated with varying concentrations of an OPRM1-specific agonist, either morphine, human β-endorphin, non-human primate β-endorphin, fentanyl, or DAMGO, and 1 µM forskolin. Luciferase activity was measured to determine ligand effects.

Results
Common polymorphisms were found in the N-terminal domain of OPRM1 in other primate species: C140T (P47L) in cynomolgus macaques, G55C (D19H) in vervet monkeys, A111T (L37F) in marmosets, and C55T (P19S) in squirrel monkeys. The human T17 allele behaves in a functionally parallel manner to the G118 allele, conferring significant differences compared to the more common C17/A118. Similar functional effects were observed for polymorphisms in other species, though there remain ligand-specific differences.

Discussion
Non-human primate models of human disease can be designed with shared molecular etiologies, allowing for separation of genetic and environmental factors in complex human association studies and greater translational relevancy in drug development. These preliminary studies in OPRM1 suggest the feasibility of the approach and set the stage for future, large-scale studies currently underway. Together this offers the opportunity for the development of shared functional polymorphisms across species as a new genetic tool for translational research.
EPIGENETIC MECHANISMS OF CHRONIC STRESS EFFECTS ON THE ANKyrIN 3 BIPOLAR DISORDER GENE
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Background
The susceptibility of bipolar disorder (BD) is known to be influenced by genetic and environmental factors. Genome-wide association studies (GWAS) of bipolar disorder case/control samples have highlighted a number of genes associated with BD including ankyrin 3 (ANK3), but the role of these genes in the pathophysiology of the disorder is largely unknown. Chronic stress triggers recurrence of BD symptoms, and dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis that controls stress reactivity is thought to contribute to BD pathogenesis. Previously, our group has shown that disruption of Ank3 expression in the brain of Ank3\(^{-/-}\) mice increases impulsivity and motivation for reward, two prominent features of BD mania. Interestingly, chronic stress reverses this phenotype to produce a depressive-like state (Leussis et. al., 2012). These data suggest that Ank3\(^{-/-}\) mice with reduced Ank3 expression have increased stress sensitivity. Here, we further explore the relationship between Ank3 and stress by investigating epigenetic mechanisms that perturb brain gene expression in the Ank3\(^{-/-}\) mouse model. Epigenetic modifications such as DNA methylation of gene promoters at CpG islands are known to be altered in rodents by stress. Methylation of promoter sequences has been shown to reduce gene expression by reducing the binding affinity of transcription factors to their specific sequence motifs. Therefore, given the apparent stress sensitivity of Ank3 \(^{-/-}\) mice, we hypothesize that stress alters expression of genes functioning in mood neural circuits through perturbed DNA methylation of gene promoters.

Methods
We are examining differential expression of genes in the hippocampus of Ank3 \(^{-/-}\) compared Ank3 \(^{+/+}\) wildtype mice under chronic stress conditions, as well as non-stress conditions, using RNA-Seq technology. RNA-Seq is an emerging method that allows detailed investigation of differential expression, novel transcripts and splice sites in the transcriptome. Genes that are found to be differentially expressed in Ank3 \(^{-/-}\) mice exposed to stress by RNA-Seq will be validated through quantitative PCR (qPCR). Since we hypothesize that DNA methylation is the mechanism by which gene expression will be altered by stress in the Ank3\(^{-/-}\) mouse model, we will investigate the methylation status of the promoter sequences of genes that are differentially expressed using bisulfite-sequencing methods.

Results
We have harvested hippocampus tissue from Ank3\(^{-/-}\) mice exposed to chronic stress (6 weeks isolation housing) and not exposed to stress (standard group housing), as well as stressed and non-stressed Ank3\(^{+/+}\) wildtype mice (\(N = 15\) mice per group). RNA and DNA for the RNA-Seq and DNA methylation bisulfite sequencing experiments (respectively) were extracted used the Qiagen AllPrep DNA/RNA mini kit. Pilot qPCR experiments suggest that Ank3 gene expression is not altered by stress in hippocampus of Ank3\(^{-/-}\) mice or Ank3\(^{+/+}\) mice compared to non-stressed mice. These data suggest the potential involvement of other gene expression changes in the stress sensitivity of Ank3 \(^{-/-}\) mice, which will be examined by our ongoing RNA-Seq experiments.
Discussion
Our results indicate that Ank3+/- deficient mice exhibiting heightened sensitivity to chronic stress do not have changes in Ank3 expression after exposure to stress. These data have led us to utilize RNA-Seq to investigate the effects of stress on expression of a broader range of genes involved in brain circuits regulating mood and the stress response pathway that may highlight the genes responsible for the heightened stress sensitivity observed in Ank3+/- mice. A better molecular understanding of the interaction between stress and Ank3 function may improve knowledge of the interplay between genetic and environmental risk factors underlying the complex brain abnormalities that occur in BD. References Leussis, M.P., et al., The ANK3 bipolar disorder gene regulates psychiatric-related behaviors that are modulated by lithium and stress. Biol Psychiatry, 2013. 73(7): p. 683-90.

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DISRUPTION OF ANKYRIN 3 IN BRAIN INCREASES IMPULSIVE-RELATED BEHAVIOR IN MICE
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Background
Ankyrin 3 (ANK3) has been identified as a risk gene for bipolar disorder (BD) in genome-wide association studies; however, the role of ANK3 in the pathophysiology of BD is largely unknown. Previously, our group reported that reduced Ank3 in brain leads to impulsive-like behavior, a classic feature of BD mania (Leussis et al., 2013). Leussis, M.P., et al., The ANK3 bipolar disorder gene regulates psychiatric-related behaviors that are modulated by lithium and stress. Biol Psychiatry, 2013. 73(7): p. 683-90.

Methods
In order to further explore the role of Ank3 in impulsivity, we tested Ank3+/+ and Ank3+/- mice in the intolerance-to-delay (ITD) task and the passive avoidance (PA) task. In the ITD task, mice must choose between a small reward delivered immediately and a large reward delivered after delay. Impulsivity is defined as a high preference for immediate over delayed rewards. In the PA task, mice learn to avoid an environment in which an aversive event (foot shock) was previously delivered. In this task, impulsivity is defined as the inability to withhold a behavioral response associated with negative consequences.

Results
Ank3+/- mice showed a greater preference for small immediate rewards than Ank3+/+ mice in the ITD task, suggesting that disruption of Ank3 increases impulsive choice. Ank3+/- mice also showed decreased latency to enter an environment previously paired with a foot shock in the PA test, suggesting that disruption of Ank3+/- results in a failure to inhibit a behavioral response associated with negative consequences. We confirmed that the impulsive behavior observed in the Ank3+/- mice was not due to impaired in memory or sensory processing.

Discussion
Collectively, our findings suggest that disruption of Ank3 increases two important features of impulsivity: the preference for immediate over delayed gratification, and the inability to
withhold a behavioral response associated with negative consequences. In future studies, we plan to investigate the neural circuits that mediate impulsive behavior in Ank3+/- mice by measuring neuronal activity (c-fos reactivity) induced by exposure to an impulsivity task. In addition, we plan to perform pharmacological studies to attenuate the impulsive behavior observed in Ank3+/- mice. These data will provide important information regarding the functional relevance of Ank3 to the pathophysiology of BD.

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PTSD SYMPTOMS ASSOCIATED WITH COMT IN A MULTIGENERATIONAL FAMILY STUDY OF ARMENIANS EXPOSED TO TRAUMA

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Background
PTSD is a disabling disorder that develops in the context of extreme traumatic circumstances. Symptoms of PTSD include recurrent intrusive thoughts and memories of the traumatic event, hyperarousal and numbness, detachment and avoidance. The genetics of PTSD is difficult to study because related individuals are rarely exposed to the similar severity of a traumatic event. This group has been studying multigenerational families in Armenia exposed to the 1988 catastrophic Spitak earthquake. They have shown that the vulnerability to PTSD is heritable (h2=42%) (Goenjian et al., 2008). We have recently reported a significant association of TPH (rs1178997) and PTSD (Goenjian et. al., 2012).

Methods
In the present study we aimed at replicating prior findings of association between Cathechol-o-methyltransferase (COMT) SNP rs4680 and PTSD (Kolassa et. al., 2010; Boscarino et. al., 2011). Additionally, we assessed the association of three other COMT SNPs (rs4633, rs4818,rs6269) and PTSD symptoms individually, and in combination with TPH2 SNP rs1178997 to the risk of PTSD symptoms . Subjects included 200 unascertained Armenians from 12 multigenerational families exposed to the 1988 earthquake in Gyumri. Symptoms of PTSD were measured using the UCLA PTSD Research Index.

Results
Significant association was observed between COMT SNP rs4633 and PTSD symptoms (p = 0.03).

Discussion
COMT SNP rs4633 is significantly associated with PTSD symptoms and together with TPH2 SNP rs1178997 contributed to 5% of the variance of PTSD symptoms

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A REPLICATION STUDY OF THE ESTROGEN RECEPTOR I GENE (ESR1) AND ANOREXIA NERVOSA
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Background
The female preponderance and onset around puberty in the majority of eating disorders, suggest that sex hormones, like estrogens, may be involved in the onset of these disorders. An eight-SNP haplotype at the estrogen receptor I (ESR1) gene was found to be associated with anorexia nervosa (Versini et al., Neuropsychopharmacology 2010) and three SNPs from this haplotype (rs726281, rs2295193 and rs3798577) were associated with anorexia nervosa and/or eating disorders. Our objective was to replicate these findings in an independent cohort of 520 patients with an eating disorder, of whom 244 had anorexia nervosa (142 restricting type), from the GenED study and 2810 random women from the Netherlands Twin Register.

Methods
The frequencies of the eight-SNP haplotype and three ESR1 SNPs were compared between patients with an eating disorder, with anorexia nervosa(restricting type) and the control women.

Results
Neither the haplotype nor the three ESR1 SNPs were associated with eating disorders, anorexia nervosa or restricting type anorexia nervosa.

Discussion
Despite sufficient statistical power, the associations reported by Versini et al. (Neuropsychopharmacology 2010) were not replicated.

DIFFERENTIAL EXPRESSION ANALYSIS OF NOVEL GENES IN BIPOLAR DISORDER USING RNA-SEQ
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Background
RNA-Seq transcriptome profiling using next generation sequencing technologies provides a far more precise measurement of levels of transcripts and their isoforms compared to the more widely used microarray technology. It provides a direct estimate of transcript abundance and can detect other characteristics such as alternative splicing, novel transcripts, and allele-specific expression. In this study, we performed deep-sequencing (110M-200M paired-end reads) of high
quality total RNA extracted from the dorsolateral prefrontal cortex of 15 cases with bipolar I disorder and 15 healthy controls.

Methods
The Stanley Medical Research Institute provided the sample and distributed the data. Paired-end sequencing was performed on the Illumina GA-II. After quality assessment using the FastX toolkit and FastQC and removal of poor reads, reads were mapped and aligned to the reference genome hg19 using two aligner applications, TopHat and GSNAP. Principal Component Analysis was performed, followed by Surrogate Variable Analysis, to control for systematic measured and unmeasured variability across samples. We then performed differential expression analysis with DESeq, edgeR, bayseq, and cuffdiff and results were combined across workflows. Gene-set enrichment analysis was then performed with DAVID and GSEA.

Results
After initial assessment, we found evidence for differential expression of 1,153 unique genes at a nominal p-value < 0. A total of 265 differentially expressed genes are identified that overlapped between two or more workflows. Preliminary gene-set analyses show evidence for the enrichment of 12 pathways. We will present on these and other analyses, including the differential expression of gene isoforms.

Discussion
Our initial results demonstrate that RNA-Seq may reveal differential expression of genes in biologically relevant pathways that may help uncover important etiological insights into the pathophysiology of bipolar disorder.

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DISTRIBUTION OF CAG EXPANSION AT THE HUNTINGTIN LOCUS IN INDIA
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Background
The prevalence of the HD varies among different populations. An estimated 2.5-10 per 100,000 people of European ancestry are affected with HD, while the Japanese and African populations show significantly lower prevalence (0.6-3.8 per 100,000). Indian and other East Asian populations are also expected to have a lower prevalence of HD; however the genetic structure of CAG expansion in the Indian population is not well described.

Methods
The clinical samples (n=256) were derived from both outpatients and inpatient referral to Genetic Counseling and Testing Clinic (GCAT) at the National Institute of Mental Health and Neurosciences (NIMHANS), Bangalore, India. The CAG expansion was characterized using standard protocols. The PCR products were subjected to fragment analysis using ABI 3500xl genetic analyzer and CAG repeat sizing was performed using GeneMapper v3.5.
Results
Genetic analysis of the HD cohort revealed expanded alleles (more than 39 CAG repeats) in 104 samples. Ten of these were clinically unaffected at the time of sampling. Fifty individuals showed choreiform movements but did not have a pathological expansion at the HTT locus. The healthy cohort (n= 102) showed a mean CAG distribution of 17.7± 2.1 [range: 11- 33] at the lower allele and 19.9 ± 3.4 [range: 15- 33] at the upper allele. The number of CAG repeats in the HD individuals ranged from 40 to a higher end of 85 repeats in the upper allele [mean: 46.1± 3.4]. The average age of onset (AAO) was 37.7± 12 years, with the CAG repeat size correlating negatively with the AAO. Further analysis of the expanded allele revealed a majority of expansions in the range of 40-50 CAG repeats (87%) with higher expansions (>50 CAG repeats) being less common. The higher expansion had a corresponding severe illness [AAO: 36.9± 12.9] with AAO as young as 4 years. A Large number of HD families showed paternal inheritance (40%) followed by maternal inheritance (25%). Sporadic mutations without any reported family history was seen in 10% of our affected cohort. All of these CAG expansions were in the 40-50 range, thus showing a multistep increase in the CAG repeat size.

Discussion
This data demonstrates a distribution of CAG repeats similar to the European population in both normal and HD affected chromosomes. Varying prevalence rates of HD are reported worldwide based on molecular genetic studies of affected individuals. The variation in the prevalence and incidence of HD can be attributed to various cis – trans mechanisms acting across populations. Further haplogroup analyses across various ethnic groups in the subcontinent are being conducted to better understand the distribution of alleles at the HTT locus in India.


GENETIC, PSYCHOSOCIAL AND CLINICAL FACTORS ASSOCIATED WITH HIPPOCAMPAL VOLUME IN A COMMUNITY- BASED SAMPLE
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**Background**
Recent genome-wide association (GWAS) meta-analyses demonstrated associations between five genetic loci and hippocampal volume. Besides direct genetic influences, conditions like overweight, type 2 diabetes, hypertension and depression have been identified as relevant factors affecting the hippocampal volume. The present study aims at investigating the putative interplay between genetic variants and clinical factors influencing the hippocampal volume in the general population.

**Methods**
Subjects from the Study of Health in Pomerania (SHIP-2; SHIP-Trend-0) who underwent whole-body MRI and genotyping were included in the analysis (n=1510). Hippocampal volumes were segmented with FreeSurfer. Associated genetic variants and clinical factors were assessed for potential interactions.

**Results**
Associations with hippocampal volume were found for rs6741949 (DPP4), rs7852872 (ASTN2), rs17178006 (MSRB3), rs7294919 (TESC, HRK, FBXW8) and smoking. Additionally we observed a significant interaction between rs7852872 (ASTN2) and rs6581612 (WIFI) (p=4.9x10^{-4}; beta=0.098).

**Discussion**
This study revealed and confirmed the impact of rs6741949 (DPP4), rs7852872 (ASTN2), rs17178006 (MSRB3), rs7294919 (TESC, HRK, FBXW8) and smoking on hippocampal volume in the general adult population. Thus, these factors might play a role in the individual susceptibility to neurodegeneration.

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**GENOME-WIDE SIGNIFICANT ASSOCIATIONS FOR GLOBAL WHITE MATTER INTEGRITY IN THE HUMAN BRAIN**
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**Background**
Post-mortem research, gene expression studies and brain imaging studies indicate that white matter structure is implicated in several psychiatric disorders including major depressive disorder, bipolar disorder and schizophrenia. Diffusion tensor imaging studies have shown that white matter integrity as indexed by fractional anisotropy (FA) is heritable and reduced in patients and their unaffected relatives, making it an excellent endophenotype candidate.
Quantitative intermediate phenotypes are assumed to be statistically more powerful and are biologically closer to the action of psychiatric risk genes than the clinical phenotype. Investigating the genetic basis of white matter integrity could therefore give important clues to the genetic and neuroanatomical basis of psychiatric disorders.

Methods
Participants included 776 Mexican-American individuals from extended pedigrees. DTI data were acquired on a Siemens 3T Trio scanner, with b-values $b=0$ and $b=700 \text{ s/mm}^2$ along 55 non-collinear directions and a spatial resolution of $1.7 \times 1.7 \times 3 \text{ mm}$. TBSS was applied to create white matter skeletons representing the centers of white matter within each subject. Heritability of mean fractional anisotropy (FA) within this skeleton was calculated using SOLAR. Genome-wide association was conducted utilizing 931,219 SNPs from Illumina microarrays under an additive genetic model. Covariates included age, sex, their interactions, and the first 4 principal components to account for population stratification.

Results
FA was significantly heritable ($h^2=0.52$, $p=1.09 \times 10^{-10}$) and a number of genome-wide significant SNPs were found. The strongest association was on 17q24.1 with the intergenic SNP rs10853057 ($p=3.18 \times 10^{-10}$) close to the gene GNA13, and the second strongest SNP rs12249377 ($p=3.26 \times 10^{-10}$) was in the serotonin receptor HTR7 gene on 10q23.31. An additional three significant hits were on chromosomes 12 (rs258415, $p=7.97 \times 10^{-10}$), 16 (rs1991867, $p=1.94 \times 10^{-8}$) and 1 (rs1361277, $p=2.00 \times 10^{-8}$). Our top two SNPs had previously shown significant associations ($0.002 < p < 0.03$) with major depressive disorder (rs10853057 and rs12249377) and schizophrenia (rs10853057) in the Psychiatric GWAS consortium datasets.

Discussion
FA is a heritable trait and a powerful quantitative phenotype to examine in genome-wide association studies. Using a completely data-driven method, we find that our top two SNPs have previously been implicated in psychiatric disorders, suggesting that the use of brain imaging endophenotypes is an effective approach to identify new candidate risk variants for psychiatric disorders. Our results encourage further research into the biological roles of these genome-wide significant SNPs in health and disease.

65 DISRUPTIONS IN WHITE MATTER INTEGRITY FOUND IN SCHIZOPHRENIC SUBJECTS ARE MODULATED BY A GENE SET OF 17 MYELIN-PROTEIN GENES.
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Background
Disruptions in white matter (WM) tract structures have been consistently implicated in the pathophysiology of schizophrenia. Recent data suggests that WM abnormalities in schizophrenia are not localized to one specific brain region, but instead reflect global low-level decreases in fractional anisotropy (FA) coupled with focal abnormalities. Global WM integrity (as measured
by FA) is highly heritable and may provide a good endophenotype for genetic studies of schizophrenia. Furthermore, it has been suggested that the same genes that control the integrity of white matter tracts also mediate the effect on cognitive performance in schizophrenia.

Methods
In this study we analyzed FA, Cognitive Functioning (WAIS-III full-scale IQ score, and verbal working memory), and genetic data from the Mind Research Network Clinical Imaging Consortium Study. We performed a functional gene set analysis to study the combined effect of multiple oligodendrocyte functional genes-sets in Schizophrenia, white matter integrity and cognitive functioning of 181 subjects (77 Cases and 104 controls).

Results
We found that FA was significantly associated with Schizophrenia in our sample. The myelin-proteins gene set (17 genes) was significantly associated with Schizophrenia (p= 0.004). In order to test if this association was mediated by FA integrity, we controlled the gene set association analysis for FA; the association between the gene set and schizophrenia remained significantly associated (p= 0.04). The myelin-proteins gene set was not associated with cognitive ability or with FA.

Discussion
In our study we applied a novel approach developed by our group to test for the combined effects of all genetic variants available within a myelination-proteins genes set of 17 genes. Our findings support the hypothesis that multiple genetic variants in myelination-proteins genes have an accumulative effect in the increases the risk for schizophrenia; this effect was mediated by fractional anisotropy, a white matter integrity measure.

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PLEIOTROPIC GENETIC EFFECTS ON OBESITY AND BRAIN ANATOMY
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Background
Obesity is a major contributor to the global burden of chronic disease and disability; though current knowledge of the biological underpinnings of obesity is comparatively poor. A significant body of literature exists supporting obesity as a disorder of hypothalamic function. This has been supported through several studies investigating cognitive dysfunction, brain atrophy and brain volume, all of which are significantly adversely influenced by overweight and obesity. While neuroanatomic variation has previously been associated with obesity, it is unclear if this relationship is due to common genetic factors. In this study, we sought genetic components that influence both brain anatomy...
and body mass index (BMI) to provide further insight into the role of the brain in energy homeostasis and obesity.

**Methods**

We acquired structural magnetic resonance images of brain anatomy from 839 Mexican American individuals from large extended pedigrees and performed bivariate linkage and quantitative analyses using SOLAR.

**Results**

Our results showed that genetic factors associated with increased BMI were also associated with reduced cortical surface area and volume. Additionally, we identified two genome-wide quantitative trait loci pleiotropically influencing BMI and brain volume on chromosomal regions 3q22.1 and 17p13.1. Region 17p13.1 harbors genes that pleiotropically influenced ventral diencephalon volume and BMI. This region has been implicated in childhood obesity, plasma leptin levels and hip and waist circumference. The ventral diencephalon houses the hypothalamus, which is involved in the regulation of eating tendencies. Although feeding behavior is complex, the lateral hypothalamus is commonly referred to as the “feeding center” of the brain because animal models show that stimulation increases food intake yet a lesion inhibits motivation to feed, suggesting functional plausibility for our finding on chromosome 17. The second QTL we identified was 3q22.1, which significantly influenced the surface area of the supramarginal gyrus and BMI. This region has also been previously associated with BMI. The supramarginal gyrus has been linked to brain function differences in overweight and healthy weight individuals. While the role of the supramarginal gyrus is poorly understood in this context, our findings in conjunction with other reports implicate its involvement in the pathogenesis of obesity due to shared influence of a region on chromosome 3.

**Discussion**

This is the first report to quantify shared genetic factors of brain anatomy and obesity and localize significant pleiotropic influence. Our results identify regions of the genome that are directly related to both brain structure and obesity. Therefore, the same causal biologic pathway likely influences the underlying function of these brain regions and the outcome measure of obesity.

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**BRAIN-DERIVED NEUROTROPHIC FACTOR AND DEFICIENT AMYGDALA HABITUATION TO UNPLEASANT EMOTIONAL STIMULI IN BORDERLINE PERSONALITY DISORDER: A RESEARCH DOMAIN CRITERIA IMAGING GENETICS STUDY**

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Background
Borderline personality disorder (BPD) patients show hyperarousal and emotion-regulation deficits and deficient amygdala habituation to repeated emotional stimuli. The Val66Met (rs6265) SNP Met allele of the brain derived neurotrophic factor (BDNF) gene increases amygdala reactivity and impairs extinction learning, a phenomenon closely related to habituation. In the present study we expand and refine our prior finding of deficient amygdala habituation in BPD by testing the impact of BDNF Val66Met genotypes on amygdala reactivity to repeated emotional and neutral pictures in BPD patients in an imaging genetics framework.

Methods
We employed event-related functional magnetic resonance imaging (fMRI) in unmedicated BPD (n=19) and schizotypal personality disorder (SPD, n=18) patients and healthy controls (HC, n=20) during a task involving viewing unpleasant, neutral, and pleasant pictures presented twice. The rs6265 or Val66Met SNP in exon 11 of the BDNF gene was one of the 130 candidate genes genotyped on a custom-designed Illumina 1536 SNP array. Genotyping was carried out following Illumina GoldenGate assay protocols and the arrays were imaged on an Illumina Beadstation GX500. Subjects were classified as Met-allele carriers or Non-Met allele carriers (ValVal homozygotes). Amygdala responses were examined with a mixed-model multivariate ANOVA including BDNF Val66Met SNP genotype (Met-carriers vs Non-Met carriers).

Results
We found a significant Diagnostic group × Genotype (BDNF Val66Met SNP Met- vs Non-Met-carriers)×Picture type (unpleasant, neutral, pleasant)×Picture repetition (Novel/Repeat)×Time interaction (F[40,64] = 1.68, p<0.04, Wilks) indicating that Met-carrying BPD patients (but not Met-carrying SPD patients or HCs) showed exaggerated amygdala reactivity to repeated, but not novel, unpleasant pictures, representing a failure to habituate.

Discussion
Using an imaging genetics approach for the first time in BPD, we found that the deficit in amygdala habituation to emotional stimuli in BPD is restricted to those carrying the BDNF gene 66Met allele, suggesting that BDNF mediates the amygdala hyper-reactivity to unpleasant emotional stimuli found in BPD, and supporting BDNF modulators as a novel therapeutic avenue for BPD.

THE COMPLEXITY OF GENETIC EFFECTS IN PHARMACOGENETICS: FOCUS ON NEUROPLASTICITY, ENVIRONMENTAL STRESS AND RESPONSE TO ANTIDEPRESSANTS
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Background
In the last years, interest has been increased in the potential involvement of neurotrophic and growth factors (neuroplasticity) in psychiatric disorders’ risk, as well as psychotropic drugs’
effects. In particular, it has been hypothesized that genetic differences related to factors involved in CNS neuroplasticity may in part explain individuals’ variation in ADs response. However, both animal and human studies have provided mixed and conflicting results so far, suggesting that the effect of neuroplastic factors may be moderated by a number of influences other than genetics, and genetics effects may be modulated by other biological, environmental, and individuals’ factors. The aim of the present study was to preliminarily evaluate the role of genetic variation within two genes involved in neuroplastic processes in early response to ADs: the Brain derived neurotrophic factor (BDNF) coding for a major prosurvival factor in the brain, and Sialyltransefarase 8B (ST8SIA2), which product modulates the adhesive properties of Neural cell adhesion molecule (NCAM). For the reasons mentioned above, we tested potential differential effect of genetic variants depending on environmental stress exposure.

Methods
The sample was composed by 114 patients affected by Mood or Anxiety disorders, enrolled for treatment with ADs, scoring 8 or more at the Hamilton Rating Scale for Depression score (HAMD), and having filled at the time of recruitment a modified version (self-report) of the Brown & Harris Stressful Life Events and Difficulties Interview (SLEDS). This modified version allows to collect stressful life events (SLEs) in young age (less than 15 years old), the year before illness onset, and the month preceding current episode. All the patients were evaluated at baseline and weekly thereafter until the fourth week by the Hamilton Rating Scale for Depression (HRSD). Subjects were genotyped for 3 single-nucleotide polymorphisms (SNPs) in BDNF and 5 in ST8SIA2. Linkage disequilibrium among SNPs was calculated by Haploview software and haplotypes were obtained by the R-software. The GLM model was employed to test the effect of alleles and haplotypes, crossed with exposure to stress, on % response at follow-up.

Results
SLEs did not impact significantly on early response to ADs. Alleles in two SNPs in BDNF (rs11030101 A-allele and rs11030104 G-allele) and alleles in two SNPs in ST8SIA2 (rs11853992-A allele and rs17522085-T) were associated to a slower response to ADs only if non-exposed to onset SLEs, whilst they had a similar response compared to the carriers of the opposite variant if exposed to onset SLEs (all p<.007). Haplotype analyses confirmed these trends.

Discussion
According to our data, variants in BDNF and ST8SIA may influence differentially the early response to ADs depending on exposure to SLEs at illness onset. Exposure to stress may impact the transcriptional activity of genes through epigenetic mechanisms, resulting in unexpected phenotypes. Further, recent animal studies reported that an over expression of BDNF may have detrimental effects (Govindarajan A, 2006), and some studies in humans found heterozygous for the most investigated BDNF rs6265 SNP having a better response to ADs (Tsai SJ et al., 2010). The complex interplay between genetic effects, environmental factors, as well as other biological systems deserves further investigation by means of sophisticated methods of investigation.
ASSOCIATION ANALYSIS BETWEEN GLUTAMATE SYSTEM GENE VARIANTS GRM2, SLC1A2, SLC6A9, GRIA1, AND GAD1 AND CLOZAPINE RESPONSE IN PATIENTS WITH SCHIZOPHRENIA

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Background
Schizophrenia (SCZ) is a debilitating mental health disorder that causes immeasurable pain and suffering to those afflicted. Response to antipsychotic treatment for SCZ is highly variable, and twin studies suggest a genetic component. Altered glutamate activity has been implicated in the etiology of SCZ, as well as in response to the atypical antipsychotic clozapine (CLZ). The aim of this study was to investigate genetic associations between glutamate system gene variants and response to CLZ.

Methods
Fifteen single nucleotide polymorphisms (SNPs) within glutamate system genes (GRM2, SLC1A2, SLC6A9, GRIA1, and GAD1) were assessed in 252 patients diagnosed with SCZ using DSM-III-R criteria. Variants were selected based on potential functionality and recent citations from the literature. Standard Taqman OpenArray® genotyping procedures were used. Power calculations were performed using Quanto 1.2.4 and linkage disequilibrium was determined using Haploview 4.2. UNPHASED 3.1.5 software was used for haplotype analysis. In terms of genetic analyses, dichotomous variables were analyzed using χ²-test and continuous variables were analyzed using analysis of covariance (ANCOVA), with baseline scores and age as covariates. All statistical analysis were performed using SPSS 20.0 and PLINK software 1.06. The Nyholt method was used to correct for multiple testing.

Results
Our subgroup of 163 European SCZ patients with categorical response data and 90 patients with continuous response data had over 80% power to detect an odds ratio of 2.75 and variance of ≥5%, respectively, at a non-responder frequency of 47.2% (α=0.05, two sided; minor allele frequency=0.123; additive model). No significant association between glutamate SNPs and CLZ response was detected after correction for multiple testing. However, a trend was observed for SLC6A9 variant rs16831558 in which CC homozygotes showed better response to CLZ than TC/TT genotypes in the BPOS subscale (p=0.008, p_corrected=0.114). In addition, AA homozygotes of GRIA1 variant rs2195350 showed better response than AG/GG genotypes in the BNEG subscale (p=0.007, p_corrected=0.100). A significant association was found between a haplotype of SLC6A9 rs16831558T-rs12037805T-rs1978195A (p=0.0012, p_corrected=0.017) and CLZ response.

Discussion
Our study supports the hypothesis that glutamate system gene variants, particularly SLC6A9, play a role in CLZ response in our sample of SCZ patients and future work to replicate these finding are warranted.
ABCB1 POLYMORPHISM PREDICTS ESCITALOPRAM DOSE NEEDED FOR REMISSION IN MAJOR DEPRESSION

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Background
The ABCB1 (P-glycoprotein) transporter is a key component of the blood brain barrier (BBB). Many antidepressants are subject to ABCB1 efflux. Functional polymorphisms of ABCB1 may influence CNS bioavailability of antidepressants subject to efflux. Single nucleotide polymorphisms (SNPs) at rs1045642 (C3435T) of ABCB1 have been associated with efflux pump efficiency. This may explain part of the inter-individual variation in antidepressant dose needed to remit.

Methods
A candidate Gene Association study of individuals (N=113) with DSM-IV major depressive disorder (MDD) was conducted. Subjects were treated with escitalopram (ESC) or venlafaxine (VEN) over 8 weeks. The 17-item Hamilton Depression Rating Scale (HDRS) was assessed serially, blind to genotype. SNP rs1045642 of ABCB1 along with two SNPs previously reported to be in linkage disequilibrium (LD) with it, (rs2032582 and rs1128503) were genotyped. Demographic features, clinical features, P450 metaboliser status, and 5-HTTLPR genotype were controlled for.

Results
Carriers of rs1045642 TT needed on average 11mg of ESC to remit whereas TC and CC carriers required 24mg and 19mg respectively (p=0.0001). This equates to a 2.0 (95% CI=1.5-3.4; p<0.001) fold greater ESC dose needed to remit for C carriers compared to TT carriers at rs1045642. Of VEN treated subjects carrying TT genotype at rs1045642, 73.3% remitted compared to 12.5% for CC genotype (OR = 6.69, 95% CI 1.72 to 25.9, p=0.006).

Discussion
These data suggest antidepressant dose needed to remit can be predicted by an ABCB1 SNP. This has potential clinical translation implications for dose selection and remission from MDD.

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GENOME-WIDE ASSOCIATION STUDY IDENTIFIES A POTENT LOCUS ASSOCIATED WITH HUMAN OPIOID SENSITIVITY

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Background
Opioids, such as morphine and fentanyl, are widely used as effective analgesics for the treatment of acute and chronic pain. In addition, the opioid system has a key role in the rewarding effects of morphine, ethanol, cocaine and various other drugs. Although opioid sensitivity is well known to vary widely among individual subjects, several candidate genetic polymorphisms reported so far are insufficient for fully understanding the wide range of interindividual differences in human opioid sensitivity.

Methods
Subjects enrolled in this multistage genome-wide association study (GWAS) were 355 healthy subjects who were scheduled to undergo cosmetic orthognathic surgery for mandibular prognathism and provided informed, written consent for the genetics studies. Postoperative pain was managed with a bolus dose of 20 μg fentanyl on demand using a patient-controlled analgesia (PCA) pump when patients felt pain, with a lockout time set at 10 min. Postoperative PCA fentanyl use during the first 24-h postoperative period was recorded, whereas venous blood (10 ml) of the subjects was sampled for preparation of DNA specimens for genotyping. Whole-genome genotyping was performed with iScan System (Illumina, San Diego, CA, USA) and mainly two kinds of BeadChip, Human1M v1.0 and Human1M-Duo v3. For additional analyses, 112 patients who underwent major open abdominal surgery, 203 patients with methamphetamine (METH) dependence, 438 patients with alcohol dependence, 228 patients with eating disorders, and 500 healthy volunteer subjects with personality profile data from the temperament and character inventory (TCI) were recruited and the TaqMan allelic discrimination assay (Life Technologies, Carlsbad, CA, USA) was mostly conducted for genotyping after total genomic DNA was extracted from whole-blood or oral mucosa samples using standard procedures.

Results
By conducting the GWAS in healthy subjects who underwent cosmetic orthognathic surgery, we found that genetic polymorphisms within a linkage disequilibrium block that spans 2q33.3–2q34 were strongly associated with the requirements for postoperative opioid analgesics after painful cosmetic surgery. The C allele of the best candidate single-nucleotide polymorphism (SNP), rs2952768, was associated with more analgesic requirements, and consistent results were obtained in patients who underwent abdominal surgery. In addition, carriers of the C allele in this SNP exhibited less vulnerability to severe drug dependence in patients with methamphetamine dependence, alcohol dependence, and eating disorders and a lower ‘Reward Dependence’ score on a personality questionnaire in healthy subjects. Furthermore, the C/C genotype of this SNP was significantly associated with the elevated expression of a neighboring gene, CREB1.

Discussion
These results show that SNPs in this locus are the most potent genetic factors associated with human opioid sensitivity known to date, affecting both the efficacy of opioid analgesics and liability to severe substance dependence. Our findings provide valuable information for the personalized treatment of pain and drug dependence.

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GENETIC TESTING OF CYTOCHROME P450 ENZYMES IMPROVES PSYCHIATRIC DRUG TREATMENT: REACHING OUT FOR THE 'IMPOSSIBLE DREAM' OF PERSONALIZED MEDICINE?
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Background
Antipsychotic and antidepressant medication are used for treatment of many psychiatric conditions such as schizophrenia, mood and anxiety disorders and obsessive-compulsive disorder (OCD) Two polymorphic enzymes, CYP2D6 and CYP2C19, metabolize a large number of these medications. Functional polymorphisms in these enzymes can confer altered enzymatic activity, typically allowing to identify poor, intermediate, normal/extensive and rapid metabolizers. Ignoring the genetic variability may potentially lead to toxic or subtherapeutic drug levels in individual patients. Previous studies have shown mixed results with respect to the role of the metabolizer status and drug response. However, most studies suffered from substantial limitations. We conducted a study in a larger set of OCD patients demonstrating that abnormal CYP2D6 activity (i.e. non-extensive metabolizer) was significantly associated with non-response to antidepressants and to significantly higher number of antidepressant trials (p<0.05; Müller et al., 2012 and Brandl et al., in press). We have started to routinely provide genetic testing of major drug metabolism genes (e.g., CYP2D6, CYP2C19, CYPC9, CYP1A2) to patients in our clinic and will here report on our experiences.

Methods
At our Pharmacogenetics Research Clinic (www.pharmacogenetics.ca), we enroll patients with a diagnosis of schizophrenia and mood disorders, mostly with complicated medication histories or who will start taking a new psychiatric drug. As a first step, we have prospectively assessed and genotyped for CYP2D6 (ten SNPs and gene duplications) as well as CYP2C19 (three SNPs). Patients participate in a structured diagnostic interview and are assessed of current and previous treatment response and occurrence of side effects. Physicians are then provided with an interpretation of the genotypic results and informed in detail about the potential clinical implications which they will discuss with their patients. Serum drug levels of the CYP2D6 and/or CYP2C19 metabolized drugs are also assessed and after 6 weeks the physician completes a questionnaire evaluating the usefulness of the genotypic information provided by the study. After 12 weeks, the clients are assessed again to monitor potential adjustments of medications and their overall treatment outcome. A major methodological challenge has been to provide rapid genotyping service (within 48 hours) and to define risk medication according to the genetic results.

Results
Overall, close to 100 patients were now enrolled in our study and successfully genotyped, detecting a relative high number of non-extensive metabolizers. Analyses of the questionnaires revealed that the genotyping service was generally very well accepted. Patients and physicians perceived the genetic information as very useful and were very satisfied to obtain genetic-based suggestions for future drug treatment. Some physicians reported that the test helped to either select medications their patients tolerated better, or to adjust doses based on genotype results and serum levels. Importantly, none of the individuals were found to have worsened following the genotype based recommendations for psychiatric drugs. Individual case reports will be presented strongly suggesting that preemptive genotyping would have helped to avoid side effects or lengthy trials with non-response.

Discussion
Our study was exclusively designed to prove the feasibility and usefulness of genetic testing in clinical practice strongly support the notion that CYP2D6 and CYP2C19 genotyping provides useful information and help physicians to improve pharmacotherapy for individual patients. Pilot data from our studies have helped to secure funding from the provincial government in order to genotype up to 20,000 individuals over the next few years across the greater Toronto area (www.im-pact.ca). We are developing a genetic test algorithm that will include clinical and demographic variables as well as gene-gene interaction analyses. Furthermore, we will expand our gene panel by including pharmacodynamic genes (e.g., SLC6A4) and are also developing a panel to predict side effects, in particular for antipsychotic-induced weight gain.

G-PROTEIN COUPLED RECEPTOR SINGLE-NUCLEOTIDE POLYMORPHISM DISTRIBUTION IN PRIMATE GENOMES
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Background
G-protein coupled receptors (GPCRs) play an inordinately large role in human health. Drugs targeting GPCRs are used to treat numerous disorders across a broad spectrum of diseases. Variation at these genes is associated with numerous disorders and pharmacogenetic effects are modulated, in part, by polymorphisms at these drug targets. Recently, non-human primate models have been developed focusing on naturally-occurring, functionally-parallel polymorphisms in candidate genes.

Methods
Our work extends those studies broadly across the roughly 400 non-olfactory GPCRs. Our initial efforts include resequencing 43 Indian-origin rhesus macaques (Macaca mulatta), 20 Chinese-origin rhesus macaques, and 34 cynomolgus macaques (Macaca fascicularis). Using the Agilent target enrichment system, we designed capture baits for GPCRs off the human and rhesus exonic sequence. We used an Illumina HiSeq with single end reads and 24x multiplexing to generate sequencing reads.

Results
Initial data analysis was done using DNAnexus. In doing so, we were able to identify non-synonymous polymorphisms throughout the GPCRome of these non-human primates. Nearly 25,000 SNPs were identified in coding exons including over 14,000 non-synonymous and over 9,500 synonymous coding SNPs. As expected, regions showing the least evolutionary constraint show greater rates of polymorphism and greater numbers of higher frequency polymorphisms. The vast majority of these SNPs are singletons and about ~1750 non-synonymous, ~2900 synonymous SNPs are found in multiple individuals.

Discussion
In all three populations, polymorphism and divergence is highly concentrated in N-terminal, C-terminal and the IC3 region of GPCRs. SNP frequencies in macaques follow a similar pattern as macaque divergence from humans. We have found several new polymorphisms in primates that parallel those seen in humans, helping to establish translationally relevant better primate models of psychiatric disease.

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TRANSCRIPTOMIC BIOMARKERS OF ANTIDEPRESSANT RESPONSE
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Background
Major depressive disorder is a disabling illness with antidepressants used as the first line of treatment. The high levels of inter-individual variability means it is currently not possible to determine who will respond and to which antidepressant. Identifying biomarkers for antidepressant response will aid in personalizing treatment, reducing both cost and suffering. It is hypothesized that levels of gene expression in peripheral blood may be markers of molecular mechanisms that underlie variability in response and therefore serve as biomarkers in personalizing treatment and understanding molecular mechanisms of drug action. We aim to determine if there exist peripheral blood transcriptomic biomarkers predictive of antidepressants response in a large sample of individuals in a prospective study using systematic measurements of gene expression.

Methods
RNA extracted from whole blood taken prior to the start of treatment (baseline/week 0) was available for 222 individuals from the Genome-Based Therapeutic Drugs for Depression (GENDEP) study. RNA samples were processed on Illumina HumanHT-12 v4 Expression BeadChip and underwent standard quality control using the lumi package in R. Linear mixed models for transcriptomic baseline predictors of antidepressant response were undertaken for the entire sample (any antidepressant, n=204), individuals taking escitalopram (n=119), individuals taking nortriptyline (n=85), and differential response to treatment with either escitalopram or nortriptyline (gene probe by drug interaction, n=204). Baseline depression severity, age, sex, microarray batch and time (linear and quadratic) were include as fixed effects in the model with individual and recruitment center included as random effects. Gene probes nominally associated at p<0.05 were subjected to gene ontology analysis using DAVID. The full list of probes passing QC was used as the background list.
**Results**
12,283 gene probes passed quality control and were used in the analysis. Gene probes with an FDR q<0.05 are reported. Two gene probes were associated with an FDR q<0.05 with response to any antidepressant (UROD, uroporphyrinogen decarboxylase enzyme, q=0.036; TPGS2, tubulin polyglutamylase complex subunit, q=0.043), and one gene probe was associated with response for the nortriptyline group only (BTN2A1, butyrophilin, subfamily 2, member A1, p=0.033). No gene probes were associated with response for the escitalopram group only or differential response at FDR q<0.05. Gene ontology analysis of nominally significant probes showed enrichment of four ontology terms for response to any antidepressant including two related to catabolic processing. Nominally significant probes in the nortriptyline group were enriched for fourteen gene ontology terms including metabolic and biosynthetic processing. Nominally associated probes for differential drug response were enriched for one gene ontology term, chromatin organization. No gene ontology terms were enriched for in the escitalopram group at FDR q<0.05.

**Discussion**
We investigated the expression of over 12,000 transcripts measured from whole blood for their relationship to antidepressant response. We identify novel biomarkers which survive correction for multiple testing for response to any antidepressant and response in the nortriptyline group only. Furthermore, gene ontology analysis showed enrichment for biological processes not previously linked with antidepressant response including catabolic, metabolic and biosynthetic processing. Previous candidate genes and pathways were not replicated. This is the largest transcriptomic study of antidepressant biomarkers undertaken to date and investigated gene transcripts in a systematic fashion. Our study identifies novel biomarkers for antidepressant response and offers new insights into the biological mechanisms of drug response.

**PREPULSE INHIBITION REQUIRES GSX1 SPECIFIED NEURONS IN ZEBRAFISH AND MICE**
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**Background**
Prepulse inhibition (PPI) is form of startle regulation in which a weak pre-stimulus suppresses the magnitude of the startle response to a subsequent intense stimulus. Deficits in PPI have been reported in a number of neuropsychiatric disorders including schizophrenia and autism. As PPI can be robustly measured in animal models, it has become a widely used endophenotype for studying genetic and environmental contributions to these disorders. Despite this, the basic neuronal circuitry that mediates PPI is poorly understood and identification of neurons required for PPI may enable subtle changes in brain connectivity to be elucidated in animal models.

**Methods**
We previously demonstrated that a form of startle inhibition, with characteristic features of mammalian PPI, is present in zebrafish. We exploited the ability to perform high throughput genetic manipulations and behavioral phenotyping in larval zebafish to identify neuronal components of the PPI circuit. We developed a novel method to enrich for brain specific
transgene expression and generated a library of more than 200 stable enhancer trap lines in which different cohorts of neurons express the Gal4 transcription factor. We then ablated the 'trapped' neurons in each line using a UAS:Nitroreductase reporter line and tested larvae for PPI.

**Results**

After ablation, line y252 showed defects in startle sensitivity and PPI. These defects were reproduced by optogenetic inhibition of neurons in y252 using a UAS:Archaerhodopsin3 reporter line arguing that the PPI defect is not due to disrupted neural development, but rather that neurons labeled in the y252 line are part of the basic PPI circuit. These neurons occupy a bilateral longitudinal column of neurons at the dorsal aspect of the hindbrain, which extend neurites across ventral commissures. These neurons co-express vglut, indicating that they are glutamatergic. Consistent with this, application of the NMDA receptor antagonist MK801 reproduces the y252 PPI phenotype. Neurites from y252 neurons are found adjacent to the Mauthner cell, the command neuron for the startle response and form synapses in close apposition to its lateral dendrite. The transgene insertion site in y252 is 21 kbp from the gsx1 gene and Gal4 expression in y252 is almost identical to that of gsx1 indicating that neurons labeled by this line are specified by gsx1. As Gsx1 in mice is expressed in a similar pattern to zebrafish, we speculated that Gsx1 may also specify neurons required for PPI in mice. Supporting this, we found that Gsx1 knockout mice showed a strong reduction in PPI.

**Discussion**

Our findings show that loss of gsx1 neurons impairs PPI in both zebrafish and mice. As gsx1 is expressed only transiently in development, it likely specifies neurons that are part of the PPI circuit. In zebrafish these neurons form synapses adjacent to command neurons for the startle response and surprisingly, are glutamatergic in character. Gsx1 is also known to be involved in specification of interneurons in the telencephalon. As a leading hypothesis for the etiology of schizophrenia is hypoglutamatergic function, we speculate that Gsx1 specified neurons in the forebrain may be involved in similar circuit motifs to those that mediate PPI in the hindbrain, and be disrupted in schizophrenia.

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**WHOLE BRAIN CONNECTIVITY STUDY IN SCHIZOPHRENIA PATIENTS AND THEIR HEALTHY SIBLINGS**

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**Background**

Schizophrenia (SCZ) is a complex disease that has been hypothesized to arise from functional dysconnectivity of the brain. However, study results discovered various inconsistent abnormal connectivity alterations in SCZ patients. Recent work used both SCZ patients and their nonpsychotic siblings in SCZ studies to seek common abnormalities as biomarkers of this disease. Yet, to date, no researchers have compared the global alterations amongst SCZ patients, their healthy siblings and healthy controls. In this study, we investigate the global brain connectivity variations in healthy siblings, compare with that of SCZ patients and healthy controls, looking into the compensative plasticity mechanism underneath the fact that those healthy siblings do not become ill despite the fact that they may share common genetic risks and environmental factors with SCZ individuals.
Methods
Resting state functional magnetic resonance imaging (fMRI) data were collected from 107 study subjects, including 44 healthy controls, and 32 schizophrenia patients that are treatment-resistant, and 31 of their healthy siblings with no SCZ history or relevant symptoms. The whole brain was parcellated into 1000 brain networks using a fix point clustering (FPC) algorithm proposed by us. Connectivity features in the number of 500500 were analyzed, including both intra- and inter-network/region connectivity. Then the ANOVA analysis was independently carried out for each connectivity feature with 4 contrasts: C1, Cases, siblings and healthy controls; C2, Case vs. sibling; C3, Case vs. control; and C4, Sibling vs. control. Significant connectivity features for each contrast were identified and compared using critical P-value thresholds corrected by a false discovery rate (FDR) approach. We used a multivariate classification method to validate the selected connectivity features and further investigated the most discriminative subsets of features for each contrast tested (C1-C4).

Results
Studies revealed that healthy siblings, when compared with the SCZ patients, showed connectivity variations involving greater than 60% of the whole brain regions. At the same level of significance, the healthy siblings exhibited little connectivity alteration in small brain regions (<3%) in comparison with healthy controls. On the other hand, SCZ patients have great than 40% brain regions that demonstrate connectivity variations in comparison with healthy controls; And those brain regions are partially overlapped with that of siblings/cases study. In addition, the changes observed between cases and controls in altered connectivity feature numbers are relatively smaller than that of cases and siblings (less than 50%). When evaluated using the multivariate classification approach, selected connectivity features gave the highest identification accuracies of 84.1%, 92.1% and 81.3% for C2, C3 and C4 respectively.

Discussion
Healthy siblings of individuals with SCZ demonstrate functional regulations within large area of brain regions. Although most of those changes are relatively moderate in comparison with healthy controls, the majority of them show significance in sibling/case study, indicating that connectivity variations in SCZ patients and their healthy siblings are in different scales or even opposite directions. As we view the connectivity alterations in SCZ patients as dysregulations that lead to mental illness, we thus postulate that those alterations in healthy siblings may be a form of virtuous compensative regulations that prevent them from becoming ill. This may support the plasticity deficits theory which hypothesize that SCZ arises from the deficit of synaptic plasticity of the brain. Results of this work may benefit novel therapeutics and drug targeting, as well as better understanding of the biological underpinning of SCZ.

THE SEARCH FOR ADDITIONAL VARIANTS RESPONSIBLE FOR PHENOTYPIC VARIABILITY OF 22Q11.21 DELETION SYNDROME
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Background
Velocardiofacial syndrome is associated with 22q11.21 deletion. This syndrome shows complex and various phenotypes. Individuals with 22q11.21 deletion have high rates of schizophrenia (SZ). However, penetrance of this deletion on SZ does not reach 100% and phenotypic variability is prominent even among patients with this deletion. It is speculated that additional variants are involved in this variability.

Methods
We conducted whole genome sequencing using Complete Genomics technology. The target samples are 5 patients with SZ and 22q11.21 deletion. These patients were various in terms of main symptoms, comorbidities, age of onset. To detect variants with phenotype-modifying roles, we applied filtering to whole genome sequencing data using Variant Analysis software.

Results
We detected around 4 million variants per sample by whole genome sequencing. After filtering by frequency, functional effect, and call quality, around 500 rare missense mutations were detected among 5 samples. Pathway analysis of these variants provided support for relevance for several neurodevelopment-related pathways in SZ. Additionally, three patients had rare missense mutations in genes on 22q11.21 (HIRA, ZNF74, SNAP29), which may have a role in phenotypic variability of SZ.

Discussion
We provided some evidence that many rare missense mutations may have a role as genetic factor for SZ and be implicated in phenotypic expressivity of 22q11.21 deletion.

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CLOZUK GWAS: GENETIC INSIGHTS INTO TREATMENT RESISTANT SCHIZOPHRENIA
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Background
Large-scale genome-wide association studies (GWAS) have provided valuable insights into the aetiology of neuropsychiatric disorders. The Psychiatric Genetics Consortium Schizophrenia group has led the field in this respect by amassing samples of over 35000 cases. Given the heterogeneity of schizophrenia it may be productive to stratify samples based on clinical characteristics and with such large sample sizes this is now feasible. Around a third of those with schizophrenia fail to respond to standard antipsychotics and are considered treatment-resistant. Treatment resistance is an attractive stratifier for genetic studies given the potential clinical impact and the fact that lack of response to dopaminergic antipsychotics may identify a genetically more homogenous group. We present a GWAS of treatment resistant schizophrenia with over 12 000 individuals and identify genetic variants specific to those who are treatment
resistant.

**Methods**
The primary GWAS was based on the CLOZUK sample of 5600 cases with a clinical diagnosis of treatment-resistant schizophrenia and 6300 UK-based healthy controls. The CLOZUK GWAS was performed as part of the PGC2 schizophrenia GWAS analysis with the same QC protocol. Data were imputed based on 1000 genomes and analysed using logistic regression with principal components as covariates. We then sought replication of the initial CLOZUK GWAS signals in 2500 independent samples of those taking clozapine. Finally in order to identify genetic variants specific to treatment resistant schizophrenia we compared the associations in the CLOZUK top SNPs with the equivalent results from the PGC (after excluding clozapine samples where possible).

**Results**
In the initial CLOZUK GWAS we identified four genome-wide significant SNPs (p<5x10^{-8}) and 22 independent polymorphisms with p<1x10^{-6}. Among these loci are regions that have been previously implicated in schizophrenia and hence we provide further support for association. We also identified several novel loci. Notable findings include strong associations for polymorphisms within BDNF and KCTD13. We are currently seeking replication of SNPs with a p value <1x10^{-4} in independent samples of 2500 of those with schizophrenia who have ever taken clozapine. Initial comparisons between the CLOZUK top hits and the equivalent results in the PGC indicate that several SNPs show associations that are specific to treatment-resistant schizophrenia. These results provide insights into the underlying biology of treatment-resistant schizophrenia.

**Discussion**
We provide evidence for genetic associations that are specific to treatment resistant schizophrenia and which provide insight into the underlying biology of treatment resistance. The results also provide supportive evidence for a number of previously associated loci with schizophrenia. These findings highlight the potential value of stratifying samples based on treatment resistance.

SCAN FOR RARE COPY-NUMBER VARIATION IN 11,850 SWEDISH SCHIZOPHRENIA SAMPLES SUGGESTS NOVEL SUSCEPTIBILITY LOCI AND PROVIDES EVIDENCE FOR CONVERGENCE WITH REGIONS OF COMMON VARIANT ASSOCIATION

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**Background**
Schizophrenia (SCZ) is a heritable disorder with substantial public health impact. Several rare copy number variants (CNVs) have been implicated in this disorder. However, prior studies indicate that larger samples are necessary for new discoveries to be made.
Methods
We conducted a genome-wide association study for schizophrenia with a Swedish national sample (4,719 cases with SCZ and 5,917 controls post-QC). All subjects from the Swedish Schizophrenia Study were born in Sweden and the schizophrenia (SCZ) cases were identified via the Swedish Hospital Discharge Register. DNA was extracted from whole blood and was genotyped at the Broad Institute using Affymetrix (5.0 and 6.0) and Illumina OmniExpress arrays. We used Birdseye to generate rare CNV calls from GWAS arrays. In addition, this Swedish sample has been well characterized for common SNPs (Ripke, O'Dushlaine, et al), enabling an examination of potential overlap in loci and pathways implicated by both sources of variation.

Results
Our results are consistent with literature reports of an increased burden of CNVs in SCZ cases. Our data confirm several known associations, including 16p11.2 duplications and 22q11.2 deletions, and also suggest a number of novel associations. Intriguingly, pathway analysis implicates several biological pathways that have also been implicated by common SNP polymorphisms, including calcium signaling and FMRP target sets. For events of >100kb, we note modest enrichment in cases for deletions in the post-synaptic density (human core) gene set, and also modest association for duplication and deletion in the NMDAR complex, known to modulate synaptic plasticity. We find evidence for modest enrichment of larger deletions in regions of SNP association.

Discussion
Together, these finding suggest both direct (loci) and indirect (pathway) convergence between these disparate sources of variation, shedding further light on core molecular components driving the etiology of this disorder.

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TRANSCRIPTOME OUTLIER ANALYSIS IMPLICATES SCHIZOPHRENIA CANDIDATE GENES AS HARBORING RARE FUNCTIONAL VARIANTS OF LARGE EFFECTS
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Background
Multiple rare and large copy number variants (CNVs, deletions and duplications) are associated with high risk for schizophrenia (SZ), and risk from common genetic variation has been uncovered by genome wide association studies. However, a substantial part of the genetic risk still remains unexplained and disease mechanisms are largely unknown. To uncover additional loci of large functional effect, we propose to sequence relevant targets derived from transcriptomic profile analyses. Most gene expression studies rely on the study of average abundances in case-control samples. Although useful, this approach assumes substantial etiologic
homogeneity, and may miss rarer genetic effects. We have employed an alternative analytical approach to identify expression outliers (i.e., transcript abundance distribution extremes) where the tails of the distribution are enriched for cases. We hypothesize that such case-enriched expression outliers are caused by rare to uncommon protein coding changes (e.g., nonsense mutations or splice site mutations) or regulatory mutations of large effect on gene expression.

**Methods**
We analyzed an RNAseq dataset comprised of lymphoblastoid cell lines (LCLs) from 312 SZ cases and 322 controls, none of which carry a known SZ-associated CNV (specifically, 1q21.2, NRXN1, 15q13.3, 16p11.2, or 22q11.21). We adjusted the gene abundances (RPKM, reads per kb per million mapped reads) for the confounding effects of a number of epidemiological and lab covariates, and calculated the Z-scores of the expression abundances for each gene for the whole sample. Expression outliers were defined as genes with abundances beyond a predefined standard deviation cutoff (2SD or 3SD); positive Z-scores beyond the cutoff were denoted as the upper tail, and negative for the lower tail.

**Results**
We identified 828 expression outlier genes with 2SD-tails (401 lower and 427 upper) enriched for SZ cases (Fisher exact test $p<0.05$). In these 828 genes we observed enrichment of brain-expressed genes, SZ-risk CNV-spanned genes (note that these results are from subjects not carrying these SZ-risk CNVs), and genes within CNVs associated with neurodevelopmental disorders, and calculated the Z-scores of the expression abundances for each gene for the whole sample. Expression outliers were defined as genes with abundances beyond a predefined standard deviation cutoff (2SD or 3SD); positive Z-scores beyond the cutoff were denoted as the upper tail, and negative for the lower tail.

**Discussion**
These results suggest that genes identified by outlier expression analyses are relevant to SZ pathogenesis. However, given the moderate sample size, multiple statistical tests, and nominal significance of the findings, we consider our results preliminary and exploratory. We are currently sequencing exons and regulatory sequences of top ranking outlier genes and will report the functional mutations we find contributing to aberrant mRNA expression in SZ cases.
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**Background**

Genome-wide significant associations of schizophrenia with eight SNPs in the **CNNM2, MIR137, PCGEM1, TRIM26, CSMD1, MMP16, NT5C2** and **CCDC68** genes have been identified in a recent mega-analysis of genome-wide association studies. To date, the role of these SNPs on gray matter (GM) volumes remains unclear.

**Methods**

After performing quality control for minor-allele frequency >5% using a JPT HapMap sample, a genotyping call rate >95% and Hardy-Weinberg equilibrium testing (p>0.01), six of eight SNPs were eligible for analysis. We used a comprehensive voxel-based morphometry (VBM) technique to investigate the effects of these six SNPs on GM volumes between major-allele homozygotes and minor-allele carriers in Japanese patients with schizophrenia (n=173) and healthy subjects (n=449).

**Results**

The rs7914558 risk variant at **CNNM2** was associated with voxel-based GM volumes in the bilateral inferior frontal gyri (right T=4.96, p=0.0088, left T=4.66, p=0.031). These peak voxels, which were affected by the variant, existed in the orbital region of the inferior frontal gyri. Individuals with the risk G/G genotype of rs7914558 had smaller GM volumes in the bilateral inferior frontal gyri than carriers of the non-risk A-allele. Although several effects of the genotype and the genotype-diagnosis interaction of other SNPs on GM volumes were observed in the exploratory VBM analyses, these effects did not remain after the FWE-correction for multiple tests (p>0.05).

**Discussion**

Our findings suggest that the genetic variant in the **CNNM2** gene could be implicated in the pathogenesis of schizophrenia through the GM volumetric vulnerability of the orbital regions in the inferior frontal gyri.

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**MULTIPLE FUNCTIONAL PATHWAY ANALYSIS OF SCHIZOPHRENIA**

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Background
Schizophrenia is a complex disorder with aetiology due to multiple genetic effects, which are likely to be embedded in functional pathways. Using experimentally-derived pathways centred around schizophrenia-associated genes in the cellular adhesion molecule (CAM), disrupted-in-schizophrenia 1 (DISC1), microRNA 137 (miR-137), neurexin 1 (NRXN1), transcription factor 4 (TCF4) and zinc finger protein 804A (ZNF804A) pathways, we assessed the pathway-specific polygenic score contribution to explaining variation in case-control status in the WTCCC2 schizophrenia case (N = 1378)-control (N = 1086) GWAS.

Methods
We created polygenic scores for each of the 6 pathways based on $p$-value thresholds and weighted by the log_{10}(odds ratio) from the PGC1 schizophrenia GWAS for the overlapping set of SNPs between the WTCCC2 and PGC1. The $p$-value thresholds considered were 0.001, 0.01, 0.05, 0.10, 0.20, 0.30, 0.40 and 0.50. After setting the per-SNP missing rate to be < 5%, the number of genes/SNPs included by pathway were: CAM (125/6332), DISC1 (130/5384), mir-137 (474/21368), NRXN1 (131/5931), TCF4 (566/9424) and ZNF804A (138/3080). The number of overlapping genes between pathways was modest and ranged from 0 to 17, with only 2 pathways containing > 5 overlapping genes (miR-137/TCF4, N overlapping = 9, and TCF4-ZNF804A, N overlapping = 17). The amount of variation explained (Nagelkerke R^2) in case-control status was assessed using standard logistic regression for individual pathways and 6 pathways simultaneously versus the standard polygenic approach, which considers all SNPs that fall under a particular $p$-value threshold. $P$-values were obtained using a likelihood ratio test (LRT) between nested models, where the full model contained the polygenic score(s), controlling for the number of missing genotypes, and the reduced model contained only the number of missing genotypes. To assess whether the pathways contained strongly-associated SNPs, we performed $\chi^2$ tests for each SNP and controlled for multiple testing using a Bonferroni correction for the number of SNPs in the pathway.

Results
Within each of the 6 pathways, single SNP tests revealed no SNP was significantly associated with schizophrenia after Bonferroni adjustment, thus indicating no pathway contained SNPs showing strong association. Using the best PGC1 $p$-value threshold for each individual pathway polygenic score, the amount of variation explained in case-control status ($R^2$, LRT $p$-value) by individual pathways were: miR-137 (2.7%, 1.1e^{-12}), DISC1 (0.95%, 2.9e^{-05}), ZNF804A (0.91%, 4.3e^{-05}), TCF4 (0.76%, 0.00018), NRXN1 (0.27%, 0.026) and CAM (0.25%, 0.031). A logistic regression model containing all 6 polygenic scores explained 5.0% of variation in case-control status ($p$-value = 1.7e^{-18}) using a total of 28702 SNPs across all pathways. For the best $p$-value threshold and using all SNPs in the GWAS, a single polygenic score based on a total of 629831 SNPs was able to explain 11% of the variation in case status.

Discussion
The use of the 6 experimentally-derived pathways explained a much larger amount of variation in schizophrenia case status than would be expected given the small number of SNPs included in each pathway; in fact, using just 4.6% of the total number of SNPs in the full-GWAS polygenic score based on $p$-values, we explained 45.5% of the total variation captured by the full-GWAS score. Predicted miR-137 targets explained the greatest amount of variation, followed by DISC1.
and ZNF804A. Both miR-137 and ZNF804A were discovered using GWAS and highlights the utility of genome-wide studies for complex disorders such as psychosis and the importance of considering genes within the context of their functional pathway. The use of pathway information enhances the interpretability of the polygenic score, as percentages of variation can be assigned to pathways and this may help to determine which pathways play key roles in the genetic architecture of psychosis. The addition of newly-discovered pathways in future may increase the amount of variation explained.

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HIGH RATE OF DISEASE-RELATED COPY NUMBER VARIATIONS IN CHILDHOOD ONSET SCHIZOPHRENIA

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Background

Copy number variants (CNVs) are risk factors in neurodevelopmental disorders, including autism, epilepsy, intellectual disability (ID) and schizophrenia. Childhood onset schizophrenia (COS), defined as onset before age 13, is a rare and severe form of the disorder, with more striking array of pre-psychotic developmental disorders and abnormalities in brain development.

Methods

Because of the well-known phenotypic variability associated with pathogenic CNVs, we conducted whole genome genotyping to detect copy number variants (CNVs) and then focused on a group of 46 rare CNV that had well documented risk for adult onset schizophrenia (AOS), autism, epilepsy and/or ID. We evaluated 126 COS probands, 69 of which also had a healthy full sibling.

Results

When COS probands were compared to their matched related controls, significantly more affected individuals carried disease-related CNVs (p=0.017). Moreover, COS probands showed a higher rate than that found in AOS probands (p<0.0001). A total of 15 (11.9%) subjects exhibited at least one such CNV and four of these subjects (26.7%) had two. Five of 15 (4.0% of the sample) had a 2.5-3Mb deletion mapping to 22q11.2, a higher rate than reported for adult onset (0.3~1%) (p<0.001) or ASD and, indeed, the highest rate for any clinical population to date. For one COS subject, a duplication found at 22q13.3 had previously only been associated with autism, for four patients CNVs at 8q11.2, 10q22.3, 16p11.2 and 17q21.3 had only previously been associated with ID.

Discussion

These findings support the well known pleiotropic effects of these CNVs suggesting shared abnormalities early in brain development. Clinically, broad CNV-based population screening is needed to assess their overall clinical burden.
GENETICS OF FUNCTIONAL DISABILITY IN SCHIZOPHRENIA AND BIPOLAR ILLNESS (VA COOPERATIVE STUDY #572)

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Background

Given the prominence of cognitive impairment and disability in both schizophrenia and bipolar disorder, substantial interest has arisen in identification of their determinants. Recent findings regarding the heritability of cognitive impairment and everyday disability has led to the suggestion that the cognitively demanding component skills that underlie disability, referred to as functional capacity, may also be heritable and associated with specific genetic polymorphisms. The current study addresses these issues, and here we are presenting initial data on recruitment and characterization of the sample. These data are particularly relevant to bipolar disorder because of reduced attention paid to heritability of cognitive deficits and related skills in bipolar disorder.

Methods

This study, VA Cooperative Studies Program #572, is recruiting and assessing as many as 9,000 Veterans with either schizophrenia (SZ) or bipolar I (BP) disorder. A related VA initiative, the Million Veteran Program, has already recruited over 100,000 Veterans that will serve a source population for psychiatrically-healthy controls. Patients with SZ or BP at 26 VA medical centers are being enrolled and evaluated regarding cognition (NP tests), functional capacity (UPSA-B), suicidality (CSSRS), and comorbid conditions such as PTSD. The functional capacity measures are the primary focus of the assessment, as they have not yet been well-examined for genetic correlates. A pilot analysis will use genotyping and exome sequencing methods on a subsample of participants.

Results

A total of 6,280 veterans (46% SZ, 54% BP) have been recruited and assessed to date. Veterans with SZ were more likely to never have been married or employed (other than military service) compared to Veterans with BP; lifetime PTSD and suicidality were more common in the BP patients. Performance on the functional capacity measures for both patient groups was, on average, within one point of all previously published studies with the UPSA-B, and the BP patients performed slightly better than SZ patients. Similarly consistent results were found for NP test performance, with mean t-scores for the Veterans with SZ of 35 (-1.5 SD) and 40 (-1.0 SD) for the Veterans with BP.

Discussion

This large and expanding sample of Veterans with schizophrenia and bipolar disorder is very representative of previous studies in terms of patients’ performance and co-morbidities. Future analyses will examine the genetic correlates of these performance-based measures of cognition
and disability. This will be the largest studies of the genetics of BPI with patients assessed in person with performance-based tests.

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TRACE ELEMENTS IN THE SERUM OF SCHIZOPHRENICS

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Background

Schizophrenia is a severe mental disorder and its social and exonomic burden on society is enormous. Trace elements are occur at less than 0.01% of the body’s weight, quantitative measurement of their changes in bloodstream may reveal substantial biological information related to the pathophysiological status of disease. Metallomics is an emerging science that primarily includes the detection, mapping, and/or quantification of trace elements in large scale.

Methods

An integrated profiling strategy to quantitate a broad range of trace elements in schizophrenia using ICP–MS was conducted in a discovery group containing 59 schizophrenia and 60 normal controls and in a validation group containing 50 schizophrenia and 48 normal controlsseperately. Multivariate statistical technique was employed to afford a global view of similarities and separation trend of trace elements of the two groups. The fold changes and P values of the detected trace elements were attained by Mann–Whitney test. Receiver operating characteristic (ROC) analysis was performed to evaluate diagnostic validity of laboratory tests. The area under the ROC curves (AUC) with 95% confidence interval (95% CI) and the corresponding P values of AUC were calculated to assess diagnostic performance of the elemental signature of schizophrenia, individual elemental markers, and other optimal combinations of elemental markers.

Results

Global elemental profiles of the healthy controls separated distinctly from the schizophrenia patients in both discovery and validation groups. A more sophisticated OPLS-DA model was achieved by maximizing the differentiation between the schizophrenia versus the healthy controls from the discovery group (R²Ycum=0.538, Q²cum=0.423). This model was further applied to the subjects in the validation group and the similar result was obtained accordingly (R²Ycum=0.575, Q²cum=0.507). According to the variable importance on a projection (VIP) from the OPLS model, Cs, Ce, and Zn were selected with a threshold of 1.5 in both discovery group and validation group. The concentrations of Cs, Ce, and Zn significantly declined in schizophrenia patients as compared to the healthy controls in the discovery group (FC=0.62, 0.69 and 0.65, respectively) . And the similar results were obtained in validation group (FC=0.69, 0.59 and 0.66, respectively). the AUC of different combinations of elemental markers was significantly higher than that of each of the three elements. And the combination of Cs, Zn and Se obtained the highest AUC value of 0.77 in discovery group and of 0.8 in validation group. Using a combination of the three elemental markers, Cs, Ce, and Zn, we were able to recognize 75 of the 100 schizophrenia patients (74.6%) and 72 of the healthy controls (71.7%) in the discovery group, respectively. In the validation group, 70.8% of the schizophrenia patients and
72% of the healthy controls were classified correctly.

Discussion
With ICP-MS-based elemental profiling strategy, it was found that there was lower level of Zn, Cs and Se in Chinese schizophrenia population than that in healthy controls and suggested that nutritional deficiency of Zn, Cs and Se may increase the risk of schizophrenia. Zinc is essential for brain development and functions in axonal and synaptic transmission and is necessary for nucleic acid metabolism and brain tubulin growth and phosphorylation. Our study supports that lower levels of Zn in patients with schizophrenia than in normal controls. And low level of Zn in schizophrenic patients is decreased antioxidative capacity and improved oxidative stress.Selenium is an essential nutrient as the amino acid selenocysteine on the active side of over 30 Se-dependent enzymes.Selenium plays a key role in the functioning of the glutathione peroxidase anti-oxidant system and has an important role in anti-oxidative protection against free radical damage to membranes, lipoproteins and nucleic acids. And it is suggested that reduced Se in schizophrenic patients is one of the cause of oxidative stress.Interestingly, we firstly reported lower levels of Cs in patients with schizophrenia than in normal controls. Previously, similar result is observed that significantly lower concentrations of both plasma Cs and cerebrospinal fluid (CSF) Cs in Alzheimer’s disease subjects. And this condition may be due to the binding to chelating proteins, e.g. Aβ and APOE, thereby detoxifying their oxidative potential. It is possible that the increased risk of developing schizophrenia may be caused by the reduced ability of some proteins to bind to Cs. However significant reduction of Zn, Cs and Se in Chinese schizophrenia population should be interpreted with caution since it is with mild sensitivity and specificity to assume elemental abnormality that could characterize schizophrenics. Further research is especially needed to ascertain the effect of trace element status.

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INDUCED PLURIPOTENT STEM CELL DERIVED HUMAN NEURONAL PLATFORM TO MODEL HERPES SIMPLEX VIRUS, TYPE 1 (HSV-1) INFECTIONS IN THE BRAIN

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Background
Herpes Simplex virus, type 1 (HSV-1), causes lifelong human infection. Recent studies have correlated HSV-1 persistent infection with cognitive impairment. Animal models of HSV-1 persistent infection do not fully recapitulate effects of human HSV-1 infection. To model persistent infection of HSV-1 during neuronal differentiation and in central neurons, we generated an induced pluripotent stem cell (iPS)-based cellular model.

Methods
iPS-derived neural stem cells (NSCs), neural progenitor cells (NPCs) and neurons were infected with a genetically engineered HSV-1 virus that expresses Enhanced Green Fluorescent Protein (GFP) and Red Fluorescent Protein (RFP). Persistent infection were induced by administration of acyclovir or (E)-5-(2-bromovinyl)-2'-deoxyuridine (5BvdU) along with IFN-α. Unfixed cells were tested for expression of EGFP and RFP, expression of latency-associated transcript (LAT),
and productive infection at day 1 and day 6. HSV-1 reactivation was induced by treatment with sodium butyrate.

**Results**

Acutely infected cultures of NSCs, NPCs and neurons showed a robust expression of EGFP and RFP. HSV-1 infection in the presence of 5BvdU along with IFN-α showed that persistent infection can be established in neurons and not in NSCs and NPCs. Persistently infected neurons showed: i) 2-3-fold decrease of GFP positive cells; ii) lack of production of infectious virions; iii) expression of latency-associated transcript (LAT). Treatment of quiescent cultures with sodium butyrate led to a significant increase of EGFP/RFP positive cells and productive infection.

**Discussion**

In this study, we showed for the first time that HSV-1 persistent infection can be consistently established in glutamateergic neurons, and not only in sensory neurons. Furthermore, our results suggest that the ability to support HSV-1 quiescence is gained during a late stage of neuronal differentiation. Our iP-based cellular model will be utilized for high-throughput screenings of small molecules to develop and effective treatment for HSV-1.

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**IDENTIFYING THE PARENT-OF-ORIGIN OF DE NOVO MUTATIONS USING PARENT-OFFSPRING TRIOS**

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**Background**

Newly arising (de novo) mutations contribute to the genetics of schizophrenia (SZ), autism (ASD) and other developmental disorders. It has been shown that de novo mutations are more frequent in SZ and ASD patients than in normal individuals. Theoretical evidence suggests that a majority of de novo mutations occur on the paternal genome (Crow, 2000). They are also positively correlated with paternal age at the time of conception of the child and the mutational burden increases with paternal age. The aim of the study is to determine the parent-of-origin of de novo mutations (SNVs, small indels) (N=1080), identified through whole-exome sequencing of 618 SZ parent-offspring trios from Bulgaria.

**Methods**

The parent-of-origin of de novo SNVs and small indels will be determined by direct exploration of the NGS runs (the BAM files) for the presence of other inherited SNPs in close proximity of the de novo mutation, that lie (or that don’t lie) on the same sequence read (or pair-end read) with the mutation. If the de novo mutation is on the same read as the inherited mutation, then it has occurred in the parent, who transmitted it (and the other way round). Alternatively, the parental origin will be determined using two stage allele-specific PCR.
**Results**
The results obtained so far confirm the original hypothesis. We have successfully identified the parent-of-origin for 97 de novo SNVs, of those 73 are transmitted from the father and 24 are transmitted from the mother (3:1 ratio).

**Discussion**
Identifying the mechanisms of mutation formation in humans is of a great theoretical value in genetics and medicine. *De novo* mutations are important both as sources of diversity in evolution and for their immediate impact on diseases.

INTEGRATED PATHWAY-BASED APPROACH IDENTIFIES GENOMIC REGIONS AT CACNB2 AND CTCF TO BE ASSOCIATED WITH SCHIZOPHRENIA
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**Background**
It has been shown that pathway-based analysis of GWA data increases the power to detect association of disease related genes and potentially single nucleotide polymorphisms (SNPs) with small genetic effects and helps to provide biological insights into the aetiology of complex diseases. Moreover, studying the cumulative effect of single nucleotide polymorphisms (SNP) assigned to functional gene-sets, such as biological pathways, may help to discover and explain the mechanisms of risk SNPs on complex diseases. Here, we used an integrated, three step approach that made use of information at the pathway, the gene, and the individual SNP level in order to identify susceptibility factors for schizophrenia.

**Methods**
For our pathway analysis we used the Global Test (Goeman et al. 2004) and discovery and replication data-sets comprising 5040 and 5082 samples of European ancestry, respectively. The functional gene-sets information was retrieved from several pathway and gene-set databases such as KEGG, Gene Ontology, and MSigDB. At the gene level we used publicly available information from the Psychiatric Genomics Consortium study on schizophrenia (Ripke et al. 2011) and computed gene-based statistics using FORGE (Pedroso and Breen, 2011) in order to identify genes of special importance to our replicated pathways. This included also identification of „hubs“, i.e. genes relevant to more then one pathway and therefore potentially relevant for the interplay of susceptibility gene-sets / pathway in schizophrenia. In a last step (at the SNP level), we focused on genes identified at the gene level and searched for evidence, that SNPs annotated
to these genes are of importance to schizophrenia susceptibility. We used different publicly available databases and tools, including RegulomeDB (Boyle et al. 2012), the Polyphen-2 (Adzhubei et al. 2010) and SIFT (Kumar et al. 2009).

Results

We discovered and replicated 14 gene-sets / pathways, which could be categorized into functional processes involved in transcriptional regulation and gene expression, synapase organization, cell adhesion and apoptosis. Furthermore, we identified two genes, namely CTCF and CACNB2, for which evidence for association with schizophrenia was available in both, our discovery study and the PGC study and that each emerged as important for 4 out of our 14 replicated pathways. Genes of further interest are FOXP2 and AKT3. Based on the functional annotation SNPs contributing to the pathway signals in CTCF and CACNB2 were associated with altered expression of the neighbouring genes RLTPR and ARLB5, respectively. Assessment of functional relevance for SNPs in CACNB2 revealed potential involvement of ARLB5.

Discussion

In our study the majority of pathways identified as significantly associated with schizophrenia are involved in transcriptional regulation and gene expression. One reason why previous pathway-based studies on schizophrenia did not track that area is that they focused mainly on the KEGG and Biocarta backbones while we applied an exhaustive number of different pathway databases, including special databases transcriptional regulation like dbTFT and dbMIR. On the gene level, CACNB2 and CTCF were the genes with the highest evidence for association with schizophrenia. CACNB2 is known to be associated with psychiatric disorders and was one of four loci found to be genome-wide significantly associated in a cross-disorder analysis of genome-wide SNP data for 5 psychiatric disorders (Cross-Disorder Group of the PGC 2013). With regards to CTCF, studies on conditional KO mice were able to show that CTCF is a key regulator of neuronal differentiation that is essential for neuronal diversity and functional neural networks (Hirayama et al. 2012). It is of note that previous studies have shown association of genetic variation in the protocadherin gene cluster with schizophrenia (Kirov et al. 2003; Gregorio et al. 2009). Our result therefore might indicate that the transcriptional regulation of genes essential for neuronal diversity like protocadherins by CTCF may alter synaptic connectivity and thus contribute to the etiology of schizophrenia. SNP level interpretation of our data revealed potential involvement of ARLB5 and RLTPR in schizophrenia. Altered ARL5B expression might be involved in dysregulations of axonal transport, while RLTPR seems to be involved in adhesion and interactions between cells as well as cell and extracellular matrix. In sum, our studies shows the necessity to include an integrative approach, that mines sophisticated information on functional gene-sets in pathway-based association analyses.

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A MOLECULAR PATHWAY ANALYSIS INFORMS THE GENETIC BACKGROUND AT RISK FOR SCHIZOPHRENIA

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**Background**

Schizophrenia is a complex mental disorder marked by severely impaired thinking, delusional thoughts, hallucinations and poor emotional responsiveness. According to the hereditability of the disease (about 80%), the biological mechanisms that lead to Schizophrenia may be related to the genetic background of patients. Thus, a genetic perspective may help to unravel the molecular pathways that are disrupted in Schizophrenia.

**Methods**

In the present work, we used a molecular pathway analysis to identify the molecular pathways associated with Schizophrenia. Data of significant associated loci with Schizophrenia was collected and a list of genes localized in those loci was compiled. First phase of analysis consisted in the identification and evaluation of the connection between the genes in list. Cytoscape software was used to analyze the genes to identify a wider group of genes linked with each other by a common molecular pathway. In particular we used “Physical”, “Genetic”, “Pathway” and “predicted interaction” to build the molecular pathway. From this analysis, genes linked with each other were selected for the identification of a pathway potentially associated with Schizophrenia. After the selection of the pathway, a permutation analysis was performed. A sample of schizophrenic patients (4,486 cases and 4,477 controls) served for the analysis.

**Results**

*Primary analysis* From first phase of analysis a pathway of 361 genes was found. This pathway was then tested for association with Schizophrenia and resisted 10^6 permutations with a permutated p = 9.9999e-06. Finally we evaluated which “common” pathways were included in the pathway under analysis and tried to identify their possible role and their connection with each other. *Secondary analysis*: We found that genes from the original pathway grouped into specific sub-pathways including ones related to the immune system and inflammation, as well as neurodevelopment and neurodegeneration. In particular the Toll-like receptor cascade seems to be a potential link between the above mentioned pathways.

**Discussion**

In the present paper we identified some of the molecular pathways whose alteration could lead to Schizophrenia. The hypothesis behind this work consists in the idea that even though every schizophrenic subject may hold an unique pattern of mutations, these mutations are on charge of single specific pathways. These findings would stress the hypothesis that the mechanism that cause Schizophrenia originates in a certain point of a pathway. This would trigger a cascade effect that causes an alteration of the normal physiology of the brain. The first alteration can occur in different points of the pathways and still can cause the same effect. This would account for the heterogeneity of results of genetic associations with Schizophrenia. This hypothesis implies that there should be a link over the possible pathways involved in Schizophrenia that connects them together. Interestingly, the Toll-like receptor family seems to play a role in the modulation/connection of various pathways whose disruption lead to Schizophrenia. These results may suggest the overall metabolic path that, if altered, could increase the risk to develop Schizophrenia.

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**GENETIC AND NON-GENETIC RISK FACTORS FOR TREATMENT-RESISTANT SCHIZOPHRENIA**
Background
Between 25 and 33% of schizophrenia patients are treatment resistant as they show only partial or no response to conventional antipsychotic medication and eventually receive clozapine treatment. However, more than 50% of these are considered to be extreme treatment resistant as they also do not respond to clozapine. To improve treatment, research efforts are needed which improve the understanding of the underlying pathophysiology and elucidate the possible predictors for response to antipsychotics and in particular clozapine. Within the CRESTAR consortium we seek to find genetic and non-genetic causes and predictors for treatment response, treatment resistance, and extreme treatment resistance. In the present preliminary study using data of the BoMa sample, we investigated, which clinical and genetic factors are different between treatment responders and treatment resistant patients. We hypothesized that patients with a poor response represent more severe cases and should thus exhibit a higher genetic load.

Methods
With respect to clinical factors we tested differences between treatment responders and treatment resistant patients (defined as those receiving clozapine) using the OPCRIT items in n=464 patients receiving clozapine and n=411 patients not receiving clozapine. To test for genetic differences we used polygenic risk scores, with the hypothesis that treatment resistant patients are less responsive to environment and have higher polygenic risk scores. As discovery sample the PCG1 schizophrenia GWAS was used. The polygenic risk score was applied to n=2172 control persons, n=1540 schizophrenia patients, n=434 patients receiving clozapine and n=370 patients not receiving clozapine. Stepwise comparisons of their polygenic risk scores were made. These analyses took into account status as regards: schizophrenia diagnosis; clozapine therapy; treatment response; and premorbid social adjustment.

Results
With respect to clinical factors we found that treatment resistant patients show poor premorbid social adjustment more often than patients not treated with clozapine (OR=2.2; CI=[1.6–3.1]). While male patients show increased history of poor premorbid social adjustment (OR=2.1; CI=[1.5–2.8]), sex itself is not associated with treatment resistance (OR=0.96; CI=[0.70–1.31]). Using polygenic risk scores we could show that patients receiving clozapine had higher scores (mean = 2.9e-4) than those with no history of clozapine therapy (mean = -3.1e-4; p = 0.025). A trend towards a higher score was observed for clozapine non-responders (mean = -2.9e-4) compared to clozapine-responders (mean = 3.1; p = 0.09). The highest scores of all were observed in clozapine non-responders with a history of poor premorbid social adjustment (mean = -2.7e-4; p = 0.06).

Discussion
Our finding that patients with poor social premorbid adjustment have worse treatment response in general and to clozapine in particular is in accordance with recent findings. Our findings applying polygenic risk scores suggest that extreme non-responders have a higher genetic load, particularly those with a history of poor premorbid social adjustment. As the sample size is very limited we intend to perform polygenic risk score analysis on scores based on schizophrenia data of PGC2 as this provides increased power, and secondly test our hypothesis in larger samples.

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DISSECTION OF QTL FOR NEUROCOGNITIVE PERFORMANCE ON CHROMOSOME 5Q: IDENTIFICATION OF TWO SCHIZOPHRENIA SUSCEPTIBILITY LOCI FROM EXOME SEQUENCE DATA

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Background
Schizophrenia is a chronic mental disorder characterized by severe breakdowns in thought, behavior and emotion, with deficits often observed in neurocognitive performance. Despite its strong heritability, much of the genetic liability for schizophrenia remains unaccounted for. In this paper, we search for susceptibility loci on chromosome 5q at a QTL previously reported for a measure of abstraction and mental flexibility (ABF), a cognitive function that is often compromised in schizophrenia patients and their unaffected relatives.

Methods
Exome sequences were generated for 134 samples from 8 multiplex, multigenerational European-American families, including 25 individuals with schizophrenia or schizoaffective disorder, using Illumina TruSeq technology on the HiSeq. The exome variant calls were filtered based on stringent GATK probabilistic quality scores (LODs ≥ 4.0) and their assigned functional relevance, retaining a total of 407 protein-altering variants at 5q32-35.3 that were tested for their association with ABF using the SOLAR software package, with the resulting top hits (P < 0.05; n = 24) analyzed for possible effects on the schizophrenia spectrum.

Results
Our association results reveal a number of compelling findings, in particular a rare SNP from the gene SYNPO, rs6579797 (MAF = 0.022), that is region-wide significant for ABF (P = 0.015) and schizophrenia (P = 0.040), as well as for the traits jointly (P = 0.0027). In addition, a second variant, common SNP rs17551608 (MAF = 0.18) in the gene WWCI, also exhibits significant, Bonferroni corrected association with schizophrenia (P = 0.038), with nominal evidence for ABF (uncorrected P = 0.041). Both the SIFT and PolyPhen algorithms predict that the WWCI variant has deleterious effects on the protein.

Discussion
Synaptopodin, the protein product of SYNPO, is believed to play a role in dendritic spine...
formation, influencing neuronal plasticity, with evidence of interaction with the scaffolding protein coded by MAGI1, a gene previously implicated in schizophrenia. Interestingly, WWC1 (also known as KIBRA) has been associated with human memory performance, with synaptopodin serving as one of its interaction partners. Therefore, our association findings suggest important roles for two genes on chromosome 5q involved in neuronal plasticity, SYNPO and WWC1, identifying a potential mechanism linking neurocognitive performance to the susceptibility of schizophrenia.

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EFFECTS OF SCHIZOPHRENIA POLYGENIC RISK ON BRAIN GENE EXPRESSION
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Background
Genome-wide association studies have discovered robust common susceptibility variants for schizophrenia as well as thousands of loci contributing to a polygenic risk score that explains a small but significant proportion of the disease liability. Polygenic risk scores have been shown to be useful for investigating the genetic basis of endophenotypes and clinical dimensions. To gain insight into the molecular mechanism of schizophrenia, we sought to investigate the relationship between aggregate risk arising from many loci, the polygenic risk score, and downstream gene expression levels in the human brain.

Methods
Gene expression array data (GSE15745) derived from 4 brain regions (frontal cortex, temporal cortex, pons and cerebellum) for 147 neurologically normal Caucasian individuals were downloaded from the Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo/). Corresponding genome-wide genotyping data were accessed through dbGAP. For each individual, schizophrenia polygenic risk scores were calculated based on five association p-value thresholds using genome wide association data from the Psychiatric Genomics Consortium. After controlling for potential confounders e.g. age, gender and post-mortem interval, the relationship between gene expression and polygenic risk score was assessed using linear regressions. Nominally significant probes (p<0.05) for each brain region were subject to gene ontology/pathway analysis using DAVID (http://david.abcc.ncifcrf.gov/home.jsp).

Results
In the temporal cortex a polygenic risk score was associated with the expression of 806 probes (p<0.05). Gene ontology analysis of these probes revealed a significant enrichment (FDR <5%) of genes belonging to biological processes such as apoptosis and signal transduction. An enrichment of proteins located at synapses and dendrites was observed. These included genes previously linked to schizophrenia such as DLG2, GRIN1 and NRXN1 as well as several GABA receptors. In the pons a polygenic risk score was associated with the expression of 1273 probes (p<0.05). Gene ontology/pathway analysis of these probes revealed a significant enrichment of genes involved in development and morphogenesis, including regulation of axon extension. Enrichment of proteins located to the axon was also significant. Additional specific terms included the KEGG pathway ‘regulation of actin cytoskeleton’ and the biological process ‘antigen processing and presentation of peptide antigen via MHC class I’.
**Discussion**

To elucidate the downstream pathways influenced by polygenic risk, we performed novel analysis of transcriptome-wide gene expression data. Our analysis shows that collectively schizophrenia risk alleles influence gene expression in the adult human brain, and the effect is brain region specific. In the temporal cortex, polygenic risk scores are associated with expression of genes located at the synapse as well as dendrites, supporting the notion of the importance of these structures in disease pathology. Genes of particular interest include DLG2 and NRXN1 that have previously been linked to schizophrenia through analysis of copy number variants, suggesting polygenic risk mechanisms converge on similar pathways to those mediated by other types of genetic variation. Gene expression correlates in the pons were enriched for proteins located to the axon. Several of these genes overlap with the significant KEGG pathway ‘regulation of actin cytoskeleton’ and together implicate control of axon function by cytoskeletal proteins as a process important to schizophrenia. Intriguingly, a set of MHC related genes were significantly overrepresented, including several HLA alleles. Genome wide association studies consistently report highly significant findings in this region. Our data suggest that the expression of HLA proteins may represent a common pathway on which many risk mechanisms converge. Our analysis gives novel insight into the molecular and biological mechanisms of schizophrenia that emerge from polygenic risk mechanisms.

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**IMPACT OF GWAS DERIVED SCHIZOPHRENIA AND BIPOLAR RISK VARIANTS ON BRAIN NEUROPHYSIOLOGY**

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**Background**

Genome-wide association studies (GWAS) have identified multiple single nucleotide polymorphisms (SNPs) as disease associated variants for schizophrenia [SCZ], bipolar disorder [BPD], or both. Although these results are statistically robust, the functional effects of these variants and their role in the pathophysiology of SCZ or BPD remain unclear. Dissecting the effects of risk genes on distinct domains of brain function can provide important biological insights into the mechanisms by which these genes may confer illness risk. This study used quantitative endophenotypes to characterize the neurophysiological effects of well documented GWAS-derived SCZ/BPD susceptibility variants in order to map gene effects onto important domains of brain function.

**Methods**

We genotyped 199 patients with DSM-IV diagnoses of SCZ or BPD and 74 healthy control subjects on 19 risk SNPs derived from previous GWAS findings and tested them for associations with five endophenotypes (P3 amplitude, P3 latency, N1 amplitude, P2 amplitude, and P50 sensory gating responses) known to be abnormal in psychosis.

**Results**
The TCF4 SNP rs17512836 risk allele showed a significant association with reduced auditory P3 amplitude (p=0.00016). The same allele was also associated with delayed P3 latency (p=0.005).

Discussion
The TCF4 rs17512836 variant could play a role in brain function relevant to attention and working memory capacity, both of which are compromised in psychotic disorders. The neurobiological basis of P3 activity may serve as a pointer to the underlying biological mechanisms for which this gene increases risk for psychotic disorder.

INCREASED COPY NUMBER OF LINE-1 IN THE PREFRONTAL CORTICES OF PATIENTS WITH SCHIZOPHRENIA
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Background
Long interspersed nuclear element 1 (LINE-1) is one of retrotransposon sequence that occupies about 20% of human genome. LINE-1 sequence encodes enzymes of reverse transcriptase and endonuclease, by which LINE-1 can retrotranspose into new site of genomic DNA. Although about 600,000 copies of LINE-1 exist in the human genome, most of them lost their enzyme activities because of their incomplete insertion and accumulated mutations. However, some of them still have the activity of retrotransposition, and can insert their new copies into genomic DNA in the germ line cells. Recently, it has been reported that LINE-1 retrotransposition also occurs in the adult neural progenitor cells, and copy number of LINE-1 in brain was increased compared to other tissues in same individual. This genome alteration by LINE-1 insertion may play important role for pathophysiology of schizophrenia. Here, we quantified LINE-1 copy number in postmortem brains of schizophrenia patients and controls.

Methods
We quantified LINE-1 copy number in genomic DNA using Taqman-PCR methods. We used two independent prefrontal cortex sample sets donated by Stanley Medical Research Institute. Samples consist of schizophrenia, bipolar disorder, major depression and controls. We calculated the relative LINE-1 content of brain/liver in the first set. In the second set, we separated brain tissue into neuronal and non-neuronal cell nuclei using the NeuN-based sorting technique, and calculated relative LINE-1 content of neuron/non-neuron.

Results
We detected significant increases in relative LINE-1 contents in schizophrenia in both postmortem brain sample sets. Confounding factors such as age, gender, postmortem interval, and sample pH did not affect the LINE-1 content in brains.

Discussion
Increased LINE-1 copy number in the neuronal cells of patients suggests the aberrant retrotransposition activity in the neural progenitor cells in patients during early developmental stage, which is well concordant with the neurodevelopmental hypothesis of schizophrenia. We will discuss some of our ongoing studies, including the measurements of LINE-1 copy number using cellular and animal models, and comparison of the LINE-1 insertion pattern between patients and controls by whole genome sequencing of brain and liver DNA.

ANALYSES OF VARIANTS OF DGCR2 AND PRODH GENES IN SCHIZOPHRENIAS AND REFRACTORINESS TO ANTIPSYCHOTIC TREATMENT

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¹Federal University of São Paulo

Background
Schizophrenia is a mental disorder arising from a complex interaction of genetic and environmental factors. One of the strongest genetic risk factors is the 22q11 deletion and it has been suggested that genes located in this region, among these DGCR2 and PRODH, might contribute to susceptibility to the disease. DGCR2 encodes a transmembrane protein with ligand-binding function and PRODH gene encodes the proline dehydrogenase enzyme that acts in proline catabolism. Proline may have a role in brain function as a modulator/precursor of neuronal glutaminergic activity. In the present study, our aim was to investigate the association between polymorphisms in DGCR2 and PRODH genes, located at 22q11.2 region, with schizophrenia and treatment resistance schizophrenia (TRS).

Methods
A total of 227 patients with schizophrenia were compared with 277 healthy controls. The patients were genotyped for rs2073776, rs807759 and rs2072123 for DGCR2 and rs4819756, rs137852934,rs16983466, rs2238731, rs2904551, rs2904552, rs3970559, rs2238730, rs2870984, rs2870983, rs4550046 and rs372055 for PRODH polymorphisms using TaqMan® PCR assay or sequencing. Chi-square test and logistic regression were used to verify Hardy-Weinberg equilibrium and to investigate the association of polymorphisms and disease or TRS respectively.

Results
All polymorphisms were in Hardy-Weinberg equilibrium. Significant associations were observed between schizophrenia and PRODH rs2904552 genotypes (p=0.004, OR=2.52, 95%CI=1.33-4.76), with higher PRODH GG frequencies in patient group compared to control group. None polymorphism analyzed was associated with TRS. Carriers of GG genotypes present a chance 2.5 fold higher of being in the schizophrenia group than in control group.

Discussion
PRODH rs2904552 is a functional polymorphism, which changes an aminoacid and modifies the protein structure, supporting a possible association between this gene and schizophrenia pathogenesis.
INVESTIGATING THE NEURODEVELOPMENTAL EXPRESSION PATTERNS OF GENES ASSOCIATED WITH NEUROPSYCHIATRIC DISORDERS

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Background
Recent evidence suggests that for susceptibility genes associated with neurodevelopmental disorders, alternatively spliced fetal transcripts expressed in the developing brain may contribute to the pathophysiology, the mechanism of risk that underlies the clinical association. In the present study we investigated risk genes systematically, as composite gene sets associated with a neuropsychiatric disorder, for the presence of fetal transcripts. A disorder hypothesized to be neurodevelopmental in etiology would be expected to be associated with genes that have a markedly elevated fetal expression pattern.

Methods
Susceptibility genes were classified into neuropsychiatric diagnostic gene sets based on literature review. The developmental expression pattern of each gene was investigated in post-mortem brains, in 269 human prefrontal cortex samples from fetal development through aging, with RNA extracted and analyzed on oligonucleotide microarrays. Linear regression was performed at 30,176 probes, modeling gene expression as the continuous dependent variable as a function of fetal stage. Statistical significance of the frequency of elevated fetal transcripts was determined by permutation analysis. Further analysis of the gene sets included a gene set enrichment analysis compared to a background of all genes expressed in the brain.

Results
Genome-wide, 47% of genes were found to have at least one transcript showing an elevated fetal expression pattern. Genes associated with syndromic neurodevelopmental disorders (n=31, p=1.2x10⁻³) and intellectual disability (n=88, p=2.1x10⁻³) were found to be consistently more transcriptionally active during fetal development while genes associated with neurodegenerative disorders (n=49, p=1.3x10⁻²) were consistently under-expressed during fetal development, compared to the genome. Genes associated with bipolar disorder were also found to be under-expressed while genes associated with schizophrenia were not consistently different than the whole genome in fetal expression activity. Interestingly, genes within CNV regions associated with ASD did not show a pattern of fetal preferential expression, though genes associated with ASD by functional biology or other association studies, appeared to show a pattern of preferential fetal expression. The gene set enrichment analysis was most significant for genes associated with ASD, which yielded multiple functional groups critical to neuronal development.

Discussion
The present analysis assessed the fetal transcriptional activity of genes associated with neuropsychiatric disorders, compared to the genome at large, by determining the fetal effect of genes expressed in the DLPFC transcriptome. An important consideration is the content, face, and predictive validity of each gene set, given the assignment of each gene to a set based on
differing levels of current genetic evidence. In addition, the microarray technology is limited by an *a priori* probe design, which may exclude the presence of novel fetal transcripts that are pathogenic, but not detectable by the probes used in the current microarray analysis. Future transcriptome level exploration of individual genes and their interaction may elucidate fetal transcriptional activity, and their impact on neuropsychiatric disorders.

**LOWER CONCORDANCE RATES AND HERITABILITY ESTIMATES FOR SCHIZOPHRENIA; REGISTER DATA FROM A DANISH TWIN STUDY**

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**Background**

The concordance rates for a diagnosis within the schizophrenia spectrum have most recently been estimated to 41-65% in monozygotic twins (MZ) and 0-28% in dizygotic twins (DZ). A meta-analysis of 12 twin studies found evidence for large additive genetic effects with heritability estimates of 81% in liability to schizophrenia. The findings of a substantial genetic contribution to the etiology of schizophrenia has been confirmed in 2 recent studies from the population-based national registers in Sweden and Denmark though the heritability estimates in these studies are lower, 64% and 67% respectively, compared to results from twin samples. Both studies are based on 1.-degree relatives, not exclusively on twins. Concordance rates and heritability estimated in twin studies may be influenced by several methodological issues. Distinct factors are heterogeneity across studies (e.g. differences in illness severity), sample selection (e.g. based on twin registers compared to admission to a psychiatric facility), and the determination of zygosity. Differences in these factors could help explain why the rates vary across studies.

**Aim:** To estimate concordance and heritability of a diagnosis within the schizophrenia spectrum based on data from the Danish Twin Register and the Danish Psychiatric Central Register. The registers: The Danish Twin Register is the oldest national twin register in the world. It was initiated in 1954 and since 2008 contains information about more than 80,000 twin pairs born after 1870. The Danish Psychiatric Central Register is a national register comprising information on every admission to psychiatric hospitals in Denmark, data which has been systematically collected since 1938.

**Methods**

A data-set was created by linking the two registers, covering twins born from 1951-1981 who has been admitted to a psychiatric hospital with a diagnosis within the schizophrenia spectrum (using in-patient data) (see Table 1, showing the number of twin pairs in the analysis):

<table>
<thead>
<tr>
<th>Zygosity</th>
<th>Discordant</th>
<th>Concordant</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>MZ</td>
<td>115</td>
<td>22</td>
<td>137</td>
</tr>
<tr>
<td>DZ same sex</td>
<td>241</td>
<td>12</td>
<td>253</td>
</tr>
</tbody>
</table>

Table 1:
We calculated probandwise concordance rates for both “narrow spectrum schizophrenia” (ICD-10: DF 20.xx and ICD-8: 29509-29599) and “broad spectrum psychosis” (ICD-10: DF 2x.xx and ICD-8: 29509-29599, 29709-29799, 29829, 29839, 29889, 29899, 29905, 29909, 30109, 30129). 95% CI was calculated using a bootstrapping method.

Results

Our results suggest that the rates of concordance and preliminary heritability estimates in our large representative twin sample are low compared to previous studies, table 2. Table 2: Number of twin pairs in the broad and narrow spectrum categories and probandwise concordance rates (CR) with 95% CI

<table>
<thead>
<tr>
<th></th>
<th>DF 2x.xx</th>
<th>DF 2x.xx</th>
<th>DF 2x.xx</th>
<th>DF 20.xx</th>
<th>DF 20.xx</th>
<th>DF 20.xx</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Discordant</td>
<td>Concordant</td>
<td>Probandwise CR</td>
<td>Discordant</td>
<td>Concordant</td>
<td>Probandwise CR</td>
</tr>
<tr>
<td>MZ</td>
<td>137</td>
<td>22</td>
<td>27.7%</td>
<td>72</td>
<td>12</td>
<td>28.6%</td>
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<td>(18,8-36,5)</td>
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<td>(15,1-42,1)</td>
</tr>
<tr>
<td>DZ</td>
<td>514</td>
<td>28</td>
<td>10.3%</td>
<td>312</td>
<td>12</td>
<td>7.4%</td>
</tr>
<tr>
<td>SS+OS</td>
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<td></td>
<td>(6,9-13,7)</td>
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<td>(3,2-11,6)</td>
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<tr>
<td>UZ</td>
<td>95</td>
<td>16</td>
<td>28.8%</td>
<td>54</td>
<td>11</td>
<td>33.9%</td>
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<td>(19,2-38,5)</td>
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<td>(18,3-49,4)</td>
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</table>

Preliminary heritability estimates:

- Heritability = 2(r(MZ) - r(DZ))
- Broad spectrum (2x.xx) = 2(27.67-10.33) = 34,7%
- Narrow spectrum (20.xx) = 2(28.57-7.41) = 42,3%

Discussion

This is a report of the preliminary analyses of concordance rates and heritability estimates in a large, representative sample from the Danish Twin Register. The data analysis is still on-going with further structural equation modelling and tetrachoric correlations. These data will be presented at the meeting.

Our findings suggest that the concordance rates and heritability estimates is lower than those found in previous twin studies. Denmark is one of the few countries in the world that has a national-based twin register, and our sample covers all twins born in Denmark from 1951-1981. These findings may indicate that heritability in previous studies have been estimated too high.
IS AKT A PATHWAY THROUGH WHICH THE ENVIRONMENT IMPACTS ON RISK OF PSYCHOISIS

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Background
In 2012, Balog et al suggested that AKT might be the pathway through which environmental effect interact with genetic predisposition to increase risk of psychosis. Two of the best replicated environmental risk factors for psychosis are cannabis use and exposure to obstetric complications. a) Ruud et al (2011) addressed the question of a gene-environment interaction between 42 SNPs and cannabis in 800 cases and controls. The only gene for which such an interaction was shown was AKT1 (especially r 2494732) and cannabis use. b) In respect of obstetric complications, Nicodemus et al (2008) examined possible interactions between OCs and 13 genes in a sample of 119 family-trios. SNPs in AKT1, BDNF, DTNBP1 and GRM3 showed significant evidence for gene-by-environment interaction.

Methods
a) In a case-control study of 489 first-episode psychosis patients and 278 control subjects, we investigated the interaction between variation at only the AKT1rs2494732 SNP and cannabis use in increasing the risk of psychosis:

b) In a case-only study we collected DNA from 212 psychotic patients whose mothers had been interviewed with the Lewis-Murray scale for obstetric complications. Genotyping was carried out for the SNPs in 4 genes which had been reported to be in interaction with obstetric complication by Nicodemus et al.

Results
a) Cannabis Use: The rs2494732 locus was not associated with an increased risk of a psychotic disorder, with lifetime cannabis use, or with frequency of use. However, the effect of lifetime cannabis use on risk of psychosis was significantly influenced by the rs2494732 locus (likelihood ratio statistic for the interaction = 8.54; $p = 0.014$). Carriers of the C/C genotype with a history of cannabis use showed a greater than twofold increased likelihood of a psychotic disorder (odds ratio = 2.18 [95% CI: 1.12, 4.31]) when compared with users who were T/T carriers. Moreover, the interaction between the rs2494732 genotype and frequency of use was also significant at the 5% level (likelihood ratio = 13.39; $p = 0.010$). Among daily users, C/C carriers demonstrated a sevenfold increase in the odds of psychosis compared with T/T carriers (odds ratio _ 7.23 [95% CI: 1.37, 38.12]). b) We tested for interaction 3 AKT1 SNPs and definite obstetric complications in 212 patients in a case only design (N=212) for psychosis. Our preliminary results using the Khoury and Flanders (1996) formula $COR = ORge / (ORe X ORg)$ for a case only study design, indicate a $COR > 1$ supporting a GXobstetric complications interaction for 2/3 AKT1 SNPs tested.

Discussion
Our findings suggest that Balog et al (2012) may be correct when they state that AKT may be the pathway via which environmental factors increase risk of psychosis. Our confirmation of previous reports of interaction with AKT1 SNPs suggests that this is the case for cannabis use and for obstetric complications.
GENOME-WIDE ASSOCIATION STUDY OF COGNITIVE DECLINE IN SCHIZOPHRENIA

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¹Osaka University, ²Fujita Health University School of Medicine, ³Osaka University, ⁴National Institute of Mental Health, NIH, ⁵Nagoya University Graduate School of Medicine, ⁶Lieber Institute for Brain Development

Background

Substantial evidence suggests that many patients with schizophrenia experience a decline in intellectual functioning. Approximately 50% of patients with schizophrenia show cognitive deterioration, with an IQ decline of 10 points from the premorbid IQ; cognitive decline in schizophrenia remains stable. As there is considerable inter-individual variation in the degree of decline, it is conceivable that genetic influences play a role in determining the severity of cognitive decline in schizophrenia.

Methods

We conducted a genome-wide association study (GWAS) of cognitive decline in 166 patients with schizophrenia (mean estimated premorbid IQ (JART: Japanese Adult Reading Test): 101.2±10.0, full scale IQ (WAIS): 85.1±16.8 and difference score (subtraction of JART from full scale IQ): -16.1±13.1). We performed a multiple linear regression analysis to compare the difference score in major allele homozygous genotypes with that in minor allele carriers, with gender and education years as covariates, using PLINK 1.07 software.

Results

Although we did not observe any association at a widely used benchmark for genome-wide significance (p=5x10⁻⁸), the strongest association was observed at rs7157599 on chromosome 14, a missense polymorphism (Asn8Ser) in the DEGS2 gene (p=5.4x10⁻⁷). The most significant 10 markers and the top 200 markers are shown. Rs17069667 is an intronic SNP in the CSMD1 gene, which has been identified as a new risk gene for schizophrenia by a recent, large-scale GWAS. Associations between the 10 SNPs and the estimated premorbid IQ were not observed (all p>0.3); however, associations between the 10 SNPs and full scale IQ were observed in all of the SNPs (Table1). Analysis using an additive model and analysis with age, gender, illness duration and antipsychotic dose as covariates also showed slightly reduced but remained significant association. Our results suggest associations at the p<1x10⁻⁵ level between difference score in schizophrenia and four genes, one of which has been identified as a new locus for schizophrenia (CSMD1). Replication analysis using the CBDB/NIMH (Clinical Brain Disorders Branch, National Institute of Mental Health) sample showed a directionally consistent, trend association of genotype for a proxy of the top SNP, rs7157599 (rs3783332: r²=0.63, one tailed p=0.03).

Discussion

Although the study should be replicated with a larger sample size, our results show that the
measurement of cognitive decline in schizophrenia as a quantitative phenotype (in conjunction with GWAS) could be a gene discovery tool. We should note, however, that we cannot rule out an interaction of our gene variants with the affect of antipsychotic drugs on cognition.

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THE GENETIC ARCHITECTURE OF SCHIZOPHRENIA: HOW DO CNVS AND POLYGENIC SCORES CONTRIBUTE TO DISEASE RISK?

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Background

Both rare CNVs and common SNPs contribute to the genetic risk for schizophrenia. Several specific CNV regions and an increased burden of large deletion CNVs have demonstrated associations with schizophrenia. A significantly higher polygenic risk score in subjects with schizophrenia has also been established, confirming that many common SNPs confer risk for this disorder as well. The relationships between these rare and common genetic risk factors have not been thoroughly investigated, and we sought to address the following questions: 1) Do cases with CNVs have lower polygenic risk scores compared to cases without CNVs? 2) Do cases with CNVs have higher polygenic risk scores compared to controls with CNVs? 3) Do controls with CNVs have lower polygenic risk scores than controls without CNVs?

Methods

We investigated the polygenic risk score differences within and between case and control groups by CNV carrier status using the Swedish Schizophrenia Consortium (N=4646) as the discovery sample to score the International Schizophrenia Consortium (ISC) (N=4921) subjects. Analyses will be extended to CNV and GWAS data from the Psychiatric Genomics Consortium. CNV carriers were defined by the two classes of CNVs conferring the greatest disease risks: 1) having one of 12 specific CNVs previously associated with schizophrenia or 2) carrying any large CNV deletion greater than 500kb.

Results

Within schizophrenia cases, CNV carriers did not demonstrate significantly lower risk scores than non-carriers. Cases with either class of CNV membership had higher polygenic scores compared to control subjects carrying CNVs. Control subjects with specific associated CNVs had lower polygenic scores than other control subjects, but controls with and without large deletions had similar scores.

Discussion

These initial results are partly inconsistent with an additive model of CNV and polygenic risk. The presence of an associated CNV alone is not sufficient to result in schizophrenia, but also requires a context of increased risk from common variants.

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EXCESS OF HOMOZYGOSITY IN THE MAJOR HISTOCOMPATIBILITY COMPLEX IN SCHIZOPHRENIA

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1Feinstein Institute of Medical Research Zucker Hillside Hospital, 2Zucker Hillside Hospital, 3Fujita Health University School of Medicine, 4Fujita Health University School of Medicine, 5Department of Genetics The Institute of Life Sciences, The Hebrew University of Jerusalem, 6Columbia University, 7Feinstein Institute of Medical Research, Zucker Hillside Hospital

Background
Recent genome wide association studies (GWAS) in schizophrenia have begun to identify loci that replicably increase risk for the illness, but GWAS to date have focused on additive allelic effects, which only account for a portion of the total genetic variance. In order to examine potential recessive effects, we applied a novel approach to identify regions of excess homozygosity in an ethnically homogenous case-control cohort.

Methods
We genotyped 904 schizophrenia cases and 1640 healthy controls drawn from the Ashkenazi Jewish (AJ) population using the Illumina HumanOmni1-Quad array. Genomewide examination of runs of homozygosity using our previously-published whole genome homozygosity association (WGHA) method. To refine this signal, we used the recently developed GERMLINE algorithm to identify chromosomal segments shared identical-by-descent (IBD) across ≥ 3 chromosomes in our dataset, and compared homozygosity at such segments in cases and controls.

Results
Genomewide examination of runs of homozygosity identified an excess in cases localized to the major histocompatibility complex (MHC). We found a significant excess of homozygosity in schizophrenia cases compared to controls in the MHC (p-value =1.59E-54). This finding was replicated in an independent cohort of 548 schizophrenia cases from Japan and 542 matched healthy controls. Most of the homozygous regions which were over-represented in cases were located within the classical MHC class I regions, with the strongest case-control differences observed near HLA-A, in a segment encompassing three poorly annotated genes, TRIM10, TRIM15, TRIM40 and TRIM31.

Discussion
Homozygosity in the classical MHC region appears to convey significant risk for schizophrenia, consistent with the ecological literature suggesting that homozygosity at the MHC locus may be associated with vulnerability to disease.

A SUMMARY OF ASSOCIATION ANALYSES BETWEEN OPIOID RECEPTOR GENETIC POLYMORPHISMS AND METHADONE TREATMENT RESPONSES
Yu-Li Liu, Ph.D.1, Sheng-Chang Wang2, Hsiao-Hui Tsou3, Hsiang-Wei Kuo2, Shu-Chih Liu2, Chiu-Ping Fang2, Yu-Huei Shih2, Yao-Sheng Chang2, Ing-Kang Ho2, Sheng-Wen Liu2
Background
Methadone is a synthetic mu-opioid receptor agonist. It has been widely used in the treatment of heroin addiction. In this study, we summarized the association results of genetic variants on opioid receptor mu, delta, kappa, and nociceptin (OPRM1, OPRD1, OPRK1 and OPRL1) and the treatment responses in a methadone maintenance treatment (MMT) cohort.

Methods
A total of 366 patients under MMT were recruited with records of clinical characters, severity of dependence, opioid withdrawal symptoms and side effects. Plasma concentrations of enantiomers of methadone and its metabolites and nicotine metabolite cotinine were measured. The single nucleotide polymorphisms (SNPs) on opioid receptors were selected and genotyped. The statistical association analyses were mainly calculated by the general linear model (GLM).

Results
The OPRM1 rs495491 genotype at intron 1 was associated with methadone side effects of change in libido and insomnia (GLM, P<0.006). SNP rs1799971 and rs12209447 at exon 1 and intron 1 were associated with nicotine metabolite cotinine plasma level (GLM, P<0.029). The OPRD1 intron 1 rs419335 and rs2236855 of genotypes (GLM, P<0.018) and allele types (GLM, P<0.003) were associated with severity of dependence and diastolic blood pressure. SNP rs419335 and rs482387 of genotype (GLM, P<0.020) and allele type (GLM, P=0.002) were associated with dry mouth. The OPRK1 SNP rs16918853 at exon 4 of genotype (GLM, P<0.005) was associated with bone joint aches and alcohol use (units/day). Intron 2 rs7843965 genotype was associated with body weight (GLM, P=0.006). The OPRL1 SNP rs4408777 at promoter (GLM, P=0.014) and rs6090043 at intron 1 (GLM, P=0.009) were associated with liver alcohol use marker γ-GT. Intron 1 SNP rs8121509 and exon 4 rs2229205 genotypes (GLM, P<0.003) were associated with fatigue.

Discussion
The genetic variants on opioid receptors are mainly associated with different side effects reported by MMT patients. The OPRK1 and OPRL1 were associated with MMT patients who had combined alcohol use.

INTERACTIONS BETWEEN ALCOHOL METABOLISM GENES AND RELIGIOUS INVOLVEMENT IN ASSOCIATION WITH ALCOHOL DEPENDENCE
Karen G. Chartier, Ph.D.1, Victor Hessselbrock, Ph.D.2, Laura Almasy, Ph.D.3, COGA Colleagues4

1University of Texas School of Public Health, Dallas Regional Campus, 2University of Connecticut, 3Texas Biomedical Research Institute, 4Collaborative Study on the Genetics of Alcoholism
**Background**

The current study tests the modifying effect of religious involvement on genetic variants for alcohol metabolism and alcohol dependence phenotypes. Previous studies show that gene variants associated with a less active rate of alcohol metabolism from ethanol to acetaldehyde correlate with increased alcohol consumption and greater risk for alcohol dependence compared to those variants that promote a faster conversion (Zakhari, 2006). Additionally, environmental factors are important to understanding the different levels of risk for alcohol dependence observed within a population. For example, weekly or more church attendance and religious affiliation are protective for alcoholism and associated with abstinence from drinking, respectively (Heath et al., 1997; Michalak et al., 2007). Few studies have tested interactions between alcohol metabolism gene variants and environmental factors in predicting alcohol dependence. Konishi et al. (2003) reported a stronger association for the ADH1C risk allele in predicting alcohol dependence for individuals with an age of drinking onset < 25 years of age. Hasin et al. (2002) showed reduced effects for the ADH1B protective allele for immigrants to Israel from heavy drinking cultures. For this study, religious involvement is expected to be protective and reduce the effects of alcohol metabolism risk variants for alcohol dependence.

**Methods**

Subjects were White (72.8%), Black (19.4%), and Hispanic (7.8%) participants (N=7977), 18 years and older (M=37.76; SD=14.72) from the Collaborative Study on the Genetics of Alcoholism. Phenotypes included the DSM-IV diagnosis for alcohol dependence and seven individual alcohol dependence symptoms. Gene variants were ADH1B (rs1229984 A/G; rs2066702 C/T), ADH1C (rs698 A/G), and ADH4 (rs1042364 A/G; rs1800759 A/C). Chi-square tests examined bivariate relationships between gene variants and phenotypes. Regression models were tested in two steps including, 1) main effects for genetic variants and religious involvement, and 2) gene-environment interaction effects. Religious involvement was based upon self-reported religious affiliation and ≥ weekly service attendance in the past year (i.e., 1) no affiliation and no attendance; 2) either affiliation or attendance; or 3) both affiliation and attendance). Genetic variants were coded as additive and phenotypes as 0 and 1. Models controlled for genetic ancestry, age, gender, and family clustering.

**Results**

Bivariate analyses identified significant associations between rs1229984 variants and alcohol dependence and each of seven alcohol dependence symptoms; subjects carrying G/G and A/G genotypes had higher rates for all phenotypes. Variants for rs2066702, rs698, rs1042364, and rs1800759 were significantly associated with the symptoms of ‘tolerance’ and ‘drinking larger amounts or over a longer period than intended’ (i.e., larger-longer). Subjects who carried the C/C genotype and the G allele, A allele, and C allele (respectively, as SNPs are listed in previous sentence) had higher rates of these symptoms. Other relationships between alcohol metabolism gene variants and the alcohol dependence diagnosis and symptoms were less consistent. Multivariate models further tested phenotypes for alcohol dependence. The main effects for (greater) religious involvement and rs1229984 variants (A/A and A/G versus G/G) were associated with reduced risk for all alcohol-related phenotypes. Variants for rs1042364 were only significant in predicting 4 of 7 alcohol dependence symptoms when modified by religious involvement; G/G versus A/G or A/A was associated with reduced risk with increasing religious involvement. Variants for rs2066702 (C/C versus C/T) were similarly modified by religious
involvement in predicting tolerance and ‘drinking despite physical or psychological problems’.

Discussion
This study provides evidence of a modifying effect for religious involvement on relationships between alcohol metabolism genes and alcohol dependence phenotypes. Greater religious involvement reduced the risk effects of $ADH4$ rs1042364 and $ADH1B$ rs2066702 variants, while main effects for $ADH1B$ rs1229984 and for religious involvement were generally associated with risk for alcohol dependence. Religious involvement may be protective for alcohol dependence via lower social norms for drinking and alternative social activities with limited drinking opportunities.

THE DOPAMINERGIC GENETIC PATHWAY AND SMOKING MOTIVES AS NOVEL INTERMEDIATE PHENOTYPES OF NICOTINE DEPENDENCE IN AFRICAN AMERICANS

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Background
This study examines the Primary Dependence Motives (PDM) of the Wisconsin Inventory of Smoking Motives (WISDM) as a novel intermediate phenotype of nicotine dependence in an African American population. Four subscales (Automaticity, Craving, Loss of Control, and Tolerance) of the WISDM have been dubbed the Primary Dependence Motives (PDM). Prior work has found that the PDM represent heavy, pervasive smoking, which is a core feature of nicotine dependence, making it a strong candidate for an intermediate phenotype (e.g. Piper et al, 2004; Piasecki, Piper, & Baker, 2010). Further supporting its role as a potential intermediate phenotype, prior research has found an association among dopaminergic (DA) genetic variants, a critical neurotransmitter system related to the pharmacodynamics of nicotine, and PDM in a large sample of European Americans (Bidwell et al., 2013). No studies have evaluated associations among these phenotypes and genetic variants in African American smokers, an ethnic subgroup that shows a unique etiology and distinctive smoking patterns.

Methods
The study used data from 268 African American daily smokers ($M(SD) = 37.9 (12.6)$ years old; 4.9 (2.1) Fagerström Test for Nicotine Dependence (FTND)) who completed a phenotypic assessment and provided a saliva sample of DNA. Based on the known pharmacodynamics of nicotine and the critical role of dopamine in smoking behavior, 13 DA loci (listed in Table 1) were tested individually, as derived haplotype blocks, and as a cumulative genetic score (CGS) for association with these novel motivational phenotypes and nicotine dependence. The characterization of the linkage disequilibrium (LD) among DA polymorphisms was performed with the Haploview program (Barrett et al. 2005). The association of FTND and PDM with the SNPs and haplotypes was evaluated by a linear regression using the PLINK program; the association with the CGS was performed in SPSS 19.0. Additive effects models were assumed and the direction of the regression coefficient represented the effect of each minor allele.
Results
See Table 1 for the list of polymorphisms tested, minor alleles, minor allele frequencies, and results of the single loci association tests. Two haplotype blocks emerged in the Dopamine D2 Receptor (DRD2) gene region comprising 8 and 3 SNPs, respectively. Using all three methods, a DA CGS, DRD2 region haplotypes, and individual loci within the Dopamine Beta Hydroxylase (DBH) gene and the DRD2 region were associated with FTND (p’s <.05). These same genetic variants were also associated, at a higher level of significance, with PDM, particularly the automaticity subscale (p’s range from < .01 to <.001). Mediation via PDM of the DA genetic pathway - FTND relationship was also supported (p’s range from < .01 to <.001).

Discussion
This study supports smoking motives related to heavy, automatic smoking as a promising intermediate phenotype for future genetic studies in African American smokers. Findings are suggestive of a causal model in which DA variants increase the likelihood that a person will become dependent via a highly automatic smoking ritual that can be elicited with little awareness. Broadly, these results support the utility of biologically-driven CGSs and also suggest a differentiated haplotype structure for the DRD2 gene region in African Americans.

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POLYMORPHISMS RELATED TO HYPOTHALAMIC-PITUITARY-ADRENAL AXIS REACTIVITY, CHILDHOOD NEGLECT AND THEIR INTERACTION ON CRACK COCAINE ADDICTION

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Background
The hypothalamic-pituitary-adrenal (HPA) axis and the childhood trauma seem to have a putative role on the establishment of substance use disorders. However, little is known about interactions between these different factors. The aim of the present study is to search for gene-gene and gene-environment interactions involved in crack cocaine addiction evaluating functional polymorphisms related to HPA axis and childhood physical neglect.

Methods
Our sample is composed by 139 crack cocaine-addicted women who completed 3 weeks of follow-up during early abstinence. Withdrawal symptoms were assessed through the Cocaine Selective Severity Assessment (CSSA) scale and the childhood adversities by the Childhood Trauma Questionnaire (CTQ). Generalized estimating equation modeling and conditional logistic regression with counterfactuals were used to test gene-gene and gene-environment interactions. Functional polymorphisms within mineralocorticoid receptor (NR3C2) and glucocorticoid receptor (NR3C1) coding genes were evaluated (rs5522 and rs6198, respectively).
Results
We found a NR3C2-NR3C1 interaction modulating the severity of withdrawal crack cocaine symptoms (P = 0.002). In the Post Hoc analysis, carriers of the NR3C2 rs5522-Val and NR3C1 rs6198-G alleles showed lower overall severity scores when compared to other genotype groups (P-values ≤ 0.035). An interaction between the rs5522-Val allele and childhood physical neglect altered the risk to crack cocaine addiction (hazard ratio = 4.0, 95%CI: 1.7-9.2, P = 0.001). In the gene-gene-environment, a ‘high risk’ genotype group interacted weakly with physical neglect conferring risk for crack cocaine addiction (hazard ratio = 1.9, 95%CI: 0.9-3.9, P = 0.083).

Discussion
These findings are consistent with the role of interactions between NR3C2 and NR3C1 genes and childhood neglect on crack cocaine susceptibility and severity. It is possible that alterations in the HPA axis reactivity caused by both physical neglect and functional SNPs in genes related to stress system change the cortisol levels and, consequently, the cocaine reinforcement properties in dopaminergic pathways. This study should be replicated in independent samples in order to confirm these interactions as relevant in crack cocaine addiction.

EVIDENCE FOR INTERACTIVE EFFECTS OF POLYMORPHISMS OF GABA, OPIOID AND DOPAMINE GENES OF REWARD SYSTEM WITH HEROIN DEPENDENCE
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Background
The GABAergic, opioidergic and dopaminergic pathways are among the key neurotransmitter systems linked to Mesolimbic-cortical reward system. The interacting effects of the genes of these pathways are likely to play a significant role in predisposition to development of substance abuse and dependence. In the current study we investigated the interacting effects of selected candidate genes of these pathways with regard to diagnosis of heroin dependence in an ethnically distinct population.

Methods
Using generalized multi dimensionality reduction (GMDR) approach we tested the interacting effects of selected polymorphisms of reward system genes in an ethnically homogenous Sinhalese male dependent heroin users (n=157) and matched controls (n=155) with virtually no life time history of smoking, alcohol or drug use.

Results
The GMDR analysis revealed statistically significant 2-loci interaction between GABRG2-rs211013 and OPRM1-rs1799971 [Testing balanced accuracy (TBA)=0.63, Cross validation consistency (CVC)=9, Permutation P value=0.03] and 3-loci interaction among GABRG2-rs211013, OPRM1-rs1799971 and COMT-rs6267 [TBA=0.60, CVC=7, Permutation P value=0.04]. However, the 2-locus model was considered the best on the basis of parsimony, highest testing balanced accuracy, highest CVC and lowest empirical P value after permutation.
Discussion
Given the importance of reward pathway in addictive behaviours and the interacting functionality of GABA, dopamine and opioid systems within the reward system, the observed interactions provide evidence for biologically plausible mechanism of genetic predisposition to heroin dependence.

IDENTIFICATION OF MICRONA EXPRESSION QUANTITATIVE TRAIT LOCI IN THE NUCLEUS ACCUMBENS OF HUMAN POSTMORTEM BRAINS FROM ALCOHOL DEPENDENT SUBJECTS AND MATCHED CONTROLS
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Background
Alcohol dependence (AD) is a chronic addiction disorder with biological, behavioral, and socioeconomic components; heritable factors account for 60% of the risk for AD. While many genetic studies have implicated numerous AD loci, the mechanisms underlying the neuroadaptations to excessive alcohol consumption are unclear. MicroRNAs (miRNAs), a species of non-coding RNAs, predominantly down-regulate gene expression and are abundantly expressed in the brain. miRNAs are implicated in brain development, plasticity, degeneration, and several neuropsychiatric disorders; however, few studies have been performed in subjects with AD. Furthermore, miRNA expression patterns in brains of AD subjects have only been assessed in the prefrontal cortex. Expression quantitative trait loci (eQTL), genomic loci regulating gene expression levels, influence phenotypes and are enriched among validated genetic signals for most commonly studied traits, including AD. However, the impact of eQTLs on miRNA expression levels in AD has not been assessed. We attempt to understand the genetic underpinning of AD in humans by integrating genome-wide miRNA expression data from the nucleus accumbens (NAc) of AD subjects and controls with genotypic data from this sample. We examined the NAc due to its role as a major addiction-related brain region within the mesocorticollimbic pathway and its involvement in drug-seeking motivation and reward.

Methods
The postmortem sample was donated by the New South Wales Brain Bank Network, University of Sydney (Sydney, Australia). NAc tissues from 18 AD subjects and 18 controls were matched on age, sex, ethnicity, brain pH, postmortem interval, and RNA quality. The miRNA expression data were generated on the Affymetrix GeneChip miRNA 3.0 array containing probes for 1733 human mature miRNAs (miRBase v.17). miRNA expression data were pre-processed with Partek Genomics Suite (v.6.6) which included probe level background correction using the robust multiarray algorithm adjusting for probe sequence (GC-RMA), quantile-normalization across all arrays, log₂ transformation, and median polish probe intensity summarization to obtain the overall score for each probe set. Normalized miRNA expression values were analyzed in a linear regression framework with case status as the main effect and smoking as a covariate.
Subjects were matched for most of the technical and biological covariates so they were not included in the final model. Approximately 1M SNP genotypes were generated on the Affymetrix SNP 6.0 array followed by imputation of an additional 3M SNPs. Potential alcohol
responsive eQTLs affecting miRNA expression were selected based on genomic proximity to the miRNA and only SNPs within 1Mb from the mature miRNA sequence (cis) were considered.

**Results**
We detected 240 miRNAs differentially expressed at p<0.05. After correction for multiple testing at a false discovery rate (FDR) <10%, 29 miRNAs were significant. Several identified miRNAs were previously reported to be involved in aging/neurodegeneration (miR-1538, -516, -34c and -487a), and neurodevelopment/function (miR-371, -154 and -1247). Interestingly, miR-154 is also reported to be associated with mesocorticolimbic pathway modulation in opiate addiction. To better understand the mechanisms contributing to the miRNA differential expression we sought to detect potential alcohol responsive eQTLs affecting miRNA expression. We detected 275 cis-eQTLs for all 29 miRNAs after correction for multiple testing (FDR<10%). Several of these cis-eQTLs are located in functionally relevant sites including transcription factor binding sites, splicing enhancers/silencers, and putative miRNA target sites.

**Discussion**
Although the analysis of our data is currently ongoing, our preliminary results support our hypothesis that miRNA expression dysregulation plays a role in AD. The identification of miRNAs reported to play a role in neural function, combined with studies examining the role of miRNAs in other addiction disorders, i.e. opiates, cocaine, and amphetamines, suggests that underlying miRNA-mediated neuropathological processes may relate these different addiction phenotypes. We must keep in mind, however, that this may be an oversimplification of the neurobiology. To explore our findings further and elucidate specific mechanisms driving AD, we will integrate mRNA expression data from our sample with the genetic and miRNA data to identify miRNA/mRNA targets and detect dysregulated cellular pathways. Further studies in animal models will be conducted to assess the behavioral effects of brain miRNA modulation on alcohol consumption.

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**GENOMEBROWSE: VISUAL ANALYTICS AND FALSE-POSITIVE DISCOVERY FOR DNA AND RNA-SEQ NGS DATA**
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¹Golden Helix

**Background**
High-throughput sequencing (HTS) has recently provided price competitive alternatives to microarrays for both RNA expression profiling with the RNA-seq protocol and DNA genotyping with whole genome and whole exome sequencing. Although the bioinformatics tools have matured for secondary analysis of sequence data, including alignment, variant calling, and gene and transcript level quantification, the outputs of these tools often require inspecting the “raw read alignments” for putative variants and genes with interesting expression profiles. Investigating these variants in their VCF format and the alignments in BAM format allows for detection of false-positives as well as aiding the interpretation process by providing a rich genomic context.

**Methods**
We introduce Golden Helix GenomeBrowse™, a free visualization tool for DNA and RNA
sequence alignment and variant calls along with annotations tracks from a rich catalog of precurated public data. GenomeBrowse is built from the ground up with the guiding principles of (1) working seamlessly with cloud-hosted data as fluidly and quickly as local files, (2) being intuitive to use for non-bioinformaticians to utilize in their research, and (3) having a multi-threaded architecture to make big-data visualization and analysis accessible to anybody capable of navigating Google Earth.

**Results**
By including integration with a rich repository of public data, users have no barriers to the process of interpreting their sequencing results. In particular, we demonstrate the ability of GenomeBrowse to stream exome sequencing of a trio from the Amazon Cloud from whole genome views down to the gene level with annotation tracks ranging from 1000 Genomes, dbSNP, genes, and miRNAs.

**Discussion**
We show how GenomeBrowse can highlight false-positive Single Nucleotide Variants and small Insertion/Deletions, confirm the inheritance pattern of putative functional variants, and aid in the interpretation of a variant’s impact.

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**GENOTYPING PORTAL: A FREELY AVAILABLE ONLINE RESOURCE FOR EXISTING METHODS FOR HUMAN MOLECULAR GENETICS**
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**Background**
A large number of experimental methods for human molecular genetics have been developed in recent years. However, there is not a comprehensive and updated website that allows an unified access to the wealth of consolidated information about available molecular genetics methods. Researchers working in neuropsychiatric genetics could benefit from the availability of such freely available resource.

**Methods**
In the present work, we present Genotyping Portal, a freely available online resource that contains comprehensive information about original papers, reviews and protocols of available methods for SNP genotyping, mutation screening, CNV genotyping, DNA sequencing, epigenetics, PCR techniques and DNA extraction.

**Results**
Currently, Genotyping Portal contains primary information for more than 110 techniques, including links to manufacturers of equipment and reagents and to genotyping and sequencing service providers. This freely available resource has been used by a large number of researchers around the world.

**Discussion**
Genotyping Portal is a very useful free resource for undergraduate and postgraduate students,
researchers in neuropsychiatric genetics and developers of new methods. It is freely available at http://goo.gl/46NSC and has been visited by more than 13,000 researchers in 85 countries.

A STRATEGY FOR PARALLEL VALIDATION OF THOUSANDS OF DE NOVO MUTATIONS USING A DESKTOP SEQUENCER

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Background

Trio-based sequencing studies allow for the detection of de novo mutations, in which the proband of a particular trio harbors a variant not seen in either the mother or the father. These mutations are thought to offer an increased likelihood of pointing to genes or regions implicated in the sporadic manifestation of disease. The detection of these variants by sequencing must be independently validated to confirm the event was not due to some systematic or experimental error. Often times techniques such as quantitative PCR (qPCR) or Sanger sequencing are used in validation because of their specificity and reliability in differentiating alleles at a locus of interest. However, these techniques do not scale well for high throughput validations primarily because of cost and impractical data formats. We have developed a method for the targeted Next Generation Sequencing (NGS) of candidate regions using a custom amplification and library construction procedure tailored for high throughput applications.

Methods

We describe our implementation of a tailed amplicon sequencing approach on the Illumina MiSeq, leveraging efficient indices and automated primer design. This technique uses amplicon specific primers with a tail sequence added on the 5’ end, which allows for sequencing adapters to be added on in a second PCR after the initial amplification of the target region. The PCR primer design was automated in Perl using Primer3 and sequence data from the UCSC browser. Only three indices are used in our approach, one each for the proband, mother, and father, to allow for pooling after initial amplification. The individuals from which a particular read came were identified based on genomic location after alignment. The purified PCR products are then sequenced on a MiSeq, and once complete, the read data is aligned with BWA-MEM. Once the reads are aligned digital counts of reads can be used to determine allele counts at each target site, allowing for very quick filtering of potentially miscalled variants in contrast to Sanger sequence data.

Results

We first piloted this method in a trio-based cohort collected from Taiwan in which over 1,500 single nucleotide variants and over 400 indels required validation after whole exome sequencing. Within this study neither of the parents had any history of mental illness, while the proband had been diagnosed with schizophrenia. In the first test batch we ran 96 samples, testing 96 regions, for 96 independent de novo calls. After sequencing, the data showed ample coverage over 89 out
of 96 sites, with 88 out of 89 having over 100 reads in all three individuals within the trio. Regions that failed to gain ample coverage were assumed to be a failure of the given primers to amplify or user error.

Discussion
The parallel sequencing of many specific amplicons on a MiSeq has allowed for great flexibility and accuracy for the validation of de novo variants. The digital readout of the data allows for a rapid and automated analysis as well as the differentiation of somatic events, which can sometimes appear to be germline de novo mutations. Furthermore, the flexibility of this method is far greater than using a qPCR assay because allele specific probes do not have to be designed over each site, only specific primers. This method offers high quality data with maximum adaptability, making it an optimal strategy for large-scale de novo validation.

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GENETIC ASSOCIATION OF MCPH1 (MICROCEPHALIN 1), SNTB1 (SYNTROPHIN, BETA 1) AND OTHER GENES WITH AUTOMATIC THOUGHTS IN A JAPANESE POPULATION
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Background
The cognitive model involving automatic thoughts has progressed in depression and anxiety. Combined studies from behavioral genetics and cognitive neuroscience launch new breaks for integrated research. The objective of this study is to investigate the genetic association of automatic thoughts with SNPs (single nucleotide polymorphisms) involved in cognitions, neurogenesis, neuronal cell structures, neurotransmitters and hypothalamus-pituitary-adrenal axis.

Methods
The number of healthy participants was 610 (363 males and 247 females, mean age = 25.0 ± 3.7). We used DACS (Depression and Anxiety Cognition Scale, Fukui, 1998), one of Japanese psychological questionnaires, to assess automatic thoughts. Twenty five SNPs containing COMT, BDNF, FKBP5, SNTP1 (rs4512418), MCPH1 (rs2911968) and others were selected, according to their minor allele frequency (MAF > 0.05). Logistic regression models were performed to test for association of the mean DACS scores with each allele (major-allele homozygote, heterozygote, and minor-allele homozygote). The significant α-value was set at α < 0.0025 (0.05/25). Statistical analysis of single-SNP or multiple-SNP was conducted using the SNPStats (Solé et al., 2006).

Results
The call rates of all genotyping were higher than 96%. Eighteen SNPs of all twenty five were not deviated from Hardy-Weinberg equilibrium (p > 0.05), and the rest seven SNPs were able to be excluded from our statistical analysis. Significant associations of SNTB1 with Interpersonal Threat (IPT) and that of MCPH1 with Future Denial (FD) were found only in female groups.
Haplotype linkage analysis of SNTB1 and MCPH1 revealed that there was a significant tendency of interaction within a CT haplotype between the FD score of males and that of females ($p=0.049$).

**Discussion**

Both SNTB1 and MCPH1 are located on chromosome 8. It has been reported that chromosome 8 may be associated with avoidant personality, neuroticism and depression (Holmans et al., 2007). Our present study suggests that the scores derived from DACS with significant interaction with those two SNPs may be regarded as appropriate traits to detect the diathesis of automatic thoughts. It has been reported that common variants of MCPH1 may be associated with brain volumes in females (Rimol et al., 2010) and that the components of dystrophin-associated protein complex like SNTB1 could be rich in postsynaptic neurons (Blake et al., 1999). In addition, linkage analysis of this present study suggests that there seems to be a potential risk haplotype consisted of SNTB1 and MCPH1. Therefore, we also consider the two SNPs may be one of the important genes on researches for the cognitive vulnerability to depression and anxiety.
FAMILIALLY ANALYSES OF BRAIN VOLUMETRIC CORRELATES OF ADHD

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Background

Attention-deficit hyperactivity disorder (ADHD) is a common and highly heritable neurodevelopmental disorder. Although ADHD has been linked to volumetric brain abnormalities, findings are often inconsistent. This study combines neuroimaging and family methods to examine brain volumetric correlates of ADHD in a large sample, and to test, for the first time, whether these correlates share familial (genetic plus shared-environmental) or non-shared (individual-specific) environmental underpinnings with ADHD.

Methods

Participants came from the NeuroIMAGE case-control project and included probands diagnosed with ADHD, their affected and unaffected siblings and healthy control sibling pairs (N>770; mean age=17 years). ADHD symptom scores of participants were obtained using ratings on the DSM-IV-based Conners’ Parent Rating Scale. SPM-based tissue-segmentation and FSL-FIRST were applied to structural MRI scans to derive total brain volume (sum of gray and white matter volumes) and subcortical regional brain volumes. Structural equation modelling using the liability threshold selected samples design was used to estimate phenotypic, familial and non-shared environmental associations. All analyses corrected for age and gender, and subcortical volumes were also corrected for total brain volume.

Results

ADHD symptom scores were significantly associated with a) total brain volume; b) gray matter volume independent of white matter volume, but not white matter volume independent of gray matter volume; c) caudate nucleus volume. Familiarity of these brain volumes was modest, familiality of ADHD symptom scores was low. Associations between ADHD symptom scores and these volumes were largely explained by non-shared environmental, but not by familial influences.

Discussion

Results suggest that ADHD symptoms are linked to a reduction in total and gray matter volumes. ADHD symptoms are also linked to a reduction in caudate nucleus volume, a subcortical region that is part of the fronto-striatal circuits. Links between ADHD symptoms and these brain volumes were largely influenced by environmental influences not shared between siblings from the same family. Finding low familiality of ADHD symptoms is consistent with the low correlations for ADHD symptoms observed within dizygotic twin pairs. Highly heritable traits can be low in familiality if non-additive genetic effects play a role. Hence, the small familial influences on ADHD symptoms and their links with associated brain volumes could be due to...
non-additive genetic effects taking place. This is a topic for future investigation through twin and molecular genetic research.

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AN EXTREME TRAIT GENOME-WIDE ASSOCIATION STUDY OF AN ADHD-RELATED ENDOPHENOTYPE IN THE GENERAL POPULATION
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Background
Attention deficit hyperactivity disorder (ADHD) is a common (5% of the general population), persistent (~50%) and impairing mental illness of childhood. It is a genetically heterogeneous and complex disorder. Meta-analyses of previously conducted GWA (genome-wide association) studies of ADHD revealed no genome-wide significant associations. The lack of genome wide findings is likely due to a number of factors including the heterogeneous nature of the behavioural phenotype and reduced power due to the difficulty and cost in ascertaining large clinical samples. Endophenotypes can provide increased power in genetic studies by pointing to a more homogeneous genetic group of individuals and measuring a process that is closer to the underlying genetic mechanism. There is evidence that response inhibition is a valid endophenotype for ADHD based on the results of clinical, family, functional imaging and preliminary genetic investigations. Response inhibition is a quantitative trait, continuously distributed in the general population, and the genetic risks underlying the variation likely reflect a subset of those involved in ADHD. Response inhibition refers to the ability to stop a speeded motor response and can be measured using a simple and quick computer based task The goal of the study was to utilize a novel and cost-effective design which combines the comprehensiveness of GWA using high-density arrays, the statistical efficiency of an extreme trait design in a general population sample, and the power of a quantitative cognitive endophenotype (response inhibition) to increase power to detect common genetic risks in ADHD.

Methods
DNA, psychiatric phenotype data and performance on a cognitive endophenotype (response inhibition) was collected on 16,099 children and adolescents from community sample. From 7545 unrelated individuals of Caucasian descent, based on performance on the cognitive endophenotype, the extreme best 10% (N=755) and extreme worst 10% (N=755) individuals were selected for GWA analysis. Genotyping is being conducted using Illumina Omni series beadchips. Quality control analyses were conducted using PLINK, including multi-dimensional scaling (MDS) for population structure and the Cochrane-Armitage trend test for detection of association.

Results
Results from the full sample will be available at the time of the presentation. We have genotyped (using the Illumina Omni1-Quad) and analyzed an initial subset of 191 individuals for quality control purposes. We first excluded samples with call rates of <98% (n=2), sex discrepancy (n=1), and non-European ancestry based on MDS plots (n=4). Association analyses were conducted in the remaining 184 individuals (92 high, 92 low). Although no results of genome-wide significance were identified in our preliminary sample, we identified SNP associations with
Discussion
This research has the potential to increase the ability to identify candidate variants for future biological investigation, to facilitate the understanding of the mechanism by which genetic risks influence brain function and result in ADHD. This will be the first report of the utility of an endophenotype in ADHD using a genome wide approach.

3
PRELIMINARY RESULTS OF AN EXOME STUDY IN ATTENTION DEFICIT HYPERACTIVITY DISORDER
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Background
ADHD is a heterogeneous disorder caused by a complex interplay of factors. In this regard, the genetic origin has been seen as an important factor, where genetic studies estimate a 76% heritability. Association studies have demonstrated a connection between genes of the dopaminergic system with ADHD, but there was little success in replicating these results. In GWAS studies no SNP reached significance level, probably because samples size were not enough. Regarding rare variants, CNVs studies have been conducted with more fruitful results. It is clear that common and rare variants are important in Psychiatric genetics and SNV have been observed in autism and schizophrenia.

Methods
In order to conduct a pathways analysis, we sequenced the whole exome of 8 selected trios with sporadic ADHD children based on clinical evaluations, pedigree structure, familial phenotype evaluation and family history. This is a pilot study where more 24 trios are being sequenced. Our sample is from a cohort of students at high risk for Developing psychopathology and resilience in childhood – the prevention study. To complete sequencing of exome we used the Nextera® Exome Enrichment Kit that targets 62Mb, using IlluminaHiScan platform for sequencing, and all the bioinformatics analysis were performed using the CLC Genomics Workbench. To maximize the efficiency and uniformity of sequencing and capture we adopted a barcoding and pooling strategy.

Results
For the 8 trios, more than 126 billions of basepair were mapped, reaching an average coverage of 38x. On a first analysis, we found more than 36k candidate SNVs. From these number, were selected only the regions with deep coverage over 40x in each trio, resulting in 8.8K SNVs. Using the 1000 genomes project (http://www.1000genomes.org/) and the 6,500 Exome Project (https://esp.gs.washington.edu/drupal/) databases, the SNVs were categorized into 3,356 Common (MAF >=5%), 2,398 Rare (MAF<5%) and 1,689 de novo. Using ANOVAR program, that gathers information from databases AVSIFT, Polyphen2, PhyloP, MutationsTaster, LRT and
SIFT, it was predicted that 125 SNVs could have a functional impact among the rare, 22 among common SNVs and 54 among de novo SNVs. After a more stringent analysis, the de novo SNVs number were restricted to 26 that could have functional impact. Pathway analysis with KEGG Pathway database (http://www.genome.jp/kegg/pathway.html) showed that those de novo SNVs may be disrupting the insulin signaling pathway.

Discussion
Those preliminary results indicates that sequencing can lead to the discovery various new variants and possible affected pathways in ADHD. For a better understanding, the sequencing of more trios and a more detailed analysis of the variants found, genes and pathways disrupted by them are currently being performed by our group.

4

EVALUATION OF CLINICAL PHENOTYPES ASSOCIATED WITH CYTOGENETIC EFFECTS AND METHYLENETETRAHYDROFOLATE REDUCTASE GENE POLYMORPHISMS IN CHILDREN WITH ATTENTION DEFICIT HYPERACTIVITY DISORDER IN COIMBATORE REGION, SOUTH INDIA.

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Background
Attention-deficit/hyperactivity disorder (ADHD) is one of the most psychiatric conditions in childhood. This disorder is defined by a combination of symptoms of inattention and hyperactivity/impulsivity. Basically a chronic psychiatric disorder seen more in boys than girls. Diagnosed in about 1 to 16% of school aged children. However, basic information about how the prevalence of ADHD varies by race/ethnicity, sex, age, and socio-economic status remains poorly described. An Indian study found 40.0% of children with ADHD to have comorbid disorder. This frontal lobe dysfunction primarily reflects the core features of ADHD, which is believed to be responsible for the ability to control and focus thinking. Hence the focal aim of the present study, To investigate the clinical phenotypic and behavioural studies on ADHD patients, Cytogenetic and Molecular analysis (Chromosomal analysis and Methylene tetrahydrofolate Reductase (MTHFR) gene polymorphism) ADHD patients by using Giemsa-Trypsin banding and polymerase chain reactions and restriction fragment length polymorphisms (PCR-RFLP) method. To understand whether the biomarkers play a vital role in clinical outcome for ADHD.

Methods
In order to investigate the possible cytogenetic damage in ADHD patients, peripheral blood lymphocyte culture (PBLC) method was carried out on the lymphocytes of 18 ADHD patients and equal numbers of controls were recruited, based on the currently applied DSM-IV diagnostic criteria was originally created for and validated among children and adolescents. In the present study volunteers provided blood samples (5 ml) to establish cell cultures at 72 h. For karyotyping, 50 complete metaphase cells from each subject were assessed using Giemsa-
Trypsin Banding method. Two Mutations in the MTHFR gene (A1298C and C677T) were investigated using PCR-RFLP.

**Results**

The present study inspected the percentage of chromosomal aberrations (CA) found in ADHD individuals and statistically analyzed the overall increased CA such as deletion, duplication, translocations, chromosomal breaks, Gaps, di-centric chromosomes and ring chromosomes. There is significant differences were found in the cytogenetic variables in the frequency of CA in ADHD patients and controls. Among the 18 ADHD patients one child had symptoms suggestive of mild autism with ring chromosome 14. The genotypic pattern of the distributions of the A1298C and C677T alleles was different between the ADHD patients and the controls (p=0.001).

**Discussion**

From the pilot study of investigations, can be concluded that population-based epidemiologic studies may lean-to important new beam on how we comprehend ADHD, its natural history, its treatment, and its consequences. Clearly, this study opens the gate for further well-built studies.

**Keywords:** Chromosomal aberrations, Gene polymorphism, ADHD.

5

ARE CNVS ASSOCIATED WITH ADHD AND NEUROPSYCHOLOGICAL MEASURES IN PRESCHOOL CHILDREN?

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**Background**

Recent studies of children and adolescents with neurodevelopmental disorders including ADHD have indicated increased rates of copy number variations (CNVs). Findings are inconsistent with regard to the specific genes involved and the types of aberrations (deletions, duplications). There are also limited knowledge with regard to the association between CNVs and neuropsychological phenotypes, including cognition, measured dimensionally across diagnostic categories. The aim of this project was to study the association between 28 candidate CNVs, selected on the basis of previous association with psychiatric disorders, and preschool ADHD and dimensionally measured neuropsychological phenotypes including IQ and executive functions in a sample from a prospective pregnancy cohort.

**Methods**

The study sample consisted of 554 children, aged 3-4 years, mean age 3.5 years, from the collaborative study “Preschool ADHD: Early characteristics, developmental trajectories, risk, and protective factors in a prospective birth cohort” (The Norwegian Longitudinal ADHD study), conducted by the Norwegian Institute of Public Health in collaboration with Oslo University Hospital. The study includes a comprehensive diagnostic and neuropsychological assessment at age 3 ½ years. DNA from cord blood collected at birth as part of the Norwegian Mother and Child Cohort Study (MoBa) were used for genetic assessment using a Multiplex Amplicon Quantification (MAQ) based CNV analysis. Twenty eight target amplicons 161 kb to
5.9 Mb long from 13 different chromosomes, in CNV regions previously associated with psychiatric disorders were included in the assay. From the clinical assessment in the ADHD study, diagnostic information from the Preschool Age Psychiatric Assessment (PAPA) interview were used in addition to data from the neuropsychological tests including verbal and non-verbal working memory and IQ from Stanford Binet as well as measures of language development.

**Results**

Of the 28 CNV regions included in the MAQ assay, no deletion or duplication was found in 16 of the regions. For the remaining 12 CNV regions, the frequency of the individual CNVs ranged from 0.2% to 2%. The frequency of CNVs in children with any diagnoses from the PAPA interview was higher than in controls, 6.4% vs 3.8%, but the difference did not reach significance. Limiting the sample to the ADHD diagnostic group, no significant difference in frequency of CNVs was found (4.1% vs. 3.8% respectively). The mean IQ did not differ between children with or without a CNV, neither did mean verbal- or non-verbal working memory. Further results from the analyses of continuous neuropsychological measures will be presented at the conference.

**Discussion**

The current preliminary findings did not reveal any significant association between the CNV regions and neurodevelopmental disorders in preschool children. However, there was a trend level increase in CNV load in the group as a whole, but not in the ADHD diagnostic category. The sample is still too small to reach conclusive results. Further analyses of continuous neuropsychological measures associated with the CNVs are currently being conducted and will be presented at the meeting.

6

THE CONTRIBUTION OF POLYGENIC RISK FOR ATTENTION DEFICIT HYPERACTIVITY DISORDER TO NEURODEVELOPMENTAL TRAITS IN THE GENERAL POPULATION

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**Background**

Attention deficit hyperactivity disorder (ADHD) can be viewed as the extreme end of a continuous distribution of traits in the general population. Epidemiological and twin studies also suggest that ADHD frequently co-occurs with and shares genetic susceptibility with autism spectrum disorder (ASD). The aims of this study were to determine whether a composite of common molecular genetic risk variants (i.e. “polygenic risk”), previously found to be associated with clinically-diagnosed ADHD, predicts ADHD and ASD-like trait variation in the general population.

**Methods**

Polygenic risk scores were calculated in the Avon Longitudinal Study of Parents and Children (ALSPAC) population sample (N=8,229), based on a discovery case-control genome-wide association study (GWAS) of childhood ADHD. Regressions were used to assess whether polygenic scores predicted ADHD and ASD-related traits (pragmatic language abilities and
social cognition) in ALSPAC. The overlap of ADHD and ASD-related traits was also confirmed.

**Results**

In terms of phenotype, ADHD diagnosis in the sample was associated with more social cognition difficulties (OR=1.36, CI=1.31-1.40) and lower pragmatic language abilities (OR=0.06, CI=0.04-0.10) and ASD diagnosis was associated with more ADHD traits (hyperactive-impulsive: OR=1.39, CI=1.31-1.47; inattentive: OR=1.45, CI=1.35-1.54). As predicted, ADHD polygenic risk showed a positive association with hyperactive-impulsive (p=0.0039) and inattentive (p=0.037) traits. ADHD polygenic risk was negatively associated with pragmatic language abilities (p=0.037), but not with social cognition (p=0.43).

**Discussion**

These results provide evidence for the dimensional nature of ADHD problems that extends to a biological (i.e. genetic) level. They further suggest that ADHD-associated genetic variants may also influence pragmatic language deficits, which characterise social-communication problems relevant to ASD. These findings imply that disorder-associated genetic risks may influence overlapping traits characterised by features beyond the core phenotype.

**POLYGENIC PREDICTION OF ADHD DIAGNOSIS, ADHD SYMPTOMS AND COGNITIVE IMPAIRMENTS**

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**Background**

ADHD is highly heritable and it is assumed that much of the variation in affection status and symptom severity can be explained by the additive influence of common genetic variants. ADHD is associated with cognitive impairments, including high reaction time variability (RTV) and poor inhibition (high commission errors, CE), both of which share familial risk factors with ADHD, but etiologically separate from one another (Kuntsi et al, 2010, Archives of General Psychiatry). Despite the evidence for shared etiology, the molecular basis for associations between ADHD as a clinical disorder, ADHD symptoms as a continuous trait and cognitive performance remains poorly understood. We examine the extent to which a polygenic score for ADHD diagnosis, comprising many thousands of SNPs, shows association with ADHD symptoms and cognitive performance in ADHD probands and individuals from the general population.

**Methods**

Analyses were conducted in four datasets. A polygenic score for ADHD was initially established using international case/control data from the ADHD working group of the Psychiatric GWAS
Consortium (Neale et al, 2010, *Journal of the American Academy of Child and Adolescent Psychiatry*). The score was trained using data from three international cohorts (N=4506) and was tested for association with ADHD affection status in probands from the International Multi-Centre ADHD Gene project (IMAGE; N=909). The score most strongly associated with ADHD was then tested for association with RTV and CE in a subset of the IMAGE probands (IMAGE 8-team; N=279). The polygenic score was regenerated using all available PGC data (N=5415) and tested for association with symptoms of hyperactivity-impulsivity and inattention in a general population sample (the Twins Early Development Study, TEDS; N=2695). The score most strongly associated with ADHD symptoms was then tested for association with RTV and CE in a subset of individuals from the Study of Activity and Impulsivity Levels in children (SAIL; N=320). Polygenic scores were generated by conducting genome-wide analysis in the training sample, weighting the log of odds ratios by the number of risk alleles carried for all SNPs at varying thresholds of significance.

**Results**
The polygenic score most strongly associated with ADHD in IMAGE was for all SNPs at the threshold \( p < 0.1 \). However, this score only explained 0.6% of the variance in ADHD. In the IMAGE 8-team data, this score was significantly associated with RTV and explained 1.5% of its variance. There was no significant association with CE. The polygenic score most strongly associated with ADHD symptoms in TEDS was for all SNPs at the threshold \( p < 0.2 \). This score was significantly associated with hyperactivity-impulsivity and explained 0.2% of the variance in these symptoms. There was no significant association with symptoms of inattention, or with RTV or CE in SAIL.

**Discussion**
Results indicate that there is a polygenic basis for ADHD as a clinical disorder and as a continuous trait. Furthermore, there is also a polygenic basis for the association between ADHD and RTV. Owing to the small effect sizes these results should be seen as preliminary. Further work is currently underway to optimise the polygenic signal for ADHD and to test if for association with cognitive performance in a larger dataset.

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**DEEP SEQUENCING OF VMAT1 (SLC18A1) IDENTIFIES NOVEL RARE FUNCTIONAL VARIANTS IN BIPOLAR DISORDERS**
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**Background**
The gene encoding the vesicular monoamine transporter 1 (VMAT1) has recently emerged as a candidate gene for bipolar disorder (BPD), schizophrenia and emotional behavior. We have shown that the common amino acid substitution polymorphism Thr136Ile leads to increased monoamine transport *in vitro* and affects negative emotion processing *in vivo*. In this study we conducted deep sequencing of patients with BPD in order to detect rare VMAT1 variants and to determine their function *in vitro*.

**Methods**
Sanger sequencing of all VMAT1 exons was carried out in 196 BPD individuals and 196 Caucasian controls. Novel rare variants that are likely to change protein function were tested for functional relevance using monoamine reuptake assays in CV-1 cells. Missense SNPs that were functional in vitro were then genotyped in a large cohort of BPD (n=4023) and normal controls (n=3305) of European descent from the NIMH Genetic Initiative using standard ABI TaqMan genotyping protocols.

Results
Sequencing of BPD patients identified several novel and rare variants. Comparison of sequencing results of rare variants in BPD individuals with normal controls from the 1000 Genome project shows that the global burden of rare variants was increased in the BPD group. Interestingly, several novel variants were only detected in the BPD group but were absent in the controls. Monoamine uptake in vitro was carried out for Gln10Arg, Phe84Ser, Ala101Pro, Arg138Leu and Leu392Val. Phe84Ser robustly increased monoamine uptake in particular for DA (p<0.001) and the three variants, Ala101Pro, Arg138Leu and Leu392Val decreased uptake, with Arg138Leu showing the largest effect for DA (P<0.001), although similar results were also obtained for 5-HT and NE. Because of the robust functional effects of Phe84Ser and Arg138Leu, we genotyped these rare variants in a large sample of BPD cases and controls. The Ser84 allele was absent in controls but present in seven BPD individuals, including one homozygote and six heterozygotes (Fisher exact test, P=0.009). The Leu138 frequency did not differ statistically between cases and controls. Haplotype analysis of the individuals with the rare variant Phe84Ser showed that all subjects had almost exclusively the same haplotype Thr–Ser–Thr, indicative of a common origin and founder population effect.

Discussion
Sequencing detected several rare and novel missense variants in BPD patients. In vitro results show that rare variants lead to “hyper or hypo” transport of monoamines. Association analyses of the rare variants Phe84Ser and Arg138Leu show that the Ser84 allele was only present in BPD but not controls. Given that the common Thr136Ile was previously shown to increased monoamine transport and has an effect on interindividual responses to medial PFC activation of negative words and threat-related amygdala reactivity, the rare Phe84Ser variant may have similar effect on these brain circuits and may contribute to the pathophysiology of BPD. Future studies are needed to comprehensively investigate common and rare SNP-dosage effects on transporter function in vivo and risk for BPD.

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ABSTRACT WITHDRAWN

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EXTREME LOW BIRTH WEIGHT IN MZ TWINS DISCORDANT FOR BIRTH WEIGHT IS ASSOCIATED WITH SHORTER TELOMERE LENGTH AND LOWER IQ, BUT NOT MENTAL WELL-BEING IN LATER LIFE
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Background
A mechanism through which environmental factors may affect the genome is reduction in telomere length (TL). Several studies have reported associations between shorter TL and both psychological stress and stress-related disorders such as major depression. An association between shorter TL and lower cognitive ability has also been reported. One twin study found better memory performance in the twins with longer TL than in their co-twins with shorter TL. However, it is unknown whether shared genetic or environmental effects account for this association and whether possible environmental effects occur pre-, peri- or postnatal. Low cognitive ability in childhood has been found to have a negative influence on the development of psychiatric disorders later in life.

Methods
We investigated the association between TL, birth weight (reported by their mother), general cognitive ability (IQ at age 16), and mental well-being (SPHERE – somatic and psychological health report questionnaire score) in a large twin and sibling sample. TL and birth weight data was available for 1424 individuals (229 MZ and 427 DZ twin pairs, 14 siblings), TL and IQ for 1391 individuals (185 MZ and 375 DZ twin pairs, 176 siblings), and TL and mental well-being for 1208 individuals (135 MZ and 301 DZ twin pairs, 253 siblings). Individuals were measured in duplicate for both the telomere (T) and 36B4 (S) assays and the T/S (TL) ratio data was obtained. The SPHERE score was based on repeated SPHERE measurements between the age of 10 and 28 years and developed according to the principles of the Item Response Theory. All analyses were corrected for the influences of sex and age at blood withdrawal.

Results
TL showed a trend for an association with IQ (p=0.052), but not with birth weight (p=0.172) or the SPHERE score (p=0.593). We also found a significant correlation between the intra twin pair difference in TL and the intra twin pair difference in IQ in the MZ (r=0.232, p=0.001), but not in the DZ twin pairs (r=−0.067, p=0.198). We hypothesized that pre- and/or perinatal environmental effects in MZ twins may account for this finding. One possible mechanism is the Twin-to-twin Transfusion Syndrome (TTTS): when MZ co-twins share placental vasculature this can result in an unequal blood supply to one of the twins, and a much lower birth weight, higher morbidity and mortality of the undersupplied MZ twin. In fact, in the MZ twins the intra pair difference in birth weight was significantly correlated with the intra pair difference in both TL (r=0.141, p=0.033) and IQ (r=0.155, p=0.035). But these associations were not found for DZ pairs (TL; r=0.047, p=0.336; IQ; r=0.013, p=0.804). In an explorative analysis, we split the MZ twin sample into two groups. We compared the twins with the highest difference in birth weight (upper 10 percent; N=24; difference in birth weight ranging between 623 g and 1200 g) with those with a low or medium difference in birth weight (lower 90 percent). We observed a high correlation between the intra twin pair difference in TL and birth weight in MZ twin pairs with the highest difference in birth weight (r=0.379, p=0.068), but no correlation in MZ twin pairs with a low or medium difference in birth weight. The correlation between the intra twin pair
difference in IQ and in TL was significant trend in the high \((r=0.407, p=0.075)\) and significant in the low to medium \((r=0.176, p=0.027)\) birth weight difference groups. The intra twin pair difference in the SPHERE score was not correlated with the intra twin pair difference in TL, either in the MZ \((r=0.048, p=0.583)\) or DZ twin pairs \((r=-0.067, p=0.243)\); however, it was negatively correlated with intra twin pair difference in birth weight in MZ twin pairs \((r=-0.196, p=0.022)\), but not in DZ twin pairs \((r=0.014, p=0.807)\).

**Discussion**

Our findings suggest that stressful environmental effects that occur pre- and/or perinatally and decrease birth weight, may have a negative influence on TL. Our findings further indicate that the association between TL and IQ may not be driven by genetic or postnatal environmental effects, but by the same stressful environmental pre- and/or perinatal effects that decrease birth weight. Finally, we consider the finding that twin pairs who are discordant for TL and BW but are not discordant for mental well-being as a positive finding. Stressful environmental pre- and/or perinatal effects such as decreased blood, oxygen and nutrition supply – which may negatively affect TL – do not appear to affect mental well-being later in life.

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**INCREASED GENETIC LOADING OF DISEASE-ASSOCIATED SNPS IN ADOLESCENT OFFSPRING AND SIBLINGS OF INDIVIDUALS WITH BIPOLAR DISORDER: A 5-SITE STUDY**

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**Background**

A positive family history in a close relative is the strongest predictor of future risk of bipolar disorder. Offspring of individuals with bipolar disorder are at 8- to 10-fold increased risk of developing bipolar disorder themselves, and are at 3-fold increased lifetime risk for major affective disorders. Longitudinal studies that ascertain young at-risk individuals and monitor them prospectively are the most effective approach for identifying early clinical and biological markers of future illness, and may facilitate the development of tools to predict which of these genetically at-risk young people will eventually develop disorder.

**Methods**

We have used identical clinical assessment protocols across 5 independent sites in the USA and Australia, to collect asymptomatic at-risk subjects (aged between 12-21 years for US samples, and 12-30 years for Australian samples). The at-risk subjects \((n=370)\) are mostly offspring of patients with bipolar I disorder, and controls \((n=229)\) are in the same age range but with no
family history of mood disorder or psychosis. Both groups have been followed longitudinally and emergence of psychiatric diagnoses assessed. Currently, 29 at-risk subjects have developed bipolar disorder (with diagnoses of BPI, BPII or SZMA). We genotyped the top 33 SNPs implicated by the PGC-BP GWAS as being associated with bipolar disorder, to assess genetic risk in at-risk participants compared to controls.

**Results**

Using polygenic risk scores derived from the 33 significantly associated SNPs, we show an increased polygenic load in at-risk individuals compared to controls. Under a dominant model, 34.5% of controls had a score of ≤20 compared to 27.6% of at-risk subjects, and 32.8% of controls had a score of ≥23 compared to 41.6% of at-risk (mean values: 21.50±2.31 vs 21.91±2.36; GEE Wald $\chi^2$=4.10, one tailed p=0.022). Under an additive model which incorporated a weighting for the frequency of the risk allele, 26.2% of controls had a score of <0.28 compared to 19.7% of at-risk, and 27.9% of controls had a score of ≥0.32 compared to 32.2% of at-risk (mean values: 0.30±0.031 vs 0.31±0.031; GEE Wald $\chi^2$=3.15, one tailed p=0.038). While the average risk scores were greater in the at-risk group than controls, an individuals’ score would not clearly identify that subject as a ‘possible converter’. The subjects who have converted to bipolar disorder (BPI, SZMA or BPII) (n=29) did not differ in risk score compared to the rest of the at-risk subjects (n=338) under any genetic model (all GEE p≥0.788).

**Discussion**

These findings suggest that increased polygenic risk load might be a useful means of identifying individuals who are at high genetic risk of bipolar disorder, although a larger number of risk variants, incorporating both common and rare variants, may prove more predictive of future illness.

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**ASSOCIATION AND FUNCTIONAL ASSAY OF POLYMORPHISMS IN NEUROCAN WITH BIPOLAR DISORDER**

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**Background**

Bipolar disorder (BPD) is a severe and complex psychiatric disorder, with high heritability around 0.6 to 0.7. A recent GWA (genome-wide association) study in Caucasian population identified a common genetic variant (rs1064395, locates in the 3’ UTR region) in Neurocan (NCAN) gene for BPD with a p-value of 2.14X10⁻⁹ [Cichon et al. 2011]. A possible explanation for the putative role of non-coding rs1064395 on BPD may be through epigenetic regulation. MicroRNA (miRNA), a post–transcriptional regulator, can modify the gene expression by base-pairing on specific target region, in particular the 3’ UTR region. In the present study, we first examined the association between rs1064395 and BPD in Taiwanese population. Secondly, we explored the potential miRNAs binding through in silico prediction. We then validated whether the candidate miRNA affects NCAN gene expression between the two alleles of rs1064395.

**Methods**
We conducted a case-control association study for rs1064395 in Taiwanese samples. Clinical patients with DSM diagnosis of BPD subtype I (N=238) and subtype II (N=80) as well as 235 healthy controls were recruited. We adopted TaqMan®SNP genotyping assay for rs1064395. miRNAs predictions were performed using PITA, miRanda, and miRWalk to evaluate binding energy and ability between the two alleles of rs1064395. A reporter constructs vector that includes the NCAN 3' UTR was used to confirm the real binding effect of a candidate miRNA in vitro.

Results
We found that rs1064395 polymorphism was significantly associated with BPD in Taiwanese, showing odds ratio (95% confidence interval) of 0.63 (0.43-0.94) with a p-value of 0.024. In searching for candidate miRNAs, we combined results from all prediction tools and created a scoring system to obtain a list of candidate miRNAs for rs1064395. Accompanying with literature review, we found a potential target to be related to BPD, miR-140-3p, which is reported to express in the prefrontal cortex. Experimental validation is underway for the miR-140-3p to evaluate the influence of two alleles of rs1064395 on gene expression.

Discussion
Genetic variant in NCAN is associated with bipolar illness in Taiwanese population. Epigenetic regulation of miR-140-3p may play a role in the etiology of BPD through influencing on the level of NCAN gene expression.

DE NOVO CNVS IN BIPOLAR AFFECTIVE DISORDER AND SCHIZOPHRENIA
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Background
An increased rate of de novo copy number variants (CNVs) has been found in schizophrenia, autism and developmental delay, but fewer studies have reported this rate in bipolar affective disorder (BD). In this study we wanted to identify de novo CNVs in BD and compare it with schizophrenia (SCZ).

Methods
We used Illumina OmniExpress microarrays to genotype 369 BD patients (256 from Bulgaria, 116 from the UK) and 78 SCZ/SA probands (61 from the UK and 17 from Bulgaria), with all their parents genotyped as well, and who passed strict QC criteria. CNVs were called by PennCNV. We excluded CNVs <10kb, covered by <10 probes, overlapping segmental duplications and with a frequency >1%. Putative de novo CNVs called by PennCNV were validated by a Z-Score calling algorithm, manual inspection of the logRratios of the trios, and subsequently with qPCR probes.

Results
We found 15 de novo CNVs in BD patients (rate of 4.1%) and 6 de novo CNVs in SCZ patients (rate of 7.7%). The rate in BD is higher than the reported ~1-2% rate in controls, but smaller than in schizophrenia and autism (as reported previously). The median size of de novo CNVs in BD was 189kb and in SCZ was 640kb, which is in accordance with what we reported in a previous study on schizophrenia (Kirov et al, 2011). One de novo deletion intersected an exon of DLG2, and one large duplication intersected 27 genes at 16p11.2, both regions having been implicated in de novo CNV studies of schizophrenia and BD. Two other de novo duplications are very large, at >3Mb, and have not been implicated before. Three of the de novo CNVs in BD did not intersect genes.

Discussion
De novo CNVs in BD are found at increased rates compared to controls, but at lower rates than in schizophrenia. Overall they tend to be smaller in BD than the ones reported in schizophrenia, and a smaller proportion of them are found at loci that have been shown to be pathogenic for neurodevelopmental disorders.
recurrent depression), BMI and genotype data for rs9939609 FTO polymorphism. The distribution of BMI was positively skewed in all studies. We therefore transformed the data to Log_{10}(BMI) to achieve a closer approximation to normal distribution. In each individual study, linear regression models for quantitative traits assuming an additive genetic model were performed to test for the interaction between rs9939609 polymorphism and depression for an effect on Log_{10}BMI. Age, sex and principal components were controlled for including them as covariates in the model. All cases and controls were of white European ancestry. A classical approach meta-analysis with effect size estimates and standard errors was performed using the statistical package METAL (http://www.sph.umich.edu/csg/abecasis/metal/).

**Results**

The results from the classical approach fixed-effects meta-analysis supported a significant interaction between FTO rs9939609 genotype and depression in relationship to Log_{10}BMI (β=0.092, SE=0.033, p=0.0058). There was significant heterogeneity among studies. The sources of heterogeneity are being investigated. The meta-analysis interaction results show that in cases with depression there is an increased of 0.092 units of BMI for each FTO rs9939609 risk allele.

**Discussion**

Although several studies have investigated the influence of FTO variants on BMI, and the association between obesity and depression separately, this is to date the first meta-analysis investigating the relationship between FTO, BMI and depression. The results confirm that a history of depression increases the effect of FTO gene on BMI. This finding suggests that FTO gene is involved in the mechanism underlying the association between obesity and depression. This finding could have implications for predicting which patients with depression are at risk of high-BMI related disorders and potentially highlights how to improve prevention, management and treatment programs.

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**SEX-SPECIFIC GWAS ANALYSIS OF SUICIDE ATTEMPT IN MAJOR DEPRESSION**

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**Background**

Suicidal behavior is a complex disorder with many contributing biological and environmental risk factors. To understand these factors on the genetic level many technologies have been employed. Genome-wide linkage studies have implicated loci on 2p11-12 and 6q25-26, gene expression analyses have identified dysregulation of genes such as SSAT, and Genome-wide Association Studies (GWAS) have identified common single nucleotide polymorphisms (SNPs) implicating the 2p25 region. Our aim is to identify common SNPs related to this phenotype in major depression which may be causing dysregulation of integral genes and pathways.

**Methods**

Using the Genetics of Recurrent Early onset Depression (GenRED) dataset and SNPs imputed from the 1000 Genomes Project, we conducted a GWAS on subjects with major depression.
containing 466 individuals with a history of suicide attempts and 1378 individuals who did not have a history of suicide attempts. Our primary analysis involved using a logistic regression-based analytic approach in PLINK to determine an association between the genotype and attempted suicide phenotype. Our secondary analysis looked for a sex-specific association using the same approach. Covariates including principal components, sex, age and study were integrated into our analysis and controlled for.

Results
In our main analysis we found eight SNPs that showed suggestive significance (P-6). The most associated SNPs are located within introns of the calcitonin receptor (CALCR) gene which is involved in calcium homeostasis. In our secondary analysis, our sex-specific approach identified one male-specific SNP on the verge of genome-wide significance (rs114536112, P=8.12 x 10^{-08}) found inside the gene Otoferlin (OTOS), which has been implicated in normal hearing. However, the sample size of the male subset may be too small to make confident assertions. Our female-specific analysis identified one SNP on the verge of genome-wide significance (rs151021389, P=8.68 x 10^{-08}) in a 16q23.1 intergenic region. The closest RefSeq gene is CNTNAP4, a member of the Neurexin superfamily involved in cell adhesion and also implicated in schizophrenia, autism and autism-related endophenotypes. In addition, four CALCR SNPs previously found in the main analysis were identified with suggestive significance (P-6). The sixth top SNP was found inside an intron of the CNTNAP2 gene again implicating the Neurexin superfamily. The final suggestive SNP was found in the intron of the MTBP gene which is thought to regulate cell cycle.

Discussion
We have used a sex-specific approach to identify SNPs associated with suicidal behavior in either males or females. Of note, three SNPs were significantly stronger in the female-specific analysis compared to the main analysis. Two of these SNPs were found in or near CNTNAP2 and CNTNAP4, members of the Neurexin superfamily. Neurexin 3 (NRXN3) and a Neurexin ligand LRRTM4, mediators of synaptogenesis, have previously been implicated in suicidal behavior in female bipolar disorder subjects. Validation of these SNPs is required to confirm their role in female subjects who attempt suicide.

NO EVIDENCE FOR ASSOCIATION BETWEEN BIPOLAR DISORDER RISK GENE VARIANTS AND BRAIN STRUCTURAL PHENOTYPES
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**Background**
Recent genome-wide association studies have identified several new bipolar disorder (BD) risk variants, and structural imaging studies have reported enlarged ventricles and volumetric reductions among the most consistent findings. In the current study, we investigated whether these genetic risk variants could explain some of the structural brain alterations in BD.

**Methods**
We tested the potential association between 9 SNPs in the genes *CACNA1C, ANK3, ODZ4* and *SYNE1* and 8 brain structural measures found to be altered in BD, in a sample of 517 individuals (N = 121 BD cases, 116 SZ cases, 61 other psychosis cases and 219 healthy controls). The polygenic effect across all these SNPs on the brain phenotypes was also assessed.

**Results**
Our most significant result was an association between the risk allele in *CACNA1C* SNP rs4775913 and decreased cerebellar volume (nominal P = 0.0075) in the total sample, which did not remain significant after multiple testing correction. There was no evidence for diagnostic specificity for this association in the BD group. Further, no polygenic effect was observed.

**Discussion**
The present findings indicate that these risk SNPs do not explain a large proportion of the structural brain abnormalities in BD. Thus, these genes which are all related to neuronal functions must be involved in other pathophysiological aspects of BD development.

**SEARCH FOR DE NOVO POINT MUTATIONS IN BIPOLAR DISORDER BY EXOME SEQUENCING IN TRIO FAMILIES**
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**Background**
Heritability of bipolar disorder calculated from the concordance rates in monozygotic and dizygotic twins is around 85% (Cardno et al, *Arch Gen Psychiatry* 1999, McGuffin et al, *Arch Gen Psychiatry* 2003). Although no specific mutation causative for bipolar disorder has been identified from initial linkage studies, recent genome-wide association studies (GWAS) successfully identified several common SNPs that are associated with bipolar disorder with genome-wide significance. However, the associated SNPs have only weak effects with odds ratios less than 1.2. Thus, currently there is no reasonable explanation for the high heritability of the disease, and this phenomenon is named as "missing heritability". Now, more attention is being paid to rare variants with larger effects. In 2012, several studies indicated that *de novo* point mutations are strongly associated with autism. Because *de novo* mutations are usually shared by monozygotic twins, heritability estimation can be inflated by the presence of *de novo* mutations. However, it is still not known whether *de novo* point mutations play a role also in bipolar disorder.

The purpose of the present study is to search for *de novo* point mutations related to bipolar disorder by whole exome analysis of trio families.
Methods
Participants volunteered to this study through the recruitment in participating hospitals or by advertisement through Bipolar Disorder Research Network Japan mail magazines, website or other resources. Probands had been clinically diagnosed as bipolar disorder and mostly medicated. Trained psychiatrists interviewed the probands and their families in person or on the phone using MINI (Mini International Neuropsychiatric Interview). Probands diagnosed as having bipolar I or bipolar II disorder were enrolled in the study. If the proband has affected siblings, they were also enrolled. We have completed interviews of 50 trios including 5 quartets so far. Probands were aged 35.7±9.1 (16-55) years old, 25 females and 25 males, 40 with bipolar I and 10 with bipolar II. In 28 families, both parents did not have any psychiatric diagnosis by MINI (“sporadic”), whereas either of parents or a sibling had mood or psychotic disorders in other 22 families (“familial”). DNA of the participants was extracted from peripheral blood or saliva. Target regions were captured by SureSelect XT All Exon ver.4 or ver.5 (Agilent Technology) and sequenced by HiSeq2000 (Illumina).

Results
We are currently sequencing 50 trios including 5 quartets. In the preliminary analysis of 10 trios/quartets, more than 99% of targeted regions were covered by 1 read and at least 83.6% were covered by 20 or more reads. Among non-synonymous single nucleotide variations (SNVs), those included in dbSNP137 database were excluded, except that the frequency was less than 1% or they were reported as disease related ones. When the SNV showed 8 or more reads in the proband and 90% or more reads in both parents showed reference allele, it was considered as the de novo mutation. The de novo mutations were also verified by visual curating. Among the 3 sporadic families subject to the preliminary analysis, de novo mutations were found only in one family. In the proband of this family, two de novo point mutations were found.

Discussion
Validation by Sanger sequencing is ongoing. If confirmed, the present results suggest that de novo point mutations might exist also in sporadic bipolar disorder patients. Data of other families will also be presented at the meeting.

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DNA METHYLATION PROFILES OF THE BRAIN-DERIVED NEUROTROPHIC FACTOR GENE AS A POTENT DIAGNOSTIC BIOMARKER IN MAJOR DEPRESSION
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Background
Since major depression has a high lifetime prevalence and is associated with a massive economic burden, the discovery of diagnostic biomarkers for major depression is an extremely important goal. The identification of accurate biomarkers for major depression could be helpful for improving patient care and useful for the development of more effective antidepressants. Based on
the results from numerous studies on the CNS mechanisms of stress and antidepressants, the brain-derived neurotrophic factor (BDNF) gene has emerged as playing an important role in the pathophysiology of major depression. Numerous clinical studies have measured blood levels of BDNF as a potential biomarker of depression, but the BDNF levels of patients with depression and healthy subjects were found to overlap.

**Methods**

As a result of recent advances in epigenetics, DNA methylation status has been shown to be tightly involved in the transcription of genes in both plants and mammals. Methylation status is reversible and is dependent on the balance between DNA methyltransferase and demethylase activity. We analyzed the DNA methylation profiles of CpG islands at the promoter of exon I and exon IV of the BDNF gene (methylation of cytosine within both regions is suggested to be important for the regulation of gene transcription) in healthy subjects (n = 18) and inpatients with major depression (n = 38) and schizophrenia (n = 40). There was no significant difference in age among these 3 groups. We used genomic DNA from peripheral blood. Methylation rates at each CpG unit was measured using MassARRAY® system (SEQUENOM), and 2-dimensional hierarchical cluster analyses were undertaken to determine the validity of these methylation profiles as a diagnostic biomarker. This study was approved by the Ethics Committee of the Hiroshima University School of Medicine. All subjects received a description of this study and gave written informed consent.

**Results**

Based on methylation profiles at the promoter of exon I, we were able to accurately distinguish subjects from all three groups in complete accordance with the clinical diagnosis based on the DSM-IV-TR criteria. At the first branch of the dendrogram, we could distinguish between healthy subjects and patients (combination of depression and schizophrenia groups) in complete concordance with classification based on clinical diagnosis. In addition, at the next branch, we could also completely distinguish between depression and schizophrenia. On the other hand, we were unable to distinguish subjects into 3 groups at any height in the dendrogram when we applied 2-way hierarchical clustering analysis of methylation rates of CpG units at the promoter of exon IV.

**Discussion**

Although only a small cohort was assessed, our study is the first to identify a reliable diagnostic biomarker for major depression. We believe the possibility that replication studies examining the DNA methylation profiles of the BDNF gene provide us with a new and potent diagnostic biomarker of major depression. If our results can be replicated, this new diagnostic approach using DNA methylation profiles could be a groundbreaking discovery in psychiatry.

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**ARE TYPE 2 DIABETES AND RELATED TRAITS CAUSALLY ASSOCIATED WITH MAJOR DEPRESSIVE DISORDER?**

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Background
The existence of an association between type 2 diabetes (T2D) and major depressive disorder (MDD) is well established. Meta-analytic results indicate that the prevalence of depression is increased up to 60% among individuals with T2D compared to controls. Despite the fact that this association has been known for decades, the nature and direction of causality remains unclear, mainly due to the cross-sectional/observational nature of most previous studies. A promising method to assess potential causality between exposures and disease outcomes is “Mendelian randomization” (MR), which uses genetic data. MR is based on Mendel’s law of random assortment, which states that the inheritance of a trait is independent of inheritance of other traits. Consequently, the random assortment of genes allows an un-confounded estimate of the association between exposure and disease outcomes, which is unaffected by concerns of reverse causality. In the present study we used MR to investigate whether there is a causal relationship going from T2D and related traits (fasting glucose and BMI) to depression.

Methods
Genetic risk scores for T2D, fasting glucose (FG), and Body Mass Index (BMI) included 47, 31 and 32 variants respectively, which had reached genome-wide levels of association with their respective traits. The association of these risk scores with MDD was investigated based on data from the MDD GWAS Mega-analysis (9240 MDD cases and 9519 controls from the Psychiatric GWAS Consortium [1]) using an indirect MR approach. Summary statistics for the association between individual SNPs and MDD were synthesised into composite risk score estimates weighted by the magnitude of the association of the SNPs with T2D, FG and BMI. The results are reported as the odds-ratio for MDD per magnitude of genetically predicted increase in the exposure (T2D, FG and BMI). For T2D vs. MDD, the association is reported per 1 unit increase in the log-odds of T2D (consistent with the increase in odds between normal weight and obese individuals), whereas the FG vs. MDD and BMI vs. MDD scores were reported per increase of 1 mmol glucose/L and 1 kg/m², respectively.

Results
A genetically predicted increase of 1 unit in the log-odds of T2D was not associated with an increase in MDD risk (OR= 1.03 (0.96-1.11); p = 0.42). Likewise, a genetically predicted increase of 1 mmol/L in FG was not associated with increased risk of MDD (OR= 0.96 (0.67-1.36); p = 0.82. However, per genetically predicted increase of 1 kg/m² (1 BMI unit) we observed a tendency towards an increase in MDD risk (OR=1.05 (0.99-1.11); p = 0.08).

Discussion
Our present results suggest it is unlikely that causal mechanisms account for a major proportion of the 60% increased prevalence of MDD in T2D and that confounding may influence association. However, for “genetically predicted higher BMI, we observed a trend towards a causal relationship with MDD. Power limitations probably reduce our ability to establish causality in this association and it is likely that either an increase in the variance in BMI explained by genetic variation or an expanded GWAS of MDD would allow this estimate to be refined. This study also emphasizes, that despite the relatively meagre direct outcome of GWAS
studies in depression so far, these large datasets may continue to provide valuable results through indirect approaches such as Mendelian randomization in the future.


INTRA-PATIENT COMPARISON OF MANIC AND EUTHYMIC PHASES IDENTIFIES STATE AND TRAIT SPECIFIC GENE EXPRESSION AND STAB1 AS A NEW CANDIDATE GENE FOR BIPOLAR DISORDER

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Background
Bipolar disorder (BD) is a highly heritable psychiatric disease characterized by recurrent episodes of mania and depression. To identify new BD genes and pathways, the present study employed a three-step approach.

Methods
Firstly, gene expression profiles of BD patients were assessed during both a manic and a euthymic phase. These profiles were then compared both intra-individually, and with the gene expression profiles of controls. Secondly, differentially expressed genes were validated using data from the Psychiatric Genomics Consortiums’ genome-wide association study of BD. Thirdly, the implicated molecular mechanisms were investigated using pathway analytical methods.

Results
In the present patients, this novel approach identified: (i) sets of differentially expressed genes specific to mania and euthymia; and (ii) a set of differentially expressed genes that were common to both mood states. In the GWAS data integration analysis, one gene (STAB1) remained significant (p = 1.9 x 10^-4) after adjustment for multiple testing. STAB1 is located in close proximity to PBMR1 and the NEK4-ITIH1-ITIH3-ITIH4 region, which are the top findings from GWAS meta-analyses of mood disorder, and a combined bipolar and schizophrenia dataset. Pathway analyses in the mania vs. control comparison revealed three distinct clusters of pathways tagging molecular mechanisms implicated in BD, e.g. energy metabolism, inflammation, and the ubiquitin proteasome system.

Discussion
The present findings suggest that STAB1 is a new and highly promising candidate gene in this region. The combination of gene expression and GWAS data may provide valuable insights into the biological mechanisms of BD.
GENOME-WIDE GENE-ENVIRONMENT STUDY IDENTIFIES REGULATOR OF G-PROTEIN SIGNALING-10 AS A SUSCEPTIBILITY GENE FOR DEPRESSION VIA STRESSFUL LIFE EVENTS

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Background
Stressful events have been recognized as a risk factor for depression. Although recent genome-wide association studies (GWAS) have identified several novel loci for depression, few reported genome-wide gene-environment interactions.

Methods
To identify genes that influence the association of stressful events with depression, we performed a genome-wide gene-environment interaction analysis. Genotyping was conducted using the Affymetrix Genome-Wide Human SNP Array 6.0 in 320 Japanese healthy subjects. Stressful lifetime events were assessed using Social Readjustment Rating Scale (SRRS) and depression was assessed with a self-rating questionnaire using Center for Epidemiologic Studies Depression Scale (CES-D). The P-values for interactions between SNPs and stressful events were calculated using linear regression model controlling for sex and age.

Results
After the data cleaning and quality control of genotype data, 534,848 SNPs on autosomal chromosomes were further analyzed. Genome-wide significant SNP was found at rs10510057 located near RGS10 on 10q25 which encodes regulatory molecules acting as GTPase activating proteins (P = 3.9 x 10^{-8}). Using a genome-wide gene-based approach, genome-wide significant gene was identified at RGS10 (gene-wide P = 3.1 x 10^{-7}). In a gene-set enrichment analysis, the most significant associations were found in two pathways, ABC transporters general and arachidonic acid metabolism (false discovery rate < 0.10).

Discussion
The present study demonstrated that RGS10, interacting with stressful life events, may be involved in depression risk. Our findings support the idea that inclusion of environmental factors may be a powerful approach to identify genes that are missed in GWAS. Replication studies with larger sample sizes will be needed to confirm our results.

GENOME-WIDE ASSOCIATION STUDY REVEALS TWO NEW RISK LOCI FOR BIPOLAR DISORDER
Background
Since the first genome-wide association study (GWAS) of bipolar disorder (BD) in 2007, the first risk loci at the widely acknowledged formal threshold of genome-wide significance (P<5E-08) could be identified which replicated in adequately sized follow-up studies, notably ANK3, NCAN, CACNA1C, and ODZ4. These findings explain only a small fraction of BD heritability. This is in line with results of recent studies that provide evidence for a strong polygenic component in BD suggesting a larger number of additional susceptibility loci, each mediating very small disease risk. One crucial step towards the identification of additional loci should be amenable by increasing the sample sizes.

Methods
As an extension of our first GWAS of BD (Cichon et al., 2011), which was included in the BD GWAS of the Psychiatric Genomics Consortium (PGC; Sklar et al., 2011), we have generated so far unpublished GWAS data from 2,266 patients with BD (~76% BD type I) and 5,028 ethnically matched controls. These data were obtained within the framework of the MooDS consortium and originate from four European countries, Canada, and Australia. To further increase the statistical power, we combined our MooDS data with published data from the PGC, resulting in the currently largest discovery sample studied in BD (9,747 patients, 14,278 controls).

Results
Overall, we detected 56 genome-wide significant single-nucleotide polymorphisms (SNPs) at five risk loci: there was support for previously identified loci at ANK3, ODZ4 and TRANK1, and two new risk loci at ADCY2 (5p15.31) and in an intergenic region (6q16.1) emerged.

Discussion
Our study implicates common variation at two novel loci as risk factors for BD. While a specific gene cannot be pinned down at 6q16.1, it harbours a genome-wide significant SNP that was previously shown to be associated with variation of “processing speed”, a cognitive function that has been postulated as a valid and highly specific cognitive endophenotype for BD differentiating both euthymic BD patients, and their healthy first degree relatives from healthy controls. ADCY2 plays a central signaling role in G protein coupled receptor (GPCR) pathways. Disturbed neurotransmission at GPCR pathways is a long-standing hypothesis in psychiatry research which has motivated multiple candidate gene studies. Most of these studies, however, focused on variation in neurotransmitter receptor and transporter genes and less on adenylate cyclase genes, located more downstream and bundeling the signals coming in from several neurotransmitter receptor types. Adenylate cyclase may therefore represent a functional
bottleneck in signal transduction pathways and genetic variation may have a more direct influence on the phenotype than at positions with higher functional redundancy (receptors/transporters). This may also explain the somewhat surprising observation that GWAS in neuropsychiatric disorders performed so far did not identify strong association signals in neurotransmitter/transporter genes.

DOPAMINE-RELATED GENETIC RISK SCORE IS ASSOCIATED WITH LEVEL OF DEPRESSIVE SYMPTOMS IN HEALTHY ADULTS AND ADULTS WITH DEPRESSION

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Background
Depression is a common source of human disability for which etiologic insights remain limited. Although abnormalities of monoamine neurotransmission, including dopamine, are theorized to contribute to the pathophysiology of depression, evidence linking dopamine related genes to depression has been mixed. While some studies find that dopamine-related variants are associated with multiple psychiatric and neurological diseases, other studies find no association. Moreover, genome-wide association studies (GWAS) of depression have not found significant signals in dopamine-related loci. One likely contributor to these inconsistent findings is that common genetic variants for complex disease tend to have small to modest effects. Thus, tests of association based on a single nucleotide polymorphism (SNP) are unlikely to yield significant effects unless very large samples are studied. We sought to provide additional evidence regarding the role of dopamine in depression by examining the combined effect of five dopamine-related polymorphisms on depressive symptomatology in both healthy individuals and individuals with depression. We used a genetic risk score approach, which sums the effects of multiple polymorphisms in the same biological system. Genetic risk score approaches have been informative in psychiatric settings, including when studying the role of dopamine. We created the genetic risk score by combining functional polymorphisms from five genes involved in both synaptic dopamine availability (COMT and DAT) and dopamine receptor binding (DRD1, DRD2, DRD3). The main hypothesis we sought to test was that the burden of common genetic variation across the dopamine system was inversely related to depressive symptoms. We further hypothesized that the genetic risk score would be more strongly associated with depression than any single polymorphism, as effects of multiple polymorphisms acting on the same neural system are hypothesized to be additive.

Methods
Data were drawn from three independent samples. Our discovery sample included 273 healthy adult participants (ages of 18–35). Depressive symptoms were measured in the discovery sample using the 20-item Center for Epidemiologic Studies Depression Scale (CES-D). We sought to replicate our findings in two replication cohorts. The first replication cohort consisted of 1,267 individuals diagnosed with major depressive disorder (MDD) from the Sequenced Treatment Alternatives to Relieve Depression Study (STAR*D). In STAR*D, depressive symptoms was
measured using the 17-item Hamilton Rating Scale of Depression. The second replication was attempted using data from 382 healthy non-depressed respondents in the Brain Genomics Superstruct Project (GSP). In the GSP, mood was assessed using the shortened-version of the Profile of Mood States, a 30-item scale designed to assess affective mood states, including depression, tension, anxiety, anger, hostility, and confusion. Respondents described their mood in the past week using a Likert-scale (0=not at all to 4=extremely). Depressed mood t-scores were obtained from the five items tapping depression/dejection.

Results
In the discovery sample, the genetic risk score was associated with depressive symptomatology (β=-0.80, p=0.003), with lower dopamine gene scores (indicating lower dopaminergic neurotransmission) predicting higher levels of depression (Figure 1). Thus, individuals with the highest possible gene score (10) were estimated to have an 8 point difference in their CES-D score. This 8 point difference is clinically relevant, as it is larger than the difference between the categories used to differentiate CES-D scores (i.e. no depression ranges from 0-9, mild depression 10-15, moderate depression 16-24, and severe depression 25+). This result was replicated in adults with depression (β=-0.51, p=0.04). Though statistically non-significant, results were of similar magnitude and in the expected direction in the replication cohort of healthy adult participants (β =-0.86, p=0.15).

Discussion
We found that a dopamine genetic risk score based on functional polymorphisms with established effects on dopamine neurotransmission was significantly associated with the level of depressive symptoms in healthy participants and those with MDD. This genetic risk score shows stronger associations with the measures of depression than did any single variant. Further studies are required to confirm the role of genetic variation in dopamine metabolism and depression.

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INTERACTION BETWEEN GENETIC VARIANTS AND EXPOSURE TO HURRICANE KATRINA ON POST-TRAUMATIC STRESS DISORDER AND POST-TRAUMATIC GROWTH: A PROSPECTIVE ANALYSIS OF LOW INCOME ADULTS
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Background
Post-traumatic stress disorder (PTSD) is a disabling psychiatric condition that occurs following exposure to a potentially traumatic life event. While large-scale epidemiological studies have found that a significant sub-set of the population report being exposed to trauma, there is considerable variation in survivors’ responses, ranging from PTSD to relatively mild responses. In light of this variation, researchers have broadened their focus to include a range of adaptive responses. Within this context, some have investigated the phenomenon of posttraumatic growth (PTG), or self-reported positive psychological changes induced by the experience and processing of a traumatic event and its aftermath. Examination of genetic variants associated with PTSD and PTG, and how genetic variants interact with environmental exposures (i.e., gene-environment
interaction; GxE) offers one promising avenue for understanding differential outcomes following trauma exposure. The goal of the current study was to examine whether ten variants in seven genes (BDNF, CACNA1C, CRHR1, FKBP5, OXTR, RGS2, SLC6A4) modified the association between exposure to Hurricane Katrina and PTSD and PTG.

Methods
Data came from a prospective study of 254 low-income, White/Non-Hispanic adults who resided in New Orleans, a city that saw widespread property damage and extensive flooding following Hurricane Katrina. Data was collected at 4 time-points: pre-Katrina baseline (2004-2005; Time 1), pre-Katrina 12-month follow-up (2005; Time 2), post-Katrina initial follow-up (May 2006 and March 2007; Time 3) and post-Katrina secondary follow-up (April 2009-March 2010; Time 4). Exposure to Hurricane Katrina was measured at Time 3 using an 8-item scale asking about experiences in the immediate aftermath of the storm (e.g., no fresh water to drink; no food to eat; felt their life was in danger). Responses to these items were categorized into three severity groups: low exposure (0 or 1 conditions), moderate exposure (2 or 3 conditions), and high exposure (4+ conditions). PTSD symptoms were measured at Time 3 and 4 using the 22-item Impact of Events Scale–Revised, which asked participants to report how often in the prior week they were distressed by experiences related to Hurricane Katrina. PTG indicators were measured at Time 4 using the 21-item Posttraumatic Growth Inventory. Participants rated the extent to which they experienced various changes as a result of Hurricane Katrina. We first examined the effect of exposure to Hurricane Katrina on our three outcomes (PTSD severity score at Time 3 and 4; PTG score at Time 4), after adjusting for covariates (i.e., gender; age; pre-Katrina social support, number of children, mental health status). We then tested the association between each variant and the outcomes using a joint test (2 df) for a main genetic effect and GxE. For each significant finding, we conducted post-hoc analyses to determine whether a main genetic effect or GxE was driving the association. Permutation was used to establish a significance threshold that accounted for multiple testing (p=0.0033 for PTSD and p=0.0046 for PTG).

Results
Level of exposure to Hurricane Katrina was significantly associated with PTSD at Time 3 (p=0.0098) and Time 4 (p=0.000002), but not PTG (p=0.17). Using the joint test, we found a significant association between RGS2 (rs4606; p=0.0037) and PTG, which was mainly driven by a GxE (p=0.0037), rather than a main genetic effect (p=0.065). (Figure 1). This result withstood a multiple testing correction. We also found an effect of two FKBP5 SNPs (rs1306780 p=0.010; rs9296158 p=0.046) on PTG, with the T allele and A allele, respectively, conferring a higher likelihood of experiencing PTG. For both SNPs, this joint effect was driven by a genetic main effect (p=0.003 and p=0.017, respectively) rather than GxE (p=0.61 and p=0.50, respectively). Finally, we found an association between CRHR1 (rs12944712 p=0.0095) and Time 4 PTSD symptoms, which was driven by a GxE (p=0.0031), not a genetic main effect (p=0.473).

Discussion
We found evidence of a significant interaction between RGS2 and exposure to Hurricane Katrina on indicators of post-traumatic growth. This finding should be replicated in a future study.
ADRB2 GENE POLYMORPHISM INTERACTS WITH CHILDHOOD TRAUMA IN CONVEYING RISK FOR ADULT POSTTRAUMATIC STRESS DISORDER (PTSD)
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Background
Posttraumatic stress disorder (PTSD) is a debilitating and highly prevalent consequence of trauma exposure, of unknown etiology. However, only a subset of trauma-exposed individuals develops PTSD, and heritable factors, in interaction with environmental exposure (trauma), have been implicated by twin and family studies. Targeted molecular genetic studies have identified a number of candidate genes in PTSD vulnerability, however large studies containing replication cohorts and detailed phenotypic data are needed to examine gene by environment interaction (G x E) models. Using two independent trauma-exposed cohorts, we report novel findings of the beta2 adrenergic receptor gene (ADRB2) SNP's conveying risk for PTSD in interaction with trauma exposure (childhood and lifetime).

Methods
Participants were selected from two independent samples. The discovery sample is a prospective longitudinal study of Ohio National Guard soldiers, primarily European-American males, recruited while in training for deployment to Iraq and Afghanistan (total cohort N=2616, genotype data for this analysis N=715 participants of European ancestry by PCA). The replication sample (N=2083) was from the Grady Trauma study, a study of predominantly African-American females in Atlanta, GA with low income and high levels of trauma exposures (Emory University). PTSD symptoms, childhood adversity, and lifetime adult trauma exposures were assessed by diagnostic interviews and self-report measures. Genotyping was performed in the discovery sample using a custom 3755 SNP Illumina Infinium genotyping array, and in the replication sample using Illumina HumanOmniExpress BeadChip. Association analyses were performed in PLINK in linear regression models including main effects of childhood adversity, lifetime adult trauma exposure, and SNP, and SNP x childhood adversity and SNP x lifetime adult trauma interaction terms. Correction for admixture and population structure was performed by PCA of 1500 markers in equilibrium (obtained through data pruning R2 <0.30) in the discovery sample and genomewide in the replication samples; Bonferroni correction for multiple comparisons (threshold 1.33 x 10^{-5}) was applied to the discovery sample.

Results
We identified a SNP within the promoter region of ADRB2 gene rs2400707 that was associated PTSD symptoms in interaction with childhood trauma (rs2400707, p=1.11 x 10^{-5}, additive genotype relative risk ~1.5), controlling for level of life-time trauma exposure and PCA factors, which was significant after Bonferroni correction. Several other ADRB2 SNPs in strong LD with rs2400707 also showed suggestive levels of significance (p < 2 x 10^{-5}) in SNP x childhood adversity interaction terms. Association of rs2400707 with PTSD in interaction with childhood adversity was confirmed in an independent, predominantly female, African American cohort (Grady Trauma Project N=2083, rs2400707 x childhood trauma interaction p=5.01 x 10^{-4}).

Discussion
Altered adrenergic/noradrenergic function has been long believed to play a key etiologic role in PTSD development, however direct evidence to this link has been missing. The ADRB2 gene rs2400707 polymorphism has been linked to function of the adrenergic system and to the development of chronic pain, however this is the first report linking the ADRB2 gene to PTSD or any psychiatric disorders. Interestingly the signal transduction of the ADRB2 gene product (beta2 adrenergic receptor) involves direct interaction with the class C L-type voltage dependent calcium channel (Ca(V)1.2). The gene for this calcium channel (CACNA1C) is among the most replicated findings in psychiatric genetics; it is thus possible ADRB2 variations may operate within the same gene network. These findings have important implications for PTSD etiology, chronic pain and stress related comorbidity, as well as for both primary prevention and treatment strategies.

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GENOME-WIDE ASSOCIATION STUDY OF PHENOTYPES IN OBSESSIVE-COMPULSIVE DISORDER
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Background
Obsessive-compulsive disorder (OCD) is a chronic and debilitating psychiatric disorder with a strong genetic etiology. A recent genome-wide association study (GWAS) reported interesting candidate gene variants potentially related to susceptibility to developing OCD. We hypothesized that genetic variation(s) may be associated with OCD phenotypes including symptom severity using the Yale-Brown Obsessive-Compulsive Scale (Y-BOCS) severity score and age at onset (AAO).

Methods
We investigated 357 individuals with OCD and their nuclear families or matched healthy controls (age, gender, and ethnicity) for an association of gene variant(s) and phenotypes including Y-BOCS severity score and AAO in a GWAS. GWAS was conducted by the OCF Genetic Collaborative Group. Quality control and analyses were conducted using PLINK and R programs.

Results
Several suggestive genome-wide association signals were detected with a p-value of 10E-6 on chromosome 3 and 22 for Y-BOCS severity score and chromosome 3 and 9 for AAO.

Discussion
Preliminary findings from our GWAS suggested possible involvement of several distinct regions in OCD symptom severity and AAO. Further analyses are required to characterize these results.

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ASSOCIATION OF LIFETIME PTSD WITH IL-18 SINGLE NUCLEOTIDE POLYMORPHISMS IN EUROPEAN AND AFRICAN AMERICAN WOMEN
Background
PTSD has been associated with increased levels of inflammation and related diseases, including autoimmune conditions, cardiovascular disease, diabetes, and obesity (Boscarino, 2004; Dobie et al., 2004; Vieweg et al., 2007). The sympathetic nervous system and hypothalamic-pituitary-adrenal (HPA) axis are activated in response to acute stress but can become dysregulated in the presence of chronic stress. This dysregulation may ultimately lead to altered immune system functioning and inflammation (Gill et al., 2009; Pervanidou et al., 2007), as demonstrated by elevated cytokine and C-reactive protein levels in individuals with PTSD (e.g., Gola et al., 2013; O'Donovan et al., 2011). However, it is unknown whether PTSD causes inflammatory changes, if inflammatory elevations increase risk for PTSD, or if a common factor accounts for the association. IL-18 is a cytokine thought to play a role in the etiology of inflammatory disease (Giedraitis et al., 2001), and a previous study found downregulated expression of IL-18 among PTSD patients (Zieker et al., 2007). In the current investigation, we assessed whether IL-18 single nucleotide polymorphisms (SNPs) were associated with PTSD. A significant association of IL-18 SNPs with PTSD might suggest a common genetic vulnerability for PTSD and inflammation.

Methods
Participants were selected from two independent samples: the Nurses Health Study II (NHSII) and the Detroit Neighborhood Study (DNHS). The NHSII cohort was established in 1989. European American women (n = 2521) with a history of trauma who participated in a diagnostic interview (Koenen et al., 2009) were selected for the current investigation. The sample included 588 women with lifetime PTSD and 1993 controls. Genotyping was performed utilizing an Illumina InfiniumSelect custom 6000 bead chip system. IL-18 SNPs included rs3882891, rs549908, rs5744258, and rs795467. The DNHS is a probability sample of 1547 adults in the Detroit area (Goldmann et al., 2011). The current investigation included trauma-exposed African American men (n = 329) and women (n = 449); there were 142 cases and 459 controls. PTSD was assessed via a telephone structured interview (Breslau et al., 1998). Genotyping was done on an Illumina HumanOmniExpress BeadChip; IL-18 SNPs included rs5744290, rs549908, rs5744259, rs5744256, rs2043055, and rs360717. All genetic analyses were performed in PLINK. Logistic regression models were used to test associations between SNPs and lifetime PTSD. The maxT permutation procedure, with 5000 permutations, was used to adjust for multiple comparisons across the gene.

Results
In the discovery sample (NHSII), three SNPs were associated with increased risk for lifetime PTSD: rs3882891 ($\chi^2 = 9.40$, OR=1.23, permutation-corrected $p = .01$), rs549908 ($\chi^2 = 7.02$, OR=1.21, permutation-corrected $p = .02$), and rs795467 ($\chi^2 = 6.90$, OR=1.21, permutation-corrected $p = .03$). Of these, only rs549908 was genotyped in the DNHS sample. The association
between this SNP and lifetime PTSD was not significant in the total sample or male subsample; however, among women, rs549908 increased risk for PTSD ($\chi^2 = 8.08$, OR=1.78, permutation-corrected $p = .02$).

**Discussion**

*IL-18* SNPs were significantly associated with lifetime PTSD among women but not men. In particular, rs549908 was shown to increase risk for PTSD among both European American and African American women. A previous study reported that this SNP was in complete linkage disequilibrium with the functional *IL-18* promoter variant rs187238, which was associated with lower transcriptional activity in Asian samples (Lingh et al., 2005). Further, rs3882891, which was significantly associated with PTSD in NHSII women but was not genotyped in DNHS, previously was found to be in complete LD with rs5744292 and was associated with metabolic syndrome and insulin sensitivity in a Caucasian sample (Presta et al., 2009). This is the first genetic investigation of *IL-18* and PTSD. A recent study found several *IL-18* SNPs that were significantly associated with depression among patients who experienced a preceding stressful life event (SLE) compared to depressed patients without a history of SLE (Haastrup et al., 2012). For this reason, we hypothesized that *IL-18* SNPs also would be associated with lifetime PTSD. In sum, inflammation is a potential mechanism for the relation between PTSD and negative health outcomes, particularly obesity and cardiovascular and metabolic disorders. Variation in *IL-18* may be one pathway that links PTSD and inflammation, and there may be important sex differences in these associations. Additional future directions include investigation of post-traumatic stress, inflammation, and health sequelae from a systems perspective.

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**ADCYAP1R1 GENOTYPE ASSOCIATES WITH POST-TRAUMATIC STRESS SYMPTOMS AND AMYGDALE RESPONSE IN HIGHLY-TRAUMATIZED AFRICAN AMERICAN FEMALES**

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**Background**

Pituitary adenylate cyclase activating polypeptide (PACAP) and its receptor (PAC1) may be critical mediators of abnormal processes following psychological trauma. Indeed, we identified that a variant, rs2267735, in the gene encoding PAC1 (*ADCYAP1R1*) is associated with post-traumatic stress disorder (PTSD) in a primarily African American cohort of highly traumatized females. We sought to extend and replicate our previous finding in a replicate sample of adult male and female patients recruited from the clinics at Grady Memorial Hospital (Atlanta, Georgia). Given that PTSD is thought to be a disorder of abnormal fear expression, our second goal was to examine how the *ADCYAP1R1* variant was associated with neural response to threat-relevant cues, using functional magnetic resonance imaging (fMRI). To this end, we examined associations between *ADCYAP1R1* genotype and blood oxygen level dependent (BOLD) response to fearful faces in the amygdala, a brain region involved with rapid threat evaluation.

**Methods**

Salivary samples were collected to obtain DNA for genetic analysis. Self-reported psychiatric measures included measures of PTSD [PTSD symptom scale (PSS), assessing PTSD symptom
severity over the prior two weeks], Beck Depression Inventory (BDI, assessing depression severity), and other measures of both adult and childhood trauma and abuse. Genotyping of rs2267735 was conducted using Taqman and Sequenom platforms. Linear regression models were used to test for association under an additive (allelic) model where the number of copies of the “C” allele was allowed to influence the outcome variable linearly. We also computed robust standard errors and verified the key result with permutation and bootstrapping to ensure that our interaction test results did not depend on linearity or distributional assumptions (replication sample set, N=858 females). A meta-analysis with the combined original and replicate sample of African Americans was also conducted (N=1424 females). As a secondary analysis, a subset of females (N=49) with significant trauma participated in an fMRI scan, viewing blocks of fearful and neutral face stimuli. We examined group differences in threat processing by contrasting activation to fearful versus neutral faces in a CC-allele group (n = 22, “risk” group), and a GG/GC-allele group (n = 27, “resilient” group).

**Results**

For the genotype analysis, we found an ADCYAP1RI genotype by trauma interaction in females (p < 0.001), but not males (p > 0.1). Moreover, this interaction remained significant in females, but not males, after controlling for age (p < 0.001), income (p < 0.01), past substance abuse (p < 0.001), depression severity (p = 0.02), or child abuse (p < 0.0005), and all five combined (p = 0.01). No significant effects of genotype (or interactions) were found when modeling depression severity when controlling for comorbid PTSD symptom severity (p > 0.1). A meta-analysis with the previously reported African-American samples revealed a strong association between PTSD symptom severity and the interaction between trauma and genotype in females (p < 0.0001). For the fMRI analysis, the CC group showed increased bilateral amygdala activation to fearful relative to neutral face stimuli relative to the GG/GC group (p < 0.05). The CC-allele group also showed increased activation of several other regions associated with threat processing, including the left posterior hippocampus and bilateral fusiform gyrus (p < 0.05).

**Discussion**

The current finding for the genotype analysis demonstrated a gene × environmental interaction of trauma with ADCYAP1RI, enhancing our understanding of a specific way the PACAP/PAC1 pathway may underlie stress-related disorders. Via fMRI analysis, we found associations between ADCYAP1RI genotype and physiological response to threat cues. Compared with the resilient group, members of the risk group demonstrated significantly greater BOLD response in limbic brain regions, particularly, the amygdala. In sum, our studies suggest that variation at a PAC1 locus impacts PTSD susceptibility in highly traumatized African American females, which may be mediated, directly or indirectly, via modulation/sensitization of amygdala response.

**THE ROLE OF GENES AND ENVIRONMENT IN SUSCEPTIBILITY TO ANXIETY-SPECTRUM PHENOTYPES**

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Background
Anxiety disorders are among the most prevalent psychiatric conditions. They are highly comorbid with one another and related (“anxiety-spectrum”) phenotypes due to both shared genetic and environmental risk factors. Replicated genetic associations between anxiety-spectrum phenotypes and several candidate genes including catecholamine-O-methyltransferase (COMT) and glutamic acid decarboxylase 1 (GAD1) have been reported. Well-established environmental risk factors include childhood adversity, lifetime trauma, and recent stressful life events. We assess the combined role of these risk factors in predicting lifetime anxiety-spectrum phenotypes.

Methods
Subjects used in this study derive from the longitudinal population-based Virginia Adult Twin Study of Psychiatric and Substance Use Disorders (VATSPSUD). First, phenotypic factor analysis was conducted to estimate a quantitative common factor score reflecting shared variance across a range of anxiety-spectrum phenotypes. Next, we chose genetic variants within COMT and GAD1 that have been robustly associated with anxiety-related disorders in this and other samples. We then analyzed the main effects of several environmental risk factors assessed in this sample. Two approaches were applied to handle multiple environmental risk factors: multivariate regression to select significant factors and partial least squares (PLS) to generate empirical weights for individual factors and the corresponding score. In PLS, split-sample cross-validation was performed to determine the number of extracted factors and verify the goodness of fit of the model. Finally, we added the effects of gene-by-environment interactions (GxE) to construct our final risk models.

Results
In multivariate analyses of all main effects, childhood sexual abuse, low parental warmth, lifetime trauma, and history of divorce together with genetic effect of both Val-Met polymorphism rs4680 of the COMT gene and a six-marker haplotype in GAD1 gene were significant. Of these environmental factors, lifetime trauma and divorce were significant only in men and women, respectively. The COMT G-A haplotype (formed by the G allele of rs4680 and the A allele of rs165599) was jointly significant with childhood sexual abuse and parental loss in women. In addition, a significant interactive effect of parental warmth and COMT marker rs4680 was detected in women when childhood sexual abuse was not included in the model.

Discussion
Main effects of candidate genetic variants and several individual environmental risk factors jointly predicted anxiety-spectrum susceptibility. PLS and gene-environment interaction analyses are in process and will be presented at the conference.

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GENOTYPES ASSOCIATED WITH TRAJECTORIES OF POSTTRAUMATIC STRESS AMONG URBAN ADULTS: MAIN EFFECTS AND INTERACTIONS WITH CHILD ABUSE HISTORY

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Background
Previous research has documented genetic variation in susceptibility to posttraumatic stress (PTS), such that rare variants of particular genes (e.g., SLC6A4, FKBP5) are associated with higher PTS. Additionally, variants have been found to interact with environmental risk factors, including childhood abuse history, such that trauma survivors with both risk alleles and more extensive abuse histories are at greatest risk for more severe PTS. The literature to date is limited, however, by a reliance on cross-sectional data that only captures PTS at a single time point. As such, less is known about how genetic variants predict posttraumatic stress trajectories over time, both independently and in tandem with childhood abuse history. The purpose of this study was to fill this gap through analysis of a large sample of urban adults (N = 981).

Methods
Participants were part of the Detroit Neighborhood Health Study (DNHS), a three-wave study of adults living in urban Detroit. At each wave, participants completed the 17-item Posttraumatic Stress Checklist-Civilian Version (PCL-C; range: 17-85) and, at Wave 2, six items from the Childhood Trauma Questionnaire assessing history of child physical abuse, emotional abuse, and emotional neglect. In the current study, we included 981 participants who reported at least one traumatic event prior to the Wave 1 (W1) assessment and who completed the PCL-C at W1 and at least one other wave. The majority of participants (84.4%) identified as non-Hispanic Black, 10.7% as non-Hispanic White, 4.4% as “other” race; 1.2% identified as Hispanic ethnicity; 59.2% were female and participants’ average age at W1 was 52.47 (SD = 16.06, range: 18-92). A subsample of participants provided blood samples for DNA extraction (n = 778). Four candidate genes were selected for the current study based on either evidence of associations with PTS in three or more studies, or in at least one study with a primarily non-Hispanic Black sample: SLC6A3, SLC6A4, RORA, FKBP5.

Latent class growth analysis (LCGA) was conducted with the full sample in Mplus to identify PTS trajectories. The solution selected as the best representation of the data was replicated on the DNA subsample. Subsequently, multinomial logistic regression models were run on the DNA subsample to assess main effects of genetic variants on the odds of being in higher PTS, versus low PTS, trajectories and interaction effects of variants with child abuse history. The analyses included age and gender as covariates and were corrected for multiple testing.

Results
A four-class model was selected as the best representation of the data. Four PTS trajectories were detected. Although the majority evidenced a trajectory of consistently few symptoms (Low PTS: 67.6%, W1 PCL-C M= 27.87 [SD = 10.06]; W2 PCL-C M = 21.29 [SD = 4.85]; W3 PCL-C M = 22.20 [SD = 8.38]), 9.1% were in trajectories with chronic posttraumatic stress disorder (PTSD) (Chronic PTSD: 6.9%, W1 PCL-C M = 49.69 [SD= 16.11]; W2 PCL-C M = 51.73 [SD = 6.44]; W3 PCL-C M = 44.92 [SD = 12.32]); Chronic Severe PTSD: 2.1%, W1 PCL-C M = 69.76 [SD = 12.14]; W2 PCL-C M = 68.33 [SD = 6.86]; W3 PCL-C M = 61.15 [SD = 10.24]). The remainder had consistently elevated, but subclinical, levels of PTS (Subthreshold PTS: 23.3%, W1 PCL-C M = 42.04 [SD = 14.82]; W2 PCL-C M = 35.22 [SD = 5.84]; W3 PCL-C M = 31.85 [SD = 9.89]). This solution was replicated in the DNA subsample. The results of multinomial
regression showed that rare variants of SNPs from each of the four genes increased the odds of being in either the Subthreshold PTS or one of the Chronic PTSD trajectories, versus, the Low PTS trajectory. Several significant interactive effects with genetic variants and child abuse history were also detected.

**Discussion**
The results provide evidence of rare variants of SLC6A3, SLC6A4, RORA, and FKBP5 in increasing susceptibility to both chronically elevated but subthreshold PTS and chronic PTSD, and the role of childhood abuse in exacerbating risk associated with these variants. Further research using a trajectory approach and exploring additional environmental factors would provide greater insight into the processes that shape psychological responses to trauma.

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**THE NOT-SO-SILENT EFFECT OF SILENCE MUTATIONS IN ASD**
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**Background**
High locus and allelic heterogeneity found in Autism Spectrum Disorders (ASD) complicate the comprehension of the genetic bases of the disorders. Thousands of clues have emerged from initial exome sequencing studies, identifying many novel *de novo* mutations in ASD family trio studies. Affected proteins seem to be highly interconnected and expressed mainly in brain. Most of the variants reported to date were found mostly in Caucasian or European descendant cohorts but Latin American cohorts have not been well studied.

**Methods**
Therefore, we decided to apply exome sequencing, at a 50X depth, in a cohort of Colombian – South American (admixed population) trios.

**Results**
Although most of exome sequencing studies focus on deleterious mutations such as non-synonymous, missense and frame shift mutations, there is still a big caveat: What about the not-so-silent effect of silence mutations might have? We not only focused on the discovery of harmful variants, but we also evaluated the possible effect of silent mutations, since it is known that synonymous mutations can actually be a cause of different diseases and syndromes altering mRNA stability or translation.

**Discussion**
The global outcomes guide to a larger range of mutations that are related to ASD.

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**A HYPOTHESIS DRIVEN APPROACH TO CHARACTERIZE ASD LIABILITY GENES DURING IN-VITRO NEURONAL DIFFERENTIATION**
Background

Autism spectrum disorders (ASD) are complex pervasive neurodevelopmental disorders, defined by deficits in social interaction and communication and repetitive behaviors. ASD are highly heritable and several susceptibility genes for ASD are known. Databases like AutismKB report on up to 3000 risk-genes associated with e.g. neuronal development, glutamatergic and gabaergic signaling. At neuropathological levels, ASD patients show a reduced development of neurons in the forebrain limbic system and a decreased number of Purkinje cells in the cerebellum. In addition, a reduced dendritic branching in the hippocampus and smaller pyramidal neurons in the Broca area of ASD patients are reported. This suggests an aberrant neuronal differentiation and development in ASD. A gene co-expression network analysis revealed that autism risk-genes are enriched in neuronal relevant networks. However, only few studies have characterized ASD susceptibility genes during neuronal differentiation: In NHNP cells (normal human neuronal progenitors) a significant number of ASD candidate genes is either induced (e.g. NRXN1, GABRB3, SHANK3) or repressed (e.g. CNTNAP2) after four weeks of neuronal in-vitro differentiation. An analysis in differentiating SH-SY5Y cells showed that genes associated with neuronal in-vitro differentiation are induced already 6 hours after application of growth factors. Thus, SH-SY5Y cell lines may present a useful model to monitor the regulation of ASD candidate genes during neuronal differentiation, to finally test the hypothesis that disturbances in early neuronal development are underlying the etiology of ASD. So far few studies have used SH-SY5Y cells in ASD research, if so, without characterizing ASD risk-genes during neuronal differentiation.

Methods

Here we aimed at testing SH-SY5Y cells as a suitable model for studying the role of defined ASD candidate genes during neuronal differentiation. Thus, we characterized mRNA and protein expression as well as morphological developments during this process. SH-SY5Y cells were differentiated using a combination of BDNF (brain derived neurotrophic growth factor) and RA (retinoic acid). We have optimized protocols to obtain a consistent differentiation and mRNA expression over time. Cells were harvested every 48h after the application of BDNF+RA over a time-course of 2 weeks. MAPT (microtubule-associated protein tau; a neuronal marker) and CDC2 (cyclin dependent kinase 1; a cell division marker) were analyzed to monitor differentiation status at mRNA and protein level. mRNA and protein expression were measured using RT real-time PCR (2^ΔΔCT method) and Western blots, respectively. Expression of specific neuronal receptors was investigated to define neurotransmitter/receptor status. Receptors to be tested were selected based on expression data available online (BrainSpan). ASD candidate genes investigated were chosen based on rankings of the AutismKB, Autworks and HuGE Network databases. Genes which were under the top 150 in all three databases were selected for the preliminary studies presented here.
Results
Here we show that the SH-SY5Y cell model is glutamatergic (GRM1, GRIN1, GRIK2), gabaergic (GABRB3) and dopaminergic (DRD4) as respective receptors were up-regulated during neuronal differentiation. However, we did not observe any changes in serotonergic receptor (HTR2A) expression. Comparing the pattern of several ASD susceptibility genes showed that expression levels of NRXN1, SHANK3 and GRIK2 were strongly correlated ($r^2 > 0.89$). Furthermore, we observed that CNTNAP2 mRNA-expression is down-regulated during early stages of neuronal differentiation and up-regulated when cells were fully differentiated. Interestingly, GABRB3 is continuously up-regulated, whereas GABRA5 is down-regulated 10 days of differentiation and exhibits normal expression levels at final stages of differentiation.

Discussion
We conclude that SH-SY5Y cells are a suitable model for the investigation of ASD cellular phenotypes and mRNA expression in-vitro as ASD-relevant signaling pathways (i.e. glutamatergic and gabaergic receptors) are expressed. Furthermore, the regulation of CNTNAP2 and NRXN1 is comparable to previous results in NHNP cells, and observed clusters of co-regulated ASD genes are in line with the co-regulated networks proposed in Konopka et al. 2012. Further studies on morphological aspects and genetic variants will allow correlating ASD-genes and mechanisms in a SH-SY5Y neurodevelopmental context.


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AUTISM SPECTRUM DISORDERS: INFLUENCE OF DIFFERENT VARIANTS AT ITGB3 GENE IN A SAMPLE OF BRAZILIAN CHILDREN
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Background
Autism Disorder is an early-onset neurodevelopmental condition, characterized by impairments in communication and social interaction and by repetitive and stereotyped behaviors. It is included in a group of diseases named Autism Spectrum Disorders (ASD) that comprehend other developmental disorders as Asperger Disorder and Pervasive Development Disorder-not otherwise specified. Although the core symptoms are the same in all ASD, they are very complex and heterogeneous diseases, with a wide variety of clinical manifestations. Both genetic and environmental factors are involved in its etiology; the high heritability (approximately 80%), however, suggests a marked genetic influence, which has stimulated a large number of studies in an attempt to identify genes involved in ASD. Results from GWAS showed that novel biological pathways, such as signaling and cellular communication, could be associated to ASD. Among neural cell adhesion molecules, integrin beta 3 (ITGB3), which mediates cell-cell and cell-matrix adhesion, seems to be an interesting component. Studies demonstrated their interaction with serotonin, leading to changes in plasma levels of this neurotransmitter. Changes in concentration of plasma serotonin are one of the most consistent results in ASD pathophysiology; therefore, ITGB3 gene, responsible for ITGB3 expression, is a suitable candidate for association studies in ASD samples. The aim of this work was to investigate the possibility of association between
Methods
The sample included 209 ASD children and their biological parents. All probands were diagnosed according to DSM-IV criteria, being fulfilled also ASQ and CARS scales. Each patient was further assessed by a questionnaire, to get information on demographic data, child behavior characteristics and family history of diseases. DNA was extracted from whole blood by salting out. Genotyping of five SNPs in ITGB3 gene (rs2317385, rs58918, rs15908, rs1260385, rs3809865) was performed by TaqMan allelic discrimination method. The software FBAT was applied to test for association hypothesis. The influences of the analyzed SNPs on specific disorder symptoms were assessed using logistic regression models. The significance level was 0.05 in all analyses.

Results
Most of our sample consisted of boys (81.3%) of European descent (76.4%), being the mean age 9.86 years. All SNPs were in Hardy-Weinberg equilibrium. The family-based analyses of individual SNPs did not detect any significant result, although a borderline P value for an increased transmission of A allele at rs15908 was detected (p=0.067). The analyses of most common symptoms showed associations of rs1260382 with echolalia (OR=2.737; p=0.008), rs5918 with aggression (OR=2.931; p=0.026), and rs2317385 with seizures (OR=2.656; p=0.031).

Discussion
According to these results, the ITGB3 gene seems to be involved with ASD and some specific clinical characteristics of ASD patients. Haplotype and bioinformatics analyzes will be conducted, attempting to better understand the possible role of this gene in the disease. Data should also be replicated in other samples, to confirm the involvement of ITGB3, thus this biological pathway, in autism spectrum.

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CONCOMITANT PRESENCE OF A BALANCED TRANSLOCATION T(4;14)(Q31.3;Q24.1) AND A MICRODUPICATION AT XP22.32-P22.31 IN A PATIENT WITH AUTISM

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Background
Autism, a childhood-onset neurodevelopmental disorder, is characterized by deficits in communication, impaired social interactions, and restricted interests/behaviors. The reported prevalence of autism is various depending on the diagnosis criteria. The commonly described number is 0.6-0.8% worldwide in preschool children with male predominance at a ratio about
 Genetic factors contribute to the incidence of ASD evidently, the highest heritability of autism was estimated about 90% in twin study; however, the genetic profile of autism remains unclear. Different aspects have been covered in genetic studies to understand the underpinnings of autism but, so far, no determined conclusions have been made due to the highly complex and heterogeneous features of study outcome. In our study design, we used various methods to discover possible pathogenic genetic factors of autism. In this study, a patient with autism who had a balanced translocation t(4;14)(q31.3;q24.1) and a Xp22.32-p22.31 microduplication was identified and these genomic alterations were further characterized.

**Methods**

We used karyotype analysis to screen our cohort of patients with autism. The breakpoints of translocation t(4;14)(q31.3;q24.1) were pinned down by fluorescent in situ hybridization (FISH) and physical mapped by long-range PCR and PCR-based autosequencing. Affymetrix Human Genome-Wide SNP 6.0 array was used to identify copy number variations (CNVs) in the patient and the suspicious CNVs were validated by real-time qPCR.

**Results**

We identified a balanced translocation t(4;14)(q31.3;q24.1) in a 18-year-old boy with autism. The clinical assessments showed that the boy has symptoms of autism and ADHD and his IQ was within the lower level of normal range. His parents did not demonstrate any apparent autistic symptoms or other psychiatric symptoms. FISH analysis pined down two breakpoints of this chromosomal rearrangement at 4q31.3 and 14q24. Furthermore, long-range PCR and PCR-based autosequencning revealed the physical positions of breakpoints at Chr4: 143604914 and Chr14: 67618633 that disrupt the gene INPP4B within intron 2 and the gene GPHN within intron 18, respectively. The same breakpoint mapping methods conducted on DNA samples of parents showed that the translocation was paternally inherited. Array-based CNV analysis did not reveal any obvious copy number change resulting from the translocation in the proband. Total 5 gene-involved CNVs were found, the size range was from around 100kb to around 726kb. The most conspicuous CNV was a microduplication of ~0.7Mb at Xp22.32-p22.31, which was the largest gene-contained fragment comprising three genes. Two genes, NIR4770 and VCX3A, were located within the duplicated region, and the NLGN4X gene was disrupted by the breakpoint of the microduplication. The results of real-time qPCR confirmed that the microduplication was transmitted from his unaffected mother; moreover, the gene NLGN4X was disrupted on intron 3.

**Discussion**

Our results suggest that the concomitant presence of both the balanced translocation and the microduplication might be associated with autistic symptoms in this boy. The genetic disruptions may result in loss of function of these two affected genes. In view of the neurobiological function of GPHN and NLGN4X, we suggest the concurrence of the dysfunction of both GPHN and NLGN4X might contribute a significant role to the pathogenesis of Asperger’s symptoms. Our data support that autism is an oligogenetic disorder and recessive compound hemizygosity model might be an important mechanism underlying the genetic complexity of autism.

**DISTINGUISHING AUTISM SPECTRUM DISORDERS FROM OTHER DEVELOPMENTAL DELAYS USING BLOOD RNA-SEQ**
Background
There is an unmet need for objective biomarkers to assist clinicians in the early diagnosis of childhood neurodevelopmental disorders. A number of investigators have reported changes in blood gene expression associated with autism spectrum disorders; Voineagu reviews this literature, while Glatt et al describe a microarray blood gene expression classification signature for distinguishing children with autism spectrum disorders from typically developing children.

Methods
The CHARGE (CHildhood Autism Risks from Genetics and the Environment) study recruited children between the ages of 2 and 5, some of whom were diagnosed on the autism spectrum, and others with other developmental delays. Subjects were grouped based on thresholds of the ADOS, ADI-R, Vineland and Mullens test into autism spectrum disorder (ASD) and other developmental delay (DD) groups to approximate the clinical use case of a secondary screen for autism in children suspected of neurodevelopmental disorders. Blood samples were acquired from each subject in RNAstabilizing PAXgene tubes. RNA was isolated and processed using the TrueSeq sequencing prep with poly- A selection for mRNA. RNASeq was then performed on an Illumina HiSeq 2000 Sequencer using 1/3 lane per sample. 174 ASD and 96 DD samples passed final QC, for a total of 270 samples. Sequence data were processed through the Tuxedo RNASeq pipeline to yield counts per gene, which were normalized by downsampling. The sample was divided into a training set (n= 153) and a holdout set (N=117), each of which was repeatedly randomly subsampled to achieve gender and age balance between the ASD and DD groups. On each iteration, informative features were selected by t-test and a support vector machine classifier was trained on a balanced subsample of the training set and tested on a balanced subsample of the holdout set; AUC’s (area under the ROC curve) were averaged across iterations.

Results
The mean AUC for the holdout set was 65.6 +/- 2.9%. When a 90% sensitivity threshold was selected on the classifier risk score, the mean specificity was 25.3, with 95% CI [13.6, 40.6%]. Gene categories found significant by ranksum test on the t-statistic include RNA processing, cell cycle, immune and inflammation-related GO categories.

Discussion
To our knowledge this represents the first report of a classification signature for ASD vs. DD using blood RNASeq. While the understanding of genetic contributions to autism spectrum disorders has been making impressive progress in recent years, genetic causes are individually rare, and are thus not sensitive in a diagnostic context. A gene expression signature with moderate AUC has potential clinical utility as a sensitive assay for identifying children at risk for ASDs within a population that is already suspected of neurodevelopmental disorders. Planned followup studies include a multicenter clinical study to further refine and validate a blood-based assay.
AUTISM CANDIDATE GENE IDENTIFICATION IN FAMILIES WITH MULTIPLE AFFECTED SIBLINGS

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Background
Autism is a common behaviorally defined neuropsychiatric disorder characterized by varied degrees of impairment in communication, social interactions, and repetitive behaviors. Despite broadness of definition autism is considered as one of the most heritable neurogenetic disorders with heritability higher than 90%. Recent studies of non-familial cases have identified novel, rare and highly penetrant de-novo CNVs and protein coding single nucleotide variants associated with autism. In aggregate the identified genes accounting for less then 25% of cases. It has been estimated that as many as 1000 genes may be responsible for autistic phenotype indicating that additional genes remain to be identified. Most of the causal genes identified so far are the result of de-novo mutations. Family studies indicate that inherited genes play important role in autism as well. Traditionally linkage analysis and positional cloning have been most successful in identifying rare, highly penetrant mutations. In autism, linkage analyses have identified more then 90 highly suggestive loci encompassing more then 10% of the genome but the attempts to identify causal variants in linkage regions have been unsuccessful. Identification of genes responsible for familial autism is important as genetic architecture of familial autism might differ from de-novo autism and understanding of the allelic spectrum of heritable variants might have important epidemiological implications. Exome sequencing offers new approach for identification of rare heritable variants. For each person the exome on average has less than 200 private (not seen before) protein altering mutations. This allows for mutational cloning as a way of identifying limited number of candidate variants in families with multiple affected cases.

Methods
Families: One family with three, three families with four and one family with five affected siblings identified in NIMH autism collection.
Exome sequencing: Nimblegen V2 capture and Illumina HIseq2000 sequencing at UW-GS.
Total of 20 cases in 5 families were sequenced.
Data processing: BWA read mapping and GATK variant calling at UW-GS.
Variant annotation: ANOVAR.
Frequency filtering: dbSNP132, 1000 genomes and ESP6500.
Bioinformatics analysis analysis: GERP, SIFT, PolyPhen.

Results
Cases sequenced – Average read depth 55, greater than 90% of exome covered with >10 high quality reads
Variant calling – 23,000 to 26,000 variants per subject
Coding functional variants – 9,100 and 10,500 coding functional variants per subject
Private, coding and functional variants (private = not present in dbSNP, 1000 genomes or ESP6500) – 88-165 variants per subject. For each family 6-27 genes are shared.
Rare, coding and functional variants (rare = not present in dbSNP, MAF< 0.01 in 1000 genomes
or ESP6500) – For each case 250-410 variants were identified. For each family 24-65 genes are shared.

Rare, coding and functional variants that are conserved and predicted to be damaging (GERP>2.0, SIFT>0.95, Polyphen>0.85) – For each case 31-59 variants were identified. For each family 3-28 genes are shared.

Genes with private, conserved and damaging variants overlapping with differentially expressed genes from Voineagu et al. (2011): FOXO1A, GADD45B, MSI2, PALLD, PLEKHC1, SDC2, YAP1, ZC3HAV1, ZFP36L.

**Discussion**

We have used exome sequencing in multiplex families with three to five affected siblings to identify novel autism candidate genes. Our approach that includes very stringent frequency filtering resulted in identification of 6-27 candidate genes per family. Approach that relaxes filtering criteria and adds bioinformatics analysis of conservation and protein function result in similar number of variants (3–28). Intersecting variants identified in our study with candidate genes identified in expression analysis narrows the candidate gene list further. It is difficult to establish ideal stringency criteria for frequency filtering but experience with Mendelian disorders indicates that filtering that results in approximately 200 private, coding and functional variants might be optimal. In the similar manner, guidelines for bioinformatics analysis of variant conversation and effects on protein function are only emerging. Our preliminary analysis and combining our approach with results of differential gene expression in autism identified nine candidate genes in 5 families. Additional bioinformatics approaches might identify different candidate genes list. Ultimately candidate genes identified in large multiplex families need to be tested for association with autism in larger gene based case control studies.

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**ON INTERACTION TERM IN LINEAR REGRESSION WITH CATEGORICAL DEPENDENT VARIABLES**

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**Background**

Classical regression with interaction term is one of the basic methods in determining common effect of two or more variables on dependent variable. Study of gene-environment interaction in genetic studies is a classical example for the application of regression with interaction term. Assessing gene-environment effect can be done using simple model

\[ y = d_0 + d_1 \text{Gene} + d_2 \text{Env} + d_3 \text{Gene} \times \text{Env}, \]

(1) where \( y \) is dependent variable (disease), \( \text{Gene} \) is categorical variable and \( \text{Env} \) is any variable corresponding to environment. In most classical applications \( \text{Gene} \) variable has two of three categories. Significant nonzero product terms’ coefficient \( d_3 \) corresponds to the existence of interaction (gene-environment) effect.

**Methods**

In this study we will discuss why interaction can be modeled as a product term and also discuss cases when use of product term can be inappropriate. In the case of binary coded gene variable linear regression with interaction term estimates interaction effect and significant interaction term coefficient reflects slope difference between two categories of gene variable. In this case
slope difference characterizes interaction effect of gene and environment variables. When having three or more category gene variable interaction term’s significant coefficient and the term itself can erroneously indicate the existence of an interaction effect. We give examples showing this misleading effect when using multicategorical gene variable. This effect is caused by insufficient number of coefficients in (1). For example, with three category Gene variable (1) has four unknown coefficients, but for each fixed level of Gene variable regression lines between y and Env variable have two unknowns (intercept and slope) totaling as six unknowns.

Results
We proved that projections of regression lines for three different Gene levels cross at one point and slope differences between regression lines are same, say if Gene levels are 0, 1 and 2. The next figure shows simulated three level Gene variable and y values mostly lined on one line for each level.

As we can see lines of four parameter model (1) do not match real sample points. They cross even the original simulation lines (six parameters model) do not cross. The lines are in the slope increasing order for Gene levels 0, 1 and 2, but original lines increase from level 0 to level 1 and then decrease from level 1 to level 2. Exact alternative formulas capable to express interaction effect with three and more category variables are derived. For example, in the case of three categories Gene variable we show that the appropriate (or equivalent) formula has the form $y = k_0 + k_1 \cdot Env + k_2 \cdot Gene + k_3 \cdot Gene \cdot Env + k_4 \cdot Gene^2 + k_5 \cdot Gene^2 \cdot Env$.

Discussion
Interaction term’s significant coefficient and the term itself can erroneously indicate the existence of an interaction effect when using product term as interaction term. Our examples show this misleading effect. This effect is caused by insufficient number of coefficients in (1). In addition, we present generalization of formulas for multicategorical interaction variable with nonlinear Env terms and interactions. Since significance of interaction effect cannot be directly derived from the significance of regression coefficients we provide an algorithm, formulas and test scheme to check for existence of interaction effect of additive interaction variable and environment based on contrasts.

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SUB-PHENOTYPIC MANIFESTATIONS OF BRAIN EXPRESSED COPY NUMBER VARIANTS IN AUTISM SPECTRUM DISORDERS AND SCHIZOPHRENIA
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Background
Emerging evidence on the association between copy number variants (CNVs), a type of DNA structural variation, and neuropsychiatric disorders provides a new vista on understanding unique and pleiotropic susceptibility to neuropsychiatric disorders such as Autism Spectrum Disorders (ASD) and schizophrenia. Specific CNVs have been associated with a range of phenotypic manifestations that characterize several neurodevelopmental disorders including ASDs, schizophrenia, bipolar disorder and attention deficit disorder. Therefore, rather than the traditional approach of attempting to identify genes for particular diagnoses, we investigate CNVs affecting brain expressed genes as risk factors for “sub-phenotypes”, or core features and
clinical correlates of these conditions.

Methods
Rare CNV and detailed phenotype data were derived from the Autism Genome Project (n=1590 cases) and Irish schizophrenia cases (n=396). Patients were classified by the presence or absence of a rare CNV that impacts genes previously implicated in: 1) ASDs or intellectual disability (ASD/ID) (Pinto, et al., Nature, 2010); or 2) that are differentially brain expressed (Raychaudhuri, et al., PLoS Genetics, 2010). Phenotypes collected include clinical characteristics and severity, measures of IQ and adaptive function, parental age at birth and other family factors. Association with candidate neurodevelopmental phenotypes were examined in each disorder sample, and then in a combined sample. Random forests and mixture models were used to explore whether phenomic features identify CNV-defined sub-groups.

Results
Paternal age was associated with deletions in brain expressed genes in both disorder samples, as well as in the combined sample. Maternal age was also associated with brain expressed deletions in the combined sample, but not in the individual disorder samples. However, maternal age was associated with brain expressed CNVs overall in the ASD sample, but not the schizophrenia sample. ASD/ID-implicated CNVs were associated with sub-phenotypes that tapped communication and language in the ASD sample. In the schizophrenia sample, CNVs were 50% less common in those with a family history of mental disorders. In the combined sample analyses, a statistically significant association between ASD/ID duplications and verbal IQ was found, but none of the other IQ measures were statistically significant.

Discussion
The paternal age association with deletions in brain expressed genes in both disorder samples, as well as in the combined sample, suggests a general influence of advanced paternal age on neurodevelopmental disorders broadly. The fact that CNVs were 50% less common in those with a family history of mental disorders in the schizophrenia sample may indicate that family history may index heritable genes rather than de novo mutations. ASD/ID-implicated CNVs were associated with sub-phenotypes that tapped communication and language in the ASD sample, which suggests some specificity to the core features of ASDs. These analyses demonstrate the importance of investigation of both common and unique genetic and environmental factors that may underlie discrete disorders such as schizophrenia and ASDs, as well as sub-phenotypes that may represent more direct links with biologic and genetic pathways underlying these disorders. Unfortunately, due to the nature of the disorders, similar phenotypic measures were not collected in ASD and schizophrenia samples, apart from general IQ measures and parental age at birth. This is a challenge for cross-disorder research, and collecting shared phenotypic measures should be a priority for future research.

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RE-CALIBRATING TEST STATISTIC DISTRIBUTIONS WHEN TESTING RARE GENETIC VARIANTS
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Background
Genome-wide interrogation of rare single nucleotide variants (SNVs) via high-throughput sequencing and custom chip arrays has become a standard method of genetic analysis. A central issue for SNV testing surrounds the interpretation of test statistic distributions when the majority of SNVs do not meet asymptotic assumptions of chi-square distributed test statistics. In turn, the detection of potential confounding elements in genotype data can be compromised.

Methods
Using control genotype data (n=2,884) from the Illumina exome chip, we characterize the test statistic properties of commonly used association tests and study designs when the majority of SNVs are rare. We then demonstrate that the use of permutation to empirically re-calibrate the null distribution of test statistics enables clear interpretation of the distributional properties of the data. To evaluate the effectiveness of this method, we simulated phenotypes confounded by various degrees of population stratification to see how well a permuted null distribution is able to pick up deviations from the null expectation.

Results
In general, the abundance of rare variants generates a distribution of test statistics in each test that do not behave with asymptotic assumptions of the single degree of freedom distribution, generally leading to a deflation in QQ plot distributions of resulting p-values. By incorporating a permuted null distribution, we are able to re-calibrate the null expectation towards a more accurate comparison to the observed test statistic distribution. For detecting effects of population stratification, the re-calibration procedure was effective for clear cases of stratification, however low levels of stratification were often un-detected using the new method.

Discussion
By using an empirical distribution of test statistics generated via permutation, we are able to re-calibrate the null distribution to reflect the observed properties of test statistics when asymptotic assumptions are not met. This method has the advantage of testing all variants, or any particular set of variants of interest, as it is conditional on the observed allele frequency spectrum within a given dataset. In cases where rare variation may be confounded by population stratification or technical artifacts, conditioning quality control checks solely on common variants may miss these effects. Furthermore, this method can be generalized to gene-based tests and collapsing methods, as permutation is applicable across any testing process.

A PERMUTATION SCHEME FOR RARE VARIANT TESTS WITH THE CORRECTION FOR POPULATION STRATIFICATION
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Background
Recent advances in genotyping and sequencing technologies have made detecting rare variants in large cohorts possible. Various analytic methods for finding disease associated rare variants have
been proposed, including C-alpha (Neale et al. 2011) and SKAT (Wu et al. 2011). However, most of these methods assume a homogenous population, which is unlikely to be true with ever increasing sample sizes. Not correcting for population stratification causes inflated p-values and false-positive associations. Here we propose a new permutation scheme that can be applied to most rare variant tests, which can correct for population stratification. This permutation scheme permutes samples based on their genetic distances so samples are more likely to be shuffled with other samples from within their own ethnicity. Using the simulated data, we have shown that this permutation scheme can successfully correct the p-values from association tests.

**Methods**

Population stratification was simulated using 6,197 samples from the Swedish control cohort (Rikke et al. 2013). Principal components were generated using LD pruned common autosomal SNPs with the minor allele frequency cutoff of MAF>5% and pairwise R2<0.5. For quantitative traits, we assume the first principal component explains 0.5% variance. For the case/control study, we assigned the samples to 2 clusters based on the 1st principal component. We randomly label 30% / 70% of samples in cluster 1 as cases/controls, and 40% / 60% of samples in cluster 2 as cases/controls. We constructed a genetic distance matrix for the samples using principal components and the samples were permuted based on their genetic distances. We implemented this method in PLINK (Purcell et al. 2007) and compared its performance with the following methods 1) Basic association test using the asymptotic chi square distribution with no population stratification correction 2) Logistic regression with the first principal component as a covariate 3) EMMAX (A mixed model association test)

**Results**

We use the QQ plot as a measure of the genomic inflation. The genomic control factor is not a good measure of the genomic inflation in this study because it only looks at the median p-value. Due to the large number of rare variants, the null distribution of p-values is not necessarily on the diagonal line. We therefore first generated the null distribution using shuffled phenotypes. Deviation from this null distribution shows a genomic inflation. Results using the simulated datasets show that our permutation method performs as well as EMMAX to correct for the population stratification. Despite the disadvantage of computation speed, our permutation method is model free and can be applied to many existing rare variant tests to correct for population stratification.

**Discussion**

We have demonstrated a permutation based method that is able to correct for population stratification in association tests. This method is compatible with both case/control studies and quantitative traits, and can be easily modified to work with existing rare variant tests.

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**INTERACTION BETWEEN POLYGENIC SCORES FOR SCHIZOPHRENIA AND INFECTION BY HERPES SIMPLES VIRUS 1 AND 2**

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Background

Ever since the aftermath of the 1918 influenza pandemic when psychoses with schizophrenia-like symptoms were linked to the influenza virus, the idea of a viral contribution to the aetiology of schizophrenia (SZ) has emerged from time to time. By now there are many well documented reports of the association of other viruses to SZ and our group recently found genome wide supported evidence of interaction with maternal infection by cytomegalovirus (CMV), a virus in the herpes family, and a SNP in the gene CTNNA3 on chromosome 10. The polygenic score introduced by Purcell and colleagues is an attempt to summon up the genetic liability in one single simple score. Selecting pruned markers based on a threshold for p-values in the training data set, the score is basically the sum of individual allele counts for these markers weighted by log of the odds ratio of each marker in the training set. Today it has proved itself as quite a good predictor of disease risk. Using the polygenic score for schizophrenia trained on the 42 data sets in PGC2-Schizophrenia complement to our own GWAS data, we conducted a study of gene-environment interaction with herpes simplex virus 1 and 2 (HSV1 and 2) and CMV.

Methods

The GWAS sample is a population based, matched case-control sample. Cases consist of all Danish citizens born from May 1981 who has a SZ (ICD-10 F20) in the Danish Psychiatric Central Registry as of May 2007. Controls were incidence density matched 1-1 to cases by gender and birth date and selected among those who were not of the same mother as the proband, resident in Denmark, alive and without a SZ diagnosis at the time the proband got hers/his first diagnosis. The Danish Newborn Screening Biobank provided neonatal dried blood spot samples from which DNA was extracted, whole-genome-amplified and subsequently genotyped on the Illumina Infinium HD Human610-Quad BeadChip. The data underwent standard PGC QC. On a subsample the same blood spots were tested for IgG antibodies to the three viruses. It should be noted that the blood samples were taken when the neonates were just 5-7 days old at which age a child has not produced any significant amount of IgG antibodies. On the other hand while in utero the maternal IgGs can pass the placental barrier. Hence, the antibodies measured are mainly of maternal origin. The measurements were dichotomized at a cut-off yielding a prevalence consistent with those measured in European populations. Standard likelihood ratio test of interaction was conducted using conditional logistic regression while adjusting for population stratification using for principal components.

Results

Polygenic scores and IgG data was present for altogether 487 cases (216 females and 271 males)
and as many controls. Of the 487 controls 57% had a mother positive for HSV1 while this was the case for 60% of the cases. For HSV2 and CMV theses numbers were 11%/16% and 71%/73%. In this preliminary analysis, the PGC2 polygenic scores corresponding to p-value thresholds of 5e-08, 1e-06 and 1e-04 all exhibited interaction with the HSV1 and 2, albeit not consistently, but none of the scores for higher thresholds showed any interaction with HSV1 and 2 and none of the scores interacted with CMV. The most robust result was the interaction between the 1e-06 polygenic score and HSV1 with an interaction incidence ratio ratio IRR=0.32, 95%-CI: (0.14 - 0.74), p=0.007.

Discussion
While the polygenic score is not localized to one functional unit in the genome but summarizes effects scattered over the genome, it is not possible to provide a functional interpretation of what may be going on at a cellular or molecular level. However, it does provide an indication that on a population level, the risk for SZ inferred by the polygenic score differs depending on maternal infection status with respect to HSV1 and 2. Such insight may eventually point at potential interventional measures, but first this rather small study must be replicated in larger samples. Moreover, a better understanding of the polygenic score in this more complex setting of GxE interaction might enable us to draw more definite conclusions.

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EVALUATING THE FIRST YEAR OF A UNIQUE SPECIALIST CLINICAL PSYCHIATRIC GENETIC COUNSELING SERVICE: UPTAKE AND IMPACT

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Background
On February 1, 2012, we launched the world’s first (to our knowledge) specialist clinical psychiatric genetic counseling (GC) service of its kind, for individuals with non-syndromic psychiatric disorders and their families. The clinical model involves all clients participating in a single GC session, followed 1 month later with a telephone call, during which any new or remaining issues are addressed. We present data from the first year of operation.

Methods
We used two instruments as clinical assessment tools to establish issues for discussion: the GC Outcomes Scale (GCOS, which measures empowerment) was completed by all clients, and those with mental illness also completed an illness management self-efficacy (ISME) scale. Both were administered at the beginning of each GC session (T1) and the routine 1-month follow-up telephone call (T2). Clients were asked for consent to allow use of these clinical data for research purposes.

Results
Between Feb 1 2012 and Jan 31 2013 we provided psychiatric GC to n=111 clients, of whom 6 were unable to consent (e.g. due to acute psychosis). Of the remaining 105, 73 (69.5%) consented to their clinical data being used for research purposes. Of these, most (86.3%) were female, self-referred (75.3%), and had a personal history of mental illness (65.7%). Between T1
and T2: mean GCOS scores increased significantly (T1: M =108.19, T2 M=127.47) t(63) = -7.973 p < 0.0001 demonstrating increased empowerment (effect size, d=1, 100% power at \( \alpha=.025 \)), and mean ISME scores increased at the trend level (T1: M=7.01, T2: M=7.37) t(36) = -1.761 p = 0.087, suggesting increased self-efficacy (d=0.33, 35% power at \( \alpha=.025 \))

**Discussion**

Among people with psychiatric disorders and their families, GC increases empowerment with large effect, and self-efficacy with small/moderate effect. These naturalistic data provide a rationale for prioritizing the establishment of specialist psychiatric GC services at other centers.

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**COPY NUMBER VARIANTS AND THEIR IMPACT ON GENETIC COUNSELING FOR MENTAL DISORDERS**

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**Background**

Several recent studies found rare and *de novo* Copy Number Variants (CNVs) to be highly associated with Bipolar Disorder, Schizophrenia and Autism Spectrum Disorders (ASD), as these structural variants have high genetic penetrance and pleiotropic effect.

**Methods**

The statistical concepts Attributable Risk in Exposed individuals (EAR) and Population Attributable Risk (PAR), as well as Bayesian probability of risk of illness, can potentially be used in clinical counseling.

**Results**

Calculation of illness risk for rare and *de novo* CNVs is higher than for the top SNPs from Genome-Wide Association Studies (GWAS), with an overall risk about 14% to develop any of these disorders for those individuals having *de novo* CNV, compared to the 1.01% to 1.10% risk yielded by the top GWAS-significant SNPs in Bipolar and Schizophrenia. Specific rare CNVs can bear very high risk to develop any of these disorders up to 82% for locus 22q11.21 and 20% for 16p11.2.

**Discussion**

A substantial minority of patients with bipolar disorder, schizophrenia, and ASD carry high-impact detectable genetic events. These results lead us to raise strategies related to patient communication and genetic counseling, including a psychotherapeutic approach that should be part of clinical practice, once demand for this type of testing develops.

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**LOW ACTIVITY ALLELES OF MAOA GENE ARE ASSOCIATED WITH MEASURES OF HOSTILITY**

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Background
Catecholaminergic systems are involved in the regulation of aggressive behavior. Most of the available evidence indicates that norepinephrine and dopamine lower the threshold for an aggressive response to environmental stimuli. A major enzyme responsible for catecholamine catabolism in the brain is monoamine oxidase A (MAOA), together with catechol-o-methyl transferase (COMT). The transcriptional activity of the MAOA gene is governed by a common functional polymorphism, a repeat polymorphism in the promoter region. Functional characterization in a luciferase assay demonstrated that the alleles 3.5r and 4r were more active than the 3r and 5r allele (Cohen et al. 2003), though more recent evidence suggests that only the 4r allele is associated to a higher expression of the gene (Guo et al. 2008). If aggressive behavior is enhanced by catecholaminergic activity, then the lower activity of MAOA (resulting in a slower inactivation of catecholamines) should indirectly enhance aggression. This prediction has been supported by most (but not all) observations in rodents and humans.

Methods
In the present sample we evaluated a large sample of male prisoners (n=752) genotyped for the MAOA repeat polymorphism. According to most recent evidence (Guo et al. 2008), we considered as low activity alleles 2r, 3r and 5r, while the 4r allele was considered the high activity allele. All subjects were evaluated for measures of aggression, hostility and anger by standardized scales and indirect indexes such as other convictions, violent behavior in jail and personality traits. Presence of a psychiatric disease was evaluated as well with a structured clinical interview according to DSM-IV criteria (SCID-I).

Results
Low activity alleles were associated with the presence of psychiatric disorder (p=0.025). Carriers of the low activity alleles, were found having low levels of “Indirect aggression” (p=0.03) and “Suspiciousness” (Buss-Durkee Hostility Inventory) but higher levels of “psychoticism” (Eynseck Personality Questionnaire) and more frequent previous convictions (p=0.041). Moreover, controlling for psychiatric status, total hostility score was significantly higher in carriers of the low activity alleles (p=0.035).

Discussion
These findings, support a role of MAOA in hostility and risk for psychiatric disease, with high activity alleles playing a protective role. The small significance of our result may be explained by the specificity of our sample, composed by only male prisoners. Indeed, in a such sample, traits of aggression and hostility are likely to be more frequent than in other populations.

ASSOCIATION OF GDNF GENE VARIANTS WITH AGGRESSION IN THREE ETHNIC GROUPS FROM INDIA
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Background
Aggression is a natural adaptive human instinct, a strategy for gaining resources, eliminating rivals, and improving one’s chances of genetic survival. Successful aggression can thus have a rewarding effect and taps the reward related neurotransmitter systems. However, human aggression is a significant health and social problem. A wide range of studies have indicated that dopamine release in the nucleus accumbens is responsible for these rewarding effects. Glial cell line-derived neurotrophic factor (GDNF) is an essential growth factor for the survival and maintenance of midbrain dopaminergic neurons. Even though several studies demonstrated association between the reward pathway and aggression, to our knowledge this is the first association study between GDNF and aggressive behavior thus far.

Methods
This study was carried out in a sample of 700 young adults (age range 18-35) in three ethnic groups from the North-Eastern region of India: Bengali (N=200, Caucasian), Hmar (N=200, Mongoloid), Khasi (N=300, Mongoloid). Data on aggression was collected using a structured questionnaire, as well as personal interview. The level of aggression was measured by self-reported methods including Buss and Perry Aggression Questionnaire. DNA was extracted from buccal swabs and genotyping of GDNF polymorphisms rs3812047, rs11111, rs2910702, rs1549250, rs1981844 was carried out by RT-PCR using TaqMan probes.

Results
In case of rs2910702 individuals with the minor allele (G) scored higher on the Buss and Perry Aggression Questionnaire (BUPE) as compared to individuals with the A allele (allele-wise: p=0.007, score with G allele: 87.83±15.1, score with A allele: 84.53±14.9). The same pattern was observed for each ethnic group (p=0.031 for genotype, ethnicity, scale interaction). BUPE has four subscales: Anger, Physical anger, Hostility and Verbal Anger. In a post hoc study analyzing the subscales, individuals with the G allele of rs2910702 scored also higher on the BUPE anger subscale (p=0.01); physical anger subscale (p=0.007); verbal anger subscale (p=0.18); for the Hostility subscale the individuals with the G allele had higher scores, but that was not significantly different.

Discussion
The G allele of rs2910702 in the GDNF gene seems to be the risk allele for Aggression in all the three ethnic groups studied. Neurobiological studies with rodent have shown dopamine and reward circuitry involved in aggression (Couppis and Kennedy, 2008; Beiderbeck et al., 2011). GDNF plays a significant role in the development and maintenance of dopaminergic neurons (Lin et al). The present study indicates that GDNF involved in human aggression.
Background
Associations between different measures of psychosis proneness have consistently been observed in both clinical psychiatry and psychiatric epidemiology studies. This link is usually suggested to be due to shared underlying biological processes between traits, despite that to date, there is limited evidence for this. Here we aimed to disentangle the underlying genetic architecture of psychosis proneness measures, through a series of comprehensive analyses of genomic data.

Methods
This study comprised 3268 individuals participating in the Northern Finland Birth Cohort 1966 study with both genomic and phenotypic data available. Psychosis proneness was evaluated in the participants using the psychological scales for: perceptual aberration (PAS), physical anhedonia (PHAS) and social anhedonia (SAS). Genome wide genetic data was available for ~365K SNPs. Phenotypic and genomic data were analyzed in three steps: univariate heritability estimates were obtained through novel IBD-based estimation methods; similar bivariate analyses were developed to obtain both genetic and environmental correlations between psychosis traits; and a series of bivariate genome-wide association analyses were developed using canonical correlation methods.

Results
Heritability estimates for the psychosis proneness scales ranged from 13% to 28%. Phenotypic correlations between traits ranged from -0.03 to 0.43, while genetic and environmental correlations did from 0.20 to 0.55, and from -0.08 to 0.40, respectively. Pair-wise GWAS analyses revealed genome wide significant loci (p-value<5·10^{-8}) on chromosomes 1q32.1, 5p13.1, 5q35.1, 6p25.2, 6q22.1, 7q11.2, 9q22.3 and 11q23.2 as underlying the association between PAS and SAS.

Discussion
This study identified a shared genetic background between PAS and SAS and between PHAS and SAS that accounts for an important part of their respective trait correlation. Preliminary genomic evidence was observed, to explain the association between PAS and SAS. These results implied that qualitatively similar biological factors underlie different psychosis proneness measures, which may provide novel insights for genetic studies of schizophrenia, depression and bipolar disorder.

NOVEL CANDIDATE GENETIC MARKERS AND CIRCADIAN PHENOTYPES IN A SOUTH AMERICAN SAMPLE
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Background
Molecular study of circadian rhythms in humans could be an excellent approach to understand the relation between genes and behavior. Polymorphisms in human clock genes have been evaluated as potential factors influencing circadian phenotypes in several populations. There are conflicting results for the association of classical markers and diurnal preference in different studies. It is possible that variations in genes involved the clock mechanism or synaptic plasticity (such as CLOCK, PER2, PER3, NPAS2, COMT and SLC6A4) could be of particular interest in understanding human circadian phenotypes.

Methods
We analyzed the possible and novel associations of the functional polymorphisms in CLOCK, NPAS2, PER2 and PER3 (Ile395Leu, Ala394Thr, Glu1244Gly and Thr1028Met) and COMT and SLC6A4 genes (Val158Met and 5-HTTLPR) and circadian phenotypes in healthy Colombian subjects. 200 university students were genotyped for these functional polymorphisms. We applied two scales to measure phenotypic patterns of human circadian rhythms: Composite Scale of Morningness (CSM) and Epworth Sleepiness Scale (ESS).

Results
We found a significant association between 5-HTTLPR polymorphism and morning preference score (CSM) (p=0.027) using an overdominant genotypic model and association of COMT Val158Met with daytime sleepiness (ESS scores) (p=0.038) in a genotypic recessive model. These results were supported by differences in genotype frequencies between circadian typologies for SLC6A4 gene (p=0.007) and categories of diurnal sleepiness for COMT gene (p=0.032). No associations were found with polymorphisms in CLOCK, PER2, PER3 and NPAS2 genes.

Discussion
Our results suggest, for the first time, a significant relationship between functional SLC6A4 and COMT polymorphisms with specific human circadian phenotypes: morning preference and diurnal sleepiness. These results need to be replicated in other populations. Further study of functional polymorphisms in other synaptic genes could be of relevance for the identification of novel candidate genes for circadian phenotypes, and related endophenotypes of neuropsychiatric importance, in healthy humans. Novel functional polymorphisms in CLOCK, PER2, PER3 and NPAS2 genes need further studies in other samples.

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HYPERMETHYLATION OF THE SLC6A4 GENE IN THE PERIPHERAL BLOOD OF PATIENTS WITH CHRONIC AND FIRST-EPISODE SCHIZOPHRENIA
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**Background**
Schizophrenia and bipolar disorder have been extensively studied through genetic approaches, but genetic factors associated with the diseases alone cannot explain their high heritability. Accumulating evidence suggests that epigenetic regulations play an important role in the pathophysiology of the psychiatric disorders. We have previously shown hypermethylation of the promoter region of SLC6A4, which encodes serotonin transporter, in peripheral blood and postmortem brains of patients with bipolar disorder. Here, we examined DNA methylation status of the same region of the SLC6A4 in the peripheral blood of patients with schizophrenia.

**Methods**
Genomic DNA was extracted from peripheral blood cells (PBC) of patients with chronic schizophrenia (N=100) and age- and gender-matched controls (N=100). Genomic DNA extracted from PBC of patients with first-episode schizophrenia (N=17) was also used. All subjects were Japanese. After bisulfite modification of genomic DNA, the promoter region of SLC6A4, which was reportedly hypermethylated in bipolar disorder, was PCR-amplified. DNA methylation level was determined by Pyrosequencer.

**Results**
Significant difference was found on one CpG site between patients and controls. The mean methylation level was 33.0% in schizophrenia and 30.6% in controls. We also found significant effect of sex on DNA methylation level of the examined CpGs in controls. Subgroup analysis considering sex revealed that altered DNA methylation was prominent in male patients compared to male controls. Significant hypermethylation in male patients was also observed in the patients with first episode schizophrenia.

**Discussion**
Similarly to bipolar disorder, patients with schizophrenia showed hypermethylation of the SLC6A4 gene. Considering that patients with first-episode schizophrenia also showed hypermethylation, epigenetic alteration in this region might occur at the very early stage of disease. Ongoing studies include the evaluation of the effect of 5-HTTLPR type on the methylation level.

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**THE CONTRIBUTION OF MEDICATION TO DNA METHYLATION LEVELS IN WHOLE BLOOD OF BIPOLAR DISORDER PATIENTS**
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**Background**
Emerging evidence suggest that DNA methylation plays a role in the pathology of psychiatric disorders. At the same time it has become apparent that psychotropic medication has a profound influence on methylation levels. In this cross sectional pilot study we therefore investigated the contribution of psychotropic medication to DNA methylation in whole blood of patients with
bipolar disorder.

Methods
A group of 131 bipolar disorder patients was recruited in the University Hospital Utrecht, Netherlands. Diagnosis, age and current medication use were obtained using the comprehensive assessment of symptoms and history (CASH). Medication type was divided in antipsychotics (typical and atypical), lithium and other mood stabilizers (including valproic acid and other antiepileptics). The methylation data was obtained using Illumina Human Methylation 27K array using standard procedures, normalization and quality control. We investigated global DNA methylation levels and the relationship to medication type using multivariate linear models with the principle component as dependent. In addition individual loci will be interrogated. All analyses were conducted in the Bioconductor framework using the methyLumi and sva packages.

Results
The clinical characteristics of 131 bipolar disorder patients were; Mean age 42.6 years (sd 11.6, range 21-77), female: 56.5 %, antipsychotic use: 38.1%, Lithium use: 65.6%, other mood stabilizers: 32.8%. Preliminary analysis focusing on relative contribution of the determinants to global methylation levels, corrected for gender, showed a large significant contribution of antipsychotic use (p=0.03, B=0.40) to methylation levels. Mood stabilizers, other than lithium, showed an opposite significant effect on methylation levels (p<0.01, B=-0.55). Age (p=0.87, r=0.00) and lithium (p=0.84, r=0.04) had a smaller contribution. Further analysis of specific compounds and analysis of specific loci and pathway will be performed.

Discussion
Our data shows that antipsychotics drug use and mood stabilizers (including valproic acid) have a large significant and opposite contribution to methylation levels. Although the results are cross sectional it points to the importance of this factor in epigenetic studies as a potential confounder and may hint to the potential role of DNA methylation as a mechanisms of action for psychiatric drugs.

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EFFECT OF SODIUM VALPROATE ON SPR GENE EXPRESSION IN A SEROTONERGIC CELL LINE
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Background
Sodium valproate (VPA) is a drug widely used in the treatment and prophylaxis of bipolar disorder and epilepsy. Despite being widely used, the mechanism of action of VPA is still unclear. Although it appears to enhance GABA-mediated neurotransmission, VPA is known to be a histone deacetylase (HDAC) inhibitor and is also reported to cause demethylation in specific genes. We studied the effect of VPA on genes involved in the tetrahydrobiopterin (BH4) biosynthesis pathway. BH4 is an essential cofactor in the biosynthesis of neurotransmitters like serotonin, dopamine and noradrenalin, and we have shown this pathway is important in mood disorders [1-3].
Methods
We used real-time quantitative PCR (qPCR) to study the gene expression changes in response to VPA in cultured rat cell lines. Three different rat neuronal cell lines (RN46A, C6 and H19-7) were treated with various concentrations of VPA for 72 hours and gene expression changes were measured by qPCR. Significant upregulation of the Spr gene, which encodes the enzyme sepiapterin reductase, was observed for all cell lines, especially the serotonergic cell line RN46A. This confirms and extends prior work from our laboratory which first demonstrated this effect in RN46A [4]. We have also tested some of the other major anticonvulsant mood drugs like carbamazepine and lamotrigine, as well as lithium, the most widely used mood stabilizer, for effects on Spr gene expression in RN46A cells. We hypothesized that the HDAC inhibitor activity of VPA leads to chromatin changes in the promoter of Spr, and this causes upregulation of the gene. Chromatin immunoprecipitation assay was used to analyse histone acetylation or methylation status at the Spr promoter after 72 h exposure to VPA. Histone modifications H3K9ac H4K8ac and H3K4me3 were studied using ChIP-qPCR. We also applied bisulphite sequencing to DNA extracted from RN46A cell lines exposed to either VPA or 5’-aza-deoxycytidine (5’azaCdR), a classical DNA methylation inhibitor, to explore the role of methylation changes in Spr induction. We have also initiated an RNA-seq study to detect differential gene expression between RN46A cells before and after treatment with valproate and lithium.

Results
An ~10-fold upregulation in Spr gene expression was observed in the RN46A cell line in response to 72 h treatment with VPA. A similar increase was observed in the C6 glial cell line, but the hippocampal cell line H19-7 did not show any significant change in Spr gene expression. Amongst other drugs, lithium and 5’azaCdR, also caused significant upregulation of Spr. Methylation analysis showed no changes in methylation patterns at the Spr promoter region before and after treatment with VPA suggesting that the observed increase in gene expression is not due to demethylation at the Spr promoter. ChIP-qPCR showed enrichment (% input method) for H3K9/K14ac and H4K8ac at Spr promoter which are both histone marks associated with active transcription. Results of the RNA-seq study are awaited.

Discussion
VPA is a standard and effective first-line treatment in bipolar disorders. Recently, several studies have focussed on the role of epigenetic changes in the pathophysiology of mood disorders and their treatment. As VPA is a well-known HDAC inhibitor and has also been shown to cause active DNA demethylation in certain genes, it is possible that these epigenetic mechanisms might contribute to its mood stabilizing effect. In the present study, we tried to investigate the role of these epigenetic mechanisms in the upregulation of the Spr gene in a rat serotonergic cell line in response to VPA. Methylation analysis suggested that the observed increase in gene expression is not due to demethylation at the Spr promoter. ChIP analysis which showed significant enrichment at H3K9/K14ac and H4K8ac at Spr promoter suggest a role for the HDAC inhibitory activity of VPA in Spr upregulation. The RNA-seq data which are awaited would shed further light on the gene expression profile of VPA (and lithium) and potential mechanism of action of these drugs in bipolar disorders including the involvement of convergent biochemical pathways.
AN INTEGRATED EPIGENOMIC-TRANSCRIPTOMIC-GENETIC ANALYSIS OF SCHIZOPHRENIA BRAIN IDENTIFIES NOVEL MOLECULAR PATHWAYS TO DISEASE

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Background

Schizophrenia (SZ) is a psychiatric disorder characterised by the presence of psychotic symptoms and altered cognition. Although SZ is highly heritable, the molecular etiology of the disease is largely unknown. In addition to genetic and structural genomic variation, recent evidence supports a role for altered epigenomic and transcriptomic processes in pathogenesis.

Methods

DNA and RNA were extracted from a large collection of human post-mortem brain samples: frontal cortex (n=47) and cerebellum (n=47) tissue obtained 23 schizophrenia patients and 24 healthy controls. Genome-wide DNA methylation, expression and SNP profiling were performed using the Illumina Infinium Human Methylation450, HumanHT-12v4 Expression, HumanOmniExpress BeadChips respectively.

Results

Our integrated multi-level analyses provide evidence of SZ-associated DNA methylation and gene expression changes at biologically relevant loci, including GABBR1, RASA3, C8A, NRN1, BNIP3, GAD1 and SERPINA3. Evidence is also found for the presence of cis-eQTLs and cis-mQTLs at SZ candidate genes nominated from published GWAS analyses, an increased burden of CNVs in patients with SZ, and a rare NRXN1 deletion in an SZ patient that was associated with altered DNA methylation. Together these results provide important insight into the biological mechanisms underlying SZ and highlight the value of taking an integrated ‘-omics’ approach to complex disease.

Discussion

Together these results provide important insight into the biological mechanisms underlying SZ and highlight the value of taking an integrated ‘-omics’ approach to complex disease.
INVESTIGATION OF THE SCHIZOPHRENIA AND BIPOLAR DISORDER ASSOCIATED BRD1 PROTEIN INTERACTION WITH HISTONES AND ITS INFLUENCE ON THE GLOBAL HISTONE MODIFICATION PATTERN

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Background
Accumulating evidence from linkage and association studies form strong evidence that the Bromodomain-containing 1 gene (BRD1) is involved in the pathogenesis of schizophrenia (SZ) and bipolar disorder (BD) (Jorgensen et al. (2002) Am J Med Genet; Severinsen et al. (2006) Mol Psychiatry; Nyegaard et al. (2010) Am J Med Genet B Neuropsychiatr Genet). Furthermore, was the BRD1 promoter SNP rs138880 found to be the highest ranking in a replication of a meta-analysis of 18 GWAS in an independent family-based replication study (Aberg et al. (2013) JAMA Psychiatry). Heterozygous Brd1 knockout mice (Brd1+/−) show affective and cognitive deficits as well as psychosis like behaviors. They also respond to anti-depressants, adding additional evidence for a role of BRD1 in the pathogenesis of SZ and BD. The BRD1 protein is known to interact with Histone Acetyl Transferases (HAT) HBO1 and TIP60 and seems to be involved in the acetylation on Histone H3 Lys14 and to a lesser extent on Histone H3 Lys9.

Results
The aim of this study is bivalent. Firstly, we aim at profiling global changes in histone modifications in mouse brain under conditions with decreased expression of the Brd1 gene by means of high resolution nanoLC-MS/MS. By doing so, we can identify major Brd1 dependent modifications on all histones. Preliminary data on 2 test samples (1 wild type (WT) and 1 Brd1+/−) show that we are able to detect all 4 core histones (H2A, H2B, H3 and H4) with between 9 and 20 different peptides of each histone isoform. The peptide coverage varies for different histone variants from 55% for histone H2B type 2E to 82% for histone H4. These numbers could increase by analyzing more samples simultaneously in the full dataset. Methylated and acetylated Lysine as well as mono- and dimethylated Arginine can be detected in a wide variety of combinations by this method. However only a few acetylation sites were specifically investigated in this pilot study: the relative ratio (acetylated/non-acetylated peptides) of H3K14Ac did not differ between WT and Brd1+/− mice whereas the relative ratio of H2AK5Ac – a known target site of TIP60 – is decreased from 0.013 in WT mice to 0.007 in Brd1+/− mice. Conclusions from this pilot study should even though be made very carefully. We aim to present the full dataset at the conference. Secondly, we aim at identifying the binding specificity of functional domains (the bromodomain, the PHD Zinc finger domain and the PWWP domain) of the BRD1 protein to histone tails using a peptide array covering the majority of known histone modifications in various combinations.

Discussion
The identification of the epigenetic targets of BRD1 in the central nervous system will help clarify its role in the pathology of SZ and BD.
A PHYLOGENETIC ANALYSIS OF PRUNING RELATED GENES, A BREAKTHROUGH FOR SCHIZOPHRENIA AND BIPOLAR DISORDER

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Background
The biological causes of Schizophrenia and Bipolar Disorder are largely unknown despite a clear heritability of the disorders. Pruning may play a fundamental role in shaping the risk for these disorders. This is consistent with the typical age at onset of the two diseases (early adulthood) and with the current etiopathologic hypotheses of the disorders that implicate neurodevelopment as a key molecular event. The aim of the present study was to identify the proteins that are involved in pruning in three different species (Humans, rats and C. elegans) and test the hypothesis that the phylogenetically new genes gather in some specific molecular aspects of pruning.

Methods
We first identified the proteins involved in pruning by a systematic literature research. Pubmed, Ensemble, Genecards were interrogated by using the following keywords “Cytogenetic band“, “Genomic views”, ”Genomic regions”, ”transcripts and products”, ”Genomic Variants”, ”Function summary”, ”Comparative genomic”, ”Gene tree”, ”Disorders/Diseases”, ”Malacards”, ”Neuron death”, ”Pruning”, ”Neurite”, ”Axon”, ”CNS”, ”Dendritic spine”, ”Glia”, ”Transcript”, ”Sequence” and ”Protein”. We then identified the molecular pathways involved in pruning by using Cytoskape (GeneMania plugin). Finally, we tested the rate of aminoacidic sequence conservation of the proteins involved in pruning in the three species under analysis.

Results
We identified N = 31 out of which 24 proteins were especially involved in pruning. Most of them are synthesized by the Glia. The analysis of the rate of aminoacidic conservation and of the cellular localization showed that these proteins gather in two clusters: less conserved proteins involved in I) extracellular modifications and more conserved proteins in II) intracellular modifications. Overall, several pruning involved genes have been related with SCZ and BD susceptibility, including EfnB2, Ncan, Thbs1.

Discussion
We provide a list of proteins related to pruning that may be candidate of investigation for Schizophrenia and Bipolar Disorder. We suggest that the higher complexity of pruning in the human brain mostly takes place in the extracellular matrix.

ALTERED SOCIAL BEHAVIORS IN MICE WITH REDUCED ANKYRIN 3 EXPRESSION ARE REVERSED BY CHRONIC TREATMENT WITH THE MOOD STABILIZER, LITHIUM

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Background
Bipolar disorder is a highly heritable psychiatric disorder that affects approximately 3% of the population. Ankyrin 3 (ANK3) is one of the most significant risk genes for bipolar disorder identified in recent genome-wide association studies (GWAS). We have previously identified that reduced Ank3 expression in mouse brain induces behavioral changes analogous to bipolar disorder mania symptoms, including increased impulsivity and motivation (Leussis et al, 2012). Although alterations in social behaviors are common in psychiatric disease, potential effects of reduced Ank3 expression on social behavior have not been investigated. Psychosocial impairments are a common occurrence in psychiatric disease, including bipolar disorder.

Methods
We tested Ank3+/− mice, which express reduced levels of brain-specific isoforms of ankyrin G, in both the social preference and social interaction paradigms. The social preference test enables mice to interact with an unfamiliar mouse in a controlled environment, such that the preference for the social (versus empty) sides of the apparatus can be assessed. In contrast, the social interaction test is based on the observation of direct interaction between two unfamiliar mice, and allows for the assessment of aggression and other specific social behaviors that cannot be observed in the social preference test.

Results
Wild-type Ank3+/+ mice exhibit normal social preference behavior, preferring the side of the 3-chamber social preference test apparatus where the unfamiliar mouse was located to the empty side of the apparatus. In Ank3+/− mice social preference was disrupted, as these mice did not exhibit a preference for the social side of the apparatus compared to the empty side, when measured as time in each chamber. This effect is completely normalized by chronic treatment with the mood stabilizer lithium, a drug commonly used in bipolar disorder treatment. Further, mild isolation stress similarly reversed the social preference deficits observed in Ank3+/− mice. This is consistent with our previous findings that the risk-taking/impulsivity phenotype of Ank3+/− mice was normalized by isolation stress (Leussis et al, 2012).

Discussion
Ongoing analysis of social interactions will help clarify whether Ank3+/− mice truly exhibit altered social behavior, including specific aspects such as aggression, or whether the altered social preference behavior of Ank3+/− mice may instead reflect differences in other domains such as exploration. By examining social behavior in mice with reduced Ank3 expression, we expect to further define the phenotype of these mice, and consequently highlight potential brain pathways or circuits that may contribute to ontogeny or symptom expression in bipolar disorder.

CIRL/LPHN3 REGULATES ACTIVITY IN A DROSOPHILA MODEL FOR ATTENTION-DEFICIT/HYPERACTIVITY DISORDER
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**Background**
Attention-deficit/hyperactivity disorder (ADHD) is a common neuropsychiatric disorder characterized by age-inappropriate, sustained hyperactivity, impulsivity and/or problems to focus attention. ADHD is amongst the most heritable neuropsychiatric disorders, but despite an increasing amount of genetic information available the etiology of ADHD remains largely unknown. To overcome this, an efficient model is required that permits generation of relevant functional information in short time.

**Methods**
The fruit fly *Drosophila melanogaster* is a cost-efficient and powerful model organism with a large repertoire of behaviors and exceptional resources to manipulate any gene of interest. We found that ADHD-associated genes are enriched among hyperactive *Drosophila* mutants, suggesting that studying phenotypes with hyperactive features is a valuable approach for studying ADHD in *Drosophila*.

**Results**
Through pan-neuronal depletion with RNA-interference, we find that dopamine transporter (DAT/SLC6A3) knockdown flies display hyperactive behaviour. We have expanded our analysis to the ADHD-associated gene Latrophilin (Cirl/LPHN3), which is highly expressed in *Drosophila* larval central nervous system and adult brain. We find that down-regulation of Latrophilin, like DAT, results in locomotor hyperactivity. We propose that this aberrant locomotor pattern represents a dopamine signature and thus a *Drosophila* ADHD-endophenotype that can be further exploited to verify novel ADHD candidates and test chemical compounds.

At the same time we are addressing the hypothesis that problems in the physical and/or functional connectivity may form the basis of ADHD, a hypothesis that fits well with neuroimaging studies of ADHD patients. We study a number of morphological synapse and dendritic parameters that we propose to be relevant.

**Discussion**
In summary, we expect *Drosophila* with its efficient genetic toolbox and genome-wide resources to significantly improve our understanding of the molecular, cellular and developmental basis of ADHD.

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**DIFFERENTIAL BEHAVIORAL RESPONSES OF ZEBAFISH LARVAE TO MK-801 TREATMENT REVEAL IMPORTANT FACTORS FOR DRUG STUDY**
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**Background**
Zebrafish is a relatively new model organism and has become a valuable tool in genetic, developmental, and pharmacological researches. Zebrafish larval, compared to adult, is particularly suitable for high-throughput screening of drug effects.
Methods
AB and TU are well established in-bred zebrafish strains. The behavioral responses to MK-801 treatments (0, 5, 20, 100, 200 uM) were studied using zebrafish larvae of both AB and TU strains at 7 dpf with ZebraLab software from ViewPoint Company. Two behavioral parameters, locomotor activity and active level, were analyzed with “Tracking” and “Activity Quantization” modes of the software respectively.

Results
Zebrafish larvae of TU strain demonstrated dose-dependent inhibitory effects in both behavioral parameters in respond to MK-801 treatment. Zebrafish larvae of AB strain showed lack of responses to MK-801 treatments in locomotor activity, and showed increased duration of burst level activity in a dose-dependent manner. Therefore, zebrafish larvae of AB and TU strains demonstrated opposite responses towards MK-801 treatment, in terms of duration of burst level activity.

Discussion
When zebrafish larvae are used in neuro-psycho-pharmacological studies, genetic background and testing parameters are important factors that need to be chosen carefully.

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FUNCTIONAL DISSECTION OF INDIVIDUAL DOPAMINERGIC NEURONS THAT MODULATE SOCIAL BEHAVIOR IN DROSOPHILA
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Background
Dopamine has a fundamental role in many aspects of behavior, ranging from motor control to mood regulation, addiction and reward. Many psychiatric diseases such as schizophrenia also link to dopamine dysfunction in particular brain regions. However, the precise functions of dopamine remain elusive due to its involvement in multiple neural circuits that regulate a wide-range of behaviors. By focusing on a simpler model organism, Drosophila, we hope to begin to unravel the function of dopaminergic circuits in relation to behavior.

Methods
In Drosophila, as in mammals, dopamine modulates a wide variety of behaviors. Among the ~200 dopaminergic neurons in the fly central nervous system, accumulating evidence shows a clear association of dopamine with reward, learning and memory, sleep and arousal, and other behaviors. With the powerful genetic tools available for use in Drosophila research, it becomes possible to manipulate gene expression in small subsets of dopaminergic neurons and begin to functionally dissect the circuits they are involved with. For these studies, we developed a novel intersectional genetic approach by combining the GAL4/UAS binary system and the flippase recombination technique that enables us to restrict the numbers of targeted dopaminergic neurons down to a single-cell level.

Results
We identified 2 pairs of dopaminergic neurons (from the T1 and PPM3 clusters) that send projection to the central complex region. Either inactivating these single neurons by expressing tetanus toxin or activating them with dTrpA1, a temperature-sensitive cation channel, leads to changes in aggression. These neurons have no effects on others behaviors involving dopamine. Manipulating the function of a different pair of dopaminergic neurons (PPL1 cluster) altered sleep, but had no aggression-related phenotype.

Discussion
These findings demonstrate a novel way to reliably dissect the function of individual identified amine neurons. They also suggest that separate dopaminergic circuits modulate clearly definable behaviors that appear to be functionally independent. Identifying the function of individual dopaminergic neurons allows us to better understand their unique roles in different behaviors and, possibly in the future, their implications in various psychiatric diseases.

RECURRENT DE NOVO COPY NUMBER VARIATION WITHIN NEURODEVELOPMENTAL LOCI IN OBSESSIVE-COMPULSIVE DISORDER
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Background
Obsessive-compulsive disorder (OCD) is characterized by recurrent, intrusive, obsessions and/or compulsions that are distressing, time consuming, or significantly impairing. Twin and family studies support a strong genetic contribution in OCD, although specific genetic risk loci have been difficult to identify. Trio and simplex designs have been particularly important for implicating de novo genomic copy number variants (CNVs) in the etiology of multiple neuropsychiatric disorders, including autism and schizophrenia. It is an important and unanswered question whether de novo CNVs also contribute to the genetic architecture of OCD. This study represents the first genome wide investigation of de novo CNVs in OCD.

Methods
Investigation of de novo CNVs in OCD cases was undertaken in 348 parent-proband trios within a larger CNV case-control study of OCD and Tourette Syndrome (TS) (N=1086 TS cases, 1613 OCD cases, 1789 controls). Two CNV calling algorithms, PennCNV and iPATTERN were used to generate CNV calls. After standard QC procedures, CNVs were filtered for rare (<1% in the population) events. Due to artifacts at smaller CNV sizes, only large CNVs (>500kb) were included in the analysis. PennCNV and iPATTERN call sets were merged using CNVision to identify overlapping calls; only CNVs calls with >50% overlap were included in the analysis. PennCNV was used to estimate the likelihood of a putative de novo deletion based on parental genotypes (i.e., in silico validation). Putative de novo CNVs identified in silico were then validated using quantitative PCR (qPCR).

Results
Five high confidence de novo deletions were identified in the OCD trios. All 5 de novo events were validated by qPCR. These CNVs occurred at 4q24, 7p21.1-7p21.2, 16p13.11, 17q12, and 22q11.21, yielding an overall de novo rate of 1.44%. Additional supporting case events were identified within the large OCD/TS case-control study at 16p13.11 (5 deletions, 2 duplications) and 22q11.21 (3 duplications). The deletions at 4q24, 7p21.1-7p21.2, and 17q12 were singleton events. The de novo events at 16p13.11, 17q12, and 22q11.21 occurred in regions identified a priori as neurodevelopmental loci because these regions have been previously associated with multiple neurodevelopmental disorders, such as autism, schizophrenia, and intellectual disability.

Discussion
The de novo rate for large CNVs (> 500kb) in this sample of OCD cases is 1.44%, which lies between published estimates for healthy controls (0.7%) and other neurodevelopmental disorders like autism (1.8%) and schizophrenia (2-3%). It is notable that 3 of 5 de novo deletions occurred in loci that have been previously associated with other neurodevelopmental disorders. The fact that such events were also identified in this OCD sample suggests that the phenotypic associations of these events may be extended to include OCD-spectrum disorders. The de novo events at 4q24 and 7p21.1-7p21.2 represent novel loci not included in our curated list of neurodevelopmental loci. Of note, the 7p21.1-7p21.2 deletion encompasses the TWIST1 locus which is implicated in Saethre-Chotzen syndrome, a syndromic form of craniosynostosis. The behavioral phenotype is variable in this syndrome, but two case studies have reported OCD (with and without TS). Overall, these results suggest that rare de novo variation may play a role in the etiology of neuropsychiatric disorders like OCD. Additional studies with larger samples, more sensitive CNV calling at all sizes, and matched case-control trios will be needed to refine this de novo estimate and test for case-control differences in the de novo rate.
Participants of European-American (EA; N=2,347) and African American (AA; N=3,272) descent were analyzed separately. Participants were recruited for genetic studies of alcohol, cocaine and opioid dependence at five US sites using similar methods. Phenotypic interviews were conducted with the Semi-Structured Assessment for Drug Dependence and Alcoholism (SSADDA), a computer-assisted measure that yields lifetime DSM-IV diagnoses of Axis I disorders and antisocial personality disorder (ASPD). In the ASPD module, participants responded to twelve items (loading on a single factor) related to lifetime history of violent/aggressive acts, such as frequently starting fights, using weapons, and physically attacking others. A quantitative trait variable for pathological aggression was derived from the number of items endorsed as occurring when a participant was not under the influence of substances. A genome-wide association was performed (low frequency SNPs imputed from 1000 Genomes) to find novel risk alleles for aggressive behavior. Generalized estimating equations were applied to account for the correlated data from individuals in the same family, and models were adjusted for age, sex, and local ancestry.

Results
Preliminary analyses in the EA and AA samples reveal genome-wide significant associations (seven SNPs, $p<5\times10^{-8}$) in genomic regions not previously implicated in studies of aggressive behavior or related psychiatric disorders. Since these variants were imputed, current efforts are underway to validate these findings with genotyping. Top hits will be examined in an internal replication sample (total N=3541), and final results will reflect necessary correction based on these refinements.

Discussion
This study provides a preliminary list of candidate genes for replication. A better understanding of the genetics and neurobiological systems involved in pathological aggression could elucidate the underlying architecture of externalizing disorders, leading to better diagnosis as well as targeted treatments.

MODELING THE ASSOCIATION BETWEEN GENETIC RISK FACTORS FOR MAJOR NEUROPSYCHIATRIC DISEASE AND TYPICAL VARIATION IN BEHAVIOR AND COGNITION

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Background
Genetic risk factors are commonly shared among neuropsychiatric disorders. For example, rare duplications and deletions at 16p11.2 create risk for each of schizophrenia (SCZ), autism spectrum disorders (ASDs), and intellectual disability; common variants near CACNA1C have been associated with five major psychiatric conditions. However, the extent to which either rare or common genetic risk factors for severe behavioral impairment are associated with typical variation in behavior and cognition in the general population is less clear. Examining these relationships could be important in understanding the model through which genetic risk for diseases like SCZ is conferred, as well as clarifying the predictive utility of emerging psychiatric
Methods
To develop expected values for these associations under various assumed conditions, we simulated mean- and variance-based models of the relationship between genetic risk factors for clinical disorders and typical variation in behavior and cognition. We then estimated empirical relationships between single SNP and polygenic risk factors for SCZ, bipolar disorder (BPD), and ASDs identified in the Psychiatric Genomics Consortium and behavioral (e.g. psychotic and autistic traits) and cognitive traits (e.g. memory, executive function, ‘g’) where available across several population samples (e.g. the Twins Early Development Study, the Avon Longitudinal Study of Parents and Children).

Results
The expected association between risk factors for neuropsychiatric diseases and behavior and cognition in the general population is impacted by the effect size of the risk factor, variation in the expected genetic correlation between the clinical disorders and general population phenotype, the heritability and measurement of the general population phenotype, and other parameters. Empirical estimates of the association between risk factors for SCZ, BPD, and ASD and general population trait variation were primarily non-significant. For example, in the Twins Early Development Study (n=2700), there was no association between polygenic risk for SCZ and either autistic traits (p=0.92) or overall behavioral difficulties (p=0.89) at age 12.

Discussion
The empirical findings are discussed in light of the expected association values as estimated under various assumed conditions. With results from the simulation study, we discuss sample size and research design considerations for studies of this type, which are likely to become common given the recent successes in SCZ and related disorders.

Gene Expression and Suicide Attempts in US Veterans
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Background
Approximately 20% of people who die from suicide in the United States have served in the military. The contributions of specific risk factors to suicidal behavior in veterans, including combat exposure and other related stressors, have not yet been determined and little is known about possible biological markers associated with suicide. Gene expression studies of suicide have examined postmortem tissue but to date, there are no published studies examining gene expression in blood samples in suicide attempters. Because the best behavioral predictor of suicide completion is a prior suicide attempt, the identification of blood biomarkers associated with suicide attempts would be a powerful agent in suicide prevention.

Methods
Twenty pairs of veterans who differed by a history of suicide attempts (never versus 1 or more
attempts) provided blood samples and completed a clinical interview to assess psychiatric history and suicide history, including lethality of attempts. The pairs were matched by age, gender and race/ethnicity. Gene expression studies were conducted to identify differences in the two groups, and such differences were followed by DNA methylation and microRNA pan-omic analyses to identify potential pathways associated with suicidality.

Results
Functional analysis of genes that were differentially expressed between attempters and non-attempters showed that genes coding for zinc finger transcription factors and proteins involved in transport were up-regulated in veterans who had attempted suicide, while genes associated with immune responsiveness, neurogenesis and neuronal signaling were down-regulated. Pan-omic analyses were conducted to identify pathways associated with gene expression differences between attempters and non-attempters.

Discussion
Gene expression is pervasively dysregulated in veterans who have attempted suicide, relative to matched psychiatric controls who never attempted suicide. Although results require replication and functional validation, the study of gene expression and pan-omics has the potential to identify molecular mechanisms involved in suicidality.

THE USE OF LONGITUDINAL DATA OF CHILDHOOD AND ADOLESCENT INTERNALIZING PROBLEMS IN GENETIC STUDIES

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Background
A next step in genome wide association (GWA) studies is to consider multivariate phenotypes, especially longitudinal where subjects are assessed repeatedly over time which enables including the information about the development of symptoms over time. Such datasets are scarce, but several birth cohorts, including twin registries, collect longitudinal data on multiple psychiatric phenotypes, including internalizing symptoms or depressive and anxiety disorders.

Methods
Data on internalizing problems from two large cohorts were analyzed. In 44,413 twins from the Netherlands Twin Register (NTR), anxious depression (AD) and withdrawn behavior (WB) were assessed repeatedly between ages 7 and 18 with the age appropriate versions of the Achenbach System of Empirical Based Assessment (www.aseba.org). In 8,236 children from the Avon
Longitudinal Study of Parents and Children (ALSPAC), depressive and anxiety disorders were assessed four times between ages 7 and 15 by the Development And Wellbeing Assessment (DAWBA). Growth Mixture Models (GMM) were fitted to the data. GMM enables identifying distinctive classes of developmental trajectories and calculating the probability for an individual to belong to each class. Concordance between monozygotic and dizygotic twin for class membership probabilities was estimated to inform heritability of the trajectories of the internalizing symptoms.

**Results**
To avoid the influence of rater effects in the NTR, maternal AD and WB reports (age 7, 10 and 12) and self reports (age 12, 14 16 and 18) were analyzed separately. Linear growth models showed no evidence for classes in maternal ratings. Self reports of AD supported a single class in males, and two classes in females. The two classes represent a smaller class of subjects with a higher starting point, and slight increase, and a larger lower class that stays essentially flat. Self reports of WB supported 3 classes in males and females. For both genders, a smaller class with a low starting point stayed flat, the largest class started slightly higher and had a small increase over time, and an intermediate size class started high and had a higher increase. Concordance of class membership was higher for MZ twins than for DZ twins. MZ twins were substantially more likely to belong to the same class compared to concordance expected by chance whereas DZ twin concordance was only slightly elevated compared to chance. The growth models fitted to the emotional disorders phenotype as measured with the DAWBA in ALSPAC identified three classes. The largest class consists of children who scored low and did not change over time. The other two classes consisted of children who scored high and showed a decrease over time and children who scored low, but whose scores increased over time. The increase in the latter group was most pronounced during adolescence (accessible via a quadratic term).

**Discussion**
The results in ALSPAC and for WB in NTR confirm the picture of a group of adolescents who have increasing internalizing problems. The twin analyses show that the trajectories are influenced by genetic factors. By including the trajectories in the analysis, more information is captured in the phenotype. The next step is to test the effect of single nucleotide polymorphisms (SNPs) on class membership, preferably genome-wide, but if that is not computationally feasible, SNPs will be selected based on earlier GWA analyses.

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**CROSS-DISORDER GENOME-WIDE ASSOCIATION ANALYSES SUGGEST SEPARATE DISTINCT GENETIC CONTRIBUTIONS TO TOURETTE SYNDROME AND OBSESSIVE COMPULSIVE DISORDER**

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**Background**
Obsessive compulsive disorder (OCD) and Tourette Syndrome (TS) are developmental neuropsychiatric disorders with overlapping clinical symptoms and high rates of comorbidity. Although definitive susceptibility genes for these highly heritable yet etiologically complex disorders remain elusive, it is widely speculated that they share genetic risk factors.
Methods
A combined genome-wide associate study (GWAS) was conducted on TS and OCD in 2723 cases (1310 OCD, 834 TS, 579 with both OCD and TS/chronic tic disorder (CT)), 5667 ancestry-matched controls, and 290 OCD parent-child trios. Imputation was carried out using the 1000 Genomes Project as the reference panel. Top single nucleotide polymorphisms (SNPs) from the combined and individual TS and OCD GWAS studies were investigated for enrichment for expression quantitative loci (eQTLs) in several brain regions (frontal cortex, parietal cortex, and cerebellum) as well as in lymphoblast cell lines (LCL). Pathway analysis was carried out using the INRICH and DAVID Bioinformatics Resources. Polygenic component analysis was applied within and cross the two disorders to explore the genetic relationship between TS and OCD.

Results
Upon 1000 Genomes imputation, no markers achieved genome-wide significance in the combined GWAS study \((p=5 \times 10^{-8})\). Among the top 9099 SNPs with \(p<0.001\), we found significant enrichment for eQTLs in all three brain regions (empirical \(p<0.001\) for frontal cortex and cerebellum, \(p=0.005\) for parietal cortex). In comparison, while top loci from individual TS and OCD GWAS studies both demonstrated brain eQTL enrichment, the specific brain regions differed between the two disorders, with TS enriched strongly with cerebellar eQTLs \((p<0.001)\) and OCD with eQTLs from frontal cortex \((p<0.001)\). Surprisingly, significant enrichment in LDL eQTLs was also found among the top SNPs of OCD GWAS, but not those of TS GWAS, though no specific pathways were found among the target genes of OCD LCL eQTLs. Polygenic score analyses identified a significant polygenic component for OCD \((p=2 \times 10^{-4})\) and a smaller polygenic component for TS \((p=0.06)\), explaining 3% and 0.6% of the phenotypic variance, respectively. Reducing the sample size of OCD to that of TS resulted in a decreased but still significant OCD polygenic signal \((p=0.01,\) explaining 1.4% of OCD phenotypic variance). Intriguingly, no shared polygenic signal was detected in cross-disorder polygenic analysis; furthermore, the OCD polygenic signal was attenuated when TS/CT cases with co-morbid OCD were included.

Discussion
Different patterns of eQTL enrichment in two disorders indicate that distinct genetic loci contribute to TS and OCD risk separately by altering gene expression levels in different brain regions in a disease-specific manner. The polygenic analysis results suggest that TS and OCD both have a polygenic component, but may have distinct genetic architectures. In addition, OCD in the presence of TS/CT may have different underlying genetic susceptibility compared to OCD alone.

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CONCURRENCE OF TWO GENOMIC COPY NUMBER VARIATIONS IN A PATIENT WITH SEVERE DEVELOPMENTAL DISORDERS
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Background
Microarray-based comparative genomic hybridization (array-CGH) has been instrumental in
identifying the genomic copy number variations (CNV) associated with idiopathic developmental disorders, such as mental retardation and autism spectrum disorders. However, the genotype and phenotype relationship of CNVs identified in patients may be more complicated than previously thought. Some CNVs need additional genetic mutations in order to cross the clinical threshold and to have clinical presentation, or the clinical presentations might be modified by another genetic mutation. Here we report the identification of two CNVs in a girl with multiple severe developmental disorders.

Methods
A girl with multiple severe developmental disorders received a series of clinical and laboratory examinations performed by a team consisting of pediatrician, clinical geneticist, ophthalmologist, child neurologist, eye, child ear, nose and throat doctor, rehabilitation doctor, and child psychiatrist. CNV analysis was conducted in this girl and her parents using Affymetrix Human Genome-Wide SNP 6.0 array, and validated by real-time quantitative PCR.

Results
The girl was found to have delay in developmental milestones, severe mental retardation, language deficit, hypotonia, impaired hearing, repetitive behavior, and lack of reciprocal interaction that meet the criteria of autism. However, she does not have seizure attack and brain magnetic resonance image showed no remarkable abnormalities. CNV analysis revealed a microdeletion of ~120 kb at chromosome 1q42.2 that disrupted the DISC1 gene and a microdeletion of ~4.8 Mb at chromosome 2q37.3. Family study showed that the microdeletion at 2q37.3 is a de novo mutation, while the chromosome microdeletion at 1q42.2 was transmitted from her unaffected mother.

Discussion
The identification of two CNVs concurrent in the affected girl demonstrates a complex genetic mechanism associated with developmental disorders. The DISC1 gene is a well-documented gene associated with various psychiatric conditions in a Scottish family. However, loss function of this gene may not lead to full penetrance as shown in the unaffected mother. Nevertheless, it may interact with the de novo microdeletion of ~4.8 Mb at chromosome 2q37.3 to modify the clinical presentations in the affected girl. Our data suggest the two-hit genetic events might be a common genetic mechanism underlying some developmental disorders.

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LOOKING FOR SHARED ETIOLOGY IN TICS AND OC SYMPTOMS: A GENETIC EPIDEMIOLOGICAL TWIN STUDY
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Background
Genetic factors are important both in the occurrence of tics and Tourette's Syndrome (TS), as shown in family studies, and in one clinical twin study of 43 twin pairs (Price et al., 1985). To date, only one genetic epidemiological twin study has investigated the relative contribution of genes and environmental factors to tics and OC symptoms in six-year old twins. This is the first large-scale genetic epidemiological twin study in adults to partial out the unique and shared
genetic contributions to tics and OC symptoms in coherence.

Methods
Genetic model fitting was conducted for tics and OC symptoms using Mplus in 8,144 adult twins from 5456 twin pairs from the Netherlands Twin Registry. Two tic variables were considered for further analyses, i.e.: any tics and childhood tics (i.e. tics occurring before age 19), and these tic dimensions were subjected to subsequent analyses. Because the traits of interest were not normally distributed, threshold liability models encompassing the entire distribution of the traits were examined.

Results
15.8% of the twin sample met criteria for any tics, 6.6% of the twins met criteria for childhood tics, and 5.7% of the sample met criteria for clinically significant OCS. In any tics, when modeled as a categorical variable, an AE model showed the best fit and a 0.35 heritability estimate. Data on childhood tics yielded similar results, but with somewhat lower genetic contribution ($h^2 = 0.29$). For OC symptoms, an AE model best fitted the data, with heritability estimates of $h^2 = 0.43$. Bivariate analyses revealed that for tics, all genetic variance was shared with OC symptoms, but reversely, for OC symptoms, only half of the genetic variance was shared with tics.

Discussion
Tic symptoms are relatively common in this population-based sample, and show -in line with other complex disorders- moderate heritability. Fully in line with previous family studies, these data suggest that, in families with tics, OC symptoms are an alternate expression of the same underlying disorder, whereas in families with OC symptoms, genetic etiology is only partly shared with tics.

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VARIATION IN THE FKBP5 GENE POLYMORPHISMS: STRESS, ANXIETY, AND CHILDHOOD-ONSET AGGRESSION.
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Background
The hypothalamic–pituitary–adrenal (HPA) axis responds to environmental stress with the release of corticotrophin-releasing hormone (CRH) which begins the neuroendocrine cascade that ends in the release of cortisol. Cortisol supports homeostasis and is adaptive in the short term but chronic exposure has been associated with an array of psychiatric disorders, such as anxiety, depression and posttraumatic stress disorder. Whereas the former disorders show cortisol hypersecretion the latter shows blunted cortisol reactivity in response to stress. Antisocial aggressive behaviour has also been associated with dysregulation of the HPA axis and with under activity of the HPA axis. In particular callous unemotional traits are thought to reflect low fear of punishment and may be associated with blunted reactivity of the HPA axis. Given the widespread and profound effects of the HPA axis we hypothesize that genetic variation in the genes of the HPA axis may be related to the development of psychiatric disorders. Recently, the FKBP5 gene has been linked to aggressive behaviour in adults with a history of childhood trauma (4). FKBP5 gene codes for FKBP506, a co-chaperone that inhibits the negative feedback
of the HPA axis. Variants of the FKBP5 gene have been consistently linked to stress-related psychopathologies, including mood disorders and PTSD (5). As well, childhood trauma was recently shown to epigenetically modify FKBP5 expression, thus FKBP5 may serve as the key mediator in the link between trauma and psychopathology (6). Here we report on the association of FKBP5 gene variants with aggression, callous-unemotional traits, physiological anxiety and an index of psychological distress.

Methods
Our sample consists of 176 Caucasian children (age 6-16) displaying extreme, persistent and pervasive aggressive behaviour, defined as a minimum 2-year history of aggressive behaviours; at or above the 90th percentile on the subscale of aggressive behaviors of the Achenbach Child Behaviour Checklist (CBCL) and Teacher Report Form (TRF) (7). Children also completed the Children’s Depression Inventory and the Revised Children’s Manifest Anxiety Scale-2, while one of the child’s parents completed the Psychopathy Screening Device. Children with chronic medical illnesses and psychiatric disorders, such as schizophrenia, mania, autism and Tourette’s syndrome were excluded. Caucasian ancestry was confirmed using an Open Array assay of 64 ancestry-informative markers (8). Genomic DNA was extracted from blood, cheek swab or saliva using commercially available kits. Genotyping was performed using PCR-based TaqMan Single Nucleotide Polymorphism (SNP) assays for 5 SNPs in the FKBP5 region that were previously implicated in psychopathology. Single-marker and haplotype associations were tested in Unphased. IBM SPSS statistical software package will be used to test the association between the maltreatment condition and the traits under study, as well as the gene-environment interaction.

Results
To date, we have genotyped five SNPs (rs1360780, rs9470080, rs9296158, rs3800373, and rs4713916) in the FKBP5 region. No significant main effects of alleles, genotypes, or haplotypes on aggression, callous-unemotional traits, physiological anxiety, or measures of psychological distress were found. Further analysis examining the association of maltreatment history with variations in the FKBP5 SNPs and psychiatric disorders is underway.

Discussion
FKBP5 SNPs may have no main genetic effect on childhood-onset aggression, physiological anxiety, or psychological distress. This finding is consistent with the previous literature that finds no main effect of FKBP5 variants on psychopathology, but does show interaction effects of FKBP5 polymorphisms and childhood trauma (4, 5). Results of our analysis of association and interaction of maltreatment with FKBP5 polymorphisms will be described and the implications of these findings will be discussed.

References:

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AUTOZYGOSITY MAPPING IN PAKISTANI INTELLECTUAL DISABILITY FAMILIES
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Background
Autosomal recessive causes of intellectual disability (ARID) have, until very recently, been under-researched due to the high degree of genetic heterogeneity. However, now that genome-wide approaches can be applied to single multiplex consanguineous families, identification of genes harboring disease-causing mutations by autozygosity mapping is expanding rapidly.

Methods
We have ascertained >165 multiplex, consanguineous ARID families from Pakistan. These families are selected for lack of obvious syndromic features. Our strategy includes genotyping family members on genome-wide single nucleotide polymorphism microarrays, looking for large regions of shared homozygosity (and haploidentity) between affected individuals (homozygosity-by-descent, or autozygosity). We also screen for potential disease-related CNVs-either as a shared homozygous genotype, or heterozygous as a potential cause of phenocopy. We firstly exclude any known ARID genes in HBD regions, then either select candidates from within the HBD region for mutation screening by Sanger sequencing, or we embark on whole exome sequencing to identify disease mutations.

Results
Our successes include a number of new genes for apparent non-syndromic ARID, such as MAN1B1, TRAPPC9, NSUN2, with evidence for an additional 6 new genes, as well as new genes for syndromic forms or ARID, such as Joubert syndrome (CC2D2A and TCTN2), and many known ARID genes (TUSC3, TPO, VPS13B, PEX1, PSPH, PMM2).

Discussion
As more and more genes for ID are identified, using these and other strategies, we are building a picture of the biological pathways that, when perturbed, may lead to intellectual disability.

PPP3CC GENE AND ANTIDEPRESSANT RESPONSE: RESULTS FROM THREE INDEPENDENT SAMPLES
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Background
The present study joined a candidate gene and genome-wide approach with pathway analysis in order to reduce the limitations of each individual methodology.

Methods
38 tag SNPs within monoamine (COMT and HTR2A), neuroplasticity (BDNF, GSK3B, PLA2G4A, PPP3CC, ST8SIA2), circadian rhythm (RORA and VIPR2), and transcription factor (ZNF804A and SP4) pathways were genotyped in two independent samples (n=369 and 93) of major depressive patients treated with antidepressants in a naturalistic setting. Phenotypes were response and remission at weeks 4 and 8 and treatment resistant depression (TRD) only in the largest sample. TRD was defined as non response to at least 2 adequate consecutive antidepressant treatments during the last episode (TRDW) or non response to at least 2 adequate consecutive antidepressant drugs of different classes administered during the last episode (TRDC). Logistic regression using appropriate covariates was carried out. Genes associated with outcomes were investigated in the in the Sequenced Treatment Alternatives to Relieve Depression (STAR*D) genome-wide level 1 (n=1861) both individually and through a pathway analysis (KEGG database: www.genome.jp/kegg/pathway.html). The genes belonging to the pathway under analysis were imputed using IMPUTE2 and CEU HapMap 1000 genomes as reference panel. Variations with p<0.01 in the index pathway were tested for a different distribution (Fisher exact test) compared to a random pathway (matched to the index pathway in terms of number of SNPs and intragenic localization). 10e-5 permutations were run.

Results
In both original samples markers associated with outcomes were concentrated in PPP3CC (rs2249098, rs7430, rs11780915, and rs10108011). The best predictor of TRD was rs7430 (TRDW: p=0.01; TRDC: p=0.008). In one original sample VIPR2 rs2657340 was associated with response (p=0.01) and remission (p=0.008), while HTR2A (rs643627; p=0.02) with remission. In the STAR*D a cluster of SNPs around rs643627 (especially rs1923888, rs1745837, and rs2296972) was associated with response. The B cell receptor signaling pathway resulted the putative mediator of PPP3CC involvement in the antidepressant effect (permutated p=0.03). The genes showing the highest proportion of associated SNPs within the pathway were PIK3AP1, PLCG2, CD22, and PPP3CA.

Discussion
The present study confirmed the role of HTR2A in antidepressant response. Among innovative candidates, PPP3CC seems promising. PPP3CC may have a role in the calmodulin activation of calcineurin, a neuron-enriched phosphatase that regulates synaptic plasticity. Further studies are needed to confirm PPP3CC involvement in the antidepressant effect.

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GENOME-WIDE ASSOCIATION STUDY SUGGESTS NOVEL LOCI FOR ANTIPSYCHOTIC DRUG INDUCED AKATHISIA IN PATIENTS WITH FIRST EPISODE SCHIZOPHRENIA

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Background

Akathisia is a common side effect of antipsychotic drugs (APD), and often causes patients to stop taking their medications. To date, there is no good clinical predictor for APD-induced akathisia. Genetic markers can be potentially useful in predicting akathisia and help in the tailoring of medication treatment for individual patients. The goal of the present study was to conduct genome-wide association study (GWAS) in first episode schizophrenia patients to explore single nucleotide polymorphisms (SNP) that may be involved in APD-induced akathisia. Due to small sample sizes in available two samples, GWAS meta-analysis was used to combine the samples.

Methods

Two samples of first episode schizophrenia patients were included in the study. Sample 1 is from the Hillside First Episode Study (Robinson et al., 2006) with 81 patients treated with either risperidone or olanzapine. Akathisia was assessed weekly using the Barnes Akathisia Rating Scale. A global rating of 2 and above was considered a case of akathisia. Sample 2 is from the European Union First Episode Schizophrenia Trial (Kahn et al., 2008) with 94 patients treated with olanzapine, quetiapine, amisulpride, or ziprasidone. Akathisia was assessed using the St Hans Rating Scale at 1 month, 3 months, and then every 3 months. Phenotype was incident akathisia in the first 3 months in both samples. Patients were genotyped on the Illumina Omni-1 Quad platform. After quality control, 876,226 SNPs in sample 1 and 710,034 SNPs were entered into a case-control genome-wide association study (GWAS) using an additive model, separately. P values from the two GWAS were combined using the METAL GWAS meta-analysis program.

Results

The incidence of akathisia was 25.9% (21/81) in sample 1 and 20.2% (19/94) in sample 2. The GWAS meta-analysis did not find any SNP that reached genome-wide significance. However, several top hit SNPs did suggest novel loci that may be involved in development of akathisia. Three closely located SNPs in chromosome 13q33.1 had p-values above 10^-6, about 500kb from DAOA (D-amino acid oxidase activator), which has been implicated in antipsychotic drug response previously. The three SNPs are in high LD with each other. The top SNP was rs1324751 with a p-value of 7.2x10^-8. 100% of minor allele homozygotes developed akathisia, whereas only 14.4% of major allele homozygotes did. Two introgenic SNPs in PARK2 (Parkinson protein 2, E3 ubiquitin protein ligase, chr6q26) had p-values above 10^-6. Major alleles of these SNPs appeared to carry akathisia risks. Other top SNPs were near or in the genes of...
Discussion
GWAS meta-analysis of two first episode samples revealed several novel loci that may be related to APD-induced akathisia. These novel findings require replication in other samples.

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DEEP RESEQUENCING OF THE HTR7 GENE IDENTIFIES VARIANTS IN THE PROMOTER ASSOCIATED WITH RESPONSE TO SSRI ANTIDEPRESSANTS
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Background
A variety of preclinical data support the role of the gene for the serotonin 7 receptor (HTR7) in the antidepressant action of both antidepressants and atypical psychotics. Genetic inactivation of HTR7 in rodents has been shown to produce antidepressant-like behaviors. HTR7 antagonists may work synergistically with other antidepressants and may increase prefrontal cortical serotonin. Some atypical antipsychotics such as aripiprazole have a high affinity for HTR7 and display antidepressant efficacy. For these reasons, we have examined whether sequence variation in the HTR7 gene influences response to antidepressants.

Methods
439 bipolar I patients were previously interviewed using the SCID or DIGS augmented so as to query their response to all past medication trials. Information from the interview, medical records and family informants was reviewed by blind clinicians who rated their response as good, partial or poor. Thirteen 10 kb amplicons were designed to tile the HTR7 gene using long PCR methods. Subject DNA was carefully quantified and pooled in pools of approximately 20 subjects each of which was amplified for each of the 13 amplicons. Sequencing was performed on an Illumina MiSeq to an average read depth per subject of 27x and analyses conducted on BaseSpace and using Fisher’s exact test. Response information was available on 4 drugs: fluoxetine, paroxetine, sertraline and citalopram. Each drug was analyzed separately and as a combination of all 4 drugs.

Results
Several SNPs in the promoter showed significant evidence for association for at least one of the four drugs. The most significant SNP of these was rs6583737 which was highly associated with response to all 4 SSRI’s combined (p=0.00016). The most significant SNP overall was rs76623564 just 3’ of the gene. Several other novel and known SNPs were also strongly associated.

Discussion
These data indicate that genetic variation in the HTR7 gene are associated with SSRI response and are consistent with the idea that HTR7 plays an important role in antidepressant response.
MICRONAS MIR-9 AND MIR-326 POST-TRANSCRIPTIONALLY REGULATE HUMAN DOPAMINE D2 RECEPTOR EXPRESSION

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Background
The human dopamine receptor D2 (DRD2) has been implicated in the pathophysiology of schizophrenia and is a pharmacological treatment target. Most antipsychotic drugs influence dopaminergic transmission through blocking dopamine receptors, primarily DRD2, and especially reduce positive symptoms (hallucinations, delusions). The role of elevated DRD2 expression in schizophrenia pathogenesis has been supported by studies of postmortem brains, in vivo human brain imaging, and DRD2-overexpressing murine models. However, the regulatory mechanism of DRD2 expression relevant to schizophrenia remains largely unknown. We report here that DRD2 is post-transcriptionally regulated by two brain-expressed microRNAs (miR), miR-326 and miR-9.

Methods
We used an in vitro luciferase reporter gene assay system in NT2 and CHO-K1 cell lines to study the post-transcriptional regulation of DRD2 expression. We assessed the endogenous post-transcriptional repression of DRD2 by the two miRs through quantifying the changes of DRD2 mRNA and protein abundances upon over-expression or knockdown of the miRs in NT2 cells.

Results
We found that miR-9 and miR-326 interact with the 3’-UTR (untranslated region) of DRD2, decreasing luciferase (reporter) activity in both NT2 and CHO-K1 cell lines. Over-expression of miR-326 in NT2 cells reduced DRD2 mRNA and DRD2 protein levels, and both antisense miR-326 and antisense miR-9 increased DRD2 protein in NT2 cells, suggesting an intracellular repression of DRD2 expression by both miRs. Of the two known variants (rs1130354 and rs6274) within the miR-targeting site at the DRD2 3’-UTR, we found the variant within the “seed sequence” of the miR-targeting site (rs1130354) to be functional. The minor allele of rs1130354 abolished the miR-326-mediated repression of target gene expression in both NT2 and CHO-K1 cells.

Discussion
We conclude that DRD2 is subject to post-transcriptional inhibition by miR-326 and miR-9, and a rare variant (rs1130354) influences this inhibition by miR-326. At the meeting, we will discuss and integrate these novel findings into the context of other aspects of regulation of DRD2, an important pharmacological target relevant to several neuropsychiatric conditions.

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MULTIDISCIPLINARY TEAM PROVIDES PHARMACOGENOMICS INTERVENTION TO IMPROVE MEDICATION BURDEN IN AN INSTITUTIONALIZED PSYCHIATRIC POPULATION

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Background
Intermediate care facility residents afflicted with psychiatric disorders, intellectual disability and debilitating syndromes have complex medication regimens. Patients with the highest use of medications, some exceeding $1,000 per month, were enrolled in this study to determine if interventions based on pharmacogenomic data would improve outcomes and reduce medication burden. The sample collection and team methodology were presented at the 2011 World Congress of Psychiatric Genetics, now we wish to provide outcomes and results of this proof of concept, feasibility study.

Methods
The study sample includes 46 subjects (30 male, 16 female) with an average age of 37 from a developmental center in rural South Dakota. Utilizing buccal or blood derived DNAs, individuals were genotyped for polymorphisms associated with drug metabolism and transport, specifically the Cytochrome P450 enzymes (CYP1A2, CYP2C9, CYP2C19, and CYP2D6). Additional data was generated to include a SNP in COMT (rs4680), the 5HTTLPR promoter polymorphism in SERT, and the DRD4 48-bp VNT-R in exon 3. A team trained in genetics, pharmacotherapy, and psychiatry evaluated current medication use, past medications, adverse effects, DSM IV diagnoses, Abnormal Involuntary Movement Scores, vital signs, and laboratory values which led to clinical recommendations guided by the knowledge gained from the pharmacogenomic data. Recommendations were sent to the treatment center twice a month for review and implementation by the treatment team (psychiatrist, medical residents, mid-level practitioners, pharmacist, nurses, and social workers). Recommendations provided detailed insight on drug-drug interactions, therapeutic duplication, pharmacogenomic interactions, medical management issues, and pharmaco-economic savings. We measured degree of implementation, medication changes, lab values, BMI, weight, medication burden, and behavior scores.

Results
A comprehensive analysis of the data for each case resulted in 444 drug recommendations on 46 subjects. Of the 444 recommendations, 125 were related to drug-drug interactions, 32 for therapeutic duplication, 90 based on pharmacogenomic metabolism status, 124 were drug monitoring recommendations, and 73 related to medical management issues and pharmaco-economic savings. Each patient had at least 5 recommendations, with a mean of 9 (max 17). Pharmacogenomic results revealed that many patients were taking a medication that was not optimally metabolized based on their genetics. Each subject used an average of 16 medications to treat 13 different conditions. We primarily focused on antipsychotics and mood stabilizers. Each individual was prescribed at least one scheduled antipsychotic (average 1.48, range 1-3) and had at least one recommendation pertaining to the antipsychotic, (e.g. impaired metabolism,
metabolic syndrome, an adverse drug reaction, drug monitoring issue, therapeutic duplication, or drug interaction).

**Discussion**
The implementation of a multidisciplinary team to evaluate an individual’s medications and pharmacogenomic results supports optimal care. Pharmacogenomic data served as the impetus for implementation to reduce the dose or change pharmacotherapy in CYP 450 non-metabolizers. Similarly it provided better effect in ultra rapid metabolizers not experiencing therapeutic benefit and helped prevent adverse effects. At this time, we are continuing to analyze the behavior scores in relation to medication burden. We hypothesize that behavior scores will be maintained or improved as unnecessary medications are discontinued and optimal regimens are implemented. Data will be described in detail. Decreasing medication burden will also reduce medication costs. Recommendations promoted indirect cost savings by reducing unnecessary laboratory assessments (i.e. creatine kinase levels, amylase levels, lipid panels, etc.) or eliminating a medication requiring intensive monitoring. Recommendations also encouraged direct cost savings through splitting pills, generic substitution, and elimination of unnecessary medications. The multidisciplinary team approach has impacted patients' lives by lessening the treatment burden and ultimately improving outcomes through personalized medicine.

**INFLUENCE OF RGS2 ON RESPONSE AND REMISSION TO SERTRALINE TREATMENT FOR SOCIAL ANXIETY DISORDER**

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**Background**
To improve the personalized medicine approach to psychiatric care, we need to identify biomarkers of response to evidence-based treatments. Most patients with social anxiety disorder (SAD) remain symptomatic after initial intervention with a selective serotonin reuptake inhibitor (SSRI). But some patients do extremely well and it would be useful to know this in advance when selecting treatments.

**Methods**
As the first phase of a subsequent randomized controlled trial, 346 patients with SAD who at three sites received protocol-driven, open-label treatment with sertraline, up to 200 mg/d over 10 weeks. Efficacy was determined using a continuous measure of outcome (Liebowitz Social Anxiety Scale [LSAS]) and dichotomous indicators of response (LSAS ≤ 50) and remission (LSAS ≤ 30). Predictors of efficacy were examined in multivariate models that included 8 polymorphic variants in 4 candidate genes (4 in RGS2, 2 in HTR1A, 1 in SLC6A2, and 1 in SLC6A4).

**Results**
A total of 113 and 45 intent-to-treat patients achieved response (33%) and remission (13%), respectively. Adjusting for non-genetic predictors of response, all 4 single nucleotide
polymorphisms (SNPs) in RGS2 were predictors of change in LSAS, at study-wise significance (p = 0.00833). For each of these variants, the minor allele was associated with less improvement over time. After adjusting for non-genetic predictors, two of the 4 RGS2 SNPs were (borderline) predictors of likelihood of remission at study-wise significance (p = 0.025), with adjusted odds ratios (AOR) and confidence intervals (CIs) as follows: rs4606 (AOR = 0.50 [95% CI 0.27 - 0.90], p=0.020), and rs1819741 (AOR = 0.51 [95% CI 0.28 - 0.92], p=0.026).

Discussion
Variation in RGS2, a gene previously shown to be associated with introversion and with brain responses to emotion processing, is a predictive biomarker of the likelihood of substantially benefiting from sertraline among patients with SAD.

Clinicaltrials.gov identifier NCT00282828

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GENETIC ANALYSIS OF A LARGE MULTI-PHENOTYPE AND ISOLATED PEDIGREE FROM NORTHERN SWEDEN

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Background
Increased genomic autozygosity arising from population isolation can facilitate the identification of novel associations to disease. We describe a dataset of 460 individuals from Northern Sweden, including into unipolar (N=28), bipolar (N=196) and schizophrenia (N=21) phenotypes.

Methods
Illumina OmniExpress arrays were used to genotype the sample. CNVs were called with both Birdseye and PennCNV algorithms. Extended runs of homozygosity and haplotypes were identified with PLINK, Germline and DASH. Polygene risk scores were generated in PLINK using publically available results from the PGC 1 Discovery schizophrenia dataset.

Results
Analysis of principal components and runs of homozygosity reveal a genetic architecture in our sample consistent with that of a extreme isolate, with the level of autozygosity (fraction of the autosome existing within runs of homozygosity) equal to about a third that of first or second cousins (about twice that of isolates such as Orkney island communities). Globally, schizophrenia cases (N=20) were significantly more autozygous than controls (p=0.04), a trend not observed for the other phenotypes. This suggests that increased numbers or burden of rare recessive variants might play a role in the etiology of schizophrenia.

To identify causal variants of interest which might not reside in the 1000 Genomes project reference panel and as such being impossible to impute, we investigated the hypothesis of whether affected individual are more likely to share regions of the genome identical by descent (IBD). Due to the isolate nature of the cohort, we hypothesized part of the increased risk could be due to one or more genetic variants inherited from a recent common ancestor. We analyzed the cohort for IBD segments using the Germline software and we further processed the generated calls using DASH (DASH Associates Shared Haplotypes) to identify long shared haplotypes.
shared among multiple individuals. After performing association analysis of these newly identified long haplotypes with PLINK, we selected a number of candidate individuals for further sequencing. CNV analysis identified modest associations at previously reported loci. CNV burden analyses, though largely non-significant, revealed a pattern of CNV enrichment in cases that is consistent with previous studies; for ‘psychotic’ and ‘mood disorder’ related phenotypes we note enrichment of rare deletions. For singleton deletions >100kb in particular, we note burden and geneic burden enrichment in cases that strongly surpass ratios seen in larger more heterogeneous samples (International Schizophrenia Consortium, Swedish Schizophrenia Consortium). Risk score profiles of individuals from the Skelleftea indicate that the pedigree may contain an elevated number of risk alleles, as compared to schizophrenia and unaffected samples collected from across Sweden.

**Discussion**

The higher level of autozygosiy and the extended pedigree nature of our sample afford us greater power to detect association to disease. Further studies exploring the genetic architecture of this sample and how findings may be extrapolated to larger psychiatric cohorts are currently underway.

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**THE GENUS CONSORTIUM: GENETICS OF ENDOPHENOTYPES OF NEUROFUNCTION TO UNDERSTAND SCHIZOPHRENIA**

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**Background**

Schizophrenia (SCZ) is a highly heritable neurofunctional disorder, and a large number of gene variants have reliably been shown to affect the risk of developing this disorder. However, how these genes influence brain functioning as indexed by neuropsychological and neuroimaging measures is less known. Although the penetrance of genetic variants may be higher on the neuroimaging and neuropsychology level, a large number of subjects with phenotype and genotype data are needed to convincingly identify genetic effects. We recently initiated a large NIMH-funded multi-national consortium entitled Genetics of Endophenotypes of Neurofunction to Understand Schizophrenia (GENUS) to facilitate the genetic mega- and meta-analysis of neuroimaging and cognitive datasets. The three main aims of this consortium are: 1) To examine genetic risk variants identified by recent large-scale SCZ and phenotype GWAS (e.g., Psychiatric Genomics Consortium [PGC]; Cognitive Genomics Consortium [COGENT]; ENIGMA) for association with impaired brain function in SCZ as indexed by neuropsychological and neuroimaging measures; 2) To clarify the role of polygenic variation in impaired brain function in SCZ as indexed by brain-based phenotypes; and 3) To identify phenotypic profiles associated with SCZ risk variants.
Methods

Sample: Currently, 15 research groups worldwide have committed samples totaling ~10,000 SCZ cases and controls with neuropsychological and/or neuroimaging data, boosting the power to detect effects that no single study alone could identify. Protocols: Phenotype and genotype processing and quality assessment protocols are being developed so that derived neuroimaging and neuropsychological measures and genotyping are maximally comparable across sites and amenable to merging across samples. Neuropsychological Phenotypes: 8-10 core cognitive phenotypes covering each of the major cognitive domains (e.g., attention, processing speed, working memory). Neuroimaging Phenotypes: Neuroanatomical imaging (e.g., global/regional gray/white matter volumes, cortical thickness) and diffusion tensor imaging traits (e.g., fractional anisotropy of major white matter tracts) which can be automatically extracted via free software packages such as FreeSurfer and FSL, and are minimally confounded by imaging parameter differences. Phenotypes should differ significantly between SCZ cases and controls. Genotypes: Samples lacking genome-wide SNP data will be genotyped for the SNPs of interest. Genome-wide SNP data will be imputed using the 1000Genomes reference panel. Genetic homogeneity of the samples will be assessed via multi-dimensional scaling. Genotypes from each sample where subject-level data are available will be pooled for meta-analysis and associations will be tested via dosage of each SNP (accounting for kinship in family-based samples). For samples where individual subject data are not available (e.g., due to IRB restrictions), results and summary statistics will be pooled for meta-analysis. Statistical analyses: Linear regression analyses will be used to investigate the association between neuropsychological and neuroimaging phenotypes and 1) SNPs with prior GWAS evidence ($p<5\times10^{-8}$) for association with SCZ risk, cognition, or neuroanatomical traits (currently ~100 SNPs); and 2) a polygenic set of SNPs identified in prior large-scale GWAS of SCZ. Multivariate regression analysis across cognitive and imaging domains will be used to 3) identify phenotypic profiles (i.e., trait clusters) associated with independent and polygenic SCZ risk variants that may point to overlapping genetic relationships between brain functions impaired in SCZ, indicating genetic modulation of a common neural mechanism that underlies the phenotypes.

Results

We are currently in the process of forming additional collaborations, and gathering and organizing the datasets. We aim to have the neuropsychological data ready for initial analyses by October 2013.

Discussion

This collaborative project, employing data sharing and mega-/meta-analysis, may contribute towards understanding the function of existing SCZ risk variants and other disease-relevant genes in specific neural circuits and processes that underlie SCZ pathophysiology.

DETECTION OF DE NOVO MUTATIONS IN SCHIZOPHRENIC PATIENTS AND THEIR UNAFFECTED SIBLINGS

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Background
Schizophrenia is a common disease, affecting ~1% of the population with an estimated heritability of up to 80%. Recent studies have shown that in addition to a polygenic model of disease, single de novo mutations may also contribute to disease susceptibility. However, these studies made use of patients and unrelated healthy controls.

Methods
In order to test whether there is an increased de novo mutation rate in patients compared to unaffected siblings, we applied whole exome sequencing in a family-based design. We included 19 families, consisting of proband with both parents, and at least one unaffected sibling. Whole exome capture was performed using Illumina TruSeq kits followed by paired end (100 bp reads) sequencing on the HiSeq2000 platform, resulting in ~30 million readpairs per sample. Reads were aligned to hg19 using BWA, PCR duplicates were removed and recalibration module of GATK was applied. Single sample variant calling was performed with SAMtools and variants were filtered on read-depth and quality. Concordance rates with SNP array for a subset of the data were ~98%. De novo variants were called per family using DeNovoGear and Polymutt. The unaffected sibling in each family served as an extra filtering step, excluding calls appearing in both siblings, which are likely to be false positives.

DeNovoGear is a Bayesian algorithm that starts with an a priori probability of 1x10^{-8}/bp/generation on haploid germline point mutation rate. Putative variants with a posterior probability of >0.98, mapping quality >20 and sequence read depth >30 across all family members were selected. Variants not overlapping between siblings and also called with Polymutt (~60%) were subsequently filtered for heterozygote genotypes in offspring.

Results
We completed the preliminary analyses using these criteria. Based on our results thus far the average predicted de novo variant is 7 per meiosis. The number of predicted de novo SNVs varied greatly by subject and family. Validation using Sanger sequencing needs to be performed on these predicted variants to exclude sequencing artifacts. The preliminary results suggest a slight overrepresentation of the overall de novo variants in coding regions in the affecteds. We did not, however, observe a difference in the distribution of nonsynonymous SNPs (damaging or benign) between probands and unaffected siblings. In both the unaffected and the affected group we find a double hit in the same gene.

Discussion
Our preliminary results indicate that although the number of de novo variants might not differ between affected and unaffected siblings, the distribution of types of SNVs seems to differ between schizophrenia patients and their unaffected siblings.

ASSOCIATION BETWEEN CATECHO-O- METHYLTRANSFERASE (COMT) POLYMORPHISM AND AGRESSION, IRRITABILITY AND SUICIDALITY AMONG SCHIZOPHRENIA PATIENTS IN MALAYSIAN

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Background
Several lines of evidence demonstrated that the low-activity homozygous genotype (Met/Met) of the catechol-O-methyltransferase (COMT) gene has been implicated to increase susceptibility for aggression and suicidal behaviour in schizophrenia patients. However, data from Malaysia is lacking. This present study aimed to determine the association between catechol-O-methyltransferase (COMT) genotype polymorphism and aggression, irritability and suicidality among schizophrenia patients in Universiti Kebangsaan Malaysia Medical Centre (UKMMC), Malaysia.

Methods
This is a cross-sectional study of 110 unrelated patients fulfilled diagnosis of schizophrenia based on the Diagnostic and Statistical Manual of Mental Disorder, Fourth Edition (DSM-IV), aged 18 and above, on treatment from psychiatric wards, clinics or community mental health service in UKMMC, Malaysia. The patients were selected randomly. Recent aggression, irritability and suicidality scores were assessed using Overt Aggression Scale -Modified (OAS-M). This questionnaire captures any aggression, irritability and suicidality past one week prior to the clinical interview. Past history of previous admissions due to aggression and suicidal behaviour were assessed through direct interview with patients and relatives and also by reviewing patients' case notes. COMT genotyping was analysed from 3-5mls patients’ whole blood. This study was approved by the UKM Research Ethics committee.

Results
We found that the mean age of diagnosis in these patients was 28.6 ±12.7 S.D. Almost half of the patients (49.1%) had family history of psychiatric illnesses. The percentage of recent aggression, irritability and suicidality were 20.9%, 20% and 15.5%, respectively. 47.3% of patients had history of previous admissions due to aggression and 17.3% due to suicidal behaviour. The frequencies of high-activity homozygous (Val/Val), heterozygous genotype (Val/Met) and low-activity homozygous genotype (Met/Met) were 28.2%, 56.4% and 15.5%. The COMT genotype frequencies were in Hardy-Weinberg equilibrium. However, no associations were found between recent aggression (p=0.169), irritability (p=0.645) and suicidality (p=0.186) with COMT genotypes polymorphism. Similarly, no associations were found between COMT genotypes with patients with history of previous admissions to psychiatric ward due to aggression (p=0.501) and suicidal behaviour (p=0.643).

Discussion
Our current findings showed that the frequency of the COMT genotypes among Malaysian patients with schizophrenia was comparable with Caucasian patients in previous studies. Nevertheless, our analysis on COMT genotypes did not indicate any robust association with recent and past history of aggression and suicidality in patients with schizophrenia. Further study with larger sample size is required to confirm the finding.

GENOME-WIDE ASSOCIATION STUDY OF ATYPICAL PSYCHOSIS
Background
Atypical psychosis with a periodic course of exacerbation and features of major psychiatric disorders [schizophrenia (SZ) and bipolar disorder (BD)] has a long history in clinical psychiatry in Japan. Based upon the new criteria of atypical psychosis, a genome-wide association study (GWAS) was conducted to identify the risk gene or variants. The relationships between atypical psychosis, SZ and BD were then assessed using independent GWAS data.

Methods
Forty-seven patients with solid criteria of atypical psychosis and 882 normal controls (NCs) were scanned using an Affymetrics 6.0 chip. GWAS SZ data (560 SZ cases and 548 NCs) and GWAS BD (107 cases with BD type 1 and 107 NCs) were compared using gene-based analysis.

Results
The most significant SNPs were detected around the CHN2/CPVL genes (rs245914, p=1.6×10^-7), COL21A1 gene (rs12196860, p=2.45×10^-7), and PYGL/TRIM9 genes (rs1959536, p=7.73×10^-7), although none of the single-nucleotide polymorphisms exhibited genome-wide significance (p=5×10^-8). One of the highest peaks was detected on the major histocompatibility complex region, where large SZ GWASs have previously disclosed an association. The gene-based analysis suggested significant enrichment between SZ and atypical psychosis (p=0.01), but not BD.

Discussion
This study provides clues about the types of patient whose diagnosis lies between SZ and BD. Studies with larger samples are required to determine the causal variant.

THE SCHIZOPHRENIA GWAS RISK GENE ZNF804A IS ASSOCIATED WITH EVENT RELATED POTENTIAL P300 AMPLITUDE
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Background
Genome-wide association studies (GWAS) of schizophrenia (SZ) have identified several genes implicated in SZ susceptibility with evidence surpassing genome-wide significance. However, the downstream pathophysiological changes conferred by these genes remain to be elucidated. One of the first genome-wide significant SZ GWAS genes was ZNF804A, which encodes a DNA-binding protein implicated in brain development. We sought to gain insight into the
function of candidate genes in SZ brain dysfunction as indexed by abnormalities in event related potentials (ERPs), which reflect pre- and post-synaptic activity in neurons.

**Methods**
We performed a genetic association study of 14 SNPs identified by prior SZ GWAS and 7 SNPs implicated in putative SZ intermediate phenotypes by multiple studies. We examined three ERP measures - mismatch negativity (MMN), amplitude of the P300 peak elicited during an auditory oddball task, and P300 amplitude during a novelty oddball task. The sample of 68 SZ cases and 75 matched controls was genotyped on the Illumina OmniExpress array. Linear regression analysis was performed using PLINK with covariates for sex, age, and population substructure captured by the first five multidimensional scaling components. The Bonferroni corrected significance threshold was set at $p = 7.9 \times 10^{-4}$ ($0.05 / 21 \times 3$).

**Results**
Initial comparison of ERP measures in cases and controls detected significantly lower amplitude in cases of the P300 peak measured at the Pz parietal electrode in the novelty oddball task, which indexes rapid orienting of attention to unexpected, salient stimuli involving the anterior cingulate, dorsolateral prefrontal cortex, and hippocampus. Novel P300 amplitude was correlated with a neurocognitive measure of auditory attention under interference conditions, supporting a link between novel P300 amplitude and higher-order attentional processes. Significant genetic association was detected between novel P300 amplitude and ZNF804A marker rs1344706 (beta = 4.38, $p = 1.03 \times 10^{-4}$) surpassing the Bonferroni threshold. Post hoc analyses revealed that the rs1344706 association was driven by the control subjects (beta = 6.35, $p = 9.08 \times 10^{-5}$), with no significant association in cases ($p = 0.21$). The rs1344706 “A” allele associated with SZ risk in GWAS was related to higher P300 amplitude in our analyses. In addition to the Pz electrode, rs1344706 was associated with novel P300 amplitude in controls at other parietal, central, and frontal electrodes ($4.2 \times 10^{-4} < p < 0.064$).

**Discussion**
Our finding of significant association between ZNF804A and novel P300 amplitude concurs with prior literature suggesting a specific role of this gene in modulating brain circuits that are neurobiological substrates of this ERP component. The divergence of ZNF804A association with novel P300 amplitude in controls but not in cases may be due to other genetic, epigenetic, and environmental risk factors, or secondary pathological or treatment effects, weakening the influence of ZNF804A on neural function in SZ cases.

**TP53 POLYMORPHISMS ARE INVOLVED IN INVERSE COLORECTAL CANCER COMORBIDITY IN CHINESE SCHIZOPHRENIA PATIENTS**
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**Background**
The inverse cancer comorbidity in schizophrenia patients may be related to the genetic factors, involving the regulation of apoptosis. The tumour suppressor gene *TP53*, involved in neural apoptosis, is one of the potential candidate genes associated with schizophrenia which might
reduce colorectal cancer risk.

**Methods**
We recruited 270 schizophrenia patients and 312 colorectal cancer patients without schizophrenia. To examine the genetic association between schizophrenia and colorectal cancer, we analysed eight SNPs (rs12951053, rs1625895, rs2909430, rs9895829, rs1042522, rs8079544, rs8064946, rs17806770) covering ~14.35kb in the region of TP53.

**Results**
We observed that one of the eight genetic polymorphisms showed statistically significant differences between the colorectal cancer subjects and the schizophrenia subjects (rs12951053, \( p=0.0001 \), OR 1.70, 95%CI 1.30-2.23). In addition, the haplotype of A-G (rs12951053-rs8064946), giving a global \( p=0.0018 \), was the most significant.

**Discussion**
Our data indicate that the polymorphisms of rs12951053 in TP53 confer reduced susceptibility to colorectal cancer and suggest a potential protective mechanism against colorectal cancer in the schizophrenia patients of Han Chinese origin.

**FUNCTIONAL CHARACTERIZATION OF RARE MUTATIONS OF THE TBX1 GENE AS A CANDIDATE GENE FOR SCHIZOPHRENIA**

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**Background**
Recent studies substantiate a higher-than-expected frequency of schizophrenia in patients with 22q11.2 deletion syndrome (22q11DS), suggesting that chromosome 22q11.2 harbors the susceptible genes related to the pathophysiology of schizophrenia. Of these genes, TBX1 (T-box 1), a member of the T-box transcription factor family, is expressed in all major forebrain subdivisions and the deletion or disruption of TBX1 may alter the expression of genes required for proper development and function of neuronal circuits in the CNS, and eventually lead to the formation of schizophrenia.

**Methods**
We used the systemic mutations detection approach to identify any disruption or rare mutations in TBX1 among 500 healthy controls and 500 non-22q11DS schizophrenic patients and conducted a case-control association study and gene functional assay.

**Results**
We identified 16 known single nucleotide polymorphisms (SNPs) in this sample. However, SNP-based analyses showed no association of these SNPs with schizophrenia. Twenty three rare variants including five missense variants were identified. Three missense mutations
(p.Asp151Glu, p.Glu257Ala, p.Arg342Gln) were only detected in schizophrenic patients, and one (p.Asp155Asn) was only detected in control group. The mutation of p.Ala393Thr was found in both groups. There was no increasing burden of these missense mutations being found in the patient group (p= 0.4812). Furthermore, we identified 4 private rare variants (c.-123G>C, c.-120G>T, c.-84G>A, c.-11delC) at the 5'UTR of TBX1 gene. c.-123G>C and c.-11delC were only detected in schizophrenic patients, while c.-120G>T and c.-84G>A were only detected in control subjects. Computer program predicts that the c.-123G>C may disrupt transcription factor binding site of EBF; the c.-120G>T may change transcription factor binding sites of EBF, TFII-I, RXR-alpha, and T3R-beta1; the c.-84G>A may create transcription factor binding sites of NFI/CTF, NF-1, and ENKTF-1. Further reporter gene activity assay demonstrates that a mutant (c.-123G>C) showed significantly decrease promoter activity compared to the wild type in SKNSH cells.

Discussion
Our results suggest that multiple private rare variants might occur in the TBX1 gene and contribute to the pathogenesis of schizophrenia in some patients.

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METFORMIN AND BERBERINE PREVENT OLANZAPINE-INDUCED WEIGHT GAIN IN RATS
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Background
Olanzapine is a first line medication for the treatment of schizophrenia, but it is also one of the atypical antipsychotics carrying the highest risk of weight gain and other metabolic disorders. A recent review of clinical trials has reported that metformin produces the most significant attenuation of antipsychotic-induced weight gain among fifteen pharmacological interventions. However, the absence of an animal study restrains the clarification of metformin’s mechanism of action. Berberine, a dietary supplement, has been shown in our previous studies to prevent fat accumulation induced by atypical antipsychotics in vitro, and attenuated weight gain induced by a high-fat diet in vivo.

Methods
We examined the efficacy and possible mechanism of metformin and berberine treatments on olanzapine-induced weight gain, utilizing a well reported female Sprague Dawley rat model. Rat body weight and white adipose tissue weight were recorded to assess the treatment efficacy. Food intake and the weight of brown adipose tissue were monitored to examine the energy intake and expenditure associated with drug treatments. The expression of genes playing critical roles in energy balance was examined using the TaqMan OpenArray gene expression platform.

Results
Our results demonstrate that two weeks of metformin or berberine treatment significantly prevented the olanzapine-induced weight gain (by 36.8% or 33.6%) and white fat accumulation (by 96.3% or 73.9%). Neither metformin nor berberine treatment demonstrated a significant
inhibition of olanzapine-increased food intake. But interestingly, a significant loss of brown fat caused by olanzapine treatment has been prevented by the addition of metformin (completely) or berberine (by 70.5%). Our gene expression analysis also demonstrated that the weight gain prevention efficacy of metformin or berberine treatment was associated with changes in the expression of multiple key genes controlling energy expenditure in brown fat tissue or skeletal muscle tissue.

**Discussion**
This study has not only demonstrated a significant preventive efficacy of metformin and berberine treatment on olanzapine-induced weight gain in rats, but also strongly suggests a potential mechanism of action by preventing olanzapine-reduced energy expenditure.

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**MITOCHONDRIAL COMPLEX I MRNA LEVELS IN SCHIZOPHRENIA SUBTYPES**
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**Background**
Mitochondrial complex I located in the electron transport chain has been identified as a potential sensitive and specific biomarker for schizophrenia. We aimed to identify the mitochondrial complex abnormalities in schizophrenia subtypes in this study

**Methods**
Hundred and thirty-eight patients with schizophrenia and forty-two healthy men were included to the study. Schizophrenia patients were divided into subtypes as paranoid (n = 32), catatonic (n = 17), disorganized (n = 39), undifferentiated (n = 26) and residual (n = 24) according to the dominance of psychotic symptoms. Subtypes of schizophrenia patients’ peripheral mitochondrial complex I gene mRNA levels were compared with control subjects.

**Results**
Complex I genes (NDUFV1, NDUFV2, NDUFS1) mRNA levels in catatonic, disorganized and undifferentiated types were significantly higher than the healthy subjects (p<0.05). There was no difference in complex I genes (NDUFV1, NDUFV2, NDUFS1) mRNA levels between paranoid, residual subtypes and healthy subjects (p>0.05).

**Discussion**
Behavioral pathology is expected to be less in paranoid and residual types than other types. The elevation of genes mRNA levels in the subtypes that the behavior pathology was more common suggested that behavior pathology could be effective in increasing the levels of mitochondrial gene mRNA levels.

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**IDENTIFYING FUNCTIONAL VARIATION IN SCHIZOPHRENIA GWAS LOCI BY POOLED SEQUENCING**
Mounting evidence supports excess rare variants in cases in GWAS-identified loci. Recent work has identified rare variants in the GWAS candidates for Inflammatory Bowel Disease (IBD) through targeted resequencing of pooled IBD cases and controls. Associations from GWAS implicate common variants in these regions in high LD with the associated SNPs. Rare functional variants in these regions are independent of the common variant signal and provide independent support for the gene as part of the disease pathway. Pooled sequencing allows efficient target capture and library construction of this large sample. A large study of this type has not yet been pursued for the GWAS loci in schizophrenia. We use this method to interrogate 500 kb of schizophrenia GWAS loci exons from the first Psychiatric GWAS Consortium (PGC) schizophrenia analysis and additional evolutionarily constrained regions in our sample of 912 cases from the Irish Case/Control Study of Schizophrenia (ICCSS) sample and 1296 Irish population controls from the Trinity Biobank in Dublin.

Methods
We sequenced 38 pools of 24 cases and 54 pools of 24 controls (total N=912 cases and 1296 controls). Samples were quantified and normalized twice using PicoGreen followed by equimolar pooling. Each pool was prepared for sequencing using the Agilent SureSelect targeted sequencing protocol and sequenced on the Illumina HiSeq platform using 2x100 bp reads. We selected targets based on exons in the regions in linkage disequilibrium in our sample with the significant SNPs in the PGC analysis. Within these areas we also selected regions with significant mammalian conservation indicating a functional region or other important unknown region. Reads were aligned to the human reference genome using BWA. We used GATK for local realignment and base quality recalibration. We used Syzygy to call variant frequencies from the sample pools. Variants will be validated using Sequenom iPLEX Gold on the MassARRAY system. For quality control of pools we compared called variant allele frequencies with known allele frequencies for the pool from imputed genotype array data we have for our sample. We considered a correlation above 0.99 to be good evidence for successful equimolar pooling. We will use the SNP-set Kernel Association Test (SKAT) to test all functional variants in a gene with MAF < 2%. We will also individually test variants above 2% MAF which are rated by PolyPhen and SIFT as the most likely to be damaging.

Results
Preliminary sequencing on the MiSeq platform at ~50x coverage per sample per pool for eight of the pools show a high correlation (> 0.99) between called allele frequencies and 1774 known allele frequencies from imputed genotype array data we have for our sample. We considered a correlation above 0.99 to be good evidence for successful equimolar pooling. We will use the SNP-set Kernel Association Test (SKAT) to test all functional variants in a gene with MAF < 2%. We will also individually test variants above 2% MAF which are rated by PolyPhen and SIFT as the most likely to be damaging.
Discussion
We demonstrated successful pooled sequencing in our sample of schizophrenia cases and controls. Our goal is to detect and validate causal rare variation in the PGC defined schizophrenia GWAS loci (MIR137, PCGEM1, TRIM26, CSMD1, MMP16, CNNM2, NT5C2, STT3A, CCDC68, TCF4, ITIH3/4, ANK3, and CACNA1C) and establish patterns of biased rare variation between cases and controls in GWAS loci using the region based test SKAT.

SHARING OF A COMMON GENETIC BASIS WITH TYPE 1-DIABETES BUT NOT TYPE 2-DIABETES INDICATIVE OF AUTOIMMUNE COMPONENT FOR SCHIZOPHRENIA
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Background
As early as 1887, Sir Henry Maudsley observed an increase of diabetes mellitus (DM) in relatives of schizophrenia patients. The aim of this study is to test whether a shared genetic basis drives the comorbidity between schizophrenia and T1D, an autoimmune-related disorder and T2D, a metabolic disorder.

Methods
In polygenic risk score analysis, SNP effect sizes (i.e., logistic regression weights) from a discovery sample are used to compute additive genetic risk scores to predict disease status in an independent validation sample. We meta-analyzed the effect sizes (using a fixed effects model) of the PGC-1 schizophrenia samples (9,528 cases and 8,922 controls; excluding the Cardiff UK sample to prevent overlap with the T1D and T2D controls) and used these to compute polygenic risk scores in T1D (1,914 cases and 2,982 controls) and T2D (1,968 cases and 2,983) quality controlled samples from the Wellcome Trust Case Control Consortium (WTCCC). The polygenic risk scores were subsequently used to predict T1D and T2D disease status, respectively, after correcting for predictive power of 10 PCA components. The analysis was performed using different significance thresholds in the schizophrenia data. E.g., $p_{\text{threshold}}=0.1$ indicates that only those SNPs with a $p$-value below 0.1 in schizophrenia data were included in the polygenic score analysis.

Results
We show that the phenotypic overlap between schizophrenia and DM observation is at least partly explained by a genetic overlap between schizophrenia and type 1-diabetes (T1D), which is traditionally categorized as an autoimmune disease. Figure 1 shows the predictive power of the polygenic risk score above and beyond the predictive power of 10 PCA components to control for differences in population stratification between cases and controls. The results imply a shared genetic basis between schizophrenia and T1D ($p=0.02$ for $p_{\text{threshold}}=0.1$, $p<0.01$ for $p_{\text{threshold}}=0.2$), whereas little evidence for genetic overlap was found for T2D ($p=0.03$ for $p_{\text{threshold}}=0.8$; $p\geq0.06$ for $p_{\text{threshold}}=0.8$). Predictive power in T1D increases from 0.2% when including SNPs with
\[ p_{\text{threshold}} = 0.1 \text{ to } 1.1\% \text{ when including SNPs with } p_{\text{threshold}} = 0.9. \]

**Discussion**
These results support a role for auto-immune processes in the etiology and pathophysiology of schizophrenia. More extensive genetic cross-disorder analyses are required to study the extent of the role of an autoimmune component in schizophrenia and whether its contribution is also found in other neuropsychiatric traits.

EXPLORING THE ALLELIC SPECTRUM OF THE SCHIZOPHRENIA CANDIDATE GENE KCTD13 ON CHROMOSOME 16P11.2
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**Background**
Rare duplications in the chromosomal region 16p11.2 are an established risk factor for the development of schizophrenia. Deletions in the same region increase the susceptibility to autism and intellectual disability. The chromosomal regions affected by these copy number variants (CNVs) are typically large and span > 20 genes. Several of these genes are interesting candidate genes. However, the underlying risk gene/genes have not yet been identified. Based on genetically modified zebrafish, Golzio et al. have recently identified \(KCTD13\) as the major driver of the neuroanatomical phenotypes of CNVs in 16p11.2 (Golzio et al., 2012). The aim of this study was to further explore \(KCTD13\) as a candidate gene in this region by analyzing whether small CNVs (affecting only parts of the coding sequence) and small changes in the DNA sequence confer risk for developing schizophrenia.

**Methods**
Identification of variation in the DNA sequence: targeted Sanger resequencing of all six exons was performed in a total of 570 patients. All patients had a DSM-IV diagnosis of schizophrenia. Of these, 285 individuals had an early age-at-onset (< 21 years) whereas the remaining 285 patients showed poor performance on a neurocognitive test battery. Publicly available data from the 1000 Genomes Project are used to determine the frequency of the identified variants in healthy individuals. Detection of small CNVs: using two commercially available TaqMan® CNV probes, quantitative PCR was performed in 500 patients with schizophrenia (part of the sequencing study) and 500 population-based controls. The TaqMan® probes were located in exon 1 and exon 6 of \(KCTD13\).

**Results**
The analyses are still ongoing. So far, we have identified in one patient an interesting point mutation in exon 5 which was predicted to be damaging by several independent mutation prediction programs. This amino acid changing mutation was not detected within the 1000
Genomes Project data. Variants predicted to be disease-causing will be followed up in a large independent sample comprising of 2000 patients with schizophrenia and 2000 population-based controls.

**Discussion**

*KCTD13*, located in the schizophrenia-associated duplicated 16p11.2 region, is a promising candidate gene for neuropsychiatric disorders. So far, no genetic study focusing on the identification of smaller CNVs/DNA sequence variants in this gene has been published. Our identification of a probably damaging mutation in a schizophrenia patient provides first support for an involvement of *KCTD13* in the development of schizophrenia. Results of more comprehensive analyses will be presented at the meeting.

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**WHAT DETERMINES CONTINUING LATERAL VENTRICLES ENLARGEMENT IN FIRST-EPIODE SCHIZOPHRENIA AND AFFECTIVE PSYCHOSIS?**

**PRELIMINARY ANALYSIS**

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**Background**

Recent neuroimaging studies reported that lateral ventricles enlargement in subjects with psychosis may be progressive after illness onset. This study aimed at investigating whether progressive lateral ventricle enlargement occur among subjects with first-episode of psychosis in contrast to controls, and which factors are associated to it (diagnosis, outcome, antipsychotic intake, SNPs).

**Methods**

Thirty-five subjects with first-episode psychosis (FEP; 21 with schizophrenia-spectrum disorders, FESZ, and 14 with affective psychoses, FEAP) and 23 controls underwent structural MRI scanning at baseline and at a 5 years follow-up. Lateral ventricles volumetry was assessed using region-of-interest manual drawing. Backward regression analyses using the univariate general linear model were employed including sociodemographic (age and gender), clinical (diagnosis, outcome, abuse or dependence of substances, duration of untreated psychosis and total exposure to antipsychotics in days between brain scans) and single-nucleotide polymorphisms (Catechol-O-Methyltransferase rs165599, Brain-derived Neurotrophic Factor rs6562, Dopamine Receptor D2 rs1799732 and SLCA63 Dopamine Transporter 3 rs6347) as independent variables. And each of four lateral ventricles measures (right and left lateral ventricles and right and left temporal horns) served as a dependent variable.

**Results**
Diagnosis, outcome or SNPs were not associated to lateral ventricles enlargement during the follow-up (all p > 0.005). However, total exposure to antipsychotics during the follow-up (in days) was positively associated to right lateral ventricles enlargement during the follow-up among FEP subjects (F: 6.167, p: 0.015, η²: 0.065), while the duration of untreated psychosis was negatively associated to left lateral ventricles enlargement during the follow-up (F: 3.962, p: 0.049, η²: 0.043).

Discussion
Our results are in line with studies that report that the progression of brain abnormalities among FEP subjects is at least partially associated to antipsychotic intake. Subjects with longer durations of untreated psychosis may have already shown important lateral ventricles enlargement at the first-episode of psychosis, so a subsequent follow-up may fail to show progression of this brain abnormality. The inclusion of more subjects with genetic data is under way, what will increase the statistical power of our analyses.

RESEQUENCING AND ASSOCIATION ANALYSIS OF PTPRA AS A POSSIBLE SUSCEPTIBILITY GENE FOR SCHIZOPHRENIA
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Background
Schizophrenia is a genetically heterogeneous disorder with heritability estimated at up to 80%. Great interest has been drawn to rare (MAF<1%) missense mutations as promising candidates to explain the ‘missing heritability’ of the disorder. The PTPRA gene encodes RPTP-α, a member of the protein tyrosine phosphatase (PTP) family, which is involved in neurodevelopmental processes such as radial neuronal migration, cortical cytoarchitecture and oligodendrocyte differentiation. Moreover, RPTP-α is functionally involved in the neuregulin 1 (NRG1) signaling pathway, one that is proven to be associated with schizophrenia. Previous linkage studies have pointed to the area which the gene is located, and association studies have indicated relations between SNPs within the gene and schizophrenia. Also, Ptpra knockout mice have shown neurodevelopmental deficiencies as well as schizophrenic behavioral patterns.

Methods
The study is conducted in two stages. During the first stage, resequencing is performed for encoding exonal regions and splicing sites of the PTPRA gene in a sample set of 190 schizophrenia patients. The aforementioned regions are divided into 10 amplicons with lengths varying between 700~3000bps for PCR amplification. The Sanger method is used for resequencing sample DNA, with BigDye Terminator v3.1 Cycle Sequencing Kit by Applied Biosystems. Plates are read on an ABI 3130xl Genetic Analyzer, the result of which is then
processed with Mutation Surveyor for SNPs detection. Only rare missense SNPs with MAF<1% are selected for association analysis in the second stage.
The second sample set includes 963 schizophrenia patients and 919 controls (total=1882, unknown=5, male 50.05%). Additionally, we also include 313 pervasive developmental disorders (PDDs) patients (male 76.68%) due to reported overlap between genetic etiologies of schizophrenia and PDDs. Custom TaqMan SNP genotyping assays are purchased from Applied Biosystems. Allelic discrimination analysis is done using ABI PRISM 7900HT Sequence Detection System.

Results
The project is currently underway. Up to the time of submission of this abstract, 95 patient samples in the first sample set have been resequenced. 2 rare missense variants, rs61742029 and 101281T>TC, 59L>L/P, were detected. Association analysis discovered 5 samples containing the rs61742029 SNP (schizophrenia case=1, control=3, PDD case=1) and 1 containing 101281T>TC, 59L>L/P (schizophrenia case).

Discussion
By the time of the conference, the resequencing of all 190 samples should be finished, and association analyses completed for all rare missense variants detected in the coding area/splicing sites. Follow-up expression study and functional analysis will be performed as well if we confirm overrepresentation of any of the variants among patients.

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COMPLEXIN2 MODULATES WORKING MEMORY-RELATED NEURAL ACTIVITY IN PATIENTS WITH SCHIZOPHRENIA
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Background
The specific contribution of risk or candidate gene variants to the complex phenotype of mental disorders such as schizophrenia is largely unknown. Studying the effects of such variants on brain function can provide insight into disease-associated changes and mechanisms on a neuroscience systems level. Common variants in the complexin2 (CPLX2) gene were found to be highly associated with cognitive functioning in schizophrenia patients. CPLX2 is a presynaptic protein regulating transmitter release and Cplx2 mutant mice show prominent cognitive loss of function if a minor brain lesion is applied during puberty (Begemann et al. 2010). We aimed to study common CPLX2 SNPs and their neurogenetic risk mechanisms by analyzing their relationship to a schizophrenia-related functional neuroimaging intermediate phenotype.

Methods
We obtained functional MRI and genotype data of 104 patients with DSM-IV schizophrenia and
122 healthy controls from the Mind Clinical Imaging Consortium study of schizophrenia. Seven SNPs distributed over the whole \textit{CPLX2} gene were tested for association with working memory-elicited neural activity in a fronto-parietal working memory network (dorsolateral prefrontal cortex and intraparietal sulcus), which is a widely acknowledged intermediate phenotype for schizophrenia.

\textbf{Results}

Three \textit{CPLX2} SNPs were significantly associated with increased neural activity in both brain regions in the schizophrenia sample, but showed no association in healthy controls.

\textbf{Discussion}

Increased working memory-related prefrontal and parietal neural activity in individuals with or at risk for schizophrenia has been interpreted as ‘neural inefficiency’. In line with the ‘second hit’ model, the combination of a \textit{CPLX2} null mutation and an environmental risk factor induces phenotypic changes specifically relevant to schizophrenia. Our findings illustrate the impact of \textit{CPLX2} risk variants on a brain-based intermediate phenotype for schizophrenia and are consistent with the aforementioned neurocognitive and animal studies.

\section*{THE SWEDISH SCHIZOPHRENIA STUDY: SAMPLE COLLECTION THROUGH GENETIC ANALYSES}

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\textbf{Background}

Schizophrenia (SCZ) is a highly heritable disease yet identification of specific risk associated genes to explain the heritability has been challenging, in part due to the limited case/control DNA cohorts available in the field. In 2004, a collaboration was formed between the Karolinska Institutet in Stockholm, Sweden, the University of North Carolina in Chapel Hill, North Carolina, and the Broad Institute in Cambridge, MA to quickly ascertain large numbers of SCZ cases and geographically matched control samples on a nationwide basis. Sweden was an ideal target population due to the genetically homogeneous population and an existing hospital discharge registry, making it possible to quickly identify and contact Swedish patients with SCZ.

\textbf{Methods}

Within a period of 6 years a total of 12K individuals, 5.5K SCZ patients and 6.5K controls, provided a DNA sample and were consented for genetic research, with the possibility to re-contact. Case inclusion criteria included ≥2 hospitalizations with a discharge diagnosis of SCZ, both parents born in Scandinavia, and age of ≥18 years. Control inclusion criteria included no hospitalizations for SCZ or bipolar disorder (given the evidence of genetic overlap with SCZ), both parents born in Scandinavia, and age of ≥18 years. DNA samples were extracted from
peripheral blood at the Karolinska Institutet Biobank, and aliquots were shipped to The Broad Institute for extensive genetic analyses. Here we report on the experiments and major findings to date, made possible by the Swedish SCZ Cohort.

Results
All 12K subjects have undergone genome-wide genotyping for common variants and rare copy number variants (CNVs). These data have led to the identification of 22 genome-wide significant regions and a novel CNV association, while highlighting several biological pathways warranting further investigation. Similarly exon variation has been exhaustively assessed using both Illumina exome array and whole exome sequencing on all samples. Analysis of exome sequence data has generated the first evidence that protein-damaging variants in certain categories of genes are enriched in SCZ cases relative to controls. Whole genome sequencing has been completed in a small number of patients and controls affected by a SCZ associated deletion at 1q21.1 to look in-depth at structural variation, revealing high variability in size and affected genes. Finally, we have begun to re-contact and collect skin biopsies from patients with large risk CNVs and controls to be used for induced pluripotent stem cell (iPSC) reprogramming and neuronal differentiation.

Discussion
Today, the Swedish cohort is one of the largest and most comprehensively characterized SCZ case/control cohorts worldwide. This cohort, rich with both phenotypic and genetic data, is better enabling the field to explain the missing heritability and elucidate underlying disease etiology.

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INTERACTION BETWEEN DRD2 AND COMT POLYMORPHISMS: ASSOCIATION WITH SCHIZOPHRENIA AND TREATMENT RESPONSE TO ANTIPSYCHOTIC DRUGS
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Background
Schizophrenia is a complex disease with genetic and environmental factors interacting to illness development. Deregulation of dopaminergic neurotransmission is described in the pathophysiology of schizophrenia. Genetic and functional evidence suggest an association of several genes, among them DRD2 and COMT, and schizophrenia, although their role in the pathogenesis is still unclear. DRD2 gene encodes a dopamine receptor and COMT gene encodes an enzyme that catalyzes the transfer of a methyl group to catecholamines, including dopamine. Interactions between genes make a substantial contribution to variation in complex traits such as schizophrenia susceptibility. We aimed to investigate the interaction between DRD2 rs1799732 (−141C Ins/Del) and COMT Val158Met polymorphisms upon schizophrenia diagnosis and treatment resistant (TR) schizophrenia.

Methods
We have analyzed 234 patients with schizophrenia and a subsample consisting of 73 TR and 73 non-TR, and 280 healthy controls. The subjects were genotyped for COMT Val158Met
polymorphism by TaqMan probe based real time PCR assay and for $DRD2$ $−141C$ $Ins/Del$ polymorphism by PCR-RFLP (polymerase chain reaction-restriction fragment length polymorphism). The effect of interaction between $DRD2$ and $COMT$ polymorphisms on schizophrenia diagnosis and TR schizophrenia was verified using logistic regression analysis.

**Results**

None of the genotype distributions deviated from those expected from Hardy–Weinberg equilibrium ($p>0.05$). There were no significant differences in the alleles and genotypes frequencies of these polymorphisms between patients and controls. Moreover, we have not found association between $−141C$ $Ins/Del$ and Val158Met genotypes or alleles and TR schizophrenia. Considering the interaction between these polymorphisms, we have not found an interaction effect between $DRD2$ and $COMT$ polymorphisms upon schizophrenia and TR schizophrenia.

**Discussion**

Although we have not found an effect of the interaction among them, many authors argue that epistasis should be accounted for in complex trait studies. Moreover, other studies have reported interaction effects between $COMT$ Val158Met and $ANKK1$ and $DAT$, which are related to schizophrenia, upon cognitive symptoms of the disease. As it is known that Val158Met polymorphism is a functional variant, which may alter dopamine levels in the prefrontal cortex and its interaction with variant of catecholamines genes beyond $DRD2$ may contribute to the risk for schizophrenia symptoms and treatment response. Therefore, our results suggest the interaction of $DRD2$ $−141C$ $Ins/Del$ and $COMT$ Val158Met polymorphisms is not an independent risk factor for the development of schizophrenia or clinical response to treatment, but may be effective over susceptibility to disease and treatment response together with other factors. Financial support: FAPESP (2011/50740-5) and CNPq.

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**METABOLIC ANALYSIS REVEALS METABOLIC DISTURBANCE IN THE CORTEX AND HIPPOCAMPUS OF SUBCHRONIC MK-801 TREATED RATS**

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**Background**

Although a number of proteins and genes relevant to schizophrenia have been identified in recent years, few are known about the exact metabolic pathway involved in this disease. Our previous proteomic study has revealed the energy metabolism abnormality in subchronic MK-801 treated rat, a well-established animal model for schizophrenia. This prompted us to further investigate metabolite levels in the same rat model to better delineate the metabolism dysfunctions and provide insights into the pathology of schizophrenia.

**Methods**

23 male Sprague-Dawley rats (220–250 g) were randomly divided in two groups. Rats in the control group (n=11) were injected subcutaneously with physiological saline 3.5 ml/kg (0.9% wt/vol NaCl [aqueous]) and those in the treatment group (n=12) with 0.7 mg/kg MK-801
(Research Biochemicals, Natick, Massachusetts) (saline as vehicle) for 10 days. Metabolomics, a high-throughput investigatory strategy developed in recent years, can offer comprehensive metabolite-level insights that complement protein and genetic findings. In this study, we employed a nondestructive metabolomic approach (1H-MAS-NMR) to investigate the metabolic traits in cortex and hippocampus of the rats. Multivariate statistics and ingenuity pathways analyses (IPA) were applied in data processing. The result was further integrated with our previous proteomic findings by IPA analysis to obtain a systematic view on our observations.

Results
The 1H MAS NMR spectra from cortex were similar to that from hippocampus. After data reduction, 325 bins (variables) were obtained from the spectra. Bins from the MK-801 treated group and the control group were compared by Student’s t-test. 48 bins had p-values lower than 0.05 in the cortex, of which 44 had q-values lower than 0.2. 34 bins had p-values lower than 0.05 in the hippocampus, of which 11 had q-values lower than 0.2. Clear distinctions between the MK-801 treated group and the control group in both cortex and hippocampus were found by OPLS-DA models (with R²X=0.441, Q²Y=0.413 and R²X=0.698, Q²Y=0.677, respectively). Further validation showed that cortex models could predict class membership well with an accuracy of 83.3% and hippocampus models with an accuracy of 82.6%. In the cortex of the MK-801 treated rats, the back-scaled loading plot shows increased levels of lactate, acetate, L-alanine, L-aspartate, GABA, NAA, scyllitol, L-serine and succinate, and decreased levels of citrate, glutamine, glutamate, myoinositol, choline, phosphorylcholine, creatine and taurine. Similar result was seen in the hippocampus but with some differences, such as acetate and L-aspartate levels which were elevated in the cortex but decreased in the hippocampus. Most of these metabolites fell in a pathway characterized by down-regulated glutamate synthesis and disturbed Krebs cycle. Our previous proteome study revealed 49 proteins altered in the cortical synaptosomes of subchronic MK-801 treated rats. We combined those differentially expressed proteins with treatment related metabolites in the cortex of this study and carried out an integrated IPA analysis. The top network function was the amino acid metabolism, molecular transport and small molecule biochemistry, while the top canonical pathway turned out to be the Krebs cycle.

Discussion
Glutamate was reduced in the hippocampus and had a trend of decrease in the cortex in our study. Comparing with related studies, it implies a potential dynamic regulation of glutamate level in response to the length of MK-801 treatment, which is suggestive for human studies since a comprehensive down-regulation of glutamate synthesis was found in chronic schizophrenia patients. GABA was up-regulated in both brain areas. An elevation of glutamate can stimulate GABAergic neurons to release GABA which inversely inhibits glutamate synthesis. This kind of negative feedback might be involved in the dynamic regulation of glutamate in respond to MK-801 treatment.

Besides neurotransmitter dysregulation, alterations in the Krebs cycle pointed to a dysfunction of brain mitochondrial energy metabolism caused by MK-801. Aspartate and alanine metabolism was also altered in the treated rats. The increase of L-alanine along with lactate indicates a stirring transamination activity in glutamatergic neurons and glials. In addition, the metabolite-protein integrated IPA analysis not only agreed well with our metabolomic conclusion that glutamate related and energy metabolism were the top altered pathways responding to
subchronic MK-801 injection but also stresses the involvement of energy metabolism such as the Krebs cycle in the MK-801 induced dysfunctions.

In conclusion, this study revealed systematic changes in metabolism in the MK-801 treated rats’ cortex and hippocampus, which could serve as a valuable reference to the etiology research of schizophrenia.

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ROLE OF DISRUPTED-IN-SCHIZOPHRENIA 1 (DISC1) IN CORTICAL INHIBITORY NEURONS.
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Background
Schizophrenia is a relatively poorly understood, debilitating psychiatric disorder affecting around 0.5% of the population worldwide. The main characteristics of the disease are hallucinations, delusions and cognitive impairment such as difficulty in learning. Recently, it has been suggested that mutations in a gene encoding Disrupted-in-Schizophrenia-1 (DISC1) might be one of the main genetic risk factors for this disease. DISC1 has been implicated in brain development, in neurite outgrowth, neural precursor proliferation/differentiation, integration of newborn neurons and neuronal migration (Chubb et al., 2008). DISC1 function in radial neuronal migration has been studied in some detail but there is little evidence implicating DISC1 in tangential migration of interneurons.

Methods
In this study, two mouse lines with point mutations in the DISC1 sequence were used: the L100P and Q31L N-ethyl-N-nitrosourea (ENU) mutant mice previously characterized as ‘schizophrenic-like’ and ‘depressive-like’ respectively (Clapcote et al., 2007). The brain tissue was collected from 21 days old mice (both sexes, 8-12 g, n=4-8 of each strain and genotype). Mice were sacrificed by injecting an overdose of anaesthetic (40 mg/kg sodium pentobarbital) and perfused with 10 ml PBS and 5 ml 4% paraformaldehyde (PFA). The brains were fixed overnight in 4% PFA, cryoprotected in 30% sucrose and cut on a Leica CM3050 S cryostat. The number and relative distribution of cortical interneuron subclasses, namely parvalbumin (PV), somatostatin (STT), calretinin (CLR); as well as total number of interneurons (GAD67 positive cells) was analysed in four 500 µm wide cortical regions: frontal and barrel-field primary somatosensory (fpSS and pSS respectively) cortices, ventral auditory cortex (vAUD) and visual cortex (VIS).

Results
There was a significant decrease in the number of PV-positive interneurons across the L100P homozygous and L100P heterozygous (L100P+/−) brains when compared to their wild-type (WT) littermates (fpSS: 67.75±4.75 WT, 50.00±2.07 L100P, p<0.01; pSS: 61.50±3.69 WT, 46.13±2.39 L100P+/−, 35.13±5.85 L100P, p<0.01; vAUD: 33.81±2.83 WT, 16.25±2.59 L100P+/−, 23.88±1.91 L100P, p<0.05; VIS: 44.17±1.69 WT, 31.5±2.51 L100P+/−, 30.00±1.00 L100P, p<0.05). However, there was no reduction in the total number of interneurons implicating that the mutation affects parvalbumin levels in the cells (fpSS: 142.67±11.42 WT, 139.50±3.37
L100P+/-, 154.8±7.47 L100P; pSS: 155.33±5.27 WT, 120.13±7.39 L100P+-, 141.50±8.97 L100P; vAUD: 128.58±5.85 WT, 123.38±4.09 L100P+-, 141.50±8.97 L100P; VIS: 95.13±10.89 WT, 91.25±9.89 L100P+-, 100.25±7.25 L100P). As expected, there were fewer cells containing PV mRNA across the L100P+/- and L100P brains, with numbers similar to those in PV expression data. Interestingly, reduction in PV was not observed in the Q31L +/- and Q31L cortices. The relative distribution of the parvalbumin positive cells across the cortex was mildly disrupted in both L100P and Q31L mutants (fpSS: 62.29±4.76 WT, 61.00±4.09 L100P+-, 60.00±3.29 L100P; pSS: 43.93±4.08 WT, 53.33±3.61 L100P+-, 41.22±3.23 L100P; vAUD: 21.29±2.66 WT, 17.17±3.42 L100P+-, 25.00±3.47 L100P; VIS: 41.38±2.91 WT, 30.17±4.01 L100P+-, 34.9±1.32 L100P). A minor change in the relative distribution of the GAD67-positive cells in pSS and vAUD cortices of L100P, but not Q31L, was also observed. There was no significant difference in the total number calretinin and somatostatin expressing interneurons in both genotypes when compared to their wild-type littermates.

Discussion
This study demonstrates that DISC1 is involved in the generation of parvalbumin expressing interneurons within the cortex. L100P point mutation in DISC1 led to downregulation of the parvalbumin, which should result in abnormal inhibitory properties of this interneuron subtype. Since parvalbumin expression has been suggested to be activity dependent (Patz et al., 2004), in utero over-expression of the L100P DISC1 constructs in the cortical excitatory cells may give further insight into how DISC1 regulates parvalbumin levels in cortical interneurons. Finally, minor changes in the distribution of interneurons across the cortex further supports DISC1 involvement in the neuronal migration and integration into the cortical circuits.

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ABSTRACT WITHDRAWN

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A CASE-CONTROL GENOME-WIDE ASSOCIATION STUDY FOR SCHIZOPHRENIA CHARACTERIZED BY EARLY ONSET AND ATTENTION DEFICIT
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Background
Schizophrenia has been considered as a polygenic disorder that is likely to have genetic heterogeneity. Early-onset and neurocognition deficit were related to more genetic loadings in
schizophrenia. A recent nested ordered subset linkage analysis has identified a subset of schizophrenia patients characterized by early age at onset and greater attention deficit that led to a linkage signal with genomewide significance. On the basis of this subset of schizophrenia, we aimed to search for potential single nucleotide polymorphisms (SNPs) associated with this subtype of schizophrenia using a genomewide approach by comparing with a community-based healthy controls group.

Methods
An age- and sex-matched case-control study of 185 schizophrenia patients and 925 community controls was used for genome-wide association study. All the patients with schizophrenia were recruited in the Taiwan Schizophrenia Linkage Study (TSLS) throughout the nation between 1998 and 2002. Initially, a subset of 95 schizophrenia patients with early age-at-onset and poorer Continuous Performance Test (CPT) results was selected according to a previous ordered subset analysis, and an opposite subset of 95 patients with late onset age and better CPT score was selected as well. The Affymetrix Axiom Genome-Wide CHB 1 Array Plate was used to determine the genotypes of 642,832 SNPs. Sample quality control was performed with sex inconsistencies, sample genotyping call rate, kinship and population stratification check. A total of 185 out of 190 patients with schizophrenia passed these quality checks and were subjected to control matching. The normal controls were selected from the community subjects of Han Chinese Cell and Genome Bank in Taiwan with age- and sex-matching in a 1:5 ratio of case-to-control to increase the statistical power of this study. Those controls had been genotyped using the same Affymetrix array plate and undergone similar quality control strategy. Associations between SNP genotypes and onset of schizophrenia were mainly assessed using multivariable logistic regression analysis with adjustment for age and sex.

Results
About 85% of the SNPs passed the quality control, including duplicate sample concordance, marker genotyping call rate, and exclusion criteria of minor allele frequency (MAF) <0.05 as well as Hardy-Weinberg equilibrium (HWE) <0.001. At first, we compared 94 schizophrenia patients with early onset and attention deficit versus community controls (n = 925) and found that the two groups had different allele frequencies for the following SNPs: 3 SNPs (rs74611150, rs4572656 and rs72626506) with P < 10^{-5}, 55 SNPs with P < 10^{-4}, and 469 SNPs with P < 10^{-3}. Among them, 228 SNPs are located on 148 genes. Then, we compared the 91 schizophrenia patients with late onset and better attention performance versus community controls and found that the two groups had different allele frequencies for the following SNPs: one SNP (rs270666) with P < 10^{-5}, 47 SNPs with P < 10^{-4}, and 438 SNPs with P < 10^{-3}. Among them, 202 SNPs are located on 150 genes. In general, the early-onset and attention-deficit subgroup of schizophrenia patients exhibit more potentially associated SNPs than the other subgroup of patients, and only 2 out of the 1010 SNPs with P < 10^{-3} overlapped. The 3 SNPs with the highest significance level for the early-onset and attention-deficit subgroup of schizophrenia, not overlapping with the other subgroup of schizophrenia, include rs74611150 in a pseudo gene on chromosome 14q11.1 and two intergenic SNPs (rs4572656 and rs72626506) located on chromosome 20q11.1, with the odds ratios of 2.5, 2.6 and 2.4, respectively.

Discussion
The results suggested that schizophrenia patients with early onset and attention deficit did exhibit
a different pattern of associated SNPs as compared to schizophrenia patients without such characteristics. Our preliminary GWAS-based search of susceptibility genes associated with age-at-onset and attention deficit identify a number of SNPs that warrants for further fine mapping.

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EXPLORING THE INDIRECT EFFECTS OF CATECHOL-O-METHYLTRANSFERASE (COMT) ON PSYCHOTIC EXPERIENCES THROUGH COGNITION AND ANXIETY DISORDERS IN CHILDREN.

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Background
Children reporting psychotic experiences (PEs) are at increased risk of developing psychosis in adulthood. Cognitive deficits and anxiety disorders often precede psychotic disorders and are also associated with higher risk of PEs. While the high activity alleles of COMT (a gene that degrades dopamine) have been associated with cognitive deficits, the low activity alleles have been associated with higher risk of anxiety disorders, but no associations of COMT with PEs have been reported. One possible explanation is that COMT is associated with PEs indirectly, through cognitive function and anxiety disorders. We set out to examine the extent to which the association between COMT and PEs in children is mediated by 1. cognition and 2. anxiety disorders.

Methods
6,784 individuals from the Avon Longitudinal Study of Parents and Children (ALSPAC) birth cohort were genotyped and completed a set of comprehensive neurocognitive assessments at ages 8 and 11, a semi-structured interview for anxiety disorders at age 10 and a semi-structured interview for psychotic experiences at age 12.

Results
We found evidence for our first hypothesis; the high activity allele of rs2097603 (a single nucleotide polymorphism located in the P2 promoter that drives transcription of the predominant form of COMT in the brain) was indirectly associated with higher risk of PEs in children though processing speed and attention. However, there was no evidence for an indirect link of the COMT genotype on PEs through anxiety disorders.

Discussion
This is the first study to examine potential indirect effects of COMT on PEs in children. Our findings suggest a potential mechanism that could underlie the emergence of PEs in children and provide additional support for processing speed and attention as potential endophenotypes in schizophrenia.

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EVIDENCE FOR THE INVOLVEMENT OF MIR185 AND ITS TARGET GENES IN THE DEVELOPMENT OF SCHIZOPHRENIA
Background

The 22q11.2 deletion syndrome (22q11.2DS), also known as velocardiofacial/DiGeorge syndrome, is a phenotypically complex syndrome caused by a hemizygous microdeletion in chromosomal region 22q11.2. It occurs approximately 1 in 2,000 - 4,000 births and about 30% of carriers develop schizophrenia, making this syndrome one of the strongest known genetic risk factors for schizophrenia. Symptoms of 22q11.2DS-related schizophrenia are largely indistinguishable from those of the idiopathic disease suggesting that the investigation of deletion-related forms may reveal further insight into the genetic mechanisms of schizophrenia in general. Most of the deletions at 22q11.2 are either 1.5 or 3 megabases in size spanning 35 and 60 known genes, respectively. Research has yet to confirm which genes within the deletion region are of importance in terms of the schizophrenia-risk effect. The minimal 1.5 megabase deletion region contains the gene MIR185, which encodes microRNA 185. This has two validated targets (RhoA, Cdc42), both of which have been associated with altered expression levels in schizophrenia.

Methods

The role of MIR185 in schizophrenia was investigated by: 1) monitoring miR-185 expression in embryonic and adult mouse brains; and 2) investigating the role of common and rare variants at this locus in humans. The latter approach involved three steps. Firstly, gene-based analyses were performed for common variants in MIR185 and its target genes using Schizophrenia Psychiatric Genomics Consortium genome wide association data (Ripke et al., 2011). Secondly, the MIR185 gene was resequenced in 1,000 schizophrenia patients and 500 controls of German origin to investigate the role of rare variants. Thirdly, promising variants were followed up by genotyping an additional 3,530 patients and 4,018 controls.

Results

In situ hybridization in mice revealed miR-185 expression in brain regions implicated in schizophrenia and other neuropsychiatric manifestations of 22q11.2DS. Gene-based tests revealed no association between schizophrenia and MIR185 at either the gene-level or the level of individual SNPs. However, we found a significant enrichment of schizophrenia-associated
genes within the targets of MIR185. After correction for multiple testing three target genes were associated with schizophrenia. Resequencing identified two rare patient-specific novel variants directly flanking MIR185. However, follow-up genotyping provided no further evidence for their involvement in schizophrenia.

Discussion
The miR-185 expression patterns in mice and the genetic association results for the three miR-185 target genes suggest that miR-185 and its down-stream pathways may be implicated in the development of schizophrenia in both 22q11.2DS patients and idiopathic cases. MicroRNA-mediated dysregulation is therefore a possible etiological mechanism in schizophrenia. AJ. Forstner and FB. Basmanav contributed equally to this work.

MICRORNA-137 IS ASSOCIATED WITH EPIGENETIC VARIATION AT THE HCG9 GENE IN THE AMYGDALA
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Background
Noncoding RNAs are widely abundant in cells and fulfill critical roles as transcriptional and post-transcriptional regulators. In addition, they have been implicated in the regulation of epigenetic mechanisms. MicroRNA-137 (miR-137) is a small non-coding RNA important for neurodevelopment. A large genome-wide association study recently suggested miR-137 mediated dysregulation as an etiologic mechanism in schizophrenia. This study aims to investigate the role of miR-137 function in epigenetic variation across regions of the human brain.

Methods
We investigated 249 postmortem brain samples originating from 44 non-demented control, 6 schizophrenia and 11 bipolar disorder individuals. Data was collected on whole genome DNA methylation and microRNA-137 expression. Linear models were used to investigate the relationship between microRNA expression and DNA methylation levels.

Results
MicroRNA-137 expression showed to be regional specific with highest expression in cortical regions, limbic system and basal ganglia and lowest expression in the cerebellum. In addition, miR-137 expression showed a strong correlation with methylation levels at a CpG site of the HLA complex group 9 gene (HCG9) in the amygdala. This correlation was significant ($b = 6.98, t = 9.52, p=0.0066$) and showed to be independent of disease status.

Discussion
This study demonstrates a relationship between the schizophrenia associated microRNA miR-137 and DNA methylation at HCG9 in the amygdala. This is a brain region involved in processing emotions and memories and reproducibly implicated in schizophrenia pathology. Interestingly, the HCG9 gene is located within the major histocompatibility complex (MHC)
class 1 region, which is also associated to schizophrenia. Future work involves investigating the effect of the miR-137 schizophrenia risk variant and miR-137 target genes on miR-137 expression levels and epigenetic variation at the HCG9 gene. Taken together, this study reveals microRNA-137 expression across the human brain and provides key insights on cross talk between this small RNA molecule and DNA methylation. In addition, these findings contribute to understanding etiologic mechanisms in schizophrenia pathology.

COMMON VARIANTS IN BCL9 GENE AND SCHIZOPHRENIA IN JAPANESE POPULATION: ASSOCIATION STUDY, META-ANALYSIS AND COGNITIVE FUNCTION ANALYSIS

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Background
Schizophrenia is a chronic, more or less enervating illness characterized by impairments in cognition, affect and behavior, all of which have a pronounced bizarre aspect. Schizophrenia is also a relatively common disorder, with a lifetime prevalence of about 1%. Family history is the most important risk factor for schizophrenia, consistent with a genetic contribution to its etiology. Recent human genetic studies reported that some common variants located within BCL9 are associated with schizophrenia in the Chinese population, but not associated with bipolar disorder in the Caucasian population. We examined the relationship of SNP variations in BCL9 and the risk for schizophrenia in a large Japanese case-control sample and conducted meta-analysis between Chinese and Japanese sample set, and explored potential relationships between variations in BCL9 and aspects of human cognitive function.

Methods
SNP prioritization sample was comprised of 575 patients with schizophrenia (43.5±14.8 years (mean±s.d.), male 50%) and 564 healthy controls with no personal or family history of psychiatric illness (44.0±14.4 years (mean±s.d.), male 49.8%). All subjects were unrelated, living in the central area of the Honshu island of Japan and self-identified as members of the Japanese population. For SNP association analysis, we used an independent Japanese sample set (replication sample) comprising 1464 cases (45.9±14.2 years, male 54.5%) and 1171 controls (48.06±14.48 years, male 47.3%). For analysis of cognitive performance, we investigated 115 cases (45.3±14.2 years, male 64.3%) and 87 controls (26.3±7.7 years, male 63.2%) using Continuous Performance Test (CPT-IP) and the Wisconsin Card Sorting Test Keio version (WCST). Meta-analysis was performed using combined Japanese total sample (N=3735) and Chinese sample (N=19088).
Results
In replication sample set, we did not detect any association in 2 SNPs (rs672607 and rs10494252) and schizophrenia. Joint analysis by PLINK also did not show significant low p-value in both SNPs. Meta-analysis of rs672607 showed significant association (p-value 0.012, odds ratio 0.855). We investigated genetic effects of rs672607 and rs10494252 on the CPT-IP and WCST. There was a significant (p<0.01) difference between A/A and G carrier group of rs672607 in CPT mean d' (p=0.0092).

Discussion
We were able to detect evidence for an association between rs672607 in BCL9 and schizophrenia in the meta-analysis of Japanese and Chinese populations. Additionally, this common variant may affect cognitive performance, as measured by the CPT-IP in schizophrenia patients. Further studies in independent populations may be needed.

Background
The neurobiological mechanisms of severe psychiatric disorders such as schizophrenia, bipolar disorder, and autism remain elusive. The aim of our project is to uncover the molecular and cellular neurobiology of psychiatric disorders by identifying rare highly-penetrant causative Mendelian genetic variants in severely affected families. A profound problem in studying the neurobiology of psychiatric disorders has always been the wide phenomenological heterogeneity [1]. In fact, there is emerging consensus that the diagnosis of psychiatric disorders reflect a multitude of different syndromes at the biological level [2]. Such limitations have likely been the principle cause of the historical difficulties in establishing causative biological factors, exemplified by recent genome-wide association studies [3]. Therefore, our study attempts to circumvent the difficulties inherent in studying genetically unrelated patients by using a family-based design, which has yielded some notable successes [4,5,6]. Further, recent advances in genetic technology, such as exome and whole-genome sequencing, have encouraged the possibility for higher specificity using a family-based gene finding approach, particularly for complex psychiatric disorders.

Methods
Families are recruited throughout the Netherlands from psychiatric clinics, national patient organizations, and the Erasmus MC department of Clinical Genetics. In particular, we have included families in which there are four or more affected family members who share a similar form of disorder. We consider a family member affected if they meet the relevant criteria established by the 4th edition of the Diagnostic and Statistical Manual (DSM-IV), confirmed by Structured Clinical Interview for DSM-IV disorders (SCID), medical record, and family history. Both affected and unaffected family members undergo a structural interview by clinically-trained staff of our research group and whole-blood DNA is obtained. DNA analysis is performed using
exome sequencing or whole-genome sequencing. In parallel, we perform linkage analysis using Illumina OmniExpress BeadChips on the affected and unaffected family members, and screen for inherited or de novo Copy Number Variants (CNVs). Called variants are filtered based on linkage regions, inheritance model, coding region, Minor Allele Frequency (MAF) <2% in public databases, and being nonsense, missense or splice-affecting. Remaining variants are Sanger-validated in the family DNA samples and candidate-variants are validated by large case-control cohort screening.

**Results**
Thusfar, we have included more than 20 families that are unique in having a high incidence of schizophrenia, bipolar disorder or autism. Next-generation sequencing methods guided by classical linkage analysis has provided a powerful framework by which family-based genetic investigations have been increasingly successful.

**Discussion**

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**EXOME SEQUENCING FOR SCHIZOPHRENIA AND ALCOHOL DEPENDENCE IN AN IRISH COHORT USING LOW-COST LIBRARY PREP AND TARGET CAPTURE**

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**Background**
Genetic variation is an important determinant of risk for many common disorders. There is mounting evidence that both common alleles of small effect and rare alleles of larger effect contribute to these risks. Although common variation is expected to account for the majority of population attributable risk, identifying specific risk alleles and elucidating their function are
difficult due to the small effect sizes and the presence of the alleles in controls. By contrast, functional variation in relevant genes may have more direct impact on trait risk and may also help to elucidate the role of variation in the gene on risk more generally. However, because of the relative rarity of such alleles, large sample sizes are required to have power to detect these effects. Sequencing remains costly for the sample sizes necessary, so approaches that reduce sequence costs are critical. Although per base costs have fallen continuously, library preparation and target capture remain expensive. We have adapted a recently published protocol (Ref 1) for reduced cost library preparation and pooled target capture that yields at least 30% reduction in the cost of sequencing an individual exome.

Methods
Samples were quantified using PicoGreen and 3 μg of DNA was sheared to a mean size of 170 bp. Fragments were ligated with indexed partial Illumina adapter sequences, nick-filled in, amplified and quantified by qPCR. After equimolar pooling and amplification, we performed target capture using the Agilent 71 Mb exome + UTR kit. Sequencing is performed on the Illumina HiSeq 2500 platform using 2x100 bp reads. Reads are aligned to the reference genome using STAMPY. GATK is used to perform local realignment, base quality score recalibration, and variant calling. We use the SNP-set Kernel Association Test (SKAT) for burden and gene-based association. Variants with MAF >2% and predicted to be damaging will be tested individually. We apply this approach to two ongoing projects using the same design. Cases of alcohol dependence (AD, N=500) and schizophrenia (SCH, N=250) will be sequenced to 50X average depth using the protocol above. Sample sizes can be further increased by imputing sequence variants into unsequenced individuals with GWAS framework data. Case variants and variants from the UK10K controls (N=2432) will be used to produce a single reference panel to impute variants into additional unsequenced cases and controls (AD total N=710 cases, 4187 controls, SCH total N=685 multiplex family members, 1606 sporadic cases, 4187 controls).

Results
We piloted this approach by comparing 1) the standard Illumina TruSeq prep and individual Agilent target capture with 2) the Rohland protocol prep and pooled target capture. Our baseline is the standard library prep, capture and sequencing of the 71 Mb exome + UTR target sequenced to 50X average cover of unique reads yielding 10x coverage for >93% of target. This baseline exome protocol shows comparable QC metrics to initial runs of the pooled capture protocol for unique reads (87.2% baseline vs. 86.1% pooled), percent of bases on or near bait (89.2% baseline vs 91.5% pooled). Our first 24 production exomes using the Rohland protocol and pooled capture are being sequenced currently. We have completed prep for 49 additional samples and we anticipate that >100 exomes will be complete and analyzed by the time of presentation.

Discussion
The cost of sequencing remains prohibitive for the sample sizes needed to have high power to detect effects, so the reduction in library prep and capture costs is an important step toward elucidating the complete spectrum of variation relevant to these traits. Coupled with imputation of sequence variants into unsequenced individuals from a single homogeneous population, this design yields significant reductions in the cost of producing a dataset of this size.
Reference:
ANALYSIS OF CNVS IN A LARGE, NEW SCHIZOPHRENIA DATASET

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Background
Copy number variants (CNVs) at 15 different loci have been suggested as susceptibility factors for schizophrenia (SCZ). However, due to their rarity, the evidence for some of them is not compelling. Additional susceptibility CNVs are also likely to exist, but very large sample sizes are needed for their discovery. In the largest independent SCZ sample to date, we set out to determine the contribution of these 15 CNVs to disease risk and identify novel susceptibility loci.

Methods
We used Illumina arrays to analyse SCZ patients from the UK and publicly available control data from individuals recruited for studies of non-neuropsychiatric phenotypes. CNVs were called with PennCNV using a consensus set of probes (520,766) found on all arrays used. After quality control filtering, we analysed a new sample of 6,882 cases and 6,316 controls. Novel findings (at \( P < 0.05 \)) were further analysed with a Cochran-Mantel-Haenszel test in an additional 14,568 cases and 15,274 controls.

Results
We found higher rates in cases than in controls for 13 of the 15 previously implicated CNVs. Six were nominally significantly associated \((P<0.05)\): deletions at 1q21.1, NRXN1, 15q11.2 and 22q11.2, and duplications at 16p11.2 and the Angelman/Prader-Willi Syndrome region. When combined with published data, 11 of the 15 loci showed highly significant evidence for association with SCZ \((P<4.1 \times 10^{-4})\). All eight Angelman/Prader-Willi duplications in cases were of maternal origin. A novel association was found for recurrent deletions at 16p12.1, a locus previously associated with developmental delay \((P=0.0017)\). Other promising candidates included deletions of SLC1A1 and duplications at 1p36.33 and CGNL1. After removing previously implicated loci, there is still an excess of 1.2% in cases of large CNVs (>500kb).

Discussion
Our study strengthens the support for the majority of previously implicated CNVs in SCZ and brings the evidence for two loci (duplications at the Angelman/Prader-Willi locus and at 16p13.11) into the genome-wide significant level. We provide strong evidence for a new risk
locus, deletions at 16p11.2. An excess of large CNVs in cases remains after removing previously implicated loci. However, as these CNVs are very rare, identifying the remaining susceptibility loci will require further large samples.

MEGABASE-SCALE DIVERSITY AMONG DISEASE-ASSOCIATED CNVS AT 1Q21.1, INVOLVING SEQUENCE MISSING FROM THE REFERENCE HUMAN GENOME

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Background

Analysis of genome biology and genome variation (such as copy number variation) generally uses the reference human genome as a framework. While a powerful, enabling tool, the human genome reference remains incomplete in potentially important ways, particularly for questions involving genome structure at large scales. Recurrent CNVs in the 1q21.1 – previously estimated at ~1.35 Mb – show variable expressivity, associating with cardiac developmental defects, schizophrenia, mental retardation, autism, congenital anomalies, and abnormal head size. Such CNVs also exhibit partial penetrance – for example it is estimated that ~10% of people with a 1q21.1 distal deletion will be diagnosed with schizophrenia, while others are born with congenital heart malformations.

Methods

We hypothesized that 1q21.1 structural variants are incompletely understood at a genomic level – that the size of 1q21.1 structural variants is underestimated and that potential missing sequence and explanatory genes are overlooked. We analyzed patients affected by deletions at the 1q21.1 distal region from two cohorts: patients with schizophrenia and patients with cardiac developmental defects. We used a combination of whole genome sequencing (WGS) and targeted digital droplet PCR to elucidate the correct extent of 1q21.1 distal deletions, paying particular attention to sequences that are missing from the human genome reference.

Results

We found that the majority of CNVs in the 1q21.1 distal region affect ~2.45 megabase-pairs of sequence, compared with ~1.35 megabase-pairs originally estimated. Surprisingly, we also found high variability in the genome sequences and genes affected by 1q21.1 distal deletions. Such deletions, which appeared identical in earlier analyses using the human genome reference, in fact exist in at least four forms, and vary by more than one megabase in the genome sequences encompassed. These alternate forms of the 1q21.1 deletions appear to have arisen from non-allelic homologous recombination among distinct sequence elements (most of which are also absent from the current human genome reference). We are currently investigating, in larger cohorts, the extent to which this cryptic structural variation helps explain the apparent variable expressivity of 1q21.1 CNVs.

Discussion

These findings highlight the value of current efforts to complete the human genome reference,
and suggest that the genomic impact of many structural variants may be less well-characterized than is currently believed.

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**ERBB4 KNOCKDOWN IN BASOMEDIAL AMYGDALA IN ADULT MOUSE RESULTED IN DECREASED SOCIABILITY**

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**Background**

Recent molecular genetics studies implicate Neuregulin 1 (NRG1) and its receptor ErbB4 in the pathophysiology of schizophrenia. The studies showed that expression of ErbB4 is restricted to interneurons in hippocampus and cortex in rodents, macaques, and humans. However, little is known about protein expression patterns and functions in other areas of the brain such as the amygdala which performs primary roles in the formation and storage of memories associated with emotional events and social interaction.

**Methods**

By using the stereotaxic apparatus, we injected shRNA-ErbB4 lentivirus into CA1 of hippocampus or basomedial amygdala (BMA) in adult mice. After two weeks' recovery we tested different behaviors: open field, novel object, sociability test, elevated plus, force swimming, Morris water maze, and fear conditioning.

**Results**

We found that knockdown of Erbb4 in CA1 lead to spatial memory impairments in the Morris water maze task. Interestingly, knockdown ErbB4 in BMA resulted in reduced sociability.

**Discussion**

These data suggested that dysfunction of ErbB4 in different brain regions might produce distinct schizophrenia related behaviors. In accordance with previous study which the function of ErbB4 in hippocampus has been confirmed, our mouse model show similar spatial memory impairments. Further more, our findings show that ErbB4 also play an important role in social related behavior through basomedial amygdala.

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**ABSTRACT WITHDRAWN**

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**ALCOHOL METABOLIZING GENES AND ALCOHOL DRINKING PATTERNS IN ASIAN ADOLESCENTS: THE ROLES OF PEER-NETWORK STRUCTURE**

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**Background**

Alcohol use and associated harms have taken an increasingly heavy toll on health globally and exact a huge burden on national productivity in diverse populations. For young people aged 10-24 years, alcohol is the leading risk factor for disease burden. The etiology of alcohol use and problems, covering environmental and genetic factors, is known to be stage dependent; for earliest stage of alcohol involvement in adolescence (e.g., alcohol initiation and continuation), peer effects have been recognized as one of strong environmental predictors shaping the transition of alcohol involvement. Meanwhile, two alcohol-metabolizing enzymes genes, ADH1B and ALDH2, have been reported in relation to reduced risk of alcoholism in Asian populations since the uncomfortable physical reaction (e.g., flush) associated with acetaldehyde accumulation may lower individual further involvement in alcohol drinking. To this point, available knowledge of GXE effects on alcohol problems has been largely derived from Western societies, and relative little evidence is targeted at earlier stages of alcohol involvement. To fill in these gaps, the present study is aimed to examine potential influences of genetic and peer factors on alcohol continuation among Asian youngsters in the transition from late childhood into adolescence.

**Methods**

The study was a subsample of the Alcohol-Related Experiences among Children (AREC) project. Via a stratified multistage probability sampling, the original sample was composed of 1630 school-attending children aged 10 (4th grade) and aged 12 (6th grade) in Taipei City in 2006 (response rate: 62%), with two consecutive annual follow-up rates of 86.6% and 80.5%. Information pertaining to sociodemographic background, alcohol drinking, parental drinking, and nominated best friends was collected via self-administered questionnaires in three waves of assessments; peer network structure was constructed via social network analyses. Among 682 children with alcohol initiation (42%), 73% (n=498) agreed to provide saliva sample for DNA retrieval at the third year upon both parental and children’s consents (positive consent is independent of alcohol initiation status at baseline). Two SNPs (i.e. rs1229984 for ADH1B gene and rs671 for ALDH2 gene) concerning alcohol-metabolizing genes were selected for this study. To understand the stability/change of alcohol consumption behaviors from late childhood into early adolescence, the alcohol-experienced children were further categorized into three subgroups on the basis of drinking behaviors in two consecutive annual follow-up: children who only drank alcohol at baseline (reference, n=173), children who consumed alcohol in less than two occasions (nonregular drinkers, n= 154), and children who drank on three or more occasions (regular drinkers, n= 170). Multinomial logistic regression analyses were performed to evaluate the association estimates.

**Results**

Among the 498 school-attending children with alcohol initiation, 54% were male, 50% were in mid- or post-pubertal stage, and 45% had their first drink prior to age 10. The allele frequencies of ADH1B*2 (rs671) and ALDH2*2 (rs1229984) were 0.74 and 0.29 respectively. After statistical controlling for sociodemographic factors, age of alcohol onset, and cumulative use, alcohol-metabolizing genes of ALDH2 appear to have significant protective effect against continued alcohol consumption in two consecutive survey years (aOR=0.84 for non-regular drinking & 0.58= for regular drinking). Youngsters occupying the bridge position (i.e., the key person who connects two peer networks) had significantly increased risk to continue regular
alcohol drinking (aOR=3.38 for non-regular drinking & 3.43= for regular drinking). In contrast, higher degree centrality (i.e., having more social connectedness with peers within a social network, or being popular) was linked with reduced risk of alcohol continuation (aOR=0.26 & 0.30), yet with no statistical significance. Possible interplay between ADH1B*2 and peer network degree centrality was also noted, indicating that the protective effect of ADH1B*2 on alcohol drinking was less salient among those children with higher social connectedness in their peer network (p-value=0.09).

**Discussion**

Alcohol-metabolizing genes and peer-network structure both play important roles influencing the earlier stages of alcohol drinking behaviors (e.g., alcohol continuation). Our results demonstrated the significant contribution of early life’s social fabric and genetic factors on alcohol involvement from late childhood to adolescence in Asian populations.

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**GENETIC AND ENVIRONMENTAL INFLUENCES ON THE RELATION BETWEEN ADHD SYMPTOMS AND PROBLEM DRINKING IN 11,545 DUTCH ADULTS**

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**Background**

Both cross-sectional and longitudinal studies have shown a phenotypic association between ADHD and problematic alcohol use. Thus far, relatively few studies have investigated the extent to which genetic and environmental factors contribute to the observed phenotypic association. The few existing studies have focused on adolescents, while the comorbidity may be stronger in adults. Using data collected in monozygotic and dizygotic twins, this study aims to investigate the phenotypic association between ADHD symptoms and problem drinking in adults and to determine the extent to which this association is explained by shared genetic or environmental factors.

**Methods**

In 2004-2005, phenotypic data were collected in 6,148 Dutch twins. The mean age of the twins is 35.04 years (SD=12.14; range=18-86 years) and 30.2% of the participants (N=1,859) is male. ADHD symptoms were assessed with the screening self-report version of Conners’ Adult ADHD Rating Scales (CAARS – S:SV). Problem drinking was defined as meeting at least one self-reported problem on the CAGE questionnaire in combination with an alcohol consumption of at least 11 drinks per week. Genetic analyses were performed in Mx, a statistical software package designed for conducting genetic analyses. As genetic modelling in Mx does not allow for a combined analysis of categorical and continuous measures, the ADHD-index scores were recoded in such a way that three thresholds divide the continuous scores into four categories. Using a liability threshold model, phenotypic correlations between ADHD symptoms and problem drinking were calculated. Genetic modelling was applied to estimate genetic and environmental influences on ADHD-symptoms, problem drinking, and on the phenotypic association of these traits.

**Results**
The best-fitting genetic model includes genetic and non-shared environmental influences. The heritability of ADHD-index scores is 38% and the remaining variance is explained by non-shared environmental influences. Problem drinking is for 63% explained by genetic factors. The phenotypic correlation \((r=0.16)\) between ADHD-index scores and problem drinking is entirely explained by genetic influences. The genetic correlation, the extent to which genes contributing to individual differences in ADHD symptoms overlap with those contributing to variation in problem drinking, is substantial \((r=0.32)\). No significant gender differences are found.

**Discussion**
In contrast with studies performed in adolescents, this study convincingly shows that ADHD symptoms and problem drinking are significantly correlated in adults and genetic factors are responsible for this correlation. Interpretation of these data in the context of existing literature suggests that subjects with ADHD symptoms which persist into adulthood have the strongest genetic vulnerability to develop problematic alcohol use.

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**SUPPORT FOR REWARD DEFICIENCY SYNDROME (RDS) AS A TRUE PHENOTYPE AND DETERMINATION OF RISK SEVERITY, USING THE GENETIC ADDICTION RISK SCORE (GARS) AND OF OUTCOME, USING THE COMPREHENSIVE ANALYSIS OF REPORTED DRUGS (CARD)**
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**Background**
Numerous studies have demonstrated an association between dopaminergic gene polymorphisms and addictive, compulsive and impulsive, reward based behaviors. These interrelated behaviors have been classified as Reward Deficiency Syndrome (RDS). We propose, a generalized RDS behavior set, as the true phenotype. There is also a need to classify patients at genetic risk for RDS behaviors, for example, prior to or upon entry to chemical dependency programs, pain clinics and bariatric surgery. We have evaluated a panel of genes and associated polymorphisms termed the Genetic Addiction Risk Score (GARS) in patients with RDS behaviors attending two treatment centers. Genotyping to determine genetic severity for patients undergoing treatment is necessary due to the danger of treatment failure and relapse. Recently in a large unpublished study of data from the Comprehensive Analysis of Reported Drugs (CARD) significant levels of both non-compliance and lack of abstinence during treatment, revealed the need for better monitoring of treatment outcomes.

**Methods**
An experimental group of 55 subjects derived from up to five generations of two separate multiple-affected families were genotyped and compared to very rigorously screened controls. Data related to RDS behaviors was collected on these subjects plus 13 deceased family members.
The potential association of dopamine D2 receptor gene (DRD2), dopamine D1 receptor gene (DRD1), dopamine transporter gene (DAT1) and dopamine beta-hydroxylase gene (DBH) polymorphisms was evaluated. In another study, to determine risk severity of addicted patients we genotyped using a 9 reward gene polymorphic (18 allele) panel including: DRD 2, 3, 4; MOA-A; COMT; DAT1; 5HTTLPR; OPRM1; and GABRA3. We calculated the percentage of prevalence of the risk alleles and genes and provided a severity score: low (LS) = 1-36%; moderate (MS) =37-50%, and high (HS) = 51-100%. We studied two treatment populations: group 1 consisted of 35 addicts from G & G Health Care Services, and group 2 consisted of 35 addicts from Malibu Beach Recovery Center, for a total of 70 participants. A statistical analysis of data from CARD on 11,403 urine specimens from 5,703 patients, in various treatment settings across six eastern states was accomplished.

Results
Among the genotyped family members, 78% carried the DRD2 Taq1 allele, 58% carried the DAT1 10/10 allele, 66% carried the DBHB1 allele and 35% carried either the DRD1 A1/A1 or A1/A2 genotypes. The experimental positive rate for the DRD2 Taq1 allele was significant ($X^2=43.6, p<0.001$), with an odds ratio of 103.9 (12.8, 843.2). All probands (n=32) from Family A that were genotyped for the DRD2 gene carried the TaqA1 allele [100%]. The experimental positive rate for the DAT1 10/10 allele was also significant ($X^2=6.0, p < 0.015$) with an odds ratio of 2.3 (1.2, 4.6). No significant differences were observed between the experimental and control positive rates of the DBH, DRD1 A1/A1 or A2/A2 genotypes. The risk stratification experiment found that of the 70 genotyped patients: 14% LS; 81% MS and 5% HS. Statistical analysis of CARD data found compliance to prescribed medications during treatment and recovery at 67 %, whereas 39% of those patients were abstinent from drugs of abuse. The analysis also found that patients in methadone opioid treatment were compliant at 92% with 49 % abstinence. Similarly in Suboxone maintenance patients 88% were compliant and 48% were considered abstinent. Surprisingly, in the Suboxone and Methadone groups we found high opioid misuse (~47%).

Discussion
The generational study demonstrated the likelihood that a nonspecific RDS phenotype exists. Utilization of a nonspecific “reward” phenotype may be more effective in furthering our understanding of psychiatric genetics while evaluating single subset behaviors of RDS, may produce misleading results. This would represent a paradigm shift in future association and linkage studies, involving dopaminergic polymorphisms and other neurotransmitter gene candidates and agrees with the view espoused recently by the NIMH arguing the new DSM-V. We are proposing that knowledge of risk severity for RDS [indeed the true phenotype] can be determined utilizing GARS and may be useful in determining treatment modalities and risk for relapse. We also propose that outcomes for treatment progress and follow-up can be assessed by utilizing CARD. Presently, we are collecting data (~ 400 subjects) for a larger multi-centered study of GARS, and exploring a potential of risk correlation with the Addiction Severity Index - Multi Media Version (ASI-MV). We encourage others to explore the RDS phenotype and clinical applications of GARS and CARD.
ASSOCIATION ANALYSIS OF POLYMORPHIC VARIANTS IN THE CLOCK GENE WITH HEROIN DEPENDENCE IN BULGARIAN AND ROMA POPULATION

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Background
Heroin addiction is a chronic complex disease with a substantial genetic contribution but the underlying genes have not been found yet. We have chosen to investigate the Circadian Locomotor Output Cycles Kaput (CLOCK) gene for association with heroin dependence. This gene, located on chromosome 4, encodes a transcription factor of the basic-helix-loop-helix (bHLH) family and a DNA binding histone acetyltransferase, involved in regulating circadian rhythm and metabolism. Genetic modifications disrupting the normal circadian gene functions have been linked to various psychiatric conditions including depression, seasonal affective disorder, eating disorders, alcohol dependence, and addiction (Albrecht U, 2012). Mice with a mutation in the Clock gene have a number of behavioral phenotypes, suggesting alterations in dopaminergic transmission such as hyperactivity, increased exploratory behavior, and increased reward value for drugs of abuse (Spencer et al., 2012). We have selected polymorphisms rs3805154 in the 5' end and rs1801260 located in the 3' untranslated region and analysed them for association with heroin dependence in Bulgarian and Roma samples. This is a part of ongoing project on genetic epidemiology of opioid dependence in Bulgaria.

Methods
In this association study 2598 heroin addicts and 1290 healthy control subjects of Bulgarian and Roma ethnicity have been included. All heroin dependent cases have been interviewed and a diagnosis made according to DSMIV. The study has been approved by the Ethics Committee of Medical University – Sofia and Washington University of St. Louis. SNP genotyping was performed using TaqMan assay (Applied Biosystems) and statistical analysis performed with PLINK (Purcell et al., 2007).

Results
There was no overall association between the selected polymorphisms and addiction. When the group was stratified by gender and ethnicity the group of Roma males showed nominally significant allelic (p=0.01) and genotypic association (p=0.006) for marker rs1801260. The less common C allele was more frequent among heroin addicts (36%) compared to controls (24%).

Discussion
Our results do not support a major role of the CLOCK polymorphisms in the opioide addiction. This polymorphism has been previously associated with schizophrenia, ADHD, sleep and activity patterns in healthy people and bipolar disorder patients as well as neuropsychological performance (Zhang et al., 2011, Xu et al, 2010, Benedetti et al, 2007). It may have relevance to some of the neurocognitive or personality traits relevant to addictive behaviours, at least in the
Roma population. However due to the small size of the healthy control group our results should be considered as preliminary and replication with a larger set of population controls is warranted.

THE ROLE OF SIRTUIN 2 GENE RS10410544 POLYMORPHISM IN ALCOHOL DEPENDENCE AND DETOXIFICATION

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Background
Alcohol dependence disorder (ADD) is a widespread burden with many liabilities factors, only partially identified. Certainly, there is an interaction among life events and genetic phenotype. Numerous neurotransmitter pathways have been implicated in genesis of dependence, particularly the serotonergic and glutamatergic pathways. Recently, sirtuins genes have roused a growing interest because of their involvement in cerebral homeostasis. Further, SIRT2 gene has been associated with mood disorders as well as with cerebral degenerative diseases. The aim of this study is to investigate the possible associations among SIRT2 rs10410544 polymorphism and psychopathological improvements before and after detoxification treatment. In addition, we performed some exploratory analyses to investigate possible associations among the rs10410544 genotype and other clinical features.

Methods
We investigated these associations in a Greek samples of 64 inpatients admitted for alcohol detoxification. Patients were evaluated twice, at admission and discharge. Depressive and anxiety symptoms were assessed through the Hamilton Depression Rating Scale (HAM-D) and the Hamilton Anxiety Rating Scale (HAM-A). Overall functioning was assessed through the Global Assessment Scale (GAS). Patients were also evaluated for social phobia by the Liebowitz Social Anxiety Scale, for general fears with the Mark & Mathews Scale, for hypochondriac symptoms by the Pilowski scale, for obsessive–compulsive dimensions with the Leyton Obsessional Inventory and alexithymia by the Schalling–Sifneos Personality Scale. To state an association among the SNP considered and ameliorations after detoxification, analysis of variance (ANOVA), repeated measures analysis of variance (R-ANOVA) and analysis of co-variance (ANCOVA) were performed when appropriate. For ANOVA analysis, considering an alpha value of 0.05, the sample had enough post-hoc power (0.80) to detect medium-large effect sizes (f= 0.36).

Results
We found a weak association between rs10410544 and general fears at the admission, measured by the Mark & Mathews Scale. We failed to find any association among rs10410544 genotype and particular psychopathological ameliorations after detoxification.
Discussion
This variant of SIRT2 gene does not appear to play a major role both in the liability for alcohol toxicity and in the determination of alcohol detoxification outcome. Clearly, considering the small sample size investigated, our results should be confirmed by further studies.

INVESTIGATION OF DNA POLYMORPHISMS LOCATED IN THE CALCIUM/CALMODULIN-DEPENDENT PROTEIN KINASE II ALPHA (CAMKIIA) IN A LARGE SAMPLE OF HEROIN ADDICTED INDIVIDUALS FROM WESTERN AUSTRALIA

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Background
The population prevalence of heroin addiction in the adult Australian population aged between 15 and 54 is estimated to be in the range of 7 per 1000. It has been shown that susceptibility to heroin dependence is strongly influenced by genetic factors with heritability estimates as high as 0.7 and most likely a number of genes as well as environmental factors contributing. The observation, that not all of the individuals who have used heroin at some stage during their life, become dependent on heroin, is puzzling and might implicate genes involved in memory formation. The calcium/calmodulin-dependent protein kinase II alpha (CAMKIIa) is one of the key players involved in long term potentiation and synaptic plasticity and might be therefore of special interest in this context. In addition, CAMKIIa has a central role in regulating neuronal excitability. Therefore, we have chosen single nucleotide polymorphisms (SNPs) located within this gene and analysed these for association with time dependent heroin usage. For our study we had access to a heroin addicted population from Western Australia who was characterized for time to regular heroin use.

Methods
We have ascertained 794 Caucasian individuals with DSMIV confirmed diagnosis of heroin dependence in Western Australia. Data on first use of heroin, switch to regular use of heroin, overall years of use, and co-morbidity with other drug abuse had been collected. This information is self-reported data, available for about 80% of the sample. In addition, DNA of all samples had been isolated. Genotyping was performed using fluorescence based TaqMan assays, PCR-RFLP, and high resolution melting (HRM). Overall, 12 single nucleotide polymorphisms located in intronic regions of the CAMKIIa gene had been selected. SPSS v20 was used for statistical analysis.

Results
The majority of the individuals started with heroin use before the age of 20 (N=498). Only a minority (N=151) did use heroin for less than 5 years. Overall, years of heroin use and age at first use were highly correlated (P<0.01). For association analysis with time to regular heroin use, we split the sample into two groups: one group which started regular heroin use only after
four years or more (N=100), and another group, which used heroin on a regularly basis within the first three years (N=499). Multiple SNPs revealed statistically significant differences in allele frequencies between the two groups (rs10066581, P=0.0009; rs6869634, P=0.02; rs4958468, P=0.0052; rs2241694, P=0.029).

Discussion
We have collected a large sample with heroin addiction with extensive phenotypic information regarding addiction related parameters. Our preliminary analysis indicates that the gene CAMKIIA might be involved in heroin addiction.

GENETIC RISK FOR NICOTINE DEPENDENCE AND DOPAMINE RECEPTOR GENE
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Background
Tobacco smoking increases the risk of various health problems, including cancer, cardiovascular and pulmonary disorders, making smoking the leading cause of preventable death in the world. Epidemiological studies have indicated significant genetic contributions to smoking behaviors. Although genetic associations to nicotine dependence (ND) have been widely investigated, the relevance of individual genetic variations for smoking behavior remains unknown. Dopamine D1 receptor (DRD1) gene is a noteworthy candidate gene for ND because it plays an important role in the brain reward system. No study has been reported regarding an association between dopamine D1 receptor (DRD1) gene polymorphism and ND using Japanese sample.

Methods
We examined the association in a total of nearly 700 healthy workers from one of the major heavy industry companies in Japan. Smoking behavior was assessed by a questionnaire including Fagerström Test for Nicotine Dependence (FTND). This study was approved by the Ethics Committee of the University of Occupational and Environmental Health and informed consent was obtained from all subjects.

Results
Although our preliminary data does not suggest a significant association, analysis with complete data set is currently undergoing to clarify the relationship between DRD1 gene and ND.

Discussion
Challenging steps towards clinical application of pharmacogenetics of ND require further studies.
EVALUATION OF SINGLE NUCLEOTIDE POLYMORPHISMS IN THE MIR-183-96-182 CLUSTER IN ADULTHOOD ATTENTION-DEFICIT AND HYPERACTIVITY DISORDER (ADHD) AND SUBSTANCE USE DISORDERS (SUDS)

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Background
Attention deficit-hyperactivity disorder (ADHD) is a neuropsychiatric disorder characterized by inappropriate and impaired levels of hyperactivity, impulsivity and inattention. Around 75% of adults with ADHD show comorbidity with other psychiatric disorders such as disruptive behavior disorders or substance use disorders (SUDs). Recently, there has been growing interest in studying the role of microRNAs (miRNAs) in the susceptibility to complex disorders. Interestingly, converging evidence suggests that single nucleotide polymorphisms (SNPs) within miRNAs or miRNA target sites may modulate the miRNA-mediated regulation of gene expression through the alteration of the miRNA maturation, structure or expression pattern as well as the silencing mechanisms of target genes. Genetic studies and animal models support the involvement of the serotonin receptor (HTR1B) in ADHD.

Methods
We evaluated the contribution of one SNP in the miR-96 target site at HTRIB and eight tagSNPs within the genomic region containing this miRNA in 695 adults with ADHD (266 and 396 subjects with and without comorbid SUD, respectively), 403 subjects with SUD without lifetime diagnosis of ADHD and 485 sex-matched controls from Spain.

Results
Single and multiple marker analyses revealed association between two SNPs located at the 3’ region of miR-96 (rs2402959 and rs6965643) and ADHD without SUD.

Discussion
Our results provide preliminary evidence for the contribution of two sequence variants at the miR-183-96-182 cluster to ADHD without comorbid SUD, and emphasize the need to take comorbidities into account in genetic studies to minimize the effect of heterogeneity and to clarify these complex phenotypes.

CANDIDATE GENETIC PATHWAYS FOR ADHD SHOW ASSOCIATION TO HYPERACTIVE/IMPULSIVE SYMPTOMS
Background

Although Attention-Deficit Hyperactivity Disorder (ADHD) is a highly heritable disorder, gene finding has been challenging. Presumably multiple genes with small effect size play a role. Compared to single-SNP or haplotype analysis, considering multiple SNPs in the same analysis might increase the explained phenotypic variance, thereby boosting the power of genetic studies. We investigated whether pathway-based analysis, considering multiple SNPs within the same biological pathway simultaneously, could bring us closer to unraveling the underlying genetic components of ADHD.

Alterations in dopamine, noradrenalin and serotonin neurotransmission have been hypothesized to play a role in ADHD, being associated to behavioral aspects of the disorder. Their importance in brain functioning, the influences on these systems by the current medications and candidate gene associations within these pathways make them prime candidates for pathway-based analysis. Genes involved in neurite outgrowth, that came up when analyzing the top results of the performed genome-wide association studies of ADHD, are also of interest.

Methods

We decided to investigating the genes underlying the biological pathways of dopamine/noradrenaline, serotonin and the neurite outgrowth genes for association to ADHD severity. Common genetic variants in these biological pathways were investigated using data from the International Multicentre ADHD Genetics (IMAGE) study. Phenotypes were DSM-IV symptom counts for inattention and hyperactivity/impulsivity (n=871) and symptom severity measured with the Conners Parent (n=930) and Teacher Rating Scales (n=916).

Selection of the genetic pathways was based on Ingenuity Pathway Analysis software (www.ingenuity.org) in combination with literature. From the imputed and pruned genome-wide data 5791 SNPs were selected and used for the association analysis. A combined analysis of the three pathways investigated the association to inattentive or hyperactive/impulsive symptom counts. The analysis consisted of SNP-by-SNP linear regression including age and gender as covariates and the estimation of the effect of the complete set of SNPs. Multiple testing correction was performed by running permutations and investigating whether the observed pathway-based statistic fell into the extreme 5% of the permutation distribution. We subsequently tested our pathway-based approach for the inattention and hyperactivity/impulsivity scales of the Conners’ Parent and Teacher Rating Scales, which assess ADHD symptom severity. For significant associations, post-hoc analysis were conducted for the separate pathways, to investigate single pathway contribution.
Results

Summing genetic effects of the variants within the pathways showed significant association with DSM-IV hyperactive/impulsive ($p_{\text{empirical}}=0.007$), but not inattentive symptoms ($p_{\text{empirical}}=0.73$). Analysis of parent-rated Conners hyperactive/impulsive symptom scores confirmed this ($p_{\text{empirical}}=0.0018$), teacher-rated Conners scores for this behavioral domain did not. Post-hoc analyses showed significant contribution of all pathways (dopamine/noradrenalin $p_{\text{empirical}}=0.0004$, serotonin $p_{\text{empirical}}=0.0149$, neurite outgrowth $p_{\text{empirical}}=0.0452$).

Discussion

The current analysis specifically finds association to the hyperactive/impulsive component of ADHD, suggesting similar underlying mechanisms for the studied pathways. Other mechanisms may be involved in the inattentive component of the disorder. These findings show that pathway-based association analyses may overcome power problems in association testing by taking into account allelic heterogeneity. Replication in other samples is necessary to confirm the current results.

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GENOME-WIDE COPY NUMBER VARIATION ANALYSIS IN ADULTHOOD ATTENTION-DEFICIT AND HYPERACTIVITY DISORDER

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Background

Attention-deficit and hyperactivity disorder (ADHD) is a common psychiatric disorder with a worldwide prevalence of 5-6% in children and 4.4% in adults. Recently, copy number variants (CNVs) have been implicated in different neurodevelopmental disorders such as ADHD. Based on these previous reports that focused on pediatric cohorts, we hypothesize that structural variants may also contribute to adulthood ADHD and that such genomic variation may be enriched for CNVs previously identified in children with ADHD.

Methods

To address this issue, we performed for the first time a whole-genome CNV study on 400 adults with ADHD and 526 screened controls. The genome-wide genotyping was performed with the Illumina HumanOmni1-Quad. CNV analysis was limited to 1109421 autosomal SNPs. BeadStudio was used to determine the Log R Ratio (LRR) amd B allele frequency BAF) at each SNP according to standard Illumina protocols. CNVs were defined by PennCNV. Significance of the burden comparisons was assessed through permutation one-sided tests (100000 permutations) using PLINK.
**Results**

In agreement with recent reports in children with ADHD or in other psychiatric disorders, we identified a significant excess of insertions in ADHD patients compared to controls. The overall rate of CNVs > 100kb was 1.33 times higher in ADHD subjects than in controls (P=2.4e-03), an observation mainly driven by a higher proportion of small events (from 100kb to 500kb; 1.35-fold; P=1.3e-03). These differences remained significant when CNVs overlapping genes were evaluated or when structural variants spanning candidate genes for psychiatric disorders were considered, with duplications showing the greatest difference (1.41, P=0.024 and 2.85-fold, P=8.5e-03, respectively). However, no significant enrichment was detected in our ADHD cohort for childhood ADHD-associated CNVs, CNVs previously identified in at least one ADHD patient or CNVs previously implicated in autism or schizophrenia.

**Discussion**

In conclusion, our study provides tentative evidence for a higher rate of CNVs in adults with ADHD compared to controls and contributes to the growing list of specific structural variants potentially involved in the etiology of the disease.

4

**COULD THE NCAM1-TTC12-ANKK1-DRD2 GENE CLUSTER CONTRIBUTE TO THE CLINICAL AND GENETIC HETEROGENEITY OF ADULT ADHD?**

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**Background**

Attention Deficit/Hyperactivity Disorder (ADHD) is a developmental psychiatric disorder that affects about 5.3% of children and its heritability is estimated at 76%. The estimated prevalence of ADHD in adults is around 4.4% and it is associated with a wide range of functional impairments across life domains and with higher occurrence of other psychiatric disorders. Both externalizing and internalizing disorders are frequently found present in ADHD patients. Converging evidence have implicated dysfunctions of the dopamine neurotransmission pathways to the etiology of ADHD. Two meta-analyses addressing the association of the dopamine receptor D2 (DRD2) gene and ADHD have shown a complex scenario, with inconclusive results due to excessive heterogeneity across studies. Most previous studies, including these meta-analyses, have analyzed the well-known ANKK1/DRD2 Taq1A (rs1800497) SNP as a marker of DRD2 gene, which actually is located in a neighbor gene (ANKK1). Together with the NCAM1 and TTC12 genes, the ANKK1 and the DRD2 genes form the NTAD cluster, which has been shown to harbor multiple independent risk variants. As an attempt to address the reasons for the high heterogeneity previously reported on DRD2 effects in ADHD, this study investigates the role of genetic variants from the NTAD cluster in the susceptibility to ADHD in adults and the modulation of associated comorbidities and personality profiles.

**Methods**

For this study a sample of 520 adults with ADHD and 630 non-ADHD controls was investigated. Diagnostic procedures were based on DSM-IV criteria and the Temperament and Character...
Inventory (TCI) was used to evaluate personality traits within the ADHD sample. Seven functional polymorphisms from the NTAD gene cluster were analysed (NCAM1 rs646558; TTC12 rs723077, rs2303380; ANKK1 rs2734849, rs1800497; DRD2 rs6277, rs2283265), both individually and in sliding-window haplotypes constructs using PLINK. Correction for multiple testing included 10,000 permutation for the SNP and global haplotype analyses ($P_{corr}$), while Bonferroni correction was used when testing the individual haplotypes.

Results
Our results show that the $DRD2$ rs2283265 $T$ allele was associated with increased risk for major depression disorder ($P=0.005$, $P_{corr}=0.023$) and with increased Harm Avoidance ($P=0.002$, $P_{corr}=0.013$) and decreased Persistence scores in ADHD patients ($P=0.002$, $P_{corr}=0.014$). Additionally, we were able to show that major depression disorder susceptibility among ADHD adults is independently associated with the $NCAM1$ rs646558 ($P=0.005$, $P_{corr}=0.032$) and the $DRD2$ rs2283265 SNPs. Furthermore, individual NTAD haplotypes, mostly centred in the ANKK1-DRD2 region, are significantly associated with internalising disorders, such as major depressive (significance ranged from $P=0.005$ to $P=0.012$) and generalized anxiety ($P=0.005$) disorders, and related personality traits, such as high harm avoidance ($P=0.002$ to $P=0.0016$) and low persistence scores ($P=0.002$ to $P=0.007$), within this sample of adults with ADHD.

Discussion
The SNPs and haplotypes associated in increased risk for such phenotypes are related to an overall low $DRD2$ expression scenario, leading to lower dopaminergic activity. Although there was no individual SNP or haplotype significantly associated with ADHD itself after correcting for multiple tests, our findings provide support to the view that this gene cluster is involved in the clinical heterogeneity of adult ADHD. It is possible that the NTAD cluster, harbouring multiple risk variants that could have additive effects, may represent a shared genetic risk factor, influencing both internalizing and externalizing disorders susceptibility, depending on additional genetic background and epistatic interactions. These effects might in turn contribute to significant heterogeneity in odds ratios reported in previous meta-analyses of $DRD2$-ANKK1 rs1800497 SNP in ADHD.

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METHYLPHENIDATE REGULATES HEY1, SLC2A3, ATXN1, GUCY1B3 AND MAP3K8 IN LYMPHOBLASTOID CELLS FROM ADULT ADHD PATIENTS

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Background
Attention-deficit/hyperactivity disorder (ADHD) is a common psychiatric condition of children with a prevalence of about 5 - 10 % worldwide. Other than previously thought up to 30% of adults with a history of childhood ADHD still display symptoms of the disorder in later life. ADHD is one of the most heritable (80%) psychiatric disorders, but gene-environment interaction also contributes to the risk of disorder. The neurobiological pathomechanisms of ADHD still remain unclear. Dysregulations in dopaminergic as well as noradrenergic neural
transmission seem to be essentially involved. Pharmaceutical treatment options include methylphenidate (MPH), which is amongst others an inhibitor of the dopamine transporter and therefore increases dopamine levels in the brain. But also the noradrenergic, serotonergic and glutamatergic systems seem to be influenced by MPH. At the moment there are no valid biomarkers for diagnosis or treatment response in the clinical routine available. Our aim was to investigate the effect of acute and chronic treatment of MPH on gene expression in human subjects.

Methods
We investigated the impact on MPH treatment of lymphoblastoid cells derived from adult ADHD patients (mean age 28.8 ±8.7) and healthy controls (36.2 ± 14.0) on gene expression levels. Lymphoblastoid cell lines (LCL) were isolated and transformed with Eppstein-Barr-virus (EBV), then grown and maintained in RPMI-culture medium. One culture of each sample was incubated with 30 ng/µL MPH. 0, 1, 6 hours, 1 and 2 weeks (t0, t1, t2, t3, t4) after MPH incubation 1mL cells were harvested for RNA isolation. For microarray hybridization RNA samples (t0 and t4) were pooled to reduce sampling of inter-individual expression differences. In total, four pools were constructed: ADHD and control samples without treatment at t0 and t4 each. The genes HEY1, SLC2A3, NAV2, MAP3K8, GUCY1B3 and ATXN1 were found to be regulated differently by MPH or showed different expression between ADHD and control. This results and investigation of all other time points were confirmed with qPCR. Expression data was edited and normalized before group and interacting effects were determined with independent and dependent t-tests, respectively.

Results
The results of a hypothesis-free microarray analysis and subsequent confirmation by quantitative Real-Time PCR analysis revealed ATXN1, MAP3K8, HEY1 and SLC2A3 expression to be influenced by MPH treatment dependent on the diagnosis. GUCY1B3 expression was different between ADHD and healthy control cells. In ADHD there were nominal higher expression in ATXN1 and lower expression in MAP3K8 one hour after MPH incubation. In contrast to this acute effects in ADHD, all significant influences of MPH in controls showed an increase in expression as a long lasting effect: ATXN1 and HEY1 were upregulated after one week of MPH treatment, MAP3K8 and SLC2A3 after two weeks.

Discussion
Our results demonstrate novel pathways for the mode of action of MPH and molecular pathomechanism of ADHD and could point to different effects of MPH in ADHD patients compared to healthy control subjects. The most significant differentially regulated genes seem to play a role in dopaminergic signaling (HEY1), central glucose metabolism (GLUT3), learning and memory formation (MAP3K8), nitrinergic signaling (GUCY1B3) and cognitive functioning and motor control (ATXN1). ADHD patients are known to have deficits in those domains and methylphenidate as one of the most effective treatment leads to improvement which is longer lasting than the known acute effects consisting of increased dopamine and norepinephrine levels. This may be due to the observed changes in gene expression. Because these genes are only one component of often long signaling cascades it’s difficult to distinguish direct or indirect gene expression changes. For further investigations it will be necessary to explore the MPH-treatment
CORTICAL THICKNESS DIFFERENCES BETWEEN ADHD PATIENTS WITH HOMOZYGOSITY FOR THE 10-REPEAT ALLELE AT DAT1 GENE AND ADHD PATIENTS WITH A SINGLE COPY OR NO COPY OF THE ALLELE

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Background
This study was conducted to explore differences in cortical thickness between children and adolescents with attention deficit hyperactivity disorder (ADHD) with the 10-repeat DAT1 allele and children and adolescents with ADHD with 1 or 0 copies of the allele.

Methods
Brain magnetic resonance images were acquired from 33 ADHD patients with homozygosity for the 10-repeat allele at DAT1 and 31 patients with a single copy or no copy of the allele. The cerebral cortex of each MRI scan was automatically parcelled into regions of interest (ROIs) based on Brodmann areas (BA). Group differences in cortical thickness within these regions-of-interest (ROI) were assessed using multiple ANCOVAs, with Group as between-subject factor (two levels: homozygous vs. non-homozygous) and subjects´ age and average thickness of each individual over the entire cortex as covariates. To prevent possible type 1 error, false positive discovery rate (FDR) correction was used.

Results
There were no significant group differences in age, sex or diagnostic subtype. Neither there were differences between groups in total mean cortical thickness. By contrast, patients with 2 copies of the 10R allele exhibited significantly decreased cortical thickness in right BA 46 and right BA 21, relative to patients with 1 or 0 copies of the allele.

Discussion
Cortical thinning was present in dorsolateral prefrontal cortex (DLPFC; BA 46) and lateral temporal cortex (BA 21) in ADHD patients with homozygosity for the 10-repeat allele at DAT1. An association between the homozygous 10/10 DAT1 allele and ADHD symptoms/neuropsychological deficits has been previously found (Cornish et al., 2005; Loo et al., 2003). Cortical thinning observed in the present study may be related to behavioral and neuropsychological differences between ADHD patients with 2 vs. 0/1 copies of the 10R allele but additional analyses will be necessary to test this hypothesis.

ASSOCIATION BETWEEN THE DAT1 GENE AND SPATIAL WORKING MEMORY IN ATTENTION DEFICIT HYPERACTIVITY DISORDER
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**Background**
An association between attention deficit hyperactivity disorder (ADHD) and the dopamine transporter gene (DAT1) has been reported in clinical samples. Our previous work showed that DAT1 gene was associated with ADHD inattentive subtype and severity of inattentive symptoms. However, little is known about its association with working memory, a promising endophenotype for ADHD. Hence, this study aimed to explore whether there was an association between the DAT1 gene and spatial working memory.

**Methods**
This family-based association sample consisted of 382 probands with DSM-IV ADHD and their family members (n = 1298) in Taiwan. The Spatial Working Memory (SWM) task of the Cambridge Neuropsychological Test Automated Battery (CANTAB) was used to measure spatial working memory of all participants. We screened 15 polymorphisms across the DAT1 gene, including 14 single nucleotide polymorphisms (SNPs) and the variable number of tandem repeat polymorphism in 3´-untranslated region. We used the Family-Based Association Test (FBAT) to test the associations of genetic polymorphisms with the SWM measures.

**Results**
In single locus association analyses, two SNPs (rs2617605 and rs37020) were significantly associated with the double errors (adjusted P = 0.03 and 0.03, respectively) after adjustment for multiple testing. In haplotype analyses, a haplotype rs403636 (G)/rs463379 (C)/rs393795 (C)/rs37020 (G) was significantly associated with total within-search errors (minimal P = 0.001), within-search errors in 8 boxes (minimal P = 0.002), total double errors (minimal P = 0.001), and double errors in 8 boxes (minimal P = 0.004).

**Discussion**
Our finding of the haplotype rs403636 (G)/rs463379 (C)/rs393795 (C)/rs37020 (G) as a novel genetic marker for spatial working memory suggests that variation in DAT1 may provide insight into the pathways leading from genotype to phenotype of ADHD.

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**OVERWEIGHT IS NOT AN IMPORTANT CAUSE OF CLINICALLY SIGNIFICANT DEPRESSION: A MENDELIAN RANDOMIZATION STUDY**

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**Background**
Obesity has been shown to be associated with depression and it has been suggested that higher body mass index (BMI) increases the risk of depression and other common mental disorders.
However, the causal relationship remains unclear and Mendelian randomization (MR), a form of instrumental variable (IV) analysis, has recently been employed to attempt to resolve this issue. Studies to date have only examined depressive symptoms rather than a clinical diagnosis of major depression. The aim of this study is to investigate whether higher BMI increases the risk of major depression using MR analyses.

**Methods**
Two IV analyses were conducted to test the causal relationship between obesity and major depression in RADIANT, a large case-control study of major depression. First, we used a single nucleotide polymorphism (SNP) in the fat mass and associated (FTO) gene and second, we used a genetic risk score (GRS) based on 32 SNPs with well-established associations with BMI.

**Results**
Linear regression analysis, as expected, showed that subjects carrying more risk alleles of FTO gene or having higher score of GRS had higher BMI. Probit regression suggested higher BMI is associated with increased risk of major depression. However, our two instrument-variable analyses did not support a causal relationship between higher BMI and major depression (FTO genotype: coefficient = 0.03, 95% CI = -0.18 to 0.13, P = 0.73; GRS: coefficient = -0.02, 95% CI = -0.11 to 0.07, P = 0.62).

**Discussion**
Our IV analyses did not support a causal relationship between higher BMI and major depression. The positive associations of higher BMI with major depression in probit regression analyses might be explained by reverse causality and/or residual confounding.

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**GENOME-WIDE ANALYSIS OF CURRENT DEPRESSIVE SYMPTOMS AND LIFETIME MAJOR DEPRESSIVE DISORDER IN PGC AND CHARGE**

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**Background**
In the last year, two independent genome-wide association meta-analyses (GWAMA) searched for common genetic variation for lifetime major depressive disorder (PGC-MDD) and current depressive symptoms (CHARGE-depressive symptoms), yet these historically large studies were both negative. This most probably reflects insufficient power in the presence of etiological heterogeneity. In the present study, we combined the PGC-MDD and CHARGE-CESD (current depressive symptoms, assessed continuously) GWAMAs to explore genetic signals of a broad depression phenotype ranging from depressive symptoms to MDD.

**Methods**
Our analyses combined results from PGC-MDD (9240 cases and 9519 controls) with CHARGE-depressive symptoms (n = 51,258). Following the sign-test, we used P-value based meta-analysis.

**Results**
These two studies had no overlap in participants. Quality control for the two studies was similar, and all samples were imputed to HapMap3. First, we evaluated the degree of similarity of these previous GWAS results, which studied different depression phenotypes, using the sign test. For this purpose, we selected SNPs overlapping in the two meta-analyses (n=1,030,530). After filtering for discordant, poorly imputed (r²<0.8) or rare SNPs (CEU MAF<0.005 or >0.995), there were 885,390 SNPs remaining for the analysis. We performed a series of sign tests in LD pruned sets using different P-value thresholds in both directions (from PGC to CHARGE and from CHARGE to PGC). The directions of associations for SNPs in the PGC-MDD meta-analysis were likely to be the same in the CHARGE depressive symptoms meta-analysis (number of successes 60,194; number of tests 118,438; P = 1.5x10⁻⁸). Similarly, pruned SNPs in the CHARGE depressive symptoms meta-analysis were more often in the same direction as PGC-MDD meta-analysis than expected by chance (number of successes 53,047; number of tests 104,545; P = 1.7x10⁻⁶). When we selected SNPs with a P-value <1x10⁻³ and top 1000 SNPs, sign tests were statistically significant in both directions. Second, we performed a P-value based meta-analysis using Stouffer’s unweighted method in METAL. We found one locus with three genome-wide significant SNPs all located in the fragile histidine triad (FHIT) gene (chr3:61 Mb, minimum P = 3.8x10⁻⁹). The top SNP (rs6445194) had a P-value of 4.6x10⁻⁵ for CHARGE depressive symptoms and P = 6.0x10⁻⁶ for PGC-MDD. The FHIT gene is involved in purine metabolism and was related to citalopram-induced side effects in STAR*D. Ongoing replication efforts are in progress.

Discussion
Although clinical heterogeneity has been thought to be a drawback to detect genetic signals in depression, these results suggest that current depressive symptoms and MDD address a similar fundamental liability.

DO SEROTONIN RECEPTOR 1A AND 2A GENE INTERACTIONS HAVE AN IMPACT ON SUICIDALITY? A EUROPEAN MULTICENTRE STUDY ON TREATMENT-RESISTANT MAJOR DEPRESSIVE DISORDER

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Background
It has been projected that Major depressive disorder (MDD) will become one of the leading contributors by disability adjusted life years (DALYs) in near future. Different lines of evidence have suggested that abnormalities in serotonergic neurotransmission pathways play a role in the pathophysiology of affective disorders, as well as in suicidality. The aim of our study was
therefore to investigate the role of gene-gene interactions of the 5-HTR1A and 5-HTR2A gene on suicide risk and personal history of suicide attempts.

Methods
In the context of a European multicentre study on treatment resistant depression (TRD) 374 patients were collected and treated with antidepressants at adequate doses for at least 4 weeks. Suicidality was assessed using the Mini International Neuropsychiatric Interview and the Hamilton Rating Scale for Depression (HAM-D) item 3 (score 0-4). Treatment response was defined as HAM-D≤17 and remission as HAM-D≤7 after 4 weeks of adequate treatment with antidepressants. To test for epistasis the logistic regression model was used on our case-control data set. Genotyping was performed for the rs 6295 (C-1019G) SNP in the 5-HTR1A- and the rs7997012, rs6313, rs643627, rs17288723 SNPs in the 5-HTR2A gene.

Results
Interaction p-values between 5HTR1A C1019G and 5HTR2A rs 6313 in suicide risk, 5HTR1A C1019G and 5HTR2A rs643627 in personal history of suicide attempts were 0.027 and 0.036, respectively, however not resisting the Bonferroni-corrected threshold for association of p=0.0125. Further, no epistatic effects could be found between 5HTR1A and 5HTR2A genes neither for suicide risk nor for personal history of suicide attempts.

Discussion
In conclusion, our study reveals no epistatic effects between 5HTR1A and 5HTR2A genes on both current suicide risk and personal history of suicide attempts. Due to sample size limitations our conclusion has to be considered with restrictions suggesting further studies on more well-powered samples.

ASSOCIATION ANALYSIS OF ANK3 AND CACNA1C GENE VARIANTS IN BULGARIAN AFFECTIVE DISORDER PATIENTS
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Background
Recent GWAS studies, subsequent meta-analysis and targeted association analysis identified the ankyrin 3 (ANK3) gene and the voltage gated calcium channel subunit 1c (CACNA1C) gene as candidate genes with genome wide significance for bipolar affective disorder, but also for major depression and schizophrenia. The functions of ANK3 are related to processes such as synapse organization and stability, cellular transport and signaling, neurogenesis and neuroprotection. The CACNA1C gene encodes the pore-forming alpha1C subunit of the voltage-gated calcium channel, which is important in mediating neuronal excitability via calcium influx in response to neuronal activity. Based on these findings a channelopathy is suggested as a biologically plausible etiologic mechanism for at least part of the bipolar disorder patients.
Methods
The current study is a replication attempt in association study of Bulgarian affective disorder patients. Both case-control and family-based association groups were collected and analysed. The sample consisted of 314 cases with bipolar disorder (BD) and 115 with major depressive disorder (MDD). The control group consisted of 205 healthy prescreened controls, matched by gender, ethnicity and age. The family association study was conducted with 119 Bulgarian families (112 BD, 45 MDD, 5 single depressive episode (SDE), 9 Schizoaffective disorder (SD), bipolar type, 3 panic disorder and 220 healthy relatives) and 56 of Roma origin (87 BD, 20 MDD, 2 SDE, 9 SD, bipolar type and 132 healthy relatives). The probands were assessed with SCAN (Schedules for Clinical Assessment in Neuropsychiatry, Wing et al., 1990) and DIP (Diagnostic Interview for Psychoses, Castle et al., 2006). After written informed consent was received, DNA was isolated and genotyped for two SNPs, rs9804190 at the 3’ and rs10994336 at the 5’end of ANK3, and rs1006737 in CACNA1C, using the TaqMan™ method (Applied Biosystems). All genetic analyses were conducted using PLINK (Purcell et al., 2007), under two affection status models. In the narrow model subjects with BP and SD, bipolar type, were considered as affected; in the broad model patients with MDD were added. Nonparametric test for significance was performed using a χ2 test. Family based association was evaluated using transmission disequilibrium test (TDT), DFAM analysis as well as haplotype based TDT analysis.

Results
Tests for HWE did not reveal any significant deviations. No LD was detected between the two ANK3 markers. In the case-control sample no significant association signals have been found for any of the studied polymorphisms. The TDT and DFAM analysis under the broad phenotype definition showed nominally significant p values for rs9804190 (p=0.01) and suggestive for rs10994336 in ANK3 gene (p=0.06). The empirical p-values after permutation testing confirmed this association. When parent of origin effect was tested, it was shown that the contribution for the TDT comes mainly from the paternally transmitted alleles. The risk T allele of rs10994336 was more often transmitted, while the rare T allele of rs9804190 was under transmitted to affected probands. Under the narrow phenotype definition, the DFAM results were in the same direction for rs9804190 (p=0.02), but not for rs10994336 (p=0.14).

Discussion
In summary, the current study adds support for the contribution of ANK3 gene to the risk of affective disorder. The effect of the markers situated about 340kb apart is independent. While the rare T allele of rs10994336 was associated with increased risk for affective disorder, the rare T allele of rs9804190 seemed to be protective. The findings are in line with published data. No significant results were obtained for the marker in CACNA1C, most likely due to the small effect of this polymorphism and the limited sample size. The same explanation is likely true for the negative results from the case-control analysis.

EFFECT OF L-METHYLFOLATE ON GENETIC MARKERS ASSOCIATED WITH MONOAMINE IMBALANCE FROM A RANDOMIZED CLINICAL TRIAL OF PATIENTS WITH MAJOR DEPRESSION: A POST HOC ANALYSIS
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**Background**

The response to L-methylfolate 15 mg as an adjunct to SSRIs was examined when stratified by baseline levels of genetic markers associated with monoamine dysregulation.

**Methods**

75 inadequate responders to SSRIs entered a 60-day, multi-center, double-blind, placebo-controlled trial. Patients received L-methylfolate 15 mg/day for 60 days, placebo for 30 days followed by L-methylfolate 15 mg/day for 30 days, or placebo for 60 days. Mean change from baseline to endpoint was evaluated by the presence of genetic markers.

**Results**

75 patients were enrolled. For pooled data, the HDRS-17 response rate with adjunctive L-methylfolate 15 mg/day vs. SSRI therapy plus placebo was 17.7% (p=0.04). Pooled differences in mean change on HDRS-17 and HDRS-28 were significantly different (p=0.05 and p=0.02, respectively). Greater mean changes from baseline on the HDRS-28 were observed with L-methylfolate vs. placebo for mutation biomarkers including FOLH1, GCH1, GCHFR, MTHFR, RFC1, CACNA1C, COMT, DNMT3B, DRD2, and MTR. No treatment effect was associated with L-methylfolate in mutation-negative (wild-type) patients.

**Discussion**

In this post hoc analysis, a robust response was observed with adjunctive L-methylfolate, and the response was enhanced in the presence of genetic markers at baseline. A priori replication in larger cohorts is needed.

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**A PREDICTIVE MODEL FOR THE MAOA PROMOTER VNTR AND ITS APPLICATION IN PUBLIC GWAS DATASETS.**

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**Background**

Monoamine oxidase (MAO) A and B are adjacent genes on chromosome X that encode the main enzymes for neurotransmitter turnover. Many drugs used as treatment for clinical depression and anxiety inhibit the MAO enzymes, this suggests that they have an important role in psychiatric traits. Both MAOA and MAOB share a common promoter where a 30bp variable number tandem repeat (VNTR) was identified 1.2kb upstream the transcription start site of the MAOA. These alleles exist in the 2, 3, 3.5, 4, and 5 repeats (R), where 3R and 4R are the most common within the population. 2R, 3R, and 5R were shown to possess low activation of MAOA, whereas 3.5R and 4R were shown to have much higher levels of activation in in vitro studies. However, the reported in vitro promoter high/low activity has poor correlation with in vivo studies examining MAOA or MAOB expression in post-mortem brain. Furthermore, these in vivo studies are
underpowered to detect significant associations.

**Methods**

We developed a predictive tool for the *MAOA* promoter VNTR based on an advanced machine learning method, multicategory vertex discriminating analysis (VDA). We PCR genotyped the VNTR in 400 individuals of European descent, including 30 HapMap trios where there was SNP genotype information within 2MB of the *MAOA* promoter region. 300 individuals were used as a training dataset to build a VDA model using only overlapping SNPs in all datasets and searched by 10-fold cross validation to optimize the accuracy rate for predicting *MAOA* promoter VNTR. A test dataset of 87 individuals was used to validate the VDA model.

**Results**

This algorithm highlighted 5 SNPs, along with gender, that correctly classified the VNTR in 97% of our training dataset, and 96.7% in our validation dataset. With the predictor tool we are now able to study the *MAOA* promoter VNTR in available GWAS data sets of neuropsychiatric traits such as major depressive disorder, bipolar disorder and schizophrenia.

**Discussion**

The predictor tool enables us to establish whether some alleles are indeed high and low expressing (as reported in previous *in vitro* studies) in large gene expression data sets of human brain. Lastly, analysis is underway to test whether specific *MAOA* VNTR alleles affect monoamine neurotransmitter turnover by examining cerebrospinal fluid (CSF) monoamine metabolite levels in healthy controls.

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**PACT: THE DEVELOPMENT OF AN INTERNATIONAL PERINATAL PSYCHIATRY CONSORTIUM**

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**Background**

Postpartum Depression (PPD) impacts at least 1 in 8 women and confers substantial morbidity and mortality. Despite its importance to the health of women and children, PPD is conspicuously under-studied (e.g., very few of the structured diagnostic instruments used in psychiatric genomics have any queries about PPD). The PACT Consortium (Postpartum Depression: Action Towards Causes and Treatment) was formed in October 2010 with the overarching goal of delivering exceptional new knowledge about the etiology of PPD in order to develop a high confidence portrait of how genes and environment interact to predispose some women to PPD and to protect others. PACT members are from 24 institutions in 7 countries. We have collected comprehensive phenotypic data on ~17,000 perinatal women, including ~9,000 cases with PPD and DNA and biological data on ~8,000.

**Methods**

PACT has multiple sequenced studies underway to elucidate the etiology of PPD. For Aim 1, we
are currently performing a rigorous delineation of the phenotypic heterogeneity and subtypes of PPD. This first step is critical in order to finalize operational definitions for cases, controls and quality standards before beginning the collection of biological samples across sites. This includes DNA sampling and biobanking and the determination of high-priority biomarkers to assess. Aim 2 of PACT will perform a comprehensive biomarker assessment based on the findings in Aim 1, as well as dramatically to increase sample sizes using identical assessments and biological sampling and to include both cross-sectional and longitudinal assessments (i.e., follow women across pregnancy).

Results
We will present results from analyses of harmonized individual-level phenotypic clinical data across the participating international sites including demography, obstetrical history, symptoms at time of diagnosis, timing of onset of symptoms and psychiatric history. Latent class analysis will be performed to examine the clinical heterogeneity of PPD.

Discussion
PACT represents an important next step toward disentangling the pathophysiology of PPD. Future work will require a sustained commitment, continuing collaborative effort and the support of both public and private sources. This knowledge can then be used to (i) predict who is at risk of PPD, (ii) to evaluate rational preventative strategies and, (iii) develop targeted treatments.

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PREDICTING AFFECTIVE SYMPTOMS: A POLYGENIC SORE ANALYSIS
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Background
Recent data provide strong support for a substantial common polygenic contribution to genetic susceptibility for schizophrenia (SCZ), bipolar disorder (BPD) and major depression (MDD). Polygenic risk scores were reported to predict disease status in independent psychiatric samples, furthermore the polygenic risk scores of SCZ was observed to relate to specific symptoms in BPD. Based on this background, we aimed to address the question: does a polygenic risk score of BPD predict bipolar-like affective symptoms in SCZ, BPD and MDD?

Methods
We have examined multiple common risk alleles en masse using polygenic score analysis. We used the published meta-analysis of the Bipolar Disorder Psychiatric GWAS Consortium (PGC-BPD) and our own German GWAS of BPD as the discovery sets on which to define polygenic scores that were assigned to each individual in independent samples of SCZ (1,531 cases; 861 controls), BPD (488 cases; 861 controls), and MDD (1,505 cases; 861 controls). Analyses were performed to assess associations of the polygenic scores with disease status and the amount of the number of affective symptoms displayed (OPCRIT items: 19-25; 30-31; 35-37; 39; 41-43; 53; 56-57).
**Results**
Categorical phenotype: Independent BPD risk alleles derived from both datasets were able to discriminate our SCZ and BPD case individuals from controls, whereas in case of MDD only the polygenic score of the PGC-BD predicted disease-status. Quantitative phenotype: The polygenic score of the PGC-BD majorly correlated with the number manic symptoms in patients with BPD, SCZ, MDD; whereas the polygenic score based on the German GWAS results predicted the number of both depressive and manic symptoms in patients with SCZ, BPD, and MDD.

**Discussion**
Individuals with higher amount of affective symptoms have more BPD risk alleles than other cases. The findings indicate that polygenic risk scores for psychiatric disorder can be usefully in order to explore phenotypic heterogeneity.

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**REGULATORY VARIANTS IN CALCIUM SIGNALING GENES UNDERLIE SUSCEPTIBILITY TO BIPOLAR DISORDER**
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**Background**
Despite several lines of evidence pointing to a role for regulatory variation in the etiology of bipolar disorder (BD) and other complex diseases, few studies have deeply characterized non-coding sequences from disease cases and controls.

**Methods**
We tested the hypothesis that uncommon, regulatory variants contribute to BD by examining whole genome sequences from a total of 358 individuals in 43 multiplex BD pedigrees and 34 control pedigrees. We identified uncommon, segregating variants in each of these pedigrees and searched for genes and pathways that contained statistically more candidate variants in BD pedigrees than controls.

**Results**
Putative regulatory variants -- defined as those that are predicted to impact the function of a promoter, enhancer, or 5’ or 3’ untranslated region – were statistically enriched within gene sets related to calcium signaling, GABA signaling, and inflammation. Although coding variants were found in some of these same genes, they were far less common. Based on these results, we selected 30 top candidate genes for re-sequencing of exons, promoters, 3’ untranslated regions, and selected enhancers in 6000 additional BD cases and controls.

**Discussion**
Our results suggest that regulatory variants within calcium signaling genes and other gene sets with neurological functions contribute to BD susceptibility.
SEARCHING FOR EPIGENETIC BIOMARKERS IN MAJOR DEPRESSION:
FOCUSBING ON THE SEROTONIN SIGNAL TRANSDUCTION
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Background
Searching for biological markers for major depression (MD) could be important for improving patient care and the development of more effective drug treatments. Since a gene-environmental interaction is closely involved in the etiology of MD, it is plausible that the analyses of DNA methylation status are useful for the diagnostic biomarkers.

Methods
To examine whether the DNA methylation profiles of the SLC6A4, serotonin (5-HT) 1A receptor, and 5-HT 2C receptor gene can be useful for a diagnostic biomarker for MD, we analyzed the DNA methylation profiles of CpG island across exon 1 of the SLC6A4 gene, CpGs at the promoter of exon 1 of the 5-HT1A receptor gene, and CpG island across exon of the 5-HT2C receptor gene in healthy subjects (n = 15) and patients with major depression (n = 15). In addition, we compared the DNA methylation profiles of CpGs of these 3 genes before and after antidepressant treatment and examined whether the DNA methylation profiles of these 3 genes can be useful for a surrogate biomarker for MD. There was no significant difference in age between these 2 groups. This study was approved by the Ethics Committee of the Hiroshima University School of Medicine. All subjects received a description of this study and gave written informed consent. Genome DNA was extracted from peripheral blood of Japanese healthy controls and patients with MD before and after treatments. DNA methylation rates at the CpG island across exon 1 of the SLC6A4 gene, CpGs at the promoter of exon 1 of 5-HT1A receptor gene, and CpG island across exon of the 5-HT2C receptor gene were analyzed using MassARRAY Compact SYSTEM (SEQUENOM). Two-dimensional hierarchical cluster analyses were undertaken to determine the validity of these methylation profiles as a diagnostic biomarker. The severity of depression was evaluated using the Hamilton Rating Scale for Depression.

Results
Two-dimensional hierarchical cluster analyses demonstrate that the DNA methylation profiles of the SLC6A4, 5-HT1A receptor, or 5-HT2C receptor gene cannot classify subjects into two groups in agreement with clinical diagnosis (MD and healthy controls). There were significant associations between the improvement ratios and methylation rates of several CpG sites in unmedicated patients with the s allele homozygote of the SLC3A4 gene.

Discussion
Different from the DNA methylation profiles of the BDNF gene, the DNA methylation profiles of the SLC6A4, 5-HT1A receptor, or 5-HT2C receptor gene may not be a potent diagnostic marker for MD. On the other hand, the DNA methylation analysis at the promoter of the SLC6A4 gene may be useful for a surrogate marker for MD in the S allele homozygote. Further studies using large scale of patients with MD are required.
A GENOME-WIDE ANALYSIS OF AFFECTIVE DISORDER SUICIDALITY WITHIN THE PGC

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Background

Suicide is estimated by the WHO to account for 1.5% of the deaths throughout the world. Psychiatric disorders, particularly affective disorders, contribute substantially to risk for suicide. Suicide attempts are reported to be moderately heritable in twin studies, with a heritability of 43%. Despite several genome-wide association studies looking at suicidality, no genome-wide replicable associations have been identified. Here we will present our analysis of suicidality in cases with either major depression (MDD) or bipolar disorder against both cases who do not report serious suicide attempts and against controls.

Methods

Our sample was drawn from the PGC’s MDD and bipolar disorder cohorts. The MDD sample contained 9,239 cases and 9,519 controls across 9 studies. Of these MDD cases 1,075 cases reported a serious suicide attempt, while 7,081 reported no such attempt. Within the bipolar disorder sample, there were 11,904 cases and 15,223 controls across 14 studies, with 2,816 of the cases reporting suicide attempts and 4,170 reporting no attempts. Two genome-wide analyses were performed: with non-suicide attempter cases vs. attempter cases; and attempter cases vs. controls. We performed these genome-wide association analyses within each disorder separately and then meta-analysed the results across the two disorders. We are also conducting polygenic scoring for the results for suicidality within MDD to predict suicidality within the bipolar samples, and from bipolar disorders into MDD, in order to examine a shared genetic aetiology. We also examine, through GCTA, the heritability of suicidality and to what extent it overlaps across bipolar disorder and MDD.

Results

The results to date have shown no genome-wide associations differentiating suicide attempters from non-attempters within a case only analysis. The top SNP for the meta-analysis was rs9428050 on chromosome 1 (p=2.3E-7, OR=1.17). However the analysis of suicide attempters vs. controls has generated several novel associations, including a top SNP on chromosome 14 for MDD suicide-attempters against controls at p=8.5E-11 (rs8013144, OR=2.22, previous p-value in total MDD sample= 1.05E-6). These results are currently being brought forward for replication in independent samples. The results of the polygenic scoring and GCTA analyses are ongoing, but will provide insight into the genetic overlap of suicidality across disorders.

Discussion

This genome-wide analysis of suicidality will provide novel insight into one of the most damaging aspects of psychiatric illness. Evidence for a shared genetic aetiology for suicidality across affective disorders would help better understand its existence across diagnoses, while novel variants would be another step on the road to greater identification of those individuals at greatest risk.
INVESTIGATING THE GENETIC VARIATION UNDERLYING EPISODICITY IN
MAJOR DEPRESSIVE DISORDER: SUGGESTIVE EVIDENCE FOR A BIPOLAR
CONTRIBUTION

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Background
Replicated significant findings in genome-wide association studies of major depressive disorder (MDD) have been scarce, partly due to its phenotypic and genetic heterogeneity. Using subphenotypes associated with increased heritability, such as recurrence and early onset, is a promising alternative research pathway. Highly recurrent and early-onset MDD have reportedly increased risk of shifting to bipolar disorder (Angst et al. J Affect Disord 2005). This study aimed to investigate the genetic underpinnings of the total number of depressive episodes (understudied to date) in recurrent MDD.

Methods
Our primary sample included 1966 cases of recurrent MDD with negative family history of bipolar disorder from the RADIANT studies DECC, DeNt and GSK (Lewis et al. Am J Psychiatry 2010). Subjects were interviewed with the Schedules for Clinical Assessment in Neuropsychiatry (SCAN). Total episode count was adjusted for the confounding effect of gender, age, MDD duration, study and center before being tested for association with genotype in a genome-wide analysis.

Results
Adjusted episode count was a significant independent index of MDD familial aggregation; intermediate episode counts (4–6) were associated with maximal familiality, in line with a previously reported ‘inverted U’ relationship (Kendler et al. Arch Gen Psychiatry 1999). A genome-wide association study in the total set and in a subset of 1364 cases with positive family history of MDD (FxD) yielded no genome-wide significant findings. The strongest suggestive-level associations were detected in the total set at MAGI1 (p=5.1x10⁻⁷), previously associated with bipolar disorder (Karlsson et al. Biol Psychiatry 2012), and in the positive FxD subset at STIM1 (p=3.9x10⁻⁶ after imputation), a calcium channel signaling gene (Soboloff et al. Nat Rev Mol Cell Biol 2012). Our top findings failed to replicate in a Munich cohort of 372 cases. In the meta-analysis of results from the RADIANT and Munich samples, no SNPs attained genome-wide significance. Polygenic risk scores were calculated from the Psychiatric GWAS Consortium MDD and bipolar disorder datasets using PLINK profile scoring procedure (SNP subset p-value thresholds <0.01, <0.05, <0.1, <0.2, <0.3, <0.4, <0.5). Both MDD (D) and bipolar disorder (B) polygenic scores significantly predicted MDD episodicity. In the total set, D scores had a stronger contribution to episodicity than B scores; however, in the positive FxD subset the
two scores ran head-to-head or B scores outperformed D scores at some SNP subsets (respective p-values 0.004 vs 0.022 for the p<0.2 SNP subset).

**Discussion**
Our findings lend preliminary support to the hypothesis that highly recurrent MDD with positive FxD is part of a ‘soft bipolar spectrum’ (Akiskal J Affect Disord 2003; Phelps et al. Bipolar Disord 2008) but await replication in larger cohorts and across a wider range of mood disorders.

**GENETIC ASSOCIATION OF EGR3 GENE WITH BIPOLAR DISORDER IN KOREA**
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**Background**
Early growth response (EGR) genes are involved in neuronal activation, brain development, and synaptic plasticity. Association between EGR3 and schizophrenia (SPR) has been reported in East Asian countries, including Japan, Korea, and China. Association between EGR3 and bipolar disorder (BPD) was also reported. EGR3 resides at the chromosomal location 8p21.3, one of the linkage regions for both SPR and BPD.

**Methods**
We investigated the association of EGR3 gene with BPD and SPR in Korea. We selected six single nucleotide polymorphisms (SNPs) in EGR3(rs17088531, rs1996147, rs3750192, rs35201266, rs7009708, and rs1008949), which have been investigated in previous studies. Participants were all ethnically Korean, including 752 controls, 632 BPD patients, 362 SPR patients.

**Results**
Three SNPs including rs35201266, rs7009708, and rs1008949 showed nominal allelic association with BPD. Haplotype analysis revealed that G-C-G-C (rs3750192-rs35201266-rs7009708-rs1008949) haplotype was over-presented (P=5.545×10⁻⁵), while G-T-G-C haplotype was underpresented (P=4.951×10⁻⁵) in BPD. Positive association of rs35201266 with SPR was repeatedly found with extended participants of our previous study. T allele of rs35201266 was over-presented in SPR, while under-presented in BPD compared to controls, resulting in the significant difference in allelic distribution between SPR and BPD (P=4.723×10⁻⁸). T allele-carrying BPD patients had more psychotic symptoms and the first manic episode history.

**Discussion**
Genetic association of EGR3 with BPD was found in Korean population. The different allelic distribution of rs35201266 in EGR3 gene between SPR and BPD was also demonstrated. Further investigations will be required to understand the pathogenetic implications of the present findings.

**GENOME-WIDE LINKAGE SCAN OF BIPOLAR DISORDER IN LATINO FAMILIES IDENTIFIES SUSCEPTIBILITY LOCI AT 8Q24 AND 14Q32**
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Background
The genetic contribution to psychiatric illnesses such as bipolar disorder (BD) has been difficult to define due to genetic heterogeneity, phenotypic overlap and lack of a simple mode of inheritance. It has been estimated that over 63,000 case-control samples are needed to identify the approximately 100 BD risk loci discoverable by genome-wide association studies (GWAS), and that these are only expected to explain <6% of the inherited risk. The remaining, unexplained variation suggests that the common disease/common variant model, on which the current GWAS analysis is based, may not adequately explain the genetic component of complex psychiatric diseases. In addition, GWAS performed to date have focused almost exclusively on persons of European ancestry and the extent to which the findings relate to minority populations remain unclear. Linkage studies in minority populations are a good alternative to traditional GWAS and may identify rare variants with larger effect sizes that underlie substantial risk for BD in other populations.

Methods
We performed a genome-wide nonparametric linkage screen to localize BD susceptibility loci in a sample of 3690 individuals of Latino ancestry. Each family had at least two siblings who had been previously diagnosed with either BD or schizoaffective disorder, bipolar type (SABD) according to inpatient or outpatient records. Final diagnoses were determined through a best estimation consensus process. The sample included 974 individuals with BD phenotype (394 sibling pairs) from 690 families recruited from the five collection sites in the US (San Antonio, TX; El Paso, TX; Los Angeles, CA), Mexico (Mexico City; Monterrey) and Central America (Costa Rica; Guatemala). Participants were genotyped from lymphoblast cell lines using the Illumina HumanLinkage-24 BeadChip (Illumina Inc., San Diego, CA), with an average genetic coverage of 0.67 cM. Non-parametric analyses were performed over a 5 cM grid using the linkage analysis software MERLIN. Multipoint analyses were conducted across the genome using non-parametric Kong & Cox LOD (NPL) scores along with Sall statistics for all relative pairs. Suggestive and significant genome-wide thresholds were calculated based on 1000 simulations generated by the genedropping algorithm in MERLIN. Single-marker association tests in the presence of linkage were performed using the LAMP software, assuming a multiplicative model with a population prevalence of 2%.

Results
Significance thresholds corresponding to a genome-wide significance level of 0.05 were NPL ≥ 3.06 (Sall ≥ 2.96) based the results of our 1,000 simulations. We identified two regions, 8q24 and 14q32, that revealed significant evidence of linkage. A third region, 2q13-q14, showed suggestive evidence of linkage. The region with the highest multipoint LOD score was on chromosome 8q24 (maximum LOD=3.528, p=2.78 x 10^-5). Results of the NPL scan are summarized in Table 1. We tested all single markers under the three linkage peaks for association with BD phenotype using the LAMP program. The most significantly associated marker was rs1847694 (p = 2.40 × 10−5). Three other SNPs in Chromosome 2 had significant p-values: rs1864474, rs2048876, and rs708670. No single marker SNP tested in the 8q24 or 14q32
linkage peak regions was significantly associated with BD phenotype after correction for multiple testing.

**Discussion**

We identified two genome-wide significant susceptibility loci for BD at 8q24 and 14q32, and a third suggestive locus at 2q13-q14. Our maximum multipoint LOD was detected at locus 8q24, which met significance thresholds for both the Kong and Cox and Sall statistics. This region is a well established BD susceptibility locus that has been previously implicated in several linkage and association studies for BD. Our largest single marker LOD within the 8q24 linkage peak was at SNP rs1488019 (LOD=2.54). We also report a significant susceptibility locus at 14q32 (Sall statistics), which has also been previously identified in linkage studies of BD.

Our top associated single marker (rs1847694, p = 2.40 \times 10^{-5}) is located 195 Kb upstream of DPP10 in Chromosome 2. DPP10 is prominently expressed in brain neuronal populations, where it has been shown to bind and regulate Kv4-mediated A-type potassium channels. Variants in DPP10 have been moderately associated with BD in previous studies. Taken together, these results provide additional evidence that 8q24, 14q32, and 2q13-q14 are susceptibility loci for BD and these regions may be involved in the pathogenesis of BD in the Latino population. Future fine-mapping studies followed by functional studies of candidate genes confirming significance are warranted.

**ASSOCIATION OF RORA AND RORB GENE POLYMORPHISMS WITH BIPOLAR DISORDER AND SLEEP FEATURES**

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**Background**

Circadian rhythm is an endogenously driven around 24-hour cycle in regulating many human physiological and behavioral processes. Disruption of circadian rhythm is often implicated in bipolar disorder (BP) and sleep-related problems, such as delayed sleep onset, shorter total sleep time and insomnia that are commonly observed in clinical patients of BP. Several circadian genes are proposed to be involved in the pathogenesis of BP and sleep disturbances. The current study aimed to examine the associations of circadian genes with BP and a number of sleep features.

**Methods**

A case-control association study was conducted for patients with BP and healthy controls. Patients’ were ascertained from three hospitals in 2008 to 2010. Healthy controls were recruited in the community and were screened for major psychiatric disorders. Sleep features were accessed by Pittsburgh Sleep Quality Index (PSQI), which includes a global score (>5: poor
sleepers; ≤5: good sleepers) and seven components: subjective sleep quality, latency, duration, efficiency, sleep disturbances, use of sleeping medication, and daytime dysfunction. Twenty six single nucleotide polymorphisms were genotyped in the NR1D1, RORA, and RORB genes in 280 patients (200 bipolar I disorder, BPI; 80 bipolar II disorder, BPII) and 200 controls using GoldenGate® VeraCode™ assays. Single marker association analyses were performed using PLINK and regression models were adjusted for age, sex, and disease diagnosis for sleep features. Generalized multifactor dimensionality reduction (GMDR) method was used to test gene-gene interactions.

**Results**

All markers were in Hardy-Weinberg equilibrium in controls (p>0.001). The strongest associations with BP were observed for rs4774388 in RORA (OR=1.53, p=0.03), and rs1327836 in RORB (OR=1.75, p<0.01) genes. For sleep features, variants in RORB showed significant associations with duration (rs7021908, OR=0.64, p=0.04), and efficiency (rs10217594, OR=1.82, p=0.03; rs2273975, OR=0.51, p=0.04). RORA exhibited association with sleep efficiency (rs809736, OR=0.75, p=0.003). Moreover, a four way gene-gene interaction was found among markers in NR1D1, RORA, and RORB on the risk of BP. The testing accuracy was 53.25% with a cross-validation consistency of 8 out of 10. Among sleep features, global score had the highest testing accuracy of 57.35% with a two way interaction in NR1D1 and RORB. Sleep duration (definition) showed a four way interaction with an accuracy of 57.2%.

**Discussion**

Our results suggest that the RORs in the circadian system show pervasive associations with BP and a number of sleep features in the Han Chinese population. The circadian pathway is an important candidate for further evaluation in studying the pathogenesis of BP and related defectiveness of sleep regulation.

**MODULATORY EFFECTS OF A DEPRESSION-ASSOCIATED SINGLE NUCLEOTIDE POLYMORPHISM (RS3923028) IN THE HUMAN ANAPLASTIC LYMPHOMA KINASE (ALK) GENE ON AMYGDALA REACTIVITY**

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**Background**

The major (C) allele of a common single nucleotide polymorphism (rs3923028) within the anaplastic lymphoma kinase (ALK) gene has been linked to major depressive disorder (MDD) via genome-wide association studies. A recent animal model study has shown that ALK knockout produces a depression-resistant phenotype, lending further support to the notion that ALK rs3923028 genotype may moderate risk for clinical depression in humans. However, the precise biological mechanisms by which this may occur are unclear.

**Methods**

We used data from the ongoing Duke Neurogenetics Study (N=419, 239 women, mean age 19.59 ± 1.27) to investigate the effect of rs3923028 on amygdala reactivity measured with a well-validated face-matching BOLD fMRI paradigm. Basolateral and centromedial divisions of the amygdala were examined independently. All analyses were conducted in a sample comprising all
Results
In a sample comprising all ethnicities, individuals homozygous for the ALK rs3923028 risk (C) allele exhibited threat-related amygdala hyper-reactivity in comparison with non-risk (T) allele carriers in all amygdala subregions (all p values < 0.05). In a Caucasian-only sample, only the effect in the left centromedial amygdala remained.

Discussion
We demonstrate that a genetic variant previously associated with increased risk of major depressive disorder results in relative amygdala hyperactivity to threat. Given that prior studies have associated depression with heightened amygdala reactivity in a state-independent manner, the current findings may provide mechanistic insight into how regulation of the ALK gene may impact depression risk by biasing neural circuit reactivity.

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PREDICTING PEDIATRIC ANXIETY FROM AN AGGREGATE OF SEROTONIN-RELATED RISK ALLELES
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Background
Anxiety disorders run in families (Low, Cui, & Merikangas, 2008) and show heritability of about 30-40% in twin studies (e.g., Hettema, Neale, & Kendler, 2001). Although the task of definitively identifying genes that increase risk for anxiety disorders remains in an early stage (Smoller, Gardner-Schuster, & Covino, 2008), genes related to serotonin availability at the synapse are frequently implicated (e.g., Strug et al., 2010). Lack of replication and the existence of several types of anxiety disorder complicate interpretation of the literature (McCarthy et al., 2008; Norrholm & Ressler, 2009). Shifting to pediatric anxiety, a modest but growing literature confirms the moderate heritability of anxiety phenotypes (Bolton et al., 2005) and suggests that it may decrease with age (Boomsma et al., 2005), with little consistent evidence for gender differences. Again, serotonin-related genes are implicated in childhood anxiety, most frequently via the serotonin transporter gene (SLC6A4) promoter polymorphism (5-HTTLPR) interacting with stressors (e.g., Stein, Schork & Gelernter, 2008) although non-replications of these interactions are also reported (e.g., Tomoda et al., 2013). Here, we take a broader perspective by studying allelic variation in a set of six serotonin-related genes aggregated to form a single genetic risk index.

Methods
From a larger sample of 7-year-old twins that was ascertained from birth records and oversampled for psychopathology symptoms, we eliminated individuals without sufficient genotyping or with other exclusions, leaving 690 Caucasian children. We assessed anxiety symptoms via standard psychiatric interviews (DISC-IV) and questionnaires completed by the mother, the father, and the child. Two observers also rated each child’s anxiety-related behaviors during a 4-hour home visit that included standardized probes meant to induce mild threat and
uncertainty or provide novel contexts. We formed dimensional composites of general anxiety (excessive worrying, obsessions or compulsions, psychosomatic complaints) and social anxiety (separation anxiety, social or performance fears) using this five-informant approach. Genotyping. We selected, a priori, genes known to affect the serotonin system from a larger set of psychopathology-related candidate genes: TPH2, MAOA, the 5-HTTLPR polymorphism of SLC6A4, and three serotonin receptor genes (5HTR1A, 5HTR2A, 5HTR2C). A single, functional polymorphism was selected for each of these six genes (rs4570625, rs6323, 5-HTTLPR, rs6295, rs6313, and rs6318, respectively) based on existing literature and transcriptional data obtained from the NIH and ENCODE databases. Having the risk allele for each gene contributed additively to scores on an aggregate genetic risk index that was scaled from 0 to 12 according to the number of risk alleles (0,1,2) at each locus that each individual possessed.

Results
Males and females did not differ on the anxiety measures at this young age, but lower social class predicted more anxiety at a highly significant (p<.001) level. After controlling for social class, the genetic aggregate significantly (p<.001) predicted general anxiety, with the model accounting for about 2.5% of the phenotypic variance (see Figure). The genetic aggregate also significantly predicted social anxiety, but with a smaller effect. When we added the interaction of social class and the serotonin aggregate to the model for general anxiety, the interaction term was significant (p=.03), and the adjusted R² for general anxiety increased to 3%. For social anxiety, the augmented model with the interaction was significant and accounted for 2% of the variance, but the aggregate by social class interaction was only significant at a trend level. Follow-up regression analyses showed that SLC6A4, 5HTR2A, and 5HTR1A carried most of the predictive power in the aggregate although only SLC6A4 interacted significantly with social class, and then only to predict social anxiety. Analyses were repeated to adjust the standard errors for clustering within families. The results were unchanged.

Discussion
Our results largely replicate prior studies but extend these findings powerfully to young children in a way that avoids method dependence. Although positive findings for SLC6A4 were expected, the contribution of allelic variation for two serotonin receptors was notable. Our using a dimensional approach to anxiety assessment shows that the impact of serotonin-related genes is not confined to more extreme, diagnosed anxiety disorders. Additional analyses of these data will examine the effect of parental standing on anxiety proneness, based on the prediction that the serotonin aggregate will predict childhood anxiety more strongly in this subgroup.

CANDIDATE GENES AND NOVEL POLYMORPHISMS FOR ANXIETY DISORDERS IN A SOUTH AFRICAN COHORT
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Background
Anxiety aetiology remains poorly understood; however clear evidence for a genetic component exists. Increasing focus concerning neurobiological and environmental interaction mediating
disorder pathogenesis has been highlighted, specifically the role of trauma in disorder susceptibility. A number of genetic studies have been conducted; however as yet, no genetic variants have been explicitly identified as being involved in disorder pathogenicity. Neural circuitry involving the striatum has been implicated in numerous anxiety disorders. Identifying mechanisms and genes responsible for changes at neuronal synapses involved in the manifestation of symptoms typical of these conditions, whilst considering trauma exposure as a contributing factor, could prove crucial to the understanding of anxiety disorders.

Methods
Adult Sprague Dawley MALE rats were subjected to maternal and/or restraint stress and used to mimic the presence of major life events (e.g. childhood trauma) and mild stress in adulthood, respectively. The expression of genes encoding components important for synaptic plasticity was investigated using pathway-based PCR array technology. Human homologue susceptibility candidate genes (selected based on aberrant gene expression observed in rats) were characterised in a case-control association study comprising patients with a primary diagnosis of obsessive-compulsive disorder (OCD), panic disorder (PD) or social anxiety disorder (SAD) for which trauma history was known. A tagSNP approach was used to genotype human homologues; alternatively targeted next-generation sequencing (tNGS) was employed. Genotyping data was statistically assessed in conjunction with trauma history to test for gene-environment interaction.

Results
Several genes (Bdnf, Mmp9, Arc, Ntf4, Egr2, Egr4 and Grm2) were identified as aberrantly expressed in rats with anxiety-like behaviours vs. controls. tNGS of ARC, NTF4, EGR2, EGR4 and GRM2 yielded 400 SNP/Indel variations, of which 42 were unique to only either patients or controls and only 4 of these were characterised on dbSNP.

Discussion
Differentially expressed genes in rats with a trauma history and anxiety-like behaviours can point to candidate susceptibility genes for anxiety disorders in humans, and significantly enhance understanding for the molecular basis of anxiety disorders. The novel variation identified within this study will be genotyped in the remainder of our cohort to assess for association, in conjunction with trauma data. This will provide proof-of-concept for use of this animal model for novel candidacy identification and elucidate any gene-environment interaction that may be occurring in relation to anxiety disorders.

CHILDHOOD ABUSE AND GENETIC RISK FOR MENTAL ILLNESS: A TEST OF THE GENE-ENVIRONMENT CORRELATION HYPOTHESIS
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Background
There is a well-established association between the experience of child abuse and increased risk for developing a wide range of mental disorders. Research in this area assumes that the association between childhood abuse and risk of mental disorders is entirely environmentally
mediated. That is, the experience of childhood abuse causes new onsets of mental disorders. However, it is also possible that the association between childhood abuse and mental disorders could partly be due to gene-environment correlation: people who have experienced childhood abuse have also inherited alleles associated with increased risk of mental illness. However, to our knowledge, this gene-environment correlation hypothesis has not been tested with genetic data.

Methods
In the present study, we examined the association of Psychiatric Genomic Consortium (PGC) risk scores for bipolar disorder, schizophrenia, and major depression with childhood abuse, measured by the physical assault subscale of the Conflict Tactics Scale and the physical and emotional abuse subscale of the Childhood Trauma Questionnaire. We used data from the case-control Posttraumatic Stress Disorder Substudy of the Nurses’ Health Study II (n=3013). We used generalized estimating equations with an identity link and a normal distribution to estimate the association between genetic risk score (independent variable) and childhood abuse (dependent variable). We weighted the data for probability of selection into the genetic study, so that our data represented the population from which the cases and controls were drawn.

Results
We found an association between physical assault in childhood and the schizophrenia PGC score (including SNPs significant at P≤.5) and physical and emotional abuse (p=0.03) and the bipolar PGC score (p=0.02), and a borderline association with the schizophrenia score (p=0.07, including SNPs significant at P≤.5). We did not find associations between abuse and the major depression score.

Discussion
Our results provide preliminary evidence that persons exposed to childhood abuse may also carry higher genetic risk for mental illness.

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REPLICATION OF GENE X ENVIRONMENT (CHILDHOOD ADVERSITY) INTERACTION IN ASSOCIATION OF FKBP5 AND 5-HTTLPR WITH PTSD IN NATIONAL GUARD SOLDIERS DEPLOYED TO IRAQ AND AFGHANISTAN:

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Background
Posttraumatic stress disorder (PTSD) is a chronic, debilitating disease that leads to substantial human suffering and costs to society. Military deployment and exposures to combat trauma are associated with high incidence of PTSD, estimates of up to 1 in 5 US soldiers and Marines deployed to Iraq developed clinically significant symptoms of PTSD. Twin and family studies suggest that PTSD is heritable, with estimates of up to 40% of variance in PTSD symptoms explained by genetic factors. Molecular genetic studies of candidate genes in trauma-exposed populations has not found “main effects” of gene association with PTSD, but rather have suggested gene x environment (G x E) interaction, specifically gene x childhood adversity, in the serotonin transporter (SLC6A4), FKBP5 and RGS2 genes, but these have not been replicated; further replication is required.
Methods
A random sample of 2616 Ohio Army National Guard (ONG) soldiers was recruited, and structured telephone interviews were conducted at three time points (pre- and post-deployment, “Waves” 1-3) to assess deployment-related and lifetime traumatic event exposure, including childhood abuse and adversity, and DSM-IV criteria PTSD (interview adapted from PTSD Checklist, PCL). Deployments included Afghanistan (OEF), Iraq (OIF), and other operations. Additional psychiatric symptoms were obtained using validated instruments, and other data using multi-measure scales adapted from the Deployment Risk and Resilience Survey. Soldiers were requested on Wave 2 interview to consent to provide saliva specimens and additional self-report measures, collection kits (Oragene) were sent to participant’s homes and shipped to our lab by return mail. Genomic DNA was purified using a semi-automated filter-based system. Genotyping was performed using PCR (serotonin transporter gene (SLC6A4) 5-HTTLPR, DRD4 VNTR), and a custom 4800 SNP Illumina array. This array maps ~120 candidate genes and genomic regions previously identified in candidate or GWAS studies of other psychiatric disorders, and includes FKBP5, RGS2, and CRHR1 genes previously implicated in PTSD, as well as ancestry informative markers. Standard Illumina clustering and data cleaning was performed, data pruning generated 1500 markers in equilibrium for principal component analyses to control for population substructure. Association of previously reported variants with level of PTSD symptoms was tested in GLM modeling of main effects of gene (SNP), controlling for levels of childhood abuse (0, 1, or 2 or more types), and lifetime trauma load (1-4 levels), and also including gene x child abuse and gene x trauma load interaction terms.

Results
The majority of soldiers (80%) agreed to be sent a genetic specimen collection kit, and 1042 returned genetic specimens and self-report data (50% return rate). 136 participants did not endorse any lifetime trauma, and were excluded from further analyses. The highest of the available PCL scores was used for association analyses (available data N = 766). As expected, PCL score was associated with report of child abuse (F[2,754] = 12.4, p <.0001) and lifetime trauma load (F[3,753] = 14.9, p <.0001). Four SNPs in FKBP5 (rs1360780, rs3800373, rs9296158, rs9470080) all showed SNP x child abuse interactions (all p < .007), but no main effect on PCL. The triallelic 5-HTTLPR also showed gene x child abuse interaction in a recessive model (p < .005), but no main effects. RGS2, DRD4, and CRHR1 all showed no main effects and no interaction effects for association with PCL.

Discussion
To our knowledge, this is the first report of genetic association with PTSD symptoms in soldiers deployed to Iraq and Afghanistan. We replicated previous findings of GxE interaction effects in FKBP5 and 5-HTTLPR. Further analyses are ongoing to identify additional PTSD associations.
A NOVEL GENOME-WIDE APPROACH TO INVESTIGATING DNA METHYLATION CHANGES IN SOCIAL DEFEAT STRESS, A MOUSE MODEL OF MOOD AND ANXIETY DISORDERS

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Background
Stress is a significant risk factor for mood and anxiety disorders. A major avenue by which stress could contribute to these disorders is through altering epigenetic patterns including DNA methylation which can influence gene expression. Through this mechanism, changes in DNA methylation patterns induced by stress can influence the likelihood of developing stress-related psychiatric illnesses such as major depression and post-traumatic stress disorder. Although previous studies have identified individual loci where changes in DNA methylation can contribute to anxiety and depression-like behavior, these studies have not done genome-wide assessment. Using a state-of-the-art genome-wide assay called Methyl-Seq, the current study investigates genome-wide DNA methylation in mice exposed to an animal model of stress called social defeat stress.

Methods
C57Bl/6 mice were exposed to 14 days of social defeat stress whereby they were daily exposed to physical aggression by CD1 aggressor mice for 10 min followed by 24 hrs sensory exposure. Anxiety behavior of social defeated animals was studied using elevated plus maze and open field test. Animals were subsequently sacrificed and hippocampus extracted from the brain. NeuN+ cells from the hippocampus were identified by immunolabelling and isolated by fluorescence activated cell sorting. DNA was subsequently extracted for Methyl-Seq. The Methyl-Seq approach involves capturing ~100MB of CpG islands and shores, promoters, and regulatory regions across the genome using the Agilent SureSelect kit. Targeted capture of these DNA sequence were subsequently bisulfite converted followed by sequencing using Illumina HiSeq2000. Data was analyzed using Bismark and GeneSpring programs.

Results
Mice exposed to 14 days of social defeat stress displayed anxiety-like behavior determined by reduced time in the open arms of the elevated plus maze. Similarly in the open field test, stressed mice moved to the center less frequently than control mice and displayed reduced exploration behavior and increased immobility times. Preliminary Methyl-Seq experiments identified an increase in DNA methylation in genes or upstream of genes involved in phospholipid metabolism (PDXDC1), appetite regulation (galanin) and histone modification (DPF3). This finding was subsequently validated by bisulfite pyrosequencing.

Discussion
The current study has identified DNA methylation changes in genes known to be involved in brain disorders. DNA methylation changes in these regions are thus candidates for mediating the effects of stress on mouse behavior. Future studies aim to validate these findings and to test larger numbers of animals for greater statistical power to detect DNA methylation in additional genes.
A GENOME-WIDE ASSOCIATION STUDY OF QUANTITATIVE OBSESSIVE-COMPULSIVE TRAITS IN CHILDREN AND ADOLESCENTS FROM THE GENERAL POPULATION

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Background
Obsessive-Compulsive disorder (OCD) is a common (1-2% lifetime prevalence), debilitating and phenotypically heterogeneous disorder which is highly heritable, particularly when symptoms begin in childhood or adolescence. OC symptoms are quantitative traits, continuously distributed in the general population and thus ignoring the quantitative nature of OCD may reduce power of genome wide association studies (GWAS) comparing OCD patients to controls. The goal of our study was to conduct a GWAS on a quantitative distribution of OC traits measured in the general population.

Methods
The sample consisted of 16,380 children and adolescents recruited from the community. Self- and/or parent-report data on obsessive-compulsive (OC) symptoms was collected from all participants. We selected 5940 individuals of European Caucasian descent for genotyping with the Illumina HumanCoreExome beadchip. Quality control analyses were conducted using PLINK, including multi-dimensional scaling (MDS) for population structure and the Cochrane-Armitage trend test for detection of association.

Results
The GWAS is ongoing and results will be available at the time of the presentation. Quality control analyses on an initial set of 191 individuals resulted in 7/191 exclusions due to call rates <98% (n=2), sex discrepancy (n=1) and non-European ancestry based on MDS plots (n=4).

Discussion
This research has the potential to increase the ability to identify candidate variants for future biological investigation, to facilitate the understanding of the mechanism by which genetic risks result in OCD. This will be the first report of the utility of performing a genome-wide study of quantitative OC traits.

PARTITIONING THE ETIOLOGY OF HOARDING AND OBSESSIVE COMPULSIVE SYMPTOMS - A GENETIC EPIDEMIOLOGICAL TWIN STUDY

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Background
Hoarding symptoms have until recently been considered to be a subdimension of obsessive-compulsive symptoms (OCS). However, evidence is mounting to suggest that these phenotypes are in fact clinically distinct, and perhaps also etiologically separate. Although both traits are
thought to have a genetic etiology, there has been little work examining the unique and shared genetic contributions to hoarding and OCS.

Methods
Univariate and bivariate genetic model fitting was conducted for OCS and hoarding symptoms using Mplus in 8,144 adult twins from 5456 twin pairs from the Netherlands Twin Registry. The rates of clinically significant symptoms using previously determined thresholds were examined, as was the role of sex. Moreover, the genetic contributions to two specific hoarding symptoms, excessive acquiring and difficulty discarding were examined. Because the traits of interest were not normally distributed, threshold liability models encompassing the entire distribution of the traits were examined.

Results
6.2% of the sample met criteria for clinically significant hoarding symptoms. Men had slightly higher rates than did women (7.5 vs 6.3). 5.7% of the sample met criteria for clinically significant OCS, with no sex differences. Genetic factors accounted for approximately 40% of the variance for OCS and 29% of the variance for hoarding. Both additive and dominance genetic effects contributed to the total genetic variance for both traits; relative contributions of dominance and additive effects differed in males and females for hoarding, but not for OCS. Approximately half of the total genetic variance was shared between the traits. There was a larger genetic contribution to difficulty discarding than to excessive acquiring, although there was some evidence for a shared genetic contribution to both symptoms.

Discussion
OCS and hoarding symptoms are common in this population-based sample, are similar to previously reported prevalence rates, and have clear genetic components. In contrast to recent clinical notions, shared genetic factors contribute substantially to the etiology of both traits.

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NEUROANATOMICAL CHARACTERIZATION OF THE CELF6 RNA-BINDING PROTEIN AND ASD-RELATED BEHAVIORAL PHENOTYPING OF CELF6 KNOCKOUT MICE
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Background
Autism Spectrum Disorder (ASD) is characterized by impaired social interactions, communication deficits and resistance to change. The prevalence of ASD is four times higher in males than females. Perturbations in the serotonergic system have long been suspected in a subset of ASD cases. Platelet hyperserotonemia occurs in about 30% of ASD individuals and depletion or augmentation of serotonin signaling can worsen or alleviate certain ASD symptoms, respectively. We identified transcripts of the gene Celf6 as enriched in serotonin cells using Translating Ribosome Affinity Purification, and analysis of genome-wide association studies suggested polymorphisms in CELF6 may contribute to ASD risk. Global deletion of this gene, which codes for an RNA-binding protein, from the male mouse brain resulted in a decrease in brain serotonin levels and a partial ASD-like phenotype. Male Celf6 knockout (Celf6−/−) mice
exhibit early communicative deficits and evidence for resistance to change (Dougherty et al. J Neurosci. 2013;33:2732-53). The first aim of the current project was to clarify regions of the brain which may be responsible for the partial ASD-like behavioral phenotype of the Celf6<sup>−/−</sup> mice. The second aim was to determine if this mutation has penetrance in both sexes and to more fully characterize the deficits of these animals, with a particular focus on the serotonergic system.

**Methods**
RT-qPCR and Western blots were used to determine if Celf6 RNA and protein are present in the mouse brain at different stages of development, including embryonic, postnatal and adult time points. Immunohistochemistry was used to identify regions of Celf6 expression in the adult and developing mouse brain, using both Celf6 antibodies and transgenic Celf6-YFP expressing animals. We also employed a battery of additional behavioral assays including novel objection recognition and social odor preference, to more fully characterize the phenotype of Celf6<sup>−/−</sup> mice, in both male and female mice. A conditional Cre/lox strategy is also being employed to specifically delete Celf6 from the serotonin producing neurons.

**Results**
High levels of Celf6 protein is expressed in neuromodulatory cell populations, including the locus coeruleus, ventral tegmental area, compact part of the substantia nigra and the raphe nuclei. Distinct thalamic and hypothalamic nuclei showed moderate to high Celf6 expression, and scattered cells of the pallidum, striatum, hippocampus, and cerebral cortex expressed low levels of Celf6 protein. Similar expression patterns were observed in the postnatal day 8 mouse brain. We further identified that Celf6<sup>−/−</sup> mice (of both sexes) fail to show normal preferences for novelty or social stimulus, and we hypothesize that the ASD-like phenotypes of the global Celf6<sup>−/−</sup> mice to be recapitulated by Celf6 deletion specifically in serotonergic neurons.

**Discussion**
Our anatomical results indicate loss of Celf6 in the neuromodulatory cell populations is likely responsible for the ASD-related behaviors observed in the Celf6<sup>−/−</sup> mice. Developmental expression of Celf6 suggests this protein is present early enough in development such that deletion may affect brain organization and maturation, contributing to the ASD-like phenotype. In addition to early communication deficits and resistance to change, male and female Celf6<sup>−/−</sup> mice demonstrate a lack of preference for novelty or social stimuli normally exhibited by mice. The behavioral findings of the conditional knockout model will clarify the influence of the serotonergic system on the ASD-like phenotype of the Celf6 model.

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**A NOVEL INBRED RAT MODEL FOR GENETIC MAPPING AND DRUG TARGETS SELECTION FOR AUTISM**
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**Background**
Animal models, especially inbred rats, play an essential role in medical research, and are of particular importance for neuropsychiatric disorders, where the affected tissue (brain) is rarely
available for study in humans. We described two closely-related substrains of Wistar-Kyoto (WKY) rats, the WKY/NCrl (US) and WKY/NHsd substrains that have been used widely and interchangeably as normal control strains for rat models of disorders. Although both are stable inbred strains and highly similar, we and others have found previously that two strains differ genetically and in their behavior. They differ only in 2.5% of their genome measured by whole-genome SNP genotyping arrays, which included variations in SLC9A9 and SLC6A3, two risk genes for both ADHD and autism. The small genetic difference has caused significant phenotypic differences that are of particular interest to studies of autism and ADHD.

Methods
We thoroughly compared these two strains in a battery of neurobehavioral testing, in particular emphasis on aspects of learning and memory, social behavior, language communication and sensory processing.

Results
We found WKY/NCrl rats demonstrate less social interactions and abnormal ultrasonic vocalization in comparison with the WKY/NHsd rats. The two strains were also found significantly different in olfactory functioning.

Discussion
We believe that these two closely related inbred rat strains provide an unique opportunity for modeling autism and may help to identify causal genetic risks and novel therapeutic targets that can be readily tested for potential in future treatment of autism.

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SCREENING FOR RARE RECESSIVES IN AUTISM SPECTRUM DISORDERS
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Background
Previously, we have shown that there is a rare recessive component in autism spectrum disorders (ASD) and estimated that there is a significant contribution from rare recessives to ASD [1]. We illustrate that if there are highly-penetrant rare recessive factors, we would not be able to detect such variants, even in 5000 cases and 5000 controls as these factors would individually account for a small fraction of the disorder. References 1. Neuron. 2013 Jan 23;77(2):235-42. doi: 10.1016/j.neuron.2012.12.029. Rare complete knockouts in humans: population distribution and significant role in autism spectrum disorders. Lim ET, Raychaudhuri S, Sanders SJ, Stevens C, Sabo A, MacArthur DG, Neale BM, Kirby A, Ruderfer DM, Fromer M, Lek M, Liu L, Flannick J, Ripke S, Nagaswamy U, Muzny D, Reid JG, Hawes A, Newsham I, Wu Y, Lewis L, Din H, Gross S, Wang LS, Lin CF, Valladares O, Gabriel SB, dePristo M, Altshuler DM, Purcell SM; NHLBI Exome Sequencing Project, State MW, Boerwinkle E, Buxbaum JD, Cook EH, Gibbs RA, Schellenberg GD, Sutcliffe JS, Devlin B, Roeder K, Daly MJ.

Methods
As such, we developed a novel statistical test, RECMOD, that utilizes the deviation from the expected homozygotes to increase the power for detecting rare recessives in a polygenic
disorder.

Results
We then applied RECMOD to screen for rare recessives in ~8000 cases with ASD and controls, where the coding variants were genotyped using the Human Exome chip at the Broad Institute and Mount Sinai School of Medicine (details described in abstract by Phil H. Lee et al.). We discovered some novel rare (~0.5% allele frequency) variants that contribute risk to ASD with large effect sizes (recessive OR>1000), and while these variants were detected with highly significant RECMOD p-values of 8e-08, a Fisher’s Exact Test on the homozygous counts for these variants showed insignificant p-value of ~0.14.

Discussion
In applying RECMOD to exome chip and exome sequencing data in autism, we hope to discover more of these novel inherited factors that contribute significantly to ASD risk.

ANALYSIS OF OXYTOCIN RECEPTOR GENE AS PUTATIVE COMPONENT OF SOCIAL BEHAVIOR IN FAMILY DOGS
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Background
Social Behaviour – similarly to several other phenotypes – is a complex trait. This means that both environmental factors and numerous genetic components contribute to the development of the given phenotype. The oxytocin system is considered to have a key function in the regulation of social behavior, and probably mediate the psychosocial effects of human-animal interactions as well. The aim of the present study was to investigate whether the genetic variations of oxytocin receptor gene are associated with dogs’ socio-cognitive skills.

Methods
Polymorphic variants were identified by direct sequencing of protein coding segment and the flanking regulatory un-translated regions of oxytocin receptor (OXTR) gene. This pilot experiment was performed on 3-3 individuals of 6 different dog breeds and wolves. The identified polymorphisms were genotyped by PCR based techniques. For the characterizing the behavioral phenotype we have constructed a test series containing five different sub-tests (greeting by a stranger, separation from the owner, problem solving, threatening approach, hiding), which revealed dogs’ socio-cognitive skills when interacting with humans. The genotype-phenotype association was assessed statistically by ANOVA.

Results
Alignment of the obtained sequence segments of the OXTR gene revealed five novel (−212AG, −93TC, −73CG, −49CG, 19131AG) and three known (rs22927829, rs8679682, rs8679684) single
nucleotide polymorphisms (SNP). The identified polymorphisms were subsequently genotyped in larger populations involving 71 German Shepherds, 104 border collies, 64 retrievers, 29 beagles and 44 wolves. The results confirmed that the identified SNPs are polymorphic not only in the dog breeds, but also in wolves, however allele frequencies differed among the investigated breeds as well as between dogs and wolves. Furthermore our preliminary results suggest that oxytocin receptor gene polymorphisms have an impact on dogs’ socio-cognitive skills.

Discussion
Our results indicate that the oxytocin receptor gene is polymorphic among the different dog breeds and may contribute to the genetic background of socio-cognitive behavior in dogs. The differences of these genetic variations between dog and wolf populations might be related to domestication process of dogs.

INDUCED PLURIPOTENT STEM CELL (IPSC)-DERIVED NEURONS FROM ONE PATIENT WITH A DELETION OF THE COLLYBISTIN GENE AS A MODEL SYSTEM TO STUDY UNDERLYING CELLULAR NEUROPATHOLOGY
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Background
Collybistin (CB) is a neuron-specific guanine nucleotide exchange factor implicated in inhibitory synapse development and plasticity that cluster and localize gephyrin and GABA_A and Glycine receptors to the postsynaptic membrane. Mutations in CB gene (ARGHF9, Xq11.2) have been associated with neurological conditions such as epilepsy, mental retardation and aggressive behavior. We have recently identified a Brazilian patient with a deletion of the entire CB gene who shows severe mental retardation, epilepsy, and autistic behavior. In order to gain further insight into the underlying cellular neuropathology, we aimed to generate iPSC-derived neural progenitor cells (NPCs) and neurons from this patient and one unaffected individual.

Methods
iPSC were generated from skin fibroblasts isolated from the patient and one control subject using standard retroviral reprogramming technology. iPSC differentiation into NPCs and neurons was performed by embryoid body and neural rosette formation and under conditions that favor the generation of either excitatory or inhibitory neurons. Expression of pluripotency-, NPC- and neuron-specific markers was determined by immunocytochemistry and/or western blotting.

Results
All control and patient iPSC clones express pluripotent markers such as Nanog, Oct4, Sox2 and Lin28. We observed that NPCs were positive for early neural precursor markers, such as Nestin and Musashi1. Mature neurons were positive for neural markers β-III Tubulin and Map2; in addition, these cells also express PSD-95 and GABARγ2 proteins, markers for excitatory and inhibitory synapses respectively. Interestingly, although CB is not expressed in fibroblast, its expression is reactivated in control iPSC, and is maintained at the same high levels in control NPCs and neurons. As expected, CB expression was not observed in iPSC, NPCs and neurons
from the patient. Another important fact is that we did not detect any difference between control and patient’ cells with respect to the reprogramming and differentiation capacities. However, we observed that iPSC and NPCs derived from the patient showed increased proliferation rate compared to control iPSC and NPCs.

**Discussion**
Our results suggest that iPSC-derived NPCs and neurons from the patient carrying a deletion of the CB gene provide very promising model systems to explore the roles of CB in human neural development and physiology as well as the mechanisms underlying the cognitive impairment in CB-deficient patients.

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**TEMPORAL PERSISTENCE AND CELL-TYPE SPECIFICITY OF COMMUNICATION DEFICITS IN CELF6 KNOCKOUT MICE.**
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**Background**
Autism spectrum disorders (ASD) have long been recognized to have a highly heritable component and the last decade has witnessed the identification of many risk alleles, often rare or de novo variants. With such complexity, it has been hypothesized that the genesis of ASD lies in the convergence of many of these genes onto similar pathways and systems. One such system is the serotonergic system, long suspected in ASD. Our lab profiled the serotonin-producing neurons of the raphe nuclei using Translating Ribosome Affinity Purification and found a number of genes with enriched expression in these neurons. Polymorphisms in one such gene, CELF6, were found to be associated with ASD risk in data from the AGRE Consortium. Global disruption of Celf6 in mice resulted in expected loss of expression of the protein from raphe neurons, reduced levels of brain serotonin, behavior resembling resistance to change, and early communication deficits as measured by levels of isolation-induced ultrasonic vocalization (Dougherty et al. J Neurosci. 2013;33:2732-53). In the current investigation, we asked whether the early communication deficits were reproducible in a new cohort of animals, and whether this deficit was also present in juvenile social encounters. We also asked whether specific loss of Celf6 protein in raphe neurons was sufficient to bring about the early communication deficits.

**Methods**
Our replication cohort of animals consists of 15 Celf6⁻/⁻ and Celf6⁺/⁺ littermate controls of both sexes, all animals on a C57Bl6/J background. For the cell-specific study, we crossed our Celf6 fl/fl line (with loxP-flanked exon 4 of Celf6) to Pet1-Cre animals, in which expression of Cre recombinase is driven by the promoter for the Pet1 transcription factor. For early communication, ultrasonic vocalization is monitored after maternal isolation on post-natal day 8 (P8). For juvenile social encounters, ultrasonic vocalization is monitored in dyads at post-natal day 25-30.

**Results**
We expect that the replication cohort of Celf6⁻/⁻ animals will reproduce the findings of our previous study. We hypothesize that the deficit will also manifest during juvenile social encounters. We hypothesize that specific loss of Celf6 protein to the serotonin producing neurons
will be sufficient to drive expression of the early communication deficit.

**Discussion**
While the number of global models of ASD risk genes continues to grow, the number of such models that investigate their role in a cell-specific manner remains few. To our knowledge, our study is the first to investigate the contribution of an ASD risk to early communication by cell-specific disruption in serotonergic neurons. Furthermore, our study explores the question of vocal communication in young, socially interacting animals, an area rarely investigated in other genetic models of ASD and one which has potentially greater relevance to the expression of an autistic phenotype.

**ACUTE PRENATAL EXPOSURE TO A MODERATE DOSE OF VALPROIC ACID INCREASES SOCIAL BEHAVIOR AND ALTERS GENE EXPRESSION IN RATS**

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**Background**
Prenatal exposure to moderate doses of valproic acid (VPA) produces brainstem abnormalities, while higher doses of this teratogen elicit social deficits in the rat. In this pilot study, we examined effects of a moderate-dose of VPA on behavior and on transcriptomic expression in three brain regions mediating social behavior.

**Methods**
Pregnant Long-Evans rats were injected with 350 mg/kg VPA or saline on gestational day 13. A modified social-interaction test was used to assess social behavior and social preference/avoidance during early and late adolescence and in adulthood. RNA from three brain regions was isolated from adult animals and used for transcriptomic analysis.

**Results**
VPA-exposed animals demonstrated more social investigation and play fighting than control animals. Social investigation, play fighting, and contact behavior also differed as a function of age; the frequency of these behaviors increased in late adolescence. Social preference and locomotor activity under social circumstances were unaffected by treatment or age. Thus, a moderate prenatal dose of VPA produced behavioral alterations that are substantially different from the outcomes that occur following exposure to a higher dose. At adulthood, VPA-exposed subjects exhibited transcriptomic abnormalities in three brain regions: anterior amygdala, cerebellar vermis, and orbitofrontal cortex.

**Discussion**
A common feature among the proteins encoded by the dysregulated genes was their ability to be modulated by acetylation. Analysis of the expression of individual exons also revealed that genes involved in post-translational modification and epigenetic regulation had particular isoforms that were ubiquitously dysregulated across brain regions. Social enhancement seen in VPA-exposed
animals may indicate that differentially expressed genes or exons act as a compensatory mechanism, working to offset the effects of VPA.

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THE POWER COMPARISON OF THE HAPLOTYPE-BASED RARE VARARANTS APPROACH AND OTHER TRANSMISSION BASED COLLAPSING METHODS IN A FAMILY DESIGN AND THEIR APPLICATIONS

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Background

It has been known that both common and rare variants contribute to complex disease etiology. Recent genome-wide association studies (GWAS) have been successfully applied to the investigation effects of common variants in development of some common diseases. However, it is still a challenge to identify the impact of rare variants, which are abundant in human population as the development in next-generation sequencing technologies. While a number of statistical tests have already been developed to analyze collapsed rare variants identified by association tests in last few years, the family-based sequence association tests received less attention. Therefore, we aim to compare our newly developed haplotype-based pedigree PDT best with others including PDT-rare, FBAT-rare and PB-STAR.

Methods

Let \( N \) be the total number of families. Let \( M \) be the total number of variants in the gene of interest, and haplotypes formed are denoted by \( h \). For the family \( i \), let be the number of informative nuclear families and be the number of informative discordant sibships. As the PDT test, the haplotype based PDT test considers the difference in the numbers of the transmitted and untransmitted haplotypes from parents to affected siblings and the difference in the numbers of haplotypes between affected and unaffected siblings. Then for \( k \)-th haplotype, the hPDT statistic for a family is defined as where \( h \) is the number of transmissions minus the number of nontransmissions on the \( h \) th haplotype in trio \( j \) and is the number of copies in the affected sib minus the number of copies in the unaffected sib in sib-pair \( j \). Without loss of generality, assume is the most frequent haplotype, and for the rest of haplotypes, the hPDT statistic for the family \( i \) is defined as , where is the weight for the haplotype \( k \). We define a similar weight function as in Madsen et al.; , where is the number of individuals in \( N \) families and is the haplotype frequency of the \( k \)-th haplotype. For \( N \) unrelated families, the hPDT statistic is defined as, The other methods compared including FBAT-rare, PDT-rare, and PB-STAR. The detailed methods are omitted here.

Results

Simulationed, in general, the IBD-based methods such as PB-STAR outperform all other methods that are transmission based.

However, among the transmission test, haplotype-based PDT and FBAR-rare are more powerful than the PDT-rare. A real data set with phenotypes of bordingle personality disorders was download from dbgap was analyzed by aboved compared methods. PB-STAR tend to give more significant p-values when the traits were dicotimized.
Discussion
There is a need to investigate power performance of approaches proposed to perform family-based rare variants analysis.

It is interesting to see that, using simulation-based methods, the PB-STAR, with a statistical core developed by Shugart et al and Zhu and Xiong, performed well under a variety of models investigated. However, it is not clear how these approaches will perform in the presence of population admixture. This will involve more realistic simulations which reflect a mixed population such as Latios and African Americans. The authors will move towards that direction though more extensive collaborations and will put more efforts on analyzing sequencing data with pedigree structure and with mixed population background.

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BAYESIAN POLYGENIC RISK PREDICTION OF SCHIZOPHRENIA USING SUMMARY STATISTICS
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Background
Despite the success of GWAS, significantly associated loci generally explain only a small fraction of total heritability and provide poor predictive accuracy. Polygenic scores using less stringent P-value thresholds do capture more heritability (Purcell et al., 2009), and allow us to gain insight into the complex genetic architecture of schizophrenia. However, as we will show here, for the purpose of risk prediction they are suboptimal under realistic genetic architectures where a given fraction of the markers are causal.

Methods
Here, we propose a computationally efficient method for Bayesian polygenic risk score prediction that uses GWAS summary statistics as training data. This avoids both logistical and computational difficulties associated with analysis of large genotype datasets. Our method adjusts the estimated marker effects by explicitly modeling the underlying distribution of causal effect as a Gaussian mixture. This results in a nonlinear Bayesian shrink, generalizing the standard thresholding with LD-pruning approach. Furthermore, our model accounts for linkage disequilibrium (LD) on indirect associations at non-causal markers and the effects of sampling noise. We also derive an efficient approximate Bayesian shrink that has linear running time, GoLD (Gaussian posterior mean with LD). Notably, GoLD assumes that truly causal markers are unlinked and does therefore not require any LD information from a reference panel.

Results
We compare our approach to the standard thresholding approach using both simulated and real disease datasets. For reasonable genetic architectures (where 1% of all SNPs are causal) and training sample sizes (20,000 individuals), our Bayesian approach significantly improves the prediction accuracy (as measured by squared correlation). Finally, we apply our methods to a schizophrenia dataset (Ripke et al., 2011) and observe a modest improvement in prediction accuracy when compared to thresholding with LD-pruning.
**Discussion**

The Bayesian polygenic risk score proposed here only requires GWAS summary statistics as training data, which allows for very large training sample sizes. This is important because large sample sizes are likely necessary for schizophrenia risk prediction accuracy to near the limit set by the heritability (Chatterjee et al., 2013). In addition, the Bayesian model allows for the inference of interesting parameters, such as the fraction of markers with causal effects, and further our understanding of the genetic architecture of schizophrenia.

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**A NOVEL HEAT SHOCK PROTEIN 8 MOLECULAR NETWORK MEDIATING RESPONSES TO STRESS AND ETHANOL-RELATED BEHAVIORS**

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**Background**

Genetics play a major role in determining an individual’s susceptibility to stress as well as responses to alcohol and other drugs of abuse. However, to date, the interplay among these factors remains poorly understood. The hippocampus is particularly vulnerable to the actions of stress and ethanol, and therefore, we examined this brain region across the highly variable genetic backgrounds of BXD mice using bioinformatic analyses. Hspa8 is an ethanol-responsive gene that encodes for the molecular chaperone heat shock cognate (Hsc) 70, which belongs to the heat shock family of proteins and is well known for its integral role in the stress response. Alterations in expression of Hspa8 are also seen following exposure to other drugs of abuse and the well-known relationship between substance abuse and stress suggests Hspa8 functions in a similar and/or overlapping molecular pathways. In this study we determined genetically variable regions of DNA that regulate Hspa8 expression, identified candidate genes at these loci that co-vary and correlate with the differential expression of Hspa8, and constructed a novel genetic network containing these genes of interest.

**Methods**

We analyzed the “Hippocampus Consortium M430v2 (Jun06) RMA” database found at www.genenetwork.org and conducted correlational and QTL analyses using strict criteria. Upstream candidate gene identification: 1) expression level over 7.0, 2) significant (p<.05) correlation with Hspa8 expression in the hippocampus, 3) probe hybridization in coding exons or the 3’UTR, and 4) non-synonymous sequence polymorphisms in coding regions of the gene. Downstream candidate gene identification: 1) gene expression modulated by the Hspa8 locus on chromosome 9, 2) expression >7.0, 3) Probe hybridization in coding exons or 3’UTR, and 4) significant correlation (p<.05) with Hspa8 expression. Network gene identification: 1) expression >7.0, 2) significant correlation (p>.05) with Hspa8 expression, and 3) literature correlation (r>.5). Gene ontology analysis determined over-represented functional classifications. Highly correlated phenotypes: We searched the phenotypic database for all traits with a Reference in to Function (RIF) containing stress or alcohol and focused our analysis on behavioral traits that were significantly correlated (p<.05) with Hspa8 expression in the hippocampus.
Results
There are three probe sets (1420623_x_at, 1420622_x_at, and 1455789_x_at) that target exons of the Hspa8 gene and 1.4-fold difference in Hspa8 transcript abundance exists between the highest and lowest values across the BXD lines. We conducted a principal component analysis (PCA) and determined the first principal component from the PCA accounts for the majority (~75%) of the variation in expression of all three probe sets. PCA interval mapping showed a significant (p<0.05) peak LRS value of 23 on chromosome 14 between 43 and 46 megabases (Mb). Our specified criteria facilitated the identification of 4 upstream candidate genes (Wdhd1, Samd4, Ktn1, and Dlgap5), four downstream candidate genes (Psme1, Vpreb3, Pdcl and Tpr), an additional 241 Hspa8 network genes, and 51 stress and/or ethanol-related phenotypes. Gene ontology analysis classifications included 26 network genes involved in unfolded protein binding (adjP=3.86E-28) and 30 involved in protein folding (adjP=1.56E-26). Two phenotypes map to the locus on chromosome 14 containing our upstream candidate and measured locomotor activity: 1) control mice 2) differences following exposure to ethanol. An additional five traits mapped to the Hspa8 locus and two measured locomotor activity: 1) baseline 2) difference following exposure to methamphetamine.

Discussion
Hspa8 is involved in a very extensive and intricate genetic network that is associated with many different stress and/or ethanol related behaviors. We hypothesize that Hspa8 mediates genetic differences in responses to stress or ethanol and plays a critical role in initiating and coordinating the appropriate responses to environmental factors.

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COMPARISON OF METHODS FOR GENOME-WIDE GENE-ENVIRONMENT INTERACTION ANALYSIS
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Background
Gene-environment interaction may be an important source of complexity to the disease etiology of psychiatric disorders and a contributing factor to the so-called hidden heritability. Nevertheless, so far only few findings have been reported, possibly due to at least two complicating factors: the need of large samples in concert with information on the environmental exposure. The comprehensive Danish study, The Initiative for Integrative Psychiatric Research, iPSYCH, will include such information drawing partly on the Danish registers and partly on the ability to extract information from neonatal dried blood spots about exposures to the fetus. The number of methods and software available for gene-environmental interaction analysis is overwhelming and there is no clear winner or gold standard. As a means of guiding decisions we therefore set up the present simulation study to compare some of the most frequently used methods with other methods, from multistep regression analyses to machine learning.

Methods
Data was simulated using a combination of Python modules: simuPOP, which is a very general
forward-time simulator, and the *simuPOP* module Gene-Environment iNteraction Simulator 2 (*GENS2*). Individual-based genetic data was generated allowing for interactions between up to two predisposing genetic markers and one environmental factor and with the possibility to include epistasis. There are no limits on the number of subjects or non-predisposing genetic and environmental factors except from hardware restrictions. A range of scenarios were chosen by varying minor allele frequencies, sample sizes and effect sizes with the intention to compare methods of the following kind: two-step analysis, multifactor dimensionality reduction (MDR), logic regression, random forests, artificial neural networks, genetic programmed neural networks. The methods are going to be compared and characterized using misclassification rate, cross validation accuracy, agreement on predictive factors, power to detect specific effects, maintenance of type I error rate, and measures of variable importance.

**Results**
We are currently generating the simulated data and setting analyses up to be run on a large cluster computing facility.

**Discussion**
Complicated problems tend to yield complicated answers and is not straight forward to compare complex approaches like machine learning methods where the performance not only depend on the often complicated problems they are applied to, but also to some extent depends on the user’s ability to tune parameters of the algorithms. Therefore, when applying machine learning methods in practice several different methods, algorithms and/or sets of parameters are often used. Our aim therefore is to characterize the performance of selected methods in a wide range of standardized scenarios to facilitate an informed choice of methods.

### POLYGENIC RISK SCORE ASSOCIATIONS WITH PSYCHIATRIC TRAITS MAY BE IMPROVED WITH SIMPLE PROCEDURES
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**Background**
Polygenic risk scores are useful tools to investigate the genetic architecture of psychiatric disease, but their utility and interpretability are likely limited by heterogeneity in both local and global ancestry. It has become standard practice to adjust for global ancestry via principle components or similar ancestry summary statistics when assessing associations between risk scores and disease. Little work, however, has been dedicated to the residual influence of local ancestry.

**Methods**
We simulate independent genetic variants with surrounding correlated SNPs to create two genetically distinct populations. Some variants are reserved for the creation of a score, while others are reserved to create principal components. Admixture is introduced in the second population from the first donor population. The disease is modeled under the null and various alternatives for each SNP, controlling the overall prevalence in each population. Pruning is carried out on the pooled data, and a polygenic risk score is summed across the set of pruned SNPs. The association of the score with disease, adjusting for principal components, is
determined for each iteration. Type I error and power is then calculating by summarizing across many iterations.

**Results**
Through simulation we show that in situations of admixture, adjustment for global ancestry in genetic risk score analysis does not fully correct for confounding by ancestry and may also limit interpretation of the score itself.

**Discussion**
We will further present and compare strategies for addressing this residual confounding via other methods of global ancestry adjustment and using estimates of local ancestry as adjustment factors in the score calculation. Our results are relevant for psychiatric genetic studies wishing to associate polygenic risk scores in populations with admixture, possibly improving type I error and/or power.

PROBING PSYCHOLOGICAL STRATA USING COMORBID ENDOPHENOTYPES IN ANOREXIA NERVOSA
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**Background**
Anorexia nervosa (AN) is a psychiatric disorder marked by extremely low body weight and intense fear of gaining weight. AN cases are categorized into restrictive (ANR) and binge-purge (ANBP) types. A number of personality features are common in AN sufferers, including perfectionism, obsessionality, anxiety, harm avoidance and low self esteem[8]. As well as these behavioural endophenotypes, studies have consistently shown a high rate of comorbidity with other DSM-IV (Diagnostic and Statistical Manual of Mental Disorders, 4th Edition) disorders; a study of nearly 10,000 participants places this estimate as high as 56.2%. The most common comorbid psychiatric disorders include depressive and anxiety disorders. Many of the previous studies into disorders comorbid with AN are small, and examine only a few traits. Further, many of the studies rely on self-reported statistics, rather than clinical diagnoses. We have access to clinically verified endophenotype data from 3,645 cases, obtained by the Wellcome Trust Case Control Consortium 3 (WTCCC3) for use in an AN genome-wide association study. The samples are taken from both discovery and replication data sets, and are taken from 15 different collection centres. We have used these data to compile information on the prevalence of psychiatric diseases among AN sufferers. We have tested the prevalence of 47 different endophenotypes, which consist of a range of anxiety disorders, compulsive disorders, depressive disorders and substance abuse disorders.

**Methods**
All samples included were diagnosed with AN by a clinician. All phenotype information was measured as either absent-0, or present-1. Samples with ‘sub-clinical’ diagnoses of a disorder were not included in prevalence calculations. Lifetime instances and current instances of a phenotype were merged. Equivalent phenotype definitions were merged. We ensured that no systematic bias was introduced due to the samples being collated across a number of populations.
This bias posed a real risk, as sample collection and phenotype recording was done separately for each population. We calculated the Pearson correlation between phenotype prevalence rates between each population. We found an average correlation of $r=0.79$ between each population and the merged population (where $r=1.0$ is a perfect correlation), and an average correlation of $r=0.62$ between populations. We performed cluster analysis on 991 samples that had passed the Sample QC. These 991 samples were taken from six populations: Boston, Canada, Leiden, Norway, Paris and NIMH.

**Results**
We have assessed the prevalence of each disorder, compared for ANR, ANBP, AN-purge only (ANP) and AN-binge only (ANB). We found a significant difference ($p<0.01$) in the prevalence of 11 disorders between ANR and ANBP. Further, we investigated mean TCI (temperament and character inventory) scores, including harm avoidance, novelty seeking, etc, stratified by ANR, ANBP and ANP. Although samples were obtained from a wide range of collection centres, we saw no systematic difference in the prevalence of comorbid disorders between populations. A key benefit of our data is that we have a wide range of phenotype information for each sample. We used these data to perform a principal components analysis (PCA) on 991 samples. We selected specifically samples that had information on at least 11/15 key endophenotypes. Five of these endophenotypes were related to substance abuse, and ten were diagnosed disorders such as Major Depressive Disorder (MDD), Obsessive-Compulsive Disorder (OCD), Bipolar 1 (BP1), etc. We noted the formation of distinct clusters of samples and calculated the prevalence of each endophenotype in each cluster. We found that PC1 vs. PC2 produced two clusters. Cluster one comprised 376 samples, none of which was diagnosed with MDD, but displayed an increased prevalence of BP1 and dysthymia compared to cluster 2. PC2 vs. PC3 produced three clusters; the first included samples with MDD, but no cases of OCD; the second included samples with both MDD and OCD, and the third had samples with OCD but no instances of MDD. We found no principal components that were stratified on the basis of substance abuse.

**Discussion**
These results suggest that meaningful clinical subgroups (restricting/binge-purge type distinction) may exist in individuals with anorexia nervosa demarcated by the presence of BP1 and dysthymia, and MDD only, OCD only and MDD plus OCD. Any large-scale genetic analysis of psychiatric disorders is likely to be complicated by a large amount of heterogeneity of the disorder. This is likely to reduce power both within a genetic study of AN, and also in any cross-disorder mega-analyses that may be carried out. We suggest that stratifying these analyses by assessing the comorbidity of samples may help to create more homogeneous groups, and may increase the power of the study.

**44 MELATONIN RECEPTOR LOCUS ASSOCIATES WITH CIRCADIAN STRESS - GENOME-WIDE STUDY AND THREE REPLICATIONS**
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Background
Shift work and irregular work create misalignment between inner clock and behavioral time which can cause sleep loss and lead to negative health consequences. The risk for circadian misalignment and sleep deprivation is especially high in certain occupations, such as aircrew who typically encounter time-zone transitions and have irregular work-rest schedules. However, inter-individual variability in response to shift work is large. Part of it can be explained with trait-like differences such as genetic factors.

Methods
We performed genome-wide association study (GWAS) with the Illumina 610K platform to explore genetic variants which associate with job-related exhaustion among shift workers using a three-stage protocol. In the first stage of the study, GWAS was performed in 179 shift workers from a Finnish population-based Health 2000 study (Gemmets sample). The initial replication analysis was performed for the best signals in shift workers the same cohort (n=241), and based on that, one variant was further studied in an occupational cohort of shift workers from the Finnish airline company sample (n=263) including aircrew (N=263) and non-flight workers (n=341) and in an occupational sample of shift-working nurses (n=73). Job-related exhaustion was assessed using the exhaustion subscale of the Finnish version of the MBI-GS. The exhaustion score was logarithmically transformed in order to follow normal distribution and then treated as a quantitative trait. Z-score normalization was performed for all the analyses The association between the genotype data and the exhaustion score was tested using the linear regression analysis of the PLINK software, additive model, with age and sex as covariates. A fixed-effect meta-analysis across all the study samples was performed by using the GWAMA software tool.

Results
The initial genome-wide association analysis in shift workers from the Gemmets sample of the H2000 study revealed suggestive association of five variants from four genomic loci to occupational exhaustion (P < 1x10^-5). Three variants were then examined among the shift workers from the rest of the Health 2000 cohort, out of which one showed association (P<0.05). The strongest signal in the Health 2000 analyses, coming from a variant close to the melatonin receptor locus, was replicated in the aircrew of the airline cohort (P<0.05), but not in the shift working non-flight workers. Comparable effect size was seen also in the small nurse cohort. When restricting the airline sample to aircrew, the meta-analysis of all the cohorts reached genome-wide significance (P<5x10^-08).

Discussion
To our knowledge, this is the first hypothesis-free genetic study exploring liability to emotional burnout related to shift work. The results suggest that genetic factors affecting inter-individual differences to tolerance to circadian stress can be identified, and at least some of them are linked to melatonin signaling.
PSYCHOMETRIC AND STATISTICAL PROPERTIES OF PERFORMANCE-BASED COGNITIVE AND FUNCTIONAL PHENOTYPES IN SEVERE MENTAL ILLNESS: IMPLICATIONS FOR GENOMIC APPROACHES

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Background
Cognitive abilities and impairments in everyday functioning appear to be heritable in families with severe mental illness. Although neurocognition is commonly described in terms of different functional domains, some factor analytic studies have suggested a simpler dimensional structure for neuropsychological (NP) tests in patients with schizophrenia. Standardized tasks of everyday functioning, or tests of “functional capacity” (FC), are viewed differently from traditional NP tests. However, FC and NP tests have been found to be highly correlated to the extent to which genetic influences on them may be difficult to separate. In fact, a recent study of ours suggested that performances on these different types of tasks constituted a single latent trait in across-sectional analysis. In this presentation we present longitudinal data on that same sample and also a replication of those findings in a larger sample of patients with schizophrenia and bipolar illness.

Methods
Sample 1 (VALERO): Patients with schizophrenia (n=195) were examined at two assessment occasions separated by periods ranging from 6 weeks to 6 months. Participants were assessed with the MATRICS Consensus Cognitive Battery (MCCB) and two performance-based assessments of functional capacity.
Sample 2 (FUNCAP). Patients with schizophrenia and bipolar illness, assessed once with the same two performance-based indices and NP tests that are essentially identical to the MCCB.

Results
The cross-sectional analyses of the FUNCAP sample suggested that a single factor solution was the best fit to the data. When two-factor and one-factor solutions were compared, they were essentially identical in fit. The same unifactorial model fit the data similarly, with the bipolar patients having better performance and an identical factor structure. The longitudinal analysis of the VALERO data, where a unifactorial model fit the data at baseline found that a single latent trait was extracted using full information maximum likelihood procedures, and its temporal stability was examined in terms of: stability of the latent trait scores, the intercorrelations of the three indicators of the latent trait, and the stability of loadings for the FC and NP items underlying the latent trait at the two measurement occasions. All indices of temporal stability were confirmed, with stability not related to follow-up duration.

Discussion
These findings raise the question of whether cognitive abilities measured by NP tests and FC instruments are tapping a single ability construct, which might have shared causal influences as well. Bipolar and schizophrenia patients' performance was very similar in terms of factor structure.
REWARD-RELATED VENTRAL STRIATUM REACTIVITY AND IMPULSIVITY MEDIATE THE EFFECTS OF A PRODYNORPHIN HAPLOTYPE ON ALCOHOL USE IN AN ASIAN-AMERICAN SAMPLE

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Background
The kappa opioid system is critical for the rewarding properties of alcohol and plays a role in responsiveness to stress. Dynorphin is the primary endogenous ligand for the kappa-opioid receptor. A functional haplotype (rs2235749, rs910079, rs910080) within the gene coding for its precursor, prodynorphin (PDYN), has been associated with altered PDYN expression in the ventral striatum and substance use disorders. Specifically, the CCT haplotype is characterized by reduced mRNA and increased vulnerability to substance use.¹ Moreover, both rs2235749 and rs910080 have been independently linked to increased risk for alcohol use disorders² and the risk allele of rs2235749 eliminates a methylation site.³ Kappa opioid signaling may affect the rewarding properties of drugs through its impact on dopamine and ventral striatum reactivity to reward. Dynorphin-related binding inhibits dopamine release; as such the reduced levels of prodynorphin conferred by the PDYN CCT haplotype may result in less dynorphin availability in the ventral striatum and hence less inhibition of dopamine. Moreover, the kappa opioid receptor is the only opioid receptor expressed within the paraventricular nucleus (PVN), the central regulator of the body’s stress response through the hypothalamic-pituitary-adrenal axis⁴ and kappa-opioid knockout mice do not show stress-induced drug reinstatement.⁵ Collectively these data suggest that the functional PDYN haplotype may influence alcohol use via effects on ventral striatum reactivity to reward that may differ in the context of stress exposure.


Methods
Genetic and neuroimaging data were available from 100 Asian-American participants who completed the Duke Neurogenetics Study, an ongoing protocol assessing a wide range of behavioral and biological phenotypes among young adult volunteers. Only Asian-Americans were included due to the elevated frequency of the CCT haplotype in this population. Using MPLus 7.1, we tested a moderated mediation pathway model in which an interaction between PDYN Haplotype (genotyped on a GWAS array through 23andMe.com) and early life adversity (as measured by the childhood trauma questionnaire) predicts reward-related ventral striatum reactivity, which in turn predicts impulsivity (as assessed by the NEO Neuroticism subscale) and alcohol use (as measured by the Alcohol Use Disorders Identification Test). Covariates included age, non-alcohol use disorders (assessed via clinical interview), and ALDH2 genotype which is prevalent in Asian-American populations and has been linked to alcohol metabolism. Indirect effects were considered significant if bootstrapped 95% confidence intervals did not contain 0.
Results

PDYN haplotype indirectly predicted alcohol use via early life stress moderational effects on reward-related ventral striatum reactivity and impulsivity (99% Boot-strapped CI for effect size: [0.030, 0.672]). Follow-up analyses revealed that in the context of elevated early life adversity, the CCT haplotype predicted blunted ventral striatum reactivity to reward, which then predicted increased impulsivity, which then predicted increased measures of problem drinking. Notably the direct path from PDYN genotype × CTQ to AUDIT scores, while significant in a simple moderation model (p=0.03), was no longer significant in the full moderated mediation model (p=0.21). This same model did not significantly predict any mood or anxiety disorders.

Discussion

The results of this study suggest that PDYN haplotype indirectly effects alcohol use through its effects on ventral striatal reactivity to reward and impulsivity in the context of stress exposure. Previous literature suggests that this may be due to impaired regulation of dopaminergic activity by kappa-opioids in response to stress. Importantly, this study is limited by its small sample size which may inflate coefficients in our path model. It will be important for future research to replicate the described model in other samples and to experimentally manipulate prodynorphin levels in non-human animals. Additionally, the sample consisted of a relatively healthy sample of young Asian-Americans; it will be important for further research to examine whether this model predicts clinically-significant levels of use and is generalizable to other populations.

ATTENUATED FLUSH RESPONSE TO NIAÇIN SKIN PATCH IN SCHIZOPHRENIA PATIENTS: RESPONSE PATTERN FROM ACUTE ADMISSION TO DISCHARGE AND ITS RELATION TO PERIPHERAL BLOOD FATTY ACIDS PROFILE

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Background

Attenuated niacin-induced flush response has been consistently observed in schizophrenia patients as well as their non-psychotic relatives. A possible mechanism underlying the attenuated niacin-induced flush has been postulated to be due to certain impairment in the prostaglandin-related microvasodilation pathway where arachidonic acid and phospholipase A2 play major roles. However, previous studies on the correlations between niacin flush response and these biochemical indexes have been inconsistent. In this study, we aimed to exam whether attenuated niacin-induced flush response is a stable trait or state-dependent marker in schizophrenia patients by comparing their niacin response in acute phase with that in remission phase. Furthermore, we applied cluster analysis using niacin response ratings in different concentration and time points
to distinguish non-flushers from flushers and explore each cluster’s relationships to fatty acids profile.

Methods
In this study, 47 schizophrenia inpatients were recruited from the acute wards of National Taiwan University Hospital and Taipei City Psychiatric Center, and were followed up for 2 months or until they were discharged from acute wards. All the measurements and blood drawings were performed both at their acute phase (baseline) and remission phase (2-month follow-up). Niacin skin tests of three different concentrations (0.1M, 0.01M and 0.001M) were conducted and a 4-point scale (0, 1, 2, and 3) was used to measure the flush response to niacin skin tests. Controls consisted of 37 frequency-matched volunteers who had similar distribution in age and sex to schizophrenia patients. The controls underwent similar niacin skin patch test and blood drawing both at baseline and 2 months later. The fatty acid composition of red blood cells for all participants were measured using gas chromatography. We used paired t test to detect the scores changes and conditional logistic regression to measure the difference of prevalence of non-flushers between baseline and two-month follow up. Then, we conducted cluster analysis using the niacin flush scores of schizophrenia patients to search for homogeneous subgroups and their relations to fatty acids profile were investigated as well.

Results
Both in baseline and 2-month follow up, the mean flush scores of all concentration and time points (except in 0.001 M, 5 min of baseline) were significantly lower in schizophrenia group compared to control group. There were no significantly changes in the niacin flush scores between baseline and 2-month follow up for schizophrenia patients, whereas the niacin flush scores (0.1M, 5min; 0.01M, 5min and 10 min and 0.001M, 5min and 10 min) in the control group increased in the 2-month follow up (a mean increase of 0.2 to 0.4 rating score). A cluster analysis in schizophrenia patients using 5 concentration-time niacin ratings at baseline revealed two clusters, one cluster (n = 18) with consistently non-flushness across the five ratings and the other cluster (n = 29) with flushness in the majority of the five ratings. When the fatty acids profile at baseline of the non-flush cluster and the flush cluster, respectively, was compared to that of the controls, seven fatty acid components were selectively alternated in the non-flush cluster of schizophrenia patients only, including higher PUFA (C18:3, n-6), MUFA (C18:1, n-9; C20:1, n-9; C16:1, n-7) and SFA (C22:0), as well as lower MUFA (C24:1, n-9) and SFA (C18:0) than the controls. The same cluster group membership was then used to examine their relationship to the fatty acids profile at the 2-month follow up. Among the seven differentially alternated fatty acids for the non-flush cluster at baseline, the increase in PUFA (C18:3, n-6) was replicated at the 2-month follow-up.

Discussion
In this study, we showed that the attenuated skin flush to niacin in schizophrenia remained at 2-months follow up, whereas the control group exhibited an increase in flush response for the same length of follow-up. One postulation for this might be that healthy controls have immune memory to niacin challenge, therefore, becoming easily be induced for the flush response, whereas such immune memory was impaired in schizophrenia patients. Aberrant fatty acids composition of RBC observed in the non-flush cluster of schizophrenia patients might be accounted for in part by a higher phospholipase a2 activity, as indicated from previous studies on
the relations of phospholipase a2 activity to fatty acids in schizophrenia patients. Whether the increase in fatty acid C18:3, n-6 in the non-flush cluster of schizophrenia patients shed some light on this postulated pathophysiological pathway warrants further investigation.

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EPIGENETIC REGULATION OF THE BDNF GENE IN MAJOR DEPRESSIVE DISORDER AND BIPOLAR AFFECTIVE DISORDER

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Background

The brain-derived neurotrophic factor (BDNF) has been implicated in the molecular mechanisms of the aetiology for affective disorders including major depression (MDD) and Bipolar Disorder (BD), among others. In recent studies, the DNA methylation level of the BDNF exon I promoter was determined in peripheral mononuclear blood cells (PBMCs) and negatively correlated with gene expression, indicating regulatory mechanisms on BDNF levels [1]. Further, a classification based on the DNA methylation status of the BDNF exon I promoter was significantly associated to the clinical diagnosis of MDD compared to healthy controls [2]. In the present study, we aimed to determine the methylation level at the BDNF exon I promoter in order to analyse if the BDNF methylation status is correlated with the clinical diagnosis of MDD in patients with affective disorders.

Methods

Overall, 563 subjects were available for this study. The clinical diagnosis was based on DSM-IV criteria from at least two independent psychiatrists. In order to determine the DNA methylation level at BDNF exon I promoter, genomic DNA from PBMCs was extracted and bisulfite conversion was performed. The percentage of methylated reference (PMR) was calculated based on results from quantitative real-time PCR following the MethyLight protocol. The results are displayed as mean ± standard error of the mean (SEM) of the PMR value. We performed analyses of groups based on clinical diagnosis and further sub-analyses of the MDD group based on treatment status. For statistical analysis one-way ANOVA followed by Bartlett’s test, and unpaired t-test were performed.

Results

Our sample consisted of 278 healthy controls and 207 subjects with a diagnosis of MDD as well as 59 subjects with a diagnosis of BD. In the analysis performed for the 3 groups based on clinical diagnosis, our results show a significantly increased PMR in MDD subjects (3.58±0.23%) but not in BD subjects (2.36±0.32%) compared with healthy controls (2.01±0.13%); p<0.0001 Bartlett’s test; ANOVA: p=0.0001; F=20.61. In a further sub-analysis of the MDD sample, a significantly increased PMR in subjects under current therapy with antidepressants (AD: 4.13±0.28%; n=140) compared to subjects without antidepressants (No-AD: 1.72±0.28%; n=19) was found; p=0.0019.

Discussion

We show that DNA methylation levels at the BDNF exon I promoter in MDD subjects are
significantly increased compared to BD subjects and healthy controls, indicating an aberrant regulation of the BDNF gene associated with the clinical diagnosis of MDD. Our results are in line with the literature postulating a relation of increased DNA methylation levels at BDNF exon I promoter with the clinical diagnosis of MDD. In a second analysis of the MDD subjects the DNA methylation levels at the BDNF exon I promoter were significantly increased in subjects under therapy with antidepressants. Without doubt, further studies in larger well-defined samples should be performed in order to dissect the influence of DNA methylation of the BDNF gene and its correlation to the clinical diagnosis of MDD.

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GENETIC EVIDENCE FOR A ROLE OF THE MLL3/MLL4 HISTONE H3K4 METHYLTRANSFERASE COMPLEXES IN BIPOLAR DISORDER.

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Background
Studies of epigenetics have suggested a prominent role for chromatin regulation in the aetiologies of the major mental disorders schizophrenia (SCZ), bipolar disorder (BPD), and major depressive disorder (MDD), as evidenced by differential epigenetic profiles in peripheral blood samples or post-mortem brain samples of patients. Given that epigenetic studies of mental disorders are hampered by limited access to the brain, and the existence of many methodological difficulties such as tissue-specificity of observed findings, time-dependent changes, confounding by medication and low power, we implemented an alternative strategy aimed at identifying genetic evidence for the involvement of chromatin regulation (CR) in these disorders.

Methods
We investigated whether variants in CR genes show associations with SCZ, BPD and MDD by using the publicly available Psychiatric GWAS Consortium dataset (pgc1project; incorporating 9,394 patients and 12,462 controls for the SCZ analysis, 7,841 patients and 9,250 controls for the BPD analysis, and 9,240 patients and 9,519 controls for the MDD analysis). The analysis focussed on the involvement of single nucleotide polymorphisms (SNPs) in a set of 473 genes known to regulate chromatin function in SCZ, BPD and MDD. The following criteria were used for inclusion in the set of chromatin regulatory genes (CR): the presence of a known chromatin interaction domain, presence of a known histone modification domain (HAT, HDAC, KMT, KDM or PRMT) or biochemical evidence of presence in a known chromatin regulatory complex. We calculated empirical p-values for the combined set of CR genes for the three disease outcomes by using permutation procedures (100,000 permutations per analysis).

Results
The set of CR genes was significantly associated with BPD (p = 0.049), showed a trend towards statistical significance for SCZ (p = 0.058) but was not associated with MDD (p = 0.48). In order to improve specificity of the findings, p-values were calculated for another set of biologically
linked genes, i.e. endoplasmatic reticulum stress which yielded no significant associations with any of the three disease outcomes (p = 0.484; 0.497 and 0.708, respectively for SCZ, BPD and MDD; Supplemental Table 1B). Within the entire set of 473 CR genes, 21 categories of genes belonging to distinct chromatin regulating pathways can be distinguished. Subsequent stratified analyses for these 21 categories in BPD identified that in particular the MLL3/MLL4 methyltransferase complex showed a significant association with BPD, which remained significant after correction for multiple testing (uncorrected p = 1.3 E-4). As BPD and SCZ show genetic overlap, we also tested the association for the category of MLL3/MLL4 histone H3K4 methyltransferase complex genes with SCZ but this was not statistically significant at conventional alpha (p = 0.07). Based on their biological action, the category of MLL3/MLL4 histone H3K4 methyltransferase complex genes may further be subdivided into genes shared with the human SET1/MLL complexes (WDR5, RBBP5, ASH2L and DPY30), and genes unique to MLL3 and MLL4. Subsequent separate analyses for these two subcategories of MLL3/MLL4 methyltransferase complex genes indicated significant associations with BPD for the subcategory of 168 SNPs in genes unique to MLL3 and MLL4 (p = 0.002; odds ratio 1.0081) as well as the subcategory of 99 SNPs in genes shared with the SET1/MLL complexes showed (p = 0.012; odds ratio 1.0079).

Discussion
Together with recent findings of pronounced age-related reorganization of H3K4 trimethylation in at least 1,340 genes (among which DARPP-32 and NRG-1 for example) in chromatin of neuronal cells in the prefrontal cortex, and findings implicating the MLL3/MLL4 histone H3K4 methyltransferase complex in psychiatric disorders, our findings suggest a role for aberrant chromatin regulation by the MLL3/MLL4 histone H3K4 methyltransferase complex in the aetiology of BPD.

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THE RIGHT NOT TO KNOW AND ITS IMPLICATIONS FOR PSYCHIATRIC GENETICS
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Background
“The right not to know”, i.e.an individual’s right to be shielded from information that might change his or her lifestyle or dramatically impact on the quality of life, is gaining increasing importance in times of sophisticated brain research and genetic sequencing projects. The “right not to know” epitomizes the core conflict of values between the two poles of "patient autonomy" and "medical care". Foreseeable technological developments result in new ethical conflicts that need to be resolved. The issue of how to deal with incidental findings may be considered the
most prominent one. However, so far, not much research has been conducted to assess both societal and individual aspects of the “right not to know.”

Methods
To fill this gap, we have established an interdisciplinary collaboration between the departments of law, human genetics, and psychiatric genetics at the University of Göttingen and the Department of Medical Ethics at the University of Ulm. This collaboration will entail several theoretical and practical research projects at the respective departments. Eventually, we aim at formulating normative statements governing our understanding and practical application of the “right not to know”. The development of an empirical questionnaire will constitute an integral part of the overall project. The aim of this questionnaire is to measure the attitudes of several groups (health professionals, patients, relatives, general population etc.) toward the “right not to know”. Therefore, we are developing useful and standardized measurement criteria to determine the ethical and legal foundations of the “right not to know”.

Results
In this presentation, we will summarize the current state of research on the ”right not to know”, introduce parts of the questionnaire dealing with the psychiatric aspects of our collaborative effort, and present first results on a pilot study based on this questionnaire.

Discussion

Literature

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GENOME-WIDE DNA METHYLATION PROFILING FURTHER IMPLICATES MAOA IN MAJOR DEPRESSIVE DISORDER
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Background
Major depressive disorder (MDD) is a heritable illness for which few candidate genes have been isolated. Traditional genome-wide association studies have failed to identify genetic variation that is significantly associated with MDD and more innovative tactics are being employed to try to identify the underlying molecular pathophysiology of this disorder.

Methods
In a cohort of 592 Mexican American individuals from large pedigrees, we performed genome-
wide DNA methylation profiling of peripheral blood samples using Illumina 450k DNA Methylation BeadChips, in an attempt to elucidate genes associated with MDD. Of the individuals used in this study, 205 (~35%) had a diagnosis of MDD. In our population, heritability of MDD is estimated at 0.393 (p=3.7x10^{-6}). DNA methylation data was normalized using inbuilt controls on the arrays, BMIQ normalization to reduce technical variance and inverse normalization to correct for residual deviation from normality. We used SOLAR to test for correlation of DNA methylation at 362,422 gene-associated CpG sites with MDD; age, sex and their interactions were incorporated in the analysis as covariates. Due to the correlative nature of CpG sites, we used a multiple testing correction threshold based on the number of transcripts represented on the array (n=29,246; p=1.71x10^{-6}). For verification of results, we also analyzed post-mortem brain tissue from five individuals with MDD and six control subjects using Illumina 450k DNA Methylation BeadChips.

Results
We identified a significant association between peripheral blood DNA methylation levels within the MAOA gene and MDD (cg04406445, p=1.28x10^{-6}); the direction of effect was negative (beta -0.24). We also identified several nominally significant associations between DNA methylation and MDD in both brain-related genes (IL2RA, p=1.36x10^{-5}; RIMS2, p=7.21x10^{-5}; HSD17B10, p=1.01x10^{-4}; SYT9, p=1.09x10^{-4}; SLC1A2, p=2.18x10^{-4}) and potentially novel genes (TTTY14, p=2.31x10^{-5}; TCEB, p=2.64x10^{-5}; CLIC4, p=3.56x10^{-5}; DOK7, p=3.62x10^{-5}; PSME3, p=5.15x10^{-5}). To further investigate the role of MAOA DNA methylation in MDD, we analyzed tissue from the dorsolateral prefrontal cortex, which has been implicated in the neurobiology of depression. Although not statistically significant in this limited sample, some evidence for decreased MAOA DNA methylation in individuals with MDD was seen in the dorsolateral prefrontal cortex (p=0.06).

Discussion
Polymorphisms within the monoamine oxidase-A gene (MAOA) have been implicated in the pathogenesis of MDD and other mood disorders in several studies, and may influence response to antidepressants. In general, those polymorphisms which are associated with increased transcriptional activity show increased frequency in MDD, particularly in females. Further, decreased MAOA methylation levels in saliva have previously been associated with depression in females, although these results were not highly significant. We find here, a highly significant association between DNA methylation within MAOA and MDD, further implicating this gene in MDD pathogenesis. We also identified a trend of decreased MAOA DNA methylation in the dorsolateral prefrontal cortex of depressed individuals versus controls, although this was not statistically significant. Increased sample sizes may allow detection of significant associations. Our results demonstrate, that for at least some genes, peripheral markers of DNA methylation may be highly relevant to major depression and may parallel changes within the brain, particularly those associated with the serotonergic system. We are currently analyzing these results further to identify cis-regulatory variants associated with DNA methylation and are further assessing DNA methylation correlations between blood and brain tissue.
CHROMATIN STATE CHARACTERIZATION OF GWAS RESULTS OF DIFFERENT NEUROPSYCHIATRIC TRAITS IS SUGGESTIVE OF BRAIN-SPECIFIC AS WELL AS NON-NEURONAL ORIGINS OF DISEASE

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Background

Large-scale GWAS studies have resulted in the identification of thousands of susceptibility alleles for common and complex traits in the human genome. In addition to disease loci with genome-wide significant evidence of involvement in disease, evidence has been accruing that a considerable part of phenotypic variation can be explained by genetic markers not achieving significance as shown by the polygenic model of disease risk. Moreover, it has been shown that top findings of GWAS studies are oftentimes enriched for expression quantitative trait loci. Thus, while most of the disease susceptibility loci have yet to be identified, it is likely that many are represented within current data sets and specific identification is largely a matter of study size. Another observation from GWAS of complex traits is that a significant proportion of identified disease variants localize outside known protein encoding regions but within regulatory elements as described through genome-wide functional data as such as generated by the ENCODE project. The Psychiatric Genomics Consortium (PGC) has been instrumental in GWAS of neuropsychiatric traits including schizophrenia, bipolar disorder and major depressive disorder (among others). Results using the tens of thousands cases and controls for the different disease groups have been encouraging with partly overlapping findings between some of these disorders. Expanding sample sizes for the different diseases are underway and are expected to uncover even more disease loci. As part of our effort to better understand the PGC GWAS findings at the functional level we set out to study the chromatin state of the top SNP findings for bipolar disorder, schizophrenia and major depressive disorder.

Methods

We obtained the PGC GWAS results of the different disorders and generated a clumped ranked list of independent SNPs based on association signal and removing any SNPs in linkage disequilibrium. The ranked list of SNPs was compared with chromatin state maps defined by NIH Epigenome Roadmap Consortium based on applying ChromHMM to multiple histone modifications mapped across 90 samples covering a wide range of different primary cell types. ChromHMM is a recently developed automated computational system for learning chromatin states. We focused the comparison on a canonical enhancer state, associated with high cell type specificity, to assess whether biologically relevant cell types were preferentially associated with GWAS prioritized SNPs. We computed the significance of the overlap of the number of GWAS SNPs overlapping the enhancer state using a binomial distribution where the probability of overlap was based on the frequency of the enhancer state among all SNPs in the clumped ranked list. Instead of focusing on one specific cut-off in the GWAS ranked list, we compared the overlap at each position within the top several thousand and ranked the cell types based on the most significant p-value obtained at any cutoff. The p-values can be corrected for multiple testing based on repeating the same procedure on randomizing the ranked ordering of SNPs.

Results

We observed enrichment of GWAS signal highlighting neuronal-derived cell types for bipolar disorder and schizophrenia. However, ChromHMM analysis of the GWAS data of major
depressive disorder completely lacked evidence of involvement of neuronal cell types.

**Discussion**

It is known that schizophrenia and bipolar disorder share a substantial burden of the genetic risk, which may explain that genetic association signal of both disorders are enriched for neuronal cells, even though our findings may indicate that different classes of neuronal cells may be implicated. If true, the lack of enrichment of neuronal cells for GWAS of major depressive disorders could imply a non-neuronal origin for the disease.

**ANTENATAL PREDICTION OF POSTPARTUM DEPRESSION WITH BLOOD DNA METHYLATION BIOMARKERS**

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**Background**

Post partum depression (PPD) affects ~10-18% of women in the general population and results in serious consequences to both the mother and offspring. While depression risk is not predicted by serum levels of gonadal hormones in humans, exposure to estrogen appears to be a key factor in establishing PPD.

**Methods**

We hypothesized that predisposition to PPD risk is due to an altered sensitivity to estrogen mediated epigenetic changes that act in a cell autonomous fashion detectable in blood. We investigated estrogen mediated epigenetic reprogramming events in the hippocampus and risk to PPD using a cross species translational design. DNA methylation profiles were generating using methylation microarrays in a prospective sample of blood from the antenatal period of pregnant mood disorder patients who would and would not develop depression postpartum. These profiles were cross referenced with syntenic locations exhibiting hippocampal DNA methylation changes in the mouse responsive to long term treatment with 17b-estradiol (E2).

**Results**

DNA methylation associated with PPD risk correlated significantly with E2 induced DNA methylation change, suggesting an enhanced sensitivity to estrogen based DNA methylation reprogramming exists in those at risk for PPD. Using the combined mouse and human data we identified two biomarker loci at the *HP1BP3* and *TTC9B* genes that predicted PPD with an area under the receiver operator characteristic (ROC) curve (AUC) of 0.87 in antenatally euthymic women and 0.12 in a replication sample of antenatally depressed women. Incorporation of blood count data into the model accounted for the discrepancy and produced an AUC of 0.96 across both prepartum depressed and euthymic women. Pathway analyses demonstrated that DNA methylation patterns related to hippocampal synaptic plasticity were enriched in gene networks co-regulated with the biomarker loci.

**Discussion**
The results of this study suggest that an increased sensitivity to E2 based epigenetic reprogramming related to hippocampal synaptic plasticity may represent a molecular mechanism of predisposition to PPD risk and that prospectively measuring epigenetic variation at these targets in peripheral tissues may be a successful strategy to identify those at risk for the disease.

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ROLE OF TRANSLOCATOR PROTEIN (TSPO) IN ANTIPSYCHOTIC-INDUCED WEIGHT GAIN
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Background
Weight gain is a common side effect of treatment with second-generation antipsychotics, contributing to patient non-compliance as well as cardiovascular and metabolic disease. While heritability estimates of antipsychotic-induced weight gain (AIWG) range from 60-80%, the genetic risk factors are still not completely understood. Translocator protein (TSPO) appears to modulate opening of the mitochondrial permeability transition pore (mtPTP), a putative multiprotein complex that regulates cytosolic reactive oxygen species (ROS) levels. As ROS generation associated with mtPTP opening is suspected to play a role in AIWG, TSPO may be involved in mediating AIWG. Furthermore, clozapine treatment enhances TSPO function, suggesting TSPO may mediate clozapine response or side effects. As genetic variation in TSPO has not been explored in relation to AIWG, the purpose of this study was to 1) investigate the role of TSPO in AIWG, and 2) explore gene-gene interactions between TSPO and other mtPTP genes (VDAC, ANT, and HK) effecting AIWG.

Methods
Genomic DNA was obtained from blood samples of schizophrenia patients with weight change observed after an average of 7±3.4 weeks of antipsychotic treatment (n=237). SNP selection was based on evidence of functional effects on gene expression or protein structure. Variants in the TSPO (rs138926, rs80411, rs6971, rs6973, rs113515, rs138911, rs5759197, rs739092), SLC25A4 (rs10024068, rs7660552), VDAC1 (rs13169435, rs4279383, rs2288834, rs2066944, rs10491289), and HK1 (rs16926246, rs7072268) gene regions were genotyped using a combination of TaqMan assays and OpenArray (Applied Biosystems, Carlsbad, CA). Selected SNPs captured 77% of the common alleles across the TSPO gene. Association between TSPO SNPs and % weight change was assessed by linear regression, with baseline weight and treatment duration as covariates. Pairwise interactions of significant TSPO markers with markers in the other mtPTP genes were assessed using model-based multifactor dimensionality reduction and tested with Wald statistic. The significance of interaction was assessed by permutation test (10,000 permutations, P<10,000).

Results
No significant association with % weight change was observed for any of the SNPs in the TSPO gene region in the total AIWG sample. In a sub-analysis of European ancestry patients on clozapine or olanzapine (n=80), drugs with the greatest propensity for weight gain, % weight
change was significantly associated with the rs6971 Thr allele after Nyholt correction for multiple testing (p=0.02). A clinically significant increase in weight gain of 4.65% (95% CI: 0.03 – 9.26%, p = 0.05) was observed for rs6971 Thr/Thr homozygotes compared to Ala/Ala homozygotes. A significant interaction was observed between TSPO rs6971 and ANT1 rs10024068 (P10 000=0.008).

Discussion
The rs6971 The allele may predispose patients of European ancestry treated with clozapine and olanzapine to AIWG. Previous functional investigations indicate that TSPO rs6971 (Ala147Thr) predicts a large portion of variance in TSPO binding, with the Thr/Thr genotype conferring lower TSPO binding affinity. rs6971 appears to interact with rs10024068, a marker 1.1kb upstream of SLC25A4, to influence AIWG. Taken together, these findings provide preliminary evidence supporting a role for the mtPTP in AIWG and suggest markers of variation in mtPTP genes may be clinically useful in predicting AIWG. Replication of these findings, in addition to functional studies clarifying the underlying mechanisms, is required to elucidate the role of these variants in AIWG.

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THE MOLECULAR INTERACTIONS OF THE SCHIZOPHRENIA SUSCEPTIBILITY GENES ZEB1 AND ZEB2 IN BRAIN DERIVED CELLS.
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Background
Schizophrenia (SZ) is a devastating psychiatric disorder affecting approximately 0.4% of the worldwide population. We recently found that the zinc finger E-box binding homeobox 1 (ZEB1) locus is associated with SZ in a region-wise genome wide association study (GWAS) and subsequent replication study, including a total of 4,969 individuals from the Danish Newborn Screening Biobank. Genome wide significant association was also recently found for the zinc finger E-box binding homeobox 2 (ZEB2) locus in a large SZ GWAS meta-analysis. ZEB1 and ZEB2 are important transcriptional repressors that regulate epithelial-to-mesenchymal transition (EMT) and several observations point toward a function of ZEB1 and ZEB2 in the development of the central nervous system. Like the E-box binding transcription factor TCF4, encoded by the SZ risk gene TCF4, ZEB1 has also been associated with Fuchs endothelial corneal dystrophy (FECD) suggesting some common regulatory functions of TCF4 and ZEB1 in some tissues. Furthermore, overexpression of TCF4 in MDCK cells up-regulates the transcription of ZEB1. ZEB1 ensures commitment to neuronal differentiation pathway by inhibiting expression of other pathways’ genes, such as RE1-silencing transcription factor, whose down regulation is essential for induction and maintenance of the neural phenotype. ZEB2 plays a role in cortical neurogenesis, hippocampal formation and myelination, and mutations in ZEB2 cause Mowat-Wilson syndrome, which is associated with mental retardation and developmental phenotypes. ZEB2 mRNA is also a predicted target for MIR137, encoded by the SZ-associated gene MIR137. Although some specific gene targets of ZEB1 and ZEB2 are known, the molecular interactions in brain derived human cells remains vaguely described.
**Methods**

We have cloned the coding regions of ZEB1 and ZEB2 with N-terminal and C-terminal epitope tags and expressed these in HEK293T and SH-SY5Y cell lines. Proteins that interact in complexes with (N- and C- terminal) epitope tagged ZEB1 and ZEB2 will be co-immunoprecipitated with anti-V5 and HA antibodies and will be identified by LTQ-Orbitrap mass spectrometry.

**Results**

Interaction partners will be validated by reciprocal immunoprecipitation, western blotting analysis, and co-immunofluorescence staining in primary mouse neurons.

**Discussion**

Our initial results will be presented at the conference.


DEVELOPMENT OF A PANEL OF SNPS IN BRAIN-EXPRESSED MIRNAS AND IN THEIR BINDING SITES: NOVEL CANDIDATES FOR NEUROPSYCHIATRIC DISORDERS

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**Background**

microRNAs (miRNAs) are a class of non-coding RNA. They have a key role regulating gene expression acting as post-transcriptional inhibitors. It is possible that single nucleotide polymorphisms (SNPs) located in brain-expressed miRNA genes or in their binding sites (3’UTRs of target mRNAs) may be novel candidates for neuropsychiatric disorders. In the field of neuropsychiatric genetics, there is a need for the creation of a panel of functional SNPs in brain-expressed miRNAs.

**Methods**

Selection of polymorphisms for inclusion in the panel was based on the following criteria: brain expression, biological function and relevance for neuropsychiatric disorders and functional impact (derived from experimental data or bioinformatic predictions). Screening of primary literature and genomic databases were used. Genotyping was carried out using TaqMan Genotyping Assays in a Bio-Rad CFX96 Real-Time PCR machine. SNPstats and PLINK were used for statistical analysis.

**Results**

A panel of functional SNPs was developed and included 12 SNPs in brain-expressed miRNAs: hsa-mir-124-2, hsa-mir-124-3, hsa-mir-125a, hsa-mir-128-1, hsa-mir-149, hsa-mir-16-1, hsa-mir-181b-2, hsa-mir-187, hsa-mir-9-2, hsa-mir-92b, hsa-miR-182 and hsa-miR-124-1. In addition, it included 10 SNPs located in binding sites for brain-expressed miRNAs: DRD1, NTRK3, GRN,
FGF20, HTR1B, ERBB4, PRKAB2, SYT14, PCDH15 and GRM6. Several of these polymorphisms have experimental evidence of having a functional allele-specific effect and others have strong *in silico* evidence. Frequencies in Colombian samples are similar to those described for populations of European descent.

**Discussion**

The panel of functional SNPs in brain-expressed miRNAs and in their binding sites developed in this work included a large number of novel variants of high interest for neuropsychiatric genetics. Future analysis of this novel SNP panel would help to understand how variants in miRNA pathways influence the susceptibility to different types of neuropsychiatric disorders.

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**HERITABILITY OF OLANZAPINE-INDUCED WEIGHT GAIN AND ALTERATIONS IN THE GUT MICROBIOME IN MICE**

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**Background**

Olanzapine, a second-generation (“atypical”) antipsychotic drug, is associated with dramatic but highly variable weight gain (5% - 40% total body weight) and the development of a metabolic syndrome resembling type 2 diabetes. Physicians currently have no way to predict which patients are likely to develop this side effect, so it is important to discover biomarkers that indicate a patient’s side effect risk before treatment. Since these studies are difficult to perform in humans, we are using laboratory mice to discover potential biomarkers worth testing in human patients.

**Methods**

First, to determine whether olanzapine-induced weight gain is in fact a heritable trait in mice, we examined the effect of chronic olanzapine treatment (seven weeks) on body weight and adiposity across a panel of eight inbred strains fed a high fat diet (N = 5 olanzapine treated, N = 5 placebo treated mice per strain). Second, since the composition of the intestinal microbiome is now recognized as a major contributor to body weight regulation, we tested whether chronic treatment with olanzapine altered the composition of bacteria in feces before and after initiation of a high fat diet and drug treatment. This was accomplished by targeted shotgun sequencing of a highly variable region (VR4) within the bacterial 16S rRNA subunit gene.

**Results**

Broad-sense heritability ($H^2$) of olanzapine-induced weight gain was 0.45. Of the eight strains tested, four experienced statistically significant weight gain (C57BL/6J, CAST/EiJ, NOD/LtJ, 129/SvImJ) and four did not (A/J, NZO/HILtJ, PKW/PhJ, WSB/EiJ). Increases in adiposity paralleled changes in body weight, with the most sensitive strain being C57BL/6J (33% increase in body weight relative to placebo treated controls). Regarding the fecal microbiome, ANOVA revealed that olanzapine significantly altered both $\alpha$-diversity (species richness within individual samples; $P = 0.003$) and $\beta$-diversity (difference in community composition between samples; $P = 0.012$). Preliminary data suggest that olanzapine potentiates shifts towards an “obesogenic” microbiota induced by a high fat diet, in a strain-dependent manner.
Discussion
The weight gain heritability data are consistent with a complex genetic trait and suggest that it may be possible to identify clinically-useful biomarkers of extreme sensitivity to this adverse drug reaction. The eight strains tested are the parental strains of two large-scale mouse populations designed for complex trait analysis, the Collaborative Cross and Diversity Outbred, so the variable responses observed here support extension to these resources. While preliminary, the bacterial sequencing results suggest that gut community composition may underlie at least some of the variation in susceptibility to olanzapine-induced weight gain.

CONTRIBUTIONS OF THE MINERALOCORTICOID RECEPTOR POLYMORPHISM (I180V) AND LIFE EVENTS TO PERFECTIONISM IN EATING DISORDERS
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Background
It is hypothesized that the balance between the glucocorticoid and the mineralocorticoid receptor determines the capacity of an individual to cope with a stressful event. Patients with an eating disorder (ED) often experience severe life events before the onset of the disorder. A personality feature that seems to precede the onset and is associated with different types of EDs is perfectionism.

Methods
It was tested if there was a gene x environment interaction between three common functional variants in the genes encoding the glucocorticoid receptor (rs41423247) and the mineralocorticoid receptor (rs5522 and rs2070951) and adverse life events on the level of perfectionism in a group (n=116) of patients with an ED.

Results
For rs5522 there was a significant interaction effect with adverse life events on MPS perfectionism score (F(2, 110)=6.69, p=0.002). Patients with an ED that carried the minor G-allele had a low perfectionism score when they had experienced only few adverse life events and showed an elevated perfectionism level if they experienced many adverse life events. These associations were not present for patients that carried the AA genotype of this SNP.

Discussion
Carriers of the minor allele of rs5522 might be less capable to cope with stressful life events, and therefore try to exercise more control over other aspects of their life and show a higher perfectionism score as a consequence.
ASSESSMENT OF GENE EXPRESSION IN PERIPHERAL BLOOD USING RNASEQ BEFORE AND AFTER WEIGHT RESTORATION IN ANOREXIA NERVOSA

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Background
Anorexia nervosa, a severe psychiatric illness marked by extremely low body weight, fear of weight gain, and inability to recognize the seriousness of the low weight, carries the highest mortality rate of any psychiatric disorder. Inpatient treatment is costly and relapse is common. There are no robust biological indices of risk, illness severity, or treatment response, and the identification of such biomarkers is an urgent area of inquiry.

Methods
We examined gene expression in the blood of six females with anorexia nervosa (AN) before and after weight restoration using RNAseq. AN cases (aged 19-39) completed clinical assessments and had blood drawn for RNA at hospital admission (T1, < ~75% ideal body weight, IBW) and again at discharge (T2, ≥ ~85% IBW). To examine the relationship between weight restoration and differential gene expression, normalized gene expression levels were analyzed using a paired design.

Results
We found 564 genes whose expression was nominally significantly different following weight restoration (p < 0.01, 231 increased and 333 decreased). With a more stringent significance threshold (false discovery rate q < 0.05), 67 genes met criteria for differential expression. Of the top 20 genes, CYP11A1, C16orf11, LINC00235, and CPA3 were down-regulated more than two-fold after weight restoration while multiple olfactory receptor genes (OR52J3, OR51L1, OR51A4, OR51A2) were up-regulated more than two-fold after weight restoration. Pathway analysis revealed up-regulation of two broad pathways with largely overlapping genes, one related to protein secretion and signaling and the other associated with defense response to bacterial regulation.

Discussion
Although results are preliminary secondary to a small sample size, these data provide initial evidence of transcriptional alterations during weight restoration in AN.

TYPE I INTERFERON SIGNALING GENES IN RECURRENT MAJOR DEPRESSION: INCREASED EXPRESSION DETECTED BY WHOLE-BLOOD RNA SEQUENCING

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Background
Altered gene expression levels in disease can reflect the effect of common and rare sequence
variation, environmental factors and their interaction with genetics, as well as the effects of the
disease processes itself. To gain insights into biological mechanisms that are relevant to major
depressive disorder (MDD) by identifying genes and pathways with altered expression levels, we
have compiled genome-wide gene expression, genotype, and physiological data in a case/control
cohort of MDD from a large population-based sample.

Methods
We analyze 922 European-ancestry individuals, including 463 non-bipolar recurrent MDD cases
and 459 never-depressed controls, that were recruited from a nationally-representative survey
research panel. We have collected RNA from whole-blood using deep RNA sequencing,
genotyped these individuals for common SNPs, and derived information on psychiatric and
medical history, childhood trauma and other environmental variables from SCID interviews and
self-report questionnaires. Using these data, we assess the association of MDD status with each
gene and with sets of genes (pathways), while accounting for the influence on expression levels
of potentially confounding variables such as environmental factors, physiological variation, and
computational estimates of cell type proportions. Our analysis consists of three components: (1)
association analysis of single gene expression levels with MDD; (2) analysis of association of
MDD with 1,325 canonical pathways using Gene Set Enrichment Analysis, and hypergeometric
tests of the enrichment of pathways in subsets of the most strongly-associated genes; and (3)
joint analysis of association of gene expression and eQTLs (SNPs associated with expression, or
expressed quantitative trait loci) with MDD.

Results
In the analysis of association between MDD and expression levels of 13,857 single autosomal
genes, a significant excess of low p-values was observed, but no single-gene association was
significant after genome-wide correction. Pathway-based analyses of expression data detected a
significant association (FDR<0.05) between MDD and the interferon α/β signaling
(REACTOME) pathway, with increased expression levels in MDD cases, where secondary
analysis did not indicate that this result was subject to additional confounding factors. Finally,
joint evaluation of gene expression and eQTL genotypes identified a significant association with
CINP, a gene involved in cell cycle arrest.

Discussion
The observed association between MDD and interferon α/β signaling genes supports hypotheses
that involve the dysregulation of cytokine activity in MDD. Because gene expression data alone
cannot conclusively resolve cause-effect relationships here, we must consider two possibilities.
First, increased type I interferon signaling may be a cause of depression in some individuals, due
to the direct effect of cytokine dysregulation, normal or abnormal response to unknown viral
infections, and/or interaction with the effects of dysregulation of glucocorticoid release. Second,
increased type I interferon signaling could also be a result of other pathogenic processes rather
than a causative factor. In conclusion, the results support the hypothesis that altered immune
signaling plays a role in the pathogenesis and/or the persistence and progression of MDD, and in
particular implicates type I interferon signaling in pathology of MDD.
CANDIDATE AND GENOME-WIDE POLYGENIC SCORING FOR A 14-YEAR LONG-TERM AVERAGE DEPRESSION PHENOTYPE

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Background
Despite moderate heritability estimates for depression-related phenotypes, few robust genetic predictors have been identified. Potential explanations for this discrepancy include the use of phenotypic measures taken from a single time-point, rather than integrating information over a longer period of time via repeated assessments, and the possibility that genetic risk is shaped by multiple loci with small effects.

Methods
We developed a 14-year long-term average depression measure based on 14 years of follow-up in the Nurses’ Health Study (NHS; N=6,989 women). We estimated polygenic scores (PS) with either candidate genes (candidate-PS, based on 45 single nucleotide polymorphisms in 9 genes) or whole-genome scoring (NHS-GWAS-PS). We also constructed PS by applying an external PS weighting algorithm previously shown to predict depression phenotypes in an independent Dutch sample (GAIN-MDD-PS). We assessed the association of all 3 polygenic scores with our long-term average depression phenotype using linear, logistic, and quantile regressions.

Results
3 PS approaches explained at most 0.2% of variance in the long-term average phenotype. Quantile regression results indicated that the effect of polygenic scores had a larger impact on the higher quantile of depressive symptoms. Quantile regression coefficients for GAIN-MDD-PS were six times larger at the 75th percentile (0.100; 95% CI: 0.012, 0.189) than that at the 25th percentile (0.014; 95% CI: -0.026, 0.053). Higher GAIN-MDD-PS was associated with larger interquartile range, with borderline statistical significance (p=0.05).

Discussion
Integrating multiple phenotype assessments spanning 14 years and applying different polygenic scoring approaches did not substantially improve genetic prediction of depression. However, quantile regressions suggested that the effect of polygenic scores may have larger impact on the higher quantile of depressive symptoms, presumably for people with more sources of vulnerability of depression.
Background
For several decades, psychiatric genetic research has focused on cross-sectionally defined phenotype definitions such as categorical diagnoses. However, course of illness in schizophrenia, bipolar, and other disorders has long been recognized to vary substantially between individuals or groups of patients. Studying the genetic underpinnings of the longitudinal course may thus serve as an avenue into disentangling the genetic heterogeneity of major psychiatric disorders. The gene CACNA1C on chromosome 12p13 encodes a subunit of the L-type calcium channel. Genome-wide association studies (GWAS) have demonstrated a role of the CACNA1C single-nucleotide polymorphism (SNP) rs1006737, in susceptibility to bipolar disorder and schizophrenia. Also, rs1006737 modulates brain activity in both healthy individuals and those diagnosed with mental disorders. Furthermore, we could recently report on a distinct sex-specific relationship with potentially illness-associated endophenotypes in the general population, such as resilience factors. Given the general role of CACNA1C in psychopathology, we hypothesized that the aforementioned SNP may also affect functional improvement following episodes of psychiatric illness across diagnostic boundaries.

Methods
Our study comprised 521 individuals suffering from major psychiatric disorder (211 bipolar disorder, 53 schizo-affective, 241 schizophrenia, and 16 schizophreniform disorder). Functional improvement was measured with the Global Assessment of Functional Scale (GAS). Based on our multi-tiered phenotype characterization inventory, we established GAS scores for different time points during the course of illness. The difference between the worst GAS value ever and the GAS level at the time of interview was used as a measure of functional improvement. Using available GWAS data, we identified rs10774035 as a proxy SNP for rs1006737 (r²=1 and d'=1). Data analysis was carried out using the R software, including a nonparametric analysis toolbox (nparcomp) accounting for imbalance of genotype categories and unequal variances. A linear model was used to globally test the effects of age, sex and the sex-genotype interaction on functional improvement score.

Results
In our data set, we observed a strong trend towards a global sex-genotype interaction (p=0.058), justifying sex-specific analyses in the 257 males and 264 females). While there was no genotypic effect on functional improvement in males (p=0.266), we observed a significant (p<0.001) additive effect in females.

Discussion
We analyzed functional improvement from episodes of psychiatric illness in a sample encompassing bipolar and schizophrenia-spectrum disorders. A SNP in CACNA1C was found to affect longitudinal improvement as measured by GAS in females but not in males. Taken together, our data indicate a sex-specific genetic effect on functional recovery across diagnostic categories. Without taking sex into account, we would not have been able to see such effect. This is in line with previous reports on CACNA1C and suggests that sex-specific analyses are warranted in complex psychiatric phenotypes.
VARIATION IN THE FKBP5 GENE POLYMORPHISMS, MALTREATMENT, AND CHILDHOOD-ONSET AGGRESSION: A CASE CONTROL STUDY

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Background
Aggressive behaviours in children are a major public health problem as well as the most common reason for referrals to mental health clinics. Childhood-onset aggression has been associated with antisocial personality disorder, substance dependence, and other antisocial behaviours later in adulthood (1). While the etiology of aggressive behaviours remains elusive, growing evidence suggests that aggression is heritable (2). Aggressive behaviour is associated with the Hypothalamus-Pituitary-Adrenal (HPA) axis, one of the two central stress response systems (1). Specifically, aggressive behaviour has been linked to reduced basal cortisol levels (1) and a flattened daily cortisol rhythm (3). However, the role of the HPA axis genes in the etiology of aggression is not well understood.

Recently, the FKBP5 gene has been linked to aggressive behaviour in adults with a history of childhood trauma (4). FKBP5 gene codes for FKBP506, a co-chaperone that inhibits the negative feedback of the HPA axis. The same variants of the FKBP5 gene have been consistently linked to stress-related psychopathologies, including mood disorders and PTSD (5). As well, childhood trauma was recently shown to epigenetically modify FKBP5 expression, thus FKBP5 may serve as the key mediator in the link between trauma and psychopathology (6).

Methods
Our sample consists 176 Caucasian children (age 6-16) displaying extreme, persistent and pervasive aggressive behaviour, defined as a minimum 2-year history of aggressive behaviours; at or above the 90th percentile on the subscale of aggressive behaviors of the Achenbach Child Behaviour Checklist (CBCL)and Teacher Report Form (TRF) (7). Children with chronic medical illnesses and psychiatric disorders, such as schizophrenia, mania, autism and Tourette’s Syndrome were excluded. These cases were matched with 176 Caucasian controls based on gender and age. Caucasian ancestry was confirmed using an Open Array assay of 64 ancestry-informative markers (8). Genomic DNA was extracted from blood, cheek swab or saliva using commercially available kits. Genotyping was performed using PCR-based TaqMan Single Nucleotide Polymorphism (SNP) assays for 5 SNPs in the FKBP5 region that were previously implicated in psychopathology. Single-marker and haplotype associations were tested in Unphased. For haplotype analyses, 2-, 3-, and 4-marker sliding window analyses were used. Two methods of correcting for multiple testing were used: the Nyholt correction (9), employing the web-based SNP Spectral Decomposition software (10), as well as the permutation-based method of correcting for multiple testing used as a built-in function in Unphased.

Results
To date, we have genotyped five SNPs (rs1360780, rs9470080, rs9296158, rs3800373, rs4713916) in the FKBP5 region. No significant main effects were found for allele, genotype, or haplotype frequencies between the aggressive cases and controls. High-aggression cases were then divided into two groups, based on a history of maltreatment. A nominal association was found between rs3800373 and the maltreatment condition (p=0.0288), which survived the 10,000
permutation tests (p=0.0342) but not the Nyholt correction (0.0288> α=0.0252). Specifically, high-aggression individuals with the C allele were about 2 times more likely to have a history of abuse (95% C.I. of the Odds Ratio = 1.058-3.059). Haplotypes analyzed containing rs3800373 also show a trend towards an association with the maltreatment condition.

Discussion
Our results suggest that allele frequencies of rs3800373 may differ in highly aggressive children depending on whether or not they have a history of maltreatment. Thus, the molecular mechanism by which these two groups of children develop aggressive behaviour may differ. It may also reflect differences in the phenotype, such as the level of callous unemotional traits, which we are currently testing and will describe. Though not evidence of an interaction, it is consistent with a study that found an interaction between the FKBP5 polymorphisms and childhood trauma and aggressive behaviour in an adult sample (4).

References:

WHOLE EXOME SEQUENCING OF CONSANGUINEOUS PAKISTANI PEDIGREES WITH RECESSIVE AUTISM SPECTRUM DISORDER AND INTELLECTUAL DISABILITY

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Background
Pakistan has the highest rate of consanguineous marriage, as well as a high fertility rate, in the world due to historical, religious, cultural and social reasons. This population characteristic makes Pakistan an excellent location for collecting large families for genetic studies, particularly for autosomal recessive traits and for severe psychiatric disorders plagued by extreme heterogeneity and reduced reproductive fitness in outbred Western populations.
Methods
In the past few years, with the cooperation from local geneticists and clinicians, we have identified and characterized 6 consanguineous pedigrees with 3-5 affected siblings/first-cousins per pedigree with variable intellectual disability and autism spectrum disorder, which fits with a recessive inheritance pattern. With the collaboration of local clinicians, we have thoroughly investigated all the symptomatic individuals, including standard clinical examinations, comprehensive psychological evaluations, routine laboratory tests, MRI and EEG investigations; and have collected blood samples from all the affected individuals, their unaffected parents and siblings. We have carried out classical linkage analysis combined with homozygosity mapping in these 6 ASD/ID pedigrees using high density DNA microarrays. Due to the extreme genetic heterogeneity of ASD/ID, as well as the efficiency and reduced cost of whole exome sequencing, we also performed whole exome sequencing in 2-3 selected affected individuals/pedigree.

Results
We have identified extensive runs of homozygosity region in each individual genome, as well as shared identical-by-descent regions among the affected individuals in each or branch of these 6 pedigrees independently. Combined with whole exome sequencing, we have so far identified a novel frameshift nonsense mutation in the VPS13B gene in one family with 3 affected brothers with atypical Cohen syndrome and autism phenotype. We have also identified another frameshift nonsense mutation in the CC2D1A gene in one family with 5 affected brothers with intellectual disability and autism behaviors. Genetic validation of potential disease-causing mutations in other 4 pedigrees is underway.

Discussion
Our results strongly indicate extensive genetic heterogeneity in these consanguineous pedigrees with ASD/ID phenotypes. Direct whole exome sequencing could be the most useful and efficient approach to identify causative disease mutations and to facilitate clinical diagnosis, as well as for much needed early intervention and genetic counseling.

ARE OBESITY-RISK GENES ASSOCIATED WITH BINGE-EATING IN ADOLESCENCE?
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Background
Binge eating disorder (BED) is characterised by episodes of overeating with loss of control (binge-eating) and it is relatively common in general population studies. There is preliminary evidence that loss of control over eating (one of the characteristics of binge-eating) is associated with a polymorphism of the FTO gene, a gene strongly related to obesity (Tanofsky-Kraff et al., 2009). In the present study we hypothesized that higher Body Mass Index (BMI) and binge-eating behaviour might share a common genetic risk. We investigated associations between the 32 GWA SNPs associated with BMI from obesity GWAS (Speliotes et al., 2010) and a polygenic risk score (weighted allelic score of the same 32 SNPs) and adolescent binge-eating in a population-based sample of adolescents from the Avon Longitudinal Study of Parents and
Methods
Binge-eating was assessed in 5,958 adolescents at age 14 and 4,949 at age 16. ALSPAC children were genotyped using the Illumina HumanHap550 quad chip genotyping platforms. Raw genome-wide data were subjected to standard quality control methods. Individuals were excluded on the basis of gender mismatches; minimal or excessive heterozygosity; disproportionate levels of individual missingness (>3%), cryptic relatedness measured as proportion of identity by descent (IBD > 0.1) and insufficient sample replication (IBD < 0.8). The remaining individuals were assessed for evidence of population stratification by multidimensional scaling analysis and compared with Hapmap II (release 22) European descent (CEU), Han Chinese, Japanese and Yoruba reference populations; all individuals with non-European ancestry were removed. Hidden population stratification was thereafter controlled for by using derived ancestry informative principal components scores (Price et al., 2006). SNPs with a minor allele frequency of < 1%, a call rate of < 95% or evidence for violations of Hardy-Weinberg equilibrium (P < 5E-7) were removed. Genotypic data were subsequently imputed using Markov Chain Haplotyping software (MACH v.1.0.16) (Price et al., 2006) and phased haplotype data from CEU individuals (Hapmap release 22, Phase II NCBI B36, dbSNP 126) based on a cleaned dataset of 9545 individuals and 464,311 autosomal SNPs. We tested associations between 32 SNPs and binge-eating (at either age 14 or 16) in crude and BMI-, age- and gender-adjusted logistic regression models. We then obtained a polygenic risk score (weighted allelic score of the same 32 SNPs) and tested its effect on binge-eating in crude and BMI-, age- and gender-adjusted linear regression models.

Results
In adjusted analyses there was a significant association between binge-eating at either age 14 or 16 and rs1558902 (FTO gene) (OR=1.3, p≤0.01), and rs887912 (FANCL-Fanconi Anemia, Complementation group L) (OR=1.4, p=0.005), and a trend for the C allele of rs10150332 (Neurexin 3 gene) being protective for binge-eating (OR=0.8, p<0.05). The weighted allelic score was also associated with binge-eating at either age 14 or 16 (coefficient: 20.14, p=0.008).

Discussion
We found evidence that genetic predisposition to higher BMI is associated with adolescent binge-eating. Although our findings need replication, there is biological plausibility given the known effect of FTO on appetite and food intake and evidence that the C allele of rs10150332 (Neurexin 3 gene) is protective for addictive behaviours.

GENES, MATERNAL SMOKING DURING PREGNANCY AND CHILD INTERNALIZING PROBLEMS: AN INVESTIGATION USING ALSPAC
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Background
The majority of research on maternal smoking during pregnancy (MSDP) and child outcomes have focused on externalizing disorders. However, findings from recent research have suggested
a potential relationship between prenatal nicotine and alcohol exposure and internalizing problems caused by disruptions in neurobehavioral and cognitive development, potentially resulting in depression, generalized anxiety, impaired memory function, lower scores on arithmetic tasks, deficits in verbal learning, auditory, and visual-perceptual processing, as well as IQ decrements and slower information processing speeds. Evidence also indicates that these adverse effects may persist into early and late adolescence, resulting in subsequent impaired executive functioning, increased incidence of substance abuse (particularly smoking) or a predisposition to early onset of smoking in offspring (after intrauterine exposure to heavy smoking). It is still unclear, however, whether these results provide sufficient evidence to suggest a cause-and-effect relationship or if the association is confounded by variables such as social background, child-rearing practices, maternal and family characteristics, or genetic factors.

**Methods**

Using data from the Avon Longitudinal Study of Parents and their Children (ALSPAC), we explore the associations between maternal smoking during pregnancy (MSDP), genetic risk, and child anxiety and depression (available at ages 7, 10, 13, 15/16). Data on MSDP was available for 13,364 mothers. Complete data on MSDP and child anxiety at age 7 were available for 8,008 children, while complete data for MSDP and child depression at age 10 were available for 6,720 children.

**Results**

Results suggest that both MSDP and maternal genetic factors are associated with increased child depression scores before additional adjustment for other parental and sociodemographic confounders, including parental education, social class, maternal psychopathology, and maternal alcohol use during pregnancy. Additional analyses considering the role of potential disruptions in executive function in the relationship between prenatal exposure, genetics, and offspring depression and anxiety will be discussed.

**Discussion**

This work adds to the growing literature on trying to disentangle genetic and prenatal environmental effects on later child behavioral and psychiatric outcomes.

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**PATHOGENIC DE NOVO SNVS, INDELS AND CNVS IN 1,000 CHILDREN WITH UNDIAGNOSED DEVELOPMENTAL DISORDERS**

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**Background**

The majority of children born with severe developmental disorders do not currently receive a definitive genetic diagnosis. To delineate the genetic architecture of severe undiagnosed developmental disorders in UK children we have deeply phenotyped over 6,000 affected children and their parents through a nationwide network of clinical geneticists, and recruited the families into a genetic research study entitled the Deciphering Developmental Disorders (DDD) study.
Seventy-five percent of the families are sporadic.

**Methods**
We are interrogating the causal roles of coding and regulatory SNVs, indels and CNVs by applying exome-array comparative genomic hybridization (exome-aCGH) to detect deletions and duplications, and exome-sequencing to detect sequence variants in all coding exons, known enhancers, and the most highly conserved non-coding elements. We have profiled over 5,000 probands using exome-aCGH, and over 1,000 parent-proband trios with exome sequencing.

**Results**
We are currently able to provide likely diagnoses for 15-20% of children. We have identified recurrent functional *de novo* mutations in 45 genes, of which only 19 are already known developmental disorder genes. The largest single contributing gene is *ARID1B*. We have identified four genes where exactly the same mutation occurs in 2 or more families, highly suggestive of gain-of-function mutations. In aggregate, these analyses have identified more than 10 likely novel developmental disorder genes. We have modeled some of these plausible candidate genes in zebrafish and identified concordant developmental phenotypes in morphant zebrafish for a subset of these.

**Discussion**
The DDD project will eventually profile 10,000 families, with the hope of substantially increasing diagnostic rate using new genomic technology. Results from the first 1000 trios studied have revealed likely novel causal genes and highlighted new mutations in known genes. The project extends from clinical recruitment, through running genomic assays and subsequent analysis to eventual reporting of likely pathogenic mutations. We hope it will serve as an example of how genomics might be incorporated into clinical practice.

PRENATAL EXPOSURE TO THE 1959-1961 CHINESE FAMINE AND THE INCREASED RISK OF EPILEPSY IN LATER LIFE: A TWO-CENTER OBSERVATIONAL COHORT STUDY

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**Background**
Epilepsy has been reported to be associated with a number of prenatal factors. Whereas the specific effects of prenatal exposure to famine or prenatal malnutrition on the lifetime risk of epilepsy has never been investigated.

**Methods**
To determine whether those who prenatally endured a massive 1959-1961 famine in China experienced increased risk of epilepsy in later life, we examined the risk of epilepsy in two different Chinese provinces that were severely affected by the Great Chinese Famine of 1959-1961. All neuro-psychiatric case records for the years 1970 through 2005 were examined, and clinical and socio-demographic information on patients with epilepsy was extracted. Data on
number of births and deaths in the famine years were available, and cumulative mortality was estimated from later demographic surveys. Evidence of famine was verified; unadjusted and mortality-adjusted cumulative incidence and mortality-adjusted relative risks of epilepsy were calculated. Potential confounding effects of family history, gender, and age of onset were estimated.

**Results**
For each of the two research regions, the decline in birth rates during the famine years was accompanied with a corresponding increase in death rates and a decline in the absolute number of cases of epilepsy born during the famine years. Among births that occurred during the famine years 1960-1961, the adjusted risks of developing epilepsy in later life increased significantly for both the Wuhu and the Liuzhou cohorts. The combined analytic results showed that the mortality-adjusted relative risks were significantly higher for exposed years of 1960 (RR:1.85; 95% CI: 1.54-2.21; p-value: 2.08×10^{-11}) and 1961 (RR:2.07; 95% CI: 1.75-2.44; p-value: 6.70×10^{-18}) compared with the un-exposed years of 1956-1958 and 1963-1965. No associations of this effect were detected with respect to family history, gender, and age of onset.

**Discussion**
We observe a 2-fold increased risk of epilepsy among those conceived during the Chinese famine. This is not biased by family history, gender, age of onset, and Chinese minorities. Further replication is warranted to verify the ethnic difference or consistency of our findings in the Caucasian population-based 1944-1945 Dutch famine cohort.

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**COMT POLYMORPHISM RS4680 MAY BE ASSOCIATED WITH REFRACTORINESS TO PHARMACOLOGICAL TREATMENT IN A SAMPLE OF DEPRESSIVE PATIENTS UNDERGOING ELECTROCONVULSIVE THERAPY**

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**Background**
Pharmacogenetics studies have demonstrated the influence of genetic variability on drug response and effectiveness. Usually, antidepressant treatments are performed on a trial and error basis, taking at least two weeks to observe appropriate response or not. Concerning the possible mechanisms of action of the antidepressants, there are some evidences that interactions between the serotoninergic and the dopaminergic systems occur. Due to the relevant role of the dopaminergic system in the pharmacodynamics of some antidepressants, proteins that regulate the availability of dopamine in the synapse may influence the response to drug treatment. One of them is the enzyme catechol-O-methyltransferase (COMT), which catalyzes the inactivation of dopamine. The polymorphism rs4680 in the COMT gene consists of a valine to methionine amino acid substitution at codon 158. The valine homozygote is four times more active in metabolizing dopamine than the methionine homozygote. Some associations have already been described between the polymorphism rs4680 (Val158Met) and response to antidepressants, with
individuals carrying the Met allele presenting the best answer. This study aims to investigate the hypothesis that refractoriness to treatment with antidepressants may be influenced by polymorphism rs4680 of the COMT gene using data of refractory patients undergoing ECT.

**Methods**
A total of 112 patients were genotyped. Among these, 50 were patients undergoing ECT treatment who met the DSM-IV criteria for refractory unipolar depression and 62 patients were patients with unipolar depression who responded to pharmacological treatment. Genomic DNA was extracted from peripheral blood. The polymorphisms rs4680 (Val158Met) of the COMT gene was determined using TaqMan® SNP Genotyping Assays. Genotyping was performed by real-time PCR allelic discrimination.

**Results**
After performing Chi-square test we found a significant difference in genotype distribution between the refractory and non-refractory groups for the rs4680 (p=0.035).

**Discussion**
Our results indicate a prevalence of the genotype GG (Val/Val) in the refractory group and also a higher prevalence of GA (Val/Met) genotype in the non-refractory group. COMT is involved in the catabolic pathways of noradrenaline and dopamine (DA) and consequently may indirect affect brain 5-HT tone because of the interactions between DA and 5-HT. Our findings suggest that the decrease of dopamine level caused by the Val/Val genotype might be related to refractoriness in antidepressant response.

**IMPLICATING RARE VARIANTS IN ANTIDEPRESSANT RESPONSE USING THE EXOME CHIP**
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**Background**
Antidepressants are used as a first line treatment of major depressive disorder, however response is highly variable with no clear path to predicting an individual’s response. Genetic markers have been proposed for personalizing treatment as genotyping technologies allow for reliable measurement of large numbers of variants. Our previous results estimate a substantial genetic contribution to antidepressant response. Previous genome wide association studies focused on common variants. By contrast, here we use genotyping arrays that focus on low frequency exonic variants enriched for functional relevance to investigate the role of rarer variants in antidepressant response.

**Methods**
We meta-analyzed exome array genotyping data from three studies; the Genome-Based Therapeutic Drugs for Depression (GENDEP) study (n=800), the Genetic and clinical Predictors
Of treatment response in Depression (GENPOD) study (n=490), and the Informatics for Integrating Biology and the Bedside (I2B2) study (n=960). Samples were genotyped using the Illumina HumanExome BeadChip. Genotype clusters were iteratively called first using Illumina’s GenCall algorithm in GenomeStudio version 2011.1 and then the zCall algorithm, designed specifically to call rare variants. Samples underwent routine GWAS quality control and were assessed for discordance with previously available GWAS data. Logistic regression were run to assess the relationship between variants <1% in allele frequency and non-responder/responder status conditioning on population structure in each study independently using PLINK. Analyses were run for response to any antidepressant, response to serotonergic antidepressants only, response to noradrenergic antidepressants only, and differential response to serotonergic or noradrenergic antidepressants. Results from the three studies were meta-analyzed. Furthermore, gene based analysis was undertaken using the SKAT-O test in each of the samples independently and jointly.

**Results**

Results from the individual studies and the meta-analysis will be presented for the single variant as well as the gene-based analyses for the four different analysis performed (response to any antidepressant, response to serotonergic antidepressants, response to noradrenergic antidepressants and differential response to serotonergic or noradrenergic antidepressants). Pathway analysis and GCTA analyses on the rare variant results will also be presented.

**Discussion**

We report the first extensive study of the role of rare variants in modulating response to antidepressants. We investigate a large number of rare polymorphisms across three different studies and meta-analyzed the results. Pathway analysis undertaken on gene-based results will provide important insight into the biological mechanism of antidepressant response and allow for genetic heterogeneity.

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**PHARMACOGENETICS OF ANTIPSYCHOTIC-INDUCED WEIGHT GAIN IN SCHIZOPHRENIC PATIENTS**

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**Background**

Antipsychotic-induced weight gain (AIWG) is a major drawback in the treatment of schizophrenic patients with second-generation antipsychotics (SGAs). Pharmacogenetic studies have established several polymorphisms in genes of different pathways contributing to the development of weight gain in schizophrenic patients. However interactions of genetic polymorphisms and clinical predictors have not been studied extensively enough to reliable predict weight gain before initiating antipsychotic treatment.

**Methods**

We analyzed several single-nucleotide polymorphisms (SNPs) of candidate genes (e.g. APOA, BDNF, DISC, GNAS, SNAP-25, LDLR, INSIG2, Resistin, TNF-a, 5HTR2C, MC4R) and clinical predictors (e.g. gender, BMI at baseline, number of episodes, pretreatment) in a sample
of 259 schizophrenic patients participating in different monotherapeutic trials of atypical antipsychotics (risperidone, olanzapine, quetiapine, amisulpride, aripiprazole) with up to six weeks of treatment. We used Univariate tests, regression analysis and Classification and Regression-Tree (CART)-analysis to determine relevant clinical and genetic predictors and their interactions.

**Results**

We found male gender, first episode of schizophrenia, smoking habits and no previous treatment before initiation of SGAs as strongest clinical predictors. BDNF Val66Met, DISC rs3738400, Resistin -420C/G rs1862513, SNAP-25 Ddel and TNF-a G-308A were associated with weight gain in the overall patient sample. We found several interactions of the clinical predictors and genetic variants or type of used SGAs. The most pronounced associations were yielded for the Resistin -420C/G rs1862513 polymorphism. Homocytotic C-allele carriers had a mean weight gain of 6.8 kg compared to 2.7 kg in homocytotic G-allele carriers (F 10,516; p < 0.0001). This effect was accentuated in male non-smoking homocytotic C-allele carriers (mean 8.7 kg) compared to female non-smoking homocytotic G-allele carriers (mean 1.7 kg).

**Discussion**

Our study confirms the contribution of several candidate genes on AIWG. CART-analysis shows interactions of several clinical and genetic predictors. Our approach of combining clinical and genetic predictors may help to identify subgroups of patients a priori of SGA treatment in order to reduce development of severe weight gain. Further investigations on larger samples are necessary to confirm our results.

**PHARMACOGENOMIC STUDY FOR LAMOTORIGINE-INDUCED CUTANEOUS ADVERSE DRUG REACTIONS**

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**Background**

Antiepileptic drugs such as Lamotrigine (LTG) and carbamazepine (CBZ) cause cutaneous adverse drug reactions (cADRs), of which the most severe forms are Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN). Recent pharmacogenetic (PGt) or -genomic (PGx) studies have suggested that particular human leukocyte antigen (HLA) alleles have been strongly associated with CBZ-induced cADRs. However, although LTG is commonly used antiepileptic drug with high prevalence of cADRs, a limited number of studies have been reported so thus; in the European cohort, the most recent PGx suggested that HLA allele were not associated with LTG-induced cADRs as well as with particular genes.
In this study, we aimed to identify susceptibility loci associated with cADRs induced by LTG in the Japanese population through PGx approach.

**Methods**
We conducted a PGx study (Affymetrix 6.0 chip) in 35 subjects with LTG-induced cADR (SJS, TEN, maculopapular exanthema) and in 151LTG tolerant controls. After the quality control, 630,378 SNPs were eligible for the association study.

**Results**
One SNP showed significant association with genome-wide significant level (rs8076290 in nucleoredoxin (NXN): \( P=4.55 \times 10^{-8} \), genomic-control adjusted). However none of the peak was observed around HLA region on chromosome 6.

**Discussion**
Although our sample size was limited, we detected risk SNP in NXN for LTG-induced cADRs. NXN is a key regulator of Wnt/β-catenin, TLR4/MyD88 pathway and Wnt/PCP pathways, however its precise mechanism linking to cADR is unclear. These results also suggest that HLA alleles do not play a major role on this condition, different from the results for CBZ. Replication study, especially using different population, will be essential for conclusive results.

AN UPDATE FROM THE CONSORTIUM ON LITHIUM GENETICS (CONLIGEN): PHENOMIC AND GENOMIC STUDIES

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**Background**
Lithium remains a mainstay in the long-term treatment of BD. Response to lithium is variable. About 30% of patients treated with lithium have fewer illness episodes over time, while about 20% have no response. The remaining 50% can be classified as partial responders. Data from pharmacogenetic studies of lithium are comparatively sparse, and these studies have generally employed small sample sizes and varying definitions of response. Genetic markers of lithium response would be valuable for treatment planning and could provide insights into the biological mechanism of lithium action. To put that idea into practice, the international Consortium on Lithium Genetics (www.ConLiGen.org) was established.

**Methods**
In a first wave analysis, ConLiGen studied 1080 European and European American lithium-treated bipolar disorder (BD) patients. All patients were characterized for lithium response with an 11-point treatment response scale (“Alda Scale”, Grof et al. 2002). The Alda Scale assesses clinical improvement attributable to lithium, taking into account the history and frequency of episodes, duration of treatment, medication adherence, and concurrent treatment. Phenotype definitions were developed by consensus within ConLiGen. 341 cases met criteria for excellent responders and 739 for partial/non-responders. The whole sample was genotyped using Illumina arrays to perform a genome-wide association study (GWAS) of lithium response.
**Results**

Inter-rater reliability of lithium response assessment was good, with kappa values >0.7. Given a responder rate of 35%, the ConLiGen sample had >80% power to detect a common allele conferring a genotype relative risk of response of 1.5, at genome-wide significance. GWAS genotyping was completed at excellent call rates (>99% of samples have a call rate >98%). While no genome-wide significant finding at the p < 5 x 10^{-8} level was observed, the top hit SNP rs17728078 in the gene SLC4A10 (p=9.59 x 10^{-6}) yielded an odds ratio of 1.58, which is quite uncommon for complex phenotypes, and represents a common allele at a minor allele frequency of ~0.4, increasing the chances at replication in an independent sample.

**Discussion**

Our finding in the SLC4A10 (solute carrier family 4, sodium bicarbonate transporter, member 10) gene is promising as this gene belongs to a small family of sodium-coupled bicarbonate transporters (NCBTs) that regulate the intracellular pH of neurons, the secretion of bicarbonate ions across the choroid plexus, and the pH of the brain extracellular fluid. However, replication of this finding in additional samples will be crucial to establish it as true susceptibility factor for lithium response. Within the framework of ConLiGen, about 2000 new samples from Europe, North America, Asia, and Australia are currently being GWAS-genotyped for that purpose. Results shall become available in the summer of 2013.

Reference


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**A HYPOTHESIS DRIVEN ASSOCIATION STUDY OF 28 NUCLEAR-ENCODED MITOCHONDRIAL GENES WITH ANTIPSYCHOTIC-INDUCED WEIGHT GAIN IN SCHIZOPHRENIA**

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**Background**

Antipsychotic-induced weight gain (AIWG) is an important phenotype that often leads to obesity and metabolic syndrome. Genetic factors play a significant role in its development and several biological systems has been showed to influence AIWG. Mitochondria are the main source of energy for neurons and play a role in many of the neuronal functions. Studies have reported that atypical antipsychotics such as clozapine and olanzapine appear to influence gene expression in mitochondria although the molecular mechanisms (including a possible genetic predisposition) by which each drug alters mitochondrial function and metabolism are poorly understood. In our study, we investigated the hypothesis that nuclear-encoded mitochondrial genes, particularly those with altered gene expression or involved in oxidative stress, mitochondrial biogenesis, inflammation and apoptosis, would be associated with antipsychotic-induced weight gain (AIWG).
Methods
In total, we selected 28 genes and analyzed 60 SNPs, most of them classified as functional or regulatory elements, in schizophrenia subjects (N=164), treated with atypical medications for up to 14 weeks. Single-SNP genetic association was tested using linear regression with percentage of weight gain from baseline as the dependent variable and treatment duration and baseline body weight as covariates. Assuming MAF of 0.15, we had more than 80% power to detect a mean difference of 2.4% between carriers and non-carriers of the risk genotype in the additive model at alpha 0.05. Several multi-SNP analyses were also carried out, including haplotype analysis (UNPHASED v3.1.5), stepwise linear regression (SPSS), and gene-gene interactions (mbmdr). The statistical strength of our biological hypothesis was measured by comparing the sum of the observed association evidence across all 60 SNPs with the value expected under the null based on a phenotype permutation method (10,000 permutation replicates).

Results
We observed a significant association between rs6435326 in the NDUFS1 gene and percentage of weight gain, even after correction for multiple testing (N= 164, b= -2.19, \( P_{corrected} = 0.02 \)). The haplotype carrying the risk alleles for rs6435326 and two other SNPs (rs1053517 and rs1801318) in NDUFS1 was also significantly protective with weight-gain (%) (T-T-G versus A-C-A, \( P = 0.005 \)). In addition, we observed a significant interaction between the TT risk genotype of rs6435326 in NDUFS1 and AG genotype of rs3762883 in COX18 (\( P_{corrected} = 0.001 \)). Finally, permutation-based test showed that the set of 60 SNPs from the 28 nuclear-encoded mitochondrial genes selected based on our hypothesis, collectively, was associated with weight gain (\( P = 0.02 \)).

Discussion
To the best of our knowledge, this is the first study to explore genetic variation in the mitochondrial genes in the context of AIWG. We observed significant association between SNPs in NDUFS1 and weight gain (%). NDUFS1 is part of the hydrophilic arm of the complex I of the oxidative phosphorylation system and it is responsible for the transfer of electrons to ubiquinone. Variation in genes of the electron transfer chain may lead to dysfunction in the cellular oxidative metabolism and increase mitochondrial ROS (mROS) production. The understanding of the effect of variants on ROS production may be of special importance since it may influence the energy homeostasis acting as “fuel sensing” in the hypothalamus. Thus, this study provides evidence implicating mitochondrial genes to be involved in the regulation of energy homeostasis and body weight in schizophrenia subjects under atypical antipsychotic treatment.

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GENETIC VARIATIONS WITHIN METALLOPROTEINASES IMPACT ON THE PROPHYLAXIS OF DEPRESSIVE PHASES IN BIPOLAR PATIENTS
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Background
Depressive bipolar phases are particularly difficult to treat. Genetic variations that segregate in responders to current treatments may inform the metabolic events that are associated with response. In the present paper we investigated the role of a set of genetic variations located in
genes that code for metalloproteinases towards the number of depressive events for visit unit in a sample of bipolar patients. Metalloproteinases are essential enzymes for communication among brain cells. They shape the extracellular environment and there is evidence that they are involved in neurodegenerative diseases such as Multiple Sclerosis, Amyotrophic Lateral Sclerosis, Alzheimer's and Parkinson's disease. STEP-BD patients (phase 1 of the study) were the sample under analysis.

Methods
Data were available from the NIMH database. The phenotype under analysis was the number of depressive events for visit unit. This was defined to limit the possible stratification of a different number of visits per patient and to enroll the higher number of patients in the analysis. Care was taken in checking the correlation between the number of depressive events for visit unit and the number of depressive events after 30, 60, 90 and so forth until 990 days from baseline. 653 subjects were analyzed. After quality control, imputation and pruning, 43 SNPs from 17 genes (MMP1 to 17) entered the model as predictors of the number of depressive events for visit unit. Clinical and sociodemographic variables entered the model when significantly associated with the outcome.

Results
rs486055 (exonic in MMP10) was associated with the number of depressive events corrected for the number of total visits during the period of observation (p=0.0004; T=3.68, corrected significant threshold=0.001). In particular, TT homozygotes had 5.08±3.51 events, CT had 3.47±3.18 and CC had 2.57±2.96 depressive events corrected for times having being assessed during the period of observation.

Discussion
We found suggestive evidence that variations located in metalloproteinases may interfere with the number of depressive events during bipolar disorder. Nevertheless, due to the limits of the present study, further independent analyses are required.

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THE SEROTONIN 2A RECEPTOR SINGLE-NUCLEOTIDE POLYMORPHISM RS7330461 IS A GENETIC BIOMARKER OF TREATMENT RESPONSE TO THE METABOTROPIC GLUTAMATE 2/3 RECEPTOR AGONIST, POMAGLUMETAD METHIONIL, IN PATIENTS WITH SCHIZOPHRENIA
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Background
Previously, we used genetic samples collected from clinical trial H8Y-BD-HBBBD to discover and then subsequently replicate, in clinical trial H8Y-MC-HBBR, a genetic association between the serotonin 2A receptor gene (HTR2A) single-nucleotide polymorphism (SNP), rs7330461, and response to treatment with the metabotropic glutamate 2/3 (mGlu2/3) receptor agonist, pomaglumetad methionil (LY2140023 monohydrate) in non-Hispanic white (NHW) patients with schizophrenia. A significantly greater response to treatment was observed in patients
carrying T-alleles for rs7330461 in HTR2A compared with A/A homozygotes. The objectives of this genetic study, which utilized patient data from HBBR, an additional Phase 2 study (H8Y-MC-HBBM), and 2 Phase 3 studies (H8Y-MC-HBBN and H8Y-MC-HBDE) were to: 1) assess the association of rs7330461 with treatment response to LY2140023; 2) assess the treatment effect (LY2140023 vs. placebo (PBO) comparison) within NHW patients carrying T/T genotype at rs7330461; and 3) evaluate the efficacy of LY2140023 versus standard of care (SOC) treatment in the same population.

Methods
We used integrated data from 4 clinical trials (H8Y-MC-HBBR, H8Y-MC-HBBM, H8Y-MC-HBBN and H8Y-MC-HBDE): a 24-week, active comparator-controlled, Phase 2 clinical trial which examined safety of a target dose of 40mg LY2140023 vs. SOC treatment in adult patients with schizophrenia (HBBR); a 6-week, double-blind (DB), placebo- and active comparator-controlled, Phase 2 clinical trial which examined efficacy of LY2140023 (40 and 80 mg BID) in adult patients with schizophrenia (HBBM); a 6-week, fixed-dose, DB, placebo-controlled, Phase 3 study of LY2140023 (10, 40, or 80 mg BID) in adults with an acute exacerbation of schizophrenia (HBBN); and a DB, Phase 3 study comparing 24 weeks of flexibly dosed LY2140023 (20, 40, or 80 mg BID) with flexibly dosed aripiprazole (10, 15 or 30 mg/day) in adult patients with schizophrenia (HBDE). The HTR2A SNP, rs7330461, was genotyped. A total of 841 NHW patients (LY2140023 40 mg, n=422; PBO, n=217; and SOC, n=202) were included in the integrated genetic analysis. Only patients treated with LY2140023 40 mg BID were included in this analysis. The primary outcome, change from baseline in the Positive and Negative Syndrome Scale (PANSS) total score, was assessed for T/T, A/T, and A/A genotype groups of the HTR2A SNP rs7330461 in NHW patients using mixed-model repeated measures (MMRM) over 6 weeks. Secondary endpoints included PANSS subscale scores and Clinical Global Impression of Severity (CGI-S). Multiple comparisons with the best was applied as a way of multiplicity adjustment to identify the most responsive genotype group. Treatment effect (LY2140023 vs. PBO and LY2140023 vs. SOC) within each genotype was also assessed based on MMRM analyses over 6 weeks.

Results
NHW T/T homozygous patients treated with LY2140023 (n=50) responded significantly better than A/A (n=184) and A/T (n=191) patients in PANSS Total score and PANSS Negative and PANSS General Psychopathology subscales at Week 6 (error rate <0.05). A statistically significant treatment effect between LY2140023 40 mg BID and PBO in PANSS Total score was observed in the T/T group (n=20 and n=29 for LY2140023 and PBO, respectively) at Weeks 4 (1-sided P=.024) and 6 (1-sided P=.002); no statistically significant treatment effects were observed in the A/T or A/A groups. Secondary analysis revealed that a statistically significant treatment effect was observed in the T/T group in PANSS General Psychopathology subscale and CGI-S at Weeks 4 and 6 (1-sided P<.024). With respect to the treatment comparison between LY2140023 and SOC in PANSS Total score, the treatment response to LY2140023 was observed to be numerically better than SOC in the T/T group, although not statistically significant (n=48 and n=24 for LY2140023 and SOC, respectively, 2-sided P=.222). In the A/T group, the treatment response to LY2140023 was significantly less effective than in SOC (2-sided P=.011). In the A/A group, the treatment response to LY2140023 was numerically, but not significantly less effective than SOC (2-sided P=.172).
Discussion
These data provide further evidence of a subpopulation defined by a genetic biomarker in which pomaglumetad methionil shows potential efficacy in the treatment of schizophrenia. Further investigation is required to fully understand the functional basis for the association between HTR2A rs7330461 and response to treatment with pomaglumetad methionil. In addition, it will be important to examine the efficacy of this compound in a prospective study conducted within patients carrying the T-allele(s).

ANALYSING EXOME CHIP GENOTYPE DATA IN SCHIZOPHRENIA
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Background
Schizophrenia is a psychiatric disorder with a lifetime risk of around 1% and a large genetic component. Previous genotyping studies have focused on common variation, but the creation of chips enriched for exome variants, many at low frequency, allows examination of association for rarer variants. A major problem when analyzing rare variants is low power.

Methods
Our study was performed on 6991 UK cases (we call these CLOZUK and Cardiff Cognition samples) and 9067 controls (UK Blood Service and additional controls from the UK exome chip consortium). The case samples were genotyped at the Broad Institute on two different versions of Illumina arrays. Quality control analysis was performed on genotypes called by the GenCall algorithm, followed by a further round of QC on those genotypes called by z-Call algorithms. Manual inspection of clusterplots revealed additional SNP filters were required to get an acceptable QC’d dataset. These included filtering by mean probe intensities or filtering by difference in mean intensities between genotype clusters. We also performed comparisons of allele frequencies in cases typed on each type of chip to identify and remove variants called differently between chips. Analyses were based on logistic regression association tests (both with and without population covariates derived from PCA) and Fisher’s exact test.

Results
After quality control, we retained 5629 cases, 8442 controls, and 142841 SNPs. One locus was genome-wide significant. 22 variants were associated at p<1x10^-5 using logistic regression and 24 with Fisher’s exact test. Most were common SNPs, but 4 variants with a minor allele frequency under 1% were associated at p<1x10^-5 using Fisher’s exact test, two of which were not significant using under logistic regression.

Discussion
Our analyses found relatively few rare variants that were individually significant. As our study had excellent power (0.83) to detect at GWAS alleles with a population frequency of 0.001 that confer an OR of 5, either such alleles are not represented on the chip or they make little contribution to schizophrenia. The standard logistic regression analysis used for common variant
GWAS does not allow for the analysis of very rare variants, and methods such as Fisher’s exact test should be considered for an exhaustive analysis of rare variants. Using collapsing or gene-based analyses to combine information from multiple SNPs may improve power, and the results of such analyses will be presented.

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**SRC KINASE IS A POINT OF CONVERGENCE FOR ABNORMAL SCHIZOPHRENIA GENE SUSCEPTIBILITY PATHWAYS LEADING TO NMDA RECEPTOR HYPOFUNCTION**

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**Background**

Multiple lines of genetic evidence support the NMDA receptor (NR) hypofunction hypothesis of schizophrenia (SCZ). Previous association studies have identified DTNBP1, DISC1, ERBB4, NRG1, and PTPRA, whose products regulate NR signaling pathways. Meta-analyses of GWAS and de novo CNV studies have shown enrichment of risk variants in the NR pathway. These findings led to the notion that various susceptibility factors may converge on the NR pathway in SCZ (Kantrowitz et al., 2009; Kirov et al., 2012; Owen et al., 2005). It is unknown, however, how these susceptibility genes and pathways conspire to induce NR hypofunction in SCZ.

Previously, we reported that ligand-induced tyrosine phosphorylation of NR2 subunits, critical for sustained enhancement for NR activity, was decreased in the DLPFC of SCZ subjects compared to controls (Hahn et al., 2006). In this study, we sought to identify the proximal molecular cause(s) of NR hypofunction in SCZ cases with particular attention to NR2 tyrosine phosphorylation and Src kinase, which are critical for synaptic plasticity.

**Methods**

We have analyzed NR function and its molecular underpinnings in postmortem brain tissues of patients with SCZ and their matched controls and of mice mutated for SCZ susceptibility genes. To overcome the technical challenges posed by postmortem tissue, we employed several novel methodologies we have recently developed to accurately quantify NR signaling molecules, their interactions in response to NMDA, and the enzyme activity of Src in human brain tissue (Hahn et al., 2009; Hahn et al., 2006; Kalia et al., 2006). A matched pairs design was used to minimize the effects of age and sex in observed differences between SCZ and control cases. We focused on the DLPFC because disruption of this brain area is common in SCZ (Glausier and Lewis, 2012; Lewis et al., 2004), contributes to deficits in selective attention, working memory, and executive function in this disorder (Glausier and Lewis, 2012; Goghari et al., 2010; Lesh et al., 2011); and has been demonstrated to display NR hypofunction in SCZ (Hahn et al., 2006). PFC tissues from homozygous sdy mice containing a spontaneous autosomal recessive mutation deletion in in Dtnbp1 (dysbindin) gene segment (Cox et al., 2009), and from ptpα-/ null mice generated by inserting a IRES-β-geo cassette on exon 3 corresponding to extracellular domain of RPTPα gene on C57Bl/6J background (Takahashi et al., 2011) were provided by Jan Sap, University of Paris.

**Results**

We observed striking hypofunction of post-receptor NR signaling in the dorsolateral prefrontal
cortex (DLPFC) of SCZ cases, which is attributable to concomitant attenuation of PKC, PLCg and Src. SCZ cases exhibit Src hypoactivity independently of other kinases, associated with decreased Src’s binding capacity for its activators and its association with binding partners. These together suggest that Src hypoactivity plays a causal role in postreceptor NR hypofunction in SCZ. To delineate how SCZ susceptibility genes impair NR function, we examined NR function and Src activity in dys/- and ptpa +/- mice as well as in postmortem brain tissues. We found that elevated neuregulin 1–erbB4 signaling, reduced dysbindin-1, and loss of RPTPα all led to decreased tyrosine phosphorylation of NR2 subunits as well as Src hypoactivity as shown in the DLPFC of SCZ cases.

Discussion
Our postmortem study results indicate that post-receptor NR function is decreased in the DLPFC of SCZ cases, for which Src hypoactivity may play a causal role. Considering that SCZ is a complex trait disorder, in which multiple etiologic factors interact and precipitate pathophysiologic substrates for the illness, NR hypofunction should be traceable to etiologic factors that may confer susceptibility for the illness. Our results indicate that at least three etiologic factors implicated in SCZ (NRG1-ErbB4, dysbindin-1, and RPTPα) decrease NR function and Src activity. Signaling mediated by each of these three risk factors has been found to be dysregulated in the postmortem brains of schizophrenia patients (Hahn et al., 2006; Takahashi et al., 2011; Tang et al., 2009). Our data therefore suggest that subtle alterations of these factors could be additive towards dysregulation of Src hypoactivity. We thus propose that Src hypoactivity is a point of convergence for multiple etiologic factors leading to NR hypofunction and be considered as a potential therapeutic target in SCZ.

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NOVEL RARE VARIANTS IN F-BOX PROTEIN 45 (FBXO45) IN SCHIZOPHRENIA
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Background
F-box protein 45 (FBXO45), known as an ubiquitin ligase, has been showed to be critical for synaptogenesis, neuronal migration, and synaptic transmission. FBXO45 is included in the 3q29 microdeletion region that has been demonstrated to confer a significant risk for schizophrenia (SCZ) by rare structural variant studies. Thus, FBXO45 can be considered as a prominent candidate for pathogenesis of SCZ. In this study, we investigated rare and deleterious single nucleotide variants, small insertions and deletions (INDELs) in FBXO45 that might contribute to susceptibility to SCZ.

Methods
We performed mutation screening in exon regions of FBXO45 using Sanger sequencing in 337 SCZ patients. Novel missense or nonsense variants were followed up by genetic association study in an independent sample set consisting of 601 SCZ patients and 916 controls, case report
for assessing the clinical consequence of the mutations, pedigree study for measuring the inheritance of the mutations in the proband’s family, bioinformatical analyses for evaluating the mutations on protein structure and function, and expression analysis for examining the transcriptional influence of the mutation on FBXO45 gene expression.

**Results**

One heterozygous, novel, and rare missense mutation (R108C) was identified in a single SCZ patient. The same mutation was detected from his healthy mother. This patient was diagnosed with paranoid type of SCZ at the age of 20 and some clinical features of 3q29 deletion phenotypes including premorbid IQ decline. By follow-up genotyping, it was found in neither SCZ group (0/601) nor healthy control group (0/916). Bioinformatical analyses predicted R108C had probably pathological impact on both structure and function of FBXO45 protein. By gene expression analysis, FBXO45’s relative expression of R108C case was extremely down-regulated in comparison to 21 SCZ patients and in comparison to 22 healthy controls.

**Discussion**

The rare and potentially deleterious R108C variant in FBXO45 was most likely to disrupt FBXO45 protein structure and function, and might be a novel genetic risk factor for SCZ. In addition, our findings pointed towards FBXO45 could be a new attractive candidate gene for SCZ.

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**MAPPING RECESSIVE RISK VARIANTS FOR SCHIZOPHRENIA IN AN INBRED POPULATION**

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**Background**

Rare genetic variants are coming to the fore as risk factors for common diseases. Ancestral chromosomal segments flanking the mutations are passed down (identical by descent, IBD). For recessive diseases, the IBD segments are by definition homozygous by descent (HBD). Genotyping affected individuals can definitively identify HBD regions harboring disease mutations. This has been used successfully for many rare Mendelian diseases. We are evaluating its utility for Schizophrenia (SZ), because our recent studies have shown that SZ is associated with consanguinity in Egypt, OR 3.53 (95% CI 1.88, 6.64).

**Methods**

We genotyped common SNPs across the genome among 94 Egyptian SZ cases and 88 Egyptian controls, using the Affymetrix 6.0 array. Genotypes and CNVs were called using Birdsuite v1.5.5 (Korn, Kuruvilla et al. 2008) and Penn CNV (Wang, Li et al. 2007). SNPs were discarded if the missing data rate exceeded 5%, individual were discarded if missing data rate exceeded 2% or if Hardy-Weinberg expectations were violated (p<0.0001). Using a total of 779,823 autosomal SNPs, we estimated HBD segments using Beagle software and computed the
frequencies of each HBD segment among cases and controls (Figure below). Using Beagle-specified default settings, a threshold score of 0.5 was used to call each HBD SNP/segment.

**Results**

Averaged across the genome, there is a twofold increase of HBD among SZ cases compared with controls (2.1% vs 1.0%). The cases also had more HBD segments on average (cases: mean 11.9, standard deviation, SD=8.7; controls mean=8.6, SD=6.7). Consistent with the analysis of overall homozygosity, consanguineous cases had more and longer segments HBD than any other group, including consanguineous controls. We also searched for CNVs among cases, using the following stringent criteria: incorporate more than 30 probes, CNVs must be longer than 10kb and detected with both Birdsuite and PennCNV software. Even in this relatively small sample, we detected CNVs known to be associated with SZ, including 15q11.2 del, 17q12 dup and CNTN4/CHL1 del (n = 1, each). We have verified the CNVs using qPCR assays.

**Discussion**

Consanguineous SZ cases are more likely to have longer homozygous segments. Our analysis showed an HBD segment on 15q region that shows intriguing case-control differences. HBD analysis may help detect recessively inherited chromosomal regions in SZ.

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**SOCIODEMOGRAPHIC AND MORBIDITY CHARACTERISTICS OF FIRST DEGREE RELATIVES OF PROBANDS WITH SCHIZOPHRENIA: A COMPARISON WITH MOOD DISORDER AND HEALTHY CONTROL**

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**Background**

Schizophrenia is a disorder which has a considerable hereditary component. However, while several studies in non-African populations have characterized the demographic and morbidity features of first degree relatives of patients with schizophrenia, there is paucity of data from African populations.

**Methods**

The study, conducted at the Federal Neuropsychiatric Hospital, Lagos, Nigeria, examined 330 first degree relatives of probands with schizophrenia (n = 50), 350 first degree relatives of probands with mood disorder (n = 50) and 387 first degree relatives of healthy control (n = 50). The Schedules for Clinical Assessment in Neuropsychiatry, SCAN was used to ascertain diagnosis in ill subjects. To each subject a sociodemographic questionnaire was administered. Family history was obtained using the Family History Schedule. Morbid risk estimates were calculated using the Weinberg shorter method.

**Results**

There was a significant difference between the mean age of relatives of schizophrenia probands compared to mood disorder (p = 0.004, 95% CI 1.34 – 9.61) and healthy control (p = 0.004, 95% CI 1.53 – 9.84). There were also significant differences between the number of children of schizophrenia probands and the number of children of normal control (p = 0.002, 95% CI -2.0 to -3.9), as well as the number of deceased first degree relatives of schizophrenia probands.
compared to normal control (p = 0.038, 95% CI 0.01 to 0.94). Finally, there was a significant difference between the number of first degree relatives of schizophrenia probands compared to the number of first degree relatives of healthy control who were below the age of risk for schizophrenia (p = 0.013, 95% CI -1.27 to -0.12). Morbid risks of 4.38 and 0.39 were obtained for schizophrenia among first degree relatives of probands with schizophrenia and mood disorder, while first degree relatives of probands with schizophrenia, mood disorder and healthy control had morbid risks for mood disorder of 0.42, 3.82 and 0.35 respectively.

Discussion
The finding of significant differences between the mean age of schizophrenia first degree relatives than the other two groups may simply be because of the tendency of the schizophrenia patients to remain single and have fewer children – another finding of significance from the study. In keeping with this also, there was a significant difference between the number of schizophrenia patients below the age of risk for schizophrenia, when compared with healthy control. This however did not apply to mood disorder. The number of deceased first degree relatives of schizophrenia probands significantly exceeded that of healthy control, which appears to support earlier assertions of excess mortality among relatives of patients with schizophrenia. With regard to morbidity, first degree relatives of probands with schizophrenia and mood disorder have higher morbid risks for these psychotic conditions than healthy control, which supports other studies which suggests that these conditions breed true. However, there is also a considerable overlap, in keeping with emerging findings of shared genetic substrate.

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TRANSCRIPTOME ANALYSIS OF LYMPHOBLASTOID CELL LINE IN THE JAPANESE SCHIZOPHRENIC PATIENTS
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Background
Schizophrenia is a chronic and disabling mental disorder with a lifetime prevalence of approximately 1% of the global population. Although a number of studies have shown several convincing candidate genes or molecules, the pathophysiology of schizophrenia has not been completely elucidated. Alternative splicing contributes to regulate cell signaling in the nervous system both during development and after maturation. Dysregulation of alternative splicing may cause abnormal neural development and could be related to the mechanism of neuropsychiatric disorders underlying both phenotypic diversity and genetic susceptibility. In the present study, we conducted transcriptome analysis of lymphoblastoid cell lines (LCLs) derived from schizophrenic patients and healthy controls to investigate dysregulation of alternative splicing and to identify genes/pathways involved.

Methods
The samples for transcriptome analysis consisted of 30 patients with schizophrenia and 30 healthy control subjects. All subjects were unrelated to each other and ethnically Japanese. The
schizophrenia diagnosis was made by at least two experienced psychiatrists and based on unstructured patient interviews and reviews of their medical records in accordance with the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision criteria. All control subjects were also psychiatrically screened based on unstructured interview. This study was approved by the Nagoya University Graduate School of Medicine and Nagoya University Hospital Ethics Review Committee. Written informed consent was obtained from each subject. Peripheral blood lymphocytes derived from 30 patients with schizophrenia and 30 healthy controls were transformed to LCLs by the widely used Epstein-Barr virus method. The transcriptome profiles of both gene- and exon-level expression were detected by using the Affymetrix GeneChip Human Exon 1.0 ST Array. To investigate the differentially expressed exons and transcripts, analyses of covariance (ANCOVA) were performed, controlling for age, gender, and batch. Differential alternative splicing patterns between schizophrenia and controls were identified using ANOVA.

Results
There were 142 differentially expressed genes (raw P < 0.01, fold change > |1.2|) between schizophrenia and controls in the gene-level analysis. In the exon-level, 197 exon probe sets were differentially expressed (raw P < 0.0001, fold change > |1.2|) between schizophrenia and controls. Although the unsupervised hierarchical cluster analysis and principal component analysis for 142 genes did not separate the schizophrenia from the control subjects, the 197 exons separated schizophrenia patients from healthy control subjects.

Discussion
We found the subtle differences in gene and exon level analysis between schizophrenic patients and healthy controls. The top hit genes were tested using pathway and hierarchical clustering analysis. In addition, we observed a number of regulatory SNPs (expression quantitative trait loci-eQTL) by integrating data from genotype and expression wise analysis. As a result, the genes which expression was affected by the disease status may be associated with molecular mechanisms that contribute to the pathophysiology of schizophrenia. These preliminary findings might provide insight into the pathophysiology of schizophrenia and potentially provide prognostic and diagnostic biomarkers. However, the findings are tempered by the small sample size and multiple comparisons and require confirmation using PCR or deep RNA sequencing and a much larger patient population.

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GENOME-WIDE ASSOCIATION STUDY OF GROWTH RATE AND ENERGY STATUS IN LYMPHOBLASTOID CELL LINES
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Background
Transformation of B-lymphocytes with Epstein-Barr virus (EBV) produces lymphoblastoid cell lines (LCLs), which are the largest renewable source of DNA for genotyping and sequencing, e.g., the NIMH collections of LCLs at Rutgers University Cell and DNA Repository. Furthermore, due largely to limitations of available living brain tissue, LCLs are commonly used
as a cellular model to study the effects of genetic variation in neurologic and in psychiatric disorders, such as for the study of gene expression profiles. The determinants of growth rate and energy status in LCLs, whether genetic or environmental (e.g., epigenetic, transformation methods), or both, remain largely unknown, though growth rate has been reported to have 30–39% heritability. However, growth rate and energy status vary across LCLs and strongly influence the transcriptional abundances of many genes (and of many other cellular phenotypes). We report here a systematic analysis of determinants of growth rate and energy status in LCLs.

Methods
We indexed growth rate by final cell count after a standardized 8-day growth protocol and normalization to equal cell counts (250,000 cells/ml) 24 hours prior to harvest and assays, and we indexed energy status by ATP level assay adjusted by cell count. To detect loci influencing the cellular traits of growth rate and energy status, we performed a GWAS (genome-wide association) study using samples (N=2,060) from the European ancestry (EA) portion of the MGS (Molecular Genetics of Schizophrenia) case-control collection with transformation site, age, EBV load, sex, caseness, and ancestry PCs (principal components) as covariates. For the top 500 most associated SNPs for each trait, we extracted their genes (or if intergenic, the genes closest on each side), resulting in 429 genes (growth rate) and 383 genes (energy status), which we then submitted to pathway analyses: Gene Ontology (GO)-term enrichment analysis using the DAVID tool.

Results
The genomic inflation factors for the growth rate and the energy status GWAS were 1.000 and 1.014, respectively, indicating little cryptic population substructure. We found some associations for growth rate with age (p=0.018), sex (p=1.7×10^{-6}), and EBV load (p=8.3×10^{-5}), and for energy status with sex (p=1.8×10^{-4}), caseness (p=0.0076), transformation site (p=0.024), and EBV load (p=0.0090), suggesting the utility of including such covariates in the analysis. We found no genome-wide significant (GWS) association for either growth rate or energy status. For growth rate, the strongest association with additional support was at 6q12 with rs7750067 (p=5.9×10^{-7}), with supportive findings (six SNPs with 5×10^{-6}<p<10^{-4}) over this intergenic region (spanning chr6:67,747,733-67,867,582; hg19). This region contains a predicted microRNA (miRNA), ENSG00000266073 (chr6:67,859,402-67,859,492). The nearest RefSeq genes are SLC25A51P1 (solute carrier family 25, member 51 pseudogene 1; 1.3 Mb centromeric), EYS (eyes shut homolog (Drosophila); 1.3 Mb centromeric), and BAI3 (brain-specific angiogenesis inhibitor 3; 1.5 Mb telomeric). EYS contains multiple epidermal growth factor (EGF)-like and LamG domains and is implicated in retinitis pigmentosa. BAI3 encodes a brain-specific angiogenesis inhibitor, which is an adhesion-G protein-coupled receptor (aGPCR) and has been shown to control dendritic arborization growth and branching in cultured neurons. Both EYS and BAI3 are expressed in various tissues, including brain and blood. Pathway analyses revealed the most enriched GO term for growth rate to be neuron projection (GO:0043005; FDR=0.08), and for energy status two were significant: regulation of cell proliferation (GO:0042127; FDR=0.0067) and neuron differentiation (GO:0030182; FDR=0.042).

Discussion
We describe the effect of epidemiological variables and of specific genomic loci on growth rate and energy status in LCLs. Our results have potential to lead to a better understanding of these
important cellular traits. We will present data on the expanded sample of LCLs at the meeting, projecting to reach ~2,500 EA subjects, along with ~1,000 African American subjects.

RELATING EMERGING SCHIZOPHRENIA GENES TO RDOC COGNITIVE SYSTEMS IN A CHILD CLINICAL COHORT

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Background
The past three years have yielded unprecedented progress in understanding the genetic bases of psychiatric illness. Because the DSM categories on which these recent studies are based contain heterogeneous and overlapping features, a critical next step for the field is to clarify the relationship between emerging genes and specific components of illness. Rigorous phenotypic dissection of this sort, particularly at different developmental periods, will help improve models of the unfolding of genetic liability, clarify targets for early intervention and contribute to a more pathophysiologically-relevant taxonomy. Recently, the NIMH has developed Research Domain Criteria (RDoC) to articulate dimensions of psychopathology that cut across traditional diagnoses and reflect extant empirical data better than DSM constructs.¹ Along with emphasizing dimensional components of the current diagnostic system, an important byproduct of RDoC and its precursors² is the elevation of cognition to a target of investigation in studies of psychopathology. The current analyses aim to examine emerging schizophrenia (SCZ) genes in relation to RDoC-informed cognitive systems. Genetic dissection of this debilitating mental illness is a public health priority, and there is a strong rationale for targeting cognition. Components of RDoC cognitive systems show large decrements in patients.³ Prospective studies suggest that such impairments emerge at the earliest phases of disease, and studies of unaffected relatives and twins suggest they reflect genetic liability.⁴-⁶ Our primary hypothesis is that, by compromising the function of the synapse, a large fraction of the genetic risk relevant to psychosis undermines aspects of higher-order cognition, particularly working memory and cognitive control, given their dependence on widely-distributed, prefrontally-mediated networks. Consistent with RDoC tenets, we contend that these impairments are not specific to a single form of psychopathology, but rather cut across conventional categories. Thus, we predict that SCZ-relevant genetic associations to cognitive systems will be evident within a clinical cohort of children and adolescents referred for evaluation of cognitive dysfunction.

Methods
Our group is collecting and characterizing a large cohort of youth uniquely suited to reveal the relationship between emerging genes for psychiatric illness and RDoC cognitive systems. To date, we have recruited over 500 youth, ages 7 to 17, with psychiatric symptomatology referred for 4 to 6 hours of neuropsychological testing due to suspected cognitive dysfunction. We have supplemented the cognitive and psychiatric measures administered as part of patients’ clinical evaluations to create a uniform data set of unprecedented depth and breadth of cognitive and
psychiatric phenotypes. Measures cover a range of cognitive systems delineated within the 
RDoC framework, including working memory and cognitive control. Using the Illumina 2.5 
array, we conducted genomewide genotyping on a subsample of 156 subjects and parents of 116 
of these patients to carry out proof-of-concept analyses. We implemented the MQFAM method² 
in PLINK to examine the multivariate association between relevant cognitive traits and genes 
emerging from recent mega-analyses of SCZ.⁸ We also assessed the ability of the SCZ polygenic 
risk score from this analysis to predict cognitive and clinical phenotypes. Finally, we examined 
rates of rare de novo copy number variants (CNVs) using a conservative method based on the 
convergence of two widely-used software tools (Birdsuite and PennCNV).

Results
In this child psychiatric sample enriched for cognitive dysfunction, common schizophrenia-
related variants showed a relationship to cognitive traits as well as indices of severity and 
psychopathology. We also found an elevated rate of de novo CNVs in the cohort.

Discussion
These preliminary data support a relationship between emerging SCZ-related risk variants and 
cognitive systems. Thus, they also corroborate a key tenet of RDoC that genetically-influenced 
dimensional components of psychopathology cut across current diagnostic boundaries. 
Additionally, these findings suggest that our growing sample of well-characterized, clinically-
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DE NOVO VARIANTS IN SYNAPTIC INTERACTION NETWORKS
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Background
Exome sequencing studies are identifying a growing number of rare de novo mutations in individuals suffering from psychiatric disorders. Only a proportion of such variants are likely to contribute to disease, being distinguished from other mutations by their convergence upon relevant functional pathways. By identifying physically interacting proteins enriched for rare de novo variants, it may prove possible to both isolate subsets of pathogenic mutations and refine our understanding of disease biology. Here we investigate the distribution of de novo mutations found in probands with schizophrenia over a set of interacting synaptic proteins. Comparisons are made with other disorders (autism and intellectual disability) where perturbation of synaptic function may play a role.

Methods
Protein-protein interactions (PPIs) involving genes hit by de novo mutations found in 623 schizophrenia proband-parent trios were identified using a public database (SynSysNet) containing 4068 non-self interactions between the unique protein products of 829 synaptic genes. Interactions were also extracted for de novo mutations uncovered by previously published studies of autism and intellectual disability. Interaction networks were constructed for the various disorders and their general properties compared.

Results
Of 463 genes hit by non-silent de novo mutations in schizophrenia probands, PPI data was available for 33 (7%). Interactions could also be identified for a similar proportion of de novo hit genes from individuals with autism (55 genes, 7%) and their unaffected siblings (24 genes, 6%), and slightly more of those from individuals with intellectual disability (18 genes, 13%). Multiple direct interactions between de novo hit genes were found for each of the disorders, but not for unaffected siblings. These interaction networks all contained elements central to the glutamatergic signalling machinery. When proteins mediating interaction between those hit by de novos were collated, virtually all de novo hits could be linked together. Schizophrenia and intellectual disability hits were found to interlink with a larger proportion of synaptic proteins than autism hits, suggesting differences in their ability to influence synaptic function.

Discussion
Our results indicate that analysis of PPI data may be of use in understanding the functional effects of variants linked to different neuropsychiatric disorders. Set-based analysis of de novo CNVs has implicated the disruption of synaptic signalling complexes in schizophrenia (Kirov et al. 2012). The interrogation of synaptic interaction networks may ultimately provide more detailed insight into disease pathogenesis.

A TWIN COHORT STUDY OF PSYCHOTIC EXPERIENCES IN ADOLESCENCE
Background
Evidence is building that both common genetic variants as well as certain environmental factors influence risk for psychosis in adulthood. Before the onset of psychosis, psychotic experiences – from mild to severe, infrequent to frequent -- occur in adolescence and can be clinically predictive, but little is known about their causes. Understanding the causes of psychotic experiences before the onset of clinical psychosis may be particularly pertinent for the development of intervention and prevention approaches. The aim of this study was to investigate the degree of genetic and environmental influences on specific psychotic experiences in adolescence in the community and in individuals at the extreme, that is, those showing many, frequent experiences (defined using quantitative cut-offs).

Methods
The classic twin design was employed. A representative, general population sample of over 5000 adolescent monozygotic and dizygotic twin pairs (M = 16.32 years; SD = 0.68 years) from England and Wales was assessed on the Specific Psychotic Experiences Questionnaire (SPEQ). The SPEQ assessed six quantitative dimensions of specific psychotic experiences: paranoia, hallucinations, cognitive disorganization, grandiosity, anhedonia (all self-report) and parent-rated negative symptoms. SPEQ subscales were derived from principal component analysis and have shown good internal consistency, retest reliability and validity against similar psychosis symptom measures. Univariate individual differences and liability threshold structural equation models were run, together with DeFries Fulker extremes analysis and Cherny regression analysis. Multivariate twin models were used to investigate the causes of the overlap between specific psychotic experiences.

Results
Liability threshold models and DeFries Fulker extremes analysis both converged to show that the genetic and environmental estimates for extreme groups (top 15%, 10% and 5%) were not significantly different to those for the whole sample, suggesting there was a genetic link between the extremes and rest of the population. Cherny analysis showed, for each of the six dimensions separately, that there were not significant linear changes in the heritability estimates across the distribution of each subscale. In the whole sample, all six types of psychotic experiences showed genetic influence. Highest heritabilities were found for paranoia (50% [95% CI .41-.54]) and parent-rated negative symptoms (59% [95% CI .54-.64]). Heritability was lowest for hallucinations, with significantly different estimates for males and females (15% [95% CI .00-.34] and 32% [95% CI .18-.46], respectively). Shared environment was only significant for hallucinations and parent-rated negative symptoms (estimates ranged from 17-24%). All self-report scales showed considerable nonshared environmental influences (49-64%), with a more modest estimate for parent-rated negative symptoms (17% [95% CI .16-.18]). The inter-subscale correlations varied from r = .00-.43, with only four relationships exceeding a correlation of .20. For these four relationships (paranoia-hallucinations, paranoia-cognitive disorganization, hallucinations-cognitive disorganization, cognitive disorganization-parent-rated negative symptoms) there was evidence for considerable shared genetic influences across subscales.
(bivariate heritabilities = .49-.64, genetic correlations = .27-.60) as well as dimension-specific genetic influences. Evidence for overlap in environmental influences was also found.

**Discussion**
These findings support the proposal that the same genetic and environmental causal factors influence extreme, frequent, psychotic experiences in adolescents and milder, less frequent manifestations. There is not a strong degree of co-aggregation of specific psychotic experiences in 16-year-olds. Where there was overlap between types of experiences, this appeared to be partly due to shared genetic influences across symptoms, but there were also experience-specific causal influences in this age group. These results support future work that will test whether specific genes and environments associated with clinical psychosis are also associated with specific adolescent psychotic experiences during the ‘prodromal’ period. These findings on adolescents also contribute to the theoretical debate concerning the accuracy and utility of categorical and continuum models of psychosis in basic research and clinical practice.

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**SEEKING SCHIZOPHRENIA CAUSATIVE VARIANTS IN ISRAELI FAMILIES.**

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**Background**
Despite an intensive search for schizophrenia genes for more than three decades, no single gene mutations of large effect have been identified. Genome-wide association studies have identified variants with very small effects on risk. Next generation sequencing studies have explored the role of highly penetrant de novo sequence variants in sporadic cases of schizophrenia. While an excess of potentially damaging variants has been reported, the high heritability of schizophrenia suggests that most cases are the result of inherited genetic variation. In a further study, genomes of schizophrenia patients were sequenced and moderately rare variants were detected; these were genotyped in a large cohort of additional cases but no significant association was found. Recent whole exome sequencing in multiplex families with schizophrenia detected rare protein-altering variants in 1 of 3 genes associated with the N-methyl-D-aspartate receptor. On this background, there is a strong basis for considering the possible contribution of rare variants with a major pathogenic effect.

**Methods**
We report the use of whole exome sequencing to seek pathogenic functional variants in Jewish Israeli families multiply affected with schizophrenia. We sequenced representative affected subjects in two families and analyzed them separately. To date, one of the families has been analyzed.

**Results**
While integrating linkage data on this family and filtering the vast number of detected variants, we identified 14 family-shared, rare (<5%), possibly functional variants. Four of the variants are located in the exon of the FAT4 gene and positioned in the best linkage region found in the same family. FAT4 was previously found to be significantly associated with bipolar disorder in a meta-analysis of genome-wide association studies and could be the best candidate for segregation
analysis. Two additional candidate variants were found in the VPS13C gene. VPS13C is a paralog of VPS13A gene. Defects in VPS13A are the cause of Levine-Critchley syndrome with psychiatric features. All interesting candidate variants will be verified by standard sequencing.

Discussion
These preliminary results support hypotheses regarding a possible role for rare variants in schizophrenia and strengthen the importance of sequencing efforts in large affected families. Supported in part by a grant from the Israel Science Foundation.

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COMPREHENSIVE ANALYSIS OF GENE-ENVIRONMENT INTERACTION AND THE GENETICS OF DISEASE HETEROGENEITY
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Background
A major overarching research question which has not been systematically evaluated in large samples is how the environment interacts with genetic risk variants to increase the risk for schizophrenia. In particular, it is possible that the small odds ratios evident for genome-wide significant findings in schizophrenia are the realization of a larger effect size in the presence of an environmental risk and a far smaller effect in the absence of an environmental risk factor. This may be particularly salient for schizophrenia given that a twin study meta-analysis, as well as a Swedish national pedigree study of 9 million individuals, both found significant shared and non-shared environmental effects. In addition, since schizophrenia is a complex disease with heterogeneous symptomatology, course and treatment outcome, there is an urgent need for improvements in predicting disease severity and drug response.

Methods
To our knowledge, the Swedish schizophrenia sample is the largest and genetically most well-characterized national sample that currently exists. Genome-wide and high-quality data are available for 5,001 schizophrenia cases and 6,243 controls, and all subjects were ascertained through a population-based sampling frame in Sweden. Genotyping for GWAS, CNVs, and exome array are available on all subjects, and exome sequencing has been completed on 5,000 subjects and will be completed for all by end of 2013. A particular strength is the availability of multiple environmental risk factors that were systematically and concurrently collected. Using the unique individual Swedish national registration number, all subjects have been linked to a range of national registers, including the hospital discharge, prescribed drug, medical birth, school grade and military enrollment registers. In addition, 1,679 original birth records have been collected.

Results
We are now analyzing a set of genetic variables (top genome-wide significant loci, common variant burden, CNV burden, exonic loss-of-function burden), and their potential interactions with established “environmental” risk factors (season of birth, urban birth, obstetric complications, fetal growth measures, parental age, school grades at age 15, cognitive function (males, conscription age 18), socioeconomic status). We approach disease heterogeneity by stratifying schizophrenia patients based on severity and treatment outcome (using hospitalization and drug prescription patterns, age at onset), and comorbid epilepsy and mental retardation, to search for genetic determinants. For example, 22% of the patients have been prescribed clozapine (which can be used as a measure of treatment resistance/severity), and 7% were identified as having comorbid epilepsy. All genomic data have been collected and quality control is completed, and the analyses to support the above analytical goals are in progress.

Discussion
We hypothesize that taking gene-environment interactions into account will lead to new discoveries. We are investigating if the effects of the many new robust SNP associations are increased in the presence of certain environmental factors, and how polygenic SNP and CNV burden is associated with such factors, which might lead to new insights into disease heterogeneity and underlying biology. Assessing environmental risk could possibly account for part of the missing heritability.

89 EFFECTS OF CACNA1C RS1006737 ON THE SCHIZOPHRENIA WITH LONG QT SYNDROME
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Background
Current treatment for Schizophrenia is mainly depends on antipsychotic drugs. However these medicatios may cause severe adverse events, such as sudden death by the acquired long QT syndrome. The Long QT Syndrome (LQTS) is mainly caused by mutations in genes encoding subunits of cardiac ion channels. One of the genes that involved in LQTS is the alpha 1C subunit of the L-type voltage-gated calcium channel (CACNA1C). Recent genetic studies found the allele of the variant rs1006737 in CACNA1C gene to be over-represented in patients with psychosis, including schizophrenia, bipolar disorder, and major depressive disorder. We used samples of Japanese schizophrenics and compared allele frequencies between the schizophrenia with long QT syndrome, schizophrenia without long QT syndrome and the normal control. We also analyzed effects of antipsychotics.

Methods
A total of 185 Japanese patients with a DSM-IV diagnosis of schizophrenic disorder and 392 controls were recruited who have electrocardiogram data. Genomic DNA was isolated from whole blood according to standard procedure. The genotyping was performed by the fluorescence resonance energy transfer method using the Rotor-Gene System, and used Fisher’s exact test for statistical Analysis. We also examined the risk for long QT syndrome from antipsychotic drugs, according to genotyping data.
Results
Comparison between the prolonged QT interval group and the normal QT interval group in the female patients with schizophrenia, we found a significant difference in a genotype (Fisher’s exact test; p= 0.024). Now we are analyzing the correlation between antipsychotics effects and long QT interval in this group.

Discussion
Our findings suggest that CACNA1C gene may be an important target to avoid sudden death in a clinical scene.

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DTNBP1 GENOTYPES AND CANNABIS-ABUSE INTERACT ON SYMPTOM SEVERITY AND AGE OF ONSET IN SCHIZOPHRENIA
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Background
Previous studies have shown that candidate gene risk polymorphisms and psychoactive substance abuse influence the patterns and severity of schizophrenia symptoms. Some of these results also supported an interactional model of these genetic and environmental effects. In this study we examined whether the most studied schizophrenia risk polymorphisms and psychoactive substance abuse interact on symptom severity in a small discovery sample and multicentric replication samples.

Methods
We analyzed the clinical data of 280 schizophrenia patients of Hungarian, Caucasian descent, together with genotyping data of the candidate genes NRG1, DTNBP1, RGS4, G72/G30 and PIP5K2A. Patients were assessed clinically by the Positive and Negative Symptom Scale (PANSS), information about substance abuse was based on self-report and reviewing patient charts. We tested for possible interactional effects using the General Linear Model (GLM) analysis.

Results
In the discovery sample 15,8% of patients reported episodic or regular substance abuse, the vast majority (92%) used cannabis or the combination of cannabis and another drug. Substance abuse was associated with higher scores of the PANSS hostility/excitement factor, independent of sex, age, or genetic results (F=4,02; p=0,04). We found significant interactional effects of the DTNBP1 gene risk polymorphisms and substance abuse on several PANSS factors: rs2619528 and substance abuse were associated with higher scores on the PANSS negative factor (F=4,6; p=0,03), and the PANSS depression factor (F=4,75; p=0,03). Moreover rs3213207 and substance abuse were associated with higher scores on the PANSS cognitive factor (F=7,55; p=0,006).

Discussion
We sought to replicate these results investigating the dbGaP Molecular Genetics of Schizophrenia (MGS) and GAIN Whole Genome Study on Schizophrenia samples. Non-matching markers were imputed using the IMPUTE software and the 1000 Genomes reference
data. Our preliminary analyses are indicative of similar gene-environment interactions after correction for demographic variables. Our results underscore the importance of gene-environment interactions in the phenotypic heterogeneity of schizophrenia.

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FIRST GWAS OF SUICIDE ATTEMPT IN SCHIZOPHRENIA REVEALS EVIDENCE OF SEX-SPECIFIC ALLELIC EFFECTS
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Background
People with schizophrenia have a lifetime risk of attempting suicide approaching 50%. While a number of candidate gene studies have been published, there have been no genome-wide association studies of suicide attempt in schizophrenia. GWAS in mood disorders revealed a different set of risk variants in men than in women (Willour et al., 2011).

Methods
We conducted a GWAS of suicide attempt in schizophrenia in the PGC Wave 1 sample. This consisted of clinical sites from the US, Norway, Denmark, Germany, Bulgaria, Portugal, the UK, and the Netherlands (N=1,689 schizophrenia cases with a history of suicide attempt and 2,919 cases with no history). Genotyping, quality control, imputation, have been previously described (Ripke et al., 2011). We used logistic regression to examine the relationship between suicide attempt history and genotype. To test for sex-specific differences, we conducted separate analyses of males and females only.

Results
There were no genome-wide significant SNPs in either the male-specific, female-specific, or combined analyses. However, we observed seven moderately significant loci (P<10^-5) in females which were not nominally significant (P<.05) in males. There were six such SNPs in males which were not nominally significant in females. Three additional loci were moderately significant in the combined analysis only.

Discussion
While we observed no genome-wide significant loci, there was no overlap between moderately significant loci in males and females, consistent with previous results in mood disorders. This suggests that genetic influences on suicidal behavior in adult psychiatric disorders are at least in part sex-specific.

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THE GENETIC ASSOCIATION STUDY AND PERIPHERAL BIOMARKER STUDY IN EARLY STAGE OF SCHIZOPHRENIA
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Background
Schizophrenia is a neuro-developmental disorder with onset during adolescent and early adulthood. There have been several hypotheses about the neural events around the onset of schizophrenia. The neurotransmitter imbalance and immune dysregulation hypothesis occupied the central role for the onset of delusion and hallucination, the key symptoms for the diagnostic criteria of schizophrenia. We clarified the genetic roles of neurotransmitter related genes and the changes of plasma cytokines in the early stage of schizophrenia.

Methods
Sample one was consisted of 136 healthy subjects and 111 patients with either ultra-high risk state of schizophrenia (UHR) or first-episode of psychosis (FEP). In sample two, 41 UHR or FEP patients, who are either drug naive or antipsychotic short exposure, were recruited to receive aripiprazole treatment for 4 weeks. Forty-one age and gender matched controls were recruited. All subjects were of Han Chinese ethnicity. Thirteen single nucleotide polymorphisms (SNPs) with evidence of association with schizophrenia from neurotransmitter related genes, including DRD2, DRD3, DRD4, DBH, HTR2A, HTR3A, GRM3, and GABRG2, were genotyped in sample one. The plasma levels of IL-1-beta, IL-2, IL-12, IL-13 and IFN gamma (IFNg) were measured in the healthy controls, the pre-treatment stage and the post-treatment stage of patients using ELISA method.

Results
We found there were significant differences for allele and genotype distribution of rs6311 and rs6313 (both SNPs in HTR2A) between the group of patients and controls. We found the plasma levels of IFNg and IL-2 reduced significantly after treatment with aripiprazole and the plasma levels of IL2 and IL-12 after treatment were significantly lower than those of normal controls. The correlation coefficients among the cytokines were not significantly in the pre-treatment stage of patients, different from those in the controls. However, the correlation coefficients among the cytokines resumed to the level of significance similar to that of controls after aripiprazole treatment.

Discussion
The study found serotonin system seemed implicated in the pathogenesis of schizophrenia. The cytokines of IL-2, IL-12, and IFNg seemed involved in the onset and treatment course of early stage of schizophrenia. The resume-to-normal correlation pattern after antipsychotics treatment implicate the antipsychotics might have modulate function upon the immune dysregulation phenomenon in early stage of schizophrenia.

AN ASSESSMENT OF TANDEM REPEAT VARIATION IN SCHIZOPHRENIA USING 700 HIGH COVERAGE WHOLE GENOMES SEQUENCES
Background
Schizophrenia is a genetically complex and clinically heterogeneous disorder. Several large-scale genome-wide association studies have been conducted which have focused on variations such as copy-number variation and single nucleotide polymorphisms. Tandem repeats have been largely neglected in this paradigm of large-scale genetic surveys, with the exception of a few candidate analyses (e.g. DRD4). This is somewhat surprising given the higher mutation rates of tandem repeats and putative functional consequences of this. The Genome Psychiatry Cohort (GPC) is a newly developed resource for large-scale genomic studies of more than 33,000 participants, including people of Caucasian, African American, and Latino ancestry, and selected for schizophrenia and bipolar phenotype, together with family members and controls.

Methods
Using the GPC cohort, we report here an initial survey of tandem repeat variation arising in 700 high coverage genomes (minimum coverage 20x), including over 500 schizophrenia/schizoaffective individuals. Focusing on 2-6mers, we used LobSTR to assess global and local patterns of variation.

Results
Globally, we quantify repeat mutation rates per genome explore how this “global burden” might influence schizophrenia susceptibility. We survey rates of variation across “functional” groups, including coding sequences and conserved non-coding elements and compare these rates between cases and controls. Locally, we report and number of repeats, coding and noncoding, enriched in cases versus controls, revealing novel candidates. We examine the spectrum of repeat variability per locus, and examine divergencies in the allelotypic spectrum between cases and controls.

Discussion
The rate at which repeats mutate throughout the genome and the potential functional, and by extension, phenotypic consequences of those mutations in relation to schizophrenia risk remain largely understudied. We present the first full-genome survey of such variation in over 700 individuals. Our results detect a large number of events, coding and non-coding, that might shed new insights into the possible roles of tandem repeat mutations in the etiology of schizophrenia, into therapeutic avenues, and into how they might rescue some of the elusive heritability that remains for this disorder.

EXAMINING DE-NOVO MUTATION RATES AND PATTERNS FROM WHOLE-EXOME SEQUENCING OF SPORADIC CASES OF SCHIZOPHRENIA FROM TAIWAN
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Background
Recent studies have implicated that exonic de-novo mutations play a convincing role in developmental disorders such as autism, intellectual disability, and epilepsy. For sporadic cases of schizophrenia, early reports have pointed towards promising de novo candidates; however, larger numbers of sequenced trios are required to identify specific genes, as de novo events arise at a low rate in the population (roughly one per trio).

Methods
Under the collaboration of multiple centers, whole-exome sequencing has been performed on 1,135 complete trios from a Taiwanese cohort, making it one the largest de novo sequencing projects to date. Exome sequencing data were generated using the Illumina HiSeq sequencing platform with the Agilent SureSelect exome capture platform, and the Illumina Miseq platform was chosen as the method for validating putative de novo calls.

Results
Preliminary results suggest that the overall rate of mutation in affected offspring falls in line with the expected mutation rate. Upon examination of high-quality putative de novo events (those with twenty or more reads in the full trio), multiple de-novo mutations have been found in thirty-nine separate genes, with multiple loss-of-function mutations found in SV2B, a synaptic vesicle glycoprotein gene primarily expressed in the brain. These findings, however, do not surpass exome-wide significance after incorporating gene size and site-specific mutation rates into expectations of de novo mutation. Further analyses examining de novo events in conserved gene sets show a 1.79-fold higher enrichment of loss-of-function events and a 1.19-fold higher enrichment of non-synonymous events. However, these levels only reach suggestive significance (p = 0.08 and 0.1, respectively).

Discussion
Overall, our findings do not clearly identify any single gene as an unequivocal risk factor for schizophrenia when disrupted by de novo mutation, although a number of genes and gene-set analyses show suggestive signals. We also integrate the results from these trios with the other available trio sequencing data from the community to provide the clearest evaluation to date of the role that de novo events play in the etiology of schizophrenia.

COPY NUMBER VARIATION ON CHROMOSOME 8P IS ASSOCIATED WITH SCHIZOPHRENIA
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Background
Multiple studies have used whole-genome genotyping methods to discover structural variations in the DNA of complex genetic disorders. In schizophrenia, for instance, the identification of novel, rare copy number variations (CNVs) has contributed to a growing database of schizophrenia-related genetic variants. The majority of these CNVs range in size from a kb to several Mb and are thought to be rare, highly penetrant, and found in only a small number of individuals (e.g., < 1% of subjects with schizophrenia). However, learning about these rare CNVs may represent an important step in understanding the underlying etiology of schizophrenia. The goal of the discovery phase of this study is to identify novel CNVs associated with schizophrenia using family-based methods rather than case-control methods.

Methods
One hundred trios (schizophrenia probands and their parents) were genotyped using the Illumina 1M array. After initial genotype cleaning, data from five trios were eliminated due to technical issues. A hidden Markov model (HMM) that jointly models all members of a trio using Mendelian inheritance principles in Penn CNV was used to identify CNVs. This inheritance model should improve the CNV genotyping of related samples because it incorporates information about an individual’s family members rather than treating that individual as an independent entity. Stringent CNV-calling criterion (i.e., 16 consecutive probes) was used to focus on large CNVs and to decrease the bias of the CNV calling, which relied on trio data. Inferred CNV genotypes were then tested for association using the transmission disequilibrium test (TDT) or the family-based association test (FBAT-CNV). Novel CNVs that showed significant overtransmission from parents to probands were identified and cataloged. CNVs that were visually convincing (through an examination of LRR and BAF plots) and statistically significant (p < 0.001) were then compared to CNVs that were shown to be associated with schizophrenia in online databases and previous studies. As a validation step, TaqMan CNV assays were used to target CNVs of interest in the discovery samples. The validated CNVs were then screened in replication cases (n = 100) and controls (n = 259), and the frequencies were compared using Fisher’s Exact Test.

Results
Schizophrenia-related CNVs were identified on chromosomes 1p, 2p, 4q, 7p, 7q, 8p, and 11q. Four of these seven CNVs were validated using TaqMan assays and were then investigated in a replication sample of cases and controls. Only one CNV (on 8p) was found to be significantly associated with schizophrenia. In the replication cases and controls, TaqMan probes in the 8p region showed that CN = 1 (1 copy) was the most frequent call in both cases and controls (~40%), not CN = 2 (diploid state). In the remaining samples, 31% of the replication cases had CN = 0, whereas only 17% of the controls had CN = 0; also, 38.6% of the replication cases had CN = 2, whereas only 29% of the controls had CN = 2 (overall p = 0.008).

Discussion
Multiple rare CNVs have been convincingly associated with schizophrenia. This study took advantage of a multifaceted approach that included a statistical and visual examination of the
results of an Illumina 1M genome-wide SNP array, the validation of those findings using TaqMan molecular genetics methods in the same samples, and a screening of the validated CNVs in a replication sample of cases and controls. The identification of CNVs is an important stepping-stone to the identification of disease-causing genes that are disrupted in schizophrenia. Additional molecular and biologic studies are necessary to identify schizophrenia-related CNVs, the putative genes within those CNVs, and the relationship between CNVs and schizophrenia.

THE GENETIC ARCHITECTURE OF A POTENTIALLY MEDICALLY ACTIONABLE MUTATION IN PSYCHIATRY
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Background
Multiple rare structural variants of relatively recent evolutionary origin are recognized as important risk factors for schizophrenia and other neurodevelopmental disorders (e.g., autism spectrum disorders, intellectual disability, epilepsy, attention deficit hyperactivity disorder, Tourette syndrome). Ideally, the identification of mutations in specific genes would result in personalized, “medically actionable” treatment interventions. We have identified such a potentially informative mutation and it has high potential to impact clinical response.

Methods
Mapping the size and breakpoints of these structural rearrangements using customized high-resolution array-based Comparative Genomic Hybridization (aCGH); bioinformatics analyses of the genomic region undergoing rearrangement in order to identify the genes involved; determining the products of recombination through direct DNA sequencing; characterizing the molecular and cellular mechanisms underlying duplications of GLDC.

Results
This rare structural rearrangement involves a duplication-triplication on chromosome 9p24.1 that segregates with psychosis in an extended family. In addition to other genes, the rearranged region contains the gene encoding glycine decarboxylase (GLDC), which affects brain glycine metabolism. This gene is an obvious ‘smoking gun,’ because glycine is a co-agonist for the NMDA receptor. Carriers of the GLDC triplication mutation would be expected to have low levels of brain glycine, resulting in NMDA receptor-mediated hypofunction, which has been strongly implicated in the pathophysiology of psychotic disorders. Carriers of this mutation, regardless of neurodevelopmental phenotype, are strong candidates to benefit from glycine augmentation of psychotropic drug treatment.

Discussion
Here we describe architectural features of this mutation based on multiple experimental molecular methods and early results of modeling this mutation using human induced pluripotent stem cells.
DECREASED MIR-937 EXPRESSION LINKED TO VERBAL MEMORY DEFICITS AND SCHIZOPHRENIA

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Background

The Multiplex-Multigenerational Genetic Investigation (MGI) of Schizophrenia is a family-based study of schizophrenia (SCZ) and related endophenotypes including measures of disordered cognition, a hallmark of SCZ. MGI families were ascertained on the basis of two affected probands and all family members over age 16 were invited to participate. Verbal memory (VMEM) is an important endophenotype for SCZ from a clinical perspective because it is a byproduct of SCZ that is resistant to current treatments and a useful endophenotype from a genetic perspective due to its relatively large heritability and genetic correlation with the disease, suggesting overlapping genetic influences.

Methods

The Penn Word Memory Test was used to assess VMEM in 419 individuals and an efficiency score was calculated as the ratio of accuracy to speed. VMEM is genetically correlated with SCZ (\(\rho_G = -0.16\)) and mean scores differ among affected individuals (\(N = 47\), mean normalized VMEM score = -0.53), unaffected relatives (\(N = 307\), VMEM = 0.01), and healthy, unrelated controls (\(N = 65\), VMEM = 0.12). While the heritability of VMEM (\(h^2 = 0.52\)) is lower than that of schizophrenia in these families (\(h^2 = 0.92\)), a substantial increase in statistical power is derived from its continuous distribution. MicroRNAs (miRNAs) were extracted from lymphoblastoid cell lines and sequenced on the Illumina GA\textsuperscript{II}x utilizing TruSeq small RNA technology. For each individual, all observed mature human miRNAs, were normalized to reads per million (RPM) and aligned with 100\% identity and a seed of 12 bases to mirBase version 17 using NextGENe software (SoftGenetics). 1,279 variably expressed miRNAs were identified with at least two RPM in five or more individuals. Of these miRNAs, 928 are heritable and association analysis was performed in SOLAR to assess the age- and sex-adjusted relationship between miRNA expression and VMEM while controlling for pedigree structure.

Results

Of the examined miRNAs, only hsa-miR-937 is significantly associated with VMEM (Bonferroni corrected \(\alpha = 5.4 \times 10^{-5}\)) and explains approximately 4.0\% of the observed variation. Expressed in 26.5\% of the sample, miR-937 has a higher mean RPM in unaffected individuals (0.81 RPM) compared to affected individuals (0.40 RPM, two-tailed t-test \(p = 9.8 \times 10^{-3}\)). Of the many available methods for identifying mRNA targets, three were selected for their complementary algorithms. Sixteen targets were identified reaching miTG scores > 0.6 in the DIANA-microT-CDS database, two targets using the MirTarget2 algorithm defaults in MIRDB, and four targets showing local conservation in TargetScan. In total, 17 genes were identified by one or more method with five of those genes showing increased expression in the brain in the PaxDb protein abundance database - \textit{ARFGAP1}, \textit{CD99 (MIC2)}, \textit{PTGFRN}, \textit{SMAD5OS}, and \textit{TNPO3}. 
Discussion
Increased expression of miR-937 is correlated with decreased likelihood of SCZ, specifically with the verbal memory impairments frequently associated with this disorder. Little experimentally validated data is available for miR-937. While the stem-loop structure can be confidently mapped to an intronic region of SCRIB on chromosome 8, the mRNA targets of miR-937 are only putative. Two targets have been previously linked with psychological disorders. Located on the pseudo-autosomal region of the X chromosome, CD99 does not undergo X-inactivation and was identified as a likely contributor to SCZ in an early linkage study. Studies of spontaneous deletions of telomeric 20q led to the association of ARFGAP1 with mental retardation, specifically the delayed acquisition of language. Additional experimental work is needed to validate the regulation of these genes by miR-937 and link them to VMEM and/or SCZ in this sample.

A POSSIBLE ROLE OF TRANSPOSABLE ELEMENTS IN DYSREGULATING THE GENOMIC ARCHITECTURE OF SCHIZOPHRENIA

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Background
Between half and two-third of our genome is composed of repetitive, low-complexity elements (e.g., LINEs, SINEs, SVAs and HERVs) collectively termed transposons or Transposable Elements (TEs). B. McClintock discovered transposons in 1948 and immediately realized that transposons might act as ‘controlling elements’ over genes in their proximity. While the notion that DNA could be mobile was accepted, the idea of control was not. The role of TEs is now more recognized, and evidence is accumulating that TEs play an essential function in regulating the human genome. TEs create variability by new retro-transpositions, mainly in germ line but also in somatic cells, and by nucleotide polymorphisms (Single Nucleotide Variants, SNVs) in “fixed” TEs. By retrotransposing to new insertional sites, TEs are also creating Copy Number Variants and Indels and can provide novel promoters, splice sites, exons or polyadenylation signals. By dispersing regulatory elements TEs can rewire transcriptional networks. However, many essential questions remain unanswered. While we are beginning to understand the specific mechanisms by which TEs are responsible for simple – single locus – diseases, we still do not know what effect TEs may have on a complex phenotype. Also, we do not know whether SNVs, or new insertions of transpositionally active TEs, have a deleterious or a neutral effect on the development of SZ.

Methods
We identified and annotated the SNVs and Retrotrasposition Insertion Polymorphisms for various classes of Transposable Elements (e.g., LINEs, SINE, HERVs and SVAs) from a subset of whole-genome sequencing data available to our consortium for a total sample of 550 SZ cases and 550 controls. In particular, we have examined TE sequence differences (including RIPS) between SZ patients and controls, using re-alignment and de novo assembly and have characterized the genomic context of RIPS (exon, introns, 5’ and 3’ UTRs, non-coding regions).
Results
Fine mapping the ~500 SZ-associated SNVs (1x10^-6 < p < 5x10^-8) from our Genomic Psychiatric Consortium (GPC) sample, we found that 36.1% of the SNVs fall into Transposable Elements and 35% into TE-derived lincRNAs. For example, the SNP rs7819570 maps a L1PA4 LINE, at approximately 230 kb from the 5’ end of the MMP16 gene, the best LD location. This L1PA4 (Divergence=5.7%, Deletions=0.3%, Insertions=0.2%) is a well-conserved L1 (SW-score > 25,000) that shows a remarkable level of brain-specific expression despite being evolutionarily ancient.

Using a small subsample of the 1,000 genomes whose whole genome sequences will be completed by summer 2013, we performed an analysis of sequence data to look for Retrotransposon Insertion Polymorphisms (RIPs). We found that RIPs are underrepresented in SZ patients compared to controls for all the TE classes that we have considered. The number of RIPs in controls ranges from as low as 3,000 to more than 4,500, within the boundaries calculated from the 1,000 Genome Project for dbRIP-reference and RIP non-reference TEs, but a larger estimate than from previous studies. SZ subjects present from 1/20 to 10/10 of RIPs compared to controls.

Discussion
The low number of RIPs in SZ subjects suggests an early embryonic neurodevelopmental defect. Since recent stem cell models indicate that a high number of RIPs for L1 (and probably Alu) elements is a key factor for neuronal development, it is possible that a reduction of RIPs in SZ at the early embryonic level may be less relevant than the somatic retrotransposition that occurs in neurons at later developmental stages. Our preliminary results may indicate a new, unexpected dimension of the regulatory genome that may play an important role in the etiology of schizophrenia. While TEs represent a controversial issue due to their biology and mechanisms of action that challenge our traditional ideas of genetics, our findings suggest that they may represent an important new genomic risk factor in schizophrenia.

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GENETIC ASSOCIATION BETWEEN NEUROPSYCHOLOGICAL TRAITS AND GENOME-WIDE SUPPORTED SCHIZOPHRENIA RISK VARIANTS IN THE BOSTON CIDAR STUDY
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Background
A large number of gene variants have reliably been shown to contribute to risk of schizophrenia (SCZ). However, how these genes influence brain functioning as indexed by neuropsychological traits is largely unclear. Furthermore, the penetrance of genetic variants may be higher on the neuropsychology level than on the disorder. The aim of this study was to test risk variants
recently identified by the Psychiatric Genomics Consortium (PGC) schizophrenia working group for association with a set of core cognitive and olfactory traits in the Boston CIDAR case/control cohort.

**Methods**

The Boston CIDAR sample consists of 110 SCZ cases (comprising clinical high-risk individuals \(n=42\), first-episode patients \(n=35\) and chronic patients \(n=33\)) and 101 healthy controls (HC) matched to cases on age, sex, and education level. Genotyping was performed on the Illumina OmniExpress BeadChip (>700,000 SNPs), and imputed to the 1000Genomes reference panel using MaCH/Minimac. The neuropsychological test battery consisted of: attention/processing speed/vigilance (Trail Making Test A; BACS Symbol Coding; Continuous Performance Test – Identical Pairs; Auditory Continuous Performance Test – QA); working memory (Auditory Continuous Performance Test – Q3A clean & Q3A interference conditions; University of Maryland Letter-Number Span; WMS-3 – Spatial Span Forward / Backward); verbal learning and memory (Hopkins Verbal Learning Test; WMS-3 or CMS-3 – Stories-Immediate and Delayed Recall); visual learning and memory (Brief Visuospatial Memory Test); visuospatial memory (WASI – Block Design); language ability (WASI – Vocabulary; Category Fluency – Animal Naming; DKEFS Proverbs Test); executive functioning (Wisconsin Card Sorting Test [WCST]; NAB Mazes); and motor control (Grooved Pegboard). Missing neuropsychological data were imputed in R. Factor analysis of 21 core neuropsychological measures was performed using maximum likelihood with varimax rotation. Analysis of variance was applied to assess phenotypic differences between diagnostic groups. Neuropsychological factors and a measure of olfaction (University of Pennsylvania Smell Identification Test - UPSIT) were tested for association with SNPs identified by the Psychiatric Genomics Consortium mega-analysis of schizophrenia GWAS data. In this interim freeze of data from analysis of 52 samples comprising 35,476 cases and 46,839 controls, 111 LD independent SNPs reached genome-wide significance \((p<5 \times 10^{-8})\). Association analysis with these 111 SNPs was carried out using quantitative regression analysis in Mach2qtl, including covariates for age, sex, and 5 multi-dimensional scaling principal components for population ancestry. In addition, analyses were run with and without dummy covariates for diagnostic group. The Bonferroni corrected \(p\)-value threshold for significance of all tests is 0.00009 (0.05 / (111 * 5)).

**Results**

Three cognitive variables showed a positive skew and were log-transformed prior to factor analysis (Trail Making Test A – time; Grooved Pegboard – time; WCST – total perseverative responses). Factor analysis extracted four neuropsychological factors with eigenvalues > 1. These four factors explained ~55% of the variance in the 21 cognitive measures, and mapped to the following cognitive domains: verbal memory (F1), visuo-spatial ability (F2), language ability (F3), and executive function / attention (F4). The four factors and the UPSIT score approximated normal distributions so transformation was not necessary. SCZ cases performed significantly worse than HC on F1 \((p<0.001)\), F2 \((p<0.001)\), F3 \((p<0.05)\), and the UPSIT \((p<0.001)\). A total of 111 SCZ risk variants were tested for association with these neuropsychological traits and olfaction. We report the results of these association analyses.

**Discussion**

Here we describe an association study of cognitive and olfactory phenotypes, which incorporates
prior knowledge from a large-scale SCZ risk GWAS mega-analysis to reduce the multiple testing burden, resulting in a more sensitive test and greater statistical power. Because there is strong prior evidence that these risk genes will influence neuropsychological performance, this approach has the potential to greatly increase our understanding of SCZ, by clarifying the neurofunctional mechanisms through which risk gene variants act to increase the risk of developing the disorder.

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MITOCHONDRIAL COMPLEX I-III MRNA LEVELS IN PATIENTS WITH SCHIZOPHRENIA
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Background
Currently, schizophrenia is diagnosed with the observations of the descriptive behaviors. A biomarker that can be obtained in peripheral tissues will help to confirm the diagnosis and monitoring of the treatment process. The aim of this study was to determine the relationship between mitochondrial complex abnormalities and schizophrenia.

Methods
Hundred and thirty-eight patients with schizophrenia and forty-two healthy men were included to the study. Peripheral mitochondrial complex I and III gene mRNA levels of schizophrenia patients compared with control subjects.

Results
Statistically significant difference between schizophrenic patients and control subjects were determined in mitochondrial complex I genes which were NDUFV1, NDUFV2, NDUFS1 (p<0.05). There was no difference in UQCR10 which was complex III gene (p>0.05).

Discussion
The result of this study suggested that NUDFS, NDUFV2, NDUFS1 gene mRNA levels in the mitochondrial electron transport chain could be used as a peripheral biomarker in the diagnosis of schizophrenia. More accurate biomarkers would be obtained in the diagnosis of schizophrenia with the increasing similar studies on this subject.

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ABERRANT DNA METHYLOME OF TGFB SIGNALING PATHWAY AND THE LOSS OF BRAIN ASYMMETRY IN SCHIZOPHRENIA
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Background
The loss of functional asymmetry of the brain is one of the most consistent endophenotypes in schizophrenia (SCZ) as determined by fMRI as well as other methods. Previously, we found that
there is lateralized DNA Methylation of the regulatory regions of genes such as \textit{MB-COMT} and \textit{HTR2A} in the left versus right brains of normal controls which were absent in SCZ, supporting a potential role for the epigenome in maintaining brain asymmetry in normal controls which is lost in SCZ.

\textbf{Methods}
We used Affymetrix U133 2.0 Plus Human Transcriptome chip containing \textasciitilde51,000 probe sets to define the brain hemisphere-specific transcriptomes in normal controls vs. SCZ and BD (a total of 30 sample, from the left or right post-mortem brain tissues). We also used the Illumina DNA methylation array technology to profile DNA methylome of the same samples.

\textbf{Results}
Our gene expression profiling studies of the left and right post-mortem brain samples from controls and patients with SCZ further support asymmetric expression of many genes linked to the TGFB super family signaling pathways which are known to establish left-right asymmetric body-plan. Examples include higher expression of Nodal, SMAD2, SMAD3 and Wnt10A in the left brain of controls (p=0.05, 0.02, 0.0006 and 0.005, respectively), while this asymmetric expression was lost in SCZ. Among other genes of TGFB signaling, TGFB2, in particular exhibited reduced expression (~40\%, confirmed by qRT-PCR analysis in a larger sets of post-mortem brain samples, including 35 SCZ and 35 control subjects) associated with higher promoter DNA methylation in the left brain of controls, but this lateralized DNA methylation and gene expression were lost in SCZ patients. Whole genome DNA methylation profiling of the same post-mortem brain samples showed that, while in general there was an inverse correlation between the expression and promoter DNA methylation of affected genes, the expression of few genes (e.g. SBNO1) showed direct correlation with DNA methylation as determined by the Illumina DNA methylation array technology. Our subsequent studies uncovered that these latter genes contain high levels of 5-hydroxymethylcytosine (5-hmc) in their corresponding Regulatory sequences which based on recent reports is abundant in the human brain and associated with the induction of gene expression. Interestingly, RNA microarray also indentified increased expression of IDH1 and IDH2 (but not TET1-3) which convert 5-methylcytosine to 5-hmc, particularly in the left brain of SCZ patients (p=0.0056 and 0.03, respectively).

\textbf{Discussion}
The evidence from developmental biology research on the role of several TGFB super family signaling proteins (e.g., lefty-1, lefty-2, nodal, ACVR2B, Smad2 and Smad5) in left-right axis determination, strongly support our observations that brain laterality is also defined by similar genes/pathways. Furthermore, the association between the loss of brain laterality in SCZ and aberrant epigenetic regulation of affected genes suggest potential avenues for prevention and novel targets for therapeutics in SCZ.

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\textbf{COMPREHENSIVE GENOMIC AND CLINICAL CHARACTERISATION OF LATE-ONSET SCHIZOPHRENIA VERSUS NORMAL- AND EARLY-ONSET: PROTECTIVE EFFECT OF PREX2}
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**Background**

Although the majority of individuals with schizophrenia experience onset before the age of 40, a sizeable minority have an onset later in life. These individuals show marked differences from individuals with earlier onset, including a greater proportion of female cases, retained cognitive function versus earlier onset cases in some domains such as vocabulary, but show larger deficits of cognitive function in others such as IQ and attention, and increased incidence of paranoid symptomology. However, little research has been conducted on late-onset schizophrenia (LOS). In addition, no one has examined the genetics of this disorder. One of the most important questions to answer in comparing LOS versus normal-onset (NOS; between 18 and 39 years) and early-onset (EOS; before the age of 18) schizophrenia is if there are protective factors - genetic, clinical or environmental - that prevent onset until later in life?

**Methods**

We used our WTCCC2 schizophrenia GWAS sample to assess genomic and clinical differences between LOS (N = 48), NOS (N = 1231) and EOS (N = 99) and used the UK ISC GWAS for genomic replication purposes, including 79 LOS, 1286 NOS and 113 EOS using a standard chi-square test or Fisher's exact test (FE), where appropriate. Analysis of clinical and cognitive differences between LOS and N/EOS were conducted using FE or Wilcoxon rank-sum tests (WRS), depending on the distribution of the outcome.

**Results**

**GENOMICS:** In the WTCCC2, an analysis of \textasciitilde600K SNPs provided a genome-wide significant hit at the intronic SNP rs12675385 on chromosome 8 in the gene phosphatidylinositol-3,4,5-trisphosphate-dependent Rac exchange factor 2 (PREX2). Cases were 5.83-fold (95\% confidence interval (CI) 2.93, 11.58; p-value = 1.3e-08) more likely to carry the minor allele of rs12675385 versus NOS. This result was replicated in comparison with the EOS cases with an odds ratio (OR) of 9.48 (95\% CI 2.46, 33.99; FE p-value = 8.4e-05). Intriguingly, the minor allele frequency was highest in the LOS cases (10\%), followed by controls (3\%), then NOS (2\%) and the lowest MAF was found in EOS (1\%), suggesting that this SNP may be acting in a protective fashion against the development of schizophrenia until later in life. These findings, including the SNP association and the pattern of MAFs, were replicated the ISC. In the ISC sample, the OR for the comparison of LOS and NOS was 4.23 (95\% CI 1.32, 13.56; p-value = 0.029) and for the comparison of LOS versus EOS (OR = 16.77 (95\% CI 1.80, 156.4; FE p-value = 0.0076). The pattern of MAFs also showed a similar pattern of frequencies found in the WTCCC2: it was highest in LOS (13\%) then controls (4\%), then NOS (3.6\%) and lowest in EOS (1\%).

**CLINICAL FEATURES:** Further, in clinical comparisons between LOS versus NOS and EOS cases 40 years and above, the distribution of positive symptoms were significantly different between LOS versus N/EOS on measures of paranoia (FE p-value = 0.042), delusions (FE p-value = 0.0012) and hallucinations (FE p-value = 0.018), with LOS patients showing a higher proportion of symptoms that were classified as marked or severe. LOS patients scored significantly lower on the mania dimension of the BADDS than N/EOS patients aged 40 or older but with onset before the age of 40; the average score among LOS was 17.68 versus 36.39 in
E/NOS (WRS p-value = 0.022). LOS cases showed a more severe psychosis but significantly less affective symptoms.

Discussion
PREX2 activates RAC1, and PREX2 interaction partners include AKT1, MTOR and CDC42, which have previously all shown association with schizophrenia in one or more studies and converge on a pathway including DISC1. Converging lines of evidence from human genetics and animal studies support the involvement of this gene in critical brain processes relevant to schizophrenia. Some evidence from genome-wide association studies suggests this gene may be involved with cognition, as SNPs in PREX2 have been listed among the top 60 most associated with processing speed and working memory from independent genome-wide association studies. Male PREX2 knockout mice show increased startle reflex, and both sexes show abnormal Purkinje cell dendrite morphology, impaired coordination and hypoactivity. Additional PREX2 interaction partners include MARK2, which regulates axon formation in the hippocampus and GNB1, which is associated with depression and anxiety phenotypes in mice. A knockout mouse model of Gnb1 showed it is required for embryonic neurogenesis, and GNB1 has shown differential expression in the anterior cingulate cortex in schizophrenia patients.

The identification of genetic factors that are protective against the development of schizophrenia until later in life will help us to understand the genetic pathways of risk for this disorder, and may help to develop drug targets to prevent the onset in at-risk individuals. Further, it will add an important, but currently missing, dimension to our understanding of the genomics of schizophrenia.

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INTERACTION BETWEEN SEX AND THE DISC1 PATHWAY AND RISK FOR SCHIZOPHRENIA
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Background
DISC1 was discovered in a large family where a balanced translocation segregated with psychiatric illness. It is a replicated schizophrenia (SZ) candidate gene; multiple reports including a meta-analysis have indicates interactions with sex. We used a recently developed DISC1 interactome (N genes = 130, N SNPs = 5384) to test the epistasis and SNP-by-sex interactions in the WTCCC2 SZ case (N = 1378)-control (N = 1086) study.

Methods
The Random Forest (RF) algorithm is a classification and regression method often used for high-dimensional data setting and had been increasingly successful in bioinformatics applications, specially in statistical genetics. We used RF to detect interactions in a training (80%) and independent test (20%) sample. We took the top10 predictors ranked in the top 10 of the training data across 100 runs of RF to follow up with logistic regression interaction models in our test sample. To determine significance, we used the likelihood ratio test (LRT) between nested models. We used Nagelkerke's R² to estimate the amount of variation in case-control status explained.
Results
We detected four SNPs in significant interaction with sex. The most significant SNP was rs495879 in MACF1 with a LRT p-value=0.006 and get the 2.2% of the variation in the case status. The SNPs rs1351998 in ANK2 significantly interacted with sex (LRT p-value = 0.015) and explained 1.7% of variation in case status, the other ones were the SNPs rs4792827 and rs7225002 both in KANSL1 and they were significant with LRT p-values 0.016 and 0.018 with $R^2$ 0.017 and 0.016, respectively.

Discussion
We found three genes (MACF1, ANK2, KANSL1) interacting with sex, similar to sex dependent effects reported for DISC1, but no epistasis based on replication in our independent test sample. The most significant genes, MACF1 and ANK2, have shown differential expression in brains of patients with schizophrenia versus controls. Mutations in KANSL1 result in 17q 21.31 deletion syndrome which features intellectual disability. Our top ranking SNP was in MACF1, and MACF1 direct interacts with DISC1. Our approach explained more significant variation in case status using interaction models with one SNP than the polygenic score containing thousands of SNPs (almost 1%, see abstract 21729).

IMPACT OF GENETIC VARIATION IN THE PROLINE DEHYDROGENASE GENE ON DEAFFERENTATION OF THE STRIATUM IN SCHIZOPHRENIA
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Background
Abnormalities in frontostriatal circuitry have been shown in schizophrenia (SZ). Using DTI, we assess the associative loop, white matter (WM) pathway connections between dorsolateral and ventrolateral prefrontal cortex (DLPFC, VLPFC) and the associative striatum, a functional loop believed to impact cognitive function. In a subset of subjects, we test a single nucleotide polymorphism (SNP), rs4819756, located in the chromosomal region of 22q11 and in the proline dehydrogenase (PRODH) gene, for its role in affecting these pathways. This gene has been identified as a schizophrenia susceptibility gene through association studies in schizophrenia patients and in the 22q11 deletion syndrome. Moreover, sMRI and fMRI studies suggest decreased frontal WM and reduced connectivity in carriers of the schizophrenia-risk variant GG of the gene. The striatum can be divided into limbic (LST), associative (AST) and sensorimotor (SMST) subregions, each of which receives input from functionally corresponding cortical areas and also from non-corresponding cortical regions, where integrative functions occur. Here, we examine in SZ and NCLs both corresponding and non-corresponding frontostriatal tracts.

Methods
DTI and sMRI images were acquired on a 3T GE Echospeed system from 27 chronic SZs and 26 matched NCLs. Associative cortex, using FreeSurfer, was parcellated into rostral MFG (rMFG) corresponding to DLPFC and inferior frontal gyrus (IFG), corresponding to VLPFC. The
striatum was manually parcellated to delineate the AST, comprised of precommissural and postcommissural caudate together with the dorsal precommissural putamen. ROIs were registered to DTI space. Two-tensor tractography fiber paths throughout the brain were obtained. Fibers tracts connecting associative cortex with AST and SMST (rMFG-AST, IFG-AST, rMFG-SMST, IFG-SMST) for each subject were extracted. DNA was extracted from saliva and genotyped using the Sequenome iPlex platform. Subjects were genotyped at the rs4819756 polymorphism in the proline dehydrogenase (PRODH) gene and divided into GG and A allele carriers (AG/AA) groups. MR-DTI measures of FA, RD, AD and fiber counts were compared.

Results
Results showed for the 4 tracts (rMFG-AST, IFG-AST, rMFG-SMST, IFG-SMST) an overall group difference in mean FA (F(1,50)=5.5, p=0.023). F/U t-tests showed that NCLs had greater FA in left, but not right, IFG-AST (t=2.0, df=50, p=0.047); and in left, but not right, IFG-SMST (t=2.4, df=50, p=0.019). Examining group differences for FA in corresponding and non-corresponding tracts separately showed for the non-corresponding tracts (rMFG-SMST, IFG-SMST), a main effect for group (F(1,50)=3.03, p=0.027) and for the corresponding tracts (rMFG-AST, IFG-AST) a trend significant main effect for group (F(1,50)=3.03, p=0.060). Similar analyses using fiber counts in tracts, instead of FA, while covarying for intracranial contents, showed similar results. In a subset of these subjects (n=22), we obtained genotyping of the proline dehydrogenase (PRODH) gene. Analyses in the 4 tracts showed findings in the left, but not right, hemisphere. Subjects from both groups with the GG genotype (n=12) compared to subjects with GA/AA genotypes (n=10) had: 1) decreased FA and trend increased RD in the corresponding rMFG-AST tract (p=0.045; p=0.7); 2) trend decrease FA in the corresponding IFG-AST tract (p=0.076); and 3) a trend increase in the non-corresponding rMFG to SMST tract (p=0.086). When we examined SZ subjects, alone, (n=13), those subjects with the GG genotype (n=9) compared with those with AG/AA genotypes, had an increased RD (p=0.049) and a trend decrease FA (0.08) in the non-corresponding rMFG-SMST tract.

Discussion
We found diminished FA and diminished fiber counts in SZ both in corresponding and non-corresponding frontostriatal associative loop tracts, possibly with stronger group differences in the latter tracts. This is consistent with a subtle somatotopic disorganization of the striatum in SZ, perhaps related to abnormal development. Consistent with the literature, those subjects with the G/G genotype, compared with those subject with AG/AA genotypes at the rs4819756 SNP of the PRODH gene showed group differences in the predicted direction in diffusion measures (decreased FA and increased RD in left hemisphere tracts originating in DLPFC and VLPFC) but not in fiber counts. This suggests that the GG genotype at rs4819756 of the PRODH gene may affect the quality of fibers, e.g., the microstructure, but not the number of fibers or the developmental somatopic arrangement of fiber connections. We plan to increase the size of our sample to strengthen these results.

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GENOMIC INFLUENCES ON ALCOHOL PROBLEMS IN A POPULATION-BASED SAMPLE OF YOUNG ADULTS
Background
Genetic influences on alcohol use disorders are substantial, with heritability estimates around 50-60%. However, standard genome-wide association studies have been largely unable to consistently identify loci that are significantly associated with an alcohol use disorder outcome. Efforts at improving our interpretation of the relevance of genome-wide association results could include gene- and network-based analyses. Furthermore, combining the results of standard association analyses with additional genomic data, such as epigenetic modifications proximal to implicated SNPs, could help prioritize loci to target in follow-up studies.

Methods
Participants in a longitudinal study of health-related behaviors reported on symptoms of alcohol misuse and problems at age 18 years 8 months. A continuous score of alcohol problems was derived for these data, which was then used in a genome-wide association analysis. Gene-based tests were conducted using VEGAS, and enrichment for gene ontologies was assessed using the online database GSEA4GWAS. Finally, we investigated the proximity of implicated SNPs to chromatin modification sites.

Results
In the initial genome-wide association analysis, no common SNP surpassed the genome-wide significance threshold. Gene-based tests were conducted for 17,871 genes, of which 28 had p<0.001. Among these 28 genes were the transcription factors CTCF and VGLL3; ACD, which is involved in telomere function; the neuropeptide CNP; and KCTD16, which interacts with the GABA neurotransmission system. Several of these genes have been previously implicated in alcohol-related phenotypes. Multiple pertinent gene ontology categories were statistically over-represented (p<0.001, FDR≤0.001), including neurogenesis, synaptic transmission, neurite development, axon guidance, behavior, transcriptional regulation, and chromatin modification. Finally, SNPs with significant p-values are proximal to the H3K36me3 histone mark in fetal brain cell lines significantly more frequently than expected by chance.

Discussion
Given the complex genomic nature of alcohol problems, moving beyond standard genome-wide association study results is necessary to interpret relevant genomic influences and prioritize loci for follow-up. The current results, from a population-based study of young adults, suggest that genes involved in transcriptional regulation, neural function, and chromatin modification impact risk for alcohol problems. Genes (e.g., CTCF) and systems (e.g., GABAergic neurotransmission) previously associated with alcohol-related phenotypes were also identified in the current study. Furthermore, mapping of SNPs to sites of known epigenetic modifications indicates that genomic loci involved in liability to alcohol problems are being actively transcribed in the fetal brain. These findings broaden our perspective on genomic influences relevant to risk for alcohol problems and suggest that genes transcribed early in development could be influential on distal alcohol-related phenotypes.
DOPAMINERGIC GENE POLYMORPHISMS IN ASSOCIATION WITH HEROIN DEPENDENCE AND THE OUTCOME OF SUBSTITUTION THERAPY

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Background

Heroin dependence as a psychiatric disorder is proven to have a complex genetic background, involving many factors, but several inconsistencies can be found in the literature regarding these variations. Thus, improving methods and increasing sample size may help us to further examine the underlying genetic factors. The goal of this study was to attempt to shed some more light on the role of dopaminergic genes involved in the reward system in context of the pathophysiology of heroin addiction using modern molecular biological technics in order to investigate the shared genetic background of common addictions.

Methods

Beside our case-control study, where we investigated genetic polymorphisms of the dopaminergic system by comparing 303 heroin dependent patients and 555 healthy Hungarian controls, an additional OpenArray study (using TaqMan® OpenArray® Genotyping System) was carried out on a sample of 317 heroin dependent patients and 577 healthy controls. The studied polymorphisms of the dopaminergic system were: the dopamine D4 receptor gene (DRD4) III. exon VNTR, the rs1800955 (-521 C/T), rs747302 (-616 C/G) and rs936462 (-615 A/G) single nucleotide polymorphisms (SNPs) and the 120bp duplication; the dopamine transporter 3’ and VIII. intron VNTRs; the rs1800497 (TaqIA) of the ANKK1 and rs1079597 (TaqIB), rs1800498 (TaqID) of the dopamine D2 gene (DRD2). In the OpenArray study we conducted an in silico selection of common addiction candidate genes including alcohol- and aldehyde dehydrogenase, acetylcholine and cannabinoid receptors, dopamine receptors and further gene variants possibly involved in the genetics of drug abuse. Our study also covered the determination of genetic factors with possible effect on the efficiency of the substitution therapy to help improve the outcome and also to help select the most appropriate therapy for the patients. Besides the conventional statistical methods, multivariate analysis of associations using Bayesian networks in Bayesian multilevel analysis was also employed.

Results

Based on our analyses all studied polymorphisms correspond to the Hardy-Weinberg equilibrium. The results of the case-control study showed significant association between the ANKK1 rs1800497 (p=0.009), the DRD2 rs1079597 (p=0.003) and the DRD4 rs1800955 (p=0.007) and heroin dependence. Additional bioinformatic analyses revealed an indirect effect of the DRD4 -521 C/T polymorphism (in the case of G allele the effect of the -521 C/T SNP is strongly significant, p=0.0013). The final OpenArray analysis included 22 of the original 32 SNPs (according to the intensity of the fluorescent signals and the efficiency of the genotyping) and found 5 nominally significant associations (ALDH2 rs886205, ANKK1 rs1800497, GABRA2 rs279858, CHRNA5 rs16969968 és rs1051730), from which only one SNP in the
ALDH2 gene (aldehyde dehydrogenase 2, rs886205, p=0.000071) survived multiple correction. ALDH2 is thought to be involved in the metabolism of dopamine so probably has a role in the pathophysiology of other addictions as well. A previous Chinese study (Wang et al., 2012) showed association between the ALDH2 and heroin dependence and now this study confirms these results on a Caucasian population. The results from the study investigating the genetic polymorphisms in the context of the efficiency of substitution therapy, the DRD4 gene 3 polymorphisms (exon III. VNTR, -521 C/T SNP and the 120bp duplication) seemed to be associated with the possible outcome based on response rates after 3 months of treatment.

Discussion
Our results show a relevant effect of the dopaminergic system in the pathophisiology of heroin dependence and due to the sample size and Bayesian multilevel analysis we were able to clarify the main effects of the possible candidate genes in the disease. Furthermore the OpenArray study confirms the role of the ALDH2 gene in the genetic background of heroin dependence in a Caucasian population.

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A FAMILY STUDY OF CANNABIS USERS WITH AND WITHOUT PSYCHOSIS
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Background
Many studies have associated schizophrenia with adolescent cannabis use; however, no causative link has been identified. Schizophrenia has a high genetic component. Thus, it is likely that people who use cannabis and go on to develop a psychosis may do so because they have an elevated familial risk for schizophrenia. However, this has never been shown.

Methods
Probands were recruited into four cohorts: Cohort 1- Controls, no drug use; Cohort 2- Controls, cannabis use; Cohort 3- Patients with a schizophrenia spectrum psychosis (SSP), no drug use; Cohort 4- Patients with SSP, cannabis use. Semi-structured interviews were used to obtain diagnostic information about all relatives from probands as well as family informants.

Results
There was an increased morbid risk (MR) for schizophrenia in relatives of both patient cohorts compared to control cohorts. There was no significant difference in MR for schizophrenia between relatives of patients who use or do not use cannabis (p=.4322).

Discussion
Schizophrenia is not a consequence of adolescent cannabis use. A genetic predisposition for schizophrenia must first be present.

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A GENOMEWIDE ASSOCIATION STUDY OF ALCOHOL DEPENDENCE IN THE IRISH AFFECTED SIB PAIR STUDY OF ALCOHOL DEPENDENCE
Background
The powerful, systematic, and unbiased genomewide association study (GWAS) has been successful in identifying replicated susceptibility variants for numerous complex diseases. We report here results from an ethnically homogeneous Irish sample (N=710 related cases, 1755 population controls) with strong supporting evidence from VCU Alcohol Research Center model organism studies.

Methods
GWAS cases from the Irish Affected Sib Pair Study of Alcohol Dependence (IASPSAD) were diagnosed using DSM-IV criteria. Genotyping was conducted using the Affymetrix V6.0 array by three separate genotyping core facilities. Because artifacts are a known issue when combining samples genotyped at multiple sites, genotypes were called using BeagleCall, which considers both allele signal intensities and linkage disequilibrium (LD) information. IMPUTE2 and the 1000 Genomes reference haplotype panel (March 2012 freeze) were used to impute unmeasured genotypes. After quality control filtering, imputation, and post-imputation filtering, the data set contained 710 AD cases, 1755 controls, and 8.2 million SNPs. Probabilities were converted to dosages (based on number of minor alleles) using MACH2. We performed the case-control association analysis with the Modified Quasi-Likelihood Score (MQLS) program, which uses relationship matrices to correct for the non-independence of siblings. A sex weighted prevalence estimate of 8.9% was used for controls. We utilized a genome-wide significance threshold of p less than 3.06 x 10^-8, which controls the false positive rate at an alpha of less than 0.05 for 1.6 million independent markers in a European population. False Discovery Rate (FDR) q-values were calculated using the QVALUE software in R.

Results
Our GWAS p-values showed no evidence of inflation (lambda = 1.05). 13 SNPs met criteria for genomewide significance with 12 falling in the collagen 6A3 (COL6A3) gene on chromosome 2 and 1 located in an intergenic region of chromosome 3. 725 SNPs had q-values less than or equal to 0.5 with a total of 103 loci represented. Preliminary experimental data using multiple model organisms support 3 of the top 5 genes: COL6A3 (top SNP p-value=6.18E-09, q-value=0.07), the Krueppel-like factor 12 (KFL12) gene on chromosome 13 (top SNP p-value=1.16E-07, q-value=0.08), and the Ryanodine receptor 3 (RYR3) gene on chromosome 15 (top SNP p-value=1.69E-07, q-value=0.08). Inactivation of one of three genes in C. elegans showing homology to human COL6A3 results in an ethanol resistance phenotype. The C. elegans klf-3 mutant (homologous to human KLF12) does not develop acute functional tolerance.
to ethanol. Furthermore, RNAi knockdown of the *D. melanogaster* homolog of *KLF12*, *luna*, results in enhanced sensitivity to ethanol. Finally, a loss of function allele of *unc-68*, the *C. elegans* homolog of *RYR3*, confers resistance to ethanol.

**Discussion**

Our case-control GWAS of AD detected a genomewide significant association signal in the collagen 6A3 (*COL6A3*) gene based on 12 non-independent SNPs. Other top signals fell within additional novel candidate genes, including those involved in neurodevelopmental disorders and cancer. Emerging evidence from the VCU Alcohol Research Center model organism investigations provides strong additional support for 3 of the top 5 genes represented in our p-value ranked SNP list. Replication is underway for our top 725 SNPs in four independent samples of European descent (N>11,000).

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**ASSOCIATION OF GDNF GENE VARIANTS WITH TOBACCO AND BETEL QUID CONSUMPTION IN THREE ETHNIC GROUPS FROM INDIA**

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**Background**

There are some culturally distinct ways of consuming tobacco along with betel quid (Areca nut and leaf), a mild stimulant among certain ethnic groups in North Eastern India. Consumption of this combination, like any other drug, is known to produce euphoria through the dopaminergic reward system. Glial cell line-derived neurotrophic factor (GDNF) is an essential growth factor for the survival and maintenance of midbrain dopaminergic neurons. Several studies have demonstrated various associations between dopaminergic genes and addictions, there are very few studies with regard to GDNF and addictive behaviors.

**Methods**

The present study was carried out in a sample of 700 young adults (age range 18-35) from three ethnic groups from the North Eastern region of India: Bengali (N=200, Caucasian), Hmar (N=200, Mongoloid), Khasi (N=300, Mongoloid). Data on tobacco and betel nut and leaf consumption was collected using a structured questionnaire, as well as personal interview. The level of addiction was measured by self-reported methods including the modified Fagerström Test for Nicotine Dependence. DNA was extracted from buccal swabs and genotyping of five GDNF polymorphism (rs2910704, rs3812047, rs2910702, rs1549250 and rs11111) was carried out by RT-PCR using TaqMan probes.

**Results**

In case of the rs3812047 A/G polymorphism the case-control analysis revealed significant differences between tobacco users vs. non-consumers (genotype-wise:p=0.0021, allele-wise: p=0.00005; OR=2.4) with A being the protective allele. The same GDNF allele was similarly associated with betel quid consumption as well, however, there were some culturally distinct differences between consumption patterns between the studied three populations.
Discussion
A study done by Yoshimura et. al in 2011 (1) reported an association between the GDNF rs2910704 variant and the severity of addiction to methamphetamine. Earlier this year our research group has found an association between GDNF polymorphisms and smoking in young Hungarian adults (unpublished data). These and the present study indicate that GDNF – probably due to its involvement in development and function of dopaminergic neurons – plays an important role in tobacco/betel consumption and the level of addiction. The two substances are most often consumed together in these ethnic groups in different combinations. The effect of genetic variations in GDNF is most likely to be a general factor related to addictive behavior due to the dopaminergic neurotransmission involved in reward, rather than a substance specific polymorphism.
A GENETIC RISK SCORE COMBINING 32 SNPS IS ASSOCIATED WITH BODY MASS INDEX AND IMPROVES OBESITY PREDICTION IN PEOPLE WITH MAJOR DEPRESSIVE DISORDER

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Background

Obesity is strongly associated with major depressive disorder (MDD) and various medical diseases. Genome-wide association studies (GWAS) have identified multiple risk loci robustly associated with body mass index (BMI). In this study, we aim to investigate whether a genetic risk score (GRS) combining multiple risk loci which achieved genome-wide significance is associated with BMI and predicts obesity in people with MDD.

Methods

The unweighted GRS was calculated by summation of the number of risk alleles. The weighted GRS was calculated by summing up the multiplication of the number of risk alleles at each locus by their corresponding effect sizes. Receiver operating characteristic curve was used to calculate the area under the curve (AUC) in order to compare the discrimination ability of obesity.

Results

A total of 2521 participants without missing data in Radiant study was included in first stage analysis. Both unweighted GRS and weighted GRS were highly associated with BMI (p<0.001) and explained approximately 1.27% of variance of BMI. Using GRS alone for discriminating obesity was not good enough but adding traditional risk factors improved the ability significantly from 0.58 to 0.66 (χ² = 27.68, p<0.0001). The best model was achieved using all genetic information, traditional risk factor and depression status (AUC = 0.71, χ² = 28.64, p<0.0001). The replication analysis using Munich study showed the similar result but smaller amount of variance of BMI (0.45%) explained by GRS.

Discussion

Using GRS alone improved modest but significant discrimination ability of obesity. Given the high prevalence of MDD and obesity, incorporating genetic information, traditional risk factors and depression status may largely improve the predicting ability for obesity. Future studies should incorporate other genetic information into GRS to improve the prediction ability of obesity.

2
A POLYMORPHIC REPEAT IN THE POLYGLUTAMINE TRACT OF THE RETINOIC ACID INDUCED 1 GENE IS ASSOCIATED WITH PERINATAL DEPRESSION AND PREMENSTRUAL/MENSTRUAL SYMPTOMS

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Background
Depression during the antenatal and/or postnatal period affects about 10% of women who undergo pregnancy and childbirth. The incidence is higher for those with family history of depression and other mental illness, suggesting the contribution of genes. One candidate gene is the Retinoic acid induced 1 (RAI1) gene which is highly active in the nerve cells in the brain. The gene is dosage sensitive and has been implicated in Smith-Magenis syndrome and Potocki-Lupski syndrome. Variants in the gene have also been associated with spino-cerebellar ataxia, non-syndromic autism and schizophrenia.

Methods
Patients who came for post-natal consultations at the obstetrics clinics and scored < 7 on the Edinburgh Postnatal Depression Scale (EPDS) were recruited via the post-natal screening programme of the hospital. Cases with confirmed diagnosis of clinical (major) depression related to pregnancy/postpartum were recruited from the outpatient clinic. Demographic information and saliva samples were collected from the participants with written informed consent. Genomic DNA was extracted from saliva and the relevant region sequenced to determine the number of CAG/CAA repeats that encodes the polyglutamine tract in the N terminal of the protein. Difference between groups was assessed by chi-square analysis for categorical variables and analysis of variance (ANOVA) for quantitative scores.

Results
For the RAI1 gene CAG/CAA repeat, the distribution in controls (n=536) ranged from 10 to 21 while the distribution for cases (n=134) were from 9 to 15. The most common was the 13-repeat allele, followed by the 14-repeat allele which was the most common in the Caucasian population. There was statistically significant difference in the distribution of this 14-repeat allele between controls and cases (Chi-Square=8.320, P-value=0.016). This allele was also marginally associated with family history of mental illness (Chi-Square=5.078, P-value=0.079). There was statistically significant association of perinatal depression with family history of mental illness (Chi-Square=131.241, P-value=0.000). Overall, patients with perinatal depression reported more frequent mood changes, cramps, nausea, vomiting, diarrhea, and headache during the premenstrual/menstrual periods (F=17.258-76.764, P-value=0.000 for all). There was also statistically significant differences in the mean scores for mood changes (F=3.027, P-value=0.049) and experience of headache (F=5.047, P-value=0.007) for patients with different copy numbers of the 14-repeat allele.

Discussion
Retinoic acid signalling in the mature brain may be important for neuronal maintenance, plasticity, repair and regeneration. The role of the RAI1 gene has not been explored in depression. In this study, we investigated the distribution of the polymorphic repeat in this gene in perinatal depression. The allele frequencies of the RAI1 gene repeat is different in our population compared to published data for Western population. The 14-repeat allele is associated
with perinatal depression and more frequent experience of physical and psychological symptoms during menstrual period. As there is evidence that retinoic acid receptor binding throughout the genome is highly coincident with estrogen receptor α binding, it may be important in the pathogenesis of perinatal depression by acting through crosstalk of retinoic acid and estrogen signaling pathways.

3

DEPRESSION AND BMI INFLUENCES THE SERUM VASCULAR ENDOTHELIAL GROWTH FACTOR LEVEL
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Background
Recent research studies suggest that the vascular endothelial growth factor A (VEGF) is involved in the pathogenesis of depression. Only a few studies have investigated the serum VEGF levels in individuals with depression as well as the possible association between genetic variants within VEGF and depression.

Methods
The purpose of the present study was to identify if depression, different socio-demographic variables, lifestyle, and health indicators as well as genotypes determine the serum VEGF level. The study population comprised 155 depressed subjects and 280 control individuals that returned a completed questionnaire and participated in a semi-structured diagnostic interview. Body mass index (BMI) was computed from measured height and weight, and included in the model as a continuous variable. The serum VEGF level was determined using the Quantikine Human VEGF Immunoassay (R&D Systems Inc., Minneapolis, USA). Eleven tagSNPs were successfully genotyped using the Sequenom MassARRAY platform and the statistical analyses were performed using PLINK. Generalized linear models were used to assess independent determinants of the serum VEGF level using Stata.

Results
In the present study the serum VEGF levels were significantly increased in depressed subjects compared to control individuals (p<0.0001). Additionally, severity of depression, previous depressive episodes, and increasing age and BMI were associated with higher serum VEGF levels. One SNP (rs10434) was significantly associated with depression after correction for multiple testing, but not with the serum VEGF level. Depression and BMI were significant determinants in the final model of the serum VEGF level.

Discussion
Our study suggests a role of circulating serum VEGF in depression. In consistence with other studies, we observed an increased serum VEGF levels in depressed individuals. Furthermore, our
data demonstrate that other factors than a diagnosis of depression influence the serum VEGF level. Potentially, several other factors not taken into account in this or previous studies are also influencing the serum VEGF level.

4

GENETIC RISK, LIFE STRESS AND DEPRESSION: SEARCHING FOR POLYGENE-BY-ENVIRONMENT INTERACTIONS

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Background

Although the heritability of depression is around 30-40\% (Sullivan et al., 2000), the nature of the relationship between genes and depression, and the extent to which this relationship depends on environmental factors, remains unclear. Previous studies suggest that certain candidate genes (e.g. 5-HTT) may interact with stressful life events (SLE’s) to predict depression (Caspi et al., 2003), however there is growing evidence to suggest that psychiatric disorders, including depression, may be polygenic in nature (Sullivan et al., 2012). The aim of this study was to extend the research on gene-environment interactions in depression by evaluating the extent to which life stress interacts with a continuous measure of polygenic risk.

Methods

The target dataset for this study was the Health and Retirement Survey (HRS), a longitudinal cohort study of \textasciitilde26,000 American adults age 50+. The HRS dataset includes genome-wide association (GWA) data for 12,507 participants, genotyped on the Illumina HumanOmni2.5-4v1 SNP array with \textasciitilde2.5 million markers. Phenotypic variables were obtained from the panel of HRS data collected in the year 2000. Participants were included in the analyses if they a) participated in the 2000 wave of data collection, b) reported no history of stroke, possible stroke, transient ischemic attack or memory related disease and c) had GWA data available. A total of 9,241 participants met these inclusion criteria. Depression was measured using an 8-item sub-scale of the CES-D, with a cut-off score of 4. Life stress was measured using the number (0, 1, 2+) of SLE’s experienced during the previous 2 years. Individual SLE’s included events such as a death in the immediate family, a significant health problem (e.g. heart attack) or a major personal life change (e.g. divorce, retirement). Polygenic risk was measured using the polygenic score approach (Purcell et al., 2009). Results from the Psychiatric GWAS Consortium (PGC) mega-analysis of major depression were used as the discovery dataset for creating the polygenic scores. Single nucleotide polymorphisms (SNPs) associated with major depression at 4 p value thresholds (.1, .2, .3, .4) were used to generate 4 separate continuous polygenic score variables (Threshold 1: 14,431 SNPs; Threshold 2: 25,475 SNPs; Threshold 3: 35,222 SNPs; Threshold 4: 44,130 SNPs). Polygenic scores were created in PLINK, and all other statistical analyses were conducted in SAS.

Results

After controlling for ancestry using the first 4 principal components, logistic regression analyses showed significant associations between polygenic scores and depression at each of the 4 significance thresholds (Threshold 1: p = .0021; Threshold 2: p = .000089; Threshold 3: p = .0002; Threshold 4: p = .0002). The polygenic scores explained \textasciitilde1\% of the variance in
depression (pseudo R$^2$ range: .0072 -.0095). Life stress was also significantly associated with depression: a single SLE in the previous 2 years increased the odds of depression by 40% (OR = 1.40, 95% CI [1.22-1.61]; p < .0001), while 2+ SLE’s were associated with a 2 fold increase in the odds of depression (OR = 1.96, 95% CI [1.55-2.49]; p < .0001). The association between SLE’s and depression persisted after controlling for age, self-reported race, gender, and education. When polygenic scores and life stress were included in the same models (along with the first 4 principal components), the magnitude and significance of the main effects remained unchanged. There was no evidence of moderation on either the additive or multiplicative scales: the interaction terms for polygenic scores and SLE’s were not statistically significant in either logistic or linear models (p value range: .25 - .95).

Discussion
Our findings suggest that polygenic risk and stressful life events are each significant, independent predictors of depression in older adults. We did not find evidence to support the hypothesis that life stress (measured using the number of SLEs) interacts with polygenic risk (measured using polygenic scores). These findings should be viewed in light of several limitations, including the age of the sample, potential lack of specificity in the outcome measure and the fact that polygenic risk was measured using markers identified by statistical significance rather than biologic plausibility.

5

THE ROLE OF MATERNAL DEPRESSION AND OXYTOCIN RECEPTOR GENE IN YOUTHS EMOTION RECOGNITION ABILITIES
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Background
Children of mothers with a history of major depressive disorder (MDD) are 3-4 times more likely to experience MDD by early adulthood, compared to children of non-depressed mothers. Despite this known risk, relatively little is known about the specific mechanism of risk for the intergenerational transmission of depression. Theorists and researchers have suggested that cognitive processing of social cues and facial expressions may play a critical role in the development and maintenance of MDD. Supporting this hypothesis, one study found that daughters of depressed mothers required greater emotional intensity than daughters of control mothers to accurately identify and detect sad facial expressions (Joormann et al., 2010). This said, not all children of depressed mothers become depressed themselves, pointing to the need to identify specific moderators of risk, which will allow a better delineation of which specific children of depressed mothers are at greatest risk for depression themselves. The goal of this study was to identify specific genetic moderators of risk. Oxytocin has been shown to improve many aspects of social cognitive functioning, including facial emotion recognition (Schulze et al, 2011). More recent research has studied the effects of genetic variations in receptors that mediate the physiological and behavioral effects of oxytocin (e.g., Bradley et al., 2011). Specifically, a polymorphism in the oxytocin receptor gene (OXTR rs53576) has been associated with emotion regulation difficulties, with individuals homozygous for the G allele being at higher risk for increased emotion dysregulation, compared to carriers of the A allele. In the current study, we predicted that the OXTR rs53576 genotype would moderate the link between maternal history of
MDD and youth’s ability to detect facial displays of emotion.

Methods
Participants were 255 mother-child pairs from the community. Children in the study were between 8 and 14 years old and 52% were female. Mothers either had a history of MDD during their child’s lifetime (n = 129) or no lifetime history of MDD (n = 126). Youth viewed faces on a computer screen that represented 10% increments of two emotions ranging from 100% neutral (0% target emotion) to 100% target emotion (e.g., 90% Neutral, 10% Sad; 80% Neutral, 20% Sad; and so on). DNA was isolated from buccal cells and used to genotype OXTR rs53576.

Results
Using generalized estimating equations (GEE), we conducted a 2 (Mom MDD: children of depressed mothers, children of non-depressed mothers) x 2 (OXTR rs53576: AA/AG, GG) x 3 (Emotion: Angry, Happy, Sad) x 9 (Morph Level: 10% to 90% morph) analysis. We found evidence for a significant Emotion × Mom MDD × OXTR rs53576 interaction, Wald = 15.95, df = 2, p < .001. Results indicated that the Mom MDD × OXTR rs53576 interaction was significant for the happy condition (Wald = 8.20, df = 1, p < .01), but not for the angry or sad condition. More specifically, we found that among children homozygous for the G allele, the children of depressed mothers had significantly lower levels of emotion identification for happy faces (M proportion endorsed as target emotion = .79, SE = .025) than children of non-depressed mothers (M = .86, SE = .016).

Discussion
The current study found that children of depressed mothers who carried the OXTR rs53576 GG genotype had more difficulty detecting happy facial expressions, compared to children of non-depressed mothers and children carrying the AG or AA genotypes. These findings provide initial support for the role of the OXTR rs53576 genotype and disrupted emotion recognition of happy faces in the intergenerational transmission of depression.

EVIDENCE SUPPORTING THE EXISTENCE OF A SPECIFIC GENETIC SUBSTRATE FOR VULNERABILITY TO THE NEW DSM-V ANXIETY-DEPRESSIVE SYNDROME DIAGNOSIS

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Background
Major depression (MD) and generalized anxiety disorder (GAD) are the most common
psychiatry disorders found among primary care attendees (King et al., 2008). Both conditions frequently co-occur, possibly as a consequence of a common psychopathology (Anderson et al., 2008) and a shared genetic substrate of vulnerability (Molina et al., 2011). Despite the frequent co-occurrence of comorbid depressive and anxiety states, no previous studies have explored the association between a wide number of potentially risk genetic polymorphisms and comorbid anxiety-depressive states. This study aimed to investigate the genetic influence of 384 candidate polymorphisms in relation to anxiety-depressive syndrome.

Methods
The sample consisted of 2546 subjects from the PREDICT-GENE study, a genetic sub-study nested in a 3-year prospective study (the predictD-Spain, Bellón et al., 2008) which was focused on the development of a risk index for the onset of major depression and other common mental disorders in primary care. Diagnoses were ascertained using the depression section of the Composite International Interview Schedule (CIDI) and the Anxiety Section of the Spanish Version of the Primary Care Evaluation of Mental Disorders Patient Health Questionnaire (PHQ PRIME-MD), respectively. DNA from all participants was obtained from blood or saliva. A total of 384 polymorphisms at 80 candidate genes for MD and/or GAD (including monoaminergic neurotransmission, plasticity and hypothalamic–pituitary–adrenal axis genes) were genotyped using GoldenGate technology (Illumina). After quality control procedures applied to individual and SNP data, 341 polymorphisms were included in the analyses. To correct for multiple testing, the significance was assessed using Single Nucleotide Polymorphism Spectral Decomposition (SNPSpD) (Nyholty, 2004) giving a threshold of $p=0.0002$. Logistic regression analyses were performed to test for the association between these polymorphisms and our outcome variable: “anxiety-depressive syndrome”, including sex and age as covariates in the models. The statistical analyses were performed using the statistical package PLINK v.107 (Purcell et al., 2007).

Results
Our results revealed 8 SNPs significantly associated ($p<0.05$) with anxiety-depressive syndrome: rs4646312 at COMT gene ($p=0.002$, OR= 1.88); rs5324 at DBH gene ($p=0.01$, OR=0.67); rs3804958 at GRM7 gene $p=0.006$, OR=0.43); rs4576167 at WWC1 gene ($p=0.016$; OR=0.68); rs2296972 at HTR2A gene ($p=0.016$, OR= 0.33); rs1875999 at CRHBP gene ($p=0.02$, OR=1.44); rs952037 at ADRA1B gene ($p=0.023$, OR=1.43) and rs8150 at RHBDF2 gene ($p=0.029$, OR= 1.43). However, they did not remained statistically significant after multiple testing correction ($p<0.0002$).

Discussion
We found associations between several SNPs in candidate genes and MD/GAD comorbid phenotype in a large sample of depressive cases and controls. The fact that these polymorphisms were not significantly associated with either MD or GAD phenotypes separately, support the hypothesis of a specific genetic substrate for vulnerability to anxiety-depressive syndrome. Although previous studies have explored the association of polymorphisms in candidate genes implicated in the atiology of MD or GAD separately, to our knowledge this is the first study investigating the relationship between these polymorphisms and MD/GAD comorbid phenotype. Our research suggests that certain variants in candidate genes play a role in the aetiology of comorbid MD and GAD conferring risk only when both disorders are present concurrently.
THE CORTISOL AWAKENING RESPONSE IN BIPOLAR DISORDER BY DISEASE STATUS AND SEVERITY IN SHORT TIME FOLLOW-UP

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Background
Dysregulation of the hypothalamus-pituitary-adrenal (HPA) axis is known to occur in bipolar disorder patients. Cortisol awakening response (CAR), a pronounced increase in cortisol level that peaks at approximately 30 to 40 minutes after waking in the morning, is an indicator of the function of HPA axis. Few previous studies that evaluated daytime cortisol level in patients with bipolar disorder at different disease status (e.g. acute episode vs. remission) did not report consistent findings. Moreover, there are only two prior studies examined the CAR level in bipolar disorder patients. The goal of the present study is to examine the level of CAR in bipolar disorder patients at different disease status and severity while compared with controls. We also attempted to evaluate the patterns of CAR level during short-time follow-up.

Methods
Longitudinal data were collected from 22 hospitalized bipolar disorder patients (12 in manic and 10 in depressive episodes) at different disease status (acute episode, remission and follow-up twice every 2-month after discharge). In addition, 13 healthy controls were recruited. Saliva samples were collected at the time of awakening (T0), 30, and 45 minutes after awakening. Two CAR indicators were calculated, delta (Peak-Baseline (T0)) and AUCi (area under the curve respect to cortisol increase). Episodic symptom severity was measured using 17-item HAM-D (Hamilton Depression Rating Scale) and YMRS (Young Mania Rating Scale). Paired t-test and student t-test were used to compare the CAR level at different disease status (within persons) and between subgroups, respectively. We calculated Pearson correlation for the relationship between CAR levels and symptom severity. Additionally, the low severity group was combined (≤ 15 for YMRS; ≤ 13 for HAMD) to compare with the high severity (moderate and severe) group.

Results
Bipolar patients showed higher CAR level in manic episode than in remission. Conversely, patients in depressive episode had lower CAR than patients in remission. Controls had higher CAR level than patients in episode and in remission. The within patients comparisons exhibited very similar results. The high severity group showed higher delta and AUCi than the low severity group. However, there was only low correlation (r= 0.03~0.30) between CAR levels and the symptom severity.

Discussion
CAR levels distinguish patients from controls. Moreover, bipolar disorder patients at different disease status and episodic types exhibit distinct CAR patterns. These findings would assist to design further studies to investigate the regulation mechanisms of the HPA axis in bipolar disorder.
PREDICTION OF LITHIUM RESPONSE IN BIPOLAR DISORDER PATIENTS USING ARTIFICIAL NEURAL NETWORK

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Background

Bipolar affective disorder (BAD) is a severe psychiatric disorder but yet the etiology of BAD is not clearly understood. Both genetic predisposition and pharmacologic maintenance treatment are crucial factors for patients' prognosis. Lithium is a mood-stabilizer which is considered as a first-line drug for treating BAD. It has also been proven to be effective in long-term prevention of recurrence and reduce the risk of suicidal behavior. Although lithium works very well in some patients, about two-thirds of lithium-treated patients are non-responders. In the past, the searching for relevant predictors for lithium response is hindered by varying measurement of lithium response, and the results are not easily compared across studies. The goal of the present study is to build prediction models for lithium response in Chinese and select important input variables for prediction. Traditional logistic regression models consider only linear effect between dependent and independent variables, which is not always true in difference cases. On the contrary, an artificial neural network (ANN) method can deal with non-linear relationship between response outcome and clinical and psychosocial predictors. Thus, we also compared the prediction accuracy using both logistic regression and ANN.

Methods

Patients who met the diagnostic criteria of DSM-IV BAD were recruited from hospitals in Taipei. There were 117 patients who took lithium regularly, including 54 males and 63 females (mean age 45±12 years). We used Alda's scale (Grof, et al. 2002) to assess lithium response. Patients were grouped into responders, whose score >= 2 and non-responders(<2). Information regarding the predictors was acquired from semi-structured interview and chart review, including clinical features (e.g. age onset, rapid cycling, family history and so on ), psychosocial features (e.g. neuroticism) and demographic (e.g. social status and employment) variables. Prediction models were constructed using ANN and logistic regression for lithium response. On the other hand, we used stepwise logistic regression and connection weight of ANN to select important variables. We also compared the prediction accuracy across different models using sensitivity, specificity and area under the curve (AUC).

Results

The ANN model yielded a much higher level of prediction rate for lithium response than did it with multiple logistic regression model. The AUC in logistic regression and ANN model were
The sensitivity in logistic regression and ANN model were 0.87 and 0.98, respectively. The sensitivity in logistic regression and ANN model were 0.87 and 0.98, and specificity were 0.41 and 0.96, respectively. The results of variable selection via ANN and logistic regression are not all consistent. In the logistic regression analysis, hospitalizations and substance use of tobacco were selected as predictors from stepwise method. On the other hand, rapid cycling, substance of tobacco, employment, family history of anxiety, sex and hospitalizations were important variables in the ANN model.

**Discussion**

A timely prediction for lithium response is one of special clinical importance to assist guiding patients’ treatment strategy. The use of ANN model provides accurate prediction and proves to be an attractive solution for this daily problems. Information regarding to substance of tobacco, rapid cycling, hospitalizations and employment play an important roles in predictive accuracy. Further research is needed to bring us closer to “achieve personalized medicine” for the use of lithium in BAD patients.

9

**IDENTIFYING POTENTIAL REGIONS OF COPY NUMBER VARIATION RELATED TO BIPOLAR DISORDER**

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**Background**

Bipolar disorder is a common mental disorder with high heritability, but its genetic determinants are still not clear. Copy number variation (CNV) is one of the sources to explain part of the heritability. Array-based technology enables fasting scanning large numbers of CNV, and many statistical strategies are developed for the estimation of copy number from experimental data. The challenge comes from estimating discrete value of the copy numbers using continuous signals calling from a set of markers and simultaneously performing association testing between CNVs and phenotypic outcomes. Recently, DNA pooling strategy is adopted to save genotyping cost. However, it faces an even bigger challenge for CNV detection using pooling data. The goal of the present study is to develop a series of procedures to identify potential CNV regions that are related to bipolar disorder

**Methods**

A total of 200 normal controls and 200 bipolar patients were included in this study, and were randomly divided into 8 control and 8 case pools. We set a series of criteria for filtering out the noise of data and to reduce false-positive findings. First, poorly performed markers were excluded based on GC score and marker intensity value. PennCNV algorithm was used to define the CNV regions for each pool. Second, after obtaining the CNV calling results, we deleted short (<20 consecutive markers) and low-confident regions (SD of Log R ratio>0.2). For neighboring CNVs with small gap length (less length 20% of the total length), we performed a gap cleaning procedure. Third, we took union of all defined CNV regions. Potential CNV regions were selected based on the following criteria, 1) the CNV regions were only in case pools but not in published Han Chinese CNV map, 2) the CNV regions were only in control pools and also reported in published Han Chinese CNV map, 3) the CNV regions were shown in both case and
control pools, but the number of difference is extreme (≥4). We conducted CNV burden analysis between cases and controls for regions with ≥100 kb or ≥500kb CNVs. In addition, we mapped genes in our selected CNV regions and performed Gene Ontology analysis. Lastly, we compared our identified CNV regions to findings from previous studies in bipolar disorder.

Results
The number of loss CNV regions (≥100 kb) in case pools is more than control pools, but this type of the CNV burden did not reach statistical significance between case and control pools (Wilcoxon p-value=0.105). 882 CNV regions were only found in case pools and not mapped to known published Han Chinese CNV map. On the contrary, 94 CNV regions were mapped to published Han Chinese CNV map and only found in our control pools. In addition, 2 CNV regions were overrepresented in cases and 26 CNV regions were overrepresented in controls. In total, 1232 genes were mapped to these selected CNV regions, and 30 of the mapped genes were in the top list of our previously prioritized candidate genes for bipolar disorder. Comparing with findings from previous studies of bipolar disorder, 28 out of 1004 CNV regions have been identified in previous studies for bipolar illness, while 976 were novel findings.

Discussion
We reported several potential CNV regions that are associated with bipolar disorder using our proposed filtering procedures in pooling data. Further experimental and replication studies could be designed for these suggestive regions.

10
MICRORNA TARGET PREDICTIONS IN CANDIDATE GENES FOR MOOD DISORDERS
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Background
Many loci from candidate genes and genome-wide association studies are reported to be associated with bipolar disorders. However, some of these single nucleotide polymorphism (SNPs) either locate in intronic regions, or do not cause amino acid change, or without apparent biological functions. Previous studies have found that SNPs located outside of exons would undergo splicing and other regulation processes during mRNA transcription. A possible mechanism to explore the actual function of these SNPs is to consider their involvement in microRNAs (miRNAs) regulation. Polymorphisms of SNPs might impact miRNAs binding ability, thereby influenced on the expression levels of targeted genes. The present study aimed to explore whether polymorphisms of several bipolar disorder-related SNPs have impacts on miRNA binding ability.

Methods
We previously found 7 SNPs in 5 genes (GAD1, SP4, COMT, RORA, RORB) that showed significant associations (P<0.05) with bipolar disorders. Two miRNA prediction software (PITA and MiRanda) were used to evaluate miRNAs binding ability. In addition, we used mirwalk, which combined the prediction of miRDB, miRWalk, RNA22, and TargetScan, to ensure if
certain miRNA binds to the genes that are predicted from PITA and MiRanda. We also developed a scoring system to incorporate information of binding position and binding energy from three software. A combined score can be calculated for each SNP-miRNA pair. The SNP-miRNA pairs with higher score and confidence were then undergone Luciferase reporter assay to validate whether there is differential gene expression levels between polymorphisms of SNPs.

**Results**

Using PITA or miRanda produced in similar results; 1476 predictive SNP-miRNA pairs (82.0%) containing 999 miRNAs were found to have dissimilar binding energy between alleles using PITA. Among 36 SNP-miRNA pairs with score greater than 4 (2.0%) in PITA, 23 pairs (63.9%) also showed different binding energy between alleles using miRanda. With our developed scoring system, 7.5 were taken as a cutoff and 7 SNP-miRNA pairs (19.4%) were in the top list. Among the 7 SNP-miRNA pairs, 4 miRNAs were ever reported to be associated with psychiatric disorders in the literature. Lastly, rs3749034 - has-miR-504 (score=7.5) pair was chosen to conduct Luciferase reporter assay to validate our prediction and the experiment is underway.

**Discussion**

The prediction of SNP-miRNAs pair can be benefit from using a combination of computational algorithms with a proper rating system. Identified miRNAs are subject to further experimental validation to evaluate whether SNP polymorphisms influence on gene expressions through miRNA regulation.

11

AN ANALYSIS OF GENETIC RISK FACTORS FOR LONG-TERM FUNCTIONAL OUTCOME IN BIPOLAR DISORDER AND SCHIZOPHRENIA

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**Background**

Various large-scale collaborative psychiatric genomic studies have suggested an overlap of genetic risk factors between schizophrenia (SZ) and bipolar disorder (BD). So far, psychiatric genetics has mainly focused on cross-sectional disease phenotypes. As the longitudinal course of SZ and BD is of utmost clinical importance due to its impact on the individual and also the societal burden of disease, there is a definite need to study the genetics of longitudinal measures. Here we focus on a comprehensive indicator for the course of illness, i.e. global level of functioning as measured by the GAF score. We assess if there is a shared genetic basis for long-term functional outcome in SZ and BD (measured by GAF score). We present a genetic analysis that investigates whether SZ and BD share a common polygenic background for the attained level of long-term functioning.
Methods
We studied samples from Germany (512 BD and 227 SZ patients) and the US (1132 European American BD patients). On a genome wide SNP panel, we analyzed GAF in BD samples for contrast of extreme outcome as well as in quantitative analyses to determine genomic regions for the composition of a genetic risk score (weighted sum score) for functional outcome in BD. Selected regions and their additive weights were used to calculate weighted sum scores from the genome wide genotypes of all SZ individuals. We investigated whether the weighted genotype risk score (as estimated for BD) has also predictive power for long-term GAF outcome in SZ subjects.

Results
We did not observe genome-wide significant findings for GAF scores in any of the three samples studied (BD-German, BD-US, SZ-German). Our finding closest to reaching statistical significance in the US sample was a region on chromosome 10 (rs11202643, p=2.7 x 10^{-7}) in the vicinity of the gene PTEN (phosphatase and tensin homolog), previously discussed within the context of psychiatric phenotypes. However, this finding did not replicate in the German sample. In the polygenic prediction analyses, we could not establish shared genetic contribution to global function for the two analyses performed (BD-US data to predict GAF in BD-German and SZ-German).

Discussion
These preliminary results suggest that it may be difficult to use long-term functional outcome as a sole phenotype for genetic investigations, as impaired levels of functioning may be influenced by different factors in BD and SZ or in populations from various ethnic backgrounds (for a complementary analysis of the influence of clinical and psychosocial variables on functional outcome see K. Gade’s contribution: “Long-term functioning in major psychiatric disorders and its clinical and psychosocial predictors: a potential cross-diagnostic phenotype for further genetic investigations”). We will present further detailed analyses on clinical subphenotypes, zooming in on specific regions highlighted by our study. Furthermore, for phenotypes like global functioning, larger sample sizes than the ones we employed may be needed.

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DRD1 POLYMORPHISM SHOWS A TREND OF ASSOCIATION WITH THE NUMBER OF MANIC PHASES IN YOUNG BIPOLAR PATIENTS WITH ALCOHOL ABUSE
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Background
A relationship between alcohol abuse and Bipolar Disorder has far been detected. Alcohol abuse may worsen the course of Bipolar Disorder, especially in younger subjects. Nevertheless, the genetic underpinnings of this comorbidity have not been completely elucidated. The aim of the present study was to investigate the impact of a set of variations harbored by genes that belong to the opioid system towards manic relapses in young (≤ 33 ys) Bipolar patients with an history of
alcohol abuse.

**Methods**
A list of candidate genes related to the opioid system were tested in a sample of 101 Bipolar patients from the public available STEP-BD. Variations harbored by these genes were checked for quality, imputed and pruned. Eventually, a set of 88 SNPs harbored by 11 genes were analyzed as predictors of the number of manic events during the period of observation (614.81+/−448.38 days). Clinical and sociodemographic variables entered the study as stratification factors when significantly associated with the phenotype under investigation.

**Results**
A trend for a positive correlation between DRD1 SNP rs11746172 and an increased number of manic phases (F=−2.477, p=0.01) was detected. This level of significance did not resist the Bonferroni correction.

**Discussion**
We found evidence for a trend of association between rs11746172 and manic relapses in young Bipolar Patients with a history of alcohol abuse. The small sample size, the natural design of the study and the high rate of false positive findings in this field of research mandate cautious interpretation and further independent investigations.

12

**COMORBIDITY BETWEEN MAJOR DEPRESSION AND TYPE 2 DIABETES IN MID-LIFE: EXPLORING CAUSAL EFFECTS USING A TWIN DESIGN**

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**Background**
Population-based cohort studies have suggested that the relationship between major depression (MD) and type 2 diabetes (T2DM) is bi-directional. However, longitudinal studies in and of themselves cannot distinguish between a direct effect (e.g., MD increases risk of T2DM) and a common cause (e.g., shared genetic or environmental factors increase risk of both MD and T2DM). Twin studies may offer a less biased estimate of the purported causal relationship between MD and T2DM because they compare individuals matched on both genetic liability and family background. There are three competing hypotheses that could explain the comorbidity between MD and T2DM: (1) direct phenotypic causation, whereby MD increases risk of T2DM (and vice versa) through biological or behavioral pathways, (2) shared genetic liability, whereby the co-occurrence of MD and T2DM is due to common genetic factors, and (3) shared environmental liability, whereby the co-occurrence of MD and T2DM is due to common environmental factors. In the latter two scenarios, there is no causal relationship between MD and T2DM; instead shared risk factors explain why these two conditions co-occur. Resolving the etiology of comorbid MD-T2DM has implications for prevention and treatment of both of these conditions.

**Methods**
Using data from the Screening Across the Lifespan Twin Study, a population-based sample derived from the Swedish Twin Registry, we examined the relationship between MD and T2DM while accounting for genetic risk among adults aged 40-55 years old (N=3,789 monozygotic and N=12,879 dizygotic (same and opposite-sex) twin pairs). MD was assessed using the Composite International Diagnostic Inventory and T2DM was assessed by self-report of physician diagnosis. Twin structural equation modeling was used to decompose the covariance of MD and T2DM into latent additive genetic (A), common environment (C) and unique environment (E) factors. The correlation between genetic and environmental components was assessed to estimate the degree to which genetic and environmental factors that increase risk of MD are shared with T2DM. Discrete-time Cox proportional hazards modeling was used to estimate the risk of T2DM associated with MD, and vice versa, while also accounting for genetic risk. In these survival analyses, genetic risk was indexed by the co-twin status (e.g., +1.0 for a monozygotic twin with a co-twin with MD, +0.5 for a dizygotic twin with a co-twin with MD, etc.) and models were adjusted for age and sex.

Results
The prevalence of T2DM was 1.8% (M, SD age of onset: 37.6, 14.3) and the prevalence of MD was 24.7% (M, SD age of onset: 36.4, 10.6). A reduced (AE) model provided the best fit for both MD (A: 0.44 and E: 0.56) and T2DM (A: 0.83, E: 0.15). There was no significant shared genetic influence between MD and T2DM (genetic correlation: 0.02). As shown by panels A and B of Figure 1, the relationship between MD and T2DM is bi-directional. The relative hazard (HR) of incident T2DM associated with MD was 1.47 (95% Confidence Interval (CI): 1.13 – 1.92), but this relationship was attenuated after accounting for genetic risk of T2DM (HR: 1.33; 95% CI: 0.93 – 1.90). The HR of incident MD associated with T2DM was 1.62 (95% CI: 1.15- 1.39), and this relationship was also attenuated after accounting for genetic risk of MD (HR: 1.39, 95% CI: 0.86 – 2.24).

Discussion
This study suggests that the relationship between MD and T2DM is bi-directional. Although both MD and T2DM each have substantial genetic components, there is little to no covariance between the genetic liability for MD and that for T2DM. There is also no evidence of shared environmental effects for either MD or T2DM. Together these findings suggest that the comorbidity between MD and T2DM is likely due to direct phenotypic causation. In this study MD was associated with approximately 50% greater risk of T2DM, but this risk was substantially attenuated after accounting for genetic liability for T2DM. Similarly, T2DM was associated with approximately 60% greater risk of MD, but this association was also attenuated after accounting for genetic liability for MD. These results indicate that MD is only a risk factor for T2DM among those with low genetic liability for T2DM, and vice versa. Although these findings suggest that the relationship between MD and T2DM is bidirectional, the processes by which MD increases risk of T2DM are likely different than the processes by which T2DM increases risk of MD. Future research should focus on understanding the biological and behavioral processes that link these conditions over the life course.

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UNRAVELING THE CAUSE OF MAJOR DEPRESSIVE DISORDER IN RHEUMATOID ARTHRITIS
Background
Comorbidity, the co-existence of two or more diseases in an individual, can provide an insight into the aetiology of many common disorders. Rheumatoid Arthritis (RA) and Major Depressive Disorder (MDD) are two complex diseases that often occur together (are co-morbid). RA patients are significantly more likely to develop MDD compared with the general population. Depression in RA is associated with worse pain, more functional disability and higher rates of healthcare utilization: establishing its cause in RA is therefore an important research goal. RA-MDD comorbidity may result from 1) shared environmental risk factors, 2) shared genetic risk loci or 3) RA causing MDD e.g. through RA related inflammation influencing networks controlling affect. The aim of our study was to establish which of these mechanisms cause MDD in RA.

Methods
The CARDERA study is a Randomized Controlled Trial (RCT) evaluating the efficacy of combination Disease Modifying Anti-Rheumatic Drugs in RA. One of the study outcomes was the SF-36 Mental Component Summary (MCS) score; this captures mental health with higher scores representing better health. Low MCS scores have been shown to correlate with MDD; the MCS thus represents a robust proxy for MDD (Silveira et al 2005). Of the 467 patients involved in the trial, 424 had their DNA genotyped (passing quality control procedures) on the Illumina ImmunoBead Chip (IChip, designed to capture variation in loci associated with common immune-mediated traits). These cases were analysed with 2900 controls from the National Blood Service (genotyped by the Wellcome Trust Case Control Consortium 2 on the IChip). A weighted genetic risk score (GRS) was calculated in PLINK for cases and controls, using odds ratios for the 51 single nucleotide polymorphisms (SNPs) attaining a genome-wide significant association with RA in the most recent GWAS meta-analysis (Eyre et al, 2012). Mendelian Randomisation (MR) was used to estimate the strength of the causal path between RA and MDD, independent of shared risk alleles / environmental risks. To investigate a genetic component to MCS variability, a second analysis was performed regressing adjusted MCS scores on SNPs in a priori gene lists. MCS scores were first adjusted for objective measures of RA severity. Three candidate SNP lists were used: (1) due to their suggested involvement in both MDD and RA, we investigated SNPs in the circadian clock genes (n\textsubscript{genes} = 13, n\textsubscript{SNPs} = 24,752) studied by Yoshida et al (2013); (2) due to the dialogue between inflammatory pathways and MDD discussed above, we studied SNPs in the anti-inflammatory cytokine genes (n\textsubscript{genes} = 25, n\textsubscript{SNPs} = 10,074) suggested by Banchereau et al (2012); (3) due to its involvement in the acquired immune system and with other psychiatric phenotypes, we investigated SNPs in the Major Histocompatibility Complex (MHC) region using the list published by Barcellos et al (2009)(n\textsubscript{SNPs} = 1,974).

Results
The causal path between RA and MCS score was estimated at -5.19 (p < 10\textsuperscript{-15}), whilst the correlational path was estimated at -7.83 (p < 10\textsuperscript{-15}). Thus the causal effect of RA was a decrease in the expected MCS score of 5.19 points, indicating lower mood. This risk was independent of shared environmental or genetic risk factors. Regression of adjusted MCS scores revealed no significant associations in genes involved with inflammatory cytokines or genes involved with the circadian clock (α = 10\textsuperscript{-5}). Although no significant associations between SNPs in the MHC...
region and adjusted MCS scores were identified ($\alpha = 10^{-5}$), there was a substantial genomic inflation ($\lambda = 1.85$) for SNPs in this region.

**Discussion**

Our results indicate that a causal path exists from RA to MDD, which is independent of any shared risk factors. This suggests that in RA-MDD comorbidity, RA may cause MDD. This could occur via biological or psychological pathways. The causal path was lower than the correlational path, however, suggesting that shared risk factors also contribute to RA-MDD comorbidity. Although we found no evidence for a contribution of the innate immune system to individual differences in MCS scores, suggestive evidence in the MHC region implies that there may be a contribution of the adaptive immune system. A lack of environmental data restricted our ability to evaluate the extent to which RA-MDD comorbidity is due to shared environmental. Our data highlights associations with the MHC region, indicating that denser imputation of MHC SNPs would enable us to better investigate which MHC alleles are responsible for individual MCS score differences. Further work in this area, using detailed environmental exposure data alongside dense MHC coverage, will better define the cause of MDD in RA.

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**GENETIC VARIANTS ON 3Q21 AND IN SP8 TRANSCRIPTION FACTOR GENE (SP8) AS SUSCEPTIBILITY LOCI FOR PSYCHOTIC DISORDERS: A GENETIC ASSOCIATION STUDY**

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**Background**

Recent genome-wide association studies (GWASs) of bipolar disorder (BD) detected a number of risk genes, as well as provided novel insight of shared genetic components between BD and schizophrenia (SCZ), two major psychotic disorders. To examine the replication of the risk variants for BD and the pleiotropic effect of the variants associated with BD, we conducted genetic association study of SNPs selected based upon previous BD GWASs targeting psychotic disorders (BD and SCZ) in the Japanese population.

**Methods**

Forty-eight SNPs were selected based upon previous GWASs. Two-stage analysis was conducted using first-set screening (for all SNPs; BD=1,012, SCZ=1,032, control=993) and second-set replication samples (for significant SNPs in the first-set screening analysis; BD=821, SCZ=1,808, control=2,149). We assessed allelic association between 1) BD, 2) SCZ, 3) Psychosis (BD+SCZ) and the SNPs selected for the analysis.
Results
Eight SNPs showed nominal association signals for either comparison (uncorrected P< 0.05). Among these, the top two SNPs (for ‘Psychosis’ association: corrected P=0.049 and P=0.038 for rs2251219 and rs2709722, respectively) were further assessed in the second-set samples, and we replicated the signals from the initial screening analysis (for “Psychosis” association: corrected P=0.0070 and P=0.033 for rs2251219 and rs2709722, respectively). The meta-analysis between the current and the previous GWAS results showed that rs2251219 in polybromo1 (PBRM1) surpassed genome-wide association level (P=5x10^-8) only for BD (P=9.4x10^-9) and ‘Psychosis’ (P=2.0x10^-10). Although the genetic association analysis of rs2709722 in Sp8 transcription factor (SP8) showed suggestive evidence in Asian population (P=2.1x10^-7 for ‘Psychosis’), this signal weakened when we increased the samples size by including data from Caucasian population (P=4.3x10^-3).

Discussion
In this study, we found 3p21.1 (including PBRM1, because of strong linkage disequilibrium around this region, thus difficult to pinpoint the susceptibility genes) and SP8 as risk loci for BD, SCZ and “Psychosis”. Further replication study will be required for conclusive results.

15 COMMON VARIANTS BETWEEN GENES HS3ST4 AND C16ORF82 ASSOCIATED WITH SEASONAL AFFECTIVE DISORDER
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Background
Seasonal affective disorder (SAD) is a mood disorder where patients experience episodes of major depression that tend to recur in the fall and winter. SAD is common, with a prevalence rate ranging from 1.4% to 9.7% in the United States. Twin studies have shown that SAD runs in families and that this is partly due to genetic predisposition. While there is modest evidence for some individual genetic variants in SAD, no gene has been definitively implicated. Further, there has been no genome-wide association study (GWAS) to date examining this phenotype.

Methods
Here we performed a GWAS with 562 seasonal major depressive disorder (MDD) cases and 1,225 comparison cases with non-seasonal MDD. Subjects were drawn from two iterations of the Genetics of Recurrent Early Onset Depression (GenRED) study. Subjects were assessed at seven sites in the U.S. Seasonal cases were those who reported their depressive episodes typically started in the fall or winter. Genome-wide single nucleotide polymorphism (SNP) genotyping was done in two rounds using the Affymetrix 6.0 and Illumina Omni1-Quad arrays, with ~700,000 SNPs and imputation was done to 1000 Genomes data using IMPUTE2.0, generating ~10 million SNPs. A mega-analysis of the two GWAS datasets was done using the SNPTEST package.
Results
We found that the imputed variants rs149882931 and rs77073398 on chromosome 16p12.1 were associated with seasonal depression, at a genome-wide significant level (OR=1.43, P=3.59 x 10^{-8} and OR=1.41, 4.76 x 10^{-8}, respectively). These SNPs, located just 7 bp apart and in high linkage disequilibrium, r^2=0.6, are located in an intergenic region between the genes HS3ST4 and C16orf82. The protein product of HS3ST4 modifies the side chains of heparan sulfate proteoglycans. We therefore tested the hypothesis that the glycosaminoglycan biosynthesis - heparan sulfate (HS) pathway is enriched in nominally significant SNPs (P<0.05) using the SNP ratio test. The result shows the HS pathway is significantly enriched (P=0.004).

Discussion
Additional study of the variants rs149882931 and rs77073398 in SAD is warranted. Replication in another major depression sample assessed for seasonality would provide increased confidence in the robustness of our results. Functional studies of the genomic region are necessary to determine the potential relevance of the genetic variation to the neurobiology of SAD.

AGE AT ONSET SPECIFIC GENETIC RISK FACTORS FOR MAJOR DEPRESSION
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Background
Age at onset (AAO) is a potential source of heterogeneity within major depression (MDD), a disorder for which few replicable genetic risk variants have been reported. Last year we presented the results of a series of genome-wide association analyses within the Psychiatric Genomics Consortium’s MDD sample, looking at AAO specific risk variants. Here we aim to summarise the discovery analysis and present the exciting results from the replication analysis.

Methods
In the Psychiatric Genomics Consortium's sample of 8,920 MDD cases and 9,521 controls from 9 studies, we split cases into 8 subsets based on AAO percentile within their study (i.e. first 8th of earliest onset, second 8th of earliest onset, etc.). These octiles (O1-8) were analysed systematically against controls to look at both early and late-onset specific risk factors (O1 only against controls, O1-2 against controls,…, O1-8 against controls, O8 only against controls, O7-8 against controls, etc.). Due to the multiple overlapping genome-wide analyses, a stringent replication threshold of p=5E-7 was used. We removed those SNPs that peaked in association when the 7 or 8 latest/earliest onset octiles were included to avoid previously reported associations with MDD at any AAO. Ten independent SNPs passed these thresholds, including one at p=2E-11, and so were tested for in a replication sample of 6 studies containing 4,922 cases and 15,141 controls. These SNPs were tested using the AAO median or quartile of cases that best matched their strongest association in the discovery sample.
Our results found 2 of the 10 SNPs at nominal significance in the replication sample, though neither passed correction for multiple testing. This included the top hit from our discovery sample, rs7647854 on chromosome 3 which was found to be associated with the 50% latest-onset MDD (p=0.01, OR=0.88; meta-analysis with discovery sample p=1.0E-11, OR=0.81). This SNP had previously been reported in the primary analysis of the PGC MDD data though had failed to replicate (p value=0.67), suggesting an increased signal when only late-onset cases were included for both the discovery and replication samples. The other nominally significant finding was rs7950328 on chromosome 11, found to be associated with the 25% latest-onset depression in males (p=0.006, OR=1.28; meta-analysis with discovery sample p=1.0E-08, OR=1.35).

Further analysis of the results from the individual replication studies showed that the effect sizes for these SNPs increased significantly with the median AAO of the study (p=0.03 and 0.001 respectively). Returning to the discovery sample showed a similar trend, with increasing effect sizes observed in each study as AAO increased. See figure for results of the top SNP (rs7647854) in non-overlapping AAO quartiles of cases, showing that the risk is specific to the late-onset MDD quartiles. As reported last year, we found no difference in the heritability of early and late-onset MDD, though did find a significantly greater burden of schizophrenia and bipolar risk variants in early-onset cases.

Discussion
In this study we have identified novel genome-wide significant replications for late-onset major depression and helped redefine its clinical picture. With a median AAO of 27, “late-onset” here reflects an adult-onset form of MDD rather than one developing in old age, with “early-onset” reflecting mostly adolescent-onset MDD. We showed that there was no increased heritability for early vs. late-onset MDD once recurrence was accounted for, suggesting that focusing on early-onset cases provides no benefit to genetic studies and may introduce additional heterogeneity in the form of greater genetic overlap with bipolar disorder and schizophrenia. Despite reduced sample sizes compared to the primary analysis of these studies, we showed considerable evidence for two SNP being associated specifically with late-onset MDD. These results suggest that as well as pursuing strategies to increase sample sizes, an alternate method of reducing heterogeneity within disorders can sufficiently increase power to identify novel risk variants.
sites in the *COMT* promoter region.

**Methods**
This study examined the potential interaction of *COMT* genotype and PTSD diagnosis on fear-potentiated startle during fear conditioning and extinction and *COMT* DNA methylation levels. Participants were recruited from medical and gynecological clinics of an urban hospital in Atlanta, Georgia. The primary outcome measures were fear-potentiated startle and *COMT* DNA methylation.

**Results**
We found that individuals with the Met/Met genotype demonstrated higher fear-potentiated startle to the CS- (safety signal) and during extinction of the CS+ (danger signal) compared to Val/Met and Val/Val genotypes. The PTSD+ Met/Met genotype group had the greatest impairment in fear inhibition to the CS- (p=.006), compared to Val carriers. In addition, the Met/Met genotype was associated with DNA methylation at 4 CpG sites, 2 of which were associated with impaired fear inhibition to the safety signal.

**Discussion**
These results suggest that multiple differential mechanisms for regulating COMT function – at the level of protein structure via the Val¹⁵⁸Met genotype and at the level of gene regulation via differential methylation - are associated with impaired fear inhibition in PTSD.

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**MOLECULAR MECHANISMS OF D-CYCLOSERINE IN FEAR EXTINCTION: INSIGHTS FROM RNA AND MICRORNA SEQUENCING**

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**Background**
Posttraumatic stress disorder (PTSD) is a severe, chronic and debilitating psychiatric disorder that can occur after a traumatic event. Dysfunctional fear extinction has been found to play a vital role in the development of the disorder. D-cycloserine (DCS), a partial N-methyl-D-aspartate (NMDA) receptor agonist, has been found to be effective in facilitating fear extinction in both animal and human studies of anxiety. However, the precise mechanism whereby DCS facilitates fear extinction is unknown. Memory consolidation and thus fear extinction requires specific gene expression and protein synthesis; however, studies investigating the molecular processes involved in the process of fear extinction, and its subsequent role in PTSD development, are still in their infancy. Furthermore, due to the dynamic nature of the transcriptome numerous factors influence gene expression profiles, including microRNAs (miRNAs), which are a class of small, noncoding RNAs that have recently drawn interest as epigenetic modulators of gene expression in psychiatric disorders. The aim of this study was to elucidate the molecular mechanism of action of DCS in facilitating fear extinction in a rat model of PTSD by investigating gene and miRNA expression profiles.

**Methods**
A PTSD animal model that distinguishes between conditioned and sensitised fear was employed. One hundred and twenty adult male Sprague-Dawley rats were grouped into four experimental groups (n=30/group), with either saline or DCS infused intrahippocampally during the fear extinction protocol. In order to provide a more focused study and to avoid potential biases, the study focused on those rats who exhibited an extreme behavioural response to the traumatic event (footshock): rats who exhibited PTSD-like behaviour were termed ‘maladapted’ and those who appeared resilient were termed ‘well-adapted’. RNA and miRNA were extracted in separate fractions from the left dorsal hippocampi (LDH), and RNAseq and miRNAseq analyses were subsequently conducted using the Illumina HiScan and MiSeq platforms, respectively. Differentially expressed genes and miRNAs between different treatment groups were identified using bioinformatics analyses. Furthermore, integrative interaction networks were constructed for differentially expressed genes to identify common pathways, diseases and functions.

Results
We compared gene and miRNA expression of rats in the groups fear-conditioned + saline (maladapted) (FSM) vs. fear-conditioned + DCS well adapted (FDW) in order to elucidate the molecular mechanism of action of DCS in facilitating fear extinction in rats exposed to a traumatic event. A previously constructed data pipeline was used to analyse the RNAseq data, and after multiple testing correction using the false-discovery rate, 108 genes were found to be significantly down-regulated in FDW compared to FSM rats. Thirty-nine of these genes were predicted to be biologically relevant to PTSD, based on their documented function. Integrative biomolecular interaction network analyses revealed that subsets of these differentially expressed genes are common between memory disorders, nervous system diseases, metabolic disorders as well as substance- and alcohol-related disorders; these included Trh, Mmp9, Mt2a, Clec7a, Il1rn, Npy, Spp1 and Cybb. Biological functions of differentially expressed genes ranged from negative regulation of glutamate secretion, response to drug or stress, behaviour and locomotion. qPCR was sensitive enough to detect significant differential expression for three of the genes (Cybb, Mt2a and Il1rn) in brain cDNA and one gene (Mmp9) in the blood cDNA.

Discussion
Differential gene expression analyses in this PTSD animal model enabled us to identify genes, networks and pathways that shed light on the molecular mechanism of action of DCS-facilitated fear extinction in PTSD. Several genes that were significantly over-expressed in FSM rats have previously been described in PTSD or anxiety research, including Npy, Trh, Il1rn, Mt2a and Grn. In addition we investigated the expression levels of miRNAs between treatment groups and will determine their effects on gene expression and ultimately fear extinction. Investigating the miRNome and transcriptome in this PTSD model will provide insight into not only which of these genes may be involved in contributing to susceptibility of PTSD, but also into the regulatory mechanisms underlying the disorder.

DISTINCT SETS OF BLOOD-BASED GENE-EXPRESSION BIOMARKERS FOR PTSD RISK AND DIAGNOSIS AMONG U.S. MARINES: A PILOT STUDY
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Background

Post-traumatic stress disorder (PTSD) is a severe anxiety disorder that is presently diagnosed based on the emergence of clinical symptoms developing in a minority of individuals who experience traumatic stress. Despite a growing understanding of the pathophysiology accompanying PTSD, a readily assessable diagnostic biomarker has yet to be validated. Similarly, because only a minority of individuals exposed to trauma develops the disorder, there is great interest in identifying those most vulnerable, yet even fewer readily assessable biomarkers for PTSD vulnerability have been validated. The identification of diagnostic and risk-assessment biomarkers for PTSD could permit earlier intervention, or outright prevention of the disorder, and may shed light on etiological mechanisms. Because PTSD appears to be influenced by both genetic and environmental factors, mRNA expression may be a sensitive biological signal. Gene expression patterns are readily assessable in blood; they have the potential to mirror gene expression in the CNS and have been shown elsewhere to be useful biomarkers for brain disorders. The present pilot studies used transcriptome-wide mRNA profiling and machine-learning techniques to identify whole-gene and exon transcripts (1) indicative of the PTSD diagnosis, based on post-deployment mRNA levels and, (2) predictive of vulnerability to developing PTSD after a future deployment, based on pre-deployment mRNA levels. Specifically, we hypothesized: 1A) post-trauma expression levels of a gene subset (particularly immune-system genes) in peripheral blood cells would differ between trauma-exposed Marines later diagnosed with PTSD and those without PTSD; 1B) a diagnostic biomarker panel for PTSD could be developed based on gene expression in readily-assessable peripheral blood cells; and 1C) a diagnostic panel based on the expression of individual exons would surpass the accuracy of a model based on the expression of full-length gene transcripts.

Similarly, we expected: 2A) pre-trauma expression levels of a gene subset (particularly immune-system genes) in peripheral blood cells would differ between trauma-exposed Marines who were eventually diagnosed with PTSD and those who did not develop PTSD; 2B) a risk-assessment biomarker panel for PTSD could be developed based on gene expression in peripheral blood cells; and 2C) a risk-assessment panel based on the expression of individual exons would surpass the accuracy of a model based on the expression of full-length gene transcripts.

Methods

Whole-gene and exon expression levels were assayed in peripheral blood samples from a small groups of U.S. Marines (n=48), half of whom went on to be diagnosed with PTSD while the other half did not meet criteria for PTSD. Gene-expression levels were assessed before deployment to warzones in Iraq and Afghanistan, and again after return from deployment. Transcripts differentially expressed between PTSD cases and comparison subjects were reported, along with significantly enriched biological annotations. Results of support vector machine models were reported, identifying subgroups of transcripts that were most predictive of PTSD diagnosis; transcripts were organized into diagnostic and risk-assessment models. These models were deployed on independent groups of subjects in order to assess their accuracy and generalizability in correctly classifying PTSD cases and comparison subjects.

Results

Preliminary results indicated, in keeping with our hypotheses, that dysregulated transcripts were
significantly enriched with immune-system annotations. The best performing risk-assessment model employed five exon transcripts and performed with 80% accuracy in an independent sample; three of 5 subjects were correctly classified as PTSD cases, five of 5 were correctly classified comparison subjects.

**Discussion**

If these profiles of PTSD-relevant mRNA-expression can be refined and replicated, and if SVM-based models are found to perform reliably in larger or more diverse populations, then these studies illustrate avenues for early detection and prevention of PTSD among trauma-exposed populations. Additionally, these studies used a data-driven approach to identify novel candidate genes that may play a role in the pathophysiology of PTSD.

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**SYMPTOMS OF THE DISSOCIATIVE SUBTYPE OF PTSD: A GENOME WIDE ASSOCIATION STUDY**

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**Background**

Psychometric (e.g., Wolf et al., 2012a, 2012b) and neuroimaging (e.g., Lanius et al., 2010, 2012) studies have identified a dissociative subtype of posttraumatic stress disorder (PTSD) which is manifested in 15-30% of individuals with the diagnosis. The subtype is defined by symptoms of derealization (i.e., feeling as if the world is not real) and depersonalization (i.e., feeling as if one’s self is not real) and it is also associated with high levels of trauma exposure, PTSD-related flashbacks, psychogenic amnesia, and psychiatric comorbidity (Stein et al., 2012; Wolf et al., 2012b). It is supported by cross-cultural research (Stein et al., 2012) and will be included alongside the PTSD diagnosis in the revised version of the Diagnostic and Statistical Manual (DSM-5). Clinically, dissociation is generally thought to interfere with PTSD treatment and is an important clinical phenomenon to attend to in its own right. From a research standpoint, dissociation represents a source of heterogeneity in the clinical presentation of PTSD that may complicate the search for biomarkers, clinical correlates, and effective treatments if not taken into account.

**Methods**

We conducted a genome-wide association study among 484 white, non-Hispanic, trauma-exposed veterans and their trauma-exposed intimate partners (mean age: 52; 314 participants were male; 60.5% met lifetime criteria for PTSD) to identify genetic variants associated with severity of derealization and depersonalization symptoms. Genotyping of DNA isolated from peripheral blood samples was performed on an Illumina OMNI 2.5 million array. Ancestry was determined using 10,000 randomly chosen polymorphisms with minor allele frequency (MAF) > .05 using the program STRUCTURE (Falush et al., 2003; Pritchard et al., 2000) in conjunction with self-report data. PTSD and dissociation severity were assessed through structured diagnostic interview with the Clinician Administered PTSD Scale (CAPS; Blake et al., 1990) and inter-rater reliability was assessed by having secondary raters score approximately 1/3 of the videotapes of these interviews (intraclass correlation coefficients were .97 and .93 for PTSD and dissociation...
severity, respectively). Single nucleotide polymorphisms (SNPs) were eliminated if they had MAF < .05 and based on a test of Hardy Weinberg equilibrium (p < 10^{-6}), leaving a total of 1,197,702 for analysis.

Results
Ten SNPs evidenced associations with dissociation in the suggestive range of p < 10^{-5} (see Figure), but no SNPs met strict genome-wide level of significance (i.e., p < 5 X 10^{-8}). The peak SNP, rs263232 (p = 6.12 X 10^{-7}), was located at 131,808,169 bp on chromosome 8 in the adenyl cyclase 8 (ADCY8) gene. A second SNP showing suggestive evidence of association, rs71534169 (p = 3.79 X 10^{-6}), was located at 154592866 bp on chromosome 7 in the dipeptidyl-peptidase 6 (DPP6) gene. The association between these SNPs and dissociation remained after controlling for PTSD severity.

Discussion
Two of the SNPs in the suggestive range of association with dissociation were located in genes that have previously been implicated in psychiatric disorder risk. ADCY8 has shown association with bipolar disorder (e.g., Zandi et al., 2008) and is expressed pre-synaptically in numerous areas of the the brain. The enzyme it produces is involved in long term potentiation, synaptic plasticity, neurogenesis, and learning and memory; processes that are relevant to dissociative phenomena. Mice who are deficient in the AC8 enzyme show deficits in retention of conditioned fear learning and appear insensitive to contextual cues (Wieczorek et al., 2010, 2012). DPP6 has previously shown association with autism (Marshall et al., 2008) and the protein encoded is involved in regulating and coordinating dendritic excitability; this also has relevance to dissociation as this phenomena involves lack of integration of cognitive processes (e.g., attentional capacity, sensory integration, memory encoding) that should be well coordinated. In conclusion, the results of this first genome-wide study of dissociation suggest that genetic variants in ADCY8 and DPP6 may be implicated in symptoms of derealization and depersonalization following trauma exposure. However, replication and additional evaluation is needed. Results also highlight the utility of parsing heterogeneous phenotypes into more homogeneous ones for the purposes of genetic association research.

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ESSENTIAL OILS OF MYRTUS COMMUNIS L. PRODUCE A NON-SEDATING ANXIOLYTIC EFFECT IN MICE MODEL OF ANXIETY
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Background
The myrtle shrub (Myrtus communis L., Myritaceae) is rich in essential oils that are used in Ethiopian traditional medicine for treatment of a variety of ailments, including anxiety. Anxiety is a common disorder that attacks many people in society and often accompanied by physiological sensations such as tachycardia, chest pain, shortness of breath, insensitivity. The present study was undertaken to evaluate the anxiolytic effect of the essential oil of M. communis using different models of anxiety.

Methods
Swiss Albino mice of either sex were randomly divided into five groups. Group 1 (control) was
administered Tween 80 (5%, v/v) in distilled water. Group II (positive control) was given diazepam (0.5 mg/kg, orally), suspended in the vehicle. Group III-VI (test groups) were given the essential oil at doses of 50, 100, 200 and 400 mg/kg, respectively. Animals were then subjected to anxiety models, including elevated plus maze (EPM), stairs case and open field, and parameters, among others, percentage of time spent in each arm and arm entries, number of steps climbed and number of rears, and number of crossings were measured.

**Results**

In EPM studies, the extract at both 100 mg/kg (p<0.01) and 200 mg/kg (p<0.05) produced a significant increase in percentage of open arm time as compared to controls. In the staircase setting, significant reduction of rearing was observed in mice treated with the essential oil at 100 mg/kg (p<0.01) and 200 mg/kg (p<0.05) compared to controls. However, at doses of 50 mg/kg and 400 mg/kg no detectable changes were noted on the measured parameters in both EPM and staircase models. The total number of entries into open field was comparable in all groups.

**Discussion**

The essential oil of *M. communis* showed better anxiolytic activity at 100 mg/kg compared to the standard drug. The possible mechanism by which the oil showed the activity could be through GABA-related mechanism. Thus, the present work holds up the traditional use of the plant for anxiety.

**GENETIC ASSOCIATION OF FKBP5 AND SALIVARY AMYLASE AND CORTISOL WITH PHYSICAL STRESS**

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**Background**

The FK506 binding protein 51 (FKBP5) genes regulate the stress hormone system and induce the risk of stress-related psychiatric disorders. They could change glucocorticoid receptor function reducing ligand binding and translocation of the receptor complex to the nucleus. Studies of human response to stressful events show that neuroendocrine indicators play an important role in starting the body’s reaction to stress. Stress responsiveness is mainly regulated by 2 neuroendocrine axes: the hypothalamic pituitary adrenocortical (HPA) and sympathetic adrenomedullary (SAM) systems (Herman and Cullinan, 1997; Isogawa et al., 2010; Tasker and Herman, 2011).

**Methods**

Some studies suggest that the associations between HPA axis responses by Trier Social Stress test (TSST) and FKBP5 polymorphisms (Ising et al., 2008). To analyze the influences of FKBP5 on stress, we examined 394 university students (162 females and 232 males) using a electrical stimulation stress test. Stress indices were derived from repeated measurements of salivary amylase, salivary cortisol, heart rate, and psychological testing during the electrical stimulation tests and all subjects were genotyped for the FKBP5 polymorphism.
Results
There was a significantly relationship between Tension-Anxiety scores on POMS and salivary cortisol levels after electrical stimulation. There was no relationship between other POMS scores and salivary cortisol levels after electrical stimulation. There was no relationship between any POMS score and sAA levels after electrical stimulation.

Stress indices were derived from repeated measurements of salivary amylase, salivary cortisol, heart rate, and psychological testing during the electrical stimulation tests and all subjects were genotyped for the FKBP5 polymorphism. Tests of within-subject effects showed a significant 3-way interaction [Time (3 levels) ×FKBP5 (rs 992105, rs 9296158, rs 9470080, rs 1360780, rs 2766534), p < 0.05], which showed different salivary amylase and cortisol responses in electrical stimulation.

Discussion
These results indicate that a common, functionally significant polymorphism in FKBP5 differently modulates hypothalamic-pituitary-adrenocortical (HPA-) axis reactivity but not sympathetic adrenomedullary (SAM-) reactivity the electrical stimulation. These findings may provide insights to reactivity and vulnerability to social stress.

A GENOME-WIDE ASSOCIATION STUDY OF THE RESPONSE TO COGNITIVE BEHAVIORAL THERAPY IN CHILDREN WITH ANXIETY DISORDERS
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Background
Anxiety disorders are the most common psychiatric disorders, with a prevalence in adults of approximately 30% (Kessler et al., 2005). Psychosocial treatments, including cognitive behavioral therapy (CBT), are the primary treatment modality for anxiety disorders in the United Kingdom. Response (at 6-12 month follow-up) for CBT is estimated at 65%, demonstrating heterogeneity (James et al., 2005). Furthermore, there is evidence that such individual differences in treatment response have a genetic basis. Recent work from our group has found preliminary evidence for beneficial effects of the 5HTT-LPR SS genotype, and for the T allele of rs6330 (NGF gene), on CBT response at 3-12 month follow-up in a cohort of children with a variety of anxiety disorders (Eley et al., 2012, Lester et al., 2012). Such therapy-genetic candidate gene studies motivated our current genome-wide association study (GWAS), examining the association of common single nucleotide polymorphisms (SNPs) with differential response to CBT.

Methods
Genetic samples were gathered from 1272 children (aged 7-13) diagnosed with anxiety disorders, undergoing CBT. The sample originated from ten different sites in the UK, USA,
Australia and Western Europe. Clinical Severity Ratings were established at baseline, upon completion of treatment, and at follow-up. Buccal swabs were used to obtain DNA. DNA was extracted using a standard protocol, concentrated by filtration and resuspension, and genotyped using the recently released Illumina HumanCoreExome array, which assays roughly 250000 SNPs and 250000 exomic variants.

**Results**
The initial outcome variable was primary disorder presence at follow-up. The SNPs most strongly associated with this outcome are presented. Additional measures include genome-wide complex trait analysis (GCTA) and quantitative trait heritability analyses. Further analyses will explore the association between SNPs and changes in Clinical Severity Ratings, and investigate response immediately post-treatment.

**Discussion**
We present a GWAS of response to CBT in a cohort of children with anxiety disorder. This is, to our knowledge, the first GWAS of response to psychosocial treatment, and the first treatment response GWAS to be performed in anxiety disorder. Past analyses in related phenotypes, particularly those of antidepressant response, suggest that the effect sizes of variants underlying treatment response are likely to be small, although probably larger than those contributing to the disorder. As the sample size of this study is relatively small, it is probably underpowered to detect such variants; power analyses estimate that the sample has 80% power to detect variants at a genotypic relative risk of 1.3 and frequency of 0.2. However, GCTA could yield valuable insight; one study that found no SNPs at genome-wide significant levels for antidepressant response gave a SNP-heritability estimate of 42% from GCTA (Tansey et al., 2013). One of the major potential benefits of the HumanCoreExome array is the usefulness of exomic tags as CNV markers, and as such, future analyses will explore the role of CNVs in marking differential responses to anxiety disorders.

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**ANALYSIS OF GENOMIC COPY NUMBER VARIATIONS IN JAPANESE AUTISM SUBJECTS**

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**Background**
It has been shown that genomic structural variants including copy number variations (CNVs) contribute to the autism etiology. There are no reports on CNVs associated with autism in Japanese population. Our aim is to study the DNA copy number variations in Japanese autism subjects using SNP microarray. The patient samples and their parents were screened with Affymetrix Genome Wide SNP 6.0 array. The data generated through this study, along with research conducted elsewhere, could provide more insights into the complex pathways involved that leads to the clinical characterization and more efficacious pharmacological involvement.

**Methods**
We have a collection of 250 ASD family samples recruited on collaboration with a non-
governmental organization, Asperger Society Japan (http://www.as-japan.jp/) and various university hospitals in central Japan. Diagnosis was made either by DSM-IV diagnostic classification of pervasive developmental disorders or by using a Japanese version of Autism Diagnostic Interview-Revised (ADIR-R). The Affymetrix Genome-Wide Human SNP Nsp/Sty 6.0 array was used to screen the samples. A total of 200 trio samples were analyzed so far. Only the samples which passed the required quality control (contrast QC >0.4) were used in subsequent analyses. PennCNV software (University of Pennsylvania) was used to call the CNVs.

**Results**

Samples with an LRR_SD [log R Ratio (log2 Copy Number – 1)] > 0.35 were omitted. Out of 200 trios, a total of 192 trio samples were available for CNV analysis after the initial QC measures. Only the CNVs with at least 20 probes within its length were considered to avoid any false positive calls. An average of 35.87 autosomal CNVs are detected per patient samples, after all the QC corrections. There is no significant difference in the total number/average size of CNVs per sample between patients and their parents. However, the percentage of deletions in the total length of CNVs is 41.16% in case of patients, while it is only 34% and 28.4% in father and mother samples respectively. Above 90% of the CNVs in children are found to be inherited; almost equally from both the parents. CNVs from 130 trios were analyzed in more detail. Among the CNVs which are spanning genes, 74.5% are deletions in the case of de novo events; it’s only 53% in inherited cases. To identify the most important de novo events, the CNVs are further screened based on the mean LRR values of all the probes within a CNV. Finally, 142 highly confident de novo CNVs were identified; among them 124 are spanning exons, and hence supposed to have some functional significance. Among these 124 CNVs, 18 are found to be rare de novo events. These 18 rare de novo events were validated with qPCR using SyBr Green. 12 of them were found to be true positive calls while remaining were false calls. A few potential candidate genes like ABR, PITPNA, YWHAE, TRAPPC9, CSMD3, BRD1, MAPK8IP2, PANX2 etc with a possible role in neurodevelopment were identified. The remaining trio samples are now being analysed. Rare inherited variants, if any, associated with autism also will be investigated. Frequency of common variants reported in other studies will be examined in Japanese samples. A gene enrichment analysis will be done to see which group of genes is over represented in CNVs and to study the role of any affected gene networks and associated biological pathways.

**Discussion**

De novo events have consistently shown the greatest genetic effect and were more frequent in ASD probands with only one affected child (the simplex families) as reported in previous studies. The burden of rare de novo CNVs (percentage of individuals carrying ≥ 1 rare de novo event) in our simplex probands (10%) is also similar to the studies reported previously (5% to 11%). Following the logic that CNV deletions should decrease the dosage of affected genes, our results that almost 75% of CNVs affecting genes are deletions, is particularly interesting. Previous studies have identified many genes enriched for CNVs which are involved in neuronal functional pathways including synaptogenesis, axon guidance and other related molecular processes. The likely morphological consequences of genes hit by rare de novo variants in the present study are now being investigated.
ANALYSIS OF THE AUTISM AND INTELLECTUAL DISABILITY GENE PTCHD1 CDNA REVEALS ALTERNATIVE SPLICING AND BRAIN-SPECIFIC ISOFORMS

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Background

Autism Spectrum Disorder (ASD) includes a range of complex neurodevelopment disorders. Children with autism spectrum disorders share features of impaired social relationships, impaired language and communication, intellectual disability (ID), repetitive behaviors and narrow range of interests. Approximately 75% have lifelong disability requiring substantial social and educational support. This study is focused on investigating the complex functional aspects of a recently identified gene -- PTCHD1, and how its disruption leads to ASD and/or ID. The insight gained will help in more precise phenotypic characterization of the individuals with disrupted PTCHD1.

Methods

We sought to identify PTCHD1 splice variants contributing different coding sequences that might have higher or more specific expression in brain, and may thus be more relevant to the neurobiology in individuals with autism and intellectual disability who have PTCHD1 mutations. We performed mRNA expression analysis using mRNA from multiple tissues, including brain regions, the mRNA from neuronally differentiated iPS cells from a PTCHD1 deletion proband and from the proband’s mother (two lines have been defined, in which the wild type PTCHD1 allele is inactive and the mutant PTCHD1 allele is active, and the other in which wild type is active, and mutant inactive). Expression analysis was performed using primer sets that discriminate the existing two PTCHD1 splice variants and were aimed at identifying new splice variants. Transcript-specific real-time quantitative assays were used to compare the expression of all transcripts in all tissues and brain sub-regions. Western blots were performed using the neuronal cell line, SK-N-SH, either undifferentiated or differentiated cells, and probed with a PTCHD1 antibody to confirm these findings. PTCHD1 encodes a Patched-related protein with 12 transmembrane domains and a sterol sensing domain, structurally similar to the Hedgehog (Hh) signaling pathway receptors PTCH1 and PTCH2, as well as the Niemann-Pick Type C1 protein (NPC1). To establish the involvement of PTCHD1 in Hh pathway, expression analysis was done with Hh pathway genes and plausible PTCHD1 partners. Immunoprecipitation studies were done to identify interacting partners. We also probed for sub-cellular localization of PTCHD1 in cilia, as this is the main site for Hh/receptor interaction.

Results

During the course of the RT-PCR studies of the PTCHD1 mRNA in human tissues, we detected an additional shorter band exclusively expressed in the cerebellum. Sequencing of the band confirmed a new transcript skipping exon2. This new transcript is predicted to encode a 542 amino acid protein in comparison to the 888 amino acid protein encoded by the PTCHD1 long isoform. Also of note, the PTCHD1 long isoform is predicted to encode a 12-transmembrane domain and a 101kDa protein, whereas this isoform lacking exon 2 has just 4 transmembrane domains, and encodes a 62kDa protein. Our preliminary results with western blots appear to confirm our findings. The RT-PCR also showed the presence of exonic sequence upstream of exon 1 which has also been sequence confirmed. The quantitative expression analysis demonstrates that expression of the two new PTCHD1 transcripts is highest in human cerebellum.
as compared to the brain sub-regions and other tissues. The quantitative expression analysis with over expression of PTCHD1 revealed increased levels in neuroligins and neurexins in two independent cell lines. This finding could indicate a plausible interaction between these genes. Preliminary western blot data confirm these results. Immunoprecipitation studies suggest plausible interactions between PTCHD1 and PSD95. The sub cellular localization of PTCHD1 is confirmed in cilia, suggesting similar function as other patches.

Discussion
The physiological significance of the new isoforms remains to be investigated. Based on the initial results with the expression of the PTCHD1 transcripts, we speculate that the new PTCHD1 transcripts have an important role to play in the brain and in its sub regions, thus, these transcripts and encoded isoforms may be more relevant to autism and intellectual disability. The interacting partners would help us elucidate the etiology underlying the disease phenotype. The Hh pathway plays an important role in embryonic development and adult stem cell functioning. Protein components of primary cilia are required for Hh signaling. We hypothesize that PTCHD1 localization to primary cilia could inhibit the Hh pathway by excluding Smo and also allows cilia to function as chemo sensors for the detection of extracellular Shh.

26 INVOLVEMENT OF SEROTONIN POLYMORPHISMS IN AUTISTIC SPECTRUM SYMPTOMATOLOGY
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Background
Twin studies have shown that Autistic Spectrum Disorders (ASD) are highly inherited, with several contributing genes and a complex interaction with environmental factors. To date, only a limited number of genetic variants have been discovered in relation to Autism, including alterations in FXMRP, Neuroligin X, SHANK2, SHANK3, CNTNAP2, EN2 and MET genes, amongst others. However, their contribution to the development of the disorder has not been clearly determined. Investigation of specific autistic symptomatology may improve the chances of identifying related genes and may help to better understand these disorders. Hypothesis: The disruption of the serotonin (5-HT) system is one of the most consistent and well replicated findings in ASD. Genetic variants in serotonin proteins may contribute to this disruption, and several studies have investigated their involvement in the development of ASD. Genetic variants in 5-HTT, 5-HT1B, 5-HT2A, 5-HT3A, 5-HT3C and 5-HT4A genes have also been linked to the predisposition to ASD. However, the results of these studies are not conclusive. Most of these findings have not been replicated in independent studies and the reported genetic effects are relatively small. To clarify the contribution of serotonergic variants, we investigated their contribution to specific phenotypes within ASD, such as intelligence quotient (IQ), and its components intellectual disability (ID) and linguistic impairment (LI). This may facilitate the identification of related genes and may help to better understand the pathophysiology of the disease.

Methods
We genotyped 80 tagged single nucleotide polymorphisms in 15 genes coding for serotonin receptor, transporter or catabolising proteins in a sample of N=141 children and young adults (121 male and 20 females, average age 14.5 ± 5.1 years), using high-throughput genotyping techniques.

**Results**
we identified novel associations between genetic variants in 5-HT2B, 5-HT6, 5-HT4 receptor genes and IQ, ID and LI, and confirmed the relevance of other serotonin proteins in the development of IQ, ID and LI in ASD patients.

**Discussion**
Our data confirm the involvement of the serotonergic variants on the pathophysiology of ASD phenotypes

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**SPLICING VARIANTS OF GLUTAMATERGIC CANDIDATE GENES AS RISK FACTORS FOR AUTISM SPECTRUM DISORDERS**

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**Background**
Autism spectrum disorders (ASD) are a heterogeneous group of highly heritable neurodevelopmental disorders with a complex genetic architecture. They are characterised by qualitative impairment in social interaction and communication, stereotyped behaviour and restricted interests. Studies on monogenetic disorders with ASD symptomatology like fragile X syndrome (FXS) have highlighted the glutamatergic signalling as a central component in the etiology of ASD. This was further underscored by several ASD-associated genes (i.e. *CYFIP1, CAMK4*) encoding for the GRM1/5 pathway. The aim of this study was to assess common functional variants of ASD candidate genes within the *GRM1/5* gene family as risk factors for ASD at genetic and functional levels.

**Methods**
We selected 10 SNPs within four glutamatergic genes (*GRM1/5, CYFIP1, CAMK4*) with a minor allele frequency (MAF) >10%. SNPs were genotyped by Real-time PCR or RFLP. 192 parent-child trios were used as a German starting set for validation of MAFs and preliminary association tests. Nine SNPs were followed up in an age-, sex- and sub-diagnosis-matched
sample of additional 254 German families. Due to the underpowered starting set, we combined both German sets for association testing to increase the power. Variants associated with ASD were replicated in a “Strict/European” ASD subsample of the AGP collection (N=1466 families) and a French Sample (531 cases/ 361 controls) if SNP data were available. Association of single variants and haplotypes was calculated using UNPHASED. To assess possible epistatic effects, we performed a scoring-based approach comparing cases and pseudo-controls. Meta-analysis including the German, the AGP and the French samples was calculated by R. Subsequently, we investigated isoform expression levels of two associated splice site variants in blood mRNA by quantitative Real-time PCR.

**Results**

In the combined German sample (N=446 families) we observed association with ASD for rs7170637 (*CYFIP1*) and rs25925 (*CAMK4*) performing single marker analysis. Our findings for rs7170637 were replicated in the AGP-sample with a suggestive p-value (<0.1). Meta-analysis supports significant association for both SNPs. An additive risk score-based approach using the German sample did not confirm our hypothesis of a synergistic effect of risk alleles on ASD liability. Database mining showed that rs7170637 and rs25925 are suggested to influence adjacent exonic splicing enhancer elements. Functional analysis revealed that carriers of the minor alleles of these SNPs significantly increased expression of an alternative isoform of the respective gene.

**Discussion**

Our results point towards the relevance of common functional variants of the glutamatergic system in the etiology of ASD. Over-transmission of the minor (G) allele of rs25925 suggests an increased risk for ASD (odds ratio (OR) >1.2), while under-transmission of the minor (A) allele of rs7170637 rather supports a protective mechanism (OR<0.9), underpinned by the ORs of respective haplotypes. Suggestive replication for rs7170637 in the AGP-sample, comparable ORs for both SNPs in the French cohort and significant results in the meta-analysis are clearly supporting our findings. Functionally, we revealed that both associated minor alleles influence alternative splicing of *CYFIP1* and *CAMK4* mRNA increasing the expression levels of a truncated mRNA-isoform without changing overall expression of the respective gene. CYFIP1 and CAMK4 play major roles in the regulation of neuronal processes and mRNA processing. The truncated isoform of CYFIP1, which is increased in rs7170637 minor allele carriers, lacks parts of the FMR1-interacting domain and may thus influence FMR1-CYFIP1-mediated translation initiation. Similarly, the increased alternatively spliced isoform of *CAMK4* mRNA may shift the availability of full length *CAMK4* transcript and subsequently may have an impact on neuronal regulation.

We conclude that effects attributed to the SNPs investigated here are too minute to act in a synergistic manner. Rather, we are suggesting an independent allele-specific mechanism. The fact that the minor alleles of rs7170637 and rs25925 were both interfering with alternative splicing and associated with ASD suggests that common functional variants do have an impact on higher order functions as cognition, social interaction or communication.
EVIDENCE OF AN ASSOCIATION OF GRIN2A WITH AUTISM IN FEMALE SUBJECTS
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Background
A wide variety of evidence, using many different behavioral paradigms, indicates a broad role for the involvement of the extracellular signaling-regulated kinases (ERK) pathway in synaptic plasticity and consequently learning and memory. Disruption of neuronal protein synthesis through ERK pathway is the shared point of several essential pathways associated with autism including the mTOR pathway. Therefore, aberrant regulation of the ERK pathway is a potential cause for deficits associated with autism. The purpose of this study was to investigate the association of the six genes in the ERK pathway, including ERK2, MNK1, eIF4E1, 4EBP2, as well as the upstream regulators GRIN2A encoding NMDA receptor 2A and GRM1 encoding mGluR1 with autism in an Iranian autism sample.

Methods
Family-based association analysis was performed using 700 individuals from 200 nuclear families from Iran with at least one autistic child. In total, 175 SNPs were genotyped for all six genes using Sequenom iPLEX. TDT analyses were performed with Plink & FBAT software. Quality control procedures applied to the data included rejection of any SNP markers with missingness greater than 0.15, MAF less than 0.01, HWE p-value less than 2.8*10^-4, and Mendelian Error rate of greater than 0.05 were also removed. After QC, 150 SNPs and 672 individuals from 194 nuclear families were analyzed. Based on the high rate of consanguineous marriage (35%) in Iranian samples and because of sex differences exist in autism, gender specified analyses were done under recessive model.

Results
We failed to identify a significant association with these six genes and ASD under recessive model on the entire sample. However, when analysis were restricted to female probands (48 families, 198 individuals), thirteen SNPs associated with ASD, of which the five best markers were in GRIN2A. After the MeffLi correction for multiple testing, one marker, rs2267784 from GRIN2A remained statistically significant (Z=-3.31, p= 0.0009). When 2-marker haplotypes including rs2267784 were constructed, three haplotypes showed significant association with ASD among girls. P-value corrected for multiple testing is 0.001068 based on the MeffLi method (SNPSpD).

Discussion
After quality control, 194 nuclear families were analyzed for 6 genes involved in local protein
synthesis. One SNP significantly and 4 SNPs were nominally significant for gene GRIN2A for affected females. One result survived correction for multiple testing. Our results support the involvement of GRIN2A in conferring risk for girls with ASD; however these results should be replicated in the same population with higher numbers of girls affected with autism.

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QUANTITATIVE REVIEW AND SYNTHESIS OF BLOOD-BASED TRANSCRIPTOMIC BIOMARKER STUDIES OF AUTISM SPECTRUM DISORDERS

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Background

Autism spectrum disorders (ASDs) comprise a group of phenotypically similar disease states that affect 1 in 88 children and are a cause of significant debilitation and distress for both affected children and their families. Efforts to discern clinically useful biomarkers have been demonstrated at many levels of potential pathophysiology, and gene-expression microarray analysis of samples obtained via peripheral blood have provided promising evidence implicating a variety of dysregulated genes. However, each study has identified and implicated different genes and pathways, and as a result the literature suffers from a lack of consensus.

Methods

In this study we pooled data from six microarray datasets in order to identify a list of genes that demonstrate concurrency and therefore serve as better predictors of the diagnosis of ASD. In addition to our own data, we identified five publicly available datasets of expression microarray data from whole-blood or peripheral blood lymphocyte samples of children with ASDs and typically developing comparison subjects. Gene Expression Omnibus accessions for the data in the public domain are GSE18123 (two samples), GSE25507, GSE26415, and GSE6575.GSE18123

Results

Employed microarray platforms included the Affymetrix Human Genome U133 Plus 2.0, Agilent-014850 Whole Human Genome Microarray 4x44K G4112F, and the Illumina WG-6/WG-12. The top 5% (by p-value) of dysregulated genes from each of the six datasets was identified by analyses of covariance (with age and sex as covariates, where available) using Partek Genomics Suite Version 6.6 (Partek Incorporated). Upstream regulators for each study's candidate biomarker set were determined by Ingenuity Pathway Analysis (IPA), and further compared across datasets to determine areas of overlap. Top concurrent upstream regulators were then matched to the top 1% of dysregulated genes across all six datasets. Those genes that were identified across more than one dataset were identified as the most reliable biomarkers of ASD. Additionally, pathway analysis was performed on the identified genes to determine potential canonical pathways as well as, potentially, implicated pathophysiology. Top biomarker genes to emerge from this analysis included transcription factor 3 (TCF3), CD44, microtubule associated protein tau (MAPT), serine/threonine-protein kinase 4 (STK4), and T-box transcription factor (TBX2), among others. Analysis of the top canonical pathways of implicated genes suggested their involvement in TGF-beta signaling, glucocorticoid receptor signaling, and acute phase response signaling.
Discussion
These results, which were obtained from peripheral blood samples, suggest immune system dysregulation at the gene expression level among children with ASDs. This result bolsters work from other studies performed at the post-transcription, protein, hormonal, and tissue levels in implicating an association of immune system dysregulation with ASDs.

HIGH-THROUGHPUT PHENOTYPING OF BIPOLAR DISORDER FOR GENETIC RESEARCH: VALIDATION
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Background
With advances in genomewide analysis, the rate-limiting step for genetic association studies has become the availability of well-characterized patient samples. The increasing utilization of electronic health records (EHR) provides new opportunities for epidemiologic and genetic research. A ready repository of phenotypic data contained in health system EHRs can enable large-scale studies of substantial size and cost-effectiveness. However, the validity of EHR-based diagnoses for genetic research has not been established.

Methods
As part of the International Cohort Collection for Bipolar Disorder (ICCCBD), a multisite genetic study using high-throughput phenotyping, we derived EHR-based diagnostic algorithms combined with collection of discarded blood samples to rapidly accrue a large case-control sample. We evaluated the diagnostic validity of informatics-based phenotyping against a gold standard of semi-structured clinician interviews. From a data registry of more than 4.2 million individuals in a large healthcare system, a datamart of 52,235 patients were identified with at least one diagnosis of bipolar disorder (BD) or manic disorder in the billing data or out-patient medical record using the i2b2 Workbench software (i2b2 v1.6.04; USA). In addition 1.2 million patients were identified as controls, defined as at least 30 years old and without any evidence of a psychiatric or neurological diagnoses or treatments related to those diagnoses. Four diagnostic algorithms for case selection were derived. One of the case algorithms utilized natural language processing (NLP) of narrative notes from the medical record. Expert clinician review and annotation of more than 200 patients from the bipolar datamart was used to classify patients as having BD (or not) using criteria adapted from DSM-IV. LASSO regression was used to train a model for predicting BD diagnosis with 95% specificity based on features selected from the manual chart review. The other three case definitions relied solely on diagnosis, encounter and medication information in the coded record. To validate the case and control algorithms, we selected a random subset of 164 cases and controls to undergo a direct-interview evaluation by blinded trained MD or PhD clinicians using the SCID-IV. An additional 27 patients with a history of major depressive disorder or schizophrenia included as “distractor” to maintain rater blinding. The validity of the diagnostic algorithms was assessed by calculating the positive predictive value (PPV) of the algorithm-based diagnosis compared to a gold standard SCID interview. In addition, rule-based algorithms for a set of subphenotypes (including history of
alcohol dependence, history of drug dependence, history of suicide attempt, history of psychosis and history of panic disorder / agoraphobia) were derived based on manual chart review by expert diagnosticians. The PPV of these additional phenotypes were also calculated based on results of the in-person SCID interviews.

**Results**
The PPV of the NLP-based algorithm (i.e., the probability of a clinical interview diagnosis given an NLP diagnosis) was 86.2%. The best performance for the coded-data algorithms was 77% (where cases were defined by having >3 BD diagnoses and either receiving care at a bipolar specialty clinic or treatment with lithium or sodium valproate). The PPV for the control selection algorithm was 100%. The PPV for subphenotypes exceeded 80% except for psychosis (40%). To date, phenotype data and DNA samples have been collected for more than 8,000 cases and controls using these definitions.

**Discussion**
Using NLP processing of EHR, we were able to derive an automated phenotyping algorithm that achieved 95% specificity and > 85% PPV compared to a gold-standard diagnostic interview. These results demonstrate that informatics-based mining of EHR data can be used to derive case and control definitions for BD research with high levels of predictive validity.

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**SNPS NOMINALLY ASSOCIATED WITH THE ACUTE RESPONSE TO AMPHETAMINE ARE ENRICHED FOR SNPS THAT CONFER PROTECTION FROM SCHIZOPHRENIA AND ADHD**

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**Background**
Genome-wide association studies (GWAS) provide an unbiased approach to investigating the contribution of genetic variation throughout the genome to a phenotype of interest. However, an underlying assumption of GWAS is that all variants (single nucleotide polymorphisms, or SNPs) are equally likely to have an effect on a phenotype, despite that fact that most SNPs do not have functional consequences.

**Methods**
We previously performed a GWAS for the acute response to d-amphetamine in 381 healthy human volunteers. In an effort to interrogate the numerous nominally significant associations we identified, we sought to identify enrichment of functional classes of SNPs among our top GWAS associations with response to d-amphetamine.

**Results**
We found that SNPs with modestly low P-values (P<0.01) for response to d-amphetamine were significantly more likely than random to have similarly low P-values in the GAIN and PGC1
schizophrenia samples and the PGC1 Attention Deficit Hyperactivity Disorder (ADHD) sample. Furthermore, the source of this enrichment was due to an excess of alleles that increased sensitivity to the subjectively positive effects of \textit{d}-amphetamine and were also associated with decreased risk for these diseases. We are currently working with newer, unpublished data in an effort to replicate these findings. We found no enrichment for negative control phenotypes such as height and inflammatory bowel disease.

**Discussion**
These results suggest that alleles identified using an acute challenge with a dopaminergic drug can be used to identify alleles that confer risk for or protection from psychiatric diseases that are associated with dopaminergic abnormalities. Moreover, they demonstrate the utility of the enrichment approach as an alternative to stringent standards for genome-wide significance.

**NET-ASSOC: A STANDALONE APPLICATION FOR THE ANALYSIS OF PROTEIN-PROTEIN INTERACTIONS IN GENETIC ASSOCIATION STUDIES**
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**Background**
The exponential increase of genome-wide data in recent years necessitates the development of novel bioinformatics tools in order to better interpret the biological context of associated genomic loci. Studying protein-protein interactions offers a molecular way of understanding how these genes are related and possibly identifying other candidates.

**Methods**
The application, NET-ASSOC, was developed in C/C++, with specific algorithms ported from the Python scripting language. This application was designed to interface seamlessly with the PLINK/Seq (\url{http://atgu.mgh.harvard.edu/plinkseq/}) toolset. We use two sources of PPI network information, STRING (Franceschini et al., 2013. PMID: 23203871) and InWeb (Lage et al., 2007. PMID: 17344885).

**Results**
We have implemented a number of PPI-network based algorithms in NET-ASSOC and expect to develop more. First, based on code from its creators, we have created a standalone version of the popular DAPPLE webservice (\url{http://www.broadinstitute.org/mpg/dapple/dapple.php}, Rossin et al., 2011. PMID: 21249183), that searches for unusually high network connectivity among genes in associated loci. We are exploring different approaches to incorporate weights (e.g. reflecting strength of association) and factors to account for potential bias arising from differential gene size. Second, we have implemented a greedy search algorithm specifically designed for rare variant association studies, that aims to find maximally associated cliques or subnetworks. Third, we have implemented a PPI-based gene prioritization scheme to re-rank and empirically evaluate gene-based association statistics in light of the statistics for other independent genes that are proximal in the network. We demonstrate these approaches with application to a large exome sequencing study of schizophrenia.
Discussion
To better understand the increasingly rich array of genetic association data from GWAS, sequencing, and CNV studies, tools that can consider collections of genes in their biological contexts, such as in terms of protein-protein interaction, will be increasingly important. Such tools can help to detect novel associations as well as to characterize sets of existing ones, and to suggest candidate loci based on a systematic assessment of the current genetic association data.

COMPARISON OF RARE VARIANT COLLAPSING METHODS FOR BINARY TRAITS IN RELATED SAMPLES
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Background
Some of the heritability for psychiatric traits may lie in rare variants, which are difficult to detect due to lack of power. To potentially increase power, methods to collapse and analyze rare variants have been developed and, to some extent, compared in unrelated samples. Less attention, however, has been devoted to methods for related samples, particularly when the phenotype is binary. Current choices include performing some of the methods appropriate for unrelated samples and then employing a corrective factor to account for relatedness, or using non-linear mixed effects models or generalized estimating equations to handle the correlation within families while making inferences across families.

Methods
We simulate several haplotypes containing a rare variant in a founder population, and gene drop these haplotypes through multilevel families. To create phenotypes under the null, affection status is randomly assigned, but under an alternative, we sample disease around the estimated probabilities from a logistic model with specified penetrance for the rare variants. We then perform a collapsing method that borrows from the generalized disequilibrium test (GDT), a family based association test that contrasts affected and unaffected members in extended pedigrees. We also create an indicator for the presence of a rare variant and perform a generalized estimating equation (GEE) model, specifying an exchangeable correlation structure that is clustered on family ID, to measure the association of this variable with disease status.

Results
We show that a rare variant collapsing method within the context of a modified GDT has an acceptable type I error, while the GEE approach tends to be inflated. Both methods have low power under an alternative, underlining the difficulty in dealing with rare variant detection.

Discussion
Our results have relevance for studies involving sequencing data in families for binary outcomes such as bipolar disorder or schizophrenia. Future work will incorporate population substructure across the simulated pedigrees and evaluate additional methods that are suitable for binary traits in family studies. We plan to apply the methods to whole genome next generation sequencing data in a set of families collected from a genetically isolated population.
MEGA-ANALYSIS OF FIVE EQTL STUDIES FROM HUMAN CORTEX BRAIN SAMPLES
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Background
GWAS for psychiatric disorders have been successful recently, with many new loci identified that met consensus criteria for significance and replication in human genetics. However, such association signals have yet to be translated into a full understanding of genetic mechanisms that mediate risk for disease at particular loci. To bridge the gap between GWAS results and disease, eQTL analysis has been utilized as a valuable and popular tool to characterize the function of loci underlying complex disease traits. Numerous eQTL studies have investigated the effects of genetic variability on gene expression in post-mortem human brain samples. However, current catalogs of eQTLs are incomplete and the findings do not replicate well across studies. In a large eQTL study in peripheral blood (6,526 subjects), we recently demonstrated the critical importance of sample size. All existing cortical eQTL studies are small and emphasize the need for a mega-analysis.

Methods
To address these issues, we performed a mega-analysis of five eQTL studies from the frontal cortex of 439 European normal brain samples with age $\geq$ 20. The five eQTL studies are Gibbs et al. (PMID: 20485568), Colantuoni et al. (PMID: 22031444), Myers et al. (PMID: 17982457), Stanley Medical Research Institute (SMRI) database, and Genotype-Tissue Expression (GTex) database. Individual genotypes and expression data were downloaded from dbGaP and GEO. We conducted extensive and rigorous Quality Control procedures for all data prior to meta-analyzing them using Matrix eQTL, R/Matlab package for ultrafast eQTL analysis.

Results
We identified 840 cis-eQTL genes in autosomes and 3782 SNP-gene pairs with FDR q < 0.05, which includes much more eQTLs than the union of individual study. To evaluate their characteristics and biomedical relevance of the findings, bioinformatics analyses are in progress.

Discussion
These systematically generated eQTL information should be useful in determining the functional mechanisms of brain gene expression and the underlying biology of associations with common psychiatric disorders.

HOW ISOLATED IS THE FAROESE POPULATION AND WHAT ARE THE PROSPECTS OF MAPPING COMPLEX PSYCHIATRIC DISORDERS?
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Background
Due to effects of bottlenecks through increased genetic drift recently founded and isolated populations typically show decreased genetic and environmental heterogeneity and may thus be advantageous for studies aiming to map genes involved in complex phenotypes. Such populations are particularly useful in identifying rare disease variants that may appear at higher frequencies or within a more clearly distinct haplotype structure compared to outbred populations. Increased levels of linkage disequilibrium in isolated populations may reduce the number of markers needed to cover the entire genome in an association mapping study. But extended levels of LD may, however, hamper the ability to discover the specific gene(s) or variants involved. A unique pattern of LD and unique haplotype frequencies may also obstruct the use of imputation algorithms based on HapMap CEU samples, and thus require the development of a population specific reference sample for imputation. Scandinavians and individuals from the British Isles settled on the Faroe Islands more than a 1000 years ago. The Faroese population may represent an isolated founder population, likely to have experienced one or more bottlenecks during its demographic history, and may therefore facilitate mapping of rare variants of complex traits. We aim to document whether the Faroese population can indeed be considered an isolated and genetic homogenous population, and discuss applications and consequences for mapping of complex disorders.

Methods
DNA was extracted from a Faroese case-control sample consisting of a total of 197 putatively unrelated individuals (106 cases with schizophrenia and 91 controls). All individuals were whole genome sequenced using the Illumina platform. Sequences were aligned and SNPs were identified using multi-sample SNP calling. Analysis are currently on-going and we aim to estimate the current effective population size based on Linkage Disequilibrium, which will reflect the recent demographic history, and will be relevant for evaluating the application of the Faroese population in mapping of complex traits. Estimated measures of LD relative to genomic distance will be compared between the Faroese and other European samples. Likewise measures of haplotype diversity will be estimated and compared with other populations of north Europe.

Results
Per sample mean depth is nowhere less than 4.3x and is on average 6.7x. Approx. 16 million SNPs were identified based multi-sample calling of the aligned sequences.

Discussion
The results of these analyses will reveal the prospects for using samples from the Faroe Islands in the identification of variants that alters the risk of complex psychiatric disorders. Estimates of effective population size, Ne, will indicate whether the Faroese population represents a true isolated and genetic homogenous population when compared to estimates of outbred populations. Results will also reveal whether LD extends further and whether the haplotype diversity is reduced in the Faeroese sample compared to European samples. Results will thus indicate whether HapMap samples can be used for imputing of missing genotypes and whether it will be more difficult to locate the actual causative polymorphisms in the Faroese sample compared to other European samples.
GENETIC COUNSELING FOR INDIVIDUALS WITH SERIOUS MENTAL ILLNESS: THE FIRST RANDOMIZED CONTROLLED TRIAL
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Background
Serious mental illnesses (SMIs) are common, complex conditions affecting ~3% of the population. Individuals with SMI have expressed interest in genetic counseling (GC), and benefits of GC for this population have been postulated but not yet well explored empirically.

Purpose: To conduct the first randomized controlled trial of the effect of GC for people with SMI.

Methods
Individuals with SMI (schizophrenia, bipolar disorder, or schizoaffective disorder, confirmed by the Structured Clinical Interview for Diagnosis), referred by self or psychiatrist, were randomized to either GC, a control intervention involving an educational brochure (EB), or a waitlist group (WL) (N=120). Participants completed a purpose-designed measure of knowledge and risk perception, the Internalized Stigma of Mental Illness scale, and the Illness Perception Questionnaire (mental illness version), at baseline (T1) and one month later (T3); those in GC and EB also completed measures immediately post-intervention (T2). The Brief Symptom Inventory was administered at T1 and T3 to control for current symptoms. Analyses included ANCOVAs for between-group effects, and nested mixed-effects linear regressions for within-group effects.

Results
Knowledge increased for GC compared to WL at T3 at the trend level (p=0.09). Risk perception accuracy increased for GC compared to WL and EB at T3 (p<0.001). Stigma scores decreased in GC and EB at T2 (p<0.01) and at T3 on the stereotype endorsement subscale for GC compared to WL (p<0.05). For perceived control, consequences subscale scores decreased in GC compared to WL and EB at T3 (p<0.05), treatment control subscale scores increased at T2 for GC and EB (p<0.01), and timeline acute/chronic subscale scores decreased for GC and EB at T2 (indicating perception of illness as less chronic) (p<0.05).

Discussion
GC improves risk perception accuracy for this population, and has potential to improve knowledge, decrease internalized stigma, and increase perceived control.

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LONG-TERM FUNCTIONING IN MAJOR PSYCHIATRIC DISORDERS AND ITS CLINICAL AND PSYCHOSOCIAL PREDICTORS: A POTENTIAL CROSS-DIAGNOSTIC PHENOTYPE FOR FURTHER GENETIC INVESTIGATIONS
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Background
Large parts of the heritabilities of psychiatric disorders have not yet been accounted for by the genomic findings of the last decade. This may in part be due to the difficulty of defining specific phenotypes. At times, it is not easy to clinically distinguish between these disorders as symptoms often overlap and vary widely during the course of illness. Therefore, researchers have attempted to identify better-defined subphenotypes sharing certain phenotypic similarities across diagnostic boundaries as these overlaps may be due to a common genetic basis. Here we study psychosocial functioning as a potential cross-diagnostic phenotype. In our study, long-term global functioning (as measured by the GAF-score) was assessed in patients with schizophrenia (SZ), bipolar disorder (BD), and major depressive disorder (MDD). We compared the predictive value of several clinical and psychosocial variables for functional outcome between these disorders and discuss the relevance of these findings for further genetic investigations.

Methods
Based on a structured interview (SCID-I for DSM-IV) and a review of clinical records using the Operational Criteria Checklist (OPCRIT), we assessed sociodemographic and clinical variables in inpatients with SZ (n=238), BD (n=533), and MDD (n=398). For each patient, we retrospectively determined global functioning (GAF) at three points in time over the course of illness: i) premorbid GAF-score, ii) worst GAF-score during course of illness, iii) GAF score right before current acute episode. The latter was analyzed as outcome parameter for long-term functioning.

Results
The average level of long-term global functioning was lowest in SZ patients (mean GAF score: 61.7± SD 16.2), and higher in MDD patients (73±15.8), while BD patients showed the least impairment of functioning (78.2±14.9). Linear regression models revealed low premorbid functioning, poor premorbid work/social adjustment, and insidious illness onset to be significantly associated with worse functional outcome in all three disorders. Additionally, lifetime occurrence of negative symptoms predicted worse functional outcome in BD and SZ patients. However, levels of functioning were also found to be significantly influenced by other factors, which differed according to diagnostic group. For SZ patients, longer duration of illness and lower levels of global functioning during episodes were found to have a major impact on long-term functioning, whereas for BD patients, lifetime alcohol, cannabis or other drug abuse, and lifetime occurrence of suicidal ideation were significant predictors for worse functional outcome. In contrast, having a premorbid personality disorder and unemployment at illness onset appeared to influence outcome in MDD.

In an additional explorative analysis, the following disorder-specific combinations of variables were identified by stepwise regression to jointly predict functional outcome. In SZ patients, the model included poor premorbid social adjustment, longer duration of illness, negative symptomatology, and lifetime single status (in total accounting for 28% of the observed variance...
of functional outcome). In BD patients, premorbid level of functioning, negative symptomatology, lifetime cannabis and other drug abuse accounted for 22% of the trait variance. In MDD patients, premorbid level of functioning alone was found to predict long-term functioning in a univariate model, accounting for 26% of the trait variance.

Discussion
Long-term functional outcome may be an important phenotype for cross-diagnostic genetic investigations. However, it may be difficult to use this as a sole phenotype, as impaired functioning seems to be influenced by different factors to a different extent in SZ, BD and MDD. Furthermore, long-term functional outcome varies between the three disorders. However, some clinical and social variables appear to predict worse outcome in SZ as well as in BD and MDD. These variables (poor premorbid functioning, poor premorbid work and social adjustment, insidious disease onset, and in BD and SZ negative symptomatology) have previously been associated with a chronic course of illness, often resulting in persistent and severe functional impairment. Therefore, it could be worth investigating whether having one or more of these clinical and psychosocial characteristics defines a cross-diagnostic subgroup of patients sharing a similar set of genetic factors. (Also see contribution by M. Budde: “An analysis of genetic risk factors for long-term functional outcome in bipolar disorder and schizophrenia”).

ASSOCIATION BETWEEN HAPLOTYPES OF BRAIN DERIVED NEUROTROPHIC FACTOR (BDNF) GENE AND COGNITIVE FUNCTIONS IN HEALTHY SUBJECTS IN POLISH POPULATION
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Background
Association studies of BDNF polymorphisms in psychiatric diseases and healthy subjects are equivocal, mostly Val66Met functional polymorphism is studied, with only few more polymorphisms or haplotypes. Haplotype structure of BDNF genomic region considerably differs between populations worldwide and explain inconsistency of the results.

Methods
The aim of the study was to assess the associations between haplotypes determined from 12 SNPs genotyped (rs2030323, rs2883187, rs11030102, rs1401635, rs2049045, rs2049046, rs2030324, rs988748, rs6265 (Val66Met), rs11030101, rs10835210, rs1013402) and cognitive functions measured by neuropsychological tests such as: Wisconsin Card Sorting Test (WCST), Trail Making Test (TMT), Stroop Test in 310 healthy volunteers. Using PLINK software (http://pngu.mgh.harvard.edu/~purcell/plink/) we identified LD blocks in a given set of markers. All from located on 11\(^{th}\) chromosome SNPs turned out to made up in a single LD block. Other statistical computations were done in R language (http://cran.r-project.org/) and appropriate package gap for genetic analyses (http://cran.r-
Results
We identified 16 haplotypes. To check potential associations between haplotypes identified and cognitive functions we applied haplotype trend regression test (HTR) with 1000 simulations as adjustment method. Test was already available in R gap package. We couldn’t use Haploview software, as our trait was quantitative, not binary as in case-control studies. No overall statistically significant association between cognition measures and all haplotypes appeared. Looking at associations between single haplotypes and trait we detected the level of conceptual responses in WCST to be related with AGCGCTGCACA haplotype (p=0.0159). TMT_B test results on the other hand were associated with AGCGCTCCTA haplotype (p=0.041). Last association was detected between AGCGCTCTACA haplotype and TMT_A test results (p=0.002).

Discussion
The results obtained showed the associations between selected haplotypes and psychomotor speed, attention, executive functions and conceptual thinking in Polish population. The results are preliminary. The analyses need to be conducted on larger group. The project has been financed from the funds of the National Science Center, granted on the basis of decision no. DEC-2011/01/B/HS6/00440.

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FAMILY-BASED GENOME-WIDE ASSOCIATION STUDY OF RESTING EEG IDENTIFIES UROC1 AND NEUROTRANSMITTER BIOSYNTHETIC PROCESS
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Background
Electroencephalography (EEG) measures are highly heritable neuroelectrical correlates of resting brain state, and contribute several markers of risk for alcoholism and related disorders. Increased beta power has been found to be a hallmark of alcoholism and related disorders and also a marker of those at risk, and is an index of neural hyperexcitability. To identify genetic variants associated with EEG, we performed a family-based genome-wide association study (GWAS) using extended multiplex families densely affected by alcohol dependence. The advantage of the design of this study is robustness against population substructure.

Methods
High beta power (20-28 Hz) of the three frontal-central bipolar electrode pairs (F3-C3, Fz-Cz, F4-C4) were calculated using standard Fourier transform methods. Samples from the Collaborative Study on the Genetics of Alcoholism (COGA) were genotyped using the Illumina Human OmniExpress array on 118 families, densely affected by alcohol use disorder. Phenotype data was derived from multivariate linear regression models which were constructed from log transformed high beta power EEGs, controlling for log-transformed age and stratified by gender. After quality control procedures, association testing was done on 1,564 samples assuming an additive model using the generalized disequilibrium test (GDT). Pathway analysis was
performed using ALIGATOR (Association List Go Annotator) to study groups of genes by testing for overrepresentation of members of those groups within lists of genes containing significantly associated SNPs from the GWAS.

Results
We found that a SNP in UROC1 (rs1687482) was significantly associated with F3-C3 high beta power EEG at a genome-wide significant level ($p = 4.8 \times 10^{-8}$). We also confirmed that SNPs in GABRA2 were significantly associated with this phenotype ($p = 9.8 \times 10^{-7}$). The most significant individual GO category from the pathway analysis is a neurotransmitter biosynthetic process with significantly associated SNPs in PAH ($p = 1.2 \times 10^{-5}$). These results suggest PAH accounts for some of the variations in high beta power EEG. PAH catalyzes the conversion of phenylalanine to tyrosine, shares physical, structural and catalytic properties with tyrosine hydroxylase and tryptophan hydroxylase that catalyze the rate-limiting steps in the biosynthesis of the neurotransmitters dopamine, noradrenaline and serotonin.

Discussion
These findings underscore the utility of using EEG phenotypes to identify meaningful genetic correlates of resting brain state with pathophysiology of neuropsychiatric conditions.

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POLYMORPHISMS IN THE CRHR1 GENE AND TEMPERAMENTAL TRAITS AS POTENTIAL ENDOPHENOTYPES FOR AFFECTIVE DISORDERS AND ALCOHOLISM

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Background
Corticotropin releasing hormone receptor 1 (CRF₁), in humans encoded by the CRHR1 gene, is a protein involved in the functioning of hypothalamic-pituitary-adrenal axis and thus it is responsible for the regulation of organism's stress response. Numerous research data shows connections between various polymorphisms in CRHR1 gene and the occurrence of different mental and behavior disorders, such as depression, anxiety disorders and alcohol abuse.

Methods
In the present study we investigated possible associations between 22 SNPs in the CRF₁ (CRHR1) gene and temperamental traits according to Strelau's theory in two groups: group I, patients with affective disorders or alcoholism (n=400) and group II, controls (n=425). Temperament traits were assessed using Polish version of Zawadzki and Strelau's Formal Characteristics of Behavior-Temperament Inventory. All diagnoses were made according to the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision with the use of WHO-CIDI.

Results
After controlling for age a main effect of rs12942300 polymorphism on Emotional Reactivity scale in subgroup of healthy women has been found. We have discovered no significant associations in patients group. Additionally haplotype analyses have been performed.
Discussion
The results of our study give evidence for the genetic basis of temperamental traits and, as rs12942300 polymorphism is strongly linked to other SNPs which have been demonstrated to have some effect on adult depression, they may indicate that Emotional Reactivity trait may be potential mechanism linking the CRF₁ (CRHR1) gene variability and proneness to mood disorders.

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CROWDSOURCING METHODS FOR CHARACTERIZING COGNITIVE ENDOPHENOTYPES IN PSYCHIATRIC GENETIC RESEARCH
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Background
Understanding the relationship between genetic variation and risk for psychiatric disorders depends on the accurate measurement and characterization of phenotypes for genetic analysis. We focus here on high throughput methods for measuring and characterizing intermediate cognitive phenotypes, using Web-based crowdsourcing in a citizen science framework.

A significant obstacle to understanding how genetic variation contributes to psychiatric disorders is the considerable heterogeneity and imprecision of DSM-defined psychiatric phenotypes. This has led to a shift towards investigations of intermediate phenotypes (also known as endophenotypes), or phenotypes that are intermediate between a disorder and underlying genetic mechanisms and contribute to pathogenesis. For any particular phenotype, the success of this approach relies on several factors, including (1) the availability of reliable, valid, and sensitive phenotype measures, (2) established estimates of heritability, (3) characterization of age-related phenotypic change, (4) identification of environmental factors that may interact with genes to influence phenotype expression, and (5) the ability to collect phenotype information in very large samples. Web-based crowdsourcing methods can address these factors by providing access to large and diverse samples at substantially reduced cost. Large web-based samples permit the rich and comprehensive characterization of phenotypes in terms of their psychometric properties and their relationship with developmental, familial, environmental, and population variables. We have shown that very large numbers of everyday people from a range of backgrounds are motivated to participate in scientific research to learn more about themselves and contribute to the advancement of science. This is the “citizen science” model of research participation.

Methods
Here, we show how this crowdsourcing approach can inform the selection, development, and characterization of basic cognitive phenotype measure, with a focus on measures of fluid intelligence, memory, and social cognition. All phenotypes were developed and characterized using data collected through TestMyBrain.org, our website for conducting behavioral experiments. Experiments on TestMyBrain are aimed at understanding the causes and
consequences of individual variation in cognitive functioning. TestMyBrain.org has been used to collect data from over 800,000 participants from all over the world with data quality comparable to data collected in traditional in-person settings.

**Results**
For specific cognitive phenotypes, we provide a comparison of (a) psychometric properties, (b) heritability, (c) age-related change, and (d) sensitivity to specific environmental factors. All results are derived from web-based measures and volunteer samples. (a) Psychometrics: We show how large samples enable the application of both classical test theory and item response theory to assess the quality of a cognitive measure and build models of how item-by-item performance predicts variations in underlying cognitive ability. This analysis shows how two tests of comparable length and difficulty can differ substantially in internal consistency (reliability) and their ability to distinguish normal from impaired performance. (b) Heritability: We show how cognitive phenotypes derived from related domains of cognitive functioning can have distinct patterns of heritability. (c) Age-related change: We show how related cognitive phenotypes can also have markedly distinct patterns of age-related change across the lifespan, highlighting the importance of a developmentally-informed approach to phenotype assessment. (d) Specific environmental influences: Finally, we show how specific environmental risk factors are linked with adult phenotype variation – information that is critical for understanding where gene-environment interactions may be most relevant.

**Discussion**
The combination of web-based methods and the participation of everyday citizen scientists can reshape the study of psychiatric genetics and vulnerability by providing new methods, tremendous power, and a leap in cost and time efficiency. Over the next few years, we will build a toolkit for high throughput cognitive phenotyping, consisting of measures that are richly characterized for genetic association analyses and available for broad use by the scientific community. Large samples can provide critical information about cognitive phenotype variation that will be essential for identifying and interpreting specific relationships between genetic variation and variations in cognitive functioning.

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**AGGREGATE GENETIC INFLUENCE MODERATES THE LINK BETWEEN WOMEN'S HISTORY OF CHILDHOOD ABUSE AND RISK FOR RECURRENT DEPRESSION AND SUICIDAL BEHAVIOR**

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**Background**
Individuals with a history of childhood abuse are at an increased risk of developing major depressive disorder (MDD) as well as suicidal behavior. Despite strong evidence for the influence of early adversity on depression and suicide in adulthood, however, many individuals who experienced abuse as children never attempt suicide or develop recurrent depression. The
goal of this study was to examine potential genetic moderators of these risks. Given the heterogeneity of MDD and the complexity of suicidal behavior, it is highly plausible that the underlying genetic influence is polygenic. In selecting candidate genes for investigation, we focused on those known to impact neural functions implicated in depression and suicide risk. More specifically, we used a biological systems approach to examine aggregate levels of influence across the two genes known to impact the activity in amygdala during emotion processing tasks and neuronal plasticity under stress. We chose variants from two candidate genes involved in regulation and stress response and amygdala reactivity—solute carrier family 6 member 4 gene, SLC6A4 (5-HTTLPR) and BDNF (Val66Met, rs6265)—to calculate an aggregate genetic score (AGS). We predicted that higher scores on our aggregate genetic variable would be positively associated with recurrent depression diagnosis and a lifetime history of suicide attempts among women with a history of childhood abuse.

**Methods**

Participants in this study were 255 women recruited from the community. Participants’ histories of MDD were assessed using a diagnostic interview (SCID-I) and women were coded as having no lifetime history of any mood disorder (n = 126), a single episode of MDD (n = 53), or a history of recurrent MDD (n = 76). Individuals’ history of suicide attempts was accessed via structured interviews and questionnaires and women were coded as either having a history of one or more suicide attempts (n = 22), or no history of suicide attempts (n = 233) in their lifetime. The Childhood Trauma Questionnaire (CTQ) was used to assess participants’ history of childhood abuse. Women were coded as having no history of abuse (n = 151), or having a history of moderate physical (n = 35), sexual (n = 46), or emotional abuse (n = 54). Women’s DNA was isolated from buccal cells and used to genotype SLC6A4 5-HTTLPR and BDNF Val66Met. The aggregate genetic score (AGS) was computed by summing the number of “more reactive” alleles exhibited across the two polymorphism (i.e., number of 5-HTTLPR S or L alleles and BDNF Met alleles).

**Results**

Consistent with our hypothesis, we found a significant history of childhood abuse by AGS interaction predicting participants’ recurrent depression (odds ratio = 2.01, β = 0.70, p<0.05). Examining the form of this interaction, we found that women’s AGS was not associated with recurrent depression among individuals who had no lifetime history of abuse (odds ratio = 0.90, β = -0.10, p<0.66). In contrast, there was a significant relation between participants’ AGS and recurrent depression among women who had a history of any childhood abuse (odds ratio = 1.81, β = 0.59, p<0.02). In addition, we found that an interaction between childhood history of physical or sexual abuse and AGS predicted lifetime history of suicide attempts (odds ratio = 2.95, β = 1.08, p<0.04). Closer examination of the interaction revealed that AGS was not associated with suicide attempts among women with no history of childhood abuse (odds ratio = 0.74, β = -0.30, p<0.36). However, consistent with our findings for recurrent MDD, there was a nonsignificant trend for the association between AGS and suicidal behavior among women with the history of physical or sexual abuse (odds ratio = 2.17, β = 0.77, p<0.07).

**Discussion**

The findings provide support for the involvement of a systems-biology defined AGS in recurrent
depression and suicidal behavior among high risk individuals and further validate the use of the combined genetic variance scoring approach.

THE UTILITY OF DNA EXTRACTED FROM SALIVA FOR METHYLATION STUDIES OF PSYCHIATRIC TRAITS
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Background
DNA methylation has become increasingly recognized in the etiology of psychiatric disorders. Because brain tissue is not accessible in living humans, epigenetic studies are most often conducted in blood. Thus, numerous studies have been published associating specific DNA methylation patterns with psychiatric conditions affecting adults. For example, epigenetic correlates of child abuse have been identified in adult samples and animal models, but comparable research is lacking in children because even a blood draw can be considered too invasive. Saliva is readily collectable, but the proportion of epithelial cells and leukocytes varies between individuals and represents a significant barrier to conducting studies in children. The goal of this study is to evaluate whether DNA isolated from saliva is comparable to DNA isolated from blood for outcomes relevant to psychopathology, using child abuse as an example.

Methods
Saliva and blood samples were collected from 64 African American participants in the Grady Trauma Project (Atlanta, GA). Child abuse was assessed using the Childhood Trauma Questionnaire (CTQ) and operationalized as a report of moderate to severe sexual, emotional or physical abuse. DNA methylation was interrogated for each sample using the HumanMethylation450 BeadChip (Illumina). The method described by Houseman and colleagues was used to estimate the proportion of epithelial cells in saliva DNA and the proportion of lymphocytes and neutrophils in blood DNA. We examined the association of each CpG site with child abuse using a linear model that adjusted confounding factors such as age, sex, race, batch effects and cellular heterogeneity. We also used linear models that adjusted for batch effects and cellular heterogeneity to test whether the DNA methylation levels of saliva predicted those of blood. For all analyses, the false discovery rate was controlled at 5%.

Results
Tissue-specific DNA methylation patterns were clearly indicated by hierarchical clustering, which segregated all blood from saliva samples using methylation probes across the genome. The average DNA methylation level at each CpG site initially appeared to be highly correlated between blood and saliva though the variance at each CpG site was high such that DNA methylation from saliva predicted that of blood in only ~10% of CpG sites. While no individual CpG site remained associated with child abuse after adjustment for multiple testing, the test statistics for comparable analyses of saliva and blood were moderately correlated (r=.21). We focused on CpG sites in genes that were reported as differentially methylated in the blood of those with a history of child abuse (CCDC85C, FANK1, FKBp5, FRG1, NR3C1, PTPRN, PTPRN2, SLC6A4, SLC29A4, TMED2 and WNT3A). With the exception of NR3C1 and TMED2, each gene contained CpG sites whose saliva methylation levels were highly predictive of those in
blood \( (p < 1 \times 10^{-7}) \) though certain areas of the gene were more likely to contain such sites.

**Discussion**

This analysis suggests that tissue-specific differences are more prominent than those related to shared genetic or environmental factors. DNA derived from saliva may be informative for research questions that can be assessed in blood, though only a small fraction of CpG sites can be considered correlative across tissues. These results have applications for longitudinal and biomarker studies as well as large-scale DNA methylation studies of childhood psychiatric disorders. Further studies will be necessary to quantify the correlation between saliva and brain methylation patterns.

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**MICRONRNAS ASSOCIATED WITH MAJOR DEPRESSIVE DISORDER AMONGST LARGE MEXICAN AMERICAN FAMILIES**

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**Background**

The debilitating symptoms of major depressive disorder (MDD) are estimated to affect several hundred million people worldwide, but the pathophysiology underlying MDD is poorly understood. Prevailing hypotheses point to disruptions in gene expression networks and cascades involving cellular plasticity. Because single microRNAs (miRNAs) may regulate hundreds of genes within a cell, and collectively miRNAs are predicted to regulate a majority of the human transcriptome, the neurological impairments associated with MDD may involve miRNA dysregulation. This study examines the association of miRNA expression with diagnoses of major depressive disorder.

**Methods**

As part of the Genetics of Brain Structure and Function (GOBSF) study, we report global microRNA expression (n = 1,733) in peripheral blood mononuclear cells (PBMCs) from a population of over 1,000 Mexican Americans individuals from approximately 50 families. The prevalence of MDD is ~33% and recurrent MDD is ~19% in this population. To date, we have analyzed miRNA expression profiles of 688 individuals using next-generation sequencing, with final analysis of >1,000 individuals currently underway. We also analyzed miRNA expression in post-mortem brain tissue of 12 individuals with MDD and 12 control subjects taken from the dorsolateral prefrontal cortex (DLPFC; BA9) and the anterior cingulate cortex (ACC; BA24).

**Results**

For the GOBSF study, 448 miRNAs were detected in at least 75% of samples and 117 of these (26%) showed strong evidence for heritability (met Bonferroni correction, \( P < 1.12 \times 10^{-4} \)); mean heritability was 0.42 (range 0.31–0.62). Expression of several miRNAs show nominal association \( (P < 0.05) \) with MDD, although none reached statistical significance after correction for multiple testing in this subset of the population. The strongest association was observed for \( miR-200b \) \( (P = 2.91 \times 10^{-4}) \). Although primarily implicated in several forms of cancer, \( miR-200b \) is also known...
to directly target CREB1. CREB1 has previously been implicated in depression, as well as antidepressant treatment response and remission. We also identified nominal associations between MDD and miRNAs whose expression has been shown to change in response to mood stabilizers and antidepressants, including let-7b ($P = 9.23 \times 10^{-3}$) and miR-151-3p ($P = 4.15 \times 10^{-2}$). Similarly, among the brain tissue samples none of the associations withstood multiple-testing correction, however we were able to replicate a previously identified association between miR-142-5p and depression within the DLPFC ($P = 2.74 \times 10^{-2}$). Several miRNAs which showed nominal significance for association with MDD in the GOBSF study (using PBMCs) have also shown nominal associations with MDD in previous studies utilizing brain tissue, including miR-142-3p, miR-494 and miR-376*.

Discussion
These results suggest that associations identified in peripheral blood tissue may represent associations that might also be seen in brain tissue, for at least some miRNAs. We anticipate that expansion to our full GOBSF cohort will yield more powerful results and are we are also continuing to expand our studies of post-mortem brain tissue.

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ABSTRACT WITHDRAWN

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A CELL EPIGENOTYPE SPECIFIC MODEL FOR THE CORRECTION OF BRAIN CELLULAR HETEROGENEITY BIAS AND ITS APPLICATION TO AGE, BRAIN REGION, AND MAJOR DEPRESSION
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Background
Many psychiatric diseases exhibit heterogeneity in brain morphology and altered neuronal to glial density ratios. Cellular heterogeneity may bias cell type specific epigenetic patterns leading to a dramatic increase in false positive or negative findings in psychiatric epigenetic studies.

Methods
We performed fluorescence activated cell sorting (FACS) of neuronal nuclei in post mortem frontal cortex of 29 major depression and 29 matched control samples followed by Illumina HM450 microarray based DNA methylation profiling.

Results
We characterized the extent of neuron and glia specific DNA methylation variation independent of disease status and identified significant cell type specific epigenetic variation at 51% of loci. Cell type specific epigenetic differences were over represented in CpG island shores and within gene bodies, 3' untranslated regions, and known enhancer sequences. Using the top cell epigenotype specific (CETS) marks, we generated an R package, ‘CETS’, capable of quantifying neuronal proportions and generating in silico neuronal profiles from DNA methylation data. We demonstrate a significant overlap in major depression DNA methylation associations between
FACS separated and CETS model generated neuronal profiles relative to bulk profiles. CETS derived neuronal proportions correlated significantly with age in the frontal cortex and cerebellum and accounted for epigenetic variation between of brain regions.

Discussion
The data presented demonstrate the utility of the CETS approach for reducing cell heterogeneity induced bias and for the identification of cortical neuron or glia specific epigenetic associations in brain related phenotypes within existing and future DNA methylation datasets. These analysis techniques will enable more robust hypothesis testing in the brain and have the potential to lead to novel epigenetic discoveries in psychiatric disease.

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COMPARATIVE APPROACH TO OCD GENETICS: CASE-CONTROL CAPTURE SEQUENCING OF DOGS AND HUMANS
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Background
Obsessive-compulsive disorder (OCD), a debilitating mental disorder manifested in time-consuming repetition of behaviors, affects 1-3% of the human population. Despite over 80 candidate gene studies and a recent genome wide association (GWA) study, no genes have been confidently associated with OCD thus far. Interestingly, compulsive disorder spontaneously occurs in dogs and strikingly resembles human OCD. As in humans, normal canine behaviors such as grooming, predation and suckling manifest in extreme repetitions i.e. acral lick dermatitis, tail chasing/fly snapping, and flank/blanket sucking, respectively. In addition, selective serotonin reuptake inhibitors (SSRIs) are effective in approximately half of affected individuals in both organisms. While the complex genetics of OCD makes it difficult to study its etiology, limited genetic diversity of the modern dog breeds resulted from strong artificial selection makes the domestic dog particularly amenable to genetic studies for complex disorders like OCD. Here, we aim to identify rare variants and genes for OCD in humans and dogs using high-throughput sequencing technology and subsequently to reveal the shared etiology between dog and human OCD.

Methods
We sequenced 8 cases and 8 breed-matched controls from four OCD enriched breeds including Doberman pinscher, German shepherd, Jack Russell terrier, and Shetland sheepdog. We selectively sequenced 25 genomic regions showing strong association signals from our GWA analysis of 83 cases and 64 control Doberman pinschers or homozygosity across OCD enriched breeds. For humans, we sequenced ~600 DSM-IV OCD cases and ~500 ancestry-matched
controls. We selectively sequenced ~600 genes (+ regulatory elements) that are involved in the cortico-striatal-thalamo-cortical neurocircuit, which may play an important role in OCD and human orthologues of canine genes within dog OCD sequencing regions.

**Results**
Targeted sequencing for dog OCD revealed >100 case-only variants within evolutionarily conserved genomic regions that are indicative of functional genomic regions. Genotyping these case-only variants in an independent set of dogs revealed that they are significantly more frequently present among OCD enriched breeds than in control breeds without known psychiatric problems (p=0.045, the Wilcoxon test), consistent with the genetic architecture of dog OCD expected from dog breed structure. In addition, gene-based analysis recurrently identified four synaptic genes to be enriched with case-only variants. Finally, luciferase enhancer assay revealed that the top candidate mutations that reside between two cadherin genes lead to significant changes in gene expression compared to the wild type. Furthermore, electrophoretic mobility shift assay showed depletion of DNA-protein binding by a nearby second candidate mutation.

**Discussion**
The significant change in gene expression level and depletion of protein-DNA binding by our candidate mutations strongly suggest that the mutations affect gene regulation by disrupting a transcription factor DNA binding site. Indeed, we identified several transcription factor binding site motifs, in which our candidate mutations were predicted to change the transcription factors’ affinity to the site. While the mutations are located between two cadherin genes, it remains to be elucidated which gene is regulated by these mutations. Importantly, the four top genes that are enriched with case-only variants have been suggested in the literature to function in concert to regulate synapse formation and adhesion, providing an insight into the neurobiology of OCD. In conclusion, our approach to selectively sequence regions driven by canine GWA study allowed an identification of several candidate genes and functional variants for OCD in spontaneous dog model. Importantly, comparison of the results from canine study with the human OCD sequencing data set has the potential to reveal the shared etiology between dog and human OCD.

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**ALLELE-SPECIFIC REGULATION OF DISC1 EXPRESSION BY MIR-135B-5P**
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**Background**
The *Disrupted-in-schizophrenia-1 (DISC1)* gene has been established as a risk factor for various neuropsychiatric phenotypes. Both coding and regulatory mutations in *DISC1* have been identified and associated to these phenotypes in genetic studies. MicroRNAs (miRNAs) are important regulators of protein coding genes. The miRNA-mRNA target recognition mechanism is vulnerable to disruption by DNA polymorphisms. We therefore investigated whether polymorphisms in the *DISC1* 3’UTR affect binding of miRNAs and lead to allele-specific regulation of *DISC1*.

**Methods**
We used bioinformatic miRNA target prediction algorithms to identify putative polymorphic
miRNA binding sites in the 3′UTR region of DISC1. We tested the effect of nine miRNAs on endogenous DISC1 expression in vitro by over-expressing these miRNAs in 293FT cells and measuring the DISC1 expression level by qPCR. To determine whether two of these miRNAs regulate DISC1 levels by targeting the predicted sites, we used a reporter gene assay, which uses luciferase activity on a protein level as the output measure. We cloned either the full length DISC1 Lv isoform 3′ UTR or the miRNA binding sites with flanking sequences into a luciferase expression vector. These constructs were co-transfected individually into 293FT cells with either the miR-135b-5p or the miR-559 precursor. We also investigated the putative allele-specificity of miR-135b-5p binding. The 293FT cell line is homozygous for the derived allele (A) of rs11122396, predicted to create a novel binding site for miR-135b-5p. We created constructs with the DISC1 (G) allele at rs11122396, to determine whether the repression of DISC1 expression by miR-135b-5p is allele-specific.

Results
Two of the nine miRNAs, miR-135b-5p and miR-559, significantly reduced endogenous DISC1 mRNA expression: miR-559 by 23.7 % (p=0.009) and miR-135b-5p by 16.2 % (p=0.039), compared to the negative control miRNA. Using the luciferase assays, we found that expression from the DISC1 full length 3′ UTR construct was reduced 32.1% (p=0.003) by miR-135b-5p, and by 10.3 %, (p=0.03) from the construct expressing the 60 nt miRNA binding site and flanking sequences. In contrast, miR-599 over-expression did not affect the expression from the DISC1 full length 3′ UTR construct or the construct with its 60 nt binding site. We next investigated the putative allele-specificity of miR-135b-5p binding. Strikingly, miR-135b-5p had no effect on the luciferase activity of either the full length 3′ UTR with G allele at rs11122396 (p=0.49), or on the construct with the ~60 nt miRNA binding site with flanking sequences insert (p=0.18), indicating that miR-135b-5p binding is specific to the derived (A) allele at rs11122396 of DISC1.

Discussion
We identified four predicted polymorphic miRNA target sites in the DISC1 3′ UTR, and demonstrated that miR-135b-5p regulates the level of DISC1 mRNA. Moreover, DISC1 regulation by miR-135b-5p is allele-specific: miR-135b-5p only binding to the major allele (A) of rs11122396, not to the minor allele (G). Thus, the G allele may be functionally related to the DISC1-associated phenotypes by abolishing regulation by miR-135b-5p, leading to elevated DISC1 levels.

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PERVASIVE ALLELIC IMBALANCE REVEALED BY ALLELE-SPECIFIC GENE
EXPRESSION IN HIGHLY DIVERGENT MOUSE CROSSES
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Background
The genetic basis of most phenotypic variation can ultimately be assigned to variation in protein sequence, RNA sequence, or regulatory sequence. The significance of the latter has become increasingly apparent in recent genomic studies comparing divergent taxa and populations and by the results of human GWAS where thousands of SNPs not predicted to change protein
structure are strongly associated with human complex diseases and biomedical traits.

**Methods**

Here we provide a detailed portrait of cis regulatory variation in mice using RNA sequencing and gene expression microarrays of multiple tissues in a full diallel of three wild-derived inbred strains (CAST/EiJ, PWK/PhJ and WSB/EiJ) representing three different subspecies (*M.m. castaneus, M.m. musculus* and *M.m. domesticus*, respectively). We generated offspring from all possible pairwise crosses to form a 3x3 diallel, including age- and sex-matched biological replicates for each of the nine possible genotypic combinations (N = 9-12 offspring per combination). Mice were aged to 23 days, sacrificed, and total RNA extracted from whole brain, liver, kidney, and lung. Brain RNA was examined by RNAseq and gene expression microarrays, while liver, kidney and lung RNA was examined by microarrays.

**Results**

Tissue was the greatest predictor of gene expression, followed by strain, parent-of-origin and sex. In brain, we observed cis regulatory effects in 11,686 genes (85% of 13,251 testable genes), exceeding all mouse expression quantitative trait loci (eQTL) studies to date, and most fit an additive pattern of inheritance. Differential expression was positively correlated with sequence diversity at multiple evolutionary scales and we estimate that at least 1 in every 1,000 SNPs create a cis eQTL, similar to the fraction of SNPs predicted to alter protein function. Regarding parent of origin effects, the number of imprinted genes in brain (N = 98, including 56 novel) is not different from historical estimates and, not only is imprinting incomplete for most genes (44% silencing on average), but cis acting mutations modified the strength of imprint for 47 genes. Regarding the X chromosome, regulation of gene expression was similar to the autosomes and we confirmed two forms of dosage compensation equalizing expression between males and females and between X and autosomes.

**Discussion**

The results of this study imply that, as with humans, complex genetic traits in mice are heavily influenced by pervasive regulatory variation. The data described here may accelerate the discovery of the precise genes that underlie mouse complex trait QTL and, to this end, we provide a web-accessible catalog of gene expression in mice ([http://csbio.unc.edu/gecco](http://csbio.unc.edu/gecco)). Informative expression patterns for genes of particular relevance to the psychiatric genetics community will be discussed.

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**INVESTIGATION OF ENCODE TRANSCRIPTIONAL REGULATION ELEMENTS IN THE CHROMOSOME 13 SLITRK GENE CLUSTER IN OBSESSIVE-COMPULSIVE-SPECTRUM DISORDERS**

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**Background**

Obsessive-Compulsive spectrum disorders, including obsessive-compulsive disorder (OCD) and
Tourette Syndrome (TS), are genetic disorders characterized by uncontrollable, repetitive, and ritualized behaviors that one feels compelled to perform. Little is known about the neurobiology of OCD and TS, although abnormal cortico-striato-thalamic circuit function has been implicated in both disorders. Current treatments, such as selective serotonin re-uptake inhibitors (SSRIs) or neuroleptics drugs, are only effective after chronic treatment in a subset of patients, highlighting the need for reliable model systems to study disease biology and to identify novel therapeutic targets to improve treatment options. Genetic studies in human and animal models have implicated three of the six members of the SLITRK gene family (SLITRK1 – 6) that are specifically expressed in brain and thought to control neurite outgrowth and inhibitory and excitatory synapse development. Rare mutations in SLITRK1 have been associated with TS and a related compulsive disorder, trichotillomania. A genome-wide association study of TS by Scharf et al. (Molecular Psychiatry 18:721-8, 2013) detected nominal association with a SNP located within the intergenic region between SLITRK1 and SLITRK6. Furthermore, a knockout mouse model in which the Slitrk5 gene is disrupted was reported to exhibit compulsive behaviors and abnormal glutamatergic circuit function. Interestingly, SLITRK1, 5 and 6 are located adjacent to each other on chromosome 13q31. We hypothesize that the GWAS variant located between SLITRK1 and 6 plays a role in transcriptional regulation of the SLITRK genes on chromosome 13.

Methods
To address the hypothesis, we first conducted an in silico investigation of the 13q31 SLITRK gene cluster using data from the Encyclopedia of DNA Elements (ENCODE) project. Construct sequences with or without the risk alleles from TS patients were cloned into a luciferase reporter vector to evaluate allele-specific transcriptional regulation in human neural progenitor cells reprogrammed from human iPSCs (induced-pluripotent stem cells). Further experiments will be performed to determine whether glutamatergic modulation could differentially affect transcription regulation, i.e., measure p300 coactivator activity in neural progenitor luciferase reporter cells carrying risk allele versus non-risk allele sequences.

Results
Using the ENCODE database, the TS GWAS sequence variant between SLITRK1 and SLITRK6, rs7336083, was found to be in perfect linkage disequilibrium with a putative regulatory enhancer element, p300, containing a SNP that may potentially regulate gene transcription specifically in neuronal cells. Transcription co-activator, p300, belongs to the p300-CBP co-activator family which interacts with transcription factors and in turn increases the expression of its target genes. Construct sequences flanking the p300-binding site with or without the risk alleles from TS patients were cloned into a luciferase reporter vector may exhibit allele-specific transcriptional regulation in human neural progenitor cells reprogrammed from human iPSCs.

Discussion
From the in silico investigation of the 13q31 SLITRK gene cluster, a p300 enhancer element was found in the SLITRK1 and SLITRK6 GWAS locus containing potential functional sequence variant that may modulate gene transcription. Luciferase reporter assay in neural progenitor cells was designed to study whether the risk allele from TS patients would differentially regulate gene transcription. The strategy described here may serve as a model to evaluate the potential
biological function of putative genetic variants found in GWAS of obsessive-compulsive spectrum and other psychiatric disorders.

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FUNCTIONAL ANALYSIS OF L-TYPE CALCIUM 2+ CHANNEL GENE CACNA1C IN MOOD DISORDER PATHOPHYSIOLOGY

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Background
The CACNA1C gene encodes the alpha-pore forming subunit of the Ca\(^{2+}\) channel CaV 1.2 (L-type voltage gated) and is located on the short arm of human chromosome 12. Results from multiple genome wide association studies (GWAS) indicate significant association between single nucleotide polymorphisms in CACNA1C and mood disorder as well as schizophrenia diagnosis. All such SNPs are located in a single intron 3 and therefore do not affect amino acid sequence of the protein. The biological implications of these genetic changes and their role in altering disease susceptibility remain to be adequately understood. This study attempts to analyze functionality of the GWAS indicated SNPs. We hypothesized that GWAS SNPs or the genomic regions in linkage disequilibrium (LD) with them harbor regulatory elements that affect CACNA1C expression.

Methods
We bioinformatically identified regions conserved across species within Intron 3 of CACNA1C. One highly conserved region is in close proximity to a GWAS significant SNP. This region includes putative binding sites for transcription factors p300 and Lhx3 among others. This ~350bp region also contains common SNPs that are in high LD with rs476513. We cloned the region containing major and minor alleles in LD with the GWAS SNP into a reporter expression vector system (pGL4, Promega) and assessed the reporter (luciferase) activation in the presence of p300 and Lhx3 in vitro in HEK293 cells.

Results
We observed a significant increase in reporter activation by the cloned region, which was allele specific. The haplotype consisting of the minor allele showed a small but significantly higher expression. While the presence of Lhx3 resulted in a robust increase in reporter activation by the haplotypes in an allele specific manner where the haplotype containing the minor allele recorded significantly higher activity, p300 did not show such activation.

Discussion
We hypothesized that the intron located genetic changes in CACNA1C reported to be significantly associated with mood disorders and schizophrenia act through regulatory changes that are allele specific. To test this we chose to focus on genomic regions that are conserved across species since such conservation likely indicates functionality. Our preliminary investigation of such a genomic region in high LD with a GWAS-identified SNP showed that the putative regulatory region affects reporter activation in an allele specific manner. While this is not an in vivo proof, the data provides basis for large-scale study of intron 3 of the CACNA1C where all the GWAS signals reside. Our laboratory is currently pursuing this line of research
whereby we expect to understand molecular mechanisms underlying pathophysiology of psychiatric disorders as modulated by CACNA1C genotype.

COPY NUMBER VARIATION IN OBSESSIVE-COMPULSIVE DISORDER AND TOURETTE SYNDROME: A CROSS-DISORDER ANALYSIS
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Background
Obsessive-compulsive disorder (OCD) and Tourette syndrome (TS) are heritable neuropsychiatric disorders with evidence supporting a partially shared genetic etiology. Patterns of TS and OCD in large family studies also suggest an important role for within-family pleiotropy in the manifestation of the disorders. More generally, genetic pleiotropy has been a prominent feature in the copy number variation (CNV) literature of neurodevelopmental disorders, where several CNV regions have been identified as risk factors for multiple disorders, such as autism, schizophrenia, and intellectual disability. Given these findings, this study addressed the important, unanswered question of whether large, rare CNVs are also relevant in the genetic architecture of TS and OCD, with a specific focus on pleiotropic effects of previously identified neurodevelopmental CNV regions. This study is the first genome-wide investigation of large (>500kb), rare (<1%) CNVs in OCD and the largest analysis in TS, to date.

Methods
Given evidence for shared genetic contributions to TS and OCD, a cross-disorder design was utilized to maximize power to detect rare events. The sample consisted of 2699 cases (1086 TS, 1613 OCD) and 1789 controls, including a subset of 348 OCD cases recruited as parent-proband trios to allow a de novo CNV analysis. CNVs were called with two algorithms (PennCNV and iPATTERN) to ensure reliability of the calls.

Results
There was no evidence for an overall increased burden of large, rare CNVs in the cross-disorder analysis or in secondary, disorder-specific analyses. However, there was a trend (p=.06) indicating a greater than 3-fold increased burden for deletions in regions previously associated with other neurodevelopmental disorders. Further examination of these deletions revealed that the 16p13.11 locus contributed disproportionately to this neurodevelopmental burden (5 case deletions: 0 control deletions, p=0.09 in current study, p=0.025 compared to published control rates). Furthermore, three of these 16p13.11 deletions were confirmed as de novo, supporting the potential etiological significance of this region.

Discussion
These results extend the phenotypic boundaries associated with previously identified neurodevelopmental deletions to include OCD- and TS-spectrum disorders. This study specifically highlighted deletions at 16p13.11 which have been previously associated with intellectual disability and autism. In this OCD/TS sample, none of the cases had autism or intellectual disability suggesting that these comorbidities cannot account for the
association. Four of the five cases with a 16p13.11 deletion had OCD (3 OCD only, 1 OCD + motor tics) whereas one case had TS only. This pattern suggests a possible pleiotropic effect of the 16p13.11 locus with alternate manifestations of TS and OCD as part of the spectrum of affectedness. Further study of large, rare CNVs in OCD and TS will be important for understanding this segment of the genetic architecture and for expanding the range of phenotypic outcomes associated with CNVs implicated across neurodevelopmental disorders.

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EVIDENCE OF ASSOCIATION OF SAT-1 GEN AND SUICIDAL BEHAVIOR IN MEXICAN POPULATION

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Background

Is knowledge the contribution genetic in suicide behavior. The possible contributions of specific genes and their different allelic variants are still in the process of ongoing study. Evidence to support the involvement dysregulation of the polyamine system in the pathogenesis of suicidal behavior has been showed. In this work, we determined the association of rs6526342 of SAT-1 in a sample of suicide attempter patients in Mexican Population.

Methods

A total of 169 patients were consecutively recruited from the outpatient service of the General Hospital of Comalcalco in the state of Tabasco, Mexico. In addition, 218 unrelated controls were recruited for this study. Each patient was given a diagnostic assessment based on the Structured Clinical Interview for DSM-IV Axis and II diagnoses in Spanish. Hardy-Weinberg equilibrium was tested using Pearson´s goodness-of-fit chi-squared test. Chi-squaredtest or Fisher´s Exact test was used to compare genotype and allele frequencies between groups.

Results

Genotype frequencies of the rs6526342 not were in Hardy-Weinberg equilibrium in all female group (p<0.05). From the 169 suicide attempters, we analyzed first the male group. Not significant differences was observed in the distribution of genotypes (χ²= 2.85, df=2, p=0.23) of alleles (q²= 0.01, df=1, p=0.91). However, when we analyzed the male group the allele A was significantly more frequent in male controls than in male cases (χ²= 4.0, df=1, p=0.04, OR 0.48; 95% CI: 0.24-0.98).
Discussion
To our knowledge, this is the first study addressing the genetic association between SAT-1 A(-1537)C in a Mexican population. A possible protection effect was observed in the allele A in male (OR 0.48 (95% CI: 0.24-0.98). In female we not find any association. Our results are in agreement with a report in the literature stating What the allele A of SAT-1 could be a factor of protection to suicidal behavior.

GENETIC FACTORS CONTRIBUTING TO LOW BODY WEIGHT IN ANOREXIA NERVOSA

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Background
Anorexia nervosa (AN) is a serious eating disorder with substantial morbidity and a lifetime of mortality as high as that associated with any psychiatric illness. Low weight or body mass index (BMI) is the *sine qua non* of AN and the primary target of initial treatment. Low weight and behaviours associated with reaching it are also the primary reason for the high morbidity and mortality in this illness. The purpose of this study is to determine the role of candidate genes selected from the leptin, melanocortin and neurotrophin systems in sustained low body weight in AN.

Methods
Only AN probands with no history of bulimia nervosa (BN) and BN probands with no history of AN are included in this analysis, reducing phenotypic heterogeneity within groups and increasing diagnostic differences between groups. The sample consisted of 745 AN probands with no history of BN, 245 BN probands with no history of AN, and 321 female nonpsychiatric controls.

Results
Our results suggest that an *MC4R* genetic variant previously linked to antipsychotic medication-induced weight gain may be underrepresented in AN probands compared to controls. Furthermore, *AGRP* gene was associated with lowest lifetime BMI in AN, and a *NTRK2* risk variant was linked to highest lifetime BMI in BN.

Discussion
To our knowledge, this is the first study to address the important issue of high crossover rates in eating disorders being a possible confounds in genetic studies. It is also the first study to explore the role of various markers with known or putative function in genetic systems known to regulate appetite and weight in AN and BN. These genetic findings associated with low body weight may serve as an important first step toward gaining a better understanding of weight regulation in AN, BN, and healthy populations, including the possible identification of genetic protective factors. These findings have the potential for developing more effective treatment options and more specifically for providing a highly specific target for the development of novel medications.
GENETIC VARIANTS ASSOCIATED WITH DISORDERED EATING
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Background
Although the genetic contribution to the development of anorexia nervosa (AN) has long been recognized, there has been little progress relative to other psychiatric disorders in identifying specific susceptibility genes. Here, we have carried out a genome-wide association study on an unselected community sample of female twins surveyed for eating disorders.

Methods
We conducted genome-wide association analyses in 2,564 female twins for four different phenotypes derived from self-report data relating to lifetime presence of 15 types of disordered eating: AN spectrum, bulimia nervosa (BN) spectrum, purging via substances, and a binary measure of no disordered eating behaviors versus three or more. To complement the variant level results, we also conducted gene-based association tests using VEGAS software.

Results
Although no variants reached genome-wide significance at the level of p \(10^{-28}\), six regions were suggestive (p \(5\times10^{-7}\)). The current results implicate the following genes: CLEC5A, LOC136242, TSHZ1, and SYTL5 for the AN spectrum phenotype; NT5C1B for the BN spectrum phenotype; and ATP8A2 for the disordered eating behaviors phenotype.

Discussion
As with other medical and psychiatric phenotypes, much larger samples and meta-analyses will ultimately be needed to identify genes and pathways contributing to predisposition to eating disorders.
Methods
To determine whether those who experienced prenatal or infant exposure to the massive 1959-1961 famine in China had increased risk of mental retardation in later life. We examined the risk of mental retardation in three clinic research centers located in three different Chinese provinces that were severely affected by the Great Chinese Famine of 1959-1961. All medical records for the years 1970 through 2005 were examined, and clinical and socio-demographic information on patients with mental retardation was extracted by well-trained medical practitioners who were blinded to the nature of exposure. Data on number of births and deaths in the famine years were available, and cumulative mortality was estimated from later demographic surveys. Evidence of famine was verified; unadjusted and mortality-adjusted cumulative incidence and mortality-adjusted relative risks of mental retardation were calculated. Potential confounding effects of family history, gender, and age of onset were estimated.

Results
For each of the three research regions, the decline in birth rates during the famine years was accompanied with a corresponding increase in death rates. Among births that occurred during the famine years 1960 and 1961, the adjusted risks of developing mental retardation in later life increased significantly for all of the three cohorts, whereas, we did not observe increased risk of mental retardation for the infant exposed years 1959 through 1958 for the Wuhu and Liuzhou cohorts. The combined analytic results only showed that the mortality-adjusted relative risks were significantly higher for the exposed years of 1960 (RR:2.53; 95% CI: 1.57-4.07; p-value: 1.26×10⁻⁴) and 1961(RR: 3.06; 95% CI: 1.73-5.44; p-value: 1.32×10⁻⁴) compared with the unexposed years of 1963-1965. No associations of this effect were detected with respect to family history, gender, and age of onset.

Discussion
We observe a 3-fold increased risk of mental retardation among those prenatally exposed to the Chinese famine in the combined analysis. This is not biased by family history, gender, age of onset, and Chinese minorities. Further studies are warranted to clarify the joint effects of specific micronutrients and related genetic pathways in pregnancy. Additional large-scale famine cohort studies are also needed to verify the infant origin.

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CONDITIONING PLACEBO ANALGESIA AND REWARD PROCESSING: INSIGHTS FROM INTRINSIC BRAIN ACTIVITY, GENE AND PERSONALITY
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Background
Our expectations about an event can strongly shape our subjective evaluation and actual experience of events. This ability as applied to the modulation of pain has the potential to affect therapeutic analgesia substantially and constitutes a foundation for non-pharmacological pain relief. Studies indicate that positive expectancy may be regarded as a reward, and brain activity in the reward system is involved in this modulation process.

Methods
In the present study, we combined resting state functional magnetic resonance imaging (rs-fMRI) measures, COMT Val158Met (rs4680) genotype, and personality measures in a model to predict the magnitude of conditioned cues to modulate subjective pain reports.

Results
We found that the regional homogeneity (ReHo) in the ventral striatum, an index of local neural coherence, was significantly associated with conditioning effects on pain ratings. We also found that the number of Met alleles at rs4680 was linearly associated with placebo response. In a regression model, the ReHo, COMT polymorphism, and Openness scores were important predictor of conditioning effect and accounted for 59% of the variance in conditioning responses.

Discussion
Our findings demonstrate the potential of combining resting state connectivity, genetic information and personality to predict conditioning placebo related and cognitive behavior.

ASSOCIATION OF TWO ERBB3 SNP GENOTYPES WITH SENSITIVITY TO CAREGIVING CONTEXT
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Background
Genetic differential susceptibility may predict response to early environments. Previous studies have demonstrated that smaller total cortical white matter volume (1, 2), corpus callosum (2, 3) and functional changes (4, 5) are associated with early severe psychosocial deprivation typical of institutional care, and associated with psychopathology (6, 7). ERBB3 is a critical component of the mechanism underlying deficits in cortical myelination following early social deprivation in rodents. (8). Based on these preclinical findings, we examined whether two polymorphic variants (SNPs) in ERBB3 would interact with early caregiving environment to predict white matter changes in children enrolled in the Bucharest Early Intervention Project (BEIP). BEIP is the only longitudinal randomized controlled trial of foster care compared to continued institutional care.

Methods
One hundred and thirty six children living in institutions in Bucharest, Romania (mean age 22 months), were randomized to continued institutional care (CAUG) or a foster care intervention (FCG). Structural MRI scans were obtained between ages 8 and 11 (mean age 9.74 years). Genotype was determined for two ERBB3 polymorphisms (rs2271189 and rs2292238). Analysis was performed on 41 children (CAUG, N=22; FCG, N=19) for whom genotype and MRI data was available. Regression analysis, controlling for gender, ethnicity, birth weight, and intracranial volume, examined the interaction between caregiving group and genotype in predicting total white matter and corpus callosum volume.

Results
Genotype was not associated with total white matter or corpus callosum volume. Consistent with previously reported data (2), caregiving group was associated with total white matter (F(1,34)=3.33, p=0.07) and corpus callosum volume (F(1,34)=3.97, p=0.05). No interaction
between genotype and caregiving group was found for total white matter volume with either SNP. For corpus callosum, a significant interaction was found between both caregiving group and rs2271189 (F(1,32)=15.91, p<0.001) and caregiving group and rs2292238 (F(1,32)=11.83, p<0.002).

**Discussion**

For corpus callosum, children with genetically defined high susceptibility had the most negative outcomes (i.e. smallest volumes) in CAUG children, but positive outcomes (i.e. largest volumes) in FCG children, suggesting an intervention effect. The differential effect of genotype on corpus callosum vs. total white matter supports previous evidence that the role of ERBB3 in the cortical encoding of early social experience is dependent on developmental period (8). These results support a model of differential susceptibility for ERBB3 and institutional care, and highlight the relevance of critical periods for the imprinting of caregiving context to distinct brain regions.

**References:**


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**ASSOCIATION BETWEEN RS10520400 AND CAUDATE NUCLEUS ASYMMETRY IN ALZHEIMER'S DISEASE AND MILD COGNITIVE IMPAIRMENT**

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Background
In Alzheimer’s disease (AD) and mild cognitive impairment (MCI), atrophy of the caudate nucleus appears in MR brain scans as reduction in volume and loss of normal asymmetry in patients compared to healthy controls.

Methods
In the present study, we extracted caudate nuclei volumes of 731 individuals from the Alzheimer’s disease Neuroimaging Initiative (ADNI) cohort. Using caudate asymmetry as a disease-associated quantitative trait, we asked whether genetic factors contribute to such inter-hemispheric differences in rates of neurodegeneration. We conducted a genome-wide association analysis of caudate asymmetry across >500,000 directly genotyped single nucleotide polymorphisms (SNPs) in two groups of patients diagnosed with either MCI (N=354) or AD (N=172), and a group of healthy elderly controls (N=204).

Results
SNP rs10520400, located in chromosome 4q34.3, was significantly associated in both patient cohorts (P=6.26 x 10^{-05} in MCI, and P= 1.77 x 10^{-05} in AD; combined P= 4.51 x 10^{-09}), but not in healthy elderly controls (P=0.13).

Discussion
Interestingly, rs10520400 had previously been reported as a cis expression QTL (eQTL) for the AGA gene, which participates in glycoprotein metabolism. Deficiencies in the AGA enzyme lead to aspartylglucosaminuria, a Mendelian condition that causes progressive decline in mental functioning. Moreover, AD-related neurodegeneration that leads to loss of normal caudate asymmetry may explain why SNP rs10520400 is specifically associated with AD and MCI patients, but not normal aging.

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GENETIC VARIATION UNDERLYING AMYGDALA-VOLUME IS HIGHLY ENRICHED WITH SCHIZOPHRENIA SUSCEPTIBILITY VARIANTS IN HEALTHY YOUNG INDIVIDUALS
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Background
Variations in amygdala volume have been implicated in the pathophysiology of schizophrenia, bipolar disorder, and autism [1-6], but the genetic basis of these volumetric differences remains undefined. Here we report genome-wide association analysis of amygdala volume in a cohort of 1,426 healthy individuals and the genetic relationship between this brain structure and major neuropsychiatric disorders.

Methods
Our study is based on the Harvard/MGH Brain Genomics Superstruct Project (GSP), a neuroimaging and genetics study of brain and behavioral phenotypes comprising more than 3,500 healthy subjects. Structural MRI images of amygdala volume were assessed for all GSP subjects using a standardized imaging protocol (3T Tim Trio scanners, 12-channel head coil, T1-
weighted magnetization). First phase genotyping was performed on 470 subjects of European ancestry using an Illumina OMNI 1M chip, yielding the discovery GWAS data of 1,140,419 SNPs. Second phase genotyping was done for additional 984 European subjects using an Illumina HumanOmniExpress array for conducting a whole-genome mega analysis. All study subjects were young adults with no history of psychiatric illnesses or major health problems (18 ≤ age ≤ 35). Imputation using the 1000 Genomes data produced the allele dosages of 8,839,342 SNPs (R²>0.3; MAF>0.01), for which single-variant association was assessed using linear-regression. Covariates included age, gender, handedness, intracranial volume, scanner, console, and multidimensional scaling factors to control for potential population sub-stratification. Genetic relationships of amygdala-volume-associated SNPs to major neuropsychiatric disorders were examined using multivariate enrichment analyses of genomic inflation factor, λ [7], as previously described by Stein et al. [8].

Results
Single-SNP-based linear-regression identified four genomic regions that show significant association with amygdala volume at P < 1e-06. While the top association locus on chromosome 3q24 (highest P-value of 4.814e-08; β=-81.33mm³;SE=14.90mm³) resides on a non-genic region, nearby brain-expressed genes of potential interest include PLOD2 (procollagen-lysine, 2-oxoglutarate 5-dioxygenase 2; ~815Kb downstream) and PLSCR4 (phospholipid scramblase 4; ~938Kb downstream). Furthermore, enrichment analyses revealed inflated association of schizophrenia susceptibility variants [9] with amygdala volume differences (λ=3.931; P<5e-05).

Discussion
Using a neuroimaging GWAS analysis of healthy young adults, we identified genetic variants influencing individual differences in amygdala volume. We also found a potential role for schizophrenia susceptibility variants in modulating normal variation of amygdala volume, suggesting an etiologic link between amygdala structural changes and emotional/cognitive abnormalities present in this serious brain disorder.

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GENETIC VARIANT IN THE PROLINE DEHYDROGENASE GENE IS ASSOCIATED WITH DEMYELINATION OF BRAIN WHITE MATTER AND SEVERITY OF CLINICAL SYMPTOMS IN SCHIZOPHRENIA PATIENTS

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Background
A single nucleotide polymorphism (SNP), rs4819756, located in the chromosomal region of 22q11 and in the proline dehydrogenase (PRODH) gene, has been observed to cause changes in activity of the protein. PRODH has been identified as schizophrenia susceptibility gene through association studies in patients diagnosed with schizophrenia and in the 22q11 deletion syndrome. Previously published structural and functional magnetic resonance imaging (MRI, fMRI) studies suggest decreased frontal white matter and reduced fronto-temporal connectivity in carriers of the schizophrenia-risk variant GG. However, the impact of rs4819756 polymorphism on specific tracts of the brain white matter has not been investigated yet. Here, we compared GG, AG and AA carriers of the rs4819756 SNP for changes in the inferior longitudinal fasciculus (ILF). The
ILF is an association fiber tract connecting temporal and occipital lobes. We used MR-Diffusion Tensor Imaging (MR-DTI) to analyze white matter microstructure of this tract and we applied a medical scale to assess the severity of the disease symptoms in schizophrenia patients.

**Methods**

MR-DTI images were acquired on 30 patients with chronic schizophrenia and 26 matched healthy controls and tractography of ILF was performed. Changes in MR-DTI measures, such as Fractional Anisotropy (FA), trace, Axial Diffusivity (AD) and Radial Diffusivity (RD), were analyzed as they might reveal microstructural changes of axons, such as changes in myelination. DNA was extracted from saliva and genotyped using the Sequenome iPlex platform. The association of the rs4819756 polymorphism and the disease symptom severity was tested using the Positive and Negative Syndrome Scale (PANSS) in patients with schizophrenia.

**Results**

Schizophrenia patients with the GG genotype (N=13) at rs4819756 exhibited significantly higher values in RD (p=0.018) and lower values in FA (p=0.047) in the ILF in the right hemisphere. Increases in RD, while no changes in AD and decreases in FA have been reported previously in animal studies as consequence of demyelination in brain white matter. Schizophrenia patients with AG/AA and the healthy control groups with either genotype did not differ in the DTI values. RD significantly and positively correlated with the total scores on PANSS (p=0.043), the global score on delusions (p=0.001), delusion of control (p=0.008), delusional jealousy (p=0.001), delusion of thought insertion (p=0.003) and delusion of mind being read (p=0.009) in the group of schizophrenia patients with the AG/AA genotype at rs4819756.

**Discussion**

Our results suggest that the GG genotype at rs4819756 of the PRODH gene is associated with demyelination of the white matter tract ILF in schizophrenia patients, and that demyelination of the white matter is associated with higher severity of the schizophrenia symptoms and delusions in patients with the AG/AA genotype.

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**CHL1: A NEW CANDIDATE GENE IN ANTIDEPRESSANT RESPONSE**

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Background
CHL1 - a gene coding for a neuronal cell adhesion protein – has been recently proposed as SSRI response DNA biomarker by using drug-effect phenotypes in human lymphoblastoid cell lines (LCLs). The aim of the present study was to investigate the effect of genetic variations within the CHL1 gene on antidepressant outcome in major depression.

Methods
6 SNPs (rs4003413, rs2133402, rs9841789, rs1516340, rs2272522, and rs1516338) in CHL1 were genotyped in two independent samples (n=368: 284 with unipolar depression and 84 with bipolar depression; and n=96: all with unipolar depression) of Caucasian major depressive patients treated with antidepressants in a naturalistic setting. Logistic regression was used to investigate associations with response/remission at week 4 in both samples and treatment resistant depression (TRD) only in the largest sample. TRD was defined as non response to at least 2 adequate consecutive antidepressant treatments during the last episode (TRDW) or non response to at least 2 adequate consecutive antidepressant drugs of different classes administered during the last episode (TRDC). Secondly, Reactome database (www.reactome.org/) was used to identify proteins that have interaction with CHL1, and a pathway analysis was performed in the Sequenced Treatment Alternatives to Relieve Depression (STAR*D) genome-wide dataset (n=1861). Genes belonging to the index pathway were imputed through IMPUTE2 taking CEU HapMap 1000 genomes as reference panel. The prevalence of variations showing p<0.05 was compared between the index pathway and a random pathway by a Fisher exact test. 5e4 permutations were run.

Results
In the largest original sample rs2133402 T allele was associated with non response (p=0.019; OR=0.55, 95% CI=0.33-0.90) and non remission (p=0.010; OR=0.48, 95% CI=0.27-0.85), while rs4003413 C allele was associated with remission only in unipolar depressed patients (p=0.012; OR=2.16, 95% CI=1.18-3.94). No marker predicted the risk of TRDW and TRDC. On the other hand, negative findings were retrieved in the smallest original sample. In the STAR*D the top CHL1 marker was about 4000 bp from rs2133402. The index pathway showed association with response and especially NRP1, ITGB1 and HSPA8 genes were responsible of the result. ITGA1 is involved in cell adhesion and migration and NRP1 is critical for the formation of neuronal circuits. HSPA8 mRNA level changes in rat frontal cortex after antidepressant treatment were previously reported.

Discussion
CHL1 and its pathway may be promising candidates for involvement in antidepressant response. Further studies should deepen their role under the perspective of the antidepressant mechanisms of action.

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ABCB1 AND HTR2A POLYMORPHISM MAY BE RELATED TO TREATMENT RESPONSE IN A SAMPLE OF MOOD DISORDERS PATIENTS UNDERGOING ELECTROCONVULSIVE THERAPY
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Background

Mood disorders represent an increasing global burden on society. Spite of the many drugs available for the treatment of depression, the results often are not as expected. Regardless of the antidepressant taken at the beginning of the treatment, about 30-50% of the patients will not respond sufficiently to this first attempt. Refractory depression is characterized by recurrent severe episodes that do not remit with the use of several classes of antidepressants. Up to 20% of these patients might need more aggressive treatments, including ECT (electroconvulsive therapy). Hence, the response to pharmacological treatment is variable and, at least in part, liable to genetic influence. Pharmacogenetics researches how different genetic factors influence response to drugs. Concerning pharmacokinetics, transport proteins carry molecules from the extracellular to the intracellular environment and vice versa, thus affecting the uptake, bioavailability, efficacy, toxicity and clearance of drugs. The ABCB1 gene, also known as MRD1 (multidrug resistance gene 1) codifies the protein that carries several classes of drugs including antidepressants to the central nervous system. A prior knowledge of the ABCB1 genotype may be relevant to drug prescription because although its plasma levels fit within the recommended therapeutic level, certain alleles of the ABCB1 gene may limit its intake to the brain through the blood-brain barrier. Concerning pharmacodynamics, the serotonergic system is widely associated with the pathogenesis of mood disorders and with some mechanisms of action of antidepressants, antipsychotics and mood stabilizers. Serotonin [5-hydroxytryptamine (5-HT)] receptors are known to be involved in the response to antidepressants and antipsychotics. There are multiple subtypes of 5-HT receptors with different affinities. Genetic polymorphisms in the HTR2A gene might be related to response to treatment with antidepressants. This study aims to investigate the hypothesis that response to treatment with antidepressants may be influenced by polymorphisms of genes related to pharmacokinetics and pharmacodynamics, using data of a very specific sample of refractory patients undergoing ECT.

Methods

A preliminary sample of 73 patients undergoing ECT treatment who met the DSM-IV criteria for refractory unipolar or bipolar depression and 76 patients with unipolar or bipolar depression who responded to treatment were genotyped. Genomic DNA was extracted from peripheral blood. Each of the following polymorphisms: rs1045642, rs1128503, rs2235040 of the ABCB1 gene and rs6311, rs6313, rs6314, rs7324218, rs7997012 of the HTR2A gene was determined using TaqMan® SNP Genotyping Assays. Genotyping was performed by real-time PCR allelic discrimination.

Results

After performing Chi-square test we observed that there was a difference in genotype distribution between the refractory and non-refractory groups for the rs6311 (p=0.038) and rs6313 (p=0.018) of the HTR2A gene. We also observed a different genotype distribution for the rs1128503 of the ABCB1 gene (p=0.023) between the mentioned groups.

Discussion
Our results point to a prevalence of the CC genotype for the rs1128503 of the ABCB1 gene among the non-refractory patients. Our results also indicate that the greater prevalence of the CC genotype for the rs6313 (HRT2A gene) is likely to predict refractoriness to treatment and TT genotype for the rs6311 (HRT2A gene) is likely to predict good response to treatment.

GENETIC DIFFERENCES IN DRUG-METABOLISING ENZYMES: CAN THEY BE USED TO PREDICT ANTIDEPRESSANT TREATMENT RESPONSE?

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Background
Response to antidepressant medication varies greatly between patients. One factor which may contribute to this variability is the rate at which the drugs are metabolised into inactive compounds, a process performed by the cytochrome P450 family of enzymes. Common genetic differences have been observed in two of the key enzymes (CYP2D6 and CYP2C19) involved in the metabolism of many antidepressants, and these polymorphisms have been linked to variation in rates of drug metabolism. Here, we examine whether circulating serum levels of antidepressant are influenced by cytochrome P450 genotype in depressed patients and, furthermore, whether treatment outcomes can be predicted by either cytochrome P450 genotype or circulating levels of antidepressant.

Methods
This study used data from GENDEP (Genome-based Therapeutic Drugs for Depression), a multi-centre pharmacogenetic project following a large sample of depressed patients who received one of two antidepressants for a period of twelve weeks. The two antidepressants studied were the selective serotonin reuptake inhibitor, escitalopram and the tricyclic antidepressant, nortriptyline. These drugs are metabolised into inactive compounds via different pathways, and so analyses were performed separately for each drug. Circulating serum levels of antidepressant (escitalopram or nortriptyline) and primary metabolite (desmethylcitalopram or 10-hydroxy-nortriptyline) were measured after eight weeks of treatment using achiral high-performance liquid chromatography. Data was available from 560 patients (314 were receiving escitalopram and 246 were receiving nortriptyline). Common variants in the genes encoding the cytochrome P450 enzymes CYP2D6 and CYP2C19 were genotyped using the micro-array based Roche AmpliChip CYP450 assay. Treatment response was measured using percentage change from baseline on the Montgomery-Asberg Depression Rating Scale (MADRS). Information was also available regarding other medication that patients were taking during the trial, so the effect of cytochrome P450 enzyme inhibiting medications could also be considered.

Results
Cytochrome P450 genotype highly significantly predicted circulating levels of both escitalopram (b=−4.23, 95% CI=−5.67 to -2.79, p=8.56 x 10⁻⁹) and nortriptyline (b=−32.18, 95% CI=−45.27 to -19.08, p=1.46 x 10⁻⁶), as well as the primary metabolite of nortriptyline, 10-hydroxy-nortriptyline (b=16.60, 95% CI=5.36 to 27.84, p=0.0038). This relationship was not influenced by the concurrent use of other medications that are known to have an effect on the cytochrome
P450. However, despite this strong link between cytochrome P450 genotype and serum concentrations of antidepressant, cytochrome P450 genotype was not predictive of antidepressant treatment response. Further investigation also demonstrated that there was no association between serum concentration of the drug and response to antidepressant treatment.

Discussion
This study investigated the effect of genetic variation in cytochrome P450 genotype on rates of antidepressant metabolism, and the potential relationship to treatment outcomes for two drugs: escitalopram and nortriptyline. These results suggest that, whilst genetic variation in cytochrome P450 enzymes does influence circulating levels of antidepressant for both of these drugs, the variability that is seen in response to treatment is not the result of individual differences in rates of drug metabolism.

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GLUCOSE-METABOLISM GENES AND THEIR IMPLICATION IN ANTIPSYCHOTIC-INDUCED WEIGHT GAIN
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Background
Atypical antipsychotic medication frequently leads to rapid changes in glucose metabolism followed by development of weight gain and/or diabetes. Recent findings from our group indicated an influence of genetic variation in TBC1D1, a protein involved in glucose trafficking, on antipsychotic-induced weight gain (AIWG). We aimed to extend our research on important glucose metabolism genes and performed a comprehensive study on the glucagon-like peptide 1 (GLP-1), the GLP-1 receptor, the peroxisome proliferator-activated receptor gamma (PPARγ) and the adiponectin gene. Our study is the first to investigate genetic variation in GCG, encoding GLP-1, and GLP1R, encoding its receptor, for its role in AIWG. Previous research on PPARG and ADIPOQ, encoding PPARγ and adiponectin, has delivered inconclusive findings, and only a limited number of polymorphisms have been investigated.

Methods
In 216 schizophrenic patients treated with various antipsychotics for up to 14 weeks, we investigated single-nucleotide polymorphisms (SNPs) in or near GCG (N=4), GLP1R (N=33), PPARG (N=24) and ADIPOQ (N=18). Statistical analysis of SNP association was done using ANCOVA with baseline weight and treatment duration as covariates. Haplotype analysis was performed in Unphased version 3.1.4. For gene-gene interaction analysis between GCG and GLP1R we used the R-package mbmdr. Multiple test correction was performed using the Nyholt method.

Results
In patients of European ancestry treated with olanzapine or clozapine (N=87), we found significant association of rs13429709 near GCG with AIWG (p(corrected)=0.008) with higher weight gain in patients carrying the C-allele. Several GLP1R polymorphisms (rs2300613, rs2268641, rs2268640, rs2268639, rs2894420, rs4714210, rs2206942, rs9296291) and
haplotypes showed a trend for an association (p<.050) with AIWG; however, none was significant after correction for multiple testing. Interestingly, we observed a highly significant gene-gene interaction between rs13429709 near GCG and rs2268639 in GLP1R (p=.0002). In ADIPOQ, rs12495941 showed significant genotype association with highest weight gain in T-allele homozygotes (p=.031). The C-allele in rs822391 was significantly associated with AIWG (p=.013); however, both did not remain significant after multiple test correction. None of the PPARG variants was significantly associated with AIWG. Despite some interesting trends (p<.050), no ADIPOQ or PPARG haplotype was associated with AIWG after correction for multiple testing.

Discussion
We could demonstrate a significant association of rs13429709 near the 3′UTR region of GCG with AIWG. Although there was no significant association of variants in GLP1R with AIWG after multiple test correction, the observed trends suggest this to be a particular interesting gene for future examination. In addition, a significant gene-gene interaction between rs13429709 near GCG and rs2268639 in GLP1R with higher weight gain in individuals carrying higher numbers of risk alleles. The negative findings for PPARG and ADIPOQ are in line with some previous studies and indicate no or only a minor influence of these genes on AIWG. Overall, the results of our study support the theory of implication of glucose metabolism genes in this severe side effect and contribute valuably towards better understanding of biological mechanisms of AIWG. However, since our study was the first to investigate GCG and GLP1R, more research is required to validate our findings.

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PRIMARY ACTION OF CLOZAPINE EXPOSURE ON ACTIVATION OF SREBP-CONTROLLED LIPOGENIC GENE EXPRESSION MAY EXPLAIN BENEFIT AND DETRIMENT

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Background
Antipsychotic drugs (APDs) are prescribed for the treatment of a broad range of psychiatric symptoms. While APDs are of value, they are often accompanied by a number of serious adverse effects. These include weight gain, metabolic adverse effects (atypical APDs) and extrapyramidal symptoms (typical APDs). Furthermore, one of the most effective atypical APDs, clozapine, can induce potentially lethal agranulocytosis in about 1% of treated patients. This study aimed to investigate the mechanisms behind the (adverse) effects of APDs by looking at gene expression profiles of exposed lymphoblast cell lines.

Methods
Four human lymphoblast cell lines were exposed to a range concentrations of clozapine (2μM to 100μM) and risperidone (0.15μM to 15μM). After exposure of 24 hours, RNA and was extracted
and gene expression profiles were generated. We studied gene expression profiles using ANOVA analysis, Weighted Gene Co-expression Network Analysis (WGCNA) and gene enrichment analyses using Ingenuity IPA.

**Results**

We found significant gene expression changes after exposure to clozapine. Additionally, using a network approach we found a gene co-expression module of genes significantly associated with clozapine. IPA enrichment analyses of both the gene expression changes and the gene co-expression module associated with clozapine showed that clozapine induces significant changes in lipid metabolism and activates Sterol Regulatory Element Binding Protein (SREBP) together with its target genes. Exposure to risperidone did not induce significant gene expression changes.

**Discussion**

We show that exposure of clozapine results in significant upregulation of gene expression in a set of genes that are part of the SREBP system. Similar patterns have been shown before in other cell types such as neuronal, liver and fat cells, but this is now also observed in blood-derived lymphoblast cells. The consistent finding of activation of the SREBP system in different cell types caused by clozapine, is suggestive of a systematic and primary lipogenic molecular underlying mechanism. Accordingly, this atypical APD could have beneficial effects on myelination in the brain and simultaneously cause metabolic side effects elsewhere in the body (e.g. changes in lipid profiles) leading to cardiovascular problems and excessive weight gain.

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**USING NEUROIMAGING ENDOPHENOTYPES TO IDENTIFY MOLECULAR MARKERS FOR TREATMENT RESPONSE TO MAJOR DEPRESSIVE DISORDER**

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**Background**

Major depressive disorder (MDD) is a highly prevalent and disabling disorder with high rates of treatment resistant and non-remission. Nonetheless biological measures to guide optimal treatment choices have been lacking. Recently, our group has described that resting state brain activity in selected brain regions can predict differential response to either antidepressant drug treatment or psychotherapy (McGrath et al., in press). The aim of this study was to identify genetic or epigenetic markers that associate with this endophenotype, in the hope to identify predictive measures that are more easily obtained in routine clinical practice than FDG-PET.

**Methods**

Patients were recruited at Emory University’s Mood and Anxiety Disorders Program. MDD patients were randomized at baseline into 12 weeks escitalopram (sCIT), or 16 sessions of CBT. Pre-treatment brain activity patterns (glucose metabolism measured by PET) of six limbic and cortical regions were significantly associated with differential response to CBT vs. sCIT and
used as the neuroimaging endophenotypes for this study.

DNA was extracted from peripheral blood drawn at baseline and genome-wide SNP genotypes (Illumina Omni-express array) and DNA methylation pattern (Illumina 450k Methylation array) were measured. Genome-wide association studies were conducted in 76 patients with MDD using the brain activity patterns from the six following regions of interest (ROIs) as endophenotype: right anterior insula, right inferior temporal cortex, left amygdala, left premotor cortex, right motor and precuneus. Univariate as well as multivariate association analyses aiming to identify not only brain region-specific associated loci but also those shared between ROIs including all possible combinations were conducted. Methylation status was interrogated at ~485,000 CpG sites across the genome. Tests for association between methylation status in white blood cells at CpG sites and baseline brain activity patterns were performed for each of the ROIs.

Results
We observed genome-wide significant association of rs34383296, \( p = 9.4 \times 10^{-9} \) in a multivariate association analysis which included the: right anterior insula, left amygdala, and left premotor cortex. Univariate analyses did not reveal genome-wide significant associations. rs34383296 is located on chromosome 9 in the NDOR1 gene locus and it is an eQTL for the ARRDC1 gene, ~400 kb downstream. The SNP locus lies in a gene dense area that includes multiple regulatory elements such as DNase hypersensitivity sites. No genome-wide significant association of DNA-methylation status was observed with any of the individual regions.

Discussion
We identified a genome-wide significant association with a particular combination of ROIs that have been shown to differentially predict response to two types of treatments for MDD. The associated variant is functionally relevant and regulates the expression of ARRDC1, a gene related to arrestin-mediated internalization of cell surface receptors. Our data suggest that a combined approach, using quantitative neuroimaging phenotypes and genomic approaches may be able to identify markers that can ultimately be used in clinical routine to guide depression therapy choices for the individual patient. Further analyses will include imputation around the associated locus. Methylation sites will also be tested as mQTLs for the significantly associated SNPs, as well for those SNPs in linkage disequilibrium with them. The associated SNPs will also be tested for association with treatment response in independent samples.

factors for this trait will enhance our understanding of the neurobiological basis of addiction. More than 10 years ago, we selectively bred mice for high and low methamphetamine (MA) sensitivity from a C57BL/6J (B6) x DBA/2J (D2) cross and identified a quantitative trait locus (QTL) on chromosome 11. Here, we utilized the power of an F₂ cross and the iterative nature of interval-specific congenic lines to replicate and fine map this QTL.

Methods
676 F₂ mice were administered saline injections (10 ml/kg, i.p.) on Days 1 and 2 in the open field (37.5 cm x 37.5 cm). On Day 3, mice were injected with MA (2 mg/kg, i.p.) and the total distance traveled was recorded over 30 min. Congenic lines containing large D2-derived portions of chromosome 11 were obtained from Dr. Aldons Lusis’s laboratory at UCLA and backcrossed to B6 to generate new recombination events. These new lines were tested alongside wildtype littermates. Congenic mice were genotyped using custom-designed fluorescent markers or PCR and traditional Sanger sequencing of genomic regions that contained SNPs. Data were analyzed using interval mapping in R (QTLRel) or repeated measures ANOVA with genotype as the factor and time as the repeated measure.

Results
We replicated a large-effect QTL in F₂ mice on chromosome 11 (50 Mb; LOD = 10; 1.5 LOD-support interval = 50-70 Mb) that was specific for MA-induced locomotor activity and was confirmed in a congenic line (0-80 Mb). The results of subcongenic lines revealed multiple, smaller-effect loci with different modes of inheritance. One QTL converged remarkably well with the F₂ peak at 50 Mb – thus, we aggressively pursued this locus via repeated backcrossing to the parental B6 strain. Owing to a fortuitous recombination event, we identified and replicated a critical interval (50,172,446-50,400,235 bp; Build 37) that contained only three protein-coding genes (Cby3, Hnrnph1, Rufy1) and one microRNA gene (Mir804). Inheritance of the B6 allele within this interval completely reversed the phenotype from D2 to B6 (see uploaded image). None of the protein-coding genes were differentially expressed in the striatum or cortex; however, Mir804 showed a significant decrease in cortical expression. Rufy1 is the only gene that contains any nonsynonymous coding polymorphisms and we confirmed all three of these SNPs via re-sequencing.

Discussion
We identified a 0.23 Mb region on chromosome 11 that influenced MA-induced locomotor activity. Rufy1 codes for an endosomal protein that interacts with Rab and could potentially affect MA-induced trafficking of monoamine transporters (the primary molecular targets of amphetamines). Interestingly, a RUFY1 polymorphism in humans was associated with the endophenotype “body dissatisfaction” in patients with eating disorders. It is worthy to note that amphetamines are the most efficacious drug class for appetite suppression and are misused precisely for this purpose by patients with eating disorders. Thus, RUFY1 could represent a common genetic mechanism for sensitivity to amphetamines and endophenotypes associated with eating disorders. Mir804 showed a 20% decrease in gene expression whereas its host gene Cby3 showed a nearly significant 70% increase (p = 0.05). One explanation is that Mir804 inhibits the expression of its own host gene and therefore a decrease in Mir804 expression would disinhibit Cby3 expression. Although there are no polymorphisms in Cby3 or Mir804, there are 300 putative, intergenic SNPs that could potentially regulate gene expression. Differential
Mir804 expression could cause global changes in the expression of gene networks that could ultimately explain the difference in behavioral sensitivity to MA. We are currently examining this possibility using RNA-sequencing and comparing these results with predicted targets of Mir804. We utilized the congeneric approach to QTL mapping by maximally leveraging our focus on a single QTL where we identified and replicated a 0.23 Mb interval for a complex trait. This is an extremely rare feat for genetic mapping studies in mice. Following nearly six years of backcrossing, phenotyping, diligence, and fortuity, we have derived a limited number of hypotheses regarding the genes and mechanisms by which this QTL regulates MA sensitivity. This level of resolution and experimental validation was made possible only by the knowledge we have from bioinformatic resources in next-generation DNA re-sequencing of inbred mouse strains and expression QTLs. Future studies involving successive gene targeting and functional characterization will define this novel genetic and neurobiological mechanism of action.

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A SYSTEMATICALLY COMBINED GENOTYPE AND PHENOTYPE ANALYSIS OF CYP2E1, CYP2D6, CYP2C9, CYP2C19 IN DIFFERENT GEOGRAPHIC AREAS OF MAINLAND CHINA: A BASIS FOR PERSONALIZED THERAPY

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Background

The cytochrome P450 is the major enzyme involved in drug metabolism. Single CYP genotypes and phenotypes have been widely studied but no combination analysis has been conducted in the context of specific populations and geographical areas.

Methods

Our study systematically analyze the combined genotypes and phenotypes of 400 samples of major CYP genes—CYP2E1, CYP2D6, CYP2C9, and CYP2C19 in four geographical areas of mainland China. Genotypes of the four genes were reviewed for each sample and rearranged as necessary for the current investigation (http://www.cypalleles.ki.se/). On the basis of each sample data, a combined genotype and phenotype analysis was performed, calculating the combination frequency of the four CYP genes found in the sample.

Results

167 different genotype combinations were identified, of which 25 had a greater than 1% frequency in the Chinese Han population. In addition, phenotypes of the four genes for each sample were in line with the predictions of previous studies for the four geographical areas. On the basis of the genotype classification, we were able to produce a systemic phenotype combination analysis for the population. 25 of the combinations detected had at least two non-wild phenotypes and four showed a frequency above 1%.

Discussion

This is the first systematic study to analyze genotype and phenotype combinations across whole Chinese population and could make a significant contribution in the field of personalized medicine and therapy.
RESEQUENCING AND ASSOCIATION ANALYSIS OF THE NDE1 GENES AND ITS CONTRIBUTION TO NEUROPSYCHIATRIC DISEASE SUSCEPTIBILITY

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**Background**

Recently there have been growing evidences showing that rare variants such as copy number variant (CNV) and rare single nucleotide variant (SNV) are closely related to the etiology of mental illness. CNV region located at chromosome 16p13.11 is implicated in several neuropsychiatric disorders namely schizophrenia (SZ), pervasive developmental disorder (PDD) and epilepsy. Regarding the aforementioned region, one of the most promising candidates is nuclear distribution factor E-homologue 1 (NDE1). It has been speculated that NDE1 has important role in neurogenesis, neural migration and mitosis by interacting with Disrupted in schizophrenia 1 (DISC1), LIS1, dynein and so on. The goal of the current study was to search for rare nonsynonymous variants within NDE1 region and evaluate its contribution to neuropsychiatric disease.

**Methods**

We used two independent samples (1) Mutation analysis of NDE1 coding regions by Sanger method on a total of 569 cases (417 patients of SZ and 152 of PDD), (2) Association analysis of novel coding variants was performed using 1511 patients of SZ and 1517 healthy controls. All cases were diagnosed according to DSM-IV-TR criteria. To discover the mutation, we used the Mutation Surveyor software. The potential influence of mutations was evaluated by using several bioinformatics tools. Written informed consent was obtained from all participants. The Ethics Committees of Nagoya Graduate School of Medicine and associated institutes and hospitals approved this study.

**Results**

We discovered three rare novel missense mutations within highly conserved region and one rare missense mutation (rs148118152). Including association sample set, two of the three novel mutations were discovered only in cases. One of the two predicted to be damaging and affecting phosphorylation status by in silico analysis.

**Discussion**

The identified missense mutations, particularly the one predicted to affect phosphorylation status of NDE1, are good candidates for further replication studies and functional evaluation.

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PLASMA MICRORNA PROFILING REVEALS ALTERED MIR-150 AND MIR-486-3P IN PARANOID SCHIZOPHRENIA

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**Background**

Schizophrenia is a mental disorder characterized by poor emotional responsiveness and thought disorder. A combination of genetic and environmental factors play a role in the development of
schizophrenia. microRNA (miRNA) is a short non-coding RNA molecule that is involved in post-transcriptional regulation. Recent studies have indicated that microRNA in plasma can served as biomarkers of cancer and other diseases. In this study, we examined the plasma microRNA expression profiling in the patients with schizophrenia.

Methods
1. Total RNA was extracted from mixing plasma samples of patients with schizophrenia and age- and sex-matched controls. RNA sequencing (solexa) data revealed a draft profiling. 2. TaqMan small RNA assays were used to exclude false positive results in a small cohort (n=50). 3. Results were confirmed in the entire sample (n=200).

Results
Quantitative PCR assessment revealed lower plasma levels of miR-150(p=0.049) and miR-486-3p (p=0.003) in schizophrenia.

Discussion
Our data demonstrate that plasma miRNA profiles are different between schizophrenia patients and health subjects.

SCHIZOPHRENIA POLYGENIC RISK SCORE PREDICTS PROCESSING SPEED & FLUID INTELLIGENCE
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Background
Common variants are important contributors to the genetic architecture of schizophrenia and cognitive ability individually. The degree of overlap between polygenic risk of schizophrenia and cognition has not been extensively studied, although initially published results show a much weaker association than might have been predicted. Our study investigated whether the schizophrenia polygenic risk score predicts cognitive ability in healthy controls across domains that are most impaired in individuals with schizophrenia. We hypothesised the polygenic risk score would predict cognitive ability on WAIS Digit Span (working memory), Digit Symbol Coding (processing speed) and Block Design (reasoning/problem solving), Verbal IQ, Performance IQ and Full IQ. We predicted the direction of effect would show increases in polygenic risk of schizophrenia would be associated with decreasing cognitive performance.

Methods
Our primary schizophrenia training dataset consisted of clumped PGC1 summary statistics. The CLOZUK sample is comprised of treatment resistant individuals with schizophrenia on Clozapine and was used for replication. It is also independent of PGC1. The cognition sample comprised of 936 healthy individuals of German origin with full information on all WAIS-R subtests. We generated lists of schizophrenia associated SNPs at p-thresholds 0.5, 0.1, 0.01 & 0.001 with their respective reference allele and log odds ratio. Polygenic scores derived from these training sets were calculated for each individual within the cognition sample. Polygenic scores were linearly regressed against WAIS tests described above. We covaried for age, gender
and the first eigenstrat principle component. We repeated these analyses by regressing the original SPRS on 10,000 permutations of the cognition data and compared our original and permuted p-values to generate empirical results.

Results
We performed 24 linear regressions (6 WAIS Tests and 4 Schizophrenia P-thresholds) each using polygenic risk scores derived from associated SNPs from PGC1 and CLOZUK datasets. Because we hypothesised a-priori the direction of the polygenic score coefficient, all reported p-values are one tailed and empirical based upon the methods described above. The PGC1 polygenic score from all training thresholds significantly predicted performance IQ (P=0.046~0.016, R2=0.003~0.005) and digit symbol coding (P=0.029~0.002, R2=0.005~0.011). Training on CLOZUK, we attempted to replicate these significant findings. CLOZUK polygenic scores were significant predictors of performance IQ at training thresholds 0.5, and 0.001 (P=0.023~0.025, R2 ~ 0.003~0.005). This also predicted digit symbol coding at the 0.1 training threshold (p=0.02, R2=0.002), with trends in the same direction of effect as PGC1 for all remaining training thresholds (p<0.12).

Discussion
Increased polygenic risk of schizophrenia was associated with worse lower scores on digit symbol coding and performance IQ in a large sample of healthy individuals. These effects for these tests were strongest when training on PGC1 schizophrenia datasets and were replicated when training on CLOZUK. Processing speed is severely impaired in individuals with schizophrenia and is an important contributor towards many other cognitive domains. This is the first study to identify a link between schizophrenia polygenic risk score and speed of processing. Furthermore, we replicate the finding by McIntosh et al (2013) showing increased polygenic risk of schizophrenia is predictive of performance IQ. Taken together, the current evidence suggests there is little overlap between the polygenic risk of schizophrenia and cognitive ability in healthy individuals on measures of crystallised intelligence. We have shown increases in polygenic risk of schizophrenia are weakly associated with worse performance on speed of processing and fluid intelligence in healthy individuals. Coincidentally, these cognitive domains are also the most severely affected in individuals with schizophrenia.

GENE EXPRESSION AND PROMOTER METHYLATION ANALYSES OF NEUROTRANSMISSON GENES IN PREFRONTAL CORTEX AND NUCLEUS ACCUMBENS OF SPONTANEOUSLY HYPERTENSIVE RATS (SHR)
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Background
Schizophrenia (SCZ) is a complex mental illness that genetic and environmental factors interact to disease development. Neurotransmission (NT) problems involving Nucleus accumbens (NAC)
are responsible for positive symptoms, while the negative and cognitive symptoms are mainly related to prefrontal cortex (PFC) deficits. Recently, our group suggested that SHR strain – mainly based on its behavior - could be a useful animal model to study several aspects of schizophrenia. The aim of the present study was to verify the expression of 84 genes and the methylation profile of promoter region of differentially expressed genes in the PFC and NAC between Wistar Rats (WS) and SHR both treated with saline (SS), and also between SS and SHR groups treated with three antipsychotics (APD): Risperidone (SR), Clozapine (SC) and Haloperidol (SH).

**Methods**

Each group was composed of 8 rats (6 months old) treated during one month. After the brain dissection, DNA was isolated and RNA was extracted and converted to cDNA. To perform the gene expression analysis we used the PCRarray technology, which verifies the expression of 84 genes related to NT plus five housekeeping genes simultaneously. We utilized the Student’s t-test to investigate the significance of each gene, and performed a False Discovering Rate correction, considering p<0.05. For methylation analysis, bisulfite sequencing was performed and we compared DNA methylation percentage values using Mann-Whitney U test.

**Results**

Four genes were significantly downregulated in the PFC of SS compared with WS: Gad2 (p=0.045), Chrnb4 (p=0.036), Slc5a7 (p=0.027), and Qrfpr (p=0.024). Five genes were downregulated in PFC of treated groups compared with SS: Brs3 in SC and SH (p=0.001 and p=0.039), Drd2 and Drd3 in SC (p=0.02 and p=0.033) and Glra1 in SH (p=0.002). Among these genes Gad2, Qrfpr and Glra1 have CpG islands in their promoter region. The promoter region of Gad2 and Qrfpr were hypomethylated in SS compared to WS. Only Glra1 promoter region showed hypermethylation in treated groups compared to non-treated (p=0.027).

**Discussion**

Regarding cholinergic pathway, we found two genes downregulated in the comparison between WS and SS, a subunit of a cholinergic receptor (Chrnb4) and the presynaptic choline transporter (Slc5a7), which is the major rate-limiting determinant of acetylcholine production in the brain. Collectively, the reduced expression of Chrnb4 and Slc5a7 suggests a deficit in cholinergic neurotransmission in the SHR PFC that could lead to cognitive deficits, such as the Contextual Fear Conditioning test deficit, found previously. The pyroglutamylated RFamide peptide (QRFP) and its receptor can influence blood pressure, so we suggest this alteration in SHR PFC could be related to its spontaneous hypertension and hyperlocomotion. Regarding Gad2, a study showed that knockout mice had prepulse inhibition (PPI) deficits characteristic of SCZ patients, as well, another study showed a reduced expression level in the PFC of SCZ patients, concordant to our results in SHR group. Since we identified downregulation and a hypomethylation of Gad2 and Qrfpr promoter regions, probably the methylation in these regions is not influencing expression of both genes. Based on APD known action, the downregulation of Drd2 and Drd3 demonstrate APD are acting in dopaminergic pathway of SHR. Bombesin is a neuropeptide that can modulate many pathways, including dopaminergic. Studies found reduced levels of bombesin in cerebrospinal fluid and urine of schizophrenia patients. Other study found that Brs3 knock-down mice have less social interaction after isolation, suggesting this receptor could affect the neural mechanism that regulates the effects of social isolation. Brs3 can influence social behavior, thus,
it can be related to higher social interaction after APD, described previously in SHR. There is no study involving Glra1 and APD, however, the downregulation in expression and hypermethylation of promoter in treated group suggest this drug can change the Glra1 expression by an epigenetic mechanism. In conclusion, our study indicated four genes differentially expressed between WS and SS, which could explain the different behavior between the strains. Besides, we found other four genes between SS and treated rats, two genes feasible related to APD action, and two pointing new pathways whereby APD may be acting. Finally, we could also suggest that the downregulation of Glra1 expression could be modulated by hypermethylation of the promoter region. Funding for this study was provided by FAPESP 2010/08968-6, 2011/50740-5 and 2012/12686-1.

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GENETIC AND EPIGENETIC ANALYSES OF THE ARC GENE AS A CANDIDATE GENE OF SCHIZOPHRENIA

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Background
Defects in NMDAR postsynaptic signaling complex, known to be important in synaptic plasticity and cognition, play a significant role in the pathogenesis of schizophrenia. ARC (Activity-Regulated Cytoskeleton-Associated Protein) is co-purified with NMDAR complex and is a critical effector molecule downstream of multiple neuronal signaling pathways that dysregulation of ARC expression can have dire consequences for learning, memory, and normal brain functions. This study aimed to investigate whether the ARC gene is associated with schizophrenia.

Methods
We re-sequenced all the exons of the ARC gene in 557 patients with schizophrenia and 521 non-psychotic controls from Taiwan and conducted a case-control association study and gene functional assay. Furthermore, 25 CpG loci at the promoter region of ARC gene were examined for DNA methylation status using the pyrosequencing in the peripheral blood lymphocytes from 62 patients with schizophrenia and 61 control subjects.

Results
We identified 13 known SNPs in this sample. SNP-based analysis showed no association of these SNPs with schizophrenia. We also detected 2 rare mutations at promoter region, 4 at 5’UTR region, 6 at coding region and 16 at 3’UTR, but no increasing burden of these mutations was found in the patient group. However, reporter gene assay demonstrated that the patient-specific alleles at the promoter region had a significantly decreased promoter activity than that of the wild type. Six CpG residues at ARC gene had an increased level in methylation status in 62 schizophrenic patients compared to 61 control subjects. The gene functional assays of the other rare variants at coding region and 3’UTR are underway.

Discussion
Our results suggest that multiple private rare variants of the ARC gene might contribute to the
pathogenesis of schizophrenia in some patients and epigenetic defects may take part in the pathophysiology of schizophrenia.

CHARACTERIZATION OF A DRD2 POLYMORPHISM THAT INCREASES RISK FOR SCHIZOPHRENIA, DISRUPTS BINDING OF A SPLICING FACTOR TO DRD2 PRE-MRNA, AND REGULATES THE EXPRESSION RATIO OF LONG:SHORT DRD2 TRANSCRIPT ISOFORMS

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Background

DRD2, which encodes dopamine receptor D2, has long been a prime candidate gene for schizophrenia, but definitive evidence of association has been lacking. Previously, we found that several polymorphisms and haplotypes spanning DRD2 exons 3-7 were associated with schizophrenia in a sample of 617 Han Chinese families from Taiwan. Concurrently, others found an intronic variant (rs1076560) in this region of DRD2 to be associated with long:short isoform-expression ratios of the gene, as well as several domains of neurocognitive functioning and accompanying brain activity levels.

Methods

Our present objective was to establish if this polymorphism was associated with schizophrenia in an expanded cohort of our original sample and in five unrelated samples of the same ancestry (additionally totaling 4,017 affected and 4,704 unaffected individuals of Han Chinese ancestry from China and Taiwan). Once association was established, we aimed to determine the molecular mechanism by which this polymorphism may increase risk for the disorder and its associated molecular, neurobiological, and neuropsychological characteristics. To test our hypothesis that some polymorphisms are affecting the affinity of nucleotide-binding proteins and, thus, altering the ability to control alternative splicing, we conducted both tissue culture and bio-layer interferometry (BLI) kinetics assays. HEK293 cells were transfected with DRD2 mini-gene constructs containing rs1076560 (G/T), rs6275 (C/T), and rs6277 (C/T) variants separately or co-transfected with the addition of the splicing factor ZRANB2. Using quantitative polymerase chain reaction (qPCR), we evaluated the ratios of DRD2 long to short mRNA isoforms. ZRANB2-RNA interactions were assessed using an Octet-RED system. We used streptavidin-coated sensors to evaluate BLI kinetics of RNA-protein binding and dissociation. Biotinylated RNA oligos with either a G (wt allele) or a U (risk allele) at the rs1076560 position were used to compare affinities to recombinant ZRANB2.

Results

Meta-analysis of the six independent samples revealed a small but reliable effect of the T allele of rs1076560 on schizophrenia susceptibility (odds ratio=1.1, p=0.004). In cultured HEK-293 cells expressing mini-gene constructs of the alternatively spliced region of DRD2, co-transfection of ZRANB2 induced exon-6 exclusion only for the G allele of rs1076560. In contrast, a mini-gene with a U at that SNP was unresponsive to ZRANB2 co-transfection, indicating that
ZRANB2 can affect alternative splicing of DRD2 only when a G allele is present at the rs1076560 position of DRD2 pre-mRNA. Octet-RED results confirmed the differential binding of ZRANB2 to the two RNA probes harboring the two different alleles at rs1076560. ZRANB2 showed stronger association to the G allele than to the U allele at all protein concentrations evaluated. Furthermore, dissociation rates were slower for the G allele at all protein concentrations.

Discussion
Collectively, this work strongly implicates rs1076560(T) as a risk factor for schizophrenia, and suggests a molecular mechanism by which it may exert such influence.

A GENOME-WIDE ASSOCIATION STUDY TO IDENTIFY GENETIC SUSCEPTIBILITY LOCI THAT MODIFY LEFT SUPERIOR TEMPORAL GYRUS THICKNESS IN PATIENTS WITH SCHIZOPHRENIA

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Background
Schizophrenia is one of the most disabling psychiatric disorders in adults. Although many studies have focused on the genetic underpinnings, we are far from understanding the complex phenotype of schizophrenia and its genetic background due to the polygenic inheritance and small gene effects. Previous studies have shown structural alterations such as left superior temporal gyrus (STG) volume decrease in patients with schizophrenia, which were also associated with hallucinations, a positive symptom of schizophrenia. We focused on left STG grey matter thickness as a dependent variable in a genome-wide association study (GWAS), because (a) cortical thickness measures were reported to have advantages over volume measures for gene discovery approaches, (b) the heritability of STG thickness (h²=0.79) is higher than for STG grey volume (h²=0.68) and (c) the aforementioned relationship between reduced left STG thickness and hallucinations was also present in our own patient sample. In order to obtain schizophrenia-specific results, we chose to analyze the SNP x Diagnosis interaction.

Methods
Genetic data (Illumina HumanOmni-Quad BeadChip), and structural MRI data (analyzed in an automated manner with the atlas-based FreeSurfer software suite) of the Mind Clinical Imaging Consortium (MCIC) study of schizophreniawere used for a GWAS. Specifically, we analysed the
interaction effects between 1,067,955 single nucleotide polymorphisms (SNPs, including 56,389 SNPs imputed with IMPUTE2) and disease status on left STG thickness in 126 healthy controls and 113 patients with schizophrenia with additive linear regression models in PLINK. In addition, we pursued a pathway approach using the gene-based analysis tool VEGAS as well as the pathway analysis tools DAVID and Gene Mania. For the pathway analysis we included all genes with a gene-based p-value ≤ 0.01 (127 genes).

**Results**
No SNP x Diagnosis interaction effect reached genome-wide significance ($5 \times 10^{-8}$) in our GWAS, but 10 SNPs reached p-values between $10^{-6}$ and $10^{-7}$. Our results remained robust with respect to statistical significance and the direction of the effects in a more homogeneous subsample of European ancestry only. Among the identified 127 genes using gene-based testing two had been associated with susceptibility to schizophrenia in previous studies. Furthermore, we identified seven pathways involved in postsynaptic modification, synaptogenesis, nervous system development and in the insulin signalling pathway.

**Discussion**
Taken together, we found supporting evidence for the left STG thickness, a known intermediate phenotype for schizophrenia, to be associated with biochemical pathways relevant to schizophrenia. The effects were specific for schizophrenia and may help to understand the underlying genetics of this burdensome disorder. In the light of limitations (heterogeneous sample, small sample size), we suggest a replication of our results in an independent, large and more homogeneous sample.

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**GENETIC EVALUATION OF SCHIZOPHRENIA USING THE ILLUMINA HUMANEXOME CHIP**
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1UPC KULeuven, Campus St. Rafaël, 2UPC KULeuven, 3Center for Human Genetics, Leuven, Belgium, 4Belgian Red Cross Flanders, 5Department of Psychiatry and Psychology, School of Mental Health and Neuroscience, Maastricht University Medical Centre, University of Maastricht

**Background**
Schizophrenia is a severe, debilitating disorder with several risk factors ranging from rare copy number variants to common SNPs. In 2011, Illumina introduced the HumanExome chip, using data from several exome sequencing projects to generate a single chip that aims to capture functional exonic variation in an European population. Due to these functional effects, it is possible that these variants confer higher effect sizes than those seen in common schizophrenia GWAS studies.

**Methods**
A sample of 1023 samples consisting of 525 cases with DSM-IV schizophrenia or schizoaffective disorder and 496 healthy controls was genotyped using the Illumina HumanExome v1.1 chip at the Center for Human Genetics in Leuven, Belgium. Diagnosis of schizophrenia was confirmed using the OPCRIT questionnaire. Control samples consisted of
healthy plasma donors without a history of psychiatric disorder and were supplied by the Belgian Red Cross Flanders. DNA quality control was done following manufacturers recommendation using GenomeStudio software (v2010.3). Genotypes were called using the manufacture supplied cluster file, with automatic reclustering of all genotypes with a call rate below 100%. Genotypes with call rates below 100% were verified manually (n=29750). Further quality control was done using PLINK v1.07. A total of 240801 SNPs, of which 87063 (36.2%) were polymorphic, passed all quality control measures (remaining call rate >99.98%). After exclusion of samples with call rate below 98% (n=2), duplicate samples (n=6), samples related up to the second degree (n=11), sex errors (n=6), samples with excess heterozygosti (n=2) and samples of non-Caucasian descent or other problems (n=29), a total of 977 samples (493 cases, 484 controls) remained. Ethnicity and relatedness was verified using the MDS algorithm in the KING software v1.4. Analysis of common (MAF >0.05) autosomal chromosomes was done using logistic regression with the first 7 principal components (PCA) generated by KING as covariates. Analysis of rare variants (MAF <0.05) was done in R v3.0 using the Rare Variant Tagging algorithms (RVT1 and RVT2) implemented in AssotesteR v0.1-7.

Results
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Discussion
Given the limited power of the current study, results should be interpreted with caution. Power limitations are clear when evaluating the rare variants, where no gene reached nominal significance. Within the common variants, again no SNPs reached genomewide significance level. The best result was rs1230345 WISP3. We were unable to find previous associations of this SNP or gene with schizophrenia. WISP3 is mainly known for its association with Juvenile Idiopathic Arthritis and cancer. CACNA2D3 belongs to a group of calcium channels modulating neurotransmission. Although no previous associations with this silent polymorphism were found, mutations within this group of calcium channels has been previously found in schizophrenia.
Lastly, no previous associations with the EBLN1 gene were found, and it is of note that a simple trend test without PCA correction did not confirm this significance. No previous associations of this gene with schizophrenia were found. **Conclusion** DNA genotyping using the Illumina HumanExome chip was done on a sample of 977 patients and controls. No SNP obtained genomewide significant p-values, but three SNPs obtained p values lower or near the $5 \times 10^{-5}$, and warrant further investigation.

**GENETIC POLYMORPHISMS ASSOCIATED TO ELEVATED FASTING GLUCOSE IN PSYCHOSIS**

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**Background**
Increased morbidity in type 2 diabetes and cardiovascular disease among patients with mental illness [1], including schizophrenia, has been observed for a long time. Mortality from cardiovascular disease among patients with schizophrenia is twice as high compared to the general population [2-4]. In addition, the prevalence of type 2 diabetes among schizophrenia patients is estimated to be two to three times that of the general population. The aim of this study was to investigate genetic associations to elevated fasting glucose in patients with schizophrenia or other psychosis, and SNPs associated to type-2 diabetes genes in population, in case-case and case-control model.

**Methods**
Patients with schizophrenia or other psychotic disorders in Sweden were sampled ($n=652$) from specialized psychosis outpatients clinics, responsible for treatment of long-term psychotic disorders. Among psychosis patients, there were patients with metabolic problem, defined by elevated fasting glucose $\geq 5.6$ mmol/L ($n=263$), and without metabolic problem, defined by fasting glucose $<5.6$ mmol/L ($n=389$). 322 subjects, defined by fasting glucose $\leq 6.0$ mmol/L, from the high risk Stockholm Diabetes Prevention Program [6], served as controls. The cut off levels for fasting glucose, and waist circumference (male $> 94$ cm, female $> 80$ cm) were defined according to the criteria for metabolic syndrome defined by the International Diabetes Federation. DNA was extracted from venous blood, and 48 SNPs were selected. The OpenArray system was used for genotyping and allelic discrimination was analyzed with the TaqMan Genotyper Software. All calculations were performed using PLINK in BC|SNPmax data management and analysis [7], using age, gender, smoking and waist circumference as a covariates.

**Results**
In the case-case model, where psychosis patients with elevated fasting glucose were compared to patients with normal fasting glucose levels, the minor allele T of rs4402960 (OR=0.71, $p=0.014$) in the **IGF2BP2** gene, was less common among the psychosis patients with elevated glucose. Thus, allele G in rs4402960 (OR=1.4, $p=0.014$) was nominally associated with elevated glucose level in psychosis patients. The allele association was further supported by genotypic association test ($p_{\text{dominant}}=0.050$, $p_{\text{trend}}=0.018$). In the case-control analysis, where psychosis patients with elevated fasting glucose were compared to normal glucose tolerance controls, the minor allele T of rs10923931 (OR=2.1, $p=0.015$) in the **NOTCH2** gene was associated with elevated glucose
levels in psychosis patients. Likewise, the minor allele C of both rs11037909 (OR=1.6, p=0.020) and rs3740878 (OR=1.6, p=0.014) in the EXT2 gene, and the minor allele G of rs10830963 (OR=1.5, p=0.025) in the MTNR1B gene were associated with elevated glucose in the psychosis sample. Further, the minor allele C of rs17228212 (OR=0.67, p=0.035) in the SMAD3 gene was less common among psychosis patients with elevated glucose level. Thus, allele T of rs17228212 (OR=1.5, p=0.035) was nominally associated with elevated glucose level in psychosis sample. Out of five allele associations, three associations were further supported by genotypic association test, either in dominant or recessive model, for rs10923931 (p_{dominant}=0.033, p_{trend}=0.012), rs11037909 (p_{recessive}=0.010, p_{trend}=0.023), rs10830963 (p_{dominant}=0.0051, p_{trend}=0.0025).

Discussion
We found genetic associations between elevated glucose levels in patients with psychosis and type 2 diabetes risk genes in the population, both in case-case and case-control.


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INTERACTION OF THE COMPOUND GENETIC RISK WITH HIGH BIRTH WEIGHT CONTRIBUTES TO SOCIAL ANHEDONIA AND SCHIZOPHRENIA RISK
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Background
Schizophrenia is a highly heritable psychotic disorder, but despite extensive study, a substantial proportion of the genetic background has remained unresolved. A recent study showed that thousands of common single nucleotide variants, each with a minor effect, might cumulatively explain one-third of the variance in the disease risk. But even these thousands of variants are far from explaining all of the variance that is estimated to be of genetic origin. Part of the unexplained variability, often termed as “missing heritability”, may be explained by gene-environment interactions. In recent studies from the population of Finns, high birth weight, reflecting pre- and perinatal conditions, was found to considerably increase the risk for familial
schizophrenia. In this study, we aimed to examine the interaction between a polygenic risk score for schizophrenia and high birth weight on social anhedonia, an intermediate phenotype reflecting schizophrenia liability, and schizophrenia diagnosis in a general population birth cohort.

**Methods**
The study sample (N = 4393) was derived from the Northern Finland Birth Cohort 1966, which is an unselected birth cohort representing the general population and prospectively followed from perinatal period to adulthood (total N = 12058). **Schizophrenia spectrum diagnosis** was assessed using several national registers (the Finnish Hospital Discharge Register and national registers of the Finnish Social Insurance Institute) and clinical interviews (N=196). **Social anhedonia** was self-rated as a part of the cohort’s 31-year follow-up in 1997 using revised Social Anhedonia Scale (Chapman et al., 1976). Information on **birth weight** was collected from child welfare clinic registries and with questionnaires filled in by the mothers during years 1965-1967. Birth weight was dichotomized as “4 kg or more” and “less than 4 kg” based on earlier findings on the association between birth weight and schizophrenia (N= 476 and 3917, respectively). **The genetic risk score** was calculated from eight best SNPs found in the recent large European genome-wide meta-analysis for schizophrenia (The Schizophrenia Psychiatric Genome-Wide Association Study (GWAS) Consortium, 2011) as a weighed sum of the risk allele count at each locus. We used reported odds ratios for each SNP as weights.

**Results**
By using multivariate linear regression model, we found that the interaction between compound genetic risk and high birth weight was associated with social anhedonia in adulthood (P for interaction = 0.04, B = 0.19). Having more risk alleles together with high birth weight was associated with higher social anhedonia scores. We also found that the interaction between high birth weight and genetic risk score increased the risk for schizophrenia diagnosis with borderline significance (p = 0.06). When males and females were analyzed separately, having more risk alleles together with high birth weight was found to increase the risk for schizophrenia two-fold in females (p < 0.01), but not in males.

**Discussion**
The interaction between risk alleles for schizophrenia and high birth weight was associated with higher social anhedonia, and in women, it also increased the risk for schizophrenia diagnosis. These results suggest that including environmental factors, such as those related to pre- and perinatal conditions, in the genetic studies of schizophrenia might be essential to understand the disease etiology.

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**LONGER TELOMERE LENGTH IN PATIENTS WITH SCHIZOPHRENIA**
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Background
Previous studies have reported an association between shorter leukocyte telomere length and schizophrenia (SCZ). The aim of the present study was to replicate this finding in a large sample of SCZ patients (n = 539) and population-based control individuals (n = 519). In addition, the possible influence of SCZ severity on telomere length - as measured by age of onset, mode of onset, and course of the disorder - was investigated.

Methods
For telomere measurement, a qPCR-based method was used as previously described (Cawthon 2002; Eerola et al. 2010; Kananen et al. 2010; Kao et al. 2008). Sex and age of the participants, as well as the batch used for telomere length measurement, were included as covariates in all statistical models in order to rule out confounding effects. Monotonic relationships between clinical status, age, lifetime smoking, severity of SCZ measures (age of onset, mode of onset, and course of the disorder) and telomere length were assessed by linear regression. All statistical analyses were performed with IBM SPSS 20.0 (Chicago, Illinois).

Results
Telomere length was negatively associated with age in both patients and controls individuals. This is a consistently reported phenomenon, related to the problem of DNA end-replication. However, in contrast to previous findings, SCZ patients displayed longer telomeres compared to control individuals (p = 0.015). No association was found with any SCZ-severity subphenotype.

Discussion
Interestingly, recent studies have reported associations between longer leukocyte telomere length and both smaller hippocampal volume, and poorer episodic memory performance. Both phenotypes are common in patients with SCZ. Further studies are warranted to investigate whether the present association between SCZ and increased telomere length was driven by such associations, or rather by association with the clinical disease per se or other associated phenotypes, endophenotypes or lifestyle factors.

COMPLETE GENOME SEQUENCE BASED FAMILY ANALYSIS OF MONOZYGOtic TWINS DISCORDANT FOR SCHIZOPHRENIA
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Background
The reality of individual genome sequencing now offers a new hope in the search of the cause of complex diseases. When combined with genetic relationships, individual sequences add an unrivaled proficiency. Given the near identical genetic structure of monozygotic twins, any difference between monozygotic twins discordant (MZD) for a disease will have a high likelihood of being causal. With this in mind we have sequenced the DNA of six individuals, which includes two pairs of MZ twins discordant for schizophrenia and one set of parents.
Methods
Sequencing was carried out using the Complete Genomics Analysis system (Drmanac et al., 2010). The sequences were further assessed for accuracy in relation to Affymetrix SNP Array 6.0 results on the same samples. Genome wide variations including SNPs, indels, and CNVs were assessed. Our approach has allowed us to evaluate the similarities and differences across unrelated individuals, parents and children, as well as between MZ twins and to identify variants which are unique to affected individuals. Variant comparisons were performed using Golden Helix SNP and Variation Suite 7.0.

Results
The results show that an individual carries approximately 3.7 million SNPs, 400,000 indels, and 150 CNVs. Also, two unrelated individuals differed for 1.5-1.8 million SNPs (~45%), a parent and child differed for 0.9-1.0 million SNPs (30%) and a pair of MZ twins differed for 100,000 (~3%) SNPs. Similarly, differences in the identity of CNVs for the three comparisons were 45%, 30% and 4%, respectively. In our family analysis, a total of 968 variants were found in the affected twin that were not present in their Mother or Father. Of these variants, 138 were also present in the unaffected co-twin. Of the 830 unique variants to the affected twin, 6 variants were found to be in coding regions. In our pair-wise analysis, 24 and 40 coding variants respectively were found in the affected patient of twin pair 1 and twin pair 2 that were not found in their co-twins. It should be noted however that a number of these differences will be the result of sequencing errors and confirmation of variants of interest will serve as the next step of this analysis.

Discussion
The results support our strategy and identify patient specific genetic changes that may lead to schizophrenia. The novel results re-enforce that individual genomes harbor extensive variability, some inherited and others acquired during parental meiosis and/or mitosis during ontogeny. There is no single human genome sequence. Even monozygotic twins are not identical and each individual may be a mosaic, potentially carrying different sequence variations in different cells. This is supported by a high mutation rate and the persistence of genetic diseases with a severely reduced fecundity in all human populations.

FUNCTIONAL ANALYSIS OF PGC2 SCHIZOPHRENIA GENETIC VARIANTS WITH EHMT REGULATED GENE SETS
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Background
de novo CNVs analysis in schizophrenia has identified euchromatic histone-lysine N-methyltransferase 1 (EHMT1) as being disrupted by two single gene CNVs, implicating its involvement in schizophrenia pathogenesis (Kirov et al. 2012). In a conditional mutagenesis
experiment in mice, Schaefer et al showed that postnatal, neuron-specific deficiency of EHMT1 or EHMT2 (with which it forms a functional complex) led to increased expression of non neuronal and neuron progenitor genes in adult neurons, and decreased transcription level of genes needed for normal functions of adult brain (Schaefer et al. 2009). In this study we investigated whether genes regulated by EHMT1 or EHMT2 are enriched for genetic variants associated with schizophrenia in the Psychiatric Genomics Consortium schizophrenia dataset (January 2013 unpublished interim freeze).

Methods
Genes showing expression changes in EHMT1 and EHMT2 deficient mice in various brain regions (hippocampus, striatum, hypothalamus and cortex) were identified and the human orthologues identified. These gene sets (and their up-regulated and down-regulated subsets) were tested for enrichment for schizophrenia association signals (after lambda correction) using a set-based test that takes into the number of SNPs per gene and the extent of linkage disequilibrium between SNPs (Moskvina et al. 2011). Genes were also assigned to GO categories and a Fisher’s Exact Test was employed to detect GO category enrichment.

Results
Genes up-regulated in striatum and hippocampus in EHMT2, and those down-regulated in cortex in EHMT1 deficient mice were enriched for association (P = 6.435 x 10^{-5}, P = 1.796 x 10^{-4} and P = 1.242 x 10^{-3}, respectively). Functional analysis showed that, relative to other gene sets regulated by EHMT1, gene set with down-regulated cortical expression in EHMT1 deficient mice were highly enriched in GO functions related to neurogenesis along with other general cell differentiation and development processes. Meanwhile, relative to other gene sets regulated by EHMT2, gene sets up-regulated in EHMT2 mutated striatum and hippocampus were both significantly enriched in GO categories involved in development and function of the immune system, even after exclusion of the extended MHC region which has large numbers of highly significant correlated SNPs.

Discussion
Our results provided additional support for the involvement of EHMT in schizophrenia. Consistent with the role of the EHMT1/EHMT2 complex in inhibiting non-neuronal genes, SZ-associated gene sets released from repression in EHMT2 knockout mice were largely immune related. Cortically expressed neurodevelopmental genes whose expression was reduced in EHMT1 mutants (possibly due to the downstream effects of increased non-neuronal gene expression) were also enriched for SZ association.

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EPISTASIS IN A FUNCTIONAL NRXN1 PATHWAY INFLUENCES RISK FOR SCHIZOPHRENIA
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Background
CNVs in NRXN1 have been shown to be strongly associated with schizophrenia (SZ). We sought to asses evidence for epistasis in a NRXN1 pathway derived from a NRXN1 knockdown
experiment using induced pluripotent/human embryonic stem cells that were differentiated into neural stem cells. The NRXN1 pathway contained 131 genes and 5931 SNPs, we used Random Forest (RF) to test for epistasis between NRXN1 pathway SNPs in the WTCCC2 SZ case (N = 1378)-control (N = 1086) study.

Methods
The Random Forest (RF) algorithm is designed for use with high dimensional data and can be use for predicting new observations. We used RF to detect epistasis in a training (80%) and independent test (20%) sample. We took the top10 predictors ranked in the top 10 of the training data across 100 runs of RF to follow up with logistic regression interaction models in our test sample. To determine significance, we used the likelihood ratio test (LRT) between nested models. We used Nagelkerke's $R^2$ to estimate the amount of variation in case-control status explained.

Results
We detected two significant interactions between the genes NPAS3 and NEBL and between NRXN1 and DSP in our independent test dataset. In NPAS3/NEBL, epistasis was detected between rs1958053 and rs4556442 (LRT p-value = 0.017 and $R^2$= 1.7%) and between rs7149368 and rs4556442 (LRT p-value = 0.012 and $R^2$ = 1.9%). Significant epistasis in DSP/NRXN1 was found between rs2237103 and the two other rs972112 and rs988179 with LRT p-values 0.044 and 0.031 with $R^2$ 0.012 and 0.013, respectively.

Discussion
We detected significant epistasis that influence risk of SZ in NRXN1 pathway in an independent test dataset. NRXN1 has been associated with schizophrenia, brain structure and cognition. NPAS3 is a transcription factor involved in neurogenesis, and a translocation breakpoint in this gene was in a family with schizophrenia. The use of RF on the training data produced a small number of interacting SNPs to assess in our test data, thus reduced multiple testing. So, the number of interactions tested in our test set was 45 vs 17585415 had we used all SNPs in the pathway.

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WHOLE-GENOME MRNA EXPRESSION PROFILING REVEALS DISTURBED GLUTATHIONE SIGNALING IN A RAT MODEL OF SCHIZOPHRENIA

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Background
Schizophrenia is a complex neurodevelopmental psychiatric disorder for which the underlying pathophysiology remains unclear and the available treatments are suboptimal. Apomorphine-susceptible (APO-SUS) rats represent an animal model displaying schizophrenia-relevant
features, while apomorphine-unsusceptible (APO-UNSUS) rats are their phenotypic counterparts. Apart from differences in the dopaminergic system, the two rat lines differ in a number of aspects. For example, APO-SUS rats exhibit a deficit in information processing (reduced prepulse inhibition) and increased locomotor activity in response to novelty. Moreover, the two lines differ in their endocrine and immune systems. To investigate the molecular pathways that are different between the two lines, we performed mRNA expression profiling of the medial prefrontal cortex of APO-SUS and APO-UNSUS rats.

Methods
Brains of APO-SUS and APO-UNSUS adult rats were collected and frozen. Coronal sections of the brains were made and punches of the medial prefrontal cortex collected. RNA was extracted from these punches and samples were subjected to microarray analysis. Quantitative RT-PCR (qPCR) was employed to confirm mRNA expression changes. To determine which molecular mechanisms underlie the observed differences, the list of differentially expressed genes was analysed using Ingenuity Pathway Analysis (www.ingenuity.com). Compound (acute) injection into APO-SUS and APO-UNSUS rats was subcutaneously. Subsequently, the phenotypes of the injected animals were investigated in three behavioral tests, namely the open field test, prepulse inhibition test and the susceptibility to apomorphine in a gnawing box.

Results
mRNA expression profiling revealed 388 genes to be significantly differentially expressed in the medial prefrontal cortex of adult APO-SUS and APO-UNSUS rats. Subsequent analysis showed only one significantly enriched canonical pathway, namely the glutathione-mediated detoxification pathway which has been previously implicated in schizophrenia. The differential expression of the six genes involved in this pathway was validated by qPCR analysis. Next, APO-SUS and APO-UNSUS rats were injected with a compound known to affect glutathione levels and subsequently behaviorally tested in the open field and for prepulse inhibition, as well as for their susceptibility to apomorphine. Intriguingly, compared to saline, the injection of the compound caused a shift of the three behavioral phenotypes of the APO-SUS rats towards those of APO-UNSUS rats.

Discussion
Taken together, our results point towards a central role of aberrant glutathione signaling in the APO-SUS rats and hence in schizophrenia etiology. Furthermore, the tested compound shows promise to be further developed as a novel treatment for schizophrenia.

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COMMON VARIANTS ON XQ28 CONFERRING RISK OF SCHIZOPHRENIA IN HAN CHINESE
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Background
Schizophrenia is a highly heritable, severe psychiatric disorder affecting approximately 1% of the world population. A substantial portion of heritability is still unexplained and the pathophysiology of schizophrenia remains to be elucidated.
**Methods**

To identify more schizophrenia susceptibility loci, we performed a genome-wide association study (GWAS) on 498 schizophrenia patients and 2,025 controls from the Han Chinese population, and a follow-up study on 1,027 cases and 1,005 controls. In the follow-up study, we included 384 SNPs which were selected from the top hits in our GWAS (130 SNPs) and from previously implicated loci for schizophrenia based on the SZGene database, NHGRI GWAS Catalog, CNV studies, GWAS meta-analysis results from the international Psychiatric Genomics Consortium (PGC) and candidate genes from plausible biological pathways (254 SNPs).

**Results**

Within the chromosomal region Xq28, SNP rs2269372 in \textit{RENBP} achieved genome-wide significance with a combined \(p\)-value of (OR of allele A = 1.31). SNPs with suggestive \(p\)-values were identified within two genes that have been previously implicated in schizophrenia, \textit{MECP2} (rs2734647, \(p_{\text{combined}} =\), OR=1.28; rs2239464, \(p_{\text{combined}} =\), OR=1.26) and \textit{ARHGAP4} (rs2269368, \(p_{\text{combined}} =\), OR=1.25). In addition, the patient sample in our follow-up study has showed a significantly greater burden for pre-defined risk alleles based on the SNPs selected than the controls.

**Discussion**

The results of the risk allele burden test indicate the existence of schizophrenia susceptibility loci among the SNPs we selected, and further support multigenic inheritance in schizophrenia. A new schizophrenia susceptibility locus on Xq28 is identified, which harbours the genes \textit{RENBP}, \textit{MECP2} and \textit{ARHGAP4}.

**CHARACTERIZATION OF A DELETION AT THE SLC1A1 GLUTAMATE TRANSPORTER GENE THAT COSEGREGATES WITH SCHIZOPHRENIA IN A 5-GENERATION FAMILY**

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**Background**

Glutamatergic systems have been strongly implicated in the pathophysiology of schizophrenia (SCZ) and related disorders. We have discovered a deletion at the SLC1A1 glutamate transporter gene, co-segregating with psychosis (schizophrenia and bipolar schizoaffective disorder). The deletion is carried by members of a 5-generation family in the Pacific island of Palau, where risk of SCZ is two to three times the worldwide rate. SLC1A1 gene encodes EAAC1, a trimeric member of the neuronal high-affinity glutamate transporter family, which are crucial in terminating the postsynaptic action of glutamate and maintaining extracellular glutamate concentrations below neurotoxic levels. Although polymorphisms in SLC1A1 have been recently associated with schizophrenia, none of the associated variants are known to influence the protein sequence. In contrast, our newly found deletion eliminates the entire promoter, start codon and N-terminal 31 amino acids from the protein, but preserves nearly all of intron 1 and the remaining structure of SLC1A1 gene. Consequently, the deletion impacts, at a minimum, the first transmembrane domain of the protein which is the Na2+/dicarboxylate symporter domain, one of the domains that perform the glutamate transport action and also participates in the
transporter trimerization process. Thus, the present studies sought to functionally characterize the SLC1A1 deletion allele.

**Methods**

(1) To determine if the sequence replacing the native promoter in the deleted allele can promote transcription, 1 Kb of the native exon 1 promoter and putative exon 2 promoter sequence were cloned into Pgl4 firefly luciferase vector (Promega). A dual luciferase assay was then used to compare the promoter activity of cloned regions transfected into HEK293 cells. A total of 9 replicates were generated for each construct.

(2) To test if the truncated EAAC1 protein is capable of transporting glutamate into the cells, Xenopus oocytes were injected with either truncated or non-truncated SLC1A1 mRNA (or water as a negative control) at a concentration of 200 ng/ul and incubated for 3 - 5 days. Western immunoblot of whole cell lysates prepared from these oocytes was used to confirm comparable expression of both wild-type (WT) and truncated EAAC1 proteins. Then, to test the function of the expressed proteins, glutamate-induced inward currents were recorded (at 1000 Hz) by two electrode voltage clamp (at a holding potential of -60 mV) following application of 2 mM glutamate. (3) Because previous studies have shown that EAAC1 localizes to both the membrane and the cytosolic compartments of neurons, our third goal was to perform localization studies using confocal microscopy to permit comparison of the membrane-associated and cytosolic expression levels of WT and truncated EAAC1 proteins. For these experiments, GFP-tagged open reading frame clones of wild-type and deleted human SLC1A1 gene were transfected into HEK293 and other cell types and high-resolution fluorescent images obtained after 2 - 4 days in vitro. (4) To determine whether the presence of the SLC1A1 deletion affects the expression of other glutamate transporter and neurotransmitter genes, we have performed next-generation sequencing using a custom-designed Targeted RNA-Sequencing (TRex) panel (Illumina) along with RT2 profiler PCR arrays (Qiagen). These studies were done using total RNA from the peripheral leukocytes of subjects with and without the deletion.

**Results**

(1) In the dual luciferase assay, the firefly to renilla luminescence ratio demonstrated that the promoter activity of the exon 2 upstream sequence was more than 7-fold higher than the native promoter (p<5.7E-8). These observations strongly support the possible expression of a truncated protein from the SLC1A1 deletion allele. (2) Comparison of the functional properties of WT and truncated EAAC1 using voltage clamp recording readily demonstrated glutamate current in the oocytes expressing WT EAAC1. However, glutamate current was absent or negligible in oocytes expressing the truncated EAAC1, and resembled the results seen following water injection. These observations strongly suggest that only the full-length WT protein functions as a glutamate transporter. (3) Comparative localization studies of the GFP-tagged WT and truncated EAAC1 are still ongoing, although it is clear that both are expressed in robust fashion in cell culture. (4) Expression profiling studies have revealed robust expression changes in a small subset of genes related to glutamate neurotransmission in the RNA from subjects with the deletion.

**Discussion**

Our discovery and initial functional characterization of a novel SLC1A1 mutation and its cosegregation with schizophrenia in a high-density multigenerational pedigree further
emphasizes the important role played by glutamatergic mechanisms in the pathophysiology and possible pathogenesis of schizophrenia.

PATHWAY ANALYSIS OF SINGLE POINT DE NOVO MUTATIONS IN SCHIZOPHRENIA

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Background
Schizophrenia (SCZ) is a severe psychiatric disorder with a lifetime prevalence of ~ 1%, and a strong genetic component. Patients with (SCZ) have a reduced fecundity rate, and so genetic susceptibility variants will be removed from the population by natural selection, and so must be replaced with independent de novo mutations. Thus, de novo mutations will be enriched for pathogenicity. Whole exome sequence data from 623 Bulgarian trios were used to identify the de novo point mutations

Methods
Assume that mutation rates are constant across the exome, so that the probability of observing a mutation in a gene is proportional to its sequence length. Assume further that mutation rates are multiplied by an enrichment factor \( \lambda \) for genes in the pathway being tested. The likelihood of the genes containing mutations can be expressed in terms of \( \lambda \) and a likelihood ratio test performed to test for pathway enrichment. Non-synonymous and loss-of-function de novo point mutations were tested for enrichment in specific synaptic pathways previously identified to be enriched in schizophrenia denovo CNVs (Kirov et al. 2012) as well as a larger set of pathways collected from the Gene Ontology database. Correction for multiple testing was performed using simulated sets of randomly placed de novo mutations. The effect on pathway enrichments of allowing mutation probability to depend on sequence context was also investigated. We also performed the same pathway analysis on previously published de novos in SCZ, as well as Autistic spectrum disorders (ASD) and intellectual disability (ID).

Results
Significant enrichment of the ARC and NMDAR pathways was observed for de novo mutations in schizophrenia, and ID. One GO term, actin filament bundle assembly (GO:0051017), was significant after multiple testing correction in SCZ, as well as 4 terms in total for ASD, and a further 65 terms for ID. However, several GO terms are nominally significant in two or more disorders, indicating shared biology. After accounting for the enrichment signal of the ARC and NMDAR pathways, actin filament bundle assembly remains nominally significant.

Discussion
Our results indicate that de novo mutations within genes encoding members of the ARC and NMDAR complexes, as well as those involved in actin filament bundle assembly may be
contributing towards the genetic susceptibility of schizophrenia and other neurodevelopmental disorders

STUDYING GENE-ENVIRONMENT INTERACTIONS IN A MOUSE MODEL OF THE SCHIZOPHRENIA RISK GENE TCF4
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Background
The basic helix-loop-helix (bHLH) transcription factor TCF4 was confirmed in the combined analysis of several large genome-wide association studies (GWAS) as one of the rare schizophrenia (SZ) susceptibility genes with population-wide significance. At risk alleles most likely cause elevated levels of gene expression, influence verbal learning and memory in humans and modulate sensorimotor gating in SZ patients. We showed recently that transgenic mice slightly overexpressing TCF4 in the forebrain (Tcf4tg) display profound deficits in contextual fear memory and sensorimotor gating. Moreover, deficits in trace fear conditioning revealed evidence for disturbed hippocampus-prefrontal cortex (PFCx) interactions.

Methods
Environmental factors interacting with genetic vulnerability (G x E interactions) play pivotal roles in development of schizophrenia. For example, social integration as well as lack of sensory stimulation have been identified as environmental risk factors and it has been shown that physical exercise can counteract some symptoms of disease in patients. We studied GxE interactions in Tcf4tg mice by housing the animals in sensory depleted conditions and upon social isolation as well as in enriched environment and upon group housing. We analyzed wild-type and TCF4 transgenic mice in a battery of behavioural tests to monitor affective and social behaviour as well as cognitive performance.

Results
Our analysis revealed profound GxE interactions at the level of behavioral flexibility reminiscent of perseveration seen in SZ that may be related to PFCx dysfunctions. High resolution STED microscopy revealed altered spine morphologies in the PFCx of Tcf4tg mice. Furthermore, by applying global proteomic as well as transcriptomic profiling techniques, we could identify changes in PFCx-associated expression profiles.

Discussion
Our study may help to delineate the function of the schizophrenia risk gene TCF4 in the brain that appears to be tightly linked particularly with cognitive symptoms of the disease.

GENOMIC PSYCHIATRY COHORT: PARTNERS IN DISCOVERY
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**Background**
The Genomic Psychiatry Cohort (GPC) is designed as a longitudinal resource to provide the necessary population for large-scale genomic studies, studies focusing on RDoC and/or other alternate phenotype constructs, clinical and interventional studies, nested case-control studies, long-term disease course studies, and genomic variant-to-phenotype studies. We provide and will continue to encourage access to the GPC as an international resource. DNA and other biological samples and diagnostic data are available through the National Institute of Mental Health (NIMH) Repository. After appropriate review and approval by an advisory board, investigators are able to collaborate in, propose, and co-lead studies involving cohort participants. (Pato et al, 2013)

**Methods**
The GPC is predicated on the ability to carefully assess for the core psychiatric disorders but optimize the ability to recontact for further definition of phenotype and follow-up studies. The participants are asked to allow ongoing access to their medical records up to 20 years +, and 88% agree to recontact. All participants receive a medical and psychiatric screening and potential affected participants are assessed with the DIPAD. (Pato et al, 2013)

**Results**
The Genomic Psychiatry Cohort (GPC) has enrolled a large clinical cohort of patients (n=33,000); suffering from schizophrenia (n=10,000), patients with bipolar disorder (n=5,000), family members (n=3,000), and control participants with no personal or family history of schizophrenia or bipolar disorder (n=15,000). The GPC was formed from an initial population of ~10,000 participants from our earlier studies. Over the past four years, we have enrolled an additional 23,000 participants (~9,000 Caucasian, ~5,000 African American, ~8,000 Latino, ~1,000 other).

**Discussion**
The GPC welcomes collaboration by a broad range of investigators. We are currently collaborating to whole genome sequence over 2,500 participants. We have genotyped over 9,000 Latino and African American participants on the Illumina 2.5 million SNP platform. A number of additional presentation on these studies are presented elsewhere at the congress.

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**A CANDIDATE GENE ASSOCIATION STUDY ON MISMATCH NEGATIVITY AND P50 OF SCHIZOPHRENIA IN TAIWAN**

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Background
Mismatch negativity (MMN) and P50 are candidate electrophysiological endophenotypes of schizophrenia. In this two-stage study, the association of schizophrenia candidate genes with MMN and P50 were analyzed in one case-control sample. Selected findings were examined further in second independent case-control sample of smaller sample size.

Methods
Sample one was consisted of 79 healthy subjects and 140 patients with schizophrenia, and most of subjects completed experiments with MMN and P50. In sample two, only less than 30 healthy subjects and around 85 patients with schizophrenia completed the experiments. All subjects were of Han Chinese ethnicity. Single nucleotide polymorphism (SNP) markers, including haplotype-tagged SNPs and SNPs with evidence of association with schizophrenia, from AKT1, CACNG2, COMT, GRM3, DISC1, DAO, NRG1, CHRNA7, GRIN1, GRIN2B, GRIN2C, GRIN2D, and GRID1 were genotyped. A total of 157 SNPs were genotyped in sample one, and 140 SNPs passed the quality control criteria. Nineteen SNPs were further genotyped in sample two.

Results
The association patterns were different in control group and case group. In sample one, CHRNA7 was associated with P50, and DAO was associated with MMN in case group. While NRG1 was associated with MMN in control group, GRIN2D and GRM3 were associated with MMN when not considering the affected status. In sample two, CHRNA7 still had borderline association with P50 in case group. DAO instead was associated with MMN in control group. GRM3 was associated with MMN in case group.

Discussion
The study found association of several schizophrenia candidate genes with electrophysiological markers. The differential association patterns related to affected status and sampling issue suggested that many uncontrolled/unknown factors exist to interact with these genes. Whether endophenotypes such as P50 and MMN are closer to the underlying genetic effects than the diagnosis of schizophrenia remains uncertain.

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EVIDENCE OF ENRICHMENT OF MICRORNAS IN SCHIZOPHRENIA AND BIPOLAR DISORDER
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Background
It has been suggested that genome-wide association studies (GWAS) have the potential to explain more of the ‘missing heritability’ of complex neuropsychiatric phenotypes. Several lines of evidence support the involvement of microRNAs (miRNA) and non-coding RNAs in both schizophrenia (SCZ) and bipolar disorders (BD). In the current study, we utilize a new analytical approach that makes use of GWAS results based on Psychiatric Genetics Consortium (PGC)
studies of SCZ and BD and miRNAs information from miRBase. We also investigated if any enrichment of miRNA is specific for SCZ and BD.

Methods
We employed a novel, recently developed genetic annotation-informed enrichment method for genome-wide association study (GWAS) that captures more of the polygenic effects in complex disorders and traits. In order to investigate if SNPs tagging miRNA regions (as a genomic category) are enriched in SCZ and BD, we first calculated the relative enrichment of miRNA tagging SNPs using the PGC summary statistics data for SCZ and BD. We then leveraged the enrichment using conditional False Discovery Rate (cFDR) to improve detection of individual miRNA SNPs associated with the disorders. Since many miRNAs have been shown to play distinct roles in somatic disorders, such as cardiovascular diseases, cancer and arthritis, we investigated if the enrichment of miRNA is specific for SCZ and BD by performing the enrichment test on twelve non-psychiatric phenotypes.

Results
We found that SNPs tagging miRNAs were more likely to be associated with SCZ and BD, discovering 19 SNPs tagging miRNA regions associated with SCZ and BD at the cFDR < 0.05 level, with 10 at cFDR < 0.01 level. The enrichment test on twelve non-psychiatric phenotypes revealed a general enrichment of miRNA SNPs across nearly all phenotypes investigated.

Discussion
SNPs in miRNA regions show a significant and consistent pattern of enrichment in SCZ and in BD. This suggests that miRNAs have an important role in the polygenic architecture of SCZ and BD, which is in accordance with the acknowledged pleiotropy between the two phenotypes. Leveraging this enrichment we identified 19 SNPs significantly associated with SCZ and BD. Further, the miRNA enrichment seems to be present across a diverse range of non-psychiatric phenotypes. This finding further underscores the important role of miRNA in complex traits and disorders. Examining miRNAs profiles in post-mortem brains and peripheral blood mononuclear cells could further elucidate their involvement in SCZ and BD. Since expression of miRNAs is influenced by environmental factors, the miRNA enrichment could help separating environmental and genetic factors involved in the development of these disorders.

A CASE-ONLY GENOME-WIDE ASSOCIATION AND SUBSEQUENT FINE-MAPPING STUDY OF SCHIZOPHRENIA CHARACTERIZED BY EARLY AGE AT ONSET AND ATTENTION DEFICIT
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Background
As schizophrenia is genetically and phenotypically heterogeneous, targeting subphenotypes with possible greater genetic loadings may help dissect genetic structure of schizophrenia. Our previous ordered subset linkage analysis, first by onset age and then by attention score, identified a subset of patients characterized by young age at onset and attention deficits that exhibited possible evidence of linkage on chromosome 2q22.1, with a maximum nonparametric logarithm of odds score of more than 7. In this study, we aimed to narrow down the linkage signal by conducting a case-only genome-wide association study by contrasting schizophrenia patients with early onset and attention deficits versus those without such features. The initial findings from this GWAS analysis were then subjected to replication in another independent sample of schizophrenia patients using a different platform as well as further fine mapping using more tag-SNPs around the initial SNPs with potential association.

Methods
This study adopted case only design by means of selecting schizophrenia patients from 557 families of co-affected sib-pairs in the Taiwan Schizophrenia Linkage Study. Two groups of affected individuals were selected for subsequent genotyping: 1) affected individuals from the subsets of families (243 families, 526 affected individuals) with earlier age at onset and more CPT deficits, which were obtained by ordered subset analysis (OSA); and 2) affected individuals in the remaining families (314 families, 681 affected individuals) with later age at onset and less CPT deficits. In total, we chose 95 schizophrenia patients with earlier age at onset and more CPT deficits and another 95 schizophrenia patients with later age at onset and less CPT deficits. These 190 subjects were then subjected to genotyping for 642,832 single-nucleotide polymorphisms (SNPs) using Affymatrix Axiom Genome-Wide CHB 1 Array Plate. Sample quality control was performed with sex inconsistencies, sample genotyping call rate, kinship and population stratification check. A total of 185 out of 190 patients with schizophrenia passed these quality checks and were subjected to control matching. For the replication of initial findings, the co-affected siblings (n = 182) of the initial 190 patients were genotyped for the selected SNPs using Illumina GoldenGate customized arrays. For the fine mapping, another 76 tag-SNPs around the initial findings were selected for genotyping in the pooled sample of original 190 patients plus their co-affected 182 siblings.

Results
A 87.7% of SNPs passed the quality control, including duplicate sample concordance, marker genotyping call rate, and exclusion criteria of minor allele frequency (MAF) <0.05 as well as Hardy-Weinberg equilibrium (HWE) <0.001. A final sample of 185 subjects (94 earlier onset patients and 91 later onset patients) and 563,740 SNPs were kept for the association analysis. A total of 17 SNPs located in 7 chromosomes were significant for the association at significance level of P <10^{-4}. On the region of 23.1Mb at chromosome 2q22.1, 4 SNPs were significant at P <10^{-3}. For the replication of initial selection of 21 SNPs (17 from genome-wide significance of P < 10^{-4} and 4 from chromosome 2q22.1 of P <10^{-3}), 20 SNPs were successfully genotyped using Illumina GoldenGate among the 182 co-affected siblings of the original 190 cases. At the significance level of P < 0.05, 7 from genome-wide and 3 from 2q22.1 region were replicated. In terms of fine mapping for the initial linkage signal at 2q22.1, 2 SNPs at HNMT exhibited
significant association (P < 0.05) in the pooled sample of original 190 patients plus their co-affected 182 siblings.

Discussion
By means of a genome-wide association analysis in a contrast of 94 patients with earlier age at onset and more CPT deficits with 91 patients with later age at onset and less CPT deficits, this study was able to identify 21 SNPs for further investigation. Among them, 10 SNPs were replicated in the co-affected siblings, with 3 around 2q22.1. Further fine mapping results implicate SNPs in HNMT as a potential modifier gene for early-onset and attention deficient schizophrenia.

POLYGENIC DETERMINANTS OF WHITE MATTER VOLUME DERIVED FROM GWAS LACK REPRODUCIBILITY IN A REPLICATE SAMPLE

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Background
A recent publication (Terwisscha van Scheltinga et al, Biological Psychiatry, 2013) reported an exciting polygenic effect of schizophrenia risk variants, identified by a large genome-wide association study (GWAS), on total brain and white matter volumes in schizophrenic patients and, even more prominently, in healthy subjects. Aim of the present work was to replicate and then potentially extend these findings.

Methods
According to the original publication, polygenic risk scores - using single nucleotide polymorphism (SNP) information of schizophrenia GWAS - (PSS) were calculated in 122 healthy subjects, enrolled in a structural magnetic resonance imaging study. These scores were computed based on p values and odds ratios available through the Psychiatric GWAS Consortium, including 9,394 schizophrenia cases and 12,462 healthy controls. Additionally, polygenic white matter scores (PWM) were calculated, using the respective SNP subset in the original publication.

Results
None of the polygenic scores, either PSS or PWM, were found to be associated with total brain, white matter or gray matter volume in our replicate sample. Minor differences between the original and the present study that might have contributed to lack of reproducibility (but unlikely explain it fully), are number of subjects, ethnicity, age distribution, array technology, SNP imputation quality and MRI scanner type.

Discussion
In contrast to the original publication, our results do not reveal the slightest signal of association of the described sets of GWAS-identified schizophrenia risk variants with brain volumes in
adults. Caution is indicated in interpreting studies building on polygenic risk scores without replication sample.

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**A GENOME-WIDE ASSOCIATION ANALYSIS OF A BROAD PSYCHOSIS PHENOTYPE IDENTIFIES THREE LOCI FOR FURTHER INVESTIGATION**

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**Background**

Genome-wide association studies (GWAS) have identified several loci associated with schizophrenia and/or bipolar disorder. We performed a GWAS of psychosis, as a broad syndrome, rather than within specific diagnostic categories.

**Methods**

1,239 cases with schizophrenia, schizoaffective or psychotic bipolar disorder, 857 of their unaffected relatives and 2,739 healthy controls were genotyped with the Affymetrix 6.0 SNP array. Analyses of 695,193 SNPs were conducted using UNPHASED, which combines information across families and unrelated individuals. We attempted to replicate signals we found in 23 genomic regions using existing data on non-overlapping samples from the Psychiatric GWAS Consortium (PGC) and SGENE-plus cohorts (10,352 schizophrenia patients and 24,474 controls).

**Results**

No individual SNP showed compelling evidence for association with psychosis in our data. However, we observed a trend for association with same risk alleles at loci previously associated with schizophrenia (one-sided \( P=0.003 \)). A polygenic score analysis found that the PGC’s panel of SNPs associated with schizophrenia significantly predicted disease status in our sample \( (P=5\times10^{-14}) \) and explained approximately 2\% of the phenotypic variance.

**Discussion**

Although narrowly-defined phenotypes have their advantages, we believe new loci may also be discovered through meta-analysis across broad phenotypes. The novel statistical methodology we introduced to model effect size heterogeneity between studies should help future GWAS that combine association evidence from related phenotypes. By applying these approaches we highlight three loci that warrant further investigation. We found that SNPs conveying risk for schizophrenia are also predictive of disease status in our data.

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**GENOME-WIDE ASSOCIATION STUDY OF FAMILIAL AND SPORADIC SCHIZOPHRENIA IN THE PSYCHIATRIC GENOMICS CONSORTIUM**

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Background
Genome wide association studies (GWAS) of schizophrenia (SZ) have yielded a burgeoning list of common susceptibility loci, and have further provided strong support for a substantial polygenic contribution of a large number of small effects. Of interest herein is the extent to which familiality of SZ is associated with enrichment for common risk variants detectable in a large GWAS.

Methods
We analyzed single nucleotide polymorphism (SNP) data for family-history positive (FH+) cases (N=1389), family-history negative (FH-) cases (N=4503), and controls (N=8285) from the Psychiatric Genomics Consortium (PGC1) study of SZ. We used a multinomial logistic regression approach with model-fitting in order to detect allelic effects specific to either the FH+ or FH- groups. We also considered a polygenic model, in which we tested whether the FH+ of FH- groups have different polygenic risk scores, on average. We contrasted these findings to those previously reported for independent samples of SZ cases and multiply-affected SZ pedigrees from Ireland.

Results
For all SNPs, the model-fitting procedure indicated that the best-fitting model did not distinguish between cases on the basis of FH. However, our analysis detected a suggestive association at 10q24.1 in BLNK (rs7099132, P< 1.94E-06) with the FH+ group, which had not previously yielded evidence of association in the original PGC study of SZ. Comparison of genome-wide polygenic risk scores based on several P-value thresholds indicated a slight enrichment for common SNP effects among FH+ compared to FH- cases (P=0.019); the score was most significant at a P-value threshold of 0.5. A similarly constructed score did not predict FH status in an independent sample of SZ cases from Ireland. However, unaffected members of multiply-affected Irish SZ families had significantly elevated scores compared to population controls.

Discussion
Our results suggest that allelic effects detectable in large GWAS of SZ do not demonstrate specificity to particular FH groups. However, consideration of a genome-wide polygenic risk score indicated a slight enrichment among FH+ cases for common polygenic effects. Given that a preponderance of affected persons are ‘sporadic’ cases, our failure to identify FH+ specific effects may reflect the fact that familial cases will be the minority in large, population-based cohorts and therefore have limited power. Alternatively, these results may simply reflect the unreliable nature of FH determination due to under-reporting of FH by both case individuals and their family members.

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A SIMPLE SPATIAL WORKING MEMORY AND ATTENTION TEST ON PAIRED SYMBOLS SHOWS DEFICITS IN PSYCHIATRIC PATIENTS

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Background

People with neuropsychiatric disorders such as schizophrenia often display deficits in spatial working memory and attention. Evaluating working memory and attention in schizophrenia patients is usually based on traditional tasks and the interviewer’s judgment. We developed a novel Spatial Working Memory and Attention Test on Paired Symbols (SWAPS) inspired by the Chinese historical jiugongtu (nine spaced grid).

Methods

The SWAPS test takes only several minutes to complete, comprising 101 trials for each subject. In this study, we tested 72 schizophrenia patients and 188 healthy volunteers in China.

Results

In a healthy control group with ages ranging from 12 to 60, spatial working memory and attention reached a peak in the 20-27 age range and then declined with increasing age. Importantly, schizophrenia patients failed to display this developmental trend in the same age range and adults had significant deficits compared to the control group.

Discussion

Our data suggests that this novel and simple SWAPS test can be a useful tool for studies of spatial working memory and attention in neuropsychiatric disorders. We also apply this test to major depressive disorders patients (n=24) from 30-40 years old finding that they present the similar deficits compared to their counterparts of control group.

INTEGRATING RARE CODING AND COMMON VARIATION IN SCHIZOPHRENIA WITH BIOLOGICAL NETWORK AND EXPRESSION DATA

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Background
In recent decades, scientists have investigated the role of various classes of inherited and de novo genetic variation in schizophrenia. On the one hand, the high heritability of schizophrenia and existing genetic data indicate the potential for many genes being involved in the mechanism of this “polygenic” disease. On the other hand, due to this same polygenicity and locus heterogeneity, it has remained quite difficult to confidently point to any one particular gene as playing a role in schizophrenia. We have recently completed our study of rare coding variation in schizophrenia, based on 2536 cases and 2543 controls from a Swedish national sample. This study could not unambiguously detect any single variant or gene as associated with disease risk, reflecting the low power of agnostic genome-wide scans in highly polygenic diseases such as schizophrenia.

**Methods**

We combine four genetically-informative sources to augment the analysis of the exome data in a principled Bayesian statistical approach: 1) association with disease from GWAS and 2) rare CNV studies, 3) protein-protein interaction network data, and 4) gene co-expression. Using these as a frame on which we place the rare genetic association data to weight and re-rank genes, we have developed statistical and graph-theoretic methods to identify individual genes (rather than broad genesets) that are likely to be involved in disease. All algorithms were programmed in C++ and added to the Plink/Seq software.

**Results**

Preliminary analyses and recent literature suggest that a rigorous statistical approach to integration of these disparate biological data sources will be helpful in teasing out genes most likely to be involved in disease. First, there has been some reported convergence of genes carrying both rare and common risk variants in schizophrenia (Gilman, 2012) and other related diseases such as autism (Ben-David, 2012), indicating that combining these signals should increase statistical power. In addition, it has been hypothesized that physical interaction between risk genes in common molecular pathways is the reason that some of these genes play a similar role in disease etiology (Rossin, 2011). Moreover, it has been shown that phenotype-associated SNPs are more likely to be eQTLs (Nicolae, 2010), suggesting that risk SNPs may mediate this risk via expression changes, making it plausible that some subset of genes with risk variants should be co-expressed. Lastly, intersecting protein-protein interaction data and tissue-specific gene expression data has proven useful in ranking disease genes (Magger, 2012).

**Discussion**

While larger exome studies may have sufficient power to discover rare variants using standard approaches, it is likely that power can be improved by augmenting the analytic approach with information from known psychiatric and neurobiological pathways and networks. Based on encouraging results in the Swedish study, we have followed this up by building a framework to take the exome sequencing statistics, in combination with external biological data, and dynamically discover genes that are coherent from a perspective of both genetic risk and brain biology.

References:

ASSOCIATION AND REPLICATION STUDY OF NPY2R PROMOTER VARIANT RS6857715 WITH ALCOHOL AND SMOKING BEHAVIOR IN PATIENTS AND THE GENERAL POPULATION

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Background

Human and animal studies respectively have shown that the neuropeptide Y (NPY)-system plays an important role in stress regulation and stress-triggered substance abuse. The COGA study, which tested the NPY and NPY receptor genes for association with alcohol and cocaine dependence in humans, found significant association between both phenotypes and the functional NPY2R promoter variant rs6857715.

Methods

In the present study, we replicated the association with alcohol dependence (AD) in 1333 male in-patients with a DSM-IV diagnosis of AD and 2168 controls (p=0.005) from Germany. To examine the contribution of this variant to substance use in the German general population, genotyping was performed in 756 individuals whose alcohol drinking and smoking behavior from early adulthood had been assessed retrospectively.

Results
Rs6857715 was not associated with alcohol consumption in either early adulthood or over the life-span. However, significant association was found with smoking quantity at age 20 years (p=0.011). This association became less significant in later life: age 30 (p=0.071); age 40 (p=0.237); and age 50 (p=0.045). Separate analysis in males and females revealed the respective p-values: 0.041, 0.025, 0.19 and 0.302 in females and 0.066, 0.479, 0.603 and 0.09 in males. We also tested a possible influence of the personality trait neuroticism on this association with smoking, as individuals who score highly for this trait are specifically sensitive to stress. This revealed a significant association between rs6857715 and neuroticism score (p=0.013), being a trend in women (p=0.087) and men (p=0.051). Risk allele carriers had higher neuroticism scores, as well as a significant interaction between rs6857715, neuroticism scores, and sex (p=0.032). Male risk allele carriers with very high neuroticism scores smoked more CPD than male non-risk allele carriers or men with low to medium neuroticism scores. We then attempted to replicate our findings in a sample of 6874 individuals of European descent from the general population of Australia. No association with drinking measures (alcohol consumption factor score and AD symptom count) was observed. However, a nominally significant association was found with the nicotine dependence (ND) factor score (p=0.05), and a trend towards significance was found for ND symptom count (p=0.085). No association with the personality trait neuroticism was observed.

Discussion
In summary, we present independent replication for association between AD and the rs6857715 variant in the NPY2R gene. We did not find an association in the Australian and German population-based samples with drinking measures. Sample characteristics may account for this observation. Neither in the COGA nor in our study AD were patients stratified for smoking. As smoking is highly co-morbid with AD, it cannot finally be decided whether the observed association in the COGA and our AD study is due to AD or smoking or to a combined phenotype. Our findings in the general population suggest that the risk variant confers a risk for increased smoking quantity. While the findings in the German population indicate a possible interaction with stress sensitivity, this could not be replicated in a much larger Australian sample. A possible reason for the lack of replication could be the age difference between the Australian and German sample. Individuals in the German sample were older than 60 years when neuroticism was assessed, while the mean age in the Australian sample was 38 years. Neuroticism scores decrease over the life-span; however, heritability estimates for neuroticism remain stable. The causal (genetic) factors of very high neuroticism scores in older age may therefore differ from the ones of high neuroticism score in adolescents and adulthood.

INTERACTION ANALYSIS BETWEEN ALPHA5 AND ALPHA7 NICOTINIC SUBUNITS IN NICOTINIC REPLACEMENT TREATMENT
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Background
Nicotine replacement therapy (NRT) in the form of patches, gum or inhaler is the most popular treatment for quitting smoking; however, NRT is effective for only a fraction of smokers. Therefore, research is needed to maximize the successful treatment of smokers who want to quit.
The purpose of this study is to examine genetic variants that may contribute to nicotine dependence and variable therapeutic response to NRT. The specific aim of this project is to examine the role of genetic variation in the α5 and α7 nicotine receptor subunits in NRT treatment outcome.

**Methods**
The effectiveness of different types of NRT was examined in a cohort of 314 Caucasian participants from an open-label smoking cessation study in Southern Ontario. Subjects received 10-weeks of NRT treatment and self-report abstinence was verified by expired carbon monoxide levels. The DNA extracted from blood samples was tested for the D398N variation (rs16969968) in the gene that encodes for the nicotinic subunit α5. We employed a logistic regression model including age and gender, coded additively for the N398 allele.

**Results**
We found a slight trend for the allele N398 associated with treatment response (95% CI OR = 1.349 [0.946, 1.923], \( p = 0.098 \)). The interaction analysis including the variant N398D and the STR D15S1360 did not produce any significant association with NRT response. Replication in a cross-population, multi-site paradigm is necessary to confirm that this marker reliably predicts NRT outcome.

**Discussion**
Replication in a cross-population, multi-site paradigm is necessary to confirm that this marker reliably predicts NRT outcome.

ASSOCIATION BETWEEN THE D4 EXON III VNTR POLYMORPHISM AND HARM AVOIDANCE LEVELS IN HEROIN-DEPENDENT PATIENTS
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**Background**
Harm Avoidance (HA) is a temperament dimension specified in Cloninger's psychobiological model of personality. However, the mechanisms leading to HA are unclear. Significantly high scores of HA are observed in heroin-addicts, in comparison with subjects with other substance addictions and controls(Grucza, Przybeck et al. 2005, Teh, Izuddin et al. 2012). In addition, heroin-addict subjects present a high level of suicidal ideation and deaths attributed to suicide range from 3–35% in these patients. Several studies suggest that genetic variants may contribute to dopamine alterations associated with heroin addiction and HA.

**Methods**
We investigated the influence of a functional VNTR polymorphism in exon III of the D4 receptor gene in a cohort of methadone maintained heroin-dependent patients (N=215) and controls (N=145) to investigate their contribution, using PCR standard techniques.

**Results**
an association between the D4 exon III VNTR polymorphism and HA levels in heroin-dependent patients was observed when considering antidepressants and duration of methadone treatment (Wald coefficient= 11.76, p=0.001).

Discussion
Our results suggest that alterations in the dopamine system contribute, at least partially, to this temperament dimension, although other neuropathways may also contribute.

COLLABORATIVE META-ANALYSIS DEMONSTRATES THAT THE OPRM1 VARIANT RS1799971 (A118G) IS MODESTLY ASSOCIATED WITH RISK FOR NON-SPECIFIC SUBSTANCE DEPENDENCE
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Background
The mu1 opioid receptor gene OPRM1 has been a high priority candidate for human genetic studies of substance dependence. Because of its potential functional significance, the non-synonymous single nucleotide polymorphism (SNP) rs1799971 (A118G, Asn40Asp) in particular has been extensively studied, yet its role in substance dependence liability in humans has remained unclear, with conflicting findings in the literature.

Methods
We conducted a collaborative meta-analysis based on new analyses of published and unpublished datasets to clarify the effect, if any, of this variant on risk for substance dependence. Starting with 25 datasets and over 28,000 subjects of European ancestry, we examined general, non-specific substance dependent cases compared to controls who are non-dependent on all assessed substances, and also five specific substance dependence diagnoses (alcohol, opioid, marijuana, and cocaine defined by DSM-IV dependent versus non-dependent; nicotine dependence proxy of heavy/light smoking (cigarettes-per-day (CPD) > 20 versus ≤ 10). Analysis scripts were developed centrally and distributed to each data-holding site.

Results
The G allele of rs1799971 shows a small protective effect on non-specific substance dependence (OR = 0.90, 95% C.I. (0.83-0.97), p-value = 0.0095, N = 16,910). This effect on non-specific substance dependence does not appear to be driven by dependence on any one particular substance: we observed a protective effect of roughly similar size (odds ratio) in analyses of each of the five substance-specific subsets of general dependence cases compared to the same controls; these were not statistically significant, likely because of reduced sample size and power.

Discussion
This project, the first collaborative genetic meta-analysis to analyze and compare these non-specific and specific substance dependence diagnoses, was able to demonstrate a significant but small effect of this variant on the endpoint of substance dependence. This work demonstrates the
importance of collaborative data sharing and meta-analyses as a powerful tool to gain genetic and biological insight into complex behavioral phenotypes such as addiction.

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ESTIMATION OF GENETIC COVARIANCE AMONG DSM-IV SYMPTOMS OF ALCOHOL DEPENDENCE
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Background
Alcohol dependence (AD) is a multifactorial disorder defined by the Diagnostic and Statistical Manual of Mental Disorders as a maladaptive pattern of drinking that leads to significant neurobiological, cognitive, and/or social impairment. Consequently, AD is a phenotypically heterogeneous disorder which poses a significant problem in the identification of genetic factors. The symptomatology of AD has been demonstrated to represent alternative forms of an underlying dimension of risk. Several studies also suggest that the genetic architecture of the underlying risk is more complex and likely involves multiple genetic factors, however, it remains unclear what proportion of variation in AD symptoms is attributable to common genetic variants across the genome; furthermore, how much of it is common across symptoms of AD.

Methods
Using DSM-IV AD symptom data ascertained from 4181 individuals participating in the Study of Addiction: Genes and Environment (SAGE; dbGAP Accession: phs000092.v1.p1), we utilized SNP-derived genomic relationship matrices to estimate the variation in each AD symptom captured by SNPs (as captured by the Illumina 1M chip), as well as the degree of genetic covariation across DSM-IV AD symptoms. All variables were adjusted for age effects, originating study sample (i.e., COGA, COGEND, or FSCD), and sociodemographic factors.

Results
The proportion of variance/covariance attributable to common causal variants (CCVs) was estimated using REML in genome complex trait analysis. CCVs (MAF > 0.01) accounted for 24% (standard error: 0.08). Further, correlations between symptoms ranged from 0.29 to 0.97, suggesting shared genetic etiology

Discussion
These results suggest that individual differences in AD and DSM-IV AD symptoms can be explained by CCVs.

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GENETIC PREDISPOSITION TO ALCOHOLISM IS HIDDEN IN JUNK DNA
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Background
Alcoholism is a complex, multigenic disorder of unclear molecular underpinnings with a strong genetic component. Analysis of protein-coding regions within alcoholism susceptibility loci only partially determined the genetic basis of alcoholism. Interestingly, the vast majority of the human genome does not encode proteins. The most prominent members of non-coding genes are microRNAs. They encode small RNA molecules, which serve as very powerful regulators of mRNA levels and protein expression. Importantly, many microRNAs can simultaneously control expression of multiple genes. We have shown previously that a particular microRNA, miR-9, is regulated by alcohol and has an essential role in the development of molecular tolerance to alcohol. Alcohol sensitivity of miR-9 has been also indicated by others. Here, we wanted to establish whether single nucleotide polymorphisms (SNPs) in regions important for miR-9 expression are associated with alcoholism in humans.

Methods
There are three distinct miR-9 genes in humans, each additionally embedded within a separate host gene. Therefore, expression of each miR-9 gene is controlled by its own promoter and also by a host gene promoter. We performed SNP discovery in all 6 regions (4-6 kb in length each). We used 282 alcoholic samples (from the Collaborative Studies on Genetics of Alcoholism (COGA) collection) and 255 non-alcoholic controls (from the NIMH control collection) to perform nested PCR and direct sequencing of PCR products (total – 50,000 sequencing reactions).Correction for ancestry was performed using ancestry informative markers obtained from Rutgers University DNA and Cell Repository, and from GWAS data. Association was determined separately for European- and African-Americans. Allele frequency, odds ratio, heterozygosity and Hardy-Weinberg equilibrium was determined for each sample. To adjust for multiple comparisons, meta-analysis by Holm’s method was performed. The effects of SNPs on transcription factors binding were determined using MAPPER2.

Results
We established that out of 142 SNPs tested, 9 SNPs are most significantly (p < 0.002) associated with alcoholism. None of these SNPs were located within any miR-9 gene itself, but rather all of them were located in the promoter regions. 2 SNPs were located in mir-9-1 host gene promoter, 2 in mir-9-1 gene promoter, 2 in mir-9-2 gene promoter and 3 in mir-9-3 host gene promoter. At each SNP, a switch from major to minor allele could significantly change binding of transcription factors, e.g. AP4, MyoD, SMAD4, Pax5 or MYC:MAX, by either decreasing binding affinity or creating a new binding site. Additionally, 4 of these SNPs are potential methylation sites, with 2 located within CpG islands.

Discussion
Our results reveal the presence of alcoholism-associated SNPs in non-coding regions. Moreover, we describe mutations in miR-9 promoters, which could change binding of specific transcription factors and thus change expression levels of mature miR-9. These mutations could lead to faster development of drug tolerance in alcoholics. In summary, these findings identify novel genetic underpinnings and molecular mechanisms of the development of alcohol addiction.