Thursday, October 17, 2013

EDUCATION DAY SESSIONS
TRACK 1
8:30 A.M. – 9:30 A.M.

THE GENETICS OF ADHD AND AUTISM SPECTRUM DISORDERS
Steve Faraone, SUNY Upstate Medical University

The existence of comorbidity between attention deficit hyperactivity disorder (ADHD) and autism spectrum disorders (ASDs) has been documented in clinical and epidemiologic studies, and in samples ascertained via ADHD or ASD diagnoses. This lecture will review the data providing evidence for the heritability of each disorder along with evidence for shared genetic risk factors. It also reviews recent genome wide association and sequencing studies and the evidenced they provide for the role of specific common and rare variants in the etiology of these disorders.

TRACK 2
8:30 A.M. – 9:30 A.M.

INTRODUCTION TO THE ANALYSIS OF RARE VARIANTS: STUDY DESIGNS AND TESTING APPROACHES
Ben Neale, ATGU, Massachusetts General Hospital

With the development of sequencing technologies near comprehensive capture of genetic variation is now a reality. Much of this newly discovered variation is rare and as a consequence requires different analytic strategies to those traditionally used to assess evidence for association for common variation. Similarly, rare variant analysis may benefit from different study designs in contrast to those used for common variants. Recent developments in the analysis of rare variants can be classed into two domains: burden approaches such as GRANVIL and the combining and collapsing method which assess differences in the rate or amount of rare variation across groups and distributional approaches such as SKAT and C-alpha which assess whether the pattern of rare variation observed in the sample correlates with phenotype. The distributional approaches are robust to variation that may increase or decrease risk or a phenotypic value while the burden approaches assume rare variation in the region affect the phenotype in a consistent direction. Beyond these methods, other classes of rare variation such as spontaneously arising mutations necessitate tailored analytic methods. In this session, all of these methods will be reviewed to provide a global view of how best to leverage rare variation for identifying regions of interest in psychiatric disease and behavioral phenotypes.

TRACK 1
9:30 A.M. – 10:30 A.M.

UPDATE ON GENETICS OF ADULT PSYCHIATRIC DISORDERS
Francis McMahon, Human Genetics Branch, NIMH, NIH

Our understanding of the genetic basis of adult psychiatric disorders such as bipolar disorder and schizophrenia has advanced substantially in recent years. Thanks to high-throughput genotyping and the accumulation of large sample sizes, we have for the first time begun to identify individual genes or genomic regions that contribute to risk. Genome-wide association studies have uncovered common genetic markers robustly associated bipolar disorder, schizophrenia, or both disorders. While no common alleles have a large impact on risk, taken together common variants could account for an important fraction of psychiatric illness in the population. Studies of copy number variants (CNVs), which involve deletion or duplication of small chromosomal segments, have found larger effects on disease risk in a small fraction of cases, but the sizes of the identified CNVs are often large enough that multiple genes are implicated. Most CNVs appear to arise de novo in affected offspring, but a few are inherited or recurrent in the population. High-throughput sequencing studies are beginning to identify additional de novo events that contribute to schizophrenia. Dr. McMahon will review these findings and give an overview of our current understanding of the genetic causes of psychiatric illnesses arising in adulthood and the implications for clinical psychiatry.

TRACK 2
9:30 A.M. – 10:30 A.M.

USING PLURIPOTENT STEM CELLS TO UNDERSTAND MAJOR MENTAL ILLNESS
John Madison, The Broad Institute

Mental illnesses such as bipolar disorder and schizophrenia are a significant burden to patients, families and society. Despite evidence for high heritability, the etiology remains poorly understood. In light of the high heritability, genome wide association studies (GWAS) have been used to identify genes and pathways associated with these mental illnesses. With what is proving to be a highly complex genetic landscape, existing cellular approaches that model changes in single or small numbers of genes across development in a disease relevant tissue are not well suited to capture all the genetic factors interacting in an individual. Human cells that capture the genetics and provide a renewable source of cells to study the pathways implicated by these genetic loci would further our understanding of the etiology of these diseases and also provide a platform to screen for novel therapeutics that ameliorate disease associated phenotypes. To investigate disease etiology of psychiatric disorders, and to eventually develop phenotype-based assays for chemical screening, biologists are turning to human pluripotent stem cells as a platform to understand the complex biology of these mental illnesses. In addition to embryonic stem cells which allow the study of the development and function of human neurons and neural progenitors in vitro, advances in stem cell biology now permit derivation of pluripotent stem cells from adult somatic tissues (induced pluripotent stem cells or iPSCs) of affected patients and unaffected individuals. Derivation of these patient specific cells allows for the identification of phenotypic differences between affected and unaffected individuals. Furthermore, recent development of genome editing tools such as TALENs or the Cas9/CRISPR system now allow for the rigorous testing of variant or gene function in cells through the introduction or repair of specific variants or mutations. Marrying genome editing tools with the ability to differentiate these modified pluripotent stem cells into subtype specific neurons now

allows for the rigorous testing of causal disease biology in disease relevant tissue. While these approaches hold great promise, ensuring the robust, reproducible differentiation at both the level of the pluripotent stem cell and the derivation of the neuronal cells still remains a challenge. Despite these challenges, human pluripotent stem cells still offer a promising approach to novel insights into the etiology of these complex psychiatric disorders and paths toward novel targeted therapeutics for their treatment.

TRACK 1
10:45 A.M. – 11:45 A.M.

AN UPDATE ON THE GENETICS OF SUBSTANCE USE PROBLEMS: AN INTEGRATED APPROACH TO FIND GENES AND UNDERSTAND THEIR IMPACT
Danielle M. Dick, Virginia Commonwealth University

Substance use disorders are common and costly, to the affected individual, their family and friends, and society as a whole. Substance use disorders represent challenging and heterogeneous conditions. In this talk I will provide an overview of what we currently know about the genetics of substance use problems, with emphasis on how different study designs can contribute to understanding substance use outcomes. While twin studies were pivotal in demonstrating the degree to which psychiatric outcomes are genetically influenced, they can also provide valuable information about how genetic influences change across development and in conjunction with the environment. This information can then be used to aid in gene identification, and in understanding the risk associated with identified genes. I will review a series of studies illustrating how this approach has contributed to our understanding of the genetics of substance use problems. I will review the current status of gene finding studies and discuss integrated efforts across human and model organism systems in understanding genetic contributions to ethanol response. Finally, I will discuss the application of gene findings to improving prevention and intervention efforts in the area of substance use.

TRACK 2
10:45 A.M. – 11:45 A.M.

AN INTRODUCTION TO ENCODE AND NONCODING GENETIC VARIATION
Kai-How Farh

Genome-wide association studies have identified thousands of genetic loci associated to human traits and diseases. However, progress towards understanding the mechanisms by which sequence variation leads to disease phenotype has been tempered by the fact that only ~12% of GWAS hits alter protein-coding sequence, indicating that most variants exert their effects through noncoding mechanisms, potentially by affecting cis-regulatory elements and gene expression. Recent studies in the field of epigenomics have identified noncoding regulatory elements in a wide range of tissue types, and have shown that noncoding variants identified by GWAS preferentially fall within the enhancers of relevant cell types. These noncoding disease-associated variants represent a unique opportunity to study how changes in regulatory DNA

sequence lead to disease, and have the potential to advance our understanding of disease pathophysiology and yield novel insights into the mechanisms of gene regulation.

TRACK 1
1:00 P.M. – 2:00 P.M.

PHARMACOGENETICS: USING GENETIC INFORMATION TO ENHANCE TREATMENT
Anil K. Malhotra, The Zucker Hillside Hospital

Pharmacogenetics offers the prospect of the identification of readily accessible biological predictors of psychotropic drug response, may provide information about the molecular substrates of drug efficacy, and guide new drug development strategies for the treatment of psychiatric disorders. In this presentation, we will review basic methodological concerns encountered in pharmacogenetic studies. These include design issues, power considerations, appropriate outcome measures, and issues pertaining to candidate gene and GWAS studies. These issues will be discussed in the context of pharmacogenetic studies of antipsychotic drug response – including data implicating the dopamine D2 receptor gene in antipsychotic drug efficacy, as well as results suggesting that genetic factors may be highly predictive of key adverse events associated with treatment. The implication of these developments for the state of the art treatment of psychiatric disorders will be discussed.

TRACK 2
1:00 P.M. – 2:00 P.M.

ADVANCES IN SEQUENCING TECHNOLOGIES
Mark Depristo, Broad Institute

The Medical and Population Genetics Program and the Genomics Platform at the Broad Institute have sequenced more than 40 thousand samples to date, across a variety of diseases from the common (Diabetes, Autism, and Heart Disease) to the uncommon or even rare such as Crohn's and other Mendelian disorders. The overarching goal has been to use NGS technologies to advance our understanding of the genetic underpinnings of human disease. Delivering on this effort requires us to analyze tens of thousands of samples, discover and genotype genetic variation accurately, and drive forward experimental technologies themselves to produce the best possible data within our budget constraints.

Variation among individuals in a population is typically split into 90% SNPs, and nearly 10% indels. However, when it comes to disease-causing variation, particularly in rare diseases, these ratios approach 50% / 50%. Today SNPs are well-handled by state-of-the-art tools like the GATK; indels, however, remain an outstanding challenge, for a variety of technical and analytic reasons which we discuss here in detail. In fact, the basic difficulties with indels provide an excellent framework to understand the limitations of today's sequencing technologies and opportunities for future advances. In this work, we discuss the steps we have been taking to overcome these challenges in the past four years. In particular, our best-practices for discovering

and analyzing SNPs and Indels from NGS data. We assess how the latest sequencing technologies, latest library construction methodologies, capture methods, and analytic tools improve our better understanding of the human genome.

TRACK 1
2:00 P.M. – 3:00 P.M.

CLINICAL GENETIC TESTING FOR NEUROPSYCHIATRIC DISORDERS
David Miller, Boston Children's Hospital Amphitheater

Genetic and genomic research on neuropsychiatric disorders is revealing numerous genes and genetic variants that contribute to disease risk. This leads to the hope that clinical genetic testing might be able to help in caring for patients with neuropsychiatric disorders. Dr. Miller will review what is known about the inheritance and "genomic architecture" of common neuropsychiatric disorders such as autism and schizophrenia, including newer research findings based on whole exome sequencing. He will provide an overview of currently available testing platforms, especially chromosomal microarray and next generation sequencing, and how they are applied for clinical genetic testing. Dr. Miller will also touch upon the issues of clinical impact of genetic testing, as well as other factors that will influence the utilization of such tests.

TRACK 2
2:00 P.M. – 3:00 P.M.
RNA-SEQ: BASICS, BRAINS AND SINGLE-CELLS
Jim Knowles, University of Southern California

The introduction of Next-Generation DNA sequencing (NGS) has enabled the facile sequencing of entire cDNA libraries. This method is called RNA-Seq and allows for whole transcriptome measurement and discovery of coding, non-coding, small (e.g., miRNA, piRNA) and novel RNA molecules. In comparison to whole transcriptome analysis using microarrays, RNA-Seq is more accurate and reproducible, and requires less pre-existing knowledge of the specific RNA transcripts which will be measured. While the laboratory cost of RNA-Seq and microarrays are similar, RNA-Seq requires substantially more post-processing of the raw data to arrive at gene expression levels, with more sophisticated analysis requiring more analytic time.

This session will cover the basic process of RNA-Seq from library preparation through data analysis. Subsequently, detailed comparisons of different modalities of RNA-Seq will be introduced and discussed, including single-cell analysis. This will be followed by a discussion of the various software packages available for the analysis of RNA-Seq with attention to the pros and cons of each. Lastly, more sophisticated analyses of RNA-Seq data will be illustrated using examples from my work (Brain Span) and the literature.

TRACK 1
3:15 P.M. – 4:15 P.M.

PSYCHIATRIC GENETIC COUNSELING: THE CLINICAL APPLICATION OF INFORMATION ABOUT THE GENETICS OF PSYCHIATRIC ILLNESS
Jehannine Austin, UBC Departments of Psychiatry and Medical Genetics

Our knowledge about the etiology of psychiatric disorders is developing rapidly. This presentation will focus on the psychosocial implications of this knowledge, and how to apply it in the clinical setting for optimal benefit of patients/families.

TRACK 2
3:15 P.M. – 4:15 P.M.

EPIGENETICS AND PSYCHIATRIC DISEASE
Schahram Akbarian, Mount Sinai School of Medicine

A comprehensive exploration of the human genome will have to extend far beyond the fine mapping of its linear sequence (6 billion basepairs in diploid cells). To this end, it is the 'epi-(greek for 'over', 'above')genome', with its rich cache of highly regulated, structural modifications of DNA cytosines, histone residues and variants, and various other determinants of chromatin structure and function, which in concert define the moldings and three-dimensional organization of the genomic material inside the cell nucleus. Epigenetics is important from the viewpoint of psychiatric genetics because (1) up to 4% of the human genome is thought to maintain some of its epigenetic decorations, including DNA methylation, during germline transmission and (2) the vast majority of genome sequence is subject to ongoing and dynamic epigenetic regulation across the lifespan, often in tissue- and cell-type specific manner and (3) promising epigenetic drug targets have been identified in preclinical models for cognitive and affective disorders.

PLENARY
5:30 PM – 6:30 PM

OVERALL ABSTRACT:
OMICS AND THE BRAIN PROJECT: INNOVATIVE NEUROTECHNOLOGIES FOR DIAGNOSES AND THERAPIES

George Church, Ph.D., Harvard Medical School

Our ability to view and alter biology is progressing at an exponential pace faster even than electronics. Next generation sequencing (fluorescent and nanopore) can be used to assess inherited, environmental and epi- genomes. We can now systematically synthesize/edit millions of genomic variants, enabling us to move from mere correlation to causality studies - connecting genomics + environments to traits. A growing set of CRISPR technologies enable similar numbers of epi-genetic variants to drive human pluripotent stem cells. We test all of these technologies and hypotheses using data and cells from the world's only fully shareable genomics resource (http://Personalgenomes.org).

Friday, October 18, 2013

PLENARY
8:30 AM – 10:30 AM

OVERALL ABSTRACT:
DEFINING MENTAL ILLNESS THROUGH GENETICS

Steve Hyman, M.D., Broad Institute

The diagnosis and classification of mental illnesses have recently generated tremendous controversy. The current system of diagnosis is based on constellations of symptoms that have been defined by a consensus of experts. While the diagnostic criteria embodied in the DSM and ICD have provided a common language for clinicians and improved the reliability of diagnosis, their validity has been widely challenged. Psychiatry remains alone among medical specialties in defining disorders without reference to etiology or pathologic findings. The best-established risk factor for major psychiatric illnesses is genetic vulnerability, but accumulating evidence suggests that genetic influences on the clinical entities defined by the DSM and ICD may cross traditional diagnostic boundaries. The National Institute of Mental Health has recently launched an effort (the Research Domain Criteria, RDoC) to reconceptualize mental illness "from the bottom up", drawing on neuroscience and genetics to provide an alternative to a purely descriptive nosology. This Plenary Panel brings together thought leaders and leading researchers to address the issue of what genetic research can tell us about the structure and boundaries of psychiatric disorders.

ADDITIONAL SPEAKERS:
Jan Buitelaar, M.D., Ph.D., Radboud University Nijmegen Medical Centre
Bruce N. Cuthbert, Ph.D., NIMH
Kenneth Kendler, M.D., Virginia Commonwealth University
Mike Owen, Ph.D., Cardiff University
Myrna Weissman, Ph.D., Columbia University

ORAL PRESENTATIONS
10:45 AM – 12:15 PM

ALCOHOL AND SUBSTANCE USE DISORDERS

INDIVIDUAL ABSTRACT:
A GENOMEWIDE ASSOCIATION STUDY OF ALCOHOL DEPENDENCE IN THE IRISH AFFECTED SIB PAIR STUDY OF ALCOHOL DEPENDENCE

Amy Adkins, Ph.D.[1], L.M. Hack, Ph.D.[2], T.B. Bigdeli, Ph.D.[3], B.T. Webb, Ph.D.[2], J.C. Bettinger, Ph.D.[4], A.G. Davies, Ph.D.[4], M.S. Grotewiel, Ph.D.[5], C.A. Prescott, Ph.D.[6], D.M. Dick, Ph.D.[7], K.S. Kendler, M.D., Ph.D.[7], B.P. Riley, Ph.D.[7]

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University of Southern California, [7]Dept. of Human and Molecular Genetics, Dept. of Psychiatry, Virginia Institute for Psychiatric and Behavioral Genetics, Virginia Commonwealth University

Background: The powerful, systematic, and unbiased genome wide 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 genome wide significance with 12 falling in the collagen 6A3 (*COL6A3*) gene on chromosome 2 and 1 located in an inter genic 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).

INDIVIDUAL ABSTRACT:

METHYLOME-WIDE ASSOCIATION STUDY IDENTIFIES CNTN4 AS AN EPIGENETIC RISK FACTOR FOR ALCOHOL USE

Shaunna Clark , Ph.D.[1], Karolina Aberg, Ph.D.[2], Gaurav Kumar, Ph.D.[2], Joseph McClay, Ph.D.[2], Andrey Shabalin, Ph.D.[2], Swedish Schizophrenia Consortium[3], Christina Hultman, Ph.D.[3], Patrik Magnussen[3], Patrick Sullivan, M.D.[4], Edwin van den Oord, Ph.D.[2]

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Background: Despite extensive anti-drinking efforts, 8.5% of US adults abuse or are dependent on alcohol. Twin studies suggest that genetic factors play a role in various aspects of drinking behavior. Identifying specific genetic variants, however, has proven difficult and the predictive power of the reported variants is typically very modest. DNA methylation studies represent a promising complement to these genetic studies focusing on sequence variation. Methylation studies of alcohol have historically been restricted to candidate genes in small sample sizes. More recent studies take a wider approach but only focus on less than 2% of all human CpG sites. This study takes comprehensive view by using next generation sequencing to screen the 28 million human CpG sites for association with alcohol.

Methods: We performed a methylome-wide association study (MWAS) of alcohol use in a sample of 619 individuals, which included 4% non-drinkers, 62% monthly and 34% weekly drinkers. To investigate all CpGs in the human genome, we used a methyl-CpG binding domain protein to enrich for the methylated genomic fraction in combination with next generation sequencing (MBD-seq). Each sample had, on average, 67.7 million fragments, for a total of 4.19 trillion sequenced fragments.

Results: After quality control and collapsing highly correlated CpGs into "blocks", 4.2 million high quality blocks were left for testing with alcohol use (λ=1.04). The MWAS showed a number of highly significant findings with 68 blocks (160 CpGs) with *p*-values < 1E-07. The top finding (*p*-value = 1.9E-09, *q*-value = 0.008) was a block with two CpGs located in an intergenic region of chromosome 20. A critical question involves the causal direction of these significant methylation-alcohol associations as methylation changes can be the result of alcohol use or methylation can contribute to susceptibility for alcohol addiction. To address this question, we combined our MWAS results with GWAS results from the same individuals to test if there is a relationship present. Our top findings in genes included *MYBPC1* (*p* = 2.09E-08; *q = 0.012*) and *CNTN4* (*p* = 2.54E-08; *q* = 0.013). We aimed to replicate our top five findings in an independent sample of 537 individuals using highly quantitative targeted bisulfite pyrosequencing, however, only *CNTN4* replicated (*p*-value= 0.041). As a final step, we fit casual models to the replicated site to test specific disease mechanisms. Specifically, we tested whether methylation mediates the effect of the SNP on alcohol use (LL=-1902, df = 5, BIC=3835), or if alcohol use causes changes in methylation (LL=-1916, df= 5, BIC = 3863). We found that methylation in *CNTN4* mediates the relationship between rs1382875 and alcohol use.

Discussion: *CNTN4* is a good candidate for futher investigation because it is known to play a role in the regulation of synaptic plasticity that can lead to reorganization of neural circuits, which is thought to contribute to addictive behavior. With this approach, we identified *CNTN4* as an alcohol use risk factor that provides insight into alcohol epigenetics.

INDIVIDUAL ABSTRACT:

INTERPLAY BETWEEN ADH VARIANTS AND SMOKING IN MILESTONES OF ALCOHOLISM DEVELOPMENT IN ADOLESCENTS AND YOUNG ADULTS

Emily Olfson[1], Howard J Edenberg, Ph.D.[2], John Nurnberger Jr., M.D., Ph.D.[2], Arpana Agrawal, Ph.D.[1], Kathleen K. Bucholz, Ph.D.[1], Victor M. Hesselbrock, Ph.D.[3], John R. Kramer, Ph.D.[4], Samuel Kuperman, M.D.[4], Jay A. Tischfield, Ph.D.[5], Laura J. Bierut, M.D.[1]

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Background: The development of alcoholism requires the initiation of a first drink followed by a chain of behavioral steps influenced by genetic and environmental factors (Bierut 2011). Variants in alcohol dehydrogenase (ADH) enzymes have been well studied in the development of alcoholism in adults, and specifically, rs1229984 (*ADH1B*2*) has been shown to be protective in European and African ancestry populations (Edenberg 2007, Bierut 2012). The next step is to understand how ADH variants influence drinking behaviors in adolescents and young adults. This is critical because drinking initiation often occurs in early adolescence. Smoking is also associated with the development of alcoholism and can alter normal brain development throughout adolescence and into young adulthood. By examining specific steps in the development of alcoholism, we clarify how rs1229984 shapes the progression to alcoholism, and how its effect can be altered by smoking.

Methods: The Collaborative Study on the Genetics of Alcoholism (COGA) is a large multi-center family study to identify genes that influence risk for alcohol dependence. Since 2005, COGA has used a prospective design focusing on adolescents and young adults. These participants are interviewed using the Semi-Structured Assessment for the Genetics of Alcoholism (SSAGA) every two years for a total of four interviews thus far. Using this longitudinal sample of 2,301 European ancestry and 917 African ancestry adolescents and young adults with an average age of first interview of 16 + 3 years, we performed Cox proportional hazards regression to model steps in the development of alcoholism. Specifically, we modeled age of first drink, age first got drunk, regular drinking onset, first symptom onset, and DSM-IV alcohol dependence onset with the covariates sex, race, study site, rs1229984, and having smoked at least one full cigarette.

Results: Rs1229984 is associated with decreased risk for first symptom onset (HR= 0.74, p=0.03), but not for earlier drinking steps, such as age of first drink (HR=1.04, p=0.64). An effect on later drinking steps but not earlier ones is consistent with the variant's hypothesized mechanism of action of increasing acetaldehyde accumulation leading to adverse effects of alcohol consumption. In contrast, smoking a full cigarette is associated with increased progression to both early and late drinking steps, including age of first drink (HR=1.05, $p<0.0001$) and first symptom onset (HR=1.24, $p<0.0001$). In addition to increasing progression to alcoholism, smoking is associated with attenuation of the protective effect of rs1229984 as demonstrated by the significant interaction term of rs1229984 and first full cigarette in the analysis of first symptom onset (p=0.05). This is clearly illustrated in Figure 1 where among individuals who have had a full cigarette, the rs1229984 protective genotype (GA or AA) is not associated with reduced progression to first symptom onset.

Discussion: Here, we demonstrate the interplay between genes and environment in the transition from early alcohol use to dependence in adolescents and young adults. Rs1229984 is not associated with first drink but has a protective effect early in the course of alcoholism development, consistent with its hypothesized mechanisms of action. Smoking is associated with

increased risk for both early and late drinking behaviors, and furthermore, smoking is associated with attenuation of the protective effect of rs1229984 in the progression to alcoholism. This provides insight into how different genotypes and environmental factors shape the trajectory of alcohol dependence development in adolescents and young adults and can inform the implementation of effective alcohol use prevention programs. References: Bierut LJ (2011) Genetic vulnerability and susceptibility to substance dependence. Neuron 69(4):618-27. Bierut LJ, Goate AM, Breslau N, et al (2012) ADH1B is associated with alcohol dependence and alcohol consumption in populations of European and African ancestry. Mol Psychiatry 17(4):445–450. Edenberg HJ (2007) The genetics of alcohol metabolism: role of alcohol dehydrogenase and aldehyde dehydrogenase variants. Alcohol Res Health 30(1):5–13.

INDIVIDUAL ABSTRACT:
SHIFT IN EPIGENETIC MECHANISMS IN HUMAN BRAIN: DYSREGULATION OF THE OPIOID GENES IN ALCOHOLICS

Georgy Bakalkin, Ph.D.[1], Igor Bazov, Ph.D.[1], Tatiana Yakovleva, Ph.D.[1], Hiroyuki Watanabe, Ph.D.[1], Olya Kononenko[1], Richard Henriksson[1], Alexandr Kuzmin, Ph.D.[1]

[1]Uppsala University

Background: Epigenetic dysregulations in the endogenous opioid system (EOS) specific for different stages of an addiction cycle may differentially contribute to the initiation and maintenance of alcohol dependence. We recently evaluated whether the EOS is altered in brain areas involved in cognitive control of addiction including the dorsolateral prefrontal cortex (dl-PFC), orbitofrontal cortex (OFC) and hippocampus, in human alcohol dependent subjects (Bazov et al., 2011; Taqi et al., 2011). Prodynorphin (*PDYN*) mRNA and dynorphin opioid peptides in dl-PFC, κ-opioid receptor mRNA in OFC, and dynorphins in hippocampus were found to be upregulated in alcoholics. No significant changes in expression of other EOS genes were evident. Human observations were replicated in rat model of cognitive impairment induced by repeated forced inragastric alcohol administration (Kuzmin et al., submitted). Dynorphins were found to be upregulated in the frontal cortex and hippocampus in alcohol treated animals, and this upregulation was paralleled by spatial memory deficit in a water maze task. Administration of selective κ-opioid antagonist norbinaltorphimine systemically or into the dorsal hippocampal CA3 area reversed the development of ethanol-induced cognitive deficit, whereas produced no effect in naive rats. Thus, in the rats, alcohol-induced impairment of spatial learning and memory may be mediated through activation of the dynorphin/κ-receptor system. In human alcoholics, upregulated dynorphins may also contribute to neurocognitive dysfunctions that may be relevant for learning and memory, and / or for addiction and disrupted inhibitory control.
Methods: DNA methylation analysis by bisulfite treatment / DNA pyrosequencing; ChIP-qPCR; EMSA; WB; immunostaining, a reporter gene assay, FACS assisted cell nuclei separation.
Results: We addressed epigenetic mechanism of *PDYN* up regulation in human alcoholics first by analysis of alterations in DNA methylation / histone modifications in *PDYN* promoter. The 1.7 kB promoter area was analyzed in post-mortem specimens, the PFC and motor cortex (MC) of human alcoholics and controls (discovery sample: n = 14 subjects in each group; replication sample: n = 8 subjects in each group) using bisulfite treatment / DNA pyrosequencing and ChIP-qPCR. The CpG-rich promoter segment was found to be strongly (~25%) and significantly ($p<0.001$) demethylated in the PFC in alcoholics. The differentially methylated region (DMR) encompassing approximately 150 nt, was apparently located within a single nucleosome. No differences were evident in the motor cortex, which showed no *PDYN* activation in alcoholics.

The DMR DNA methylation was associated with a substantial decrease in histone H3K27-trimethylation across the analysed 1.7 kB promoter area. Search for transcription factor (TF) binding sites found canonical E-box in the DMR, and then Upstream Regulatory Factor-2 (USF2) with strong histone-modifying activities was identified as a dominant E-box binding factor in the human PFC by EMSA. In this brain area, USF2 was found i) to be colocalized with PDYN protein using immunostaining; and ii) to be bound to the *PDYN* DMR using ChIP-qPCR. *PDYN* mRNA / peptides, DMR CpG methylation and USF2 were significantly correlated with each other. However, these correlations differed between controls and alcoholics suggesting transition to a new epigenetic state in alcoholics.
Discussion: We hypothesize that the *PDYN* DMR functions as the brain-area specific "epigenetic switch" affected by alcohol; DMR demethylation may promote USF2-mediated recruitment of histone modifiers to the promoter resulting in *PDYN* activation in alcoholics.

INDIVIDUAL ABSTRACT:
UNDERSTANDING THE RISK PATHWAY FROM GABRA2 TO ALCOHOLISM: EFFECTS OF PERSONALITY, BRAIN, DEVELOPMENT AND GENE X ENVIRONMENT INTERACTIONS

Margit Burmeister, Ph.D., Sandra Villafuerte, Ph.D., Elisa Trucco, Ph.D., Mary Heitzeg, Ph.D., Robert Zucker, Ph.D.

University of Michigan

Background: For more than 10 years, SNPs within *GABRA2* have been known to be associated with alcohol use disorder and EEG phenotypes. We used the Michigan Longitudinal Study (MLS) to understand HOW this association comes about. The MLS is a longitudinal, ongoing family-based population enriched for families at high risk for alcoholism. The MLS consists of 463 families who were recruited >20 years ago with a 3-5 year old boy (offspring) and his parents, later adding other siblings. ~40% of fathers had a drunk driving conviction, ~30% met criteria for alcoholism without such conviction, and ~30% are control families from the same neighborhoods. All were extensively assessed every 3 years, offspring at ages 11-20 every year. Genotypes are available for about ~1300 of the >2000 MLS subjects.
Methods: Impulsiveness in adults was measured using the NEO-PI-R. fMRI was measured in a subset of the young adult offspring (age 18-22) during a modified incentive monetary delay task. Alcohol use problems were measured with the Life-time Alcohol problem scale, LAPS. Parental monitoring was assessed using the Parental Monitoring Youth Form and externalizing behavior was assessed using the Young Self Report. *GABRA2* genotypes were obtained using Taqman and Golden Gate Illumina genotypes. Genetic associations were tested by regression analysis (i.e. additive genotypes). Mediation was tested using bootstrapping procedures in AMOS. Growth mixture modeling (GMM) was used to identify externalizing trajectory classes. Class labels were regressed on *GABRA2*, parental monitoring, and their interaction using multinomial linear regression via GMM.
Results: 9 of 11 *GABRA2* SNPs were in strong LD and formed two major (Yin/Yang) haplotypes. The most significant in most analyses, and most likely functional (as it is near a splice site) SNP, rs279827, was picked to tag the haplotypes. The G (minor) allele of this SNP represents the risk haplotype with regard to alcoholism in the literature which is confirmed in our data. We identified three trajectory classes of externalizing behavior across adolescence, a low, a developmentally limited, and a high risk class. Parental monitoring but not genotype predicted lower levels of externalizing behavior. Our significant findings are: 1) *GABRA2* risk alleles are

associated with impulsivity, particularly in females. 2) fMRI shows that *GABRA2* risk alleles increase insula activation during monetary reward anticipation. 3) *GABRA2* risk alleles' effect on Alcohol Problems is mediated by Impulsiveness, which is also associated with insula activation during monetary reward anticipation. 4) We observed a *GABRA2* x parental monitoring interaction effect on trajectory class membership, consistent with differential susceptibility. While A-carriers' trajectory class membership was largely unaffected by parental monitoring, those with the risk (G) genotype were affected by parental monitoring, both positively and negatively.

Discussion: Our results demonstrate that subjects who carry the risk (minor) haplotypes of *GABRA2* are more impulsive, their brain reacts stronger when anticipating a reward, and, when not monitored by parents, are more likely to engage in problem behaviors ("act out") during adolescence, and later become more likely to have alcohol problems . With GWAS and sequencing ongoing in large meta-analyses, most current psychiatric genetic findings are with broad, dichotomous phenotypes, necessitated to identify small effects in large studies. Our study demonstrates how a better understanding of the path from SNP to final phenotypic outcome can be achieved in smaller, well characterized longitudinal samples. Our studies are starting to pull genetic findings into the existing psychosocial literature, merging studies of nature with nurture in a meaningful way to explain some aspects of human behavior.

INDIVIDUAL ABSTRACT:
BRAIN MORPHOLOGY IN ADOLESCENTS AND YOUNG ADULTS AT HIGH AND LOW-RISK FOR ALCOHOL DEPENDENCE

Shirley Hill, Ph.D.

University of Pittsburgh Medical Center

Background: Familial risk for alcohol dependence appears to be accompanied by genetic, behavioral, and brain morphological characteristics that differ from those seen in individuals without a family history. Emerging evidence suggests that brain morphology is a robust neurobiological feature of offspring from alcohol dependent families that may represent an intermediate phenotype forecasting increased susceptibility to develop alcohol and drug use disorders. Some volumetric differences appear to exist prior to the initiation of drinking. Abnormalities in brain circuits involved in emotion regulation, impulse control, and executive functioning and its attendant impact on decision-making capacity appear to be good candidates for investigation into the neurobiological underpinnings of addictive disorders and represent a major focus of our work. Uncovering the familial-genetic aspects of these circuits is complicated by the effect of alcohol and drug use on brain morphology. Both animal and human studies have found that the neurotoxic effects of alcohol may be accentuated in adolescent and young adult binge drinkers because the developing brain may be more sensitive to the deleterious effects of alcohol. With prospective data that spans adolescence and young adulthood, we have attempted to capture the influence of environmental exposure in order to separate the effects of familial/genetic underpinnings for addiction associated with a multiplex family history and those that may be due to personal alcohol and drug exposure. The interaction of genetic and environmental effects is especially important to uncover.

Methods: A longitudinal follow up of third generation adolescents and young adults from either multiplex alcohol dependence families or control families that spans over 20 years and involves yearly clinical diagnosis has offered the opportunity to perform repeated magnetic resonance imaging (MRI) scans of a sub-sample of these individuals from 2004-2013. The MRI data set

now includes 160 first-time scans, with a total of 380 scans that include second, third, and fourth repeated scans for sub-sets of individuals. Neuropsychological testing includes administration of the Iowa Gambling Task (IGT) that measures effectiveness of decision making. Genotyping has been done for candidate genes.

Results: Familial risk effects are seen for the amygdala, and orbitofrontal (OFC) cortex, regions involved in emotion regulation. OFC volume in the right hemisphere was found to be associated with MPQ Control (impulsivity). Because the OFC appears to modulate the neural activity of the amygdala, the ratio of OFC volume to amygdala volume was tested for its relationship to age of onset for developing a substance use disorder with a significant effect on survival time observed. Additionally, DTI analyses revealed alterations in white matter integrity that include the inferior longitudinal fasiculus (ILF), a major pathway between the temporal lobe and the occipital lobe that is thought to be involved in visual processing including emotionally salient information. Genetic variation in these third generation offspring that appears to be linked to behavioral outcomes include DRD2 variation (rs6277) which predicts substance use outcome by age 20, and 5HTTLPR variation which is associated with IGT performance. Behavioral differences include earlier onset to develop a substance use disorder among high risk offspring from the multiplex families and significantly worse performance over trials on the IGT indicating diminished ability to profit from past experience in choosing reward/non-reward-based outcomes.

Discussion: Neural circuitry nodes (OFC and amygdala) involved in emotion regulation and the ILF tract connecting the temporal lobe and occipital tract involved in processing the salience of visual emotional stimuli may differ in those at risk for alcohol dependence. Genetic variation in the DRD2 and 5HTTLPR genes are linked to behavioral traits associated with risk and resilience.

ORAL PRESENTATIONS

10:45 AM – 12:15 PM

BIOMARKERS OF DISEASE RISK AND TREATMENT RESPONSE

INDIVIDUAL ABSTRACT:
GENOME WIDE ASSOCIATION STUDY OF UK PATIENTS WITH CLOZAPINE-INDUCED NEUTROPENIA

Sophie E. Legge[1], Marian Hamshere, Ph.D.[2], Michael Owen, M.D., Ph.D.[2], Michael O'Donovan, M.D., Ph.D.[2], Dan Rujescu, M.D., Ph.D.[3], Stephan Ripke, Ph.D..[4], Jennifer Moran, Ph.D..[4], Steven McCarroll, Ph.D.[4], Benjamin Neale, Ph.D.[4], Kimberly Chambert, MSc.[4], James Walters, M.D., Ph.D.[2]

[1]Cardiff University, [2]MRC Centre for Neuropsychiatric Genetics and Genomics, Cardiff University, [3]Ludwig-Maximilians-University, [4]Broad Institute

Background: Clozapine has been demonstrated to be more effective than other anti-psychotics in patients with treatment resistant schizophrenia (TRS). Nevertheless, clozapine is widely under prescribed. A contributing factor is a rare hematological side effect of the rapid reduction of white blood cells, specifically neutrophils. Neutropenia is characterised by a decrease in neutrophil count to below 1500 cells/mm^3 and agranulocytosis below 500 cells/mm^3. Clozapine-induced agranulocytosis (CIA) usually occurs within the first 18 weeks of treatment initiation and has a prevalence of 0.8%. If undetected, CIA can be fatal and hence clozapine treatment is accompanied by mandatory blood monitoring in the UK. If neutropenia is detected in the UK, a 'red alert' is given and clozapine treatment discontinued. The aim of the present study was to

investigate genetic causes of these red alert cases of clozapine induced neutropenia in two genome wide association analyses. 5469 controls who took clozapine for at least a year without developing this rare side effect were compared firstly to cases who developed neutropenia and received a 'red alert' (N=64) and in a more stringent analysis, those with an ANC below 1000 cells/mm^3 (N=18).

Methods: 58 'red alert' cases and 5469 clozapine treated controls were obtained anonymously from the CLOZUK sample, though partnership with Novartis. 6 cases and 150 controls were obtained from the Cardiff COGS (Cardiff cognition in schizophrenia) sample. All cases and controls had a diagnosis of schizophrenia or schizoaffective disorder. The Cardiff COGS sample and part of the CLOZUK sample (38 cases and 3547 controls) were genotyped on Illumina Combo and the remainder of the CLOZUK sample (26 cases and 2072 controls) were genotyped on Illumina Omni. Quality control and logistic regression using PLINK was performed separately on each chip for 577,356 SNPs and meta analyzed.

Results: No single variant achieved a genome wide significance level of $P < 5x10^{-8}$ in either analysis. The two top hits from the analysis with neutropenia cases (N=64) were rs2228563 (p=5.86x10-7, OR=12.4, 3.91% of cases vs. 0.38% of controls) a missense variant in *COL7A1* and rs7571379 (p=7.02x10-7, OR=3.6, 14.84% of cases vs. 4.48% of controls) an intron variant in *ALK*. The top two hits from the analysis only including cases with an ANC below 1000 cells/mm^3 (N=18) were rs2196239 (p=6.83x10-8, OR=6.78, 41.67% of cases vs. 9.83% of controls) a variant near *DEFB125*, and rs4808550 (p=1.58x10-7, OR=8.61, 50% of cases vs. 17.3% of controls) an intron variant in *CPAMD8*. These variants were associated in the neutropenia analysis at p=.0002 and p=.02 respectively.

Discussion: We present a genome wide association study investigating the possible genetic causes of clozapine induced neutropenia in patients with a red alert from the UK. No single variant reached genome wide significance with an ANC cutoff of 1500 cells/mm^3 or 1000 cells/mm^3. Our failure to find any genome wide significant associations suggest that it is unlikely a single or small number of genes of large effect are the cause and genetic underpinnings of this rare hematological side effect may be more complex. The top signals from each analysis are noted. Mutations in *COL7A1* have been associated with dystrophic epidermolysis bullosa and *ALK* is implicated in neurodevelopment. Interestingly, *DEFB125* and *CPAMD8* have both been implicated in immunologic response. We are seeking a replication sample to validate the signals noted and completing additional analyses within our sample, such as imputed genotypes, exome chip, pathway analysis and gene based tests. Provisional results of analysis with the 64 neutropenia cases in imputed genotypes show several genome wide significant SNPS, although these results are yet to be validated. Although our analyses were limited to cases with neutropenia, it should provide clues to mechanisms of CIA. Searching for genetic associations with CIA is important because a specific and sensitive predictive test would be an invaluable tool for clinicians and could conceivably lift the burden of blood monitoring.

INDIVIDUAL ABSTRACT:
LONG-TERM LITHIUM TREATMENT IN BIPOLAR DISORDER IS ASSOCIATED WITH LONGER LEUKOCYTE TELOMERES

Lina S.C. Martinsson, M.D.[1], Yabin Wei, Ph.D. student[2], Dawei Xu[2], Philippe A Melas, Ph.D.[2], Aleksander Mathé[2], Martin Schalling[2], Catharina Lavebratt[2], Lena Backlund, M.D., Ph.D.[2]

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Background: Lithium is the first-line mood stabilizer in bipolar disorder and has a unique role in suicide prevention. However, 40–50% of bipolar patients relapse within 2 years of lithium treatment. Lithium response clusters in families to a large extent, but it is difficult to identify these responders among patients because of the lack of predictive biomarkers. Lithium is thought to stabilize neuronal activities and to promote neural plasticity and neuroprotection. Lithium has probably several additional modes of action that are important for its therapeutic effects, but these mechanisms remain poorly understood. Telomere shortening is a hallmark of aging and has been associated with oxidative stress, inflammation and chronic somatic, as well as psychiatric disorders, including schizophrenia and depression. Furthermore, early life stress, depression and hypocortisolemia have also been negatively correlated with leukocyte telomere length. In contrast, factors like physical activity and effective pharmacological treatment have been associated with increased leukocyte telomere length and telomerase activity, respectively. Additionally, antidepressants have been found to protect against telomere shortening. However, pharmacological telomere studies are lacking in bipolar disorder. Therefore, the objective of this study was to explore leukocyte telomere length in patients with bipolar disorder in the context of lithium treatment.
Methods: Bipolar patients all with therapeutic serum concentration of lithium (0.5-0.9 mmol/L) in a period of at least three months before DNA sampling, were randomly selected from the Unit of Affective Disorders at Psychiatry Southwest, Karolinska Huddinge Hospital, Stockholm (n=256). Healthy controls were selected from the longitudinal population-based studies Stockholm Diabetes Prevention Program and the PART study (n=139). Retrospective case–control and case–case study designs were applied. Lithium response was scored using the Alda-Scale. Leukocyte telomere lenght was determined by quantitative real-time PCR using peripheral blood leukocytes.
Results: Lithium-treated bipolar disorder patients overall, as well as those on lithium monotherapy, had 35% longer telomeres compared with controls (P<0.0005, partial η^2=0.13). In patients with a lithium treatment duration >30 months, leukocyte telomere length correlated positively with lithium treatment duration (P=0.031, R^2=0.13). Leukocyte telomere length was negatively associated with increasing number of depressive episodes (P<0.007). Bipolar disorder patients responding well to lithium treatment had longer telomeres than those not responding well.
Discussion: This is the first study to report a positive effect of long-term lithium treatment on leukocyte telomere length. Importantly, longer leukocyte telomere length was also associated with a better lithium response in bipolar disorder patients. These data suggest that lithium exerts a protective effect against telomere shortening especially when therapeutically efficacious. We hypothesize that induction of telomerase activity may be involved in lithium response in bipolar disorder.

INDIVIDUAL ABSTRACT:
COMBAT TRAUMA EXPOSURE AND PTSD:A PROSPECTIVE DNA METHYLATION STUDY

Marco P.M. Boks, M.D., Ph.D.[1], Erik Vermetten, M.D., Ph.D.[2], Elbert Geuze, Ph.D.[3], Christiaan Vinkers, M.D., Ph.D.[4], Bart Rutten, M.D., Ph.D.[5]

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Background: Numerous animal studies have indicated that the epigenetic mechanism of DNA methylation is a prime molecular candidate for mediating the impact of environmental exposures on gene transcription, and thereby for vulnerable phenotypes for development of psychopathology. Various cross-sectional studies in humans have observed striking alterations in DNA methylation profiles in different tissues of patients with psychiatric disorders. Yet, evidence from prospective epidemiological studies covering the period of risk exposure or the onset of psychopathology, although highly warranted, is currently very limited.
Methods: We analyzed longitudinal changes in DNA methylation in selected subgroups from a large, prospective cohort of Dutch military personnel deployed to Afghanistan. This high risk cohort for trauma exposure showed differential susceptibility for traumatic stress-related psychopathology. Blood samples and standardized measures of Posttraumatic Stress Disorder (PTSD) symptoms were collected before and 6 months after deployment. The Self-Rating Inventory for PTSD was used to measure the presence of PTSD symptoms, while exposure to combat trauma during deployment was assessed with a 19-item deployment experiences checklist. From the entire cohort, three equally sized subgroups (total n=96) were selected based on level of traumatic stress exposure and presence of PTSD symptoms: i) a group with high combat trauma exposure (7.3 ±2.9) and high levels of post-deployment PTSD symptoms (45.3 ±8.6), ii) group with high combat trauma exposure (8.7 ±2.2) and low levels of PTSD symptoms (26.0 ± 3.7), and iii) group with low combat trauma exposure (0.4 ± 0.5; levels of post-deployment PTSD symptoms 25.4 ± 3.4). These groups did not differ for age, gender, alcohol consumption, cigarette smoking, military rank, length, weight, or medication use. DNA was isolated from whole blood and bisulphite conversion was performed. Longitudinal changes in DNA methylation profiles (captured with Illumina 450K arrays) were compared between the groups.
Results: Participants did not differ between inclusion and follow up regarding cell count and medication use. Quality control showed absence of type I inflation (see figure of QQ plot). We found a substantial but not genome wide effect of combat exposure pointing to alterations in GABA metabolism. The later development of PTSD was associated with genome wide significant alterations in methylation at two loci. These differences were in the opposite direction as the impact of trauma, suggesting that maladaptation is a core feature of PTSD.
Discussion: Later development of PTSD is associated with altered DNA methylation at at least two CpG loci. The data from this unique, prospective collected cohort form an important step forward in elucidating the epigenetic mechanisms underlying differential susceptibility for traumatic stress-related psychopathology in humans.

INDIVIDUAL ABSTRACT:
DISCOVERY AND VALIDATION OF BLOOD BIOMARKERS FOR SUICIDALITY

Alexander B. Niculescu, III., M.D., Ph.D.

Indiana University School of Medicine

Background: Suicides are a leading cause of death in psychiatric patients, and in society at large. Developing more quantitative and objective ways (biomarkers) for predicting and tracking suicidal states would have immediate practical applications, and positive societal implications. We undertook such an endeavor.
Methods: First, building on our previous blood biomarker work in mood disorders and psychosis, we decided to identify blood gene expression biomarkers for suicidality, looking at differential expression of genes in the blood of subjects with a major mood disorder (bipolar

disorder), a high risk population prone to suicidality. We compared no suicidal ideation states and high suicidal ideation states using a powerful intra-subject design, as well as an inter-subject case-case design, to generate a list of differentially expressed genes. Second, we used a comprehensive Convergent Functional Genomics (CFG) approach to identify and prioritize from the list of differentially expressed genes biomarkers of relevance to suicidality. CFG integrates multiple independent lines of evidence- genetic and functional genomic data, as a Bayesian strategy for identifying and prioritizing findings, reducing the false-positives and false-negatives inherent in each individual approach.
Results: We subjected our findings to the ultimate test, examining the expression levels of biomarkers identified by CFG in blood samples from a cohort of consecutive suicide completers collected from the coroner's office, and validated these possible markers. We also show that levels of SAT1, the top biomarker identified by us, differentiated future hospitalizations due to sucidality in a cohort of bipolar disorder subjects. Lastly, we conducted bioinformatic analyses to identify biological pathways, mechanisms, and medication targets.
Discussion: Taken together, our results have implications for the understanding of suicide, as well as for the development of objective laboratory tests and tools to track suicidal risk and response to treatment. More work needs to be done to examine potential gender differences. Our current work is based on male subjects only. Given the fact that approximately one million people die of suicide worldwide each year, and this is a potentially preventable cause of death, the need for and importance of efforts such as ours cannot be overstated.

INDIVIDUAL ABSTRACT:
CHEMICAL GENOMICS AND PSYCHIATRIC NEUROBIOLOGY: IDENTIFYING DISEASE SIGNATURES FOR SCHIZOPHRENIA AND BIPOLAR DISORDER USING IPSC-DERIVED NEURONAL CELLS

Rakesh Karmacharya, M.D., Ph.D.[1], Shaunna Berkovitch, Ph.D.[2], Steven Sheridan, Ph.D.[2], Sabine Bavamian, Ph.D.[2], Joanne Huang, B.S.[2], Elizabeth O'Brien, A.B.[2], Jonathan Iaconelli, B.S.[2], Dost Ongur, M.D., Ph.D.[3], Bruce Cohen, M.D., Ph.D.[3], Stuart Schreiber, Ph.D.[4], Stephen Haggarty, Ph.D.[2]

[1]Harvard Medical School, [2]Massachusetts General Hospital, [3]McLean Hospital, [4]Broad Institute of Harvard and MIT

Background: Schizophrenia and bipolar disorder are complex genetic disorders with high heritability. Postmortem brain studies and imaging studies suggest deficits in the neuronal biology in patients with schizophrenia and bipolar disorder. However, the study of the neurobiological underpinnings of these disorders has been hindered by the inability to study live neuronal tissue from patients. Advances in stem cell research that enable reprogramming of induced pluripotent stem cells (iPSCs) and neural prognitor cells (NPCs) from human fibroblasts present an exciting opportunity to generate live neuronal cells that carry the patients' genetic backgrounds.
Methods: We are reprogramming fibroblasts from subjects with schizophrenia and bipolar disorder as well as matched controls using transduction of four genes (OCT4, SOX2, KLF4 and C-MYC). We differentiate the iPSCs to establish self-renewing NPCs that grow as adherent monolayers. We further differentiate NPCs along different neuronal and glial lineages, including directed differentiation to specific neuronal subtypes that are hypothesized to be involved in disease biology, with the goal of culturing patient-specific live neuronal cells *in vitro*. We plan to acquire high-content images of neuronal cells labeled with neuronal subtype-specific markers as

well as a set of cellular stains. In addition to images acquired under normal conditions, we will culture the neuronal cells in the presence of an annotated library of 300 small-molecule perturbagens that modulate specific signaling pathways. We will use machine-learning algorithms to identify features that distinguish neurons derived from schizophrenia and bipolar iPSCs from neurons derived from healthy controls, in order to identify disease-specific cellular signatures.
Results: We have reprogrammed iPSCs and NPCs from subjects with schizophrenia and bipolar disorder as well as matched controls. We have developed methodologies to diferentiate them along the neuronal lineage and have successfully generated mature neuronal cultures that have neuronal subtypes with markers specific for upper layer cortical projection neurons. We have also developed a set of assays using a range of cellular stains to study cellular phenotypes and gene-expression in the presence of specific small-molecule perturbations in a 384-well format. These studies have laid the groundwork to carry out image-based and gene-expression based profiling in order to identify "disease signatures" for schizophrenia and bipolar disorder.
Discussion: The challenge of modeling neurodevelopmental psychiatric disorders of complex three-dimensional brains in two-dimensional neuronal cultures is immense. In addition, gene-environment interactions are posited to play a major role in the disease biology of schizophrenia and bipolar disorder. We hypothesize that the vulnerability for disease will be reflected at the cellular level when we examine specific neuronal subtypes. We further attempt to emulate gene-environment interactions *in vitro* by studying the biology of these cells in a range of small-molecule perturbations. By studying the subtle morphology and gene-expression of specific neuronal subtypes in the setting of various perturbations, we hope to identify disease signatures for schizophrenia and bipolar disorder.

INDIVIDUAL ABSTRACT:
REPLICATION AND EXTENSION OF BLOOD-BASED BIOMARKERS AND CLASSIFERS OF AUTISM SPECTRUM DISORDERS, DEVELOPMENTAL AND LANGUAGE DELAYS, AND TYPICAL DEVELOPMENT

Stephen J. Glatt, Ph.D.[1], Nicholas Schork, Ph.D.[2], Mary Winn, Ph.D.[2], Sharon Chandler, Ph.D.[3], Ming Tsuang, M.D., Ph.D.[3]

[1]SUNY Upstate Medical University, [2]The Scripps Research Institute, [3]University of California, San Diego

Background: We previously constructed and cross-validated a support vector machine-learning algorithm that successfully distinguished children with an autistic spectrum disorder (ASD) from typically developing (TD) children based on the expression levels of just 48 genes in peripheral blood. Subsequently, we have collected and assayed gene expression in peripheral blood samples from approximately 200 additional subjects. Here we report on the classification accuracy of our previously derived gene-expression signature of ASDs in this new independent sample, as well as an expanded set of classifiers distinguishing between children with ASDs, children with developmental delay (DD) or language delay (LD), and TD children.
Methods: We employed analyses of covariance to identify genes differentially expressed between training subsamples from each diagnostic group. Expression levels of these genes were then employed in the construction and optimization of support vector machines classifying subjects in training subsamples into clinically derived diagnostic categories. The optimal classifier derived for each comparison of diagnostic groups was then deployed in an independent test subsample to evaluate the classifier's sensitivity, specificity, positive predictive value,

negative predictive value, and area under the receiver-operating characteristic curve.
Results: The identical support vector machine that obtained approximately 70-90% accuracy in distinguishing ASD subjects from TD subjects our initial study attained an accuracy of 58% in the newly collected sample, with a corresponding sensitivity of 55%, specificity of 62%, positive predictive value of 65%, and an area under the receiver operating characteristic curve or 0.59. This model performance, while far from perfect, was significantly better than chance expectation (p=0.012). Newly derived classifiers distinguishing ASDs from clinically informative combinations of DD, LD, and TD children performed particularly well within the 12-24-month age range, with AUCs ranging from 0.70-0.90 in independent test subsamples of each diagnostic group.
Discussion: These results suggest that the continued pursuit of a blood-based biomarker of early autism is warranted. Additional analyses—including further cross-validation, re-optimization of classifier parameters, and more precise quantification of distinct mRNA isoforms—should yield more accurate, stable, and generalizable classifiers of ASDs and other developmental delays and disorders, which may pave the way for molecular diagnostic testing.

ORAL AND POSTER PRESENTATIONS
10:45 AM – 12:15 PM

SCHIZOPHRENIA: GENETICS AND FUNCTIONAL GENOMICS

INDIVIDUAL ABSTRACT:
COMPARISON OF THE PENETRANCE OF COPY NUMBER VARIATIONS FOR SCHIZOPHRENIA AND NEURODEVELOPMENTAL DISORDERS

George Kirov[1], Elliott Rees, Cardiff University[1], James Walters[1], Valentina Escott-Price[1], Lyudmila Georgieva[1], Kimberly Chambert[2], Jenniefer Moran[2], Steven McCarroll[2], Michael O'Donovan[1], Michael Owen[1]

[1]Cardiff University, [2]The Broad Institute

Background: Several recurrent copy number variants (CNVs) have been shown to increase risk to develop schizophrenia (SCZ) or a range of neurodevelopmental disorders, such as developmental delay (DD), autism spectrum disorders (ASD) and various congenital malformations (CM). The increase in risk to develop SCZ has been estimated to be modest for some of these CNVs. Comparisons between their penetrance for the development of SCZ or DD/ASD/CM have not been made yet.
Methods: We use data from the largest available studies on SCZ and DD/ASD/CM to estimate the frequencies of 70 implicated CNVs, in carriers with these disorders, in healthy controls and in the general population. On the basis of the frequencies of these CNVs we estimate their penetrance.
Results: The rates of nearly all CNVs are higher in DD/ASD/CM, compared to SCZ. The penetrance of CNVs is at least several times higher for the development of a disorder from the group of DD/ASD/CM. This applies even for CNVs that are strongly associated with SCZ. The overall penetrance for developing any disorder for the list of SCZ-associated CNVs is high, ranging between 10.6% and 100%.
Discussion: CNVs associated with SCZ have high pathogenicity for a neurodevelopmental disorder. The majority of the increased risk conferred by CNVs is towards the development of an earlier-onset disorder, such as DD/ASD or CM, rather than SCZ. Many highly pathogenic CNVs do not increase risk for SCZ, but their carriers develop DD/ASD or CM earlier in life. The

improved estimates of penetrance presented here will provide crucial information for genetic counseling.

INDIVIDUAL ABSTRACT:
A METHYLOME-WIDE INVESTIGATION IN 1500 SCHIZOPHRENIA CASE-CONTROL SAMPLES IDENTIFIES AND REPLICATES PROMISING BIOMARKERS FOR SCHIZOPHRENIA

Karolina Aberg, Ph.D.[1], Joseph L. McClay, Ph.D. [1], Shaunna L. Clark, Ph.D.[1], Gaurav Kumar, Ph.D. [1], Andrey Shabalin, Ph.D.[1], Daniel E. Adkins, Ph.D.[1], Swedish Schizophrenia Consortium[2], Patrick F. Sullivan, Ph.D.[3], Patrik KE Magnusson, Ph.D.[4], Edwin JCG van den Oord, Ph.D.[1]

[1]Center for Biomarker Research and Personalized Medicine, School of Pharmacy, Virginia Commonwealth University, [2]VCU, [3]Department of Genetics, University of North Carolina School of Medicine, [4]Department of Medical Epidemiology and Biostatistics, Karolinska Institutet

Background: The methylation of DNA cytosine residues at the carbon-5 position (5mC) is a common epigenetic modification that is most often, although not exclusively, found in the sequence context CpG. Investigations of these marks provide a promising complement to schizophrenia (SZ) studies of DNA sequence variation.
Methods: We performed a methylome-wide association study (MWAS) of DNA extracted from whole blood in 1,500 SZ case-control samples from Sweden. We applied methyl-binding domain 2 protein enrichment to extract the methylated fraction of the genome followed by next-generation sequencing (MBD-seq). This approach allows for investigation of all ~27 million CpGs in the human reference genome. Furthermore, using GWAS genotyping in combination with 1000-genomes imputation we investigate the methylation status of an additional ~1.2 million common CpGs (MAF > 5%) created by SNPs (SNP-CpGs). We also used the SNP data to identify methylation quantitative trait loci (meQTLs) (i.e. SNPs that are associated with methylation status in a cis- or trans-acting fashion) that showed altered effects in SZ cases versus controls. Top findings were replicated with targeted bisulfite pyrosequencing in up to 1,100 independent SZ case-control samples from the same Swedish population. To analyze this massive dataset, we have developed an analysis pipeline specifically for MBD-seq that includes alignment, quality control, estimators for calculating CpG coverage, data reduction combining highly correlated CpGs into blocks, principal component analysis, association testing, bioinformatics annotation and network analysis and have used the software package Matrix eQTL.
Results: Our MWAS suggested a considerable number of effects with 141 loci, considered methylome-wide significant using a stringent threshold for false discovery rate (FDR) of 0.01. Our MWAS top finding ($p = 6.28\times10^{-11}$) was located in *FAM63B,* a gene regulated by miRs that can be linked to neuronal differentiation and dopaminergic differentiation and expression. This finding was replicated in independent samples ($p = 2.3\times10^{-10}$). Other replicating top findings (e.g. CREB1, SMAD3 and ARNT) were linked to hypoxia, possibly suggesting that biomarkers of environmental insults may be preserved in the methylome. Furthermore, the SNP-CpG analysis identified highly significant differences in methylation levels, conditional on genotype, and meQTL analysis suggested that disrupted control of the methylome might contribute to SZ. Among the replicated top findings was TET1 (replication p = of 4.66×10^{-09}), a gene of major importance for transcriptional regulation by converting 5mC to 5-hydroxymethylcytosine

(5hmC).
Discussion: This study represent one of the first sets of genome-wide analysis that identifies and replicates methylation sites that are of potential use as biomarkers to improve treatment, diagnosis and disease etiology for SZ patients.

INDIVIDUAL ABSTRACT:
PSYCHIATRIC GENOMICS CONSORTIUM QUADRUPLES SCHIZOPHRENIA GWAS SAMPLE-SIZE TO 35,000 CASES AND 47,000 CONTROLS

Stephan Ripke, M.D.[1], Schizophrenia Working Group[2]

[1]The Broad Institute; Massachusetts General Hospital, [2]Psychiatric Genomics Consortium

Background: The PGC (Psychiatric Genomics Consortium) is an international group of researchers whose major aim is to maximize the utility of extant psychiatric GWAS through mega-analysis. In a previous study, our first wave of genome-wide schizophrenia association analysis identified multiple loci involved in this genetically complex and clinically heterogeneous disorder (*Nature Genetics*, 2011). While around 20,000 individuals were necessary to achieve this result, detailed analysis of the data suggested that there are many more genes to discover, and that this should be possible by further increase of sample size.
Methods: Here we present an update of this international endeavor, which now comprises 35,476 schizophrenia cases and 46,839 controls coming from 52 sub studies. The presented data is imputed into 1000 Genomes (Aug, 2012) and analyzed using standard logistic regression with ancestry components as covariates. All index SNPs with a p-value smaller than $1x10^{-6}$ were used for replication lookup in an independent GWAS analysis with 1,500 cases and 66,000 controls.
Results: The number of independent genome-wide significant regions in this newest round of meta-analysis increased to 108 ($P < 5x10^{-8}$). These results increase the number of loci strongly implicated in schizophrenia by more than 80. The loci implicated include prior targets (*MIR137, CACNA1C, ZNF804A*) along with a host of new targets many of which are now implicated by multiple lines of genomic evidence (*DRD2, KCTD13*).
Discussion: These results are in line with prior predictions and developments in other complex disease GWAS with sufficiently large samples like Crohn's disease. They provide new insights into the biology of schizophrenia.

INDIVIDUAL ABSTRACT:
A NOVEL METHOD FOR DISSECTING GENETIC HETEROGENEITY ACROSS SUBJECTS AND GENES IN SCHIZOPHRENIA

Danielle Posthuma, Ph.D.[1], Frank Koopmans[2], Christiaan de Leeuw[2], Anna Kähler [3], Christina Hultman[3],
Patrick Sullivan[4], The Psychiatric Genomics Consortium – Schizophrenia Group

[1]VU University, [2] Netherlands, VU, [3] Sweden, KI, [4]UNC

Background
Schizophrenia (SCZ) is a highly polygenic disorder influenced by thousands of genetic variants each of small effect. Gene-set or biological pathway approaches have proven to be particularly useful in understanding genomic results for polygenic diseases. Gene-set analyses are similar in kind to a uni-dimensional cluster analysis (i.e. along the dimension of the gene). A natural extension would be an attempt to cluster both subjects and genes. Such an analysis could directly

identify or index genetic heterogeneity with a heterogeneous disorder like SCZ by identifying which subsets of cases had higher genetic loadings for which classes of genetic variation.

Methods

The PGC-SCZ sample (excluding all subjects from Sweden) was used as training set to identify for every SNP the risk allele and the log of the odds ratio. Polygenic risk scores and risk allele-burden across 1028 genes expressed in synapses1 were then calculated in a subset of cases (N=1769) from the Sweden SCZ study2 and used as input for a two-dimensional hierarchical cluster analysis.

Results

The two-dimensional cluster analysis was conducted across genes and subjects. There were 3 main classes of genes: one class of genes that did not show any association with SCZ for most of the subjects, a second class of genes for which some cases carried risk alleles, but others did not, and a third class of genes for which nearly all cases carried risk alleles. Simultaneous clustering of cases showed potentially important heterogeneity and multiple classes of cases. Clustering on risk-allele burden resulted in fewer classes of cases, although each class still represented less than 2% of the sample.

Discussion

Two-dimensional hierarchical clustering along genes and cases provides a promising novel method to dissect genetic heterogeneity in polygenic disorders. Our preliminary analyses are based on a subset of genes (1028 out of ±22,000 and a subset of available SCZ cases). Increase of sample size (using the full Swedish sample) and prioritizing genes are planned subsequent steps that may further improve dissecting subject heterogeneity. Identifying latent classes of cases and genes for SCZ could provide important clues for etiological heterogeneity and could be used to guide the selection of genes and cases for functional analyses.

References:

1. Lips ES et al. Mol Psychiatry. 17, 996-1006
2. Ripke, S. et al. Nat Genet, in press

INDIVIDUAL ABSTRACT:
A HIGH-RESOLUTION SURVEY OF STRUCTURAL VARIATION IN 500 SCHIZOPHRENIA CASES FROM WHOLE GENOME SEQUENCING IN THE GPC SCHIZOPHRENIA COHORT

Bob Handsaker[1], Liz Bevilacqua[2], Giulio Genovese[2], Colm O'Dushlaine[2], Ayman Fanous, M.D.[3], Helena Medeiros[4], Jennifer Moran[5], Jim Knowles[4], Michele Pato[4], Carlos Pato[4], Steven McCarroll[5]

[1]Harvard Medical School, Department of Genetics, [2]Stanley Center for Psychiatric Research, Broad Institute of MIT and Harvard, [3]Mental Health Service Line, Washington VA Medical Center; Georgetown University School of Medicine; University of Southern California, [4]Department of Psychiatry and the Behavioral Sciences, University of Southern California, Los Angeles, California, [5]Stanley Center for Psychiatric Research, Broad Institute of Harvard and MIT

Background: Sensitive ascertainment and accurate genotyping of structural variation in disease studies is a challenging problem. 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. From deep whole genome sequencing on

a subset of the GPC cohort, we have created a detailed map of structural variation and CNVs that is more complete and at finer scale than other available resources. This map will inform our interpretation of association results in the GPC cohort and serve as a high-resolution resource for imputing common structural variants in other studies.
Methods: We have performed deep whole genome sequencing (>30x average coverage) on a subset of the GPC schizophrenia cohort (500 affected individuals and 200 controls). We have further developed our published methods for structural variation analysis in whole-genome sequencing data (Genome STRiP; Handsaker, Korn, Nemesh, and McCarroll, Nature Genetics 2011). These methods were used in the 1000 Genomes Project in 2012 to genotype all deletion variants for that project, and we have subsequently extended our methods to reach all forms of CNV and to increase power to discover rare, singleton variants. We further phase the genotyped CNVs onto SNP haplotypes of these individuals from the GPC cohort to create a reference panel for imputation.
Results: This study allows us to survey the spectrum of copy number variation in unprecedented physical resolution and sequence detail, and with genotype-quality inference about loci that were previously only known to be variable. The high depth of sequencing allows us to identify over 10,000 CNVs and to interrogate CNVs as small as 1 kb with more precision than is obtainable with microarrays. Furthermore, the broad ascertainment (of 1000 diploid genomes) allows us to characterize low-frequency CNVs that segregate in populations at allele frequencies of 0.05-1%. We find that genes that exist in large numbers of copy number states (e.g. genes with copy number of 4-12) can be assigned an integer copy number with near-perfect accuracy. We can for the first time identify the copy-number alleles that are segregating at each site and relate these alleles to SNPs and haplotypes. The set of phased SNP and CNV haplotypes creates a reference panel we can use to impute these CNVs into the rest of the GPC cohort and enables the imputation of these CNVs in other studies. We show that a substantial fraction of loss-of-function mutation involves structural variants that are invisible to exome sequencing and SNP arrays but are prominent in high-coverage WGS data.
Discussion: We present the results of our CNV survey in this cohort and our analysis of cryptic structural variation in genomic regions associated with psychiatric illness. The results of this analysis provide a useful resource for genotype-to-phenotype studies in the GPC and for imputation into genome-wide SNP data for all phenotypes.

INDIVIDUAL ABSTRACT:
LARGE-SCALE RNA-SEQUENCING OF SCHIZOPHRENIA BRAINS BY THE COMMONMIND CONSORTIUM

Menachem Fromer, Ph.D.[1], for the CommonMind Consortium (CMC)[2]

[1]Icahn School of Medicine at Mount Sinai, [2]CommonMind Consortium

Background: Schizophrenia is a severe psychiatric disease with ~1% lifetime prevalence in the general population. And, although it is a highly heritable disease, a picture of the genetics is only now emerging from large-scale studies (of both common and rare variation) but is nowhere near complete. That is, the exact genes and pathways involved are still largely unknown, and no individual variant explains even a moderate fraction of the total genetic variance. Thus, two things have become increasingly clear: i) large studies are needed due to disease heterogeneity and polygenicity, and ii) study of any single level of biology (genetics, expression, imaging, etc.) will be insufficient to fully unravel the biology of disease, but rather information must be collected (and then combined) across multiple levels. The CommonMind Consortium (CMC,

http://commonmind.org) is a public-private pre-competitive consortium that brings together disease area expertise, large and well-curated brain sample collections, and data management and analysis expertise. The goal of the CMC is to generate and analyze large scale data from human subjects with neuropsychiatric and neurodevelopmental disorders and to make this data and the analytical results broadly available to the public as a free resource. This is based on the belief that development of new therapies is best met by pooling resources and by sharing data with the entire research community. The consortium consists of five academic groups, two pharmaceutical companies, and one non-profit group.

Methods: Phase I of the CMC project is generating whole-genome transcriptome data on the prefrontal cortex as well as high-density SNP genotypes from ~600 postmortem brain samples from schizophrenia and control tissue collections. Currently it is envisaged that this data will become available to the public through Synapse (www.synapse.org) in 2014. Subsequent phases of the project will expand the molecular data for the brain collection to include new brain regions and new types of information beyond transcriptomic data (exome sequencing, epigenetic marks, etc.). Here we describe this Phase I large-scale study of RNA-sequencing-based gene expression in prefrontal cortex in brains of individuals with schizophrenia, as compared to control subjects. The full sample is composed of post-mortem brain tissue of schizophrenia patients (N~300) and controls (N~300). The utility of the data being generated here, beyond the large size of the sample, is that the data will be processed concurrently at the same site (Icahn Medical School at Mount Sinai), which permits randomization of case and control samples into batches for sample preparation and sequencing (e.g., across different sequencing lanes, etc.). Having a uniform data generation and analysis pipeline from start to finish will allow us to be as highly powered as possible to draw conclusions about functional changes at the molecular level in the brains of individuals with schizophrenia.

Results: This project will generate a gene expression dataset for schizophrenia far larger than any currently available to date. RNA sequencing is currently underway and expected to be completed late 2013, so we present preliminary results on the first half of the dataset (~150 cases, ~150 controls). We performed extensive QC of pilot samples (N=14 controls), including 5 samples for which RNA was prepared using both polyA isolation and RiboZero depletion of ribosomal RNA, in order to calibrate the amount of sequencing necessary to detect both low- and high-expression transcripts. Similarly, we carried out an analysis of power for differential expression to inform our decision of how deeply to sequence. The analysis of the expression data will aim to identify particular genes and pathways with differential expression between cases and controls, while correcting for available clinical (age at onset, medications) and technical (post-mortem interval, collection site) covariates. We will also explicitly search for more homogeneous subsets of affected individuals, as compared to controls. Moreover, we will curate a catalogue of brain-expressed genes and their splice forms in case and control individuals.

Discussion: The CommonMind Consortium (CMC) is poised to expose signals of functional changes in the expression levels of genes in the human brain as related to schizophrenia. Leveraging a large and uniformly processed and analyzed dataset that will also be made public, the schizophrenia research community will be empowered to make novel discoveries relating neurobiology to risk of disease.

SYMPOSIA
2:15 PM – 4:15 PM

OVERALL ABSTRACT:
NETWORK APPROACHES AND SYSTEMS BIOLOGY IN PSYCHIATRIC DISORDERS

Manuel Mattheisen[1], Danielle Posthuma, Ph.D.[2], Danielle Posthuma, Ph.D.[2], Albert-László Barabási[3], Kristen Brennand[4], Olaf Sporns[5], Dennis Vitkup[6]

[1]Aarhus University, [2]VU University, [3]Center for Complex Network Research, Northeastern University, [4]Icahn School of Medicine at Mount Sinai, [5]Indiana University, [6]Department of Systems Biology, Department of Biomedical Informatics

Recent genome-wide association analyses provided strong evidence for the involvement of common (and rare variants) in the susceptibility of psychiatric disorders. In case of schizophrenia this lead to identification of an astonishing number of risk loci implicating hundreds of genes in its etiology. Irrespective of the unquestionable importance of such findings little is still known about their interplay in the diseaseome and the specific genetic risk architecture of these disorders. Starting from the understanding that a disease is rarely a consequence of an abnormality in a single gene, but reflects the perturbations of the complex intracellular network this symposium will focus on network and systems approaches lately introduced to the field of psychiatric genetics. We will start with a theoretical overview of network medicine. This will be followed by the practical application of network medicine approaches designed to define disease neighborhoods and identify parts of the shared genetic risk architecture of psychiatric disorders. Through recent developments in generating induced pluripotent stem cells (iPSCs) the validation of disease modules / neighborhoods in psychiatric illnesses now also may relay on expression data from disease relevant tissue. We will report on such a study that provided neurons derived from fibroblasts of individuals diagnosed with schizophrenia. Furthermore, we will give an example for an integrated analysis of diverse disease-related genetic data (NETBAG+). This analysis did not only allow the identification of a disease module for schizophrenia (that was validated using the aforementioned iPSC data) but also helped to dissect which of the genes in previously identified risk loci (GWAS hit regions) are of importance. Finally, the symposium will focus on the intersection of network science and human neuroimaging. Here we will report on recent progress in mapping human brain networks and in modeling the relationship between network structure and dynamics in health and disease.

INDIVIDUAL ABSTRACT:
NETWORK MEDICINE: FROM CELLULAR NETWORKS TO THE HUMAN DISEASOME

Albert-László Barabási

Center for Complex Network Research, Northeastern University

Given the functional interdependencies between the molecular components in a human cell, a disease is rarely a consequence of an abnormality in a single gene, but reflects the perturbations of the complex intracellular network. The emerging tools of network medicine offer a platform to explore systematically not only the molecular complexity of a particular disease, leading to the identification of disease modules and pathways, but also the molecular relationships between apparently distinct (patho)phenotypes. Advances in this direction are essential to identify new disease genes, to uncover the biological significance of disease-associated mutations identified

by genome-wide association studies and full genome sequencing, and to identify drug targets and biomarkers for complex diseases.

INDIVIDUAL ABSTRACT:
MODELING NEURODEVELOPMENT OF SCHIZOPHRENIA

Kristen Brennand

Icahn School of Medicine at Mount Sinai

Though the characteristic symptoms of schizophrenia (SZ) generally appear late in adolescence, it is now thought to be a neurodevelopmental condition, often predated by a prodromal period that can appear in early childhood. To test if the basic molecular mechanisms underlying this disease occur prior to neuronal maturation, we differentiated SZ-specific hiPSCs into forebrain neural progenitor cells (NPCs). Our unbiased genomic and proteomic analysis observed altered cellular adhesion and oxidative stress proteins in forebrain SZ hiPSC NPCs. Consistent with this, we observed aberrant migration and increased oxidative stress in forebrain SZ hiPSC NPCs.

INDIVIDUAL ABSTRACT:
MAPPING AND MODELING COMPLEX NETWORKS OF THE HUMAN BRAIN

Olaf Sporns

Indiana University

Recent advances in network science have greatly increased our understanding of the structure and function of many networked systems, ranging from transportation networks, to social networks, the internet, ecosystems, and biochemical and gene transcription pathways. The advent of new noninvasive imaging tools now allows the creation of comprehensive network maps of structural and functional connectivity of the human brain. The confluence of network science and human neuroimaging has given rise to a new field, human connectomics (Sporns, 2011; 2012). My talk will focus on recent progress in mapping human brain networks and in modeling the relationship between network structure and dynamics in health and disease. I will give an overview of recent work characterizing the structure of complex brain networks, with particular emphasis on studies demonstrating how the brain's structural topology constrains and shapes its capacity to process and integrate information. Early studies in this field have focused on mapping brain network topology and have identified some of its characteristic features, including small world attributes, modularity and hubs. An important recent discovery is the existence of a "rich club", a set of highly connected and centrally positioned collective of mostly multimodal or transmodal brain regions. Network analysis strongly suggests a crucial role of the rich club in global brain communication and other integrative functional processes. Another important line of work attempts to link brain network topology to brain dynamics, the patterns of functional interactions that unfold during both rest and task conditions. Community detection methods have proven particularly fruitful in that regard. Studies have revealed a set of dynamic resting-state networks that engage in coherent neural activity and whose activity is shaped by the underlying scaffold of the human connectome. Analytic and computational models have helped to make further inroads in our understanding of how structural networks shape and constrain spontaneous and evoked brain activity. In a clinical context, disturbances of both rich club organization and of the brain's community structure are potential biological network substrates for brain and mental disorders.

INDIVIDUAL ABSTRACT:
GENE NETWORKS UNDERLYING COMMON PSYCHIATRIC DISORDERS

Dennis Vitkup

Department of Systems Biology, Department of Biomedical Informatics

Identification of complex molecular networks underlying common human phenotypes is a major challenge of modern genetics. Despite the identification of many relevant loci, molecular mechanisms of many common human diseases remain largely unclear. We have developed several computational approaches that allow an integrated analysis of diverse disease-related genetic data using a unified statistical framework. The application of these approaches to several psychiatric diseases (autism, schizophrenia) allowed us to implicate several molecular processes involved in synapse development, axon targeting, neuronal mobility, and chromosomal modification. The obtained results reveal an amazing phenotypic and genetic diversity of the psychiatric disorders. The networks associated with distinct psychiatric disorders significantly overlap in terms functional modules and biological pathways. A comparative analysis of copy number variants associated with autism and schizophrenia suggests that although the molecular networks implicated in these distinct disorders may be related, the mutations associated with each disease may lead, at least on average, to different functional consequences.

SYMPOSIA
2:15 PM – 4:15 PM

OVERALL ABSTRACT:
RETURN OF RESULTS IN GENETICS: INTEGRATION WITH CLINICAL CARE

Scott Roberts, PhD, Robert C. Green, MD, MPH[1], Sarah Hartz[2], Sarah Hartz[2], David Kaufman, Isaac Kohane

[1]Department of Medicine, Division of Genetics, Brigham and Women's Hospital and Harvard Medical School, [2]Washinton University in St. Louis School of Medicine

The science of genetics is rapidly advancing towards personalized medicine. In this symposium, we highlight empirical research that focuses on clinical integration of genetics, an essential step towards effective and evidence-based integration of genetics into clinical medicine. Dr. Robert Green will start the symposium by discussing an evaluation of direct-to-consumer genetic testing in clinical medicine. Then Dr. Scott Roberts will discuss implications of genetic risk disclosure in Alzheimer's disease. Dr. Sarah Hartz will then present a study of return of genetic results to an underserved population of African American smokers. Finally, Dr. Isaac Kohane will present how integration of genomic and clinical data can lead to improved characterization of Autism Spectrum Disorders. Our discussant, Dr. Laura Bierut, will synthesize these translational studies and lead the discussion regarding the clinical and research implications.

INDIVIDUAL ABSTRACT:
MOTIVATIONS AND IMPACT OF CUSTOMERS USING PERSONAL GENOMICS SERVICES

Robert C. Green, M.D., MPH

Department of Medicine, Division of Genetics, Brigham and Women's Hospital and Harvard Medical School

The Impact of Personal Genomics (PGen) Study is an NIH-funded collaboration between academic researchers and two consumer genetic testing companies: 23andMe and Pathway Genomics. New customers of these companies were recruited in 2012-2013 for the study at the time they submitted DNA samples to assess how consumer genetic testing influence their risk perceptions, psychological health, health behaviors, healthcare utilization, and communication about results. Participants agreed to share their actual genetic results with the researchers, and filled out surveys prior to receiving their genetic testing results (baseline), 3 weeks post-results, and 6 months after receiving results. To date, 1737 participants have been enrolled and of these, the mean age was 47 years (SD=15.6), they were 60.2% female, 89.5% white, 48.1% college educated and 55% rated their health as excellent or good. Analyses will be presented describing predictors of interest in genetic testing for specific conditions, of perceived medical utility, of anxiety after testing, of willingness to communicate results to others and of behavioral change after receiving results.

INDIVIDUAL ABSTRACT:
GENETIC RISK DISCLOSURE IN ALZHEIMER'S DISEASE: FINDINGS FROM THE REVEAL STUDY

Scott Roberts, Ph.D., University of Michigan

The rapid identification of genetic risk factors for common diseases allows for susceptibility testing of at-risk populations, but many questions remain about whether and how best to implement such programs. How should we communicate this often ambiguous information? How will patients and families respond, both in terms of psychological distress and health behavior changes? What are the broader ethical and policy issues raised by the increased availability of genetic testing in medical and consumer genomics formats? To help address these questions, the author will draw upon his work over the last decade as part of the NIH-funded Risk Evaluation and Education for Alzheimer's disease (REVEAL) Study. The REVEAL Study is a series of three multi-site randomized clinical trials (N = 720 participants) that have examined different methods for providing genetic risk information for Alzheimer's disease (AD) to asymptomatic individuals. The e4 allele of the Apolipoprotein E (*APOE*) gene increases AD risk but is neither necessary nor sufficient to cause the disease. *APOE* disclosure serves as an instructive paradigm for examining the benefits and risks of communicating personalized genetic information. Study outcomes include 1) psychological adjustment to risk information, 2) behavior changes following testing, 3) comprehension of test results, and 4) impact across various genetic counseling methods (e.g., extended vs. condensed vs. telephone protocols). Findings include:

- Participants sought testing for reasons of personal (vs. clinical) utility, such as financial planning and a sense that "knowledge is power"
- Participants did not generally experience adverse psychological outcomes following testing, even when they received e4 results
- Participants generally understood that "genetics is not destiny," but their risk perceptions suggested an overrating of "negative" test results
- Genetic risk information prompted behavioral responses among higher risk groups, including long-term care insurance changes and addition of dietary supplements not proven to reduce disease risk

The study's implications for practice and policy will be discussed, including how findings inform debates over the benefits and harms of genetic susceptibility testing for other common diseases including those in psychiatry.

INDIVIDUAL ABSTRACT:
RETURNING INCIDENTAL GENETIC RESULTS TO MINORITY PARTICIPANTS

Sarah Hartz, M.D., Ph.D.

Washington University in St. Louis School of Medicine

Due to the generosity of participants in genetic studies, our understanding of genetic risk factors for disease has improved exponentially over the past decade. However, there is no system in place for reporting genetic results back to study participants. To address this issue, we sampled 50 subjects from a genetic study of nicotine dependence and returned incidental genetic results to these subjects. We followed them to evaluate smoking behavior, healthcare utilization, and psychological symptoms. This study is a paradigm shift by offering genetic information to community-based study participants, an essential step towards wide-scale personalized medicine.

INDIVIDUAL ABSTRACT:
BIG ELEPHANTS NEED WIDE ANGLE LENSES: WHY ASD REQUIRES AN EXPANSIVE INTEGRATIVE PERSPECTIVE

Isaac Kohane, M.D., Ph.D.

Boston Children's Hospital

There are several parallel and often quite distinct investigational approaches to understanding ASD. This is a necessary and Salutory development because of the considerable heterogeneity in both the etiologies and the manifestations of ASD. I will review this heterogeneity and outline an integrative framework for the diagnosis of ASD.

SYMPOSIA
2:15 PM – 4:15 PM

OVERALL ABSTRACT:
ROLE OF METHYLATION AND CHROMATIN MODIFICATION IN SUBSTANCE ABUSE AND BEHAVIOR

Jonathan Pollock[1], Deborah Mash, Ph.D.[2], Anne E. West, M.D., Ph.D.[3], Chris Pierce[4], Jian Feng[5]

[1] NIDA, [2]University of Miami Miller School of Medicine, [3]Neurobiology, [4]University of Pennsylvania, [5]Mount Sinai School of Medicine

Over the past several years there has been a revolution in understanding how the genome is modified by the environment through epigenetic changes. These epigenetic modifications of the genome produced by the environment are mediated through DNA methylation and modification of histone proteins. Some epigenetic modifications are transmitted through the genome while others produce long lasting changes in behavior. This symposium reviews recent advances in how drugs of abuse modify the epigenome to produce addictive behavior in animals and in human beings. Limits of epidemiological designs in identifying epigenetic changes in human beings will also be discussed.

INDIVIDUAL ABSTRACT:

THE EPIGENETIC REGULATION OF COCAINE ACTION IN MOUSE NUCLEUS ACCUMBENS

Jian Feng

Mount Sinai School of Medicine

Increasing evidence supports a role for altered gene expression in mediating the lasting effects of cocaine on the brain, and recent work has demonstrated the involvement of chromatin modifications in these alterations. We utilize next generation sequencing technology, mRNA-seq and ChIP-seq, to obtain an unprecedented view of cocaine-induced changes in gene expression and associated adaptations in numerous modes of histone modification in mouse nucleus accumbens, a key brain reward region. We map at a genome wide scale RNA polymerase II and several histone modifications including H3K4m1/3, H3K9m2/3, H3K36m3 and H3K27me3 after chronic cocaine administration. We then superimpose these data with mRNA-seq transcriptome alterations. We identify unique combinations of chromatin changes, or signatures, that accompany cocaine's regulation of gene expression, including the dramatic involvement of pre-mRNA alternative splicing in cocaine action. In addition, we identify one splicing factor A2BP1 (Rbfox1/Fox-1), which is enriched at genes that display certain chromatin signatures and contributes to drug-induced behavioral abnormalities. Together, this delineation of the cocaine-induced epigenome in the nucleus accumbens reveals several novel modes of drug regulation, thereby providing new insight into the biological basis of cocaine addiction. More broadly, the combinatorial chromatin and transcriptional approaches that we describe serve as an important resource for the field, as they can be applied to other systems to reveal novel transcriptional and epigenetic mechanisms of neuronal regulation.

INDIVIDUAL ABSTRACT:
ROLES FOR THE METHYL-DNA BINDING PROTEIN MECP2 IN ADDICTIVE-LIKE BEHAVIORS

Anne E. West, M.D., Ph.D.

Neurobiology

The methyl-DNA binding protein MeCP2 is emerging as an important regulator of drug reinforcement processes. Psychostimulants induce phosphorylation of MeCP2 at Ser421 (pMeCP2), however the functional significance of this posttranslational modification for addictive-like behaviors was unknown. We find that MeCP2 Ser421Ala knockin mice display both a reduced threshold for the induction of locomotor sensitization by investigator-administered amphetamine and enhanced behavioral sensitivity to the reinforcing properties of self-administered cocaine. Behavioral differences are accompanied in the knockin mice by an enhanced psychostimulant-dependent reduction of medium spiny neuron excitability, which is a neural adaptation strongly associated with the rewarding properties of these drugs. We propose that pMeCP2 functions to limit the circuit plasticities in the nucleus accumbens that underlie addictive-like behaviors.

INDIVIDUAL ABSTRACT:
FORGIVING THE SINS OF THE FATHER: EPIGENETIC INHERITANCE OF A COCAINE RESISTANCE PHENOTYPE

Chris Pierce

University of Pennsylvania

A rat model was developed in order to delineate a heritable phenotype resulting from the self-administration of cocaine. Delayed acquisition and reduced maintenance of cocaine self-administration was observed in male, but not female, offspring of sires that self-administered cocaine. Brain-derived neurotrophic factor (BDNF) mRNA and protein were increased in the medial prefrontal cortex (mPFC) and there was an increased association of acetylated histone H3 with BDNF promoters only in the male offspring of cocaine-experienced sires. Administration of a BDNF receptor antagonist (the TrkB receptor antagonist ANA-12) reversed the diminished cocaine self-administration in male cocaine-sired rats. In addition, the association of acetylated histone H3 with BDNF promoters was increased in the sperm of sires that self-administered cocaine. Collectively, these findings indicate that voluntary paternal ingestion of cocaine results in epigenetic reprograming of the germline resulting in profound effects on mPFC gene expression and resistance to cocaine reinforcement in male offspring.

INDIVIDUAL ABSTRACT:
EPIGENETICS MARKS OF COCAINE ADDICTION IN DOPAMINE PATHWAYS

Deborah Mash, Ph.D.

University of Miami Miller School of Medicine

Multiple interacting genes and environmental factors underlie the risk of drug addiction. High throughput technologies are used to develop epigenome maps of human brain, which are essential for understanding the role of epigenetic mechanisms in the neurobiology of drug abuse. In this study, we aimed to investigate DNA methylation patterns associated with chronic cocaine use in dopamine pathways. To this end, we used reduced representation bisulfite sequencing (RBBS) to detect DNA methylation at single-nucleotide resolution coupled with RNA-Seq analysis in the dorsal and ventral striatum from chronic cocaine abusers compared to age-matched control subjects who had no history of drug or alcohol abuse. We will report on the development of protocols optimized and fit for use on postmortem brain, which afford near-complete bisulfite conversion and largely unbiased representation of RRBS libraries. These first-time studies in human brain provide support for the role of epigenetic regulatory responses at the level of the genome in cocaine addiction.

Saturday, October 19, 2013

PLENARY
8:30 AM – 9:30 AM

OVERALL ABSTRACT:
WORLD-WIDE, AND GENOME-WIDE, STUDIES OF AUTISM SPECTRUM DISORDERS (ASD)

Christopher A. Walsh, M.D., Ph.D.

Boston Children's Hospital

Although ASD is among the most highly heritable of neuropsychiatric disorders, causal genes are typically only defined in 15-20% of patients. Growing evidence suggests that ASD can arise from a diversity of rare mutations. De novo mutations (i.e., present in an affected child but absent from parents) both copy number variants (CNVs) and point mutations appear to be important risk factors for ASD. However, de novo mutations do not substantially contribute to the heritability of ASD. In order to define heritable rare causes of ASD, our lab has focused on identifying recessive mutations associated with ASD. We have recruited > 200 families with one or more children affected with ASD (>40 with 2 or more affected children) in which parents share ancestry (typically as first cousins), focusing recruitment on countries where cousin marriage is common, including Kuwait, Saudi Arabia, Turkey, the United Arab Emirates, and Pakistan. Consanguineous families show many notable differences in ASD causation compared to typical nonconsanguineous American families, including a lower male:female ratio among probands (<3:1 versus >4:1), and a notable lack of de novo CNVs in affected patients compared to nonconsanguineous families. On the other hand, ASD patients from consanguineous marriages show a much higher rate of homozygous CNVs (deletions) inherited from parents-- compared to nonconsanguineous families, suggesting recessive mutations. These homozygous deletions often do not remove genes, but often occur near genes that are regulated by neuronal activity, deleting key regulatory sequences. Whole exome and whole genome sequencing has also identified several genes with homozygous mutations in ASD patients, some of which also cause recessive ASD in American patients. Our data suggest that partial loss of gene function (either by complete loss of one allele, or partial impairment of two alleles) may be an important mechanism in ASD. Recent work also implicates recessive mutations in nonconsanguineous families as well.

PLENARY
9:45 AM – 10:45 AM

OVERALL ABSTRACT:
NOVEL USES OF IPS CELLS

Kevin Eggan, Ph.D., Harvard University

In principal, stem cell and reprogramming technologies allow unprecedented access to significant quantities of specific well-defined, human neural subtypes relevant to psychiatric disease. Given that the genetics community has delivered a wealth of genetic variants that need to be systematically interrogated for their effects on human neurobiology, the unification of these platforms offer an opportunity to advance hypotheses concerning the biology of these conditions. Efforts to advance in these goals will be discussed.

SYMPOSIA
10:30 AM – 12:30 PM

OVERALL ABSTRACT:
GENE PATHWAYS: TRANSCENDING DISORDERS

Aiden Corvin[5], Dick McCombie, Ph.D.[1], Patrick Sullivan, M.D., FRANZCP[2], Dennis Vitkup[3], Daniel Geschwind, M.D., Ph.D.[4], Michael Gill, M.D. [5]

[1]Cold Spring Harbor Laboratory, [2]UNC/Genetics, [3]Department of Systems Biology, Department of Biomedical Informatics, [4]Departments of Neurology, Psychiatry and Human Genetics, [5]Trinity College Dublin

Risk genes have no respect for clinical diagnostics. There is compelling evidence for shared molecular etiology across psychotic disorders with emerging evidence for overlap between schizophrenia and other neurodevelopmental disorders (e.g. Autism, schizophrenia and Intellectual disability).Individual gene findings cannot be seen in isolation. To establish coherence, systematic approaches are required to dissect the molecular mechanisms or pathways involved. In this symposium we review emerging support for specific pathways and the challenges that face this burgeoning field.

INDIVIDUAL ABSTRACT:
POTENTIAL OVERLAP OF GENES INVOLVED IN AUTISM AND SCHIZOPHRENIA: A LINK TO CHROMATIN REMODELING

Dick McCombie, Ph.D.

Cold Spring Harbor Laboratory

Autism and Schizophrenia represent complex phenotypes which likely have underlying complex genetic contributors. We have carried out sequencing on trios consisting of unaffected parents and a child with schizophrenia. Analysis of these data show interesting potential overlaps with genes associated with autism that have recently been published by a variety of groups. Among these are genes involved in chromatin remodeling and regulation of transposition and epigenetic function in cells. We are more deeply analyzing these associations with both functional and additional genetic studies. This class of genes may represent one of the important functions that are perturbed leading to a diverse range of psychiatric illnesses.

INDIVIDUAL ABSTRACT:
PATHWAYS AND CROSS DISORDER ANALYSIS, PSYCH CHIP AND BEYOND

Benjamin Neale

ATGU, Massachusetts General Hospital

The psych chip is a purpose-built assay designed for the identification and validation of loci for psychiatric illness. The goals of this platform are to provide an array that can be used across a range of psychiatric illnesses and thus enable assessment of shared and specific genetics factors across these illnesses. In addition, the design of the psych chip incorporates a range of elements aimed at revealing novel associations beyond those chosen for replication. In addition to a description of the psych chip proposal, this session will describe emerging pathway and cross-disorder analyses integrating results from a variety of different approaches to the identification of risk loci, genes, and pathways for psychiatric illness. In particular, an assessment of the overlap between autism and schizophrenia will be described leveraging genome-wide association and

next-generation sequencing data. By integrating across psychiatric illnesses, we demonstrate greater power to identify the underlying genetic risk factors as well as highlight emerging pathways from this work.

INDIVIDUAL ABSTRACT:
GENE NETWORKS UNDERLYING COMMON PSYCHIATRIC DISORDERS

Dennis Vitkup

Department of Systems Biology, Department of Biomedical Informatics

Identification of complex molecular networks underlying common human phenotypes is a major challenge of modern genetics. Despite the identification of many relevant loci, molecular mechanisms of many common human diseases remain largely unclear. We have developed several computational approaches that allow an integrated analysis of diverse disease-related genetic data (CNVs, SNVs, GWAS) using a unified statistical framework. The application of these approaches to several psychiatric diseases (such as autism, schizophrenia) allowed us to implicate several molecular processes involved in synapse development, axon targeting, neuronal mobility, and chromosomal modification. Our results reveal an amazing phenotypic and genetic diversity of the psychiatric disorders. Notably, the networks associated with distinct psychiatric disorders significantly overlap in terms functional modules and biological pathways. A comparative analysis of copy number variants associated with autism and schizophrenia suggests that although the molecular networks implicated in these distinct disorders may be related, the mutations associated with each disease may lead, at least on average, to different functional consequences.

INDIVIDUAL ABSTRACT:
INTEGRATIVE, SYSTEMS LEVEL ANALYSIS OF THE TRANSCRIPTOME IN ASD

Daniel Geschwind, M.D., Ph.D.

Departments of Neurology, Psychiatry and Human Genetics

We have previously defined a shared molecular pathology in ASD using microarray gene-expression profiling. Large scale gene discovery studies including whole exome sequencing have also identified dozens of genes that are high probability candidate genes. We have integrated these different forms of data to identify both convergent molecular pathways and brain circuits. We have also extended our previous transcriptional profiling using RNAseq in a new independent cohort of ASD cases and controls, validating the previous findings based on microarrays. These analysis point to distinct epochs early in development for ASD etiology, as well as dysfunction in specific interhemispheric and cross collosal pathways as one area of convergence. Specifically, rare variants implicate early transcriptional dysregulation during the phases of progenitor proliferation and early cell fate decisions during corticogenesis. Common variants are more likely to target early postnatal processes involved in neuronal and synaptic maturation. ASD risk genes also appear to be enriched in superficial cortical laminae, which implicates cortical cortical interconnectivity, which is especially striking when compared to intellectual disability, where risk genes are enriched in deep layers.. Both ASD risk genes and differentially expressed genes in brain are enriched in GABA-ergic neurons consistent with interneuron dysfunction as a potential ahred mechanism in a subset of ASD.

SYMPOSIA
10:30 AM – 12:30 PM

OVERALL ABSTRACT:

GENETICS OF POST-TRAUMATIC STRESS DISORDER (PTSD): RECENT ADVANCES IN THE SEARCH OF GENETIC DETERMINANTS OF PTSD FROM GENOME-WIDE ASSOCIATION STUDIES

Karestan C. Koenen, Ph.D.[1], Joel Gelernter[2], Guia Guffanti, Ph.D.[3], Kerry Ressler, M.D., Ph.D.[4], Patrick F. Sullivan, M.D., FRANZCP[5], Murray B. Stein, M.D.[6]

[1]Columbia University Mailman School of Public Health, [2]Yale Univ. School of Medicine, [3]Columbia University, [4]Emory University, [5]University of North Carolina, [6]Departments of Psychiatry and Family & Preventive Medicine

Posttraumatic stress disorder (PTSD) is a common and debilitating mental disorder with heritability estimated between 30 and 72%. The lifetime prevalence of PTSD is 7.6% in the United States and more than doubled in special populations: 13-20% among the 2.6 million soldiers who have served in Operation Enduring Freedom, 30% among Katrina survivors and 17% in an epidemiologic sample from urban Detroit. PTSD has been identified as a serious public health problem, which compels the search for biomarkers that distinguish between persons at high and low risk of developing PTSD following trauma exposure. It is well known that the majority of persons who are exposed to even a severe traumatic event do not develop PTSD. Why some individuals are vulnerable and others are resilient remains an open question; genetic factors are hypothesized to play an important role PTSD. The Institute of Medicine, Department of Defense and the National Institute of Mental Health have identified the identification of robust genetic risk variants for PTSD, together with other types of biomarkers, as a priority research goal. Until very recently, genetic association studies of PTSD were limited to candidate genes. A recent review of the biology of PTSD concluded that candidate gene studies had not produced robust results. Large genome-wide association studies (GWAS) of PTSD are needed to identify novel regions in the genome. GWAS allows a comprehensive scan of the genetic risk landscape in an unbiased fashion for not being anchored to literature-based selection of candidate genes. Thus, GWAS provides a critical hypothesis-generating tool for identifying totally unprecedented genes contributing to PTSD. In 2012, the first GWAS of PTSD has been published using a sample of 295 cases and 196 controls and implicated retinoid-related orphan receptor alpha gene (RORA). Results have been replicated in at least one independent sample at this writing. The most recent results of GWAS for other mental disorders such as schizophrenia and bipolar disorder have shown that very large sample sizes produce many, over 60 in the case of schizophrenia (S. Ripke, WCPG oral presentation, October 2012), robust genetic associations. Growing evidence suggests psychiatric disorders are highly polygenic and that very large samples sizes are required to detect weak effects on disease. The genetic architecture of PTSD may be similar, but the architecture will be determined with large scale GWAS of PTSD that will require pooling data across studies. Numerous research groups, such as Army STARRs (http://www.armystarrs.org), have large PTSD GWAS under review or in preparation. This symposium will present and discuss the most recent results of four GWAS of PTSD in four different and independent populations. It will review the intrinsic biology emerging from the results of these first GWASs as well as common challenges encountered in the design and analysis of GWASs of PTSD. There is great interest in the genetics of PTSD and we expect that the growing number of risk variants identified in these GWASs will greatly enhance our understanding of PTSD's etiology leading to more effective pharmacological interventions.

INDIVIDUAL ABSTRACT:
GENOME-WIDE ASSOCIATION STUDY IMPLICATES A NOVEL RNA GENE, THE LINCRNA AC068718.1, AS A RISK FACTOR FOR POST-TRAUMATIC STRESS DISORDER IN WOMEN

Guia Guffanti, Ph.D.

Columbia University

Posttraumatic stress disorder (PTSD) is a common and debilitating mental disorder with a particularly high burden for women. Emerging evidence suggests PTSD may be more heritable among women and evidence from animal models and human correlational studies suggest connections between sex-linked biology and PTSD vulnerability, which may extend to the disorder's genetic architecture. We conducted a genome-wide association study (GWAS) of PTSD in a primarily African American sample of women from the Detroit Neighborhood Health Study (DNHS) and tested for replication in an independent cohort of primarily European American women from the Nurses Health Study II (NHSII). We genotyped413 DNHS women - 94 PTSD cases and 319 controls exposed to at least one traumatic event - on the Illumina Human Omni Express Bead Chip for > 700,000 markers and tested 578 PTSD cases and 1963 controls from NHSII for replication. We performed a network-based analysis integrating data from GWAS-derived independent regions of association and the Reactome database of functional interactions. We found genome-wide significant association for one marker mapping to a novel RNA gene, lincRNA *AC068718.1,* for which we found suggestive evidence of replication in NHSII. Our network-based analysis indicates that our top GWAS results were enriched for pathways related to telomere maintenance and immune function. Our findings implicate a novel RNA gene, lincRNA *AC068718.1,* as risk factor for PTSD in women and add to emerging evidence that non-coding RNA genes may play a crucial role in shaping the landscape of gene regulation with putative pathological effects that lead to phenotypic differences.

INDIVIDUAL ABSTRACT:
GENOMEWIDE ASSOCIATION STUDY IDENTIFIES TLL1 AS A PTSD RISK LOCUS

Joel Gelernter

Yale Univ. School of Medicine

Genetic factors influence the risk for posttraumatic stress disorder (PTSD). Candidate gene association studies and gene-by-environment interaction studies have identified several genetic variants that contribute to PTSD risk, and so has a prior GWAS. We conducted a GWAS in 4344 subjects -- 1578 European Americans (EAs) including 300 PTSD cases, and 2766 African Americans (AAs) including 444 PTSD cases -- to identify novel common risk alleles of PTSD. Subjects were recruited originally for studies of substance dependence genetics. Samples were genotyped on the Illumina HumanOmni1-Quad v1.0 microarray containing ~1M 900k autosomal SNPs. In EAs, we observed that one SNP on chromosome 7p12, rs406001, exceeded genomewide significance. A SNP that maps to the first intron of the tolloid-Like 1 gene (*TLL1*) showed the second strongest association signal, although no SNPs at this locus reached genome wide significance initially. We then tested six SNPs in an independent sample of nearly 2000 EAs, and successfully replicated the association findings for two SNPs in the first intron of *TLL1*, with P values of 6.3×10^{-6} and 2.3×10^{-4}. In the combined sample, rs6812849 had a p-value of 3.1×10^{-9}. No significant signals were observed in the AA part of the sample. This study identified *TLL1* as a new susceptibility gene for PTSD.

INDIVIDUAL ABSTRACT:
GENETICS OF RISK AND RESILIENCE IN PTSD: UPDATES FROM A LARGE TRAUMATIZED CIVILIAN COHORT

Kerry Ressler, M.D., Ph.D.

Emory University

Background: We interviewed >6000 highly traumatized civilians from inner city Atlanta. In addition to detailed phenotype data on their trauma history and trauma-related psychopathology, we have gathered genetic data on all subjects and and neuroimaging data on a subset.
Methods: We extended our previous candidate gene findings, as well identified new target pathways, in a trauma exposed, sample of ~3000 African American subjects. Self-reported psychiatric measures were collected, and DNA was obtained for genetic analysis. Genomic DNA was genotyped on the Illumina 1M Omni Quad array as well as specific candidate genes analyzed via ABI Taqman and Illumina Sequenom Massarray.
Results: Our follow up data examining the PACAP receptor SNP rs2267735, resulted in a genotype x trauma interaction in females ($p<0.001$), but not males ($p>0.1$). A meta-analysis with our previously reported samples revealed a strong association between PTSD severity and the interaction between trauma and genotype in females ($N=1424$, $p<0.0001$). Regarding FKBP5, we report replication and extension of the prior PTSD risk findings using MRI, hippocampal activity, shape, and connectivity effects as a function of the FKBP5 risk allele in traumatized subjects. We will also present updates related to quality control and early findings from our GWAS study.
Discussion: We report follow up replications of our prior findings in PTSD as well as new data from a GWAS of civilian PTSD. While further replications and additional samples await addition, these data provide hope for a future understanding of the molecular genetic pathways involved in mediating risk vs. resilience and PTSD.

INDIVIDUAL ABSTRACT:
POSTTRAUMATIC STRESS AND RELATED SYMPTOMS FOLLOWING DEPLOYMENT: GWAS FINDINGS FROM ARMY STARRS

Murray B. Stein, M.D.

Departments of Psychiatry and Family & Preventive Medicine

Army STARRS is the largest study of mental health risk and resilience ever conducted among military personnel. One of the most important foci of Army STARRS is on risk and protective factors for posttraumatic stress (PTS) and related symptoms. This presentation will feature preliminary findings from a new GWAS study of approximately 8,000 members of Army Brigade Combat Teams who were evaluated shortly prior to deployment to Afghanistan and then within the first month following their return to the US and again 2- and 6-months later. This presentation will emphasize genome wide findings pertinent to outcomes including posttraumatic stress symptoms and disorder, major depressive symptoms and disorder and, time permitting, measured traits and latent construct(s) relevant to these symptoms and disorders.

SYMPOSIA
10:30 AM – 12:30 PM

OVERALL ABSTRACT:
NEW DATA ABOUT THE GENETICS OF ATTENTION DEFICIT HYPERACTIVITY DISORDER

Stephen V. Faraone, Ph.D.[1], Benjamin Neale, Ph.D.[2], Peter Holmans, Ph.D.[3], Andreas Reif, M.D.[4], Anita Thapar, F.R.C.Psych., Ph.D.[5], Barbara Franke[6]

[1]SUNY Upstate Medical University, [2]ATGU, Massachusetts General Hospital, [3]Cardiff University, [4]University Clinic of Wuerzburg, Dept. of Psychiatry, [5]MRC Centre for Neuropsychiatric Genetics and Genomics, Cardiff, University, [6]Departments of Human Genetics and Psychiatry, Donders Institute for Brain, Cognition & Behaviour, Radboud University Medical Centre, Nijmegen, The Netherlands

With a heritability of .70 across twenty twin studies, ADHD has been viewed as a suitable candidate for molecular genetic studies. To date, genome-wide association studies have not discovered any common DNA variation that has achieved genome-wide significance, but have documented an excess of rare, large duplications and deletions through analyses of copy number variations (CNVs). This symposium will provide new data about the genetics of ADHD and its relationship with other psychiatric disorders. Peter Holmans will present pathway analyses on a multi-centre sample of over 10,000 rare ADHD CNVs, and on a GWAS combining nine different studies, totaling 5,621 cases and 13,589 controls. Both of these samples are the largest of their kind ever assembled for ADHD. A comprehensive set of pathways was used, comprising Gene Ontology, KEGG, PANTHER, Mouse Genome Informatics, Reactome and Biocarta). A significant excess of pathways enriched for association signal was observed in both the GWAS and the CNV samples separately. The overlap in pathways enriched in both samples was also significantly larger than expected. Ben Neale will present the results of an ongoing exome chip study of ~10,000 ADHD and control samples. The design of the exome chip was to aggregate ~12,000 samples with exome sequencing from a variety of disease studies and cross-sectional cohorts to identify rare coding variation in the population. This platform contains ~240,000 missense, nonsense, and splice site rare variants, with allele frequency less than 0.02%. We present single variant analysis and rare variant burden analysis for each of the genes. Barbara Franke will discuss Drosophila melanogaster as a model system for ADHD. Her drosophila study knocked down the expression of the DAT1 homologue. The study used activity monitors to record 24-hour activity rhythms in a quantitative manner and was able to show mutant flies to have a very specific pattern of hyperactivity. This behavioral effect could be rescued by methylphenidate, the most frequently used stimulant treatment for ADHD. At the cellular level, mutant flies showed clearly altered synapse morphology at the larval stage. A knock-down of the LPHN3 homologue showed very similar results, which allows conclusions to be drawn about the mode of action of this largely uncharacterized ADHD risk gene. Andreas Reif and Alejandro Arias Vasquez will present a cross-disorder GWAS study of ADHD and bipolar disorder (BPD) which are known to co-occur and to be transmitted together in families. The study aims to identify both shared as well discriminating risk genes by analyzing the ADHD and BPD GWAS datasets from the Psychiatric Genomics Consorium (PGC). Bipolar cases have been restricted to an age of onset less than 21. Hypothesis-driven analyses investigated mutual risk genes by specifically testing the top 1000 SNP hits from either GWAS in the respective other sample. This was done on the single SNP and on a gene based level. Hypothesis-free analyses were run by meta-analytic treatment of the data identifying novel loci below genome-wide significance, but with a p>10-5. Currently, polygenic analyses of genetic components of the two psychiatric diseases were performed, training on one and using the other as target set.

INDIVIDUAL ABSTRACT:
PATHWAY ANALYSIS OF LARGE MULTICENTRE ADHD GENOME-WIDE DATASETS

Peter Holmans, Ph.D.

Cardiff University

Previous studies have shown an enrichment of large, rare CNVs in ADHD cases compared to controls, suggesting a role for such CNVs in the aetiology of ADHD (Williams et al. 2010). There is some evidence (Elia at al. 2012) that CNVs in ADHD cases preferentially hit genes in particular biological pathways, giving insight into the processes underlying ADHD susceptibility. GWAS studies of ADHD have failed to show conclusive evidence of association to any one gene. However, pathway analyses of such data may prove valuable. We have performed pathway analyses on a multi-centre sample of over 10,000 rare ADHD CNVs, and on a GWAS combining nine different studies, totaling 5,621 cases and 13,589 controls. Both of these samples are the largest of their kind ever assembled for ADHD, and will thus be more powerful than previous studies. A comprehensive set of pathways was used, comprising Gene Ontology, KEGG, PANTHER, Mouse Genome Informatics, Reactome and Biocarta). A significant excess of pathways enriched for association signal was observed in both the GWAS and the CNV sample separately. The overlap in pathways enriched in both samples was also significantly larger than expected. Interestingly, this overlap contains pathways relating to nicotinic acetylcholine receptors, which have been highlighted by previous studies. A significant overlap in enriched pathways is also observed between ADHD and other psychiatric disorders, such as autism and schizophrenia. Analysis of all CNVs >100kb yielded several pathways showing significant enrichment for genes hit by case CNVs after correction for multiple testing of pathways. Several clusters of enriched pathways were observed, relating to brain morphology, the complement cascade, acetylcholine receptors, B-cells, and SNARE/syntaxin binding. Subdividing the CNVs into deletions and duplications yielded further significant areas of biological interest.

INDIVIDUAL ABSTRACT:
CROSS-DISORDER GWAS OF ADHD AND BIPOLAR DISORDER

Andreas Reif, M.D.

University Clinic of Wuerzburg, Dept. of Psychiatry

There is considerable evidence that ADHD and bipolar disorder (BPD) can be co-morbid conditions. Coming from primary bipolar samples, the co-morbidity between BPD and ADHD has been estimated to be between 9 and 18%. Coming from primary ADHD samples, comorbidity rates vary more, although the mean seems to be between 9% and 19% as well. Also, family-based studies argue for a shared genetic liability between both disorders which however has not yet been addressed in depth. We have thus aimed to identify both shared as well discriminating risk genes by analyzing the ADHD and BPD GWAS datasets from the PGC; bipolar cases have been restricted to an age of onset before the 21st birthday. The main analysis focuses on the PGC BPD and ADHD GWAS datasets on the categorical level. Datasets were examined in separate ways: first, hypothesis-driven analyses were performed, investigating mutual risk genes by specifically testing the top 1000 SNP hits from either GWAS in the respective other sample. This was done on the single SNP and on a gene based level (also testing imputed SNPs). Furthermore, hypothesis-free analyses were run by meta-analytic treatment of

the data identifying novel loci below genome-wide significance, but with a $p>10^{-5}$. The top loci of each individual analysis were still retained in this meta-analysis. Currently, polygenic analyses of genetic components of the two psychiatric diseases are performed, training on one and using the other as target set. In these analyses, we test the hypothesis that risk alleles – as a group – influence the other phenotype using a polygenic score test. Additionally, we aim to identify risk genes separating both disorders by contrasting ADHD and BPD (as opposed to comparing them to control samples). Further description of the genetic architecture underlying the co-morbidity of ADHD and BPD will aid in identifying mechanisms of disease and hopefully also biomarkers in order to enable better differential diagnosis of these conditions.

INDIVIDUAL ABSTRACT:
MODEL SYSTEMS FOR MAPPING BIOLOGICAL PATHWAYS FROM GENE TO DISEASE IN ADHD

Barbara Franke

Departments of Human Genetics and Psychiatry, Donders Institute for Brain, Cognition & Behaviour, Radboud University Medical Centre, Nijmegen, The Netherlands

By collaborating in large, international consortia, psychiatric genetics research has finally started to pick up speed in identifying novel risk factors for mental disorders. However, we now often find ourselves faced with the fact that the genes implicated are uncharacterized, and the way in which they contribute to disease is unclear. Also for most known ADHD genes, the mechanistic biological pathways leading from affected gene to psychiatric phenotype still need clarification. This is rapidly developing into the next bottleneck in our ability to understand disease etiology and translate findings from genetics into clinical practice.

Valid model systems are dearly needed to improve this situation. In this presentation, we will show two complementary models:

The fruit fly Drosophila melanogaster is an ideal model system to study behavioural and cellular consequences of ADHD genes. This model system can be easily genetically manipulated and shows behaviour related to traits underlying ADHD, i.e. (hyper)activity and (in)attention. These behaviours can be measured quantitatively and are genetically sensitive. Importantly, we can also easily assess the effects of ADHD genes at the cellular level. We will show examples of such research based on knock-down of the DAT1 homologue as a proof of concept for the use of Drosophila melanogaster in ADHD research, and the LPHN3 homologue.

Neuroimaging genetics offers a second opportunity to unravel the mechanisms underlying the effects of genes on ADHD. This model has the advantage that the exact genetic variant causing increased ADHD risk is modeled in the human genetic background. We will show results for DAT1 and a recently observed variant in AS3MT, a recently discovered risk gene for several forms of psychiatric disease.

Our results indicate that work in Drosophila in combination with neuroimaging genetics work offer a powerful approach for mapping risk pathways from gene to disease and for testing new candidate risk genes. Importantly, these models can be used at a throughput that can match the increasing pace of gene-finding in psychiatric genetics.

INDIVIDUAL ABSTRACT:

ANALYSIS OF RARE CODING VARIATION IN A SAMPLE OF 10,000 CASES OF ATTENTION DEFICIT/HYPERACTIVITY DISORDER AND CONTROLS

Benjamin Neale, Ph.D.

ATGU, Massachusetts General Hospital

Attention Deficit/Hyperactivity disorder (ADHD) is characterized by difficulties in attention and impulse control. ADHD is one of the most common psychiatric disorders in children, with estimates of prevalence ranging from 4 to 16% and is correlated with a range of outcomes including poorer school performance, increased incidence of drug use and abuse, and higher rates of sexually transmitted disease. Slightly less than half of individuals diagnosed with ADHD will persist into adulthood. Estimates of heritability for ADHD have ranged form 60-95% with a mean estimate of 76% (Faraone et al, 2005). Thus far, genome-wide association has not identified any single SNP meeting significance threshold, but ADHD has been shown to be heritable directly as estimated by SNP data. Copy number variation polymorphisms, however, have been identified as risk factors to ADHD. In an attempt to identify further risk variants for ADHD, we have genotyped approximately 10,000 samples on the exome chip. The design of the exome chip was to aggregate ~12,000 samples with exome sequencing from a variety of disease studies and cross-sectional cohorts to identify rare coding variation in the population. This platform contains ~240,000 missense, nonsense, and splice site rare variants, with allele frequency less than 0.02%. We present single variant analysis and rare variant burden analysis for each of the genes.

ORAL AND POSTER PRESENTATIONS
2:30 PM – 4:00 PM

AUTISM AND NEURODEVELOPMENTAL DISORDERS

INDIVIDUAL ABSTRACT:
THE ROLES OF FMRP-REGULATED GENES IN AUTISM: SINGLE- AND MULTI-HIT GENETIC AETIOLOGIES

Julia Steinberg[1], Caleb Webber, Ph.D.[2]

[1]University of Oxford, [2]MRC Functional Genomics Unit, University of Oxford

Background: Autism spectrum disorder (ASD) is a highly heritable complex neurodevelopmental condition characterized by impairments in social interaction and communication, and restricted and repetitive behaviors. Although roles for both *de novo* and familial genetic variation have been documented, the underlying disease mechanisms remain poorly elucidated. In this study, we defined and explored distinct aetiologies of genetic variants in individuals with ASD that affect genes regulated by FMRP, a protein with key roles in neuroplasticity and neuronal translation.

Methods: We exploited detailed spatio-temporal maps of gene expression within the human brain to identify discrete subpopulations of FMRP targets, and used functional genomics approaches to elucidate the biological functions of each subpopulation. Subsequently, we examined the contribution of the FMRP target subpopulations to ASD by considering damaging *de novo* mutations in ASD probands, rare copy number variants (CNVs) in ASD probands and controls, as well as single nucleotide polymorphism (SNP) data from ASD probands and controls. To better understand the role of inherited variation, we developed a statistical Trend test that is both sensitive to a multi-hit disease aetiology model and demonstrates an increase in

power to detect pathway associations compared to existing approaches.
Results: We identified four discrete subpopulations of FMRP targets and showed that different subpopulations contribute to ASD via different types of genetic variation. We found that genes disrupted by damaging *de novo* mutations in ASD are significantly enriched in an embryonically-upregulated subpopulation of FMRP targets that is involved in transcriptional regulation. By contrast, ASD rare copy number variants (CNVs) preferentially disrupt a subpopulation of FMRP targets with synaptic functions; this same subpopulation of FMRP targets is also associated with ASD diagnosis based on single nucleotide polymorphism (SNP) data. Employing our novel Trend test, we demonstrated that individuals carrying multiple disruptions of FMRP targets, particularly those with synaptic functions, by rare CNVs are at a significantly higher risk for ASD.
Discussion: Our results suggest that mutations in genes regulated by FMRP contribute to ASD via two distinct genetic aetiologies:

1. single disruptions of embryonically-upregulated FMRP targets that are likely to be highly penetrant and ultra-rare ("single-hit aetiology"), or
2. less-penetrant, multiple disruptions of non-embryonic, synaptic FMRP targets, which act in combination to give rise to ASD ("multi-hit aetiology").

Aetiologies that lie between these two extremes may well be found, and it will be of interest to investigate to what extent less penetrant disruptions of synaptic genes can modify phenotypes caused by highly penetrant disruptions of embryonic transcription factors, for example. Notably, the Trend test is applicable to a wide range of disorders which exhibit a strong polygenic component and easily extendible to combine information from multiple types genetic variation (i.e. CNVs and exome variants).

INDIVIDUAL ABSTRACT:
META-ANALYSIS OF EUROPEAN ANCESTRY INDIVIDUALS WITH AUTISM SPECTRUM DISORDER REVEALS STRONG ASSOCIATION 3' OF THE ASTROTACTIN 2 (ASTN2) GENE LOCUS ON CHROMOSOME 9

Richard J.L. Anney, Ph.D.[1], Stephan Ripke[2], Dan Arking[3], Ed Cook[4], Elise Robinson[2], Verneri Anttila[2], Michael Gill[1], Susan Santangelo[5], James Sutcliffe[6], Mark Daly[2], Bernie Devlin[7]

[1]Neuropsychiatric Genetics Group & Department of Psychiatry, Trinity College Dublin, [2]Analytic and Translational Genetics Unit, MGH & Harvard Medical School & Stanley Center for Psychiatric Research, Broad Institute of MIT and Harvard, [3]Johns Hopkins University, [4]University of Illinois, [5]MGH, [6]Vanderbilt University, [7]University of Pittsburgh

Background: Autism Spectrum Disorder (ASD) affects social communication, social interaction and is accompanied by restricted and repetitive behaviour. Twin-studies have highlighted a strong genetic underpinning in these disorders and it is estimated that between to 5 and 40% of individuals with ASD carry pathogenic structural or sequence rearrangements in the genome that are causative. Recent estimation of SNP-heritability also suggests that common variation also harbours some of the liability to ASD. In an effort to elucidate specific common risk we conducted a large high-density meta-analyses combining data from multiple studies including 6500 individuals with ASD.
Methods: All raw genotype and phenotype data for the Psychiatric Genomics Consortium (PGC) Collection were uploaded to a central server and processed through the same quality control,

imputation, and analysis process. The PGC Autism study comprised of data from six studies across five different genotyping arrays. All studies included in these analyses were family-based, containing genotype data on parent-child trios. These trio data was converted to case and matched pseudo-controls using PLINK_v1.07. Imputation was performed using SHAPE-IT/IMPUTE routines against the 1000 genome project (build v3.Aug2012). Individuals were excluded from analyses if they were assessed at less than 36 month old or if there was any evidence contradictory to an ASD diagnosis from the Autism Diagnostic Interview (ADI) or the Autism Diagnostic Observation Schedule (ADOS). The primary analyses explored individuals with any ASD diagnosis restricted to "European" ancestry. "European" ancestry was defined as close similarity in genotype to the CEPH HapMap population and not geographic location. A total of 5305 individuals met study inclusion and ancestry criteria. Secondary analyses explored association with stricter autism diagnosis, cognitive ability, verbal status and gender. Association was tested for using logistic regression of imputed dosages. All association analyses were performed using PLINK v1.07. Fixed effect meta-Analysis was performed using METAL, weighted for the inverse standard error of the effect.
Results: In the European ASD GWAS we observed a genome wide significant association for the SNP rs7026354 (OR=1.17; $p=6.7x10^{-9}$) which is located 3' to the *ASTN2* gene. Each of the five studies reported an association in the same direction. Likewise five smaller replication samples also showed the same direction of over-transmission; however, for the largest replication sample, transmission was almost 1:1, which shrinks the combined association signal just below the genome wide significant threshold. In addition to the association signal observed at *ASTN2*, other strong associations were observed within previously implicated ASD genes, *EXT1* (rs7836146; OR=0.85; $p=9.16x10^{-7}$) and *MACROD2* (rs6079556; OR=0.88; $p=2.18x10^{-6}$). Additional exploratory phenotype results will also be presented. Using an independent dataset of 1500 cases and 51K controls from 5 distinct datasets, we observed 73% same-direction effects for the 26 SNPs passing $p < 1x10^{-5}$. This accumulation is significantly different from chance (p=0.014).
Discussion: We report on the largest genome-wide association analyses in ASD to date. We observed a single GW-significant finding at the *ASTN2* locus on chromosome 9. However, the associaton was tempered when combined with the replicatoin data. *ASTN2* (astrotactin 2) is a cell adhesion molecule expressed in the brain and is thought to have a role in neuronal migration. *ASTN2* has been previously implicated in ASD via the observation of rare copy number deletions. In a replication study we show a significant direction effect for highly associated SNPs. These data along with those supporting SNP-heritability indicate that common variation studies will be important in explaining genetic liability in ASD. This work is presented on behalf of the Psychiatric Genomics Consortium: Autism Spectrum Disorder Working Group

INDIVIDUAL ABSTRACT:
DELETIONS BETWEEN 1 AND 30 KB ASSOCIATE WITH AUTISM

Christopher S. Poultney, Ph.D.[1], Arthur Goldberg, Ph.D.[1], Menachem Fromer, Ph.D.[2], Elodie Drapeau, Ph.D.[1], Hala Harony-Nicolas, Ph.D.[1], Yuji Kajiwara, Ph.D.[1], Silvia de Rubeis, Ph.D.[1], Simon Durand[1], Joseph Buxbaum, Ph.D.[3]

[1]Seaver Autism Center, Department of Psychiatry, Icahn School of Medicine at Mount Sinai, [2]Division of Psychiatric Genomics, Icahn School of Medicine at Mount Sinai, [3]Seaver Autism Center and Division of Neurodevelopmental Disorders, Department of Psychiatry, Icahn School of Medicine at Mount Sinai

Background: Autism spectrum disorders (ASD) are highly heritable neurodevelopmental disorders. Genetic studies have shown a strong association between ASD and both inherited and *de novo* copy number variation (CNV). Studies to date focused on CNV that were >30kb. In the current study, we investigated the role of smaller exome-targeted CNV.
Methods: Whole exome sequencing (WES) was performed on 811 subjects (432 ASD cases, 379 controls) using the Agilent Whole Exome 1.1 capture kit and a mixture of Illumina GA2 (495 samples) and HiSeq (316 samples) systems to generate 75 bp paired-end reads (Neale et al. 2012, Nature, 485:242-5). XHMM (eXome Hidden Markov Model; Fromer et al. 2012, Am J Hum Genet, 91:597-607) was used to call CNV from exome read depths, employing an algorithm that: 1) Removes batch effects by filtering higher-order principle components, and 2) computes the highest likelihood series of states (diploid, deletion, duplication) for each sample across all exons. XHMM made 4608 putative CNV calls, which were then filtered to retain CNV with XHMM quality score ≥ 65, number of exons spanned ≥ 3, CNV length ≥ 1 kb, per-sample number of CNV ≤ 55, per-sample total kb CNV ≤ 18 Mb, and MAF ≤ 1%. The XHMM quality score cutoff was the 50% transmission threshold for a separate set of 225 families (201 quads, 24 trios) sampled to similar depth on similar platforms. The filtered set contains 1386 CNV calls (803 case, 583 control) in 559 samples (299 cases, 260 controls). These results were further stratified on type (deletion/duplication) and CNV size (1-10 kb, 10-30 kb, 30+ kb). Size ranges were specifically chosen to focus on CNV too small to detect with array-based CNV detection methods. Burden analyses were performed with PLINK on each subset to assess the CNV called per sample, the proportion of samples with CNV, and the number of genes hit by CNV per sample. After finding increased burden for all three measures in the 1-30 kb deletions, we chose a subset of those to validate using qPCR and/or Sanger sequencing.
Results: Strong enrichment was found in the 1-30 kb deletion subset, as measured by the number of CNV calls per sample (p=0.0037), proportion of samples with CNV (28% in cases vs. 21% in controls, p=0.017), and number of genes hit by CNV per sample (p=0.041). Figure 1 shows values and significance for cases and controls for these three metrics. We used qPCR to attempt to validate 66 of the 219 deletions in the 1-30 kb range, spanning a range of XHMM quality scores and lengths. Of these, qPCR confirmed 55 (84.8%) deletions overlapping the deletion predicted by XHMM. Further validation of 5 deletions was attempted with Sanger sequencing: three of these showed deletions very close to the extent predicted by XHMM, while two were technical failures.
Discussion: We investigated CNV deletions of between 1 to 30 kb in ASD. The validation of 85% of these deletions by qPCR showed that XHMM can accurately call small CNV. We found a significant (p=0.017) burden of these deletions in ASD cases, which, if replicated, represents a genetic finding that would be made in 7% of individuals with ASD

INDIVIDUAL ABSTRACT:
EXOME ARRAY ANALYSES OF AUTISM SPECTRUM DISORDER REVEAL AN ETIOLOGIC ROLE OF LOW FREQUENCY PROTEIN CODING VARIANTS

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Background: Autism spectrum disorder (ASD) is a pervasive neurodevelopmental disorder, featured by high heritability and heterogeneous clinical presentation. Recent genome-wide

genetic studies have established an important role of rare and/or *de-novo* copy number and single nucleotide variants in ASD, but a substantial fraction of its genetic basis remains undefined. Here we examined an etiologic role of low-frequency/rare variants (MAF<1%) in ASD using the exome array data of 12,510 subjects.

Methods: Genotyping was conducted for 12,510 subjects using the Illumina HumanExome bead chip. Family data included: (1) 5,489 subjects from NIMH multiplex and SSC simplex families (3,018 unaffected parents, 2,471 ASD offspring) and (2) 480 subjects from Taiwanese trios (304 unaffected parents, 152 ASD offspring). Case/control data included a total of 6,513 subjects from NIMH/SSC/MGH/MSSM (2,139 ASD cases, 4,374 controls). The zCall algorithm was used to call low frequency heterozygous variants that are missed by Illumina Beadstudio genCall algorithm. A series of rigorous quality control (QC) procedures were applied including: calling rates (> 99.5%), outlier heterozygous call rates, sex inconsistency, spurious relatedness, HWE, and Mendel errors when applicable. For case/control data, multidimensional scaling (MDS) analysis was additionally conducted to include European, non-Hispanic subjects that are clustered with HapMap III CEU/TSI subjects. After QC, 4,190 and 5,057 subjects were retained in the case/control and family data, respectively. We performed logistic regression on case/control data while including gender and 10 MDS factors as covariates, and TDT tests on family data using PLINK. Lastly, fixed-effects-based meta-analysis was performed on the two studies to estimate the combined effect sizes of 52,258 low-frequency/rare variants with MAF<1%. Significance of meta association statistics was assessed using a simulation of 1,000,000 null datasets by randomly permuting case/control status and transmitted/non-transmitted alleles.

Results: We identified 9 low-frequency/rare single nucleotide variants associated with ASD at *P*<1e-03. Eight of the variants reside on protein-coding genes: *MYH13, COLEC12, SRRM5, ZNF428, TET2, MYCBP, GJA9, RRP8, STX5,* and *ANO1*. We also observed significant inflation of test statistics for low-frequency variants (0.5%<MAF<1%; lambda=1.038), which is highly unlikely to occur by chance (empirical *P*<1e-05). This inflation was not observed for rare variants (MAF≤0.5%; lambda=0.704), which suggests a lack of power to detect the genetic effects.

Discussion: Using exome array genotyping data of 9,247 subjects, we demonstrated that low-frequency nonsynonymous variants contribute to the genetic etiology of ASD. With increased power from larger sample sizes, this targeted exome genotyping strategy will provide us with a valuable approach to further our understanding of the genetic basis of this complex brain disorder. Ongoing analyses include gene-based tests and pathway/network analyses.

INDIVIDUAL ABSTRACT:
THE TRANSCRIPTIONAL CONSEQUENCES OF 16P11.2 MICRODELETION/MICRODUPLICATION SYNDROME IN MULTIPLEX AUTISM FAMILIES AND MOUSE CORTEX

Ian Blumenthal[1], Ashok Ragavendran, Ph.D.[1], Serkan Erdin, Ph.D.[1], Lambertus Klei, Ph.D.[2], Jolene Guide, VET[1], Matt Stone[1], Carl Ernst, Ph.D.[1], Joshua Levin, Ph.D.[3], Vanessa Wheeler, Ph.D.[1], Kathryn Roeder, Ph.D.[4], Bernie Devlin, Ph.D.[2], James F. Gusella, Ph.D.[5], Michael Talkowski, Ph.D.[5]

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Background: Autism spectrum disorder (ASD) is a complex, heterogeneous developmental disorder affecting 1-2% of the population, with recurrent reciprocal copy number variants (CNVs) mediated by non-allelic homologous recombination representing one of the largest known risk factors. Of these, the 593 kb region of 16p11.2 is among the most frequent contributors to ASD, but despite recurrent breakpoints, it also confers risk to diverse phenotypic outcomes including schizophrenia, bipolar disorder, seizures, obesity, and numerous other traits, suggesting modifying factors that affect penetrance and expressivity. We investigated the global transcriptional consequences of reciprocal 16p11.2 CNVs using a customized strand-specific RNA sequencing protocol on lymphoblasts from a unique cohort of 35 individuals from 7 multiplex ASD families, each harboring a segregating 16p11.2 CNV and displaying heterogeneity of both genotype (ASD individuals discordant for CNV) and phenotype (subjects with CNV but discordant for phenotype). We also sequenced RNA from the cortex of 8 mice with the 16p11.2 syntenic region (7qF3) either deleted or duplicated and 8 gender-matched wild type littermates.

Methods: Synthetic RNAs provided by the External RNA Control Consortium (ERCC) were spiked into the libraries to calibrate the experiment, set empirical thresholds for detection of gene expression, and model any between library artifacts. We analyzed gene expression using custom generalized linear models, which can allow for the addition of kinship relationships to the model. We first fit a linear model regressing gene expression as a function of the number of copies of the 16p11.2 region (or 16p11.2 syntenic region) within an individual to assess linear trends associated with dosage imbalance. The second method fit a factorial ANOVA with genotype and gender as the factors. The model included both main effects as well as interactions. Pairwise differences among means and linear combinations of model parameters were used to evaluate specific hypotheses.

Results: For genes within the CNV region, we observed a highly significant effect with deletions yielding a relatively uniform mRNA reduction (~0.5X normal expression), and duplications having a consistent but more heterogeneous outcome, (1.2-2X normal expression) in both human and mouse datasets (Figure A). For genes outside the CNV region, we detected presumed positional effects in the CNV's proximity as well as dysregulation of genes elsewhere in the genome. In the human dataset, the most significant results from gene ontology analysis were pathways important to chromatin structure and remodeling. Notably, 27 genes previously associated with ASD (from SFARIgene and AutismKB) were nominally significant and 9 appeared in a protein interaction network (generated with DAPPLE) connecting them to 3 genes in the CNV. The mouse cortex data identified 42 ASD associated loci among the nominally significant results, and a similar DAPPLE interaction network revealed a large sub network of 190 genes connecting 7 of the CNV region genes to 16 ASD loci outside the region (Figure B). Gene ontology analysis of this sub network highlighted WNT signaling, melanogenesis, and MAPK signaling. Interestingly, there were 126 genes outside of 16p11.2 in which both deletion and duplication resulted in parallel changes in gene expression (e.g. reduced expression was observed from both deletion and duplication).

Discussion: Our findings reveal interconnected networks of genes whose expression is disrupted by 16p11.2 dosage imbalance, suggesting potential convergence on common ASD pathways due to different mutational mechanisms. The genes whose expression is altered in the same direction by either genomic dosage imbalance warrant further consideration as they suggest the potential

for a common mechanism of ASD risk conferred by both deletion and duplication through genes that require tight dosage control.

INDIVIDUAL ABSTRACT:
INDIVIDUAL GENE DISRUPTIONS FROM BALANCED CHROMOSOMAL REARRANGEMENTS DEFINE NOVEL NEURODEVELOPMENTAL LOCI AND GENOMIC DISORDERS

Harrison Brand, Ph.D.[1], Vamsee Pillalamarri[1], Ian Blumenthal[1], Matthew Stone[1], Shahrin Pereira[2], Cynthia Morton[3], James Gusella[1]

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Background: Cytogenetically defined balanced chromosomal aberrations (BCAs), including translocation, inversions, and excision/insertion events, represent substantial contributors to neurodevelopmental disorders (NDD), but have only been routinely detected at extremely low resolution by karyotyping as deep, high resolution whole-genome sequencing (WGS) is cost prohibitive. Recent studies from our laboratory have delineated BCAs at high resolution using a large-insert WGS, or 'jumping library' approach to derive nucleotide resolution of BCA breakpoints at a cost comparable to conventional cytogenetic methods. We previously reported sequencing of 38 NDD subjects and identified disruption of novel genes in NDD that conferred risk across formal diagnostic boundaries by multiple mutational mechanisms (Talkowski et al., 2012, Cell).

Methods: We created customized jumping libraries, which contain short 300-500 bp DNA fragments manipulated so that the paired ends are separated by ~3-4 kb in genomic distance and sequenced with Illumina chemistry. Bioinformatic analysis aligned reads with BWA, followed by processing of BAM files using BamStat, a program we developed to identify anomalous mapping inserts and cluster paired-end reads using a single linkage clustering. BCAs are identified by investigating unusual clusters near the karotypically defined breakpoints, which are confirmed through PCR and Sanger sequencing (Talkowski et al., 2011, American Journal of Human Genetics; Chiang et al., 2012, Nature Genetics). We sequenced a total of 56 subjects independent of previous studies with congenital anomalies that harbored a karyotypically identified BCA, >70% of which present with a NDD.

Results: We identified complex rearrangements (>3 breakpoints) in 15 subjects (26.8%) disrupting 45 genes, consistent with our previous findings and dramatically higher than the 2.8% predicted by cytogenetic estimates. Of the 41 cases with canonical BCAs, we found disruption of 27 genes among 26 subjects, many of these representing putative pathogenic loci. In DGAP055, sequencing identified disruption of *CDK6*, a locus within the 7q21 micro-deletion syndrome often associated with mental retardation, microcephaly, dysmorphism, and short stature, but where no causative gene has been previously identified. Interestingly, this disorder includes many symptoms overlapping with DGAP055's mental retardation, microcephaly, dysmorphism, and short stature. Two other genes (*CACNA1C, CTNND2*) found to be disrupted in cases with congenital anomalies demonstrating the variable expressivity of NDD, even from known 'causal' loci, as these genes have been previously associated with the Mendelian disorder Timothy syndrome (*CACNA1C*) and cri-du chat syndrome (*CTNND2*), but neither of the cases associated with these genes present the hallmark symptoms of each disorder. Another intriguing class of

loci involves genes disrupted in subjects with a complex NDD, including autism spectrum disorder, that are also associated with other psychiatric disorders: *TRANK1* (bipolar disorder), *DOCK9* (bipolar disorder), *TCF4* (schizophrenia), and *ZNF804A* (schizophrenia), to name just a few.

Discussion: This study emphasizes the significance of cytologically visible chromosomal abnormalities as a reservoir of strong effect mutations with a significant impact in human development and psychopathology. It implicates several novel genes (e.g. *CDK6, CTNND2*) contributing to NDD. The identification of loci previously associated with other psychiatric disorders further supports a growing role of shared genetic etiology among numerous neuropsychiatric and neurodegenerative diseases. These data and our previous findings argue strongly for the wide-spread adoption of methods capable of delineating BCAs in routine genetic studies.

ORAL AND POSTER PRESENTATIONS

2:30 PM – 4:00 PM

GENETIC ARCHITECTURE: STATISTICAL AND GENOMIC DISSECTION

INDIVIDUAL ABSTRACT:
THE GENETIC ARCHITECTURE OF TOURETTE SYNDROME AND OBSESSIVE COMPULSIVE DISORDER

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University of Chicago

Background: Tourette Syndrome (TS) and obsessive-compulsive disorder (OCD) are two phenotypically overlapping neuropsychiatric disorders. TS is characterized by the presence of tics while OCD results in recurrent unwanted thoughts or repetitive behaviors. Very little is known about the genetic architecture of these two frequently co-morbid early-onset neuropsychiatric phenotypes. Recently, quantitative methods from animal breeding genetics have been adapted to large scale human genomic data and provide a means to quantify and partition heritability attributable to all interrogated variants and to specific subsets of variants. The novelty of this approach lies in the ability to use large samples of ostensibly unrelated individuals to quantify excessive and ancient risk allele sharing identifiable due to categorization according to phenotype.

Methods: We have used Genetic Complex Trait Analysis (GCTA) to quantify the heritability of TS and OCD in the largest collections of OCD and TS samples available to date. After extensive quality control and filtering of the data, we proceeded with analysis on a final data set of 617 TS cases and 4,116 TS controls genotyped on 393,387 SNPs, as well as 1,061 OCD cases and 4,236 OCD controls genotyped on 373,846 SNPs. In order to facilitate cross disorder comparisons, the cases were randomized on the same plates and genotyped together and the controls were shared between the two data sets. Analyses were conducted using both directly genotyped data and imputed data. To reduce effects of population stratification, subjects were limited to those with genetically defined European ancestry. Each analysis also included the top 20 principal components as covariates. We conducted global heritability analyses on each phenotype and subsequently conducted multiple genomic partitioning analyses to identify regions of the genome and subsets of variants that contributed disproportionately to the heritability of each phenotype. We examined the genomic architectures of TS and OCD by chromosome, by minor allele frequency, and by annotation of variants that regulate gene expression in two regions of the

brain (parietal eQTLs and cerebellar eQTLs). In addition, we assessed heritability for early onset and adult onset OCD. A total of 732 cases were diagnosed or reported symptom onset prior to age 16 and were considered early onset. A total of 267 cases were diagnosed or exhibited symptoms later than age 16 and were classified as adult onset. The remainder were unclassified due to missing age data.

Results: Our analysis yielded a heritability point estimate of 0.58 (se = 0.09, p=5.64e-12) for TS, and 0.37 (se = 0.07, p=1.5e-07) for OCD. Among other notable results, we discovered a disproportionately large contribution to OCD heritability originating from chromosome 15. Greater than expected heritability per chromosome was also discovered in the TS data for chromosomes 2, 5, 11, 16 and 20. Additionally, results showed that parietal eQTLs accounted for significantly more TS heritability ($p=5.36e^{-46}$) and OCD heritability ($p=2.80e^{-16}$), and cerebellum eQTLs accounted for significantly more TS heritability ($p=4.02e^{-15}$) and OCD heritability ($p=1.37e^{-14}$) than expected based on the number of SNPs tested under a uniform distribution model. We also found that SNPs with a minor allele frequency of less than 5% accounted for 21% of the TS heritability and 0% of the OCD heritability. It has been observed that early-onset OCD is more heritable (45-65%) than adult onset OCD (27-47%). Our results were consistent with this observation as the heritability for early-onset OCD was 0.43 (se=0.10) and for adult-onset was 0.26 (se = 0.24).

Discussion: The overall GCTA heritability calculations for TS and OCD, using large samples of unrelated individuals, are very similar to twin and family studies. This finding suggests that 1) very little, if any, heritability is truly missing (i.e., unassayed) from TS and OCD GWAS studies of common variation and 2) shared environment does not result in excessive bias in twin and family studies of TS and OCD. While previous TS and OCD GWAS have been underpowered to identify individual susceptibility variants with modest effect sizes, our results highlight the polygenicity of both TS and OCD and suggest that future GWAS in much larger samples will be successful in identification of individual risk variants. The results also suggest that SNPs associated with gene expression in the parietal and cerebellar cortex capture a significant proportion of heritability for both TS and OCD. Despite these similarities, TS and OCD appear to have distinct genetic architectures. Specifically, we identified a striking difference between TS and OCD in the proportion of heritability accounted for by variants with MAF < 0.05 and in the pattern of chromosomal contributions to heritability. In conclusion, we present the first systematic analysis of the genetic architecture of OCD and TS.

INDIVIDUAL ABSTRACT:
BAYESR POLYGENIC RISK PREDICTION FOR SCHIZOPHRENIA

Gerhard Moser, Ph.D.[1], Sang Hong Lee[1], Michael E. Goddard[2], Christina M. Hultman[3], Pamela Sklar[4], Patrick F. Sullivan[5], Peter M. Visscher[1], Naomi R. Wray[1]

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Background: Profile scoring is a polygenic method for prediction of genetic risk for an individual. A “profile" is generated from the sum of the risk alleles they carry, with the allelic effect sizes estimated in an independent discovery sample. To date, profile scoring is based on SNP effects estimated one at a time in a standard GWAS analysis, followed by an arbitrary selection of a SNP set based on association p-value and linkage disequilibrium. Here we report results from a new analysis method that can jointly performs GWAS, select SNPs, estimate genetic variance and predict SNP effects, using all SNPs simultaneously.

Methods: We investigate the potential to increase prediction performance using a Bayesian regression approach (BayesR) for estimation of polygenic risk scores from genome-wide SNP data with all SNPs considered simultaneously. BayesR uses a mixture of normal distributions as the prior of SNP effects, including one distribution that sets SNP effects to zero, thus facilitating SNP selection. To assess the performance of BayesR polygenetic risk scores we used a Swedish SCZ case/control data set comprising 4,227 controls and 2,991 cases and 1,034,503 HAPMAP3 imputed SNPs. Lower-density SNP sets were generated by pruning based on LD between SNP ($r^2 < 0.8$, N = 471,237; $r^2 < 0.5$, N = 280,266; $r^2 < 0.25$, N = 145,672). We estimated parameters from the training data treating the binary disease labels as quantitative traits and used the correlation between realized and predicted phenotype as measure of the accuracy of risk prediction. Predictive performance was estimated by 10-fold cross-validation. Accuracy of prediction of BayesR was compared with other methods that utilize information from all SNPs for risk prediction (ridge regression, RBF kernel regression, Bayesian sparse linear mixed model).
Results: As expected, accuracy of prediction increased with the number of SNPs in the training data from 0.146 (N =145,672) to 0.214 (N =1,034,503). We calculated the number of SNPs in distributions explaining 0, 0.01, 0.1 or 1% of the genetic variance. On average only between 2 and 7% of all SNPs contributed to the risk prediction score, and the largest proportion of genetic variance (> 93%) was explained by SNPs with small effect. Estimates of the proportion of variance explained by SNPs derived from BayesR were similar to the estimates of 'chip-heritability' using GCTA.
Discussion: We used a Bayesian mixture model with discrete distributions to evaluate polygenic risk models that could be used to predict future cases of schizophrenia from genome-wide genotype data. Although our polygenic scores were based on specific priors for the effect-size distribution, accuracy of prediction was similar to those observed with other algorithms. The effect-size distribution suggest that very large sample sizes will be required to increase power of polygenic models for risk prediction.

INDIVIDUAL ABSTRACT:
QUANTIFYING MISSING HERITABILITY FROM CODING VARIATION IN SCHIZOPHRENIA

Alexander Gusev, Ph.D.[1], Benjamin Neale[2], Gaurav Bhatia[3], Noah Zaitlen[4], Bjarni Vilhjalmsson[5], Bogdan Pasaniuc[6], Patrick Sullivan[7], Alkes Price[5]

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Background: As analyses of disease-causal variants increasingly focus on protein-coding mutations, quantifying the overall contribution to phenotype made by exonic variants is crucial for understanding the effectiveness of such studies and the broader genetic architecture of complex disease. We explore these components of heritability in a cohort of 2,500 Schizophrenia cases and 3,875 controls which have been typed for both GWAS and exome chip variants. The genotyped and imputed GWAS variants can explain 60-90% (2-3%) of trait variation that stems from common (rare) coding variants, depending on disease architecture, motivating specific investigation of rare coding variants as potential sources of "missing" heritability.
Methods: We propose methods to jointly estimate these components of heritability using a multivariate variance-components model corresponding to rare coding, common coding, and common non-coding variants. We demonstrate the importance of adjusting for LD within/between variant classes, which can significantly bias variance component estimates even

if all causal variants are typed. In instances where causal variants within a class exhibit systematically lower LD, their contribution will be unrepresented and the estimate will be biased unless within-component LD is adjusted for. Alternatively, in instances where causal variation is tagged by multiple variant classes, univariate models will inflate the corresponding estimate of heritability due to tagging. After accounting for both types of bias our simulations on real genotypes show that our methods produce unbiased estimates regardless of the underlying disease architecture. To reduce the noise around our heritability estimates, we also propose and evaluate an alternative strategy that estimates heritability from variants collapsed by gene, conceptually similar to gene-based rare variant association tests.
Results: We partitioned total heritability explained by all typed SNPs of 0.38±0.04 into 0.09±0.03 from coding SNPs and 0.30±0.03 from noncoding GWAS-chip SNPs, demonstrating significant heritability present in exons. However, the contribution of 0.04±0.03 from rare coding SNPs (after adjusting for LD between variants) was non-significant, and remained so when collapsing rare variants to reduce statistical noise. The remaining contribution from common coding SNPs was largely tagged by common GWAS-chip variants.
Discussion: Our results shed light on components of missing heritability for schizophrenia and provide insights into the genetic architecture of this complex disease.

INDIVIDUAL ABSTRACT:
STATISTICAL EVALUATION OF DE NOVO VARIATION IMPLICATES A DISTINCT ETIOLOGIC SUBTYPE OF AUTISM

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Background: Recent exome sequencing studies of patients with autism spectrum disorders (ASDs) have indicated a significant role for *de novo* loss of function variants (Neale et al. 2012; Sanders et al. 2012; O'Roak et al. 2012a; Iossifov et al. 2012; O'Roak et al. 2012b). Fragile X syndrome, while primarily an intellectual disability phenotype, features ASD-like symptomology in 25-40% of cases. Fragile X syndrome is caused by activity loss of the FMRP protein, a critical neuronal RNA-binding protein that affects the translation of genes with which it interacts (targets). Darnell and colleagues identified 842 targets of FMRP *in vivo* (Darnell et al. 2011); the purpose of this study was to investigate if *de novo* variation in those genes plays a role in ASDs.
Methods: We used a sequence context based model of *de novo* mutations to create per-gene probabilities of synonymous, missense, and loss of function mutations. These probabilities were used not only to evaluate the overall excess of loss of function *de novo* variants in ASD cases, but also to determine if the genes harboring such variants were enriched for genes that could potentially be relevant to ASD. To do this, we determined the total probability of mutation for all genes on the gene list of interest. The list total was compared to the total probability of mutation for all genes. This percentage became the expected overlap of *de novo* mutations with the gene list. We used the binomial distribution to evaluate the number of observed mutations overlapping the list compared to the established expectation.
Results: As previously reported in the four large ASD exome sequencing studies, we noted a significant excess of *de novo* loss of function mutations in ASD cases, but not in any of the unaffected siblings sequenced ($p = 2.05x10^{-7}$ for ASD; $p = 0.451$ for unaffected siblings). This excess was not seen for either missense or synonymous mutations. Further analysis of the set of

genes containing *de novo* loss of function variants in cases revealed a significant enrichment with 842 *in vivo* targets of the FMRP protein (2.3-fold enrichment, $p < 0.0001$; Darnell et al. 2011). Roughly 3% of the ASD cases had a *de novo* loss of function mutation in a target of FMRP. Compared to the remainder of sequenced cases in the sample, these individuals were significantly less likely to have average or above average intellectual functioning ($IQ \geq 100$, Fisher's exact $p = 4.01 \times 10^{-4}$), and were significantly more likely to be female (Chi square $p = 0.02$).
Discussion: Leveraging our per-gene probabilities of mutation allowed for the identification of a distinct etiologic subset of the sequenced ASD cases. This subset shows an underrepresentation of typical cognitive function as well as an overrepresentation of females, both of which are associated with case severity in ASD (Fombonne 2003). Further characterization of this subset of individuals may allow for insights into ASD and intellectual disability.

INDIVIDUAL ABSTRACT:
THE GENETIC ARCHITECTURE OF SCHIZOPHRENIA IN THE SWEDISH SCHIZOPHRENIA STUDY

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Background: Schizophrenia has long been recognized to be familial, and more recently to be highly polygenic. Quantitative inference of its genetic architecture using genome-wide data is an active area of research, including methods development. The substantial contribution to phenotypic variance of SNPs falling short of genome-wide significance in GWAS is well appreciated, particularly in schizophrenia. Here we describe inference analyses of schizophrenia genetic architecture using the comprehensive genomic data collected on this sample.
Methods: We analyzed GWAS data of 5,001 schizophrenia cases and 6,243 healthy controls from the Sweden, imputed into the 1000 Genomes (1KG) reference panel (5.7M SNPs after QC). In polygenic risk score (PRS) analyses, the 2011 PGC schizophrenia GWAS meta-analysis results (with all Swedish subjects removed) were used as training data and the Swedish subjects were used for testing. Approximate Bayesian Polygenic Analysis (ABPA) was performed on PRS variance explained, using training and test data simulated under a mixture of independent associated and null SNPs (Stahl et al PMID 22446960). Linear mixed models of normally distributed effects were fit using GCTA (Yang et al PMID 21167468, Lee et al PMID 21376301), with and without LD-based SNP weighting (using LDAK, Speed et al PMID 23217325). Results were compared using the actual genotype data, using real and simulated phenotypes under a range of heritability, and number and frequencies of causal variants.
Results: ABPA yields an estimate of 8,300 independent SNPs (95% credible interval 6,300-10,200 SNPs) underlying risk of schizophrenia, the great majority of which are common (MAF>0.1), and together account for 52% (95% CI 0.45-0.54) of the variance in liability for disease (i.e. heritability). Using GCTA, we estimated somewhat lower SNP heritability, 33% (0.27-0.39), still a majority of heritability estimated in a Swedish national pedigree study (64%; Lichtenstein 2009). Differences in the methods appear to stem from linkage disequilibrium,

particularly given the dense genotyping of the 1KG-imputed data, and how linkage disequilibrium is handled in the analysis. Simulation analyses were used to assess the accuracy and precision of the methods under a range of genetic architectures.
Discussion: These results answer a question that has been debated most of the past century: the majority of the phenotypic heritability of schizophrenia can be explained by GWAS data, with the associated SNPs being overwhelmingly common and of small effect. These results strongly suggest that additional GWAS will yield many more additional genome-wide significant discoveries. Furthermore, the estimate of thousands of risk alleles is consistent with very high locus and allelic heterogeneity for schizophrenia, and suggests that schizophrenia may be more polygenic than other common, complex diseases. We compare the methods in the same real and simulated data, and assess them in terms of accuracy, robustness to assumptions as well as computational demands.

INDIVIDUAL ABSTRACT:
DISCOVERY OF CRYPTIC CHROMOSOMAL ABNORMALITIES IN CLINICALLY-REFERRED YOUTH WITH NEUROPSYCHIATRIC DISORDERS

Michael E. Talkowski, Ph.D.[1], Vamsee Pillalamarri, M.S.[2], Alysa Doyle, Ph.D.[3], Harrison Brand, Ph.D.[4], Matthew Stone[2], Ian Blumenthal, B.S.[2], Colm O'dushlaine, Ph.D.[5], Ellen Braaten, Ph.D.[6], Jill Rosenfeld, M.S.[7], Steven McCarroll, Ph.D.[5], Jordan Smoller, Ph.D.[8]

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Background: A significant portion of the genetic etiology of neurodevelopmental disorders (NDD), including autism spectrum disorder (ASD), and other neuropsychiatric disorders (NPD) remains unexplained, though in each there is unequivocal evidence to suggest that rare structural variation (SV) is among the strongest predisposing genetic risk factors. Unfortunately, the vast majority of SV studies to date evaluated copy number variants (CNV), generally encompassing large genomic segments and many genes. We recently showed that balanced chromosomal abnormalities (BCAs) that were detected by conventional cytogenetic methods represent a unique class of highly penetrant SV that can result in heterozygous inactivation at a single point in the genome. To date, the impact of submicroscopic cryptic BCAs have not been routinely investigated in genetic studies; they remain intractable to all conventional technology other than deep sequencing. Further, our study of BCAs, and many other CNV and GWAS studies, also provide strong evidence of a shared genetic etiology between NDD and NPD, with risk conferred by multiple mutational mechanisms. In this study, we defined the landscape of cryptic BCAs in a unique longitudinal cohort of individuals with comorbid ASD and early onset NPD phenotypes.
Methods: We investigated cryptic BCAs in 33 subjects from our LOGIC (Longitudinal study of genetic influences on cognition) cohort using large-insert or "jumping" library whole-genome sequencing. We sequenced the 33 libraries to a median insert coverage of ~60x with an insert

size of ~2.6kb. We optimized our algorithms using a deep sequencing training set and find 98.1% sensitivity to detect cryptic BCAs at five standard deviations outside of the median insert. We used a convergent genomic approach to validate the broader significance of associated loci in this small cohort by comparing subjects to a database of over 34,000 individuals from clinical diagnostic CNV testing, 14,000 controls from various GWAS studies, and existing GWAS and exome sequencing data.

Results: Our approach uncovered a spectrum of BCAs including *intra-chromosomal* excision, inversion, and insertion events, *inter-chromosomal* excision / insertion as well as reciprocal chromosomal exchange events, and *semi-balanced* BCAs with small CNVs at the breakpoint. Of note, and as we have shown previously, ~55-65% of all cryptic BCAs represent reference assembly artifacts and are seen in all individuals. Across 33 subjects, we detected about 50 balanced exchanges per individual, with most events being polymorphic. When events were found to be private to an individual in the LOGIC set after filtering against these data and data from other sequencing studies, we identified 10 genes and one microRNA (mir1256) that were disrupted by a cryptic BCA that was private to that individual. All events were validated by PCR and the parental origin was evaluated. Of interest, convergent genomics and network analyses implicated four of these loci, each with a direct, first-order interaction to a previously implicated ASD locus (*AKAP13*, *IQGAP1*, *ETV4*, *CTNNA3*), as well as a putative novel locus that was not connected to existing loci in the network (*UBE2F*, which encodes a ubiquitin conjugating enzyme; CNV burden $p = 8.3x10^{-4}$). For one of these loci, *CTNNA3*, we discovered a disruption upstream of the locus by a reciprocal translocation in an additional case, DGAP126, with ASD and self-injurious behavior.

Discussion: We show here that cryptic BCAs represent an important component of the genetic architecture of NDD and NPD. We identified as many as five putative contributors to the phenotypes of 33 subjects, each warranting further study, and several of which interact with known ASD loci. These data suggest that opening access to cryptic BCAs into routine genetic studies could have a significant impact in developmental and psychiatric disorders, and larger cohort studies are ongoing.

ORAL AND POSTER PRESENTATIONS
2:30 PM – 4:00 PM

GENETICS OF MOOD DISORDERS

INDIVIDUAL ABSTRACT:
MAJOR DEPRESSION GENES IDENTIFIED USING WHOLE GENOME SEQUENCING IN EXTENDED PEDIGREES

Emma E M. Knowles, Ph.D.[1], Marcio de Almeida, Ph.D.[2], Jack Kent, Ph.D.[3], Joanne Curran, Ph.D.[2], Melanie Carless, Ph.D.[2], Rene Olvera, M.D.[4], Reese McKay, Ph.D.[5], Ravi Duggirala, Ph.D.[3], Laura Almasy, Ph.D.[3], John Blangero, Ph.D.[3], David Glahn, Ph.D.[5]

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Background: MDD is a common and costly disorder. While effective treatments exist many patients fail to receive them. Identifying genetic markers for depression may provide a reliable indicator of depression risk, which would substantially improve detection, and in so doing enable

earlier more effective treatment. The aim of this study was to identify genes for depression modeled as a continuous trait, using whole genome sequence data.
Methods: The sample comprised 530 Mexican-American individuals from extended pedigrees. Whole genome sequence (WGS) data with a 60-fold coverage were available for the entire sample amounting to ~3.4M SNPs per individual. Association testing was performed using a unitary factor score derived by applying CFA to all items from the Major Past Depressive Episode section of the Mini-International Neuropsychiatric Interview. Genome wide-significant hits were followed up using gene-specific analyses.
Results: WGS analysis revealed two variants on chromosome 3 that were significantly associated with depression, plus a number of variants that reached a suggestive level of significance. The first was located at ~188.0 Mb ($X^2 = 40.15$, $p = 2.35 \times 10^{-10}$) and the other at ~67.0 Mb ($X^2 = 35.21$, $p = 2.96 \times 10^{-09}$). Post-hoc analysis revealed a number of interesting candidate genes.
Discussion: Using a continuous measure of depression combined with WGS data we have identified genetic influences for depression. These genetic influences are located on chromosome 3 and overlap, in part, with identifications from linkage studies and also from the GWAS for depression carried out by the PGC.

INDIVIDUAL ABSTRACT:
BIPOLAR DISORDER GWAS OF 13,741 CASES AND 19,762 CONTROLS IDENTIFIES EIGHT GENOME-WIDE SIGNIFICANT HITS AND IMPLICATES GENES TARGETED BY FMRP PROTEIN.

Alexander W. Charney, M.D.[1], Douglas Ruderfer, MS[1], Jennifer Moran, Ph.D.[2], Sarah Bergen, Ph.D.[3], International Cohort Collection for Bipolar Disorder (ICCBD)[4], Jordan Smoller, M.D., ScD[5], Carlos Pato, M.D., Ph.D.[6], Christina Hultman, Ph.D.[3], Mikael Landen, M.D., Ph.D.[7], Shaun Purcell, Ph.D.[1], Nick Craddock, MA, MMedSci, Ph.D., FRCPsych[8], Pamela Sklar, M.D., Ph.D.[1]

[1]Icahn School of Medicine at Mount Sinai, [2]Broad Institute, [3]Karolinska Institutet, [4]Multisite, [5]Massachusetts General Hospital, [6]University of Southern California, [7]University of Gothenburg, [8]Cardiff University School of Medicine

Background: Bipolar disorder (BPD) is a common, highly heritable disorder affecting nearly 1% of the population. Recent genome-wide association studies (GWAS) have identified common variants contributing to disease risk, however the bulk of the heritability remains to be explained. In many neuropsychiatric diseases – such as autism and schizophrenia – rare CNVs have been implicated, but the role these variants play in BPD is less clear. We performed a GWAS on the largest BPD data set to date (13,741 BPD cases and 19,762 controls), and in a subset of this sample (3,682 BPD cases and 4,187 controls) further examined the role of rare CNVs in BPD.
Methods: We conducted a meta-analysis combining 4 independent GWAS datasets with the previously published PGC BPD dataset, totaling 13,741 cases and 19,762 controls after accounting for overlaps. Individually the sample consisted of a Swedish national sample (2,121 cases, 5,894 controls), a UK sample (2,595 cases, 5,645 controls), a sample of mixed European ancestry (1,512 cases, 1,338 controls) and the PGC BPD sample (7,481 cases, 9,250 controls). Quality control (QC) was performed using Plink. Large, rare CNVs (<1%, >20kb) were called using Bird suite, and CNV QC was performed using Plink.
Results: In total, we identified eight regions meeting genome-wide significance, more then any single previous study. We tested if these regions were enriched for genes annotated to a

particular biological function. We defined LD independent regions with association significance below $p < 1x10^{-5}$ and identified four gene sets significantly enriched for these regions, including a set of voltage-gated calcium channel genes (p=0.004) and a set of FMRP target genes (p=0.008). CNV analyses revealed at most a limited contribution to BPD, with large deletions (> 500kb) that occurred only once in the dataset having marginally higher rates in cases than controls (p=0.03).
Discussion: As seen in other disorders, the increase in GWAS sample size for BPD will increase power to detect common SNPs of smaller effect. Here, we identify twice as many genome-wide significant loci as previously presented in the most recent, largest GWAS. We identify particular biological processes enriched for genes associated with BPD, in particular genes targeted by FMRP and calcium channel genes. Consistent with previous studies, we show a limited contribution of CNVs to risk for BPD.

INDIVIDUAL ABSTRACT:
ANALYSIS OF ~48,000 PSYCHIATRIC GENOMIC CONSORTIUM (PGC) BIPOLAR CASE-CONTROL SAMPLES

Pamela Sklar, M.D., Ph.D.

Icahn School of Medicine at Mount Sinai

Background: The purpose of the Psychiatric Genomics Consortium (PGC) is to conduct meta-analyses of genome-wide genetic data for psychiatric disease. Recognizing that individual GWAS studies are often too small to have adequate power for gene discovery, an international PGC Working Group has focused on extending their meta-analysis of bipolar disorder. Recently, we reported a combined GWAS of bipolar disorder in a sample of 16,731 individuals that identified two genome wide-significant loci (Nature Genetics, 2011).
Methods: Relative to our 2012 WCPG presentation, which was based on approximately 10,988 cases and 14,139 controls we now update those findings using additional GWAS data from approximately 8,643 cases and 13,949 controls from Germany, Sweden, Norway and the US. Thus we have a total of 47,719 samples: 19,631 cases and 28,088 controls. Quality control was performed using the PGC central analytic pipeline. We imputed genotypes from 1000 Genomes Project haplotypes and analyzed the data using logistic regression with MDS components as covariates.
Results: We will report on analyses of the entire dataset and demonstrate that there are additional genome-wide significant findings, as well as strong support for prior loci. We will also report on sub phenotype and pathway exploration of the dataset.
Discussion: In conclusion, we provide support for the importance and utility of continued GWAS exploration in bipolar disorder in efforts to increase the number of genetic loci with compelling association to bipolar disorder.

INDIVIDUAL ABSTRACT:
NEXT-GENERATION SEQUENCING OF SYNAPTIC GENES IN FAMILIAL MAJOR DEPRESSIVE DISORDER

Fernando S. Goes, M.D.[1], Mehdi Pirooznia, Ph.D.[1], Peter Zandi, Ph.D.[2], Tao Wang, M.D.[1], James Potash, M.D.[3]

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Background: Major depression is a common, complex disorder that is among the leading causes of disability worldwide. Despite substantial heritability, GWA studies have yet to uncover robustly associated variants, suggesting the need for larger samples and complimentary approaches. In this study, we perform a preliminary study of rare variation in the subset of genes expressed in the synapse. Synaptic pathophysiology has been previously implicated in the etiology of major depression and represents one of the most potentially "druggable" targets for psychiatric illness.
Methods: We have performed next-generation sequencing of all exons in 2011 genes that comprise the vast majority of genes expressed in the synapse. Sequencing was performed in 350 cases from the Genetics of Recurrent Early-Onset Depression family collection and 350 screened controls, all of European American ancestry. We used Agilent's solution based target capture technology followed by high-throughout sequencing using the Illumina Hiseq. Sequence alignment, variant calling and annotation were performed with Bowtie, GATK and Annovar.
Results: After initial quality control, 697 individuals were successfully sequenced with at least 70% of all bases sequenced at 6X coverage. There were over 40,000 exonic variants called, the vast majority being rare (78% with a MAF < 1%). We will present gene-based and pathway based mutational burden analyses using a number of established and novel statistical tests. We will also cross-reference our results to ongoing exome sequencing studies in bipolar disorder cases and controls.
Discussion: We will report on one of the first next-generation sequencing studies of major depression using an early onset, familial sample. Our analysis will aim to identify rare variants in in synaptic genes and pathways that influence susceptibility to depression.

INDIVIDUAL ABSTRACT:
GENOME-WIDE ASSOCIATION STUDY OF MAJOR DEPRESSION IN THE KAISER PERMANENTE/UCSF GENETIC EPIDEMIOLOGY RESEARCH ON AGING COHORT

Catherine Schaefer, Ph.D.[1], Ling Shen, Ph.D.[2], Thomas Hoffmann, Ph.D.[3], Mark Kvale, Ph.D.[3], Lori Sakoda, Ph.D.[2], Yambazi Banda, Ph.D.[3], Pui-Yan Kwok, M.D., Ph.D.[3], Pui-Yan Kwok, M.D., Ph.D.[3], Neil Risch, Ph.D.[3], Eric Jorgenson, Ph.D.[2]

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Background: Major depression is the most common major psychiatric disorder, with a lifetime prevalence of about 15%. The genetic factors that influence its occurrence have remained elusive despite multiple genome-wide association studies (GWAS) and meta-analyses. We conducted a GWAS of major depressive disorder (MDD) in a cohort of 78,697 non-Hispanic white individuals with comprehensive longitudinal electronic medical records linked to genome-wide genotype data, obtained using a custom Affymetrix Axiom array for European ancestry with 674,518 SNP markers.
Methods: We identified a total of 7,380 cases of MDD who had a history of 2 or more visits to outpatient psychiatric clinics with a diagnosis of MDD and a minimum of 1 year of filled prescriptions for antidepressant medications, establishing a homogeneous phenotype for analysis. We excluded those who did not meet these criteria, as well as those with 1 or more diagnoses of other Axis I disorders, and those with less than one year's prescriptions of antidepressant medications. Individuals with any history of MDD or other Axis I disorders, or with prescriptions for antidepressant or antipsychotic medications were excluded from the controls. A

total of 41,239 controls were identified after screening. MDD cases were 71.6% women, compared with 50.9% women among controls. On average MDD cases had 13.8 MDD diagnoses (SD = 20.1). The GWAS analysis was conducted using Plink v1.07 using an additive logistic regression model that controlled for age, gender and ancestry principal components.
Results: A single SNP, rs35350027, located in an intergenic region near the SHROOM3 gene on chromosome 4, reached a genome-wide level of statistical significance (OR =0.85; 95% confidence interval: 0.81, 0.90; p = 5.14*10^-9). Eight other SNPs were associated with MDD with p-values less than 10^-5, including a second chromosome 4 SNP in LD (r^2=0.73) with rs35350027. In analyses stratified by gender, no associations reached genome-wide significance.
Discussion: This is the first GWAS of MDD to report an association that reached genome-wide significance. The SNP with the strongest association in this study, rs35350027, was not genotyped or imputed in the MDD Working Group of the Psychiatric GWAS Consortium study, nor did we observe suggestive associations when comparing our results to those of the Consortium. However, an analysis in men only identified a suggestive association with a single SNP on chromosome 7 that is near a suggestive signal in the male-only analysis reported by the Consortium.

INDIVIDUAL ABSTRACT:
GENOMEWIDE ASSOCIATION STUDY OF RECURRENT EARLY-ONSET MAJOR DEPRESSIVE DISORDER (GENRED)

Douglas F. Levinson, M.D.[1], Jianxin Shi, Ph.D.[2], Myrna Weissman, Ph.D.[3], James Potash, M.D., M.P.H.[4], William Scheftner, M.D.[5], William Coryell, M.D.[4], James Knowles, M.D., Ph.D.[6], Stephan Ripke, M.D.[7], William Lawson, M.D., Ph.D.[8], J. Raymond DePaulo, M.D.[9], Janet Sobell, Ph.D.[6], Pablo Gejman, M.D.[10], Alan Sanders, M.D.[10], Carlos Pato, M.D.[6], Peter Holmans, Ph.D.[11], PGC MDD Workgroup[12]

[1]Stanford University School of Medicine, [2]National Cancer Institute, Bethesda, MD, [3]Columbia University, New York, NY, [4]University of Iowa, Iowa City, IA, [5]Rush University Hospital, Chicago, IL, [6]University of Southern California, Los Angeles, CA, [7]Broad Institute of Harvard and MIT, Cambridge, MA, [8]Howard University, Washington, DC, [9]Johns Hopkins University, Baltimore, MD, [10]University of Chicago, Chicago, IL, [11]Cardiff University, Cardiff, [12]Psychiatric Genomics Consortium

Background: The Genetics of Recurrent Early-Onset Depression study (GenRED) recruited cases of major depressive disorder (MDD) with a restricted, familial phenotype for genetic studies. The phase 1 GWAS detected no genome-wide significant associations (Shi et al., Mol Psychiatry 2011;16:193) . Here we report on meta-analyses of GenRED phases 1+2, and on polygenic score analyses predicting GenRED case status from PGC MDD, schizophrenia (SCZ) and bipolar disorder (BIP) results.
Methods: The sample included 1,847 MDD cases and 2,566 controls in two phases. Phase 1 included 1,020 cases and 1,636 controls (MGS) (Affymetrix 6.0 array; part of the PGC_MDD discovery sample). Phase 2 included 827 cases and 930 controls (Mayo and GPC) (Illumina Omni1-Quad array; a PGC replication sample). Best estimate consensus diagnoses were assigned based on DIGS 3 interviews and other available information. Cases had recurrent MDD (or one episode of >3 years) with at least one episode without substance dependence; onset < age 31; and DIGS or FIGS evidence of recurrent/chronic MDD in a parent or sibling (onset < 41), and no suspected BIP-I parent or sib. QC procedures were applied separately to the two phases. 1000 Genomes (06/2011) SNPs were analyzed if imputation r^2 > 0.3 (IMPUTE2). Association

analyses were by logistic regression (PLINK) in each phase separately (corrected for principal components reflecting ancestry). The two phases were combined by inverse-variance-weighted meta-analysis. We also meta-analyzed GenRED1+2 plus the PGC_MDD sample (cross-disorder version) after removing GenRED1 and STAR*D (which used MGS controls). For polygenic score analyses, GenRED genotypes for independent SNPs were weighted by the log(OR) for each test allele in each of three separate association analyses of the PGC (CDG) MDD, MDD-recurrent-early-onset, SCZ and BIP datasets. Prediction of case status by polygenic score was analyzed by linear regression, corrected for ancestry covariates. SNP heritability was estimated with GCTA. Genetic pathway analysis was based on MSigDB canonical pathways.
Results: In single-SNP analyses, genome-wide significant association was observed for 17 SNPs in an intergenic region (chr21:25141353-25144575, HG19; min p=6.2E-09). This signal was not replicated by PGC_MDD, nor were the three genic regions with the strongest evidence for association: in SLC28A1 (chr15: 85428103, rs12910396, GR_p=6.8E-08, PGC_p= 0.99); SP4 (chr7:21504427, rs17144465, GR_p= 8.2E-07, PGC_p=0.3); and AMPD2 (chr1:110172362 , rs2269341, GR-P=2.0E-06, PGC_p[closest proxy]= 0.58). We then meta-analyzed the GenRED1+2 and PGC_MDD datasets (i.e., a subset of the published PGC MDD replication analysis), to further evaluate the best GenRED results. The most interesting result was for the best SNP (rs1969253) in the PGC discovery+replication analysis (p=3.4E-06) and the PGC female-only discovery sample analysis (p=1.0E-07). In GenRED+PGC, we find greater support for males+females (p=7.92E-07); and significant evidence for association in females (p=6.44E-09; ORs 1.15 in GenRED, 1.17 in PGC). The LD block on chr3 spans DVL3 (disshevelled 3, involved in cell proliferation), ABCF3 (involved in antiviral mechanisms, interacting with OAS1), EIF2B5 (mutations produce leukoencephalopathy) and AP2M1 (recycling of pre-synaptic vesicle membranes). In polygenic score analyses, the best p-values (% of "best" training SNPs) were: PGC_MDD: p=2.4E-08 (20%); PGC_SCZ: p=7.5E-07 (40%); PGC_BIP: p=3.6E-03 (60%).Prediction was much stronger for PGC_MDD than for (the smaller) PGC_MDD-REO dataset (best p=7.12E-03); and for females (p=1.84E-04) vs. males (n.s.). The SNP heritability estimate was 0.374 (0.06) for GenRED males+females and 0.503 (0.105) for females. No genome-wide significant pathway association was found.
Discussion: The only genome-wide significant signal was in an intergenic region that did not replicate in the independent part of the PGC_MDD dataset. The support for SP4 (observed in a previous meta-analysis of GenRED1+STAR*D) was increased by GenRED2, but not supported by PGC_MDD. Significant association was observed in females in a meta-analysis of GenRED and PGC_MDD data, across DVL3, ABCF3, EIF2B5 and AP2M1, but the larger PGC_MDD replication analysis produced a weaker signal. Polygenic prediction of GenRED case-control status was observed for PGC_MDD and much less so for the (smaller) PGC_MDD-REO dataset. This supports the current PGC strategy of substantially enlarging the MDD GWAS dataset using a broad definition of MDD. However, larger numbers of narrow-phenotype cases would be needed to determine whether they make unique contributions to geneticstudies. SNP heritability was substantial in women, supporting the use of sex-specific analyses. Some polygenic overlap was observed between MDD and both SCZ and BIP.

PLENARY
4:00 PM – 4:45 PM

OVERALL ABSTRACT:
GENETICS AND NEUROBIOLOGICAL INSIGHTS OF ADDICTION

Yasmin Hurd, Ph.D., Friedman Brain Institute

Drug addiction continues to be of substantial public health concern worldwide. In the United States, this burden is evident not only economically with over half trillion dollars expended yearly on medical and criminal costs, but also to the poor quality of life incurred by the individual, family and community. Significant knowledge has been obtained regarding the pharmacological actions of drugs on multiple neurobiological systems, but this insight has unfortunately not helped to fully address the complex nature of addiction disorders in which individual vulnerability is central to the disease as many young people experiment with drugs, yet only a select number will ultimately progress to abuse. Prevention and intervention of substance use disorders is contingent on being able to identify these vulnerable individuals. Multiple factors including genetics, behavioral traits, psychiatric comorbidity, as well as environmental conditions are now acknowledged to contribute to drug addiction risk. These factors can influence not only the initiation of drug use and the transition from controlled to compulsive use that characterizes addiction, but also the effectiveness of treatment and relapse vulnerability that maintains the insidious cycle of abuse disorders. A significant body of genetic epidemiological and candidate gene research over the past decade have clearly emphasized the importance of heritable influences on addiction demonstrating high heritability for most substance use disorders. A critical step is determining what neurobiological insights such genetic variants identified in substance abuse populations can help to uncover about distinct components of neural systems underlying addiction risk. In contrast to most neuropsychiatric illnesses, neurobiological investigations that explore the molecular underpinnings of addiction disorders have traditionally not been grounded on knowledge garnered from neuropathogical information directly obtained from the human brain. Our research has focused on investigating molecular disturbances in the human brain related to genetic variants of the opioid neuropeptide system, a proposed final common neurobiological pathway dysregulated by many drugs of abuse including opioids, psychostimulants and cannabinoids. Despite the challenges of studying the human brain, especially in relation to the complex nature of genetics and other risk factors, such investigations have begun to reveal discrete disturbances of neuronal systems relevant to inhibitory control, negative affect and reward choice that contribute to the motivational drive for drug-seeking and relapse behaviors. These investigations also provide an important foundation for reverse translational experimental animal studies in which causal relationships to known molecular disturbances documented in humans can be mechanistically established. These include integrating molecular and in vivo neuroimaging strategies that provide significant translational insights. Elucidating neurobiological substrates in the human brain related to individual genetic variability, and other risk factors of addiction, is important to advance insights about addictive disorders.

Sunday, October 20, 2013

PLENARY
8:30 AM – 10:30 AM

OVERALL ABSTRACT:
PERSONAL AND CLINICAL GENOMICS: THE OPPORTUNITIES AND RISKS

Robert C. Green, M.D., M.P.H.

Department of Medicine, Division of Genetics, Brigham and Women's Hospital and Harvard Medical School

The availability of powerful genomic technologies and the ever-growing catalogue of identified risk variants have created new opportunities and dilemmas for researchers, clinicians and consumers. In the clinical and research realms, these advances have raised a host of questions that require careful consideration. How is genetic information best incorporated into clinical care? When and how should genetic risk information be shared with patients? Should incidental findings that emerge through genotyping or sequencing be returned to research subjects, patients and families? We have also now entered an era when the public can search the internet and find several companies that will provide direct-to-consumer genotype or sequence data, not only providing information about their ancestry, but risk for many diseases. The cost may be as little as $99 per genome, making these services widely accessible. Regulation of such "personal genome" information is often lacking, and the validity and utility of the tests are debatable. In addition, it is unclear that clinicians are prepared to deal with the results and how they should counsel their patients about them. In this important plenary session we have brought together experts from diverse perspectives to address the research, clinical and policy issues raised by personal and clinical genomics and, in particular, the implications for neuropsychiatric genetics.

ADDITIONAL SPEAKERS:
Paul Appelbaum, M.D., Columbia University
Atul Butte, M.D., Ph.D., Stanford University and Lucile and Packard Children's Hospital
Cecile A. Janssens, Ph.D., Emory University
Francke Uta, M.D., Stanford University School of Medicine

SYMPOSIA
1:15 PM – 3:15 PM

OVERALL ABSTRACT:
INDUCED PLURIPOTENT STEM CELLS: TOOLS FOR THE INVESTIGATION OF NEUROPSYCHIATRIC DISORDERS

Jay Tischfield, Ph.D., FFACMG[1], Jay Tischfield, Ph.D., FFACMG[1], Michael Sheldon, Ph.D.[1], Flora Vaccarino[2], Stephen J. Haggarty[3], Vishwajit Nimgaonkar[4], David M. Panchision[5]

[1]RUCDR Infinite Biologics, [2]Yale University, [3]Harvard Medical School and Massachusetts General Hospital, [4]Psychiatry and Human Genetics, [5]National Institute of Mental Health

The pursuit of the etiologic factors behind neuropsychiatric disorders, be they genetic, environmental, or via pathologic organisms such as viruses, has been hampered by the lack of availability of relevant living cells from affected individuals. The development of methods to reprogram differentiated somatic cells, such as fibroblasts and lymphocytes, to yield induced

pluripotent stem cells (iPSC), with the concurrent development of protocols for directed differentiation of iPSC to a number of cell types, such as neurons, has laid the foundation for great advances in this field. This symposium will address a number of critical issues in this emerging field. In an important validation of iPSC methodology, **Flora Vaccarino** will present evidence that genomic instability, measured as CNVs, detected in iPSCs can be attributed to the inherent mosaicism present in the somatic cells used as the source of the iPSCs rather than as a consequence of reprogramming itself. She will also present data from genomic and transcriptomic comparisons of probands with autism spectrum disorder and macrocephaly versus unaffected individuals, using iPS and derivative cells. **Vishwajit Nimgaonkar**will describe his use of neural progenitor cells and neurons, derived from human iPSCs, to establish a human in vitro model for herpes virus (HSV-1) infection that will enable understanding of HSV-1 latency mechanisms. As infected and uninfected cells are compared, artifacts introduced during iPSC transformation can be controlled for. We will also consider the potential of iPSCs in the development of therapeutic approaches, as **Stephen Haggarty** will speak about the collaborative, multidisciplinary program he and his colleagues have established to collect and reprogram primary source cells from individuals affected with Fragile X syndrome, Pitt-Hopkins syndrome and bipolar disorder, to name a few. His group has focused on the development of iPSC and differentiated cells as tools for use in a variety of phenotypic assays to enable high-throughput screening of small molecules. No discussion of the role of iPSCs would be complete without consideration of the establishment of resources for the dissemination of high quality cell lines to the broadest possible segment of the research community. There are numerous precedents for the utility of such centers: in 1998 the NIMH funded the Center for Collaborative Genomic Studies on Mental Disorders (https://www.nimhgenetics.org/) at the RUCDR, with the mission of providing high quality centralized processing and distribution of biospecimens collected as part of NIMH funded investigations. **Michael Sheldon** will describe the NIMH Stem Cell Resource (http://nimhstemcells.org/) that was subsequently established at the RUCDR with the mission of providing a resource for human control and patient-derived somatic cells and their reprogrammed derivatives and to support stem cell research relevant to mental disorders. The capabilities of the Resource range from derivation and/or banking of primary source cells from the tissues of human subjects tissue to more comprehensive banking and validation of iPSCs or similar reprogrammed/de-differentiated cells.

INDIVIDUAL ABSTRACT:
GENOME AND TRANSCRIPTOME ANAYLSES OF INDUCED PLURIPOTENT STEM CELLS IN AUTISM SPECTRUM DISORDERS

Flora Vaccarino

Yale University

Human induced pluripotent stem cells (hiPSCs) are a promising model for diagnostics, drug discovery, therapeutics and personalized medicine. However, hiPSCs have come under intense scrutiny because of questions about their genomic stability and the robustness of their differentiation. For example, clonal lines derived from the same individual can have a different neuronal differentiation potential. Two approaches that can be helpful in interpreting such variability are: i) the careful characterization of the genomic structure and gene expression of hiPSC lines, ii) the use of robust neuronal differentiation protocols to minimize line-to-line differences. We performed whole-genome and transcriptome analyses of 20 human iPSC lines derived from primary skin fibroblasts of 7 individuals using next-generation sequencing. We

found that, on average, a hiPSC line manifests two copy number variations (CNVs) not detected in the fibroblast culture of origin, and that the majority of these events are already present as low frequency somatic genomic variants in parental fibroblasts. We estimate that 30% of human fibroblasts carry large CNV not present in the germline. Thus, there is extensive somatic mosaicism in somatic cells, although the amount of variation that it confers to human cells is overall less than 10% of the interindividual genomic variation transmitted through the germline. Despite a clear tendency for increase in expression for genes in duplicated regions and decrease in expression for genes in deleted regions in the same hiPSC lines, this correlation was not universal. Furthermore, the hiPSC lines did not significantly differ from human embryonic stem cells on the transcriptome level, and their differentiation potential was not altered by the presence of these CNVs. To evaluate the ability of hiPSCs to model complex neuropsychiatric disorders of unclear etiology, we are analyzing the genomes, transcriptomes, and biological characteristics of hiPSCs, neuronal progenitors and neurons of patients with autism spectrum disorder and macrocephaly, as compared to their unaffected family members. Our dataset currently comprises 45 different iPSC lines obtained from 15 individuals in 5 families. Preliminary analyses using EdgeR and a generalized linear model to account for family differences suggest that we can detect differences in gene expression in probands' hiPSC and their neuronal progeny. Pathway analyses of these differentially expressed reveal biofunctions mostly related to *Cell death/Cell cycle/Cell proliferation* and *Embryonic Development.* Furthermore, these differences in transcriptome were validated biologically and functionally using assays for cell proliferation and differentiation in non-dissociated, rosette-based protocols. We suggest that hiPSCs can be useful as a model to elucidate the neurobiological basis of neuropsychiatric disorders of unclear etiology. Nonetheless, it is important to evaluate somatic mosaicism as a potential source of variability among hiPSC lines, and for the correct interpretation of results obtained using the hiPSC model, especially when only one hiPSC line is used as representative of an individual.

INDIVIDUAL ABSTRACT:
INDUCED PLURIPOTENT STEM CELL DERIVED NEURONAL LINEAGES AS HUMAN CELLULAR MODELS FOR HERPES SIMPLEX VIRUS, TYPE 1 (HSV-1) INFECTIONS

Vishwajit Nimgaonkar

Psychiatry and Human Genetics

BACKGROUND: Herpes Simplex virus, type 1 (HSV-1) causes lifelong human infection in human sensory ganglia, with occasional lytic lesions in the mucosa/cornea and rarely in the brain, presenting as encephalitis. It is associated with cognitive impairments that are untraceable to acute encephalitis, suggesting a pathogenic role for persistent / latent infection. Available information regarding HSV-1 latency derives primarily from studies in rodent and rabbit models that do not fully recapitulate all the hallmarks of the human disease. Hence we used neurons derived from human induced pluripotent stem (iPS) cells to investigate molecular features of HSV-1 lytic and latent infections, as iPS-derived cells potentially provide limitless quantities of human neurons. **METHODS:** We generated human induced pluripotent stem cells (iPSCs) from fibroblasts obtained through a skin biopsy of adult individuals. iPSCs were differentiated into neurospheres and expanded as monolayer cultures consisting of neural progenitor cells (NPCs) or further differentiated into neurons. NPCs were infected with a genetically engineered HSV-1 virus that expresses green fluorescent protein (GFP) and red fluorescent protein (RFP) at

an MOI of 0.3 in the presence or absence of viral replication inhibitors acyclovir (50 mM) or (E)-5-(2-bromovinyl)-2'-deoxyuridine (5BVdU, 30 mM)) together with interferon alpha (IFN-a, 125 IU). Unfixed cells were tested for expression of GFP and RFP at day 1 and day 6. Establishment of HSV-1 latency was analyzed by RT-PCR using latency-associated transcript (LAT)-specific primers. Localization of HSV-1 genome in the infected nuclei was investigated by 3D-Fluoresscent in situ hybridization (3D-FISH). HSV-1 reactivation in quiescent cultures wad induced by culturing the cells in neurobasal medium supplemented with sodium butyrate (NaB, 5 mM). **RESULTS:** HSV-1-infected neuronal cultures showed features of viral replication and cellular lysis documented in prior studies. Cultures treated with viral replication inhibitors showed features of quiescent infection previously documented in animal models: i) a significant reduction in the percentage of GFP and RFP positive cells; ii) localization of HSV-1 genome at the nuclear periphery; iii) expression of LAT transcripts; (iv) lack of productive infection 5 days after removal of 5BVdU and IFN-a; (v) absence of infectivity in cellular supernatants. Furthermore, treatment of the quiescently infected NPCs and neurons with NaB caused production of infective virions, indicating HSV-1 reactivation. **CONCLUSIONS:** Our results show for the first time that the ability of HSV-1 to establish quiescent infection in human central glutamatergic neurons. They support the utility of iPS-based models to elucidate mechanisms of HSV-1 latency in human neurons. As the models involve comparison of infected and uninfected cells, artifacts introduced during iPS transformation can be controlled for.

INDIVIDUAL ABSTRACT:
CHEMICAL GENOMICS: TARGETING NEUROPLASTICITY PATHWAYS WITH PATIENT-SPECIFIC STEM CELL MODELS

Stephen J. Haggarty

Harvard Medical School and Massachusetts General Hospital

One of the major obstacles to the identification of therapeutic interventions for neuropsychiatric disorders has been the difficulty of studying the step-by-step development of pathophysiology in experimental systems amenable to functional genomic and drug screening. Recent advances in human stem cell biology and the advent of somatic cell reprogramming technology now enable the generation of patient-specific, induced pluripotent stem cells (iPSCs) that can be differentiated *in vitro* into an increasingly diverse array of cell types of the nervous system. Accordingly, the use of these patient-derived iPSC models provide a means to: i) recapitulate the step-by-step development of disease; ii) discover the underlying molecular mechanisms involved in disease pathophysiology; and iii) apply existing and emerging chemical genomic technologies for discovering novel therapeutics that target early steps in disease pathogenesis. Here, I will summarize the recent efforts of our collaborative, multidisciplinary program to assemble a collection of fibroblasts from clinically well phenotyped patients with neuropsychiatric disorder with known single gene causes, such as Fragile X syndrome and Pitt-Hopkins syndrome, as well as complex polygenic causes, including bipolar disorder as part of an experimental therapeutic clinical trial. Using these iPSC models and directed differentiation methods, we have begun developing a panel of high-throughput, neuronal cell-based, phenotypic assays that report on diverse aspects of neuroplasticity, including neurogenesis, Wnt/GSK3 signaling, and global transcriptome changes upon exposure to diverse small-molecule probes. Collectively, the cellular and molecular tools emerging from these studies will advance our understanding of genotype-phenotype relationships and begin to address the critical goal of identifying and validating novel targets for developing disease-modifying therapeutic interventions.

INDIVIDUAL ABSTRACT:
THE NIMH CENTER FOR COLLABORATIVE GENETIC STUDIES OF MENTAL DISORDERS STEM CELL RESOURCE

Michael Sheldon, Ph.D.

RUCDR Infinite Biologics

The study of human neuropsychiatric disorders is particularly suited to the use of induced pluripotent stem cells (iPSC) due to the combination of an established history of genetic studies and the difficulty in obtaining functional tissue samples from affected subjects. The capacity of reprogrammed human cells to generate neurons and glia makes them potentially groundbreaking tools to study cellular mechanisms underlying human brain development and disorders, to identify novel molecular targets and screen candidate therapeutics to treat these disorders. Realization of the full potential of these tools requires free and open sharing of cellular material with associated phenotypic and genotypic data among researchers. This is necessary to achieve sample sizes with adequate statistical power for robustly detecting phenotypic differences among diverse patient populations. Several efforts are currently underway at NIH to enhance research through the standardization, collection, and improved distribution of resources, including biomaterials and protocols. The genetic component of this resource, the NIMH Center for Collaborative Genomic Studies of Mental Disorders (CCGSMD), established at RUCDR in 1998, currently banks and distributes over 100,000 subject samples of blood, lymphoblastoid cell lines, and DNA, along with a bioinformatics infrastructure housing genetic and clinical phenotyping data. The CCGSMD has supported work leading to over 500 publications to date. In 2011, NIMH established the new Stem Cell Resource at RUCDR to make source cells and iPSC lines more widely accessible to the research community, to facilitate data replication, and to ensure standardization. It is designed to integrate the banking of human primary and reprogrammed cells with the comprehensive genetic and clinical data capabilities of the CCGSMD. The NIMH Stem Cell Resource has begun banking iPSC source cells (fibroblasts, olfactory epithelial cells, and lymphocytes) from a variety of disorders, including autism, schizophrenia, and bipolar disorder. The Resource is also engaged in converting source cells into iPSC for distribution to the scientific community. Although initial protocols were developed to reprogram fibroblasts, we have developed optimized protocols to reprogram blood cells. These have several advantages over skin fibroblasts, including less invasive collection, less post collection manipulation, and a large number of samples in existing collections with substantial clinical data. In addition to expansion for distribution, the Resource performs a full range of quality control tests, including microbiological, Immunocytochemistry and FACS analysis of pluripotency markers, whole transcriptome expression analysis, genomic stability testing, and embryoid body formation. In addition to the NIMH, RUCDR has recently entered into an agreement with the NIH Center for Regenerative Medicine (CRM) to distribute its iPSC control and reporter lines. The mission of the CRM is to pilot new technologies and disseminate procedures throughout the community. CRM efforts include optimizing iPSC and cell screening methods, generating reference and reporter cell lines, harmonizing informed consent language, and working with reagent vendors to enhance freedom to operate. CRM is also coordinating with the new National Center for Advancing Translational Sciences (NCATS) to integrate iPSC technology into the therapeutic pipeline.

SYMPOSIA
1:15 PM – 3:15 PM

OVERALL ABSTRACT:
NEW FINDINGS ON THE GENOMIC BASIS OF ALCOHOL USE DISORDERS

John I. Nurnberger, Jr., M.D., Ph.D.[1], Arpana Agrawal, Joel Gelernter[2], Kenneth Kendler, M.D.[3], Abbas Parsian, Ph.D.[4], R. Dayne Mayfield[5]

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A heritability of 40-60% has been found in twin/family studies for most substance use disorders (dependence or abuse of alcohol, nicotine, and/or illicit drugs), as reviewed in Ducci and Goldman, 2012. Specific genetic factors have been identified for many of these disorders; general liability to addiction has also been investigated, and does appear to account for a substantial part of the heritability. In this symposium, we will discuss some of the specific genetic factors involved in alcohol use disorders, as well as more general factors involved in substance use and abuse. *Joel Gelernter* (Yale University) describes genome-wide association studies (GWAS) in subjects meeting criteria for alcohol and/or drug use disorders, investigating populations of multiple ethnicities. He finds a common genetic factor in variants of alcohol metabolizing enzymes. *Arpana Agrawal* (Washington University in St. Louis) presents GWAS data from the largest meta-analysis of alcohol dependence to date, including 6k cases and 21k controls. Early results suggest that methodologic changes may be necessary to address heterogeneity. Additional analyses using quantitative phenotypes, genome-wide complex trait analysis (GCTA) methods, and gene-based association testing, are underway. *Dayne Mayfield* (University of Texas at Austin) reports integrated studies of microRNAs (miRNAs), messenger RNAs, and protein networks in brain areas of mice exposed to ethanol. The findings suggest adaptive changes in immune, synaptic, and epigenetic signaling in response to ethanol; in particular, findings suggest "cross-targeting" responses by groups of miRNAs in ways that maximize cellular efficiency. Variations in miRNA levels in the brains of human subjects with alcohol dependence will also be described. *John Nurnberger* (Indiana University) reports on an ongoing Prospective Study within the COGA (Collaborative Study of the Genetics of Alcoholism) consortium. This longitudinal follow-up of 3460 US adolescents and young adults will track the effect of risk and protective factors on development of drinking problems in persons passing through the age of greatest liability for new disorders. Subsamples carrying protective variants on *ADH4* (relatively specific for alcohol use disorders), or risk variants on *GABRA2*(which confers liability to abuse of multiple substances), are being compared with other adolescents at risk.

INDIVIDUAL ABSTRACT:
GWAS OF ALCOHOL DEPENDENCE TRAITS IN THREE POPULATIONS

Joel Gelernter

Yale Univ. School of Medicine

We conducted a GWAS of alcohol dependence (AD) in which the discovery sample included a total of 5,697 U.S. subjects (1432 AD-affected EAs and 1731 AD-affected AAs). A second identically ascertained dataset comprising 2,551 U.S. subjects was used for replication. All of our U.S. subjects were recruited for studies of the genetics of drug (cocaine or opioid) or alcohol dependence. We did additional analyses including the public-domain SAGE data set. Samples from individuals in the discovery sample were genotyped on the Illumina HumanOmni1-Quad v1.0 microarray containing ~900k autosomal SNPs. Follow-up genotyping in the replication

sample was performed using a custom Illumina GoldenGate microarray assay. Additional SNPs were genotyped individually using the TaqMan method. We did a separate study in a sample of 569 individuals recruited in China, who were genotyped with the Illumina Cyto12 array containing ~300k markers. There were genome wide significant (GWS) findings in both the EA and the AA parts of the U.S. sample– the associated SNPs corresponded to different functional variants in the same risk locus (*ADH1B*). When our sample was analyzed together with the public domain SAGE data (http://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs000092.v1.p1), there were additional GWS findings mapped to the alcohol dehydrogenase gene cluster on chromosome 4–both within genes (*ADH1C, ADH4*) and in intergenic regions. In the Chinese sample, the trait of MAXDRINKS was GWS-associated with a SNP at the *ALDH2* locus. The alcohol metabolizing enzymes have long been known to influence risk for AD; these GWAS studies show convergence in three populations on the genes encoding those enzymes as an AD risk pathway, but with different genes or SNPs involved in different populations.

INDIVIDUAL ABSTRACT:
META-ANALYSIS OF GENOMEWIDE STUDIES OF ALCOHOL DEPENDENCE

Arpana Agrawal

About 50% of the variation in alcohol dependence is attributable to additive genetic influences. Some of the most promising candidate genes in this area have been genes in the alcohol and aldehyde dehydrogenase systems, however, effect sizes in non-Asians are modest and require very large samples. Thus, it is expected that alcohol dependence, in non-Asians, is highly polygenic and in need of considerable gene discovery. Yet, several genome wide association studies of Caucasian populations have, with rare exception (see Frank et al., 2012), had limited success with gene identification at levels of statistical significance. Concerns regarding the expected small effect sizes and the relatively low power of each individual study, coupled with the growing success of other psychiatric consortia, has generated considerable excitement for meta-analysis. We combined effect sizes across multiple Caucasian samples resulting in 6957 DSM-IV alcohol dependent cases and 21777 controls to conduct a genome wide meta-analysis. Data were analyzed on imputed genomic data by the individual study sites and results were meta-analyzed using inverse-variance weighting in METAL after alignment of strands and quality control. No single variant surpassed genome wide statistical significance. Additional analyses examining gene systems via pathway analysis and examining the aggregate effects of all variants in explaining heritable variation in alcohol dependence is also under way. Overall, these analyses indicate that substantially larger sample sizes might be required to attain the power required to detect genetic variants in these Caucasian samples. Heterogeneity across the samples, in ascertainment strategy and phenotype definition was also evident and implicated in loss of statistical power. For instance, whether all alcohol dependence cases, regardless of the symptoms they endorse, are created equal will be explored, as twin studies indicate substantial variability in the magnitude of genetic influences on individual dependence criteria. Additionally, quantitative indices (e.g. symptom counts) may facilitate gene identification as they better account for severity of disorder. Similar exciting developments for substance use disorders (e.g. cannabis) will also be discussed.

INDIVIDUAL ABSTRACT:

VARIATION IN PHENOTYPE FOR SINGLE GENES RELATED TO ALCOHOL DEPENDENCE BASED ON DEVELOPMENTAL STAGE, GENDER, AND ETHNICITY

John I. Nurnberger, Jr., M.D., Ph.D.

Indiana University

It has been known for several decades that specific single genes coding for the enzymes that metabolize alcohol (ALDH and ADH*)* have variants that affect risk for alcohol use disorders that are common in East Asian populations. In recent years, evidence has accumulated that some genetic variants coding for ADH are important modulators of drinking behavior in populations of European ancestry as well. The Collaborative Study of the Genetics of Alcoholism (COGA) has previously presented evidence that *ADH4* in particular is associated with risk for alcohol dependence (Edenberg et al, 2006). We now show that *ADH4* variants are associated with a number of alcohol-related phenotypes as part of an ongoing study of ~3600 adolescents/young adults at risk for alcohol use disorders because of membership in a family with one or more affected members. Specific genotypes (CC on rs4148886, CC on 3762894, or TT on rs4148884) are associated with decreased risk for alcohol-related disorders. In particular, genotype CC on rs44148886, found in 9% (61/693) of COGA European-American adolescents, is associated with a significant decrease in risk for alcohol dependence ($p<.0001$; OR=.0005, CI .0002-.001). In the larger COGA sample we have identified 775/6674 subjects (11.6%) with one or more of the protective *ADH4* genotypes. These genotypes are more common in African-Americans (29.2%) than in European-Americans (5.8%) (chi-square = 24.7, $p<.001$). We have also recently shown that *AHD1B* is associated with alcohol dependence and alcohol consumption in populations of European and African ancestry (Bierut et al, 2012). In addition, new evidence is available on the relationship between *GABRA2* alleles and alcohol use disorders. Aside from the alcohol metabolic enzymes, association with the gene *GABRA2* is probably the single most replicated finding in molecular genetic studies of alcohol use (see summary in Edenberg et al, 2012). SNPs in this gene have been associated with alcohol dependence; however, expression of GABRA2-related phenotypes is clearly under developmental regulation – adolescents in COGA families carrying the *GABRA2* risk allele do not show alcohol use disorders, but they do show increased symptoms of conduct disorder. Recent studies show evidence of gender-specific gene by environment interaction in the expression of *GABRA2* alleles. For men with high risk genotype AA on SNP rs279871, positive daily life events are associated with decreased rates of alcohol dependence (OR = 0.37, CI: 0.22 - 0.64, $p < .001$); no such relationship is seen for women (or for either men or women with the low-risk genotype) (Perry et al, submitted for publication). Bierut LJ et al ADH1B is associated with alcohol dependence and alcohol consumption in populations of European and African ancestry. Molecular Psychiatry 2012 Apr;17(4):445-50. doi: 10.1038/mp.2011.124. Epub 2011 Oct 4. Edenberg HJ, et al, Association of alcohol dehydrogenase genes with alcohol dependence: a comprehensive analysis. Hum Mol Genet 15(9):1539-1549, 2006. doi:10.1093/hmg/dd1073. Edenberg HJ. In Nurnberger Jr JI and Berrettini W (eds.) Principles of Psychiatric Genetics. Cambridge University Press Perry, B et al, Gender-specific gene-environment interaction in alcohol dependence: The impact of daily life events and GABRA2, submitted for publication

INDIVIDUAL ABSTRACT:
INTEGRATED MIRNA, MRNA, AND PROTEIN CO-EXPRESSION NETWORKS IN BRAIN OF ETHANOL-TREATED MICE.

R. Dayne Mayfield

University of Texas at Austin

Alcohol abuse causes dramatic neuroadaptations in the brain, which contribute to tolerance, dependence, and behavioral modifications. Previous microarray/proteomics studies in human alcoholics and animal models have identified candidate microRNAs (miRNAs), genes, and proteins associated with alcohol abuse; however, no single approach can fully account for the impact of these changes on the complex interactions that regulate brain function. We recently reported the first comprehensive study of alcohol on miRNA levels in the brain of human alcoholics (Front Genet. 2012; 3:43). In the present study, mice were exposed to high drinking protocols (two-bottle choice/drinking-in-the-dark and chronic intermittent ethanol/two-bottle choice) and tissue was dissected from different regions of the brain: frontal cortex (FCtx), infralimbic/prelimbic cortex (ILPrL), nucleus accumbens (NAc), and basolateral/central amygdala (Amy). Exiqon miRCURY LNA(tm) microRNA, Illumina MouseRef-8 microarrays, and two-dimensional differential in-gel electrophoresis (2D-DIGE) proteomics were used to assess RNA/protein expression levels. Gene and miRNA differential expression analysis and weighted gene coexpression network analysis were utilized to provide an integrated view of negatively and positively correlated miRNA-mRNA network relationships. We found that, as in FCtx of human alcoholics, miRNAs are predominantly upregulated in FCtx of alcohol-drinking mice, with 52 miRNA families being upregulated in mouse brain. We also found that a highly significant number of miRNA families that are upregulated in human alcoholics (14 out of 32 miRNA families) are also upregulated in the FCtx of ethanol-treated mice ($p < 1x10^{-5}$; hypergeometric function). Genes changing expression in response to alcohol treatment were over targeted by differentially expressed miRNAs, suggesting a potential combinatorial mode of action of miRNAs. The findings suggest system-wide neuroadaptive alterations of innate-immune, synaptic, and epigenetic signaling in response to alcohol consumption. An important concept emerging from our analyses is that miRNAs appear to have evolved "cross-targeting" capabilities that allow them to regulate gene and possibly protein expression in distinct cellular pathways in such a way that promotes molecular efficiency and cellular economy.

SYMPOSIA
3:30 PM – 5:30 PM

OVERALL ABSTRACT:
ENHANCING NEURO IMAGING GENETICS THROUGH META-ANALYSIS: RESULTS FROM THE ENIGMA CONSORTIUM

Sarah E. Medland, Barbara Franke[1], Ole A. Andreassen, M.D., Ph.D.[2], Jessica Turner, Ph.D.[3], Jason Stein, Derrek Hibar[4], Neda Jahanshad[4],

[1]Departments of Human Genetics and Psychiatry, Donders Institute for Brain, Cognition & Behaviour, Radboud University Medical Centre, Nijmegen, The Netherlands, [2]University of Oslo, [3]Mind Research Network (MRN), [4]University of California Los Angeles

The ENIGMA consortium (http://enigma.loni.ucla.edu) was founded in 2010 and brings together researchers in imaging genomics, to understand brain structure and function, based on MRI, DTI, fMRI and genome-wide association scan (GWAS) data. In this session, we will provide an overview of the consortium's work on structural MRI and DTI. The motivation behind the development of the consortium and the philosophy underlying the approach will be briefly introduced by Sarah Medland (Symposium Chair). As an initial flagship project, we undertook a GWAS meta-analysis of hippocampal volume, intracranial volume, and total brain volume. This

highly successful project resulted in a meta-analysis of data from 28 groups that span 5 continents, including 16,125 subjects. We identified several genome-wide significant variants that influence hippocampal and intracranial volumes. We recently extended the scope of the initial project to include the examination of genetic influences on the volumes of subcortical structures, i.e., caudate, putamen, pallidum, thalamus, nucleus accumbens, amygdala, as well as hippocampus and intracranial volume (ICV). This second project involves additional participating groups and imputation to the 1000 Genomes reference set. This project is currently nearing completion and is already showing exciting new results that will be presented by Derrek Hibar. In addition, the collaboration has led to the creation of working groups addressing a range of important topics, including the integrity of the brain's white matter. The DTI Working Group was formed with the initial goal of developing a validated protocol to obtain reliable and consistently heritable measures from images. This protocol is now easily implementable at the many ENIGMA sites that have DTI and genomic (GWAS) data. The results of this work and first GWAS findings will be presented by Neda Jahanshad. Given the focus on neuroimaging as an endophenotype for psychiatric disease we have established a cross-consortium collaboration with the Psychiatric Genetics Consortium to explicitly examine the overlap in genetic variants between brain MRI measures and psychiatric disease. Results from this work will be presented by Barbara Franke (Moderator). This work also led to the formation of working groups performing phenotypic meta-analyses examining the association between brain measures and psychiatric disease. Ole Andreassen will present results from the meta-analysis of structural variation in bipolar disorder and Jessica Turner will present results from the meta-analysis of structural variation in schizophrenia. The implications of these findings for psychiatric research using imaging endophenotypes will be discussed (Jason Stein, Discussant).

INDIVIDUAL ABSTRACT:
ENIGMA2: GENOME-WIDE SCANS OF SUBCORTICAL BRAIN VOLUMES IN 16,125 SUBJECTS FROM 28 COHORTS WORLDWIDE

Derrek Hibar, University of California Los Angeles

Introduction: Neuroimaging genetics has the potential to identify genetic contributions to disease pathology by discovering both common and rare genetic variants that relate to brain structure and function. Many studies have identified significant genetic associations with brain measures, but effect sizes are generally small so vast samples are needed to find and replicate genetic associations (Stein et al., 2012). In addition to small effect sizes, data collection is expensive and small datasets are common. To maximize power to detect genetic effects on brain measures, we formed the ENIGMA Consortium (http://enigma.loni.ucla.edu/) to help coordinate and harmonize neuroimaging genetics efforts at sites around the world. Methods: The ENIGMA Consortium is comprised of 28 groups that span 5 continents, including 16,125 subjects. ENIGMA follows a meta-analysis framework, where analyses are conducted at local sites and group-level, de-identified statistics are contributed for meta-analysis. To harmonize analyses across sites, we developed standardized protocols for image analysis, imputation of genetic data, and genetic association analysis (http://enigma.loni.ucla.edu/protocols/). Image analysis was conducted using fully-automated and validated neuroimaging segmentation algorithms (either FSL FIRST or FreeSurfer). The heritability of each structure was estimated using structural equation modeling (SEM) from 801 twins and siblings from the QTIM study. Genetic data were imputed to the latest 1000 Genomes reference panel (phase I, version 3) using MaCH and minimac. The imputation results were cleaned to remove poorly imputed SNPs (Rsq < 0.3) and

SNPs with low minor allele frequency (MAF < 0.01). Association testing was conducted using mach2qtl for samples with only unrelated subjects and merlin-offline for samples with a family design. Association tests conducted at each SNP controlled for age, sex, 4 MDS components, intracranial volume (ICV), site variables (for multi-site studies) and disease status (if applicable). Association was conducted separately for each brain structure phenotype. Data were combined across sites using the inverse variance-weighted meta-analysis. Results: Heritability analysis indicates that several of the structures under examination in our study are very highly heritable including the caudate ($a^2 = 0.8$), pallidum ($a^2 = 0.79$), putamen ($a^2 = 0.84$), and thalamus ($a^2 = 0.85$). After combining association results across thirteen sites, preliminary results suggest genome-wide significant hits at previously unidentified loci for the caudate (chr10), putamen (chr14), and pallidum (chr7). Discussion: Inverse variance-weighted meta-analysis combines effect sizes (regression coefficients) across sites in a way that penalizes SNPs with an inconsistent direction of effect. In this way, we minimize the chance of finding false-positive results while boosting power to detect even small genetic effects across sites. In future, the ENIGMA Consortium will examine genetic influences on cortical surface phenotypes, 3D morphometry, white-matter integrity, and many other brain-derived measures. In addition, we are examining disease-specific hypotheses in bipolar disorder, schizophrenia, and major depressive disorder. New groups with neuroimaging data are encouraged to join in or propose new projects; many ongoing projects do not require genotyped cohorts.

INDIVIDUAL ABSTRACT:
MULTI-SITE GENETIC ANALYSIS OF DIFFUSION MRI SCANS FROM THE ENIGMA DTI WORKING GROUP

Neda Jahanshad, University of California Los Angeles

Introduction: Human brain structure is genetically influenced, and many neuro-imaging measures are heritable, i.e., a proportion of their variance is due to differences in the human genome. Recently, the Enhancing Neuroimaging Genetics through Meta-Analysis (ENIGMA) Consortium created a Diffusion Tensor Imaging (DTI) Working Group to study genetic influences on white matter micro-architecture and integrity. This integrity as measured through DTI fractional anisotropy (FA), have been shown to be highly heritable. Variations in FA are also strongly linked with disorders such as schizophrenia, Alzheimer's disease and other diseases with known genetic associations. While several risk genetic variants such as those in *CLU, DISC1, APOE4, NTRK1* have already been strongly associated to changes in FA, much of the genetic influence that puts our brain's micro-architecture at risk for disease is still unknown and large sample sizes are needed to make such discoveries. To make it efficient to analyze DTI on a large scale from many cohorts, the ENIGMA-DTI working group first set out to define reliable, and heritable, measures from DTI scans of cohorts of different ages and ethnicities. We prioritize the most promising brain measures for genetic analysis, based on their consistently high heritability in different cohorts. **Methods** Two ethnically different family-based datasets, QTIM (N=292) and GOBS (N=859) (the Queensland Twin Imaging and the Genetics of Brain Structure and Function Studies) with different imaging protocols were used to estimate heritability on a white matter tract based level. Standardized imaging and genetic protocols are made publicly available. To calculate heritability across images, we used a variance components method, as implemented in SOLAR (http://www.nitrc.org/projects/se_linux). GWAS was performed on the FA values of each heritable white matter tract. Heritability and GWAS results were meta-analytically combined. **Results:** FA values for most tracts were highly heritable in both cohorts

individually and when combined using meta-analysis. The most highly heritable tracts overall were regions of the corpus callosum and the superior longitudinal fasciculus, while the fornix and cortical-spinal tract showed the least heritability. Meta-analyzed GWAS results show SNPs that associate to brain microstructure. **Conclusion:** By harmonizing protocols and initially estimating heritability of the FA across the entire scan, we are able to prioritize the regions on the FA map by selecting those tracts that are most heritable and reliably measured across populations. Initial meta-analyzed GWAS results with 1159 subjects from two groups show promise for further genetic discovery. Currently the combined number of subjects with DTI scans and genotypes from interested groups exceeds 5000, and ENIGMA-DTI is actively recruiting other interested research groups to increase sample size and increase power to collectively detect more genetic variants affecting brain structure, and possibly put the brain at risk for disease.

INDIVIDUAL ABSTRACT:
META-ANALYSIS OF STRUCTURAL BRAIN DIFFERENCES IN BIPOLAR DISORDER: THE ENIGMA-BIPOLAR DISORDER PROJECT

Ole A. Andreassen, M.D., Ph.D.

University of Oslo

Bipolar disorder (BD) is a heritable brain disorder, with a large 'missing heritability'. Neuroimaging analyses of BD have reported significant structural differences in volumes of subcortical brain structures including the amygdala (DeBello 2004; Strakowski 1999), hippocampus, thalamus, accumbens and lateral ventricles (Rimol 2010). However, meta-analysis identified only the lateral ventricle as consistently reported across studies to be significantly different between patients with BD and controls (Kempton 2008), while a recent mega-analysis found that the right lateral ventricle, left temporal lobe, and right putamen differed in volume between BD patients and controls (Hallahan 2011). The inconsistency across sites and studies, and the relative small number of subjects per site makes a combined meta-analytic approach advantageous. This will also improve the probability to identify gene-brain structure associations. Here we used a standardized image analysis and consensus quality control protocol at a large number of international sites, the Enhancing Neuro Imaging Genetics Through Meta-Analysis (ENIGMA) Consortium, ENIGMA-Bipolar Disorder Working Group. The aim was to first determine patient vs. control effect sizes for subcortical volume differences, in the largest neuroimaging study of BD to date, and secondly, explore common genetic variants associated to the brain structure abnormalities. Methods: Current members of the ENIGMA Bipolar Working Group include TOP, BFS, UCLA, Yale, GIG, NIMH-IRP, NUIG, Landen, Cincinnati, Pittsburgh, and London (1221+ patients and 1241+ controls, a large proportion with GWAS data). High-resolution structural MR images were processed with fully automated, validated segmentation software (FSL FIRST or FreeSurfer) to extract a set of subcortical structures: caudate, hippocampus, putamen, pallidum, thalamus, nucleus accumbens, lateral ventricles, as well as intracranial volume. We obtained Cohen's *d* effect size estimates for the left, right, and average volume of each subcortical structure. Effect size estimates were calculated per site and combined meta-analytically, weighted by each site's total sample size. Results: The magnitude of case-control effect size differences varied across subcortical and intracranial volumes. Pilot analyses at the individual cohort level suggested disease effects for the pallidum, lateral ventricles, and intracranial volume. We are currently preparing for gene-brain structure analysis. Conclusions: The heterogeneity of results across previous BD studies has created the

need for an international effort to examine the effects of BD on the brain through meta-analysis. The ENIGMA framework allows new sites to participate in analyses without requiring them to share raw scan data, just pre-agreed summary statistics for meta-analysis. The ENIGMA-Bipolar Disorder Project will examine cortical phenotypes, 3D surface morphometry, and genetic influence on brain-derived measures, and we welcome additional projects. Results of first phase analysis will be presented.

INDIVIDUAL ABSTRACT:
IDENTIFYING IMAGING PHENOTYPES: A PROSPECTIVE META-ANALYSIS OF SUBCORTICAL BRAIN VOLUMES IN SCHIZOPHRENIA VIA THE ENIGMA CONSORTIUM

Jessica Turner, Ph.D.

Mind Research Network (MRN)

Introduction: Schizophrenia patients show significant subcortical brain abnormalities but there is considerable heterogeneity of findings across studies. There is also considerable genetic contribution to risk for the disease, as well as the course of the disease and response to treatment. Identifying the most robust imaging phenotypes is a first step for identifying potential genetic modulation. We present a coordinated, large-scale meta-analysis with the same quality assurance (QA) metrics and statistical models across independent datasets, with the goal of identifying the strongest effect sizes across the various subcortical abnormalities in schizophrenia. Retrospective meta-analyses on published results suffer from variations in image segmentation methods and analysis techniques across the reported studies. Using the methods developed by the Enhancing Neuro Imaging Genetics Through Meta-Analysis (ENIGMA) Consortium, participating researchers can provide their analysis results for prospective aggregation and meta-analysis. These results provide effect sizes for subcortical volume differences between almost 1,000 schizophrenia patients and an equal number of healthy controls. **Methods:** The ENIGMA schizophrenia study consortium currently comprises the TOP, FBIRN, UMCU, MCIC, NU, HMS, CliNG, and GOBS datasets (~990 patients and 1037 controls, age range=17-65) and encourages other sites to join ongoing collaborative analysis efforts. At each site, total intracranial and subcortical volumes (for pallidum, hippocampus, putamen, lateral ventricle, amygdala, caudate, thalamus, nucleus accumbens) were extracted using FreeSurfer from high-resolution structural MRI scans of schizophrenia patients and healthy volunteers of similar mean age and sex distribution. The analyses in each study included age, gender, and total intracranial volume as covariates, and dummy variables for site effects for the multi-site datasets. For each subcortical region, we computed Cohen's *d* effect sizes within each study, and weighted mean effect sizes for group differences across all studies. **Results:** Effect sizes for group differences varied for subcortical and intracranial volumes. Initial analysis indicate that the largest effect sizes in terms of *deficit* and *excess* volume in schizophrenia patients compared to healthy volunteers were obtained for the hippocampus and pallidum, respectively. **Conclusions:** Combining data using harmonized methods from many large cohorts through consortia such as ENIGMA can provide robust effect size estimations. This helps focus on the most consistent imaging measures for imaging genetics analysis; ongoing analyses are exploring the potential causes for variations in effect sizes across studies. Such meta-analyses may be particularly useful in research areas where study samples are traditionally small (e.g., high-risk, first-episode and medication studies in schizophrenia). Future work will explore clinical and cognitive factors that influence disease effects within the consortium infrastructure. These more granular analyses can

only be addressed by various subsets of the samples that collected common relevant measures. The ENIGMA-Schizophrenia group is actively encouraging other participating research groups to contribute their analyses and collaborative efforts, as these next meta-analyses are being developed.

SYMPOSIA
3:30 PM – 5:30 PM

OVERALL ABSTRACT:
THE BIOLOGY OF NEW MUTATION AND ITS IMPACT ON THE BRAIN

Christopher A. Walsh, M.D., Ph.D.[1], James R. Lupski, M.D., Ph.D., D.Sc. (hon)[2], Jonathan Sebat, Steven A. McCarroll, Ph.D.[3], Steven A. McCarroll, Ph.D.[3], Jonathan Sebat[4], Joris A. Veltman[5]

[1]Boston Children's Hospital, [2]Baylor College of Medicine, [3]Stanley Center for Psychiatric Research, Broad Institute of MIT and Harvard, [4]UC San Diego, [5] Radboud University Nijmegen Medical Centre

Rationale Neuropsychiatric disorders are highly heritable, but not always inherited. There is a growing appreciation for the role of de novo mutation in disorders of the brain. But there remains a limited understanding of the underlying biological processes that shape patterns of mutation in the genome and influence risk for human disease. We will explore a variety ways in which spontaneous germline and somatic mutation can contribute to psychiatric disorders. **Abstract** Mutation in the human genome is a major force driving biological innovation and disease susceptibility in humans. In disorders of the brain, the clinical impact of new mutation is particularly evident. De novo germline mutation is an important contributor to disease risk in common disorders, including autism and schizophrenia. In addition, somatic mosaicism of new mutations has been found to contribute to a variety of rare syndromes. A key to understanding the genetic susceptibility for neurodevelopmental and neuropsychiatric disorders is to understand the nature of mutation in the germline and in the brain. This symposium will address the underlying genetic mechanisms of new mutation and how they are shaped by intrinsic properties of the genome and extrinsic factors such as parental age and environment. Presentations will cover a variety of disorders and a variety of mutation types, including CNVs, retransposition events and point mutations. We will discuss how patterns of mutation are shaped by the intrinsic mutability of the genome. We seek to explore the question: to what extent are disorders of the brain programmed into our DNA? Presentations: **Jonathan Sebat** – Whole Genome Sequencing Identifies Hotspots for germline mutation in Autism Spectrum Disorders **James Lupski**- Genomic architecture and Genomic disorders **Steve McCarroll** – Patterns of somatic mutation in the genome **Christopher Walsh** – Somatic mutation, transposition and somatic disorders of the brain **Joris Veltman** - Discussant

INDIVIDUAL ABSTRACT:
MECHANISMS FOR HUMAN GENOME REARRANGEMENTS: INSIGHTS FROM PATIENTS WITH GENOMIC DISORDERS

James R. Lupski, M.D., Ph.D., D.Sc. (hon)

Baylor College of Medicine

Copy number variants (CNV) are now a well-established cause of neuropsychiatric diseases including autism, schizophrenia, attention deficit hyperactivity disorder (ADHD), and intellectual disability (ID). CNV often occur *de novo*. The mechanisms by which genomic rearrangements leading to CNV form and the properties of the human genome rendering genomic instability are still being elucidated after >20 years of effort. Remarkably, such genomic rearrangements are often more complex than perhaps initially appreciated and these allow a remarkable plasticity of our genome likely facilitating evolutionary processes to an extent potentially greater than base pair changes of DNA. We present recent data that provide further insights into both recombination based mechanisms such as NAHR and the replicative mechanisms including FoSTeS/MMBIR. NAHR is a well-established mechanism for recurrent rearrangements associated with genomic disorders. The mechanism for NAHR appears to occur because of an ectopic synapsis that 'sets-up' the chromosome for an ectopic crossing over. However, the frequency at which duplication can convert to triplication, the mechanisms by which that occurs, and whether that can convey a more severe clinical phenotype within a family are not understood. We studied families with the CMT1A triplication to try and address these questions. In a separate set of experiments we investigated patients with complex genomic rearrangements to further elucidate the FoSTeS/MMBIR mechanism. We provide evidence that MMBIR is associated with a > 1000 fold increase in point mutations in the vicinity of breakpoint junctions likely reflecting the fidelity of the replicative polymerase and that it also can be associated with a genomic segment of copy number neutral absence of heterozygosity (AOH) when the template switch occurs to the chromosome homologue versus the sister.

INDIVIDUAL ABSTRACT:
WHOLE GENOME SEQUENCING IDENTIFIES HOTSPOTS FOR GERMLINE MUTATION IN AUTISM SPECTRUM DISORDERS

Jonathan Sebat

UC San Diego

De novo mutation plays an important role in autism spectrum disorders (ASDs). Notably, pathogenic copy number variants (CNVs) are characterized by high mutation rates. We hypothesize that hypermutability is a property of ASD genes and may also include nucleotide-substitution hot spots. We investigated global patterns of germline mutation by whole-genome sequencing of monozygotic twins concordant for ASD and their parents. Mutation rates varied widely throughout the genome and could be explained by intrinsic characteristics of DNA sequence and chromatin structure. Hypermutability was a characteristic of genes involved in ASD and other diseases. In addition, genes impacted by mutations in this study were associated with ASD in independent exome-sequencing data sets. Our findings suggest that regional hypermutation is a significant factor shaping patterns of genetic variation and disease risk in humans

INDIVIDUAL ABSTRACT:
MUTATIONAL HOTSPOTS AND GENETIC ANALYSIS OF PSYCHIATRIC ILLNESS

Steven A. McCarroll, Ph.D.

Stanley Center for Psychiatric Research, Broad Institute of MIT and Harvard

New mutations shape the landscape of genetic variation in genomes and create the scientific opportunity to relate variation in genes to variation in phenotypes. And yet, we know

surprisingly little about the biology of mutations – why they arise where they do, and what context this creates for genetic analysis. New mutations show a profoundly non-random distribution across the genome, but we are only beginning to understand the forces governing this distribution. A substantial fraction of new mutations appear to arise during the process of DNA replication, a structured, choreographed program in which genomic loci replicate in a specific temporal order. We recently developed approaches to profile replication timing at extremely high resolution, by sorting S- from G1-phase cells and measuring locus-specific copy number in these two cellular fractions by whole genome sequencing. This allows us to create high-resolution replication timing maps of each chromosomes and to compare these maps to the distributions of new mutations in trio-based sequencing studies. We find that distinct forms of mutations – including transitions, transversions, and structural mutations – partition differently into the early- and late-replicating parts of the genome, suggesting that the replication process itself changes dynamically in ways that favor the generation of particular kinds of mutations in particular genomic regions at particular times. We further developed these analytical methods to profile replication timing in a more high-throughput way in more than 100 individuals. We find that the genome's replication timing program varies among individuals at hundreds of genomic loci – that specific genomic loci replicate earlier in some individuals than in others, and that this variation is heritable. Thus, genetic variation may itself shape the distribution of mutations in dividing cells, providing new potential mechanisms by which genetic variation, somatic mutation, and phenotype relate to one another. An interesting set of genomic loci appear to undergo structural rearrangement at surprisingly high frequency, segregating in human populations in many different structural forms. After struggling for many years to characterize these complex and multi-allelic forms of genome structural variation, which are not well-analyzed by arrays or exome sequencing, we have finally developed a molecular and statistical toolkit for analyzing these regions in a rigorous way. I will describe how the complex patterns of genetic variation at such loci create new scientific opportunities for relating genome variation to phenotypes.

INDIVIDUAL ABSTRACT:
SOMATIC MUTATION AND GENOMIC DIVERSITY IN THE DEVELOPING HUMAN CEREBRAL CORTEX

Christopher A. Walsh, M.D., Ph.D.

Boston Children's Hospital

Somatic mutation and genomic diversity in the developing human cerebral cortex Chris A. Walsh, Ann Poduri, Xuyu Cai, and Gilad Evrony, Division of Genetics and Howard Hughes Medical Institute, Boston Children's Hospital and Departments of Neurology and Pediatrics, Harvard Medical School Formation of the human brain requires coordinated cell division, and genes mutated in human "microcephaly," (a severe reduction of cortical neurons) have identified important mechanisms of neurogenesis. Recent studies have identified somatic, "brain only" mutations affect human brain size and structure, in which mutations that apparently occur during neurogenesis are present in neural cells but are rare or undetectable outside the brain. A major question in neuroscience is whether similar mutations cause "complex" neurogenetic disease, and how commonly neuronal diversity reflects genomic diversity in individual neurons. We have developed methods for isolating single neurons from the postmortem human brain, and amplifying and sequencing the genomes of single CNS neurons, with coverage of >90% of the

genome in most single cells. Sources of human CNS genomic diversity include spontaneous mobilization of L1 retrotransposons, spontaneous point mutations and copy number variation.

SYMPOSIA
3:30 PM – 5:30 PM

OVERALL ABSTRACT:
THE BIPOLAR DISORDER SEQUENCING CONSORTIUM: A PROGRESS REPORT

Francis J. McMahon, M.D.[1], Jared Roach, M.D., Ph.D.[2], Pamela Sklar, M.D., Ph.D.[3], Guy A. Rouleau, M.D., Ph.D., FRCP(C), O.Q. [4], Gerome Breen[5], Nelson B. Freimer[6], Peter Zandi, Ph.D., M.P.H., M.H.S.[7]

[1]NIMH Intramural Research Program, National Institutes of Health, [2]Institute for Systems Biology, [3]Icahn School of Medicine at Mount Sinai, [4]McGill University, [5]Institute of Psychiatry, King's College London, [6]UCLA, [7]Johns Hopkins Bloomberg School of Public Health

The Bipolar Disorder Sequencing Consortium arose in April, 2012, with the goal of bringing together the many groups that are pursuing exome or whole-genome sequencing in bipolar disorder. Currently, 13 groups comprising close to 18,000 cases have joined the Consortium. In this Symposium, we will describe the Consortium and provide a progress report with preliminary findings. Speakers will address: systems biology validation of candidate variants, rare deleterious alleles shared by distant relatives, linkage and sequencing in a single large family with many mood disorder cases, exome sequencing analysis of lithium responsive families, and a combined analysis of data from two whole exome sequencing studies. During the discussion, members of the Consortium will outline future goals, including data sharing mechanisms for the broader scientific community.

INDIVIDUAL ABSTRACT:
SYSTEMS BIOLOGY VALIDATION OF CANDIDATE VARIANTS FOR BIPOLAR DISORDER

Jared Roach, M.D., Ph.D.

Institute for Systems Biology

Family genomics has been applied to simple Mendelian diseases such as Miller syndrome. The power of family genomics arises from two pillars. First: a near perfect combination of data and algorithms such as ISCA for linkage analysis. Second: analysis of all rare variants in each genome. The solidity of interpretations of whole-genome family studies is increasing due to improving allele-frequency databases (e.g., KAVIAR), improving algorithms and databases for evaluation of the functional impact of both novel and known variation, and empirical experience in detecting false-positive molecular and bioinformatic error. Bipolar disorder (BD) hypotheses must be tested on eroded pillars of family genome analysis. First, BD is common. Therefore, constraints on allele frequency cannot confidently be applied. Second, BD may be influenced by multiple independently segregating alleles, broadening the scope of linkage hypotheses. Despite these limitations, family genomics may currently be the most powerful method for genetic analysis of BD. In particular, affected individuals in multiplex families are more likely to share a causative allele than unrelated individuals. Therefore detection of allele sharing between affected members of a multiplex family will enrich for causative variants. To test this hypothesis we sequenced 200 whole genomes from 43 families. In such an analysis, all of the power of family genomics to exclude false positive genomic signals is retained. We leveraged data from 158

personal genome sequences from 34 non-BD pedigrees to eliminate false positive results at the variant, gene, and network levels of analysis. Under our hypothesis of oligogenic or even more highly multigenic complexity, analysis of a sufficient number of BD families may detect the same causative variant in different families, could detect a set of distinct causative variants in the same gene, and is likely to detect common networks driving causality. To date, our analysis has revealed gene networks including voltage-gated calcium channels ($p = 0.0002$), calmodulin-dependent protein kinase signaling components ($p = 0.004$), and GABA receptors ($p = 0.0001$). These *p*-values suggest that our analyses are sufficiently powered to detect some of the stronger signals emanating from BD genetics. Validation of these variants, genes, and networks requires cycles of new hypothesis generation, prediction, and testing. Although particular causative variants may be private to a family, affected genes should be more broadly shared. Therefore, deep sequencing of these genes should demonstrate an excess of detrimental variation in cases versus controls. We are deeply sequencing ~20 genes in thousands of cases and controls to confirm their association with BD. Analysis of additional distinct sets of families should result in similar sets of candidate genes and networks. Candidate genes from family studies should belong to similar networks as candidates from GWAS studies. Haplotypes containing rare candidate variants from family studies may be tagged by a set of common SNPs, and these haplotypes should be enriched upon retrospective analysis of GWAS studies. Integration of expression and epigenetic data with genomic data should yield a coherent regulatory network through systems biology analysis. Analyses of larger sets of families should detect fainter genetic signals. Ultimately, specific variants and genes can be tested with in vitro, induced stem cells, and model systems.

INDIVIDUAL ABSTRACT:
EXOME SEQUENCING APPROACH TO IDENTIFY RARE SUSCEPTIBILITY VARIANTS FOR FAMILIAL BIPOLAR DISORDER

Guy A. Rouleau, M.D., Ph.D., FRCP(C), O.Q.

McGill University

Bipolar disorder (BD) is a complex psychiatric condition characterized by both manic and depressive episodes. Previous studies strongly support the role of genetics in BD, with heritability estimates as high as 80%, but likely due to genetic and phenotypic heterogeneity, there has been minimal replication across studies. To address this problem we have been focusing on a well-defined sub-phenotype of BD, positive response to Lithium (Li) therapy, and shown that Li-response clusters in families. Research in BD genetics to date has consisted of linkage and genome-wide association studies, which presume that common variants in a small subset of genes are the cause for BD. However, findings from these studies only explain a fraction of the predicted BD heritability, suggesting that BD a causal role for highly penetrant rare variants in many different genes across the population. Our approach focuses on a well-defined clinical subtype of BD (Li-responsive) to minimize clinical heterogeneity, and we are using massively-parallel DNA sequencing to re-sequence the exomes of all affected individuals from 80 multi-generational family units, for a total of up to 250 individuals. To identify relevant BD susceptibility genes we are prioritizing rare variants that segregate with affected status within each family. To further explore the mechanisms by which these variants could lead to pathology we explore their expression in post-mortem brain samples and patient-specific lymphoblastoid cell lines. In each family we are prioritizing on average 12 potentially highly penetrant (e.g. protein-truncating, missense, or frameshift) or functionally relevant (e.g. 3'UTR, 5'UTR,

splicing) variants. Some of the pathways that emerged from this analysis are involved in brain development and neurogenesis, inflammation, and epigenetic regulation – all processes that have been suggested to be dysregulated in mood disorders including BD. By focusing on rare variants in a familial cohort we hope to explain a significant portion of the missing heritability in bipolar disorder, as well as to have narrowed in on the key biochemical pathways that are implicated in this complex condition.

INDIVIDUAL ABSTRACT:
IDENTIFYING LOCI AND MUTATIONS IN A BRAZILIAN BIPOLAR FAMILY WITH 111 MOOD DISORDER CASES

Gerome Breen

Institute of Psychiatry, King's College London

Very large families pose unique oportunities and analytical challenges for psychiatric research. We have identified a family with over 100 mood disorder cases from a rural village in Brazil. The pattern of inheritance is complex and included multi-generation transmission, 32 Bipolar I cases, 11 Bipolar II and 59 Depression case, 5 Parkinson's disease cases and pediatric mood disorder cases are frequent in the younger generations. Anticipation is observed in the pedigree with 5 inbreeding loops or marriage loops. 333 DNA samples were obtained from a broader pedigree of over 900 subjects. Non-parmetric linkage was carried out via MERLIN with exponsential calulation of the LOD score (--exp) and parametric with MERLIN and MCLINKAGE (which can take the entire pedigree without breaking loops). For the MERLIN analyses the pedigree was broken into sub-families and dummy individuals introduced to break loops. Simulations were carried out to validate thresholds and examine the performace of statistics. Our analysis revealed 4 linkage regions (2 Bipolar, 2 Depression) passing multiple testing corrected genome-wide significance (a LOD of 3.83). We conducted exome sequencing of bipolar cases and I will present our analysis and followup in sporadic cases of the mutations identified for one of the regions.

INDIVIDUAL ABSTRACT:
A COMBINED ANALYSIS OF DATA FROM TWO WHOLE EXOME SEQUENCING STUDIES

Pamela Sklar, M.D., Ph.D.

Icahn School of Medicine at Mount Sinai

Here we present data from two large-scale whole exome sequencing studies of bipolar disorder. The first, the BLISS study, is 497 bipolar disorder cases that were sequenced along with 470 controls at Cold Spring Harbor Laboratories. In the second study Swedish bipolar disorder patients (N=1,195) and matched Swedish controls (N=2,477) were sequenced at the Broad Institute. Previous genome-wide association studies (GWAS) focusing on common single-nucleotide polymorphisms have pointed to a complex genetic basis for bipolar disorder, in which a large number of loci are implicated in determining disease risk, although no individual variant explains even a moderate fraction of the total genetic variance. In this study, we focus on the role of rare coding variation (single point mutations, indels and structural variation), as assayed by high-depth next-generation sequencing. We will describe analysis of each dataset, as well as the combined analysis of 4,639 individuals. The analysis will aim to map specific variants, genes and/or networks related to rare alleles of moderate or large effect. We will

discuss the methodological challenges in analyzing meta-analysis of exome sequencing datasets and interpret the results in terms of emerging views on the genetic architecture of bipolar disorder and other neuropsychiatric disease.

Monday, October 21, 2013

PLENARY
8:30 AM – 11:00 AM

OVERALL ABSTRACT:
WHERE DO WE GO FROM HERE?

The field of neuropsychiatric genetics has been transformed in recent years by the identification of scores of common and rare variants that confer risk to a broad range of psychopathologies. This transformation has been enabled by advances in high-throughput genotyping and, more recently, sequencing coupled with a remarkable cultural shift toward collaborative research. Despite the tremendous progress that has occurred in a relatively short time, much work remains to be done if we are to fully characterize the genetic architecture of neuropsychiatric disorders and translate genetic discoveries into clinical practice. A substantial degree of "missing heritability" remains to be explained, and the functional characterization of established risk variants has just begun. The role of novel forms of genomic variation and regulatory mechanisms (including epigenetic variation) along with epistasis, gene-environment interaction, and pleiotropy are still relatively uncharted. Newer approaches--including transcriptomics, stem cell biology, connectomics, and systems biology--offer unprecedented opportunities for the genetic dissection of neural function and the identification of new treatment strategies. What lies ahead for psychiatric genetics? What approaches are most likely to advance the field in the coming years and where should we focus our scientific investments? In this closing plenary session, we have brought together international leaders in the fields of genomics and psychiatry to discuss the most important challenges and opportunities for the future of psychiatric genetic research.

ADDITIONAL SPEAKERS:
Mark Daly, Ph.D., Broad Institute
Insel Thomas, M.D., NIMH
Patrick Sullivan, M.D. FRANZCP, University of North Carolina at Chapel Hill
Peter Donnelly, FRS, Wellcome Trust Centre for Human Genetics

ORAL AND POSTER PRESENTATIONS
11:15 AM – 1:00 PM

CROSS-DISORDER GENETICS

INDIVIDUAL ABSTRACT:
USING BRAIN MOLECULAR QTLS TO IDENTIFY NOVEL RISK GENES SHARED BY MULTIPLE PSYCHIATRIC DISEASES

Chunyu Liu, Ph.D., Chao Chen, Judith Badner, Elliot Gershon, Ney Alliey-Rodriguez, Eric Gamazon, IOCDF-GC, TSAICG, Nancy Cox

[1]University of Illinois at Chicago

Background: Genome-wide association studies (GWASs) have detected some common variants associated with psychiatric diseases. While these variants explain a small proportion of disease heritability, their functional effects, largely unknown, may hold clues to disease etiology. Many common SNPs have been found to be associated with gene expression or DNA methylation levels in human brain by quantitative trait loci (QTL) mapping. We and others have shown that

SNPs associated with expression or DNA methylation were enriched in GWAS signals.
Methods: Using expression QTL (eQTL) and DNA methylation QTL (mQTL) mapping results from multiple postmortem brain collections, we studied QTL SNPs ($p < 0.001$) in GWAS signals ($p<0.01$) of bipolar disorder (BD), schizophrenia (SCZ), major depression (MDD), autism (ASD), attention deficit and hyperactivity disorder (ADHD), obsessive compulsive disorder (OCD) and Tourette syndrome (TS) for their abilities explaining disease heritability. We further compared lists of eSNPs in disease GWAS signals across two or three diseases to identify putatively functional SNPs that are shared across multiple diseases. ENCODE epigenomic data and mouse phenotype data, behavioral data were used to further distill most possible novel risk genes.
Results: After we showed that GWAS signals of psychiatric diseases were significantly enriched with brain eQTL and mQTL SNPs, we further found that 19-50% of psychiatric disease heritability captured by GWAS could be explained by brain eQTLs. Different diseases shared different amount of expression QTL SNPs (eSNPs) in their GWAS signals. SCZ and BD shared the most comparing to other disease combinations. Cerebellum, parietal, and temporal cortex data, gene level and exon level analyses showed consistent pattern of sharing. The pattern clearly indicated a spectrum of genetic relatedness among the seven psychiatric diseases. By examining eSNPs shared by three diseases, we found one eSNP shared by SCZ, BD and MDD; and eight eSNPs shared by SZ, BD and OCD. Fifteen genes were regulation targets of these SNPs. Most of these genes were associated with mouse behavioral phenotypes that may link to psychiatric diseases. Interestingly, type II diabetes, as a control disease, has the fewest brain eSNPs in its GWAS signals than psychiatric diseases. But it still shares one eSNP with SCZ. The shared SNP is associated with expression of CACNA1C, which is a known risk genes of both SCZ and BD. Brain eQTL and mQTL data helped to identify novel functional SNPs and their target genes for multiple diseases.
Discussion: Brain eQTL and mQTL data helped to identify novel functional SNPs and their target genes for multiple diseases. Different diseases share different amount of eSNPs that can be captured by GWAS. Multiple novel risk genes were identified as shared risk genes of psychiatric diseases, even non-psychiatric diseases.

INDIVIDUAL ABSTRACT:
COPY NUMBER VARIATIONS (CNVS) IN A NOVEL LARGE BIPOLAR DISORDER SAMPLE IN COMPARISON TO SCHIZOPHRENIA

Elaine Green, Ph.D.[1], Elliot Rees[2], James Walters[2], Jennifer Moran[3], Pamela Sklar[4], Ian Jones[5], Lisa Jones[6], Michael Owen[2], Michael O'Donovan[2], George Kirov[2], Nick Craddock[2]

[1]Plymouth University, [2]Cardiff University, [3]Broad Institute, [4]Mount Sinai, [5]Cardiff University, [6]University of Birmingham

Background: Large rare CNVs have been implicated in the aetiology of schizophrenia, however their role in bipolar disorder has been less well studied and remains unclear with some studies reporting an increased incidence of CNVs and others not. We sought to compare the incidence of rare (< 1% in general population) CNVs (>100Kb in size) in bipolar disorder with that reported in large datasets of patients affected with schizophrenia from the UK(Rees *et al* 2013).
Methods: The samples consisted of UK bipolar cases that are completely independent of those previously published by our group (Grozeva *et al* 2010). The schizophrenia cases came from 2 UK-based samples, (i) CLOZUK (individuals taking the antipsychotic clozapine) and (ii) Cardiff COGS (Cardiff Cognition in Schizophrenia). The samples were genotyped using Illumina arrays

(OmniExpress and Combo arrays), and a total of approx. 700,000 probes were analysed common to both Illumina arrays. CNVs were called with PennCNV. After quality control filtering a total of 6,882 schizophrenia samples and 2,591 bipolar disorder samples were analysed. CNV burden analysis was performed using PLINK (Purcell *et al* 2007) comparing bipolar disorder versus schizophrenia using permutation for all CNVs (duplications and deletions), and separately for deletions and duplications. The CNV burden was also examined at varying CNV sizes ranging from 100 Kb to greater than 1Mb.
Results: There were significantly fewer deletions >1Mb observed in bipolar disorder cases (p=0.0012). Deletions >1 Mb were present in 0.66% of bipolar disorder and in 1.53% of schizophrenia cases. We also noted fewer duplications in bipolar disorder cases 500 to 1Mb in size (p=0.012)(present in 5.3% of bipolar disorder and in 6.7% of schizophrenia cases).These finding were driven to some extent by deletions at known schizophrenia loci, in particular deletions >1Mb at the following loci; *NRXN1*, 3q29, 15q13.3, 17p12, 17q12 and 22q11.2. Removing the 15 known schizophrenia CNV loci from the burden analyses indicated there was no significant difference between the burden of deletions greater 1 Mb in size in bipolar disorder and schizophrenia (p=0.178).
Discussion: Our findings are consistent with our previous findings (Grozeva *et al* 2010) indicating that schizophrenia and bipolar disorder differ with respect to CNV burden in general, and in particular in the possession of large, rare deletions. References

1. Rees E, et al., (2013) Analysis of copy number variations at 15 schizophrenia-associated loci in a large independent cohort. Under review
2. Grozeva, D et al., (2010) Rare copy number variants: a point of rarity in genetic risk for bipolar disorder and schizophrenia. Arch Gen Psychiatry 67:318-27
3. Purcell S et al., (2007) PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 81:559-7

INDIVIDUAL ABSTRACT:
SHARED BURDEN OF ULTRA-RARE DISRUPTIVE MUTATIONS BETWEEN BIPOLAR DISORDER AND SCHIZOPHRENIA FURTHER IMPLICATES CALCIUM CHANNEL FUNCTION

Douglas Ruderfer[1], Eli Stahl, Ph.D.[1], Menachem Fromer, Ph.D.[1], Jennifer Moran, Ph.D.[2], International Cohort Collection for Bipolar Disorder and Sweden Schizophrenia Consortia, Christina Hultman, Ph.D.[3], Mikael Landen, Ph.D.[3], Steven McCarroll, Ph.D.[2], Patrick Sullivan, M.D.,FRANZCP[4], Pamela Sklar, M.D., Ph.D.[1], Shaun Purcell, Ph.D.[1]

[1]Division of Psychiatric Genomics in the Department of Psychiatry, Icahn School of Medicine at Mount Sinai, [2]Stanley Center for Psychiatric Research, Broad Institute, [3]Department of Medical Epidemiology, Karolinska Institutet, [4]Departments of Genetics, Psychiatry, and Epidemiology, University of North Carolina at Chapel Hill

Background: Schizophrenia (SCZ) and bipolar (BP) are common, complex diseases with a strong and partially shared genetic basis. Beyond the shared common-variant polygenic component of risk, specific loci including several calcium channel genes have been significantly associated to both disorders. However, the contribution of rare single nucleotide variations (SNV) to these disorders, as well as whether such variants will point to the same underlying biology as common variants, remains largely unknown. Here, we combine exome sequences of 2,536 SCZ cases, 1,048 BP cases and 2,546 controls of Swedish descent to assess the overlap in

contribution of rare variants to BP and SCZ.
Methods: Exome sequences for all samples were obtained using Agilent Sure Select hybrid selection and Illumina GAII and HiSeq sequencing technology. Read data were processed using the Picard/BWA/GATK pipeline resulting in SNV/Indel calls. Samples were matched for ancestry and sequence metrics including number of alternate-allele variants and proportion of variants in dbSNP. Association and enrichment analyses were performed using the Plink/Seq package.
Results: We separately matched SCZ and BP cases to the same pool of controls. We observed a significant excess of both BP and SCZ singleton disruptive mutations compared to their respectively matched control sets in voltage gated calcium channel genes (SCZ 12:1 p=0.0021, BP 7:0 p=0.0006). In particular, for both disorders we predominantly observed mutations in α_1, α_2d and b subunits and not in g subunits, consistent with associated loci identified in large GWAS of both BP and SCZ. Furthermore, preliminary results point to a significant enrichment in BP cases of singleton disruptive mutations within genes carrying only singleton disruptive mutations exclusive to SCZ cases, compared to genes carrying only control-exclusive mutations (521 mutations in 458 case-only genes, 374 mutations in 345 control-only genes, one-sided Fisher's p=0.00019) suggesting that further loci with shared rare variant burdens are likely to be discoverable in larger samples.
Discussion: Multiple studies have shown considerable overlap of common polygenic risk between BP and SCZ. Our results indicate that this overlap is also likely to exist in rare deleterious variations of relatively large effect. Additionally, our results provide further support for the role of calcium channel genes in the shared pathophysiology of these two disorders.

INDIVIDUAL ABSTRACT:
THE BRAINSTORM PROJECT; A CROSS-DISORDER APPROACH TO THE GENETICS OF COMMON NEUROLOGICAL AND PSYCHIATRIC DISEASES

Verneri Anttila[1], Stephan Ripke, M.D.[1], Alessandro Biffi, M.D.[2], Rainer Malik, Ph.D.[3], Phil Hyoun-Lee, Ph.D., M.S.[2], Jeremiah Scharf, M.D.[2], Aarno Palotie, M.D., Ph.D.[4], Jordan Smoller, M.D., Sc.D.[5], Mark Daly, Ph.D.[1], Jonathan Rosand, M.D., M.Sc.[1], Benjamin Neale, Ph.D.[1]

[1]MGH / Broad Institute, [2]MGH, [3]University of Munich, [4]Sanger Institute, [5]Harvard University

Background: Many neurological and psychiatric diseases have considerable co-morbidity with each other, and the strength of the etiological boundaries is a topic for an active debate. While it is unknown whether this co-morbidity extends to the genomic level, the recent work by the Cross-Disorder Group of the Psychiatric Genomics Consortium (CDGPGC, 2013) has suggested that pleiotropic effects are present in many psychiatric diseases. The classic example of the overlap between the moderate genetic association signals between bipolar disorder and schizophrenia (Purcell et al 2009) may suggest a way to overcome difficulties due to still insufficient sample sizes by leveraging genomic information across disorders. The objective of the study is to explore whether a cross-disorder approach to common genetic variation of common psychiatric and neurological diseases would reveal more of the underlying processes and pathways involved in the disease pathophysiology.
Methods: We are undertaking cross-phenotype analyses between summary-level data from genome-wide association studies of roughly 100,000 cases and 100,000 controls of eight neurological and psychiatric diseases (migraine, stroke, ADHD, bipolar disease, schizophrenia, major depression, autism, Tourette syndrome) from the Psychiatric Genetics Consortium, the International Headache Genetics Consortium and the METASTROKE Consortium. Analysis

methods include a Bayesian conditional analysis allowing for multiple effects for different haplotypes and phenotypes, cross-disorder bivariate heritability estimation, multi-directional polygenic prediction, tissue-of-effect analyses using ENCODE and GTEX data as well as functional interpretation approaches.
Results: Previously, gene sets such as the calcium channels and the APOE-related genes have been reported to play a role in multiple phenotypes, across neurology and psychiatry. We will assess the impact of specific gene sets identified in single disorders across multiple domains, as well as take a comprehensive approach to integrate genome-wide results.
Discussion: The results from cross-disease analyses can shed light on to our ability to prioritize follow-up studies of genetic loci that predispose to multiple neurological and psychiatric diseases, as well as the ability to highlight functional connections that transcend clinical boundaries.

INDIVIDUAL ABSTRACT:
GENOME-WIDE ASSOCIATION STUDY OF SCHIZOPHRENIA AND BIPOLAR DISORDER IN AFRICAN AMERICAN AND LATINO INDIVIDUALS

Giulio Genovese, Ph.D.[1], Robert Handsaker[2], Richard Belliveau[2], Elizabeth Bevilacqua[2], James Knowles[3], Jennifer Moran[2], Carlos Pato[3], Michele Pato[3], Steven McCarroll[4]

[1]Stanley Center / Broad Institute, [2]Broad Institute, [3]University of Southern California, [4]Harvard Medical School

Background: Large-scale studies of schizophrenia and bipolar disorder have been successfully performed in populations of European descent, leading to the discovery of several loci harboring common single-nucleotide polymorphisms and rare copy number variants playing a role in the etiology of these psychiatric diseases. Genome-wide association studies (by arrays or sequencing) have tended historically to focus on populations with simple ancestral histories and to avoid admixed populations due to concerns that population stratification could confound analysis. 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: We genotyped African American and Latino participants of the GPC with the Illumina Omni2.5 array, and imputed this dataset using the 1000 Genome Project Phase 1 reference panel. We were able to correct for population stratification using principal components demonstrating that population stratification can be dealt with even in largely admixed populations.
Results: We were also able to investigate the degree of shared genetic components in these differently ascertained cohorts and we identified, within individuals of African descent, a significant global burden towards susceptibility ("sign test") among variants confidently known to associate with disease at a genome-wide level in individuals of European descent. We replicated CNV findings at known disease-associated loci as 1q21.1, 22q11.2, and 16p11.1 and we evaluated the ability of polygenic score computed from European studies to predict risk of schizophrenia and bipolar illness in African American and Latino populations.
Discussion: Overall, our results underline the importance of expanding the study of schizophrenia and bipolar disorder to populations that trace ancestry to continents other than Europe, and confirm the presence of shared genetics factors in the etiology of disease.

INDIVIDUAL ABSTRACT:

AN EMPIRICAL BAYES STRATEGY IDENTIFIES HUNDREDS OF SPECIFIC VARIANTS ASSOCIATED WITH SCHIZOPHRENIA AND BIPOLAR DISORDER

Mark Reimers, Ph.D.[1], Kenneth Kendler, Ph.D[2]

[1]Virginia Commonwealth University, [2]VCU

Background: The risk for psychiatric disorders contributed by common genetic variants appears to be spread over a large number of variants, each with a very small effect size. Thus association studies would require enormous sample sizes to identify a majority of these variants. Furthermore association alone cannot resolve SNPs in strong LD. High-throughput DNaseI sequencing data recently generated by the ENCODE consortium revealed that, for a variety of complex traits, a large fraction of the most highly associated SNPs are located within putative genomic regulatory elements under DNaseI peaks. Careful integration of these novel genomic data with GWA studies should provide additional power to identify previously undetected variants and tease apart the effects of distinct SNPs in strong LD.
Methods: We have developed an Empirical Bayes strategy to integrate genomic conservation and DNase hypersensitivity measures with the PGC phase I and II data on schizophrenia and bipolar disorder. We estimate the Bayes Prior and the necessary conditional probabilities from the data.
Results: Using this strategy we identified over 200 hundred distinct SNPs with a high posterior probability (>0.9) of association with schizophrenia and over 20 SNPs associated with bipolar disorder. We find a large overlap between SNPs implicated in the two disorders, but that the SNPs implicated for schizophrenia are enriched in regulatory regions active during fetal development, whereas those for bipolar disorder are not.
Discussion: An Empirical Bayes strategy can incorporate a variety of sources of information in a consistent framework, making it well suited for leveraging current GWAS data sets to the many specific variants most likely to be associated with disease. Our results highlight meaningful distinctions between the genetic risk for schizophrenia and bipolar disorder.

ORAL AND POSTER PRESENTATIONS
11:15 AM – 1:00 PM

FUNCTIONAL GENOMICS

INDIVIDUAL ABSTRACT:
CELL-TYPE SPECIFIC EXPRESSION ANALYSIS TO IDENTIFY CELLULAR DISRUPTIONS FROM PSYCHIATRIC GENETIC DATA

Joseph Dougherty, Ph.D.[1], Xiaoxiao Xu[2], Arye Nehorai[2]

[1]Washington University School of Medicine, [2]Washington University

Background: The brain contains hundreds of distinct cell types, each with unique morphologies, projections, and functions. Yet, there are clear examples of neurological disruptions caused by deficiencies in just one cell type or circuit – such as motor neurons in Amyotrophic Lateral Sclerosis (ALS), striatally projecting dopaminergic neurons in Parkinson's disease, or hypocretinergic (Hcrt) neurons in narcolepsy. Clearly, distinct cell types in the nervous system contribute to different behaviors. However, the cellular disruptions that lead to the behavioral abnormalities in many disorders, including autism, are not clear. If there were a method to identify the cell types that serve as the intermediaries between a set of genetic lesions and a particular behavioral disruption, then one could identify cellular targets for treatment.

Importantly, insights into the cell types responsible for a disorder create more obvious routes to treatment, as exemplified by the diverse strategies adopted for Parkinson's disease. We have developed and validated an approach, Cell-type Specific Expression Analysis (CSEA), for identifying candidate cell populations likely to be disrupted across sets of patients with distinct genetic lesions.
Methods: In previous work (eg. *Cell* 135(4):749-762, *Journal of neuroscience* 33(7):2732-2753), we generated bacTRAP transgenic mouse lines specifically with the goal of systematically examining the expression of all genes in dozens of targeted cell types across the CNS. Here, we use this gene expression data to develop a Gene Ontologies type tool that will identify cell types from user-provided candidate gene lists by the statistical over-representation of cell-type specific and enriched genes. The tool is efficiently implemented in python on a straightforward public web interface. We validate the sensitivity and specificty of this tool with publically available human genetic data with known cellular causes such as retinopathies (n=120 genes with Mendelian inheritance patterns) as well as published port-mortem expression data from narcoleptic patients. We then apply this tool to mutlple published sources of autism data, including postmortem expression data, curated human genetics data, and recent exome sequencing studies to determine: 1) whether CSEA may identify cell types relevant to mechanisms of this disorder; 2) whether this is a single cell type or multiple cell types; 3) whether these results were reproducible across multiple studies and data types.
Results: Positive controls: CSEA correctly identifies retinopathies as diseases of rods and cones, and narcolepsy as a disease of hypocretin neurons.Sensitivity and Specificity: Permutation analyses with random subsets suggest that roughly 30/120 genes are required to properly ascribe cell types for retinopathies. Contamination of this list with up to 70% random genes from the genome does not disrupt this detection, yet rarely (<1%) results in any false positive cell type associations. Negative control gene lists for attributes unrelated to the CNS (height) do not map to CNS cell types.Autism: CSEA suggests that multiple cell types are likely to be disrupted in autism, including striatal cell types, cortical interneurons, and astrocytes, and immune cells.
Discussion: Two of three transcriptional studies suggest there is an increase in glia in the brains of individuals with autism, perhaps reflecting some kind of gliosis. It is also very interesting to note that both a transcriptomic study and the human genetics database converge on a cortical interneuron for autism. Given the divergent sources of the information, this convergence is surprising and unlikely due to chance. This supports proposals that one of the cellular mechanisms of autism may be a relative deficit in the function or presence of interneurons. However, the results are not entirely convergent across the transcriptomic studies and the human genetics analysis. Compared to human genetics, gene expression is likely to show signal derived both from the causes and the consequences of the disease. While we tested our CSEA approach with autism candidate genes, this approach could be applied to other disorders of the nervous system as well. Therefore, we have provided a web server (http://genetics.wustl.edu/jdlab/csea-tool-2/) that will permit other investigators to examine potential selective expression patterns for any candidate gene lists across cell types. We hope that the identification of candidate cell types from the genetic information will suggest novel routes to treatments in these disorders.

INDIVIDUAL ABSTRACT:
INVESTIGATING THE SYNAPTIC FUNCTION OF THE BIPOLAR DISORDER GENE ANKYRIN 3.

Melanie P. Leussis, Ph.D.[1], Omer Durak[2], Mai Saito[3], Erin Berry-Scott[4], Froylan Calderon de Alda[2], Li-Huei Tsai[2], Tracey Petryshen[3]

[1]Emmanuel College, [2]Picower Institute of Learning and Memory at Massachusetts Institute of Technology, [3]Center for Human Genetic Research at Massachusetts General Hospital, [4]The Broad Institute

Background: Genome-wide association studies (GWAS) have identified ankyrin 3 (ANK3) as one of the most significant risk genes for bipolar disorder, yet the genetic variation contributing to disease risk is unknown. Despite the fact that bipolar disorder is a highly heritable disease, the underlying neurobiology is still poorly understood. Investigating the disease-relevant function of ANK3 in brain may increase our understanding of the pathological processes implicated in bipolar disorder. Several functions of the ankyrin G protein encoded by ANK3 have been well documented, such as scaffolding of ion channels at the axon initial segment. Still other functions of ankyrin G have been less well characterized, especially in brain. There is some evidence to suggest ANK3 may have a synaptic function. For example, drosophila Ank2 (which is homologous to mammalian Ank3) plays a role in synapse stability (Koch et al, 2008, *Neuron* 58:210) while in rats, mood stabilizer treatment alters Ank3 expression in hippocampal postsynaptic fractions (Nanavati et al, 2011, *J Neurochem* 119:617). We hypothesize that ankyrin G has a synaptic function in the mammalian brain, perhaps through scaffolding critical proteins to the post-synaptic membrane, similar to its role at the axon initial segment. This study therefore sought to establish the presence of ankyrin G at the post-synaptic density and to begin to evaluate whether reductions in Ank3 expression could alter synapse-associated markers.
Methods: Based on the hypothesis that ankyrin G is involved in synaptic function, we examined whether ankyrin G could be detected by immunoblotting of post-synaptic fractions from C57BL/6 mouse forebrain. Further, we assessed the levels of synaptic-associated proteins including PSD-95 using immunohistochemical methods and assessed dendritic synaptic spine density of neurons in mice with decreased Ank3 expression by either RNA interference or conventional transgenic knockout of Ank3. Since changes in dendritic spine density are often associated with altered dendritic morphology, we also conducted Scholl analyses to evaluate differences in dendritic branching patterns following Ank3 reduction in mouse brain.
Results: We detected several isoforms of ankyrin G in postsynaptic fractions prepared from C57BL/6 mouse forebrain, indicating that ankyrin G is present at the synapse and may have several functions mediated by different isoforms. Decreased ankyrin G levels, by RNA interference or in Ank3+/- mice, were associated with altered dendritic synaptic spine density and expression of the synapse associated proteins PSD95 (postsynaptic) and synapsin (presynaptic) in the hippocampus. The changes in spine density were dependent on the location along the dendritic arbor, suggesting these changes may have different effects on neuronal activity. Further, alterations in synaptic proteins were largely normalized by chronic treatment with the mood stabilizer lithium. In addition to the changes in synaptic protein expression and spine density, we observed a decrease in mice with reduced Ank3 of dendritic branching complexity in neurons of the granule cell layer of the hippocampal dentate gyrus.
Discussion: The presence of ankyrin G at the postsynaptic density implies a synaptic role for this protein in this dynamic neuronal structure. This is further supported by the changes observed in synaptic-associated proteins in mice with reduced Ank3 expression. Whether these findings are related to bipolar disorder is yet to be determined, however reversal of the synaptic changes by chronic lithium treatment strongly supports such a potential role. Synaptic disturbances have been increasingly implicated in numerous psychiatric diseases including bipolar disorder.

Extending the known functions of ANK3 in the brain may help elucidate the neurobiological processes contributing to bipolar disorder.

INDIVIDUAL ABSTRACT:
ALTERATIONS IN TELENCEPHALIC NEURONAL FATE, NEURONAL CALCIUM SIGNALING AND NEUROTRANSMITTER RELEASE IN ISPC MODELS OF BIPOLAR DISORDER

Melvin G. McInnis, M.D., Haiming Chen, M.D., Cynthia DeLong, Ph.D., Monica Bame, Ph.D., Todd Herron, Ph.D., Omar Mabrouk, Ph.D., Robert Kennedy, Ph.D., Sue O'Shea, Ph.D.

University of Michigan

Background: A major challenge in studying complex, human neuropsychiatric disorders such as Bipolar Disorder (BPD) has been the lack of reliable cell models. Patient-derived induced pluripotent stem cells (iPSC) now offer the remarkable opportunity to study a full range of neural tissues and the exciting prospect of identifying novel disease mechanisms in BPD. We have derived fibroblast cell lines from15 patients with BPD and 6 Controls, reprogrammed them into iPSC, which have been extensively characterized.
Methods: Fibroblasts were differentiated into neurons by growth in suspension culture in medium containing nodal and BMP inhibitors, followed by plating on polyornithine/laminin-coated coverslips. With reprogramming, pluripotency factors were induced, while levels of fibroblast-restricted genes were down-regulated. With neuronal differentiation, expression of transcripts for membrane receptors and ion channels were significantly increased in BPD-derived neurons compared to controls, and expression of transcription factors involved in the specification of neuronal identity altered. To simultaneously measure action potential (Vm) and calcium wave propagation, we employed Fluo-4 AM optical mapping of BPD and C neurons after four and eight weeks of differentiation + 24h pre-treatment with 1mM lithium chloride, the most common and effective treatment for BPD. To determine what neurotransmitters the neurons released, we carried out nanoscale mass spec analysis of 21 analytes in supernatants from the calcium imaging experiments, as well as from pooled culture medium from BPD or C neurons collected over a 12 week period.
Results: Control neurons expressed transcripts that confer dorsal telencephalic fate, while neurons derived from BPD iPSC expressed genes involved in the differentiation of ventral (LGE) regions. Exposure of BPD neurons to lithium significantly decreased their calcium transient ($p < 0.007$) and wave amplitude ($p < 0.018$) following stimulation with 50 mM KCl, compared with lithium-exposed Control neurons. Levels of serotonin and the dopamine metabolite DOPAC were significantly higher in media from unstimulated BPD vs. C neurons, while both adenosine and glutamine were significantly increased in stimulated BPD neurons following lithium treatment.
Discussion: Since current evidence suggests that subtle alterations in neurodevelopmental pathways can produce consequences that only become apparent much later in life, iPSC lines provide a unique opportunity to develop models of neuroaffective disorders such as BPD, with the long term goals of identifying alterations in their differentiation and thereby novel treatment approaches.

INDIVIDUAL ABSTRACT:
DISSECTING THE CIS REGULATION OF GENE EXPRESSION IN SCHIZOPHRENIA

Panos Roussos, M.D., Ph.D.[1], The Psychiatric Genomics Consortium – Schizophrenia Group[2], Schahram Akbarian, M.D., Ph.D.[3], Menachem Fromer, Ph.D.[3], Shaun Purcell, Ph.D.[3], Eric Schadt, Ph.D.[3], Pamela Sklar, M.D., Ph.D.[3]

[1]Icahn School of Medicine at Mount Sinai School, [3]Icahn school of Medicine at Mount Sinai

Background: Schizophrenia (SZ) is a highly polygenic disorder that perturbs molecular and cellular networks underlying disease pathophysiology. The most recent Psychiatric Genomic Consortium genome-wide association study (GWAS) reported more than a hundred linkage disequilibrium independent loci as risk factors for SZ. However, because the majority of SNPs reside within non-coding regions of genes or in intergenic regions, it has been difficult to determine the causal genetic variants, and there is limited knowledge about the regulatory mechanisms by which they act. In this study, we conduct a multi-scale integration of high dimensional datasets [genomic; brain tissue expression quantitative trait loci (eQTL), *cis*-regulatory elements (CREs) annotations], combined with gene coexpression network analysis to identify putative causal SNPs and genes.

Methods: Gene coexpression network and high density eQTL analyses were conducted using transcriptome profiling in two human postmortem brain non-disease cohorts [BrainCloud[1] (*N*=269) in the dorsolateral prefrontal cortex (DLPFC) and NIH[2] (*N*=150) in the DLPFC, superior temporal gyrus, pons and cerebellum]. A variety of publicly available CRE annotations for promoters, enhancers or open chromatin (DNase hypersensitivity regions) was used[3,4]. Furthermore, we used in-house generated CRE (promoter) annotations for neuronal cells sorted from the DLPFC of controls and cases with SZ. We examined whether functional SNPs, defined as loci that affect gene expression and/or lie within CREs: (i) increase the proportion of significant PGC2 SNPs; (ii) can better predict which SNPs will become significant when GWA sample size increases; (iii) affect genes that cluster in specific gene coexpression networks, where abnormalities have been shown in SZ.

Results: PGC2 SNPs are enriched for eQTLs [average odds ratio (OR): ~3.2] and CREs [$OR_{promoter}$: ~1.5; $OR_{enhancer}$: ~1.7; $OR_{open\text{-}chromatin}$: ~1.2]. Combined analysis of eQTL and CRE annotations showed a further increase in the PGC2 SNPs enrichment [$OR_{promoter}$: ~5.3; $OR_{enhancer}$: ~5.9; $OR_{open\text{-}chromatin}$: ~4.6]. Higher enrichment of significant PGC2 SNPs was observed when neuron-specific [$OR_{neuron\text{-}specific}$: ~7.0] than homogenate tissue [$OR_{homogenate}$: ~4.6] promoter annotations were used. Functional SNP annotations more efficiently predict which non-significant SNPs (based on first PGC GWA study) will meet genome-wide significance when sample size increases (based on the larger PGC2 dataset). Using a stepwise approach, we identified a subset of *N*=151 linkage disequilibrium independent putative causal SNPs affecting the gene expression of *N*=103 genes. Overlap analysis demonstrated that putative causal genes cluster in gene coexpression networks related to neuronal function and synaptic neurotransmission.

Discussion: A major challenge in the post-GWA era is how to prioritize GWA significant loci and identify SNPs and genes that carry a higher probability for causality. In this study, we applied a stepwise approach to identify a subset of putative causal SNPs and genes and then examined their distribution in gene coexpression networks. Overall, these results support the existence of convergent genetic abnormalities in SZ that could potentially drive the disease leading to molecular and cellular alterations.Refs: 1. Colantuoni, C. *et al. Nature* **478**, 519-23 (2011). 2. Gibbs, J.R. *et al. PLoS Genet* **6**, e1000952 (2010). 3. Maurano, M.T. *et al. Science* **337**, 1190-5 (2012). 4. Zhu, J. *et al. Cell* **152**, 642-54 (2013).

INDIVIDUAL ABSTRACT:
DRUG-INDUCED PHENOTYPE CHANGES IN IPSC AND IPSC NEURAL DERIVATIVES TO IDENTIFY NOVEL THERAPEUTIC TARGETS FOR BIPOLAR DISORDER

Sevilla D. Detera Wadleigh, Ph.D.[1], Xueying Jiang, Ph.D.[2], Barbara Mallon, D.Phil[2], Liping Hou, Ph.D.[2], Nirmala Akula, Ph.D.[2], David Chen, M.D.[2], Winston Corona, M.S.[2], Layla Kassem, Ph.D.[2], Nahid Tayebi, Ph.D.[2]

[1]National Institute of Mental Health/National Institutes of Health, [2]NIH

Background: Bipolar disorder (BD) is a debilitating disease, characterized by severe episodes of mania and depression that incurs enormous cost in morbidity and lost productivity. Available treatments can ameiorate symptoms and reduce episodes, but even the most effective, lithium, does not work for everyone and is not curative. BD has a complex genetic architecture and genome-wide association studies have detected low-risk variants indicating that the majority of risk alleles remain to be discovered. Reprogramming technology enables derivation of living neuronal cells that may provide a cellular template to gain functional support for genetic findings, clarify salient aspects of the molecular mechanisms involved in BD, and potentially reveal factors amenable for drug discovery.
Methods: We used a established fibroblast cell lines from Amish BD patients and generated iPSC clones using the STEMCCA OKSM polycistronic lentiviral construct. Spectral karyotyping was performed on selected colonies. Differentiation into neural stem cells (NSC) and neurons was done following protocols described by Stem Cell Tech and the NIH Stem Cell Unit. Pluripotency was assessed using established pluripotency markers and scored using PluriTest. Markers for NSCs and neurons were employed. Expression profiling was done using the Illumina HT12 v4 microarrays.
Results: iPSC clones that displayed a normal karyotype scored positive for established pluripotency markers (Oct4, Nanog, TRA 1-60 and TRA 1-81). NSCs showed strong binding to nestin and Sox2 antibodies. Differentiation produced neurons that were positive for TuJ1 and MAP2, and a small proportion showed binding to TH antibody. Microarray profiling in neural derivatives displayed a strong perturbation of expression of four genes previously found to harbor rare nonsynonymous variants in the source individual. In addition, we found a significant overlap of >200 neutrally regulated genes with published differntiallye xpressed genes in iPSC-derived schizophrenia neurons (hypergeometric p=7E-08), including loci previously implicated in these disorders, e.g., *ODZ4*, *NRG1* and *TCF4*. We have initiated cell toxicity assays to explore protective effects of mood stabilizers, antidepressants and antipsychotics.
Discussion: To identify novel therapeutic targets in BD we are investigating phenotype changes associated with drug and other exogenous factor challenges in iPSC and iPSC neural derivatives. The effect of mood stabilizers, antidepressants and antipsychotics on these phenotypes will be exploited to develop assays for high throughput small molecule screening for lead compounds. This study is being extended to additional samples.

INDIVIDUAL ABSTRACT:
GENOME CHARACTERIZATION IN A PANEL OF INDUCED PLURIPOTENT STEM CELLS WITH THE VCFS DELETION IN 22Q11

Alexander E. Urban, Ph.D.[1], Carolin Purmann, Ph.D.[2], Sergiu Pasca, M.D.[2], Hui Gai[2], Anna Krawisz[2], Xiaowei Zhu, Ph.D.[2], Judith Rapoport, M.D.[3], Jon Bernstein, M.D., Ph.D.[2], Joachim Hallmayer, M.D.[2], Ricardo Dolmetsch, Ph.D.[2]

[1]Psychiatry and Behavioral Sciences, Genetics (secondary), Center for Genomics and Personalized Medicine, [2]Stanford University, [3]NIH

Background: The 3 Mbp heterozygous deletion on chromosome 22q11 is common and associated with multiple disease phenotypes, most notably with behavioral and neuropsychiatric abnormalities as well as with malformations of the cardiovascular system. Here we report the characterization, on the level of the genomic sequence, of a panel of iPS cell lines from probands with the typical 22q11 deletion and matched controls.
Methods: The iPSC lines are derived from fibroblast biopsies from 7 patients with the deletion and 7 matched controls. Multiple iPSC lines were created from each fibroblast sample and we characterized between one and three lines per proband with SNP arrays, for a total of 26 iPSC lines analyzed. We used the new Illumina HumanOmni5+Exome SNP array. This array probes 4.3 million markers genome wide and an additional 300,000 exonic SNPs and the resulting data can be used to detect and characterize duplications and deletions in the genomic sequence with high accuracy.
Results: For each iPSC line derived from a patient we determined the exact extent of the main deletion. We also determined for all lines the overall complement of CNVs and SNPs, in each iPSC line, including in a small subset of the lines a few additional, smaller, CNVs that would have been missed with karyotype analysis but that are large enough that they should be taken into consideration while using the iPSC lines as a model system. The iPSC lines are showing full differentiation potential along the neuronal trajectory. For a subset of the lines we used RNA-Seq analysis and detected gene expression changes in a large number of the genes within the deletion region.
Discussion: These iPSC lines will be a highly valuable resource for the analysis of this important microdeletion syndrome as well as in general for the elucidation of molecular effects of large copy number aberrations on the genomic control of cellular differentiation and functioning. Furthermore our analysis highlights that high-resolution genome analysis in iPSC based model systems should be standard practice.

ORAL AND POSTER PRESENTATIONS

11:15 AM – 1:00 PM

GENOMIC AND NEURAL BASIS OF EARLY-ONSET DISORDERS

INDIVIDUAL ABSTRACT:
A GENOME WIDE APPROACH TO CHILDHOOD AGGRESSION

Irene Pappa, M.D.[1], Marinus Van Ijzendoorn, Ph.D.[2], Henning Tiemeier, MD, Ph.D.[3], Andrew Whitehouse, Ph.D.[4], Beate St.Pourcain, Ph.D.[5], Christel Middeldorp, M.D., Ph.D.[6], Alana Cavadino, MSc[7], Joachim Heinrich, Ph.D.[8], Maciej Trzaskowski, MSc[9], Ilja Nolte, Ph.D.[10], Christian Hakulinen, MA[11]

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Background: Aggression is a key component in child psychopathology, implicated in the diagnosis of conduct disorder (CD) and oppositional defiant disorder (ODD). Childhood aggression can persist into adulthood, although diminishing by age, and there is considerable variation in both childhood and adult levels of aggression. Previous twin and adoption studies

have attributed half of the variance in aggression to genetic factors. However, there are often contradicting findings between studies based on parental reports and observational studies. The search for genetic variants associated with childhood aggression has not yielded consistently replicated associations of high significance and the biological pathways leading to aggression remain largely a mystery. In this study, we used a genome- wide approach (GWAS) to explore the continuum of childhood aggression. Furthermore, Genome wide Complex Trait Analysis (GCTA) will be used for more precise estimations of heritability, captured by common SNPs on genotyping arrays.
Methods: In the discovery phase, we combined the GWAS results of five birth cohort studies, comprising a total of 11,606 children of Caucasian descent, age 3 to 18 years. Childhood aggression was assessed by the aggression scale of the Child Behavior Checklist (CBCL), or the conduct problems scale of the Strengths and Difficulties Questionnaire (SDQ), both of which were parent-reported. We analyzed our data in two age strata, preschool and school age, based on previous literature of more prominent genetic influences with older age. We used a continuous score of childhood aggression, with higher scores indicating more aggressive symptoms. Since the distribution of aggression scores is highly skewed, a quasi-Poisson regression was performed on total aggression score, adjusted for age and gender. To our knowledge, this is the first time that such an approach has been followed at a genome-wide level. In the replication phase, five additional birth cohorts assessed childhood aggression with other instruments. A combined meta-analysis of the discovery and replication phase will be performed in a total of N= 22,142, constituting the largest healthy, population based sample of childhood aggression, analyzed to date. In addition, GCTA based on at least one cohort study (Generation R) will provide us with a DNA-driven estimate of heritability.
Results: In the discovery phase, two independent SNPs in chromosome 18 reached genome wide significance (rs7504612, p= 4.79^{-8} and rs7236288, p=5.56^{-7}). Regional plots of chromosome 18 did not reveal any known gene, in ±500bp distance of the top hits.
Discussion: In this genome- wide association effort, data from 29,474 children will be used in the total meta-analysis. We will attempt to replicate the top hits from the discovery phase and contribute to a more precise estimation of heritability of childhood aggression, by using the GCTA method. Other possibilities will be explored, such as GxE interaction in childhood aggression.

INDIVIDUAL ABSTRACT:
A GENOME-WIDE ASSOCIATION STUDY OF ANOREXIA NERVOSA: WTCCC3 AND GCAN

Cynthia M. Bulik, Ph.D.[1], Vesna Boraska, Ph.D.[2], Patrick Sullivan, M.D., FRANZCP[3], David Collier, Ph.D.[4], Eleftheria Zeggini, Ph.D.[2], Wellcome Trust Case Control Consortium 3, Genetic Consortium for Anorexia Nervosa

[1]University of North Carolina at Chapel Hill, [2]WTSI, [3]UNC Chapel Hill, [4]King's College London

Background: Anorexia nervosa (AN) is a complex and heritable eating disorder characterized by the maintenance of dangerously low body weight.
Methods: We established the Genetic Consortium for Anorexia Nervosa which is an unprecedented worldwide collaboration combining existing DNA samples of AN patients into a single resource. As part of the Wellcome Trust Case Control Consortium 3 we conducted the largest genome-wide association study of AN to date combining 2,907 AN cases, originating

from 15 different countries of European ancestry, and 14,860 ancestrally matching controls. Individual association analyses were carried out in each ancestry stratum and then meta-analysed across all 15 strata. Seventy-six SNPs were taken forward for *in silico* and *de novo* replication in another 15 datasets of European ancestry and from Japan. Global meta-analysis across discovery and replication datasets, comprising a total of 5,551 AN cases and 21,080 controls, was then performed.
Results: Suggestively associated SNPs were rs9839776 ($p=3.01x10^{-7}$) within *SOX2OT* and rs17030795 ($p=5.84x10^{-6}$) within *PPP3CA* in the main analysis and rs1523921 ($p=5.76x10^{-6}$) located between *CUL3* and *FAM124B* and rs1886797 ($p=8.05x10^{-6}$) located near *SPATA13* in Europeans only. In comparing the discovery to the replication results, 76% of the effects were in the same direction, an observation highly unlikely to be due to chance ($P = 4x10^{-6}$).
Discussion: This suggests that many true findings exist but that our sample, the largest yet reported, was underpowered for their detection at the genome-wide level and that accrual of large genotyped AN case-control samples should be an immediate priority for the field. Under the auspices of the PGC, a mega-analysis of this GWAS and previous smaller GWAS of anorexia is underway as are cross disorder analyses. A large-scale collection, the Anorexia Nervosa Genetics Initiative (ANGI) is proceeding in the US, Sweden, Australia, and Denmark.

INDIVIDUAL ABSTRACT:
BRAIN IMAGING STUDIES AND GENETIC CORRELATIONS IN A YOUTH COHORT AT GENETICALLY DEFINED HIGH RISK FOR BIPOLAR DISORDER

Philip Mitchell, M.D., Ph.D.[1], Gloria Roberts, PhD[2], Michael Breakspear, MB BS, PhD[3], Melissa Green, PhD[2], Andrew Frankland, PhD[2], Phoebe Lau, BA (Psych Hons)[2], Cassandra Joslyn, BSc (Hons)[2], Clare McCormack, BPsychol[2], Peter Schofield, DSc[4], Jan Fullerton, PhD[4]

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Background: Prospective study of young individuals at genetically defined high risk of developing bipolar disorder enables potential elucidation of both endophenotypes and predictors of risk of "conversion" to this condition. To identify such endophenotypic and predictive factors we have recruited three cohorts aged 12-30 years : i)150 unaffected first-degree relatives (mostly offspring) of subjects with DSM-IV bipolar I or II disorders (mostly bipolar I); 125 controls (no family history of bipolar disorder or psychosis); and 65 subjects with DSM-IV bipolar I or II disorder (mostly bipolar I). Recruitment is still ongoing.
Methods: Our recently published research (Roberts et al, Biological Psychiatry, e-published Dec 2012) has suggested that a cortical region implicated in bipolar disorder (the inferior frontal gyrus, IFG) is inadequately engaged during emotional inhibition in non-affected first degree relatives. Given that the inhibition of a response to an emotionally salient stimuli requires coordination between the circuits required for emotion processing and those for cognitive control, it is possible that this deficit does not arise in the IFG *per se*, but is rather a reflection of the network dynamics underlying this coordination. Here we analyze effective connectivity in first degree relatives of bipolar patients compared to a well matched control group. We also examine resting state fMRI (using network based statistic) and diffusion tensor imaging (tractography). Polygenic risk scores using the top 34 risk alleles from the PGC-Bipolar Disorder report (Sklar et al Nature Genetics 2011) were determined for at-risk and control subjects.
Results: We first compared three candidate classes of models that might underlie the coordination of emotional and control circuitry and find that a nonlinear hierarchical model provides stronger empirical support than models that embody either (bilinear) serial or parallel

processing. Crucially, the nature of this arrangement differs between the two groups: In the control group, the circuit for cognitive control "gates" activity arising from emotional processing, whereas the hierarchical order is reversed in the at-risk group such that emotional salience gates the influence of cognitive control. Resting state fMRI and tractography analyses are currently in progress. Correlations of polygenic risk scores with imaging findings (dynamic causal modelling, resting state fMRI and DTI) will be presented. (A comparison of polygenic risk scores between the at-risk and control groups has been submitted as a separate presentation by Fullerton et al).

Discussion: Whilst the notion of network dynamics underlying emotional and cognitive disturbances in psychiatric disorders finds widespread and intuitive support, this is the first empirical finding of a specific network dysfunction in those at genetic high risk of bipolar disorder. This study is consistent with our prior fMRI report in suggesting that brain imaging studies of unaffected young individuals at high genetic risk for bipolar disorder enable identification of potential endophenotypes for this condition. Ongoing prospective studies will confirm if such imaging findings predict later 'conversion' to bipolar disorder.

INDIVIDUAL ABSTRACT:

ATTENTION DEFICIT HYPERACTIVITY DISORDER AND BIPOLAR DISORDER SHARE OVERLAPPING GENETIC BACKGROUNDS

Kimm van Hulzen, Ph.D.[1], Claus-Jürgen Scholz[2], Barbara Franke[3], Stephen V. Faraone[4], Heike Weber, Ph.D.[2], Alejandro Arias Vasquez, Ph.D.[3], Andreas Reif, Professor[2]

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Background: Attention Deficit Hyperactivity Disorder (ADHD) and Bipolar Disorder (BD) both show symptoms of impulsivity, hyperactivity and irritability, with impairments in social relations, increased substance use and underachievement. However, no consistent polygenic overlap has been yet detected between the two disorders. For most patients with ADHD, the onset of disease takes place during childhood, whereas for BD the average age of onset differs between patients. We performed a genome-wide meta-analysis of association data from patients with ADHD and patients with BD with an age at onset below 21 years to provide more information about the biological underpinnings of the shared symptoms between the two disorders.

Methods: We used the results of the PGC's ADHD (N= 5840 cases and 13648 controls across nine studies) and PGC's BD genotype and phenotype data (N= 6077 cases with age at onset before 21 years and 15223 controls across fourteen studies). For the BD samples, we had to run genome-wide association analyses first, and then performed a disease-specific meta-analysis. For ADHD, a meta-analysis could be retrieved from the PGC. We combined them in a cross-disorder meta-analysis using fixed-effects modeling.

Results: We detected a genome-wide significant association for several SNPs at two loci on chromosome 10 (p-value$\leq 5\times10^{-8}$). We also detected fifteen new suggestive loci (p-value$\leq 1\times10^{-6}$) on chromosomes 1-6, 10, 11, and 14-16.

Discussion: Our results suggest that the cross-disorder meta-analysis of ADHD and BD at an early age at onset yields new information regarding a shared genetic background of the two disorders.

INDIVIDUAL ABSTRACT:

THE ROLE OF DNA METHYLATION IN DEVELOPMENT AND NEUROPSYCHIATRIC DISORDERS OF THE HUMAN BRAIN

Andrew E. Jaffe, Ph.D., Jooheon Shin, Ph.D., Yuan Gao, Ph.D., Thomas M. Hyde, M.D., Ph.D., Joel E. Kleinman, M.D., Ph.D., Daniel R. Weinberger, M.D.

Lieber Institute for Brain Development

Background: DNA methylation (DNAm) is an important epigenetic mark associated with development, tissue differentiation, and cancer. We sought to characterize the epigenetic landscape associated with brain development and identify which, if any, regions in the epigenome are associated with neuropsychiatric illness.
Methods: We measured DNAm across the lifespan in 355 human post-mortem dorsolateral prefrontal cortex (DLPFC) non-psychiatric control samples from the second trimester of fetal life to the aged using the Illumina HumanMethylation450 microarray. We then examined DNAm differences between the adult control samples (n=255 of 355) and brain samples from additional individuals diagnosed with schizophrenia (n=202). Lastly, enrichment for these two classes of differentially methylated regions (DMRs) within the single nucleotide polymorphisms (SNPs) significantly associated with clinical risk for schizophrenia from the latest freeze (May 2013) of the Psychiatric Genetics Consortium (PGC) was assessed.
Results: There are thousands of significant differentially methylated regions (DMRs) with large changes in DNAm (20+%) that occur at birth or in the third trimester of life. These DMRs were explored functionally using a combination of microarray- and RNA sequencing-based gene expression measurements. We find a striking balance between DMRs with the expected negative correlation between DNAm and gene expression, and almost half of the DMRs that have DNAm unexpectedly positively correlated with gene expression. There were also thousands of small (< 5%) but significant ($p < 1e-12$) differences in DNAm between patients with schizophrenia and controls. There was also a small but significant increase in relatively neuronal composition in samples diagnosed with schizophrenia that we estimated using novel methods in composition estimation and regression calibration. Lastly, we identified significant enrichment for developmental DMRs, but not the schizophrenia DMRs, within the significant clinical risk SNP regions.
Discussion: DNAm plays a crucial role in the developing human brain, and likely a small but significant role in schizophrenia, although we cannot disentangle its role in initiation, progression or treatment of the illness. Enrichment for schizophrenia risk variants in developmentally important regions, but not diagnosis differences, suggests that genetic risk for schizophrenia is influenced by early epigenetic regulation of brain development.

INDIVIDUAL ABSTRACT:
LONGITUDINAL STUDIES OF ADOLESCENTS AT FAMILIAL RISK FOR SCHIZOPHRENIA

Matcheri Keshavan, M.D.

Harvard Medical School

Background: Familial high risk studies offer a valuable way to elucidate the premorbid pathophysiology of schizophrenia. In this presentation, I will present data from an ongoing follow-up study of adolescent and young adult relatives(HR) of patients with schizophrenia and schizoaffective disorder.
Methods: We longitudinally followed up a series of young relatives of patients with

schizophrenia or schizoaffective disorder. Neurocognitive, psychopathological and neuroimaging assessments were carried out at baseline, and annually thereafter for up to 3 years.
Results: Our baseline assessment revealed that about 60% of HR subjects have axis I psychopathology, notably developmental disorders such as ADHD, conduct disorders and learning disorders, mood and anxiety disorders. Mild elevations are seen in prodromal symptoms, and these symptoms appear to increase over time in a subset.HR subjects show significant elevations in schizotypy, soft neurological signs and cognitive impairments similar to but less prominent than those with schizophrenia. MRI assessments reveal widespread, albeir subtle structural brain changes as well alterations in brain chemistry as assessed by Magnetic Resonance Spectroscopy. Follow-up studies suggest that about 14% of subjects develop psychotic disorders during follow-up.
Discussion: Schizotypy appears to be significant predictor of transition to psychosis. The premorbid alterations observed in this population will be compared and contrasted with those observed in other at-risk populations in psychiatry.

SYMPOSIA
2:30 PM – 4:30 PM

OVERALL ABSTRACT:
GENETICS OF SMOKING BEHAVIORS - DISCOVERY, FUNCTION, AND TRANSLATION

Laura J. Bierut, M.D., Laura J. Bierut, M.D., Eric Johnson, Ph.D.[1], Alison Goate, D.Phil.[2], Jerry A. Stitzel[3], Nancy L. Saccone, Ph.D.[2]

[1]RTI International, [2]Washington University School of Medicine, [3]Institute for Behavioral Genetics, University of Colorado, Boulder

Tobacco use, primarily through cigarette smoking, is the largest cause of preventable mortality, and each year, over 400,000 people in the United States die of a smoking related illness. Large-scale genome-wide association studies of nicotine dependence report that variants in the chromosome 15 region including the α5 nicotinic receptor gene increase a smoker's risk for nicotine dependence. The variant, rs16969968, results in an amino acid change in *CHRNA5*, the gene encoding the α5 nicotinic receptor subunit, and it is unequivocally associated with nicotine dependence and heavy smoking. Importantly, rs16969968 is also the strongest genetic risk factor for lung cancer and chronic obstructive pulmonary disease, which demonstrates the convergence of the genetic studies of nicotine dependence and smoking related diseases. Variants in other nicotinic receptor subunit genes are similarly emerging as important factors influencing nicotine dependence. This symposium will pursue the theme of Genetic Discovery, Function, and Translation in nicotine dependence and smoking cessation. Once an association is identified, we must obtain a deeper understanding of a gene's role through refining association signals, functional experiments, and animal models. Discovery studies will highlight identification of variants in nicotinic receptor subunit genes, refinement of initial signals, and extension of those findings for discovery of new variant associations with nicotine dependence. Functional studies that determine the underlying molecular and cellular pathways form an essential biological bridge between genetic associations and behavioral phenotypes. We will describe functional studies that improve our understanding of the biology underlying these genetic risks through alteration of receptor function and differential expression of nicotinic receptor subunit genes. Importantly, we have engineered a mouse model with the human amino acid change

caused by rs16969968 (a knock-in model mouse) so that we can study translational measures of behaviors in response to nicotine. These studies provide mechanistic insights into the role of this nicotinic receptor gene in smoking behaviors in humans. Finally, we will describe how variants in *CHRNA5* influence smoking cessation and the effect of cessation treatments, which translates this work to the clinic. Thus in this symposium we discuss ongoing research that completes the circuit from discovery and replication, to function and mechanism, to clinical translation. Given that over 400,000 people in the U.S. die each year from smoking related illnesses, an improved understanding of the mechanisms underlying smoking behavior and smoking cessation must be a high public health priority. Our goal is to use genetic and functional approaches to push the boundaries of our understanding of the fundamental neurobiological underpinnings of nicotine dependence. These studies require a multidisciplinary approach so that we can make faster progress towards understanding one of the most important public health problems of our time: nicotine dependence and failed smoking cessation.

INDIVIDUAL ABSTRACT:
DISCOVERING GENETIC VARIANTS UNDERLYING NICOTINE DEPENDENCE: PAST, CURRENT, AND FUTURE CONTRIBUTIONS OF GENETIC EPIDEMIOLOGY

Eric Johnson, Ph.D.

RTI International

Cigarette smoking, and its more severe manifestation - nicotine dependence, remain critically important public health problems world-wide, resulting in more than 400,000 deaths annually in the United States and more than 5 million deaths annually worldwide. Since the application of genome-wide association study (GWAS) methods to nicotine dependence and heaviness of smoking phenotypes, unequivocal genetic associations have been identified with single nucleotide polymorphisms (SNPs) in the nicotinic receptor gene cluster *CHRNA5-CHRNA3-CHRNB4* on chromosome 15q25, as well as replicable findings on chromosomes 8p11 (*CHRNA6*) and 19q13 (*CYP2A6*). Follow-up fine-mapping and functional studies have identified multiple independent and biologically plausible variants for roles in ND, notably rs16969968 having a functional effect on the α5-containing receptor and rs588765 being associated with mRNA levels in brain and lung tissues. Translation of these discoveries to clinical studies has begun to reveal gene by treatment interactions that effect cessation and may realistically form the basis for personalized treatment decisions. However, together the currently identified genetic associations account for 10% or less of the phenotypic variance. It is very likely that additional genetic contributions to cigarette smoking and nicotine dependence remain to be discovered, understood, and translated to cessation treatment. Since the initial wave of GWAS of cigarette smoking phenotypes were published, statistical imputation of ungenotyped SNPs based on the 1000 Genomes Project reference panel has shown validity for common SNPs and become widely used to substantially enhance coverage of the genome and discover novel genetic associations. Similarly, the value of cross ancestry comparisons using differential linkage disequilibrium patterns as a method for narrowing association signals to the most promising loci is increasingly evident. Lastly, the recent publications from the ENCODE group highlight the incredible utility of the developed bioinformatics to interpret and refine GWAS findings in light of functional potential. In this presentation we review core results of the first wave of GWAS for cigarette smoking phenotypes, report discovery of novel variant associations with nicotine dependence from the first study to apply these three new tools for GWAS, and look to the future role of next generation sequencing.

INDIVIDUAL ABSTRACT:
FUNCTIONAL STUDIES IMPLICATE COMMON AND RARE VARIATION IN NICOTINIC RECEPTORS IN RISK FOR NICOTINE DEPENDENCE

Alison Goate, D.Phil.

Washington University School of Medicine

GWAS for nicotine dependence and other smoking related traits have consistently observed a strong association with multiple SNPs within the *CHRNA5-CHRNA3-CHRNB4* gene cluster on chromosome 15. Follow up studies have demonstrated that this is a complex GWAS signal resulting from several independent associations. Our functional studies have demonstrated that a common missense variant (rs16869968, D398N) in *CHRNA5* likely explains at least part of this association signal. Haplotypic analyses show that further common variation in *CHRNA5* also contributes to risk for nicotine dependence and that this signal is not associated with common coding variation. We performed quantitative allele specific gene expression using brain tissue derived from African and European Americans and identified 6 highly correlated variants, located in a region of ~14 kb upstream of *CHRNA5*, that are associated with a two-fold difference in *CHRNA5* mRNA expression in frontal cortex strongly suggesting that another component of the risk for nicotine dependence in this region is explained by differences in CHRNA5 expression. To evaluate the role of rare variation in this region in risk for nicotine dependence we have performed large-scale next-generation sequencing in samples from the COGEND study and have followed this up by genetic and functional studies. Our genetic studies indicate that rare variants in CHRNB4 reduce risk for nicotine dependence and are associated with lower mean cigarette consumption values. Cell surface ELISAs demonstrate that many of these variants are associated with lower cell surface expression of functional receptors. Furthermore, electrophysiological experiments show that some of these variants also impact the EC50 for nicotine but do not affect the EC50 for acetylcholine. Together these results demonstrate that both common and rare variation in nicotinic receptors can influence risk for nicotine dependence.

INDIVIDUAL ABSTRACT:
THE CHRNA5 D398N VARIANT AND NICOTINE DEPENDENCE: IN VITRO AND IN VIVO FUNCTIONAL STUDIES

Jerry A. Stitzel

Institute for Behavioral Genetics, University of Colorado, Boulder

The CHRNA5 D398N polymorphism is a non-synonymous polymorphism in the nicotinic receptor alpha5 subunit that repeatedly has been associated with various measures of smoking behavior including nicotine dependence, heaviness of smoking, and smoking relapse. These associations are compelling and strongly suggest a role for CHRNA5 in smoking behaviors. However, validating the role of this genetic variant in smoking related behaviors beyond a statistical genetic association and ascertaining the behavioral, cellular and molecular consequences of this genetic change is essential for improving our understanding of the relationship between genetic variation, neurobiology and nicotine dependence.

It is the goal of the research in our laboratory to utilize both in vitro and in vivo tools to understand the mechanisms through which the Chrna5 D398N variant alters risk for nicotine dependence. Towards this goal, we have utilized in vitro assays to demonstrate that the

polymorphism affects the function of the major alpha5 containing nicotinic receptor in brain, alpha4beta2alpha5, and the major alpha5 containing nicotinic receptor present in the peripheral nervous system, alpha3beta4alpha5. To explore the effect of the Chrna5 D398N variant in vivo, we have engineered a mouse that possesses the risk variant. We currently are evaluating the effect of the polymorphism on receptor function, behavior and physiology in this mouse model. Initial studies with the D398N mice demonstrated that animals possessing the "risk" variant for nicotine dependence consumed significantly more nicotine by choice than did littermates with the non-risk allele. Across the nicotine concentration range of 100-400 micrograms/ml, the risk variant mice consumed twice as much nicotine as did their littermate controls. This finding confirms that the risk variant contributes to individual differences in nicotine intake and validates this mouse model as a valuable tool for dissecting the behavioral and molecular mechanisms through which the genetic variant alters nicotine intake in humans.

Other studies with these mice have shown that the Chrna5 D398N variant affects brain neurochemistry in the ventral tegmental area and nucleus accumbens under baseline conditions and during nicotine withdrawal. The observation that the risk variant of CHRNA5 alters brain neurochemistry in the reward pathway under baseline conditions and during withdrawal suggests that the CHRNA5 D398N polymorphism may impact the risk for nicotine dependence on two levels. The altered baseline neurochemistry may predispose individuals to increased risk for developing nicotine dependence if they initiate smoking while the altered neurochemistry during withdrawal may exacerbate withdrawal symptoms and contribute to relapse. These studies as well as ongoing studies on brain function and behavior related to the CHRNA5 D398N variant will be discussed in the context of the human studies.

INDIVIDUAL ABSTRACT:
TRANSLATING GENETIC FINDINGS FOR NICOTINE DEPENDENCE TO CESSATION, CESSATION TREATMENT, AND PREVENTION

Nancy L. Saccone, Ph.D.

Washington University School of Medicine

Large-scale genetic association studies have demonstrated that multiple genes and gene clusters harbor variants associated with risk of nicotine dependence and heavy smoking. Genes identified with genome-wide significant evidence include nicotinic receptor subunit genes (*CHRNA5-CHRNA3-CHRNB4*, *CHRNB3-CHRNA6*) and nicotine metabolism genes (*CYP2A6*). It is now important to understand how these genes affect the clinically important phenotype of smoking cessation. Also, interactions with environmental factors are important to characterize as they may provide additional important routes by which individuals can ameliorate genetic risks for nicotine dependence. Nicotine dependence predicts smoking cessation, but until recently it has been unclear whether genes involved in nicotine dependence risk also exert detectable effects on smoking cessation success. We will present evidence that multiple genetic variants influence smoking cessation, especially through interactions with pharmacologic treatments. For example, *CHRNA5* risk variants delay smoking cessation in population samples. Even more striking results are seen in smoking cessation treatment trials. When pharmacologically treated, genetically high-risk smokers more than doubled their rate of abstinence compared to those who receive no active medication; in contrast, in smokers with low risk genetic variants, treatment did not alter the rate of abstinence. These findings are an important step towards personalized medicine where genetic information can differentiate between patients who are likely to respond

strongly to pharmacologic treatment and those who receive no benefit. Genetic risk variants also interact with modifiable factors such as age of onset and peer smoking to alter risk for nicotine dependence. These findings for nicotine dependence and cessation are relevant for psychiatric genetics research, given the high prevalence of smoking amongst psychiatric patients. Also, as demonstrated by these smoking genetic findings, treatment-by-genotype and environment-by-genotype effects will be important to examine for other psychiatric diseases for which genetic risk variants have been identified.

SYMPOSIA
2:30 PM – 4:30 PM

OVERALL ABSTRACT:
THE PSYCHIATRIC GENOMICS CONSORTIUM: MARKED PROGRESS VIA COLLABORATION

Patrick F. Sullivan, M.D., FRANZCP[1], Michael O'Donovan, FRCPsych, Ph.D.[2], Shaun M. Purcell[3], Peter M. Visscher[4], Benjamin Neale, Ph.D.[5], Mark Daly, Ph.D.[6]

[1]University of North Carolina, [2]Medical Research Council Centre for Neuropsychiatric Genetics and Genomics, Cardiff University, [3]Mount Sinai School of Medicine, [4]University of Queensland, [5]ATGU, Massachusetts General Hospital, [6]Massachusetts General Hospital

Psychiatric disorders are compelling targets for genomic studies owing to their high public health impact and strong evidence for a genetic component. As with other areas of human genetics, historical progress has been hampered due to inadequate sample sizes and suboptimal evaluation of genetic variation across the allelic spectrum. The Psychiatric Genomics Consortium (PGC) has been conducting large-scale and integrated mega-analysis to attempt to understand the genetic architecture of schizophrenia, bipolar disorder, autism, ADHD, and major depressive disorder. Approximately 125,000 cases and controls are now included. This work has revealed a considerable body of new knowledge about the genetics of these disorders. For example, a new and unpublished schizophrenia GWAS of 31K cases and 38K controls identified 78 regions meeting genome-wide significance (almost all are novel). Multiple loci are directly involved in calcium neurobiology including genes that encode multiple components of calcium channels. Multiple large studies of exonic variation have also been conducted, along with integrated analysis of copy number variation. The purpose of this symposium is to describe the empirical findings about variation across the allelic spectrum (common, rare exonic, and rare copy number variation), to consider what this tells us about the genetic architectures of these disorders, and to anticipate the "post-GWAS" implications for researchers and clinicians.

INDIVIDUAL ABSTRACT:
COMMON GENETIC VARIATION IN PSYCHIATRIC DISORDERS

Michael O'Donovan, FRCPsych, Ph.D.

Medical Research Council Centre for Neuropsychiatric Genetics and Genomics, Cardiff University

This talk will describe what we now know about the role of common variation based on new data. Large-scale analyses have provided considerable insight. For example, a recent and as yet unpublished analysis of over 31,000 schizophrenia cases and 38,000 controls identified 78 genomic loci that met genome-wide significance, and far larger GWAS mega-analyses for bipolar disorder and autism will soon be completed. The schizophrenia loci have definite

translational significance including: multiple components of neuronal calcium channels that physically interact (*CACNA1C*, *CACNB2*) and other genes central to calcium signaling in neurons (*CACNA1I*, *CMMT*); the dopamine type 2 receptor (*DRD2*, the site of action for most approved antipsychotics); and *KCTD13* which may be the gene important to brain phenotypes in the rare 16p11.2 CNV that confers risk for schizophrenia and autism. This talk will cover new and unpublished findings for schizophrenia, bipolar disorder, autism, ADHD, and major depressive disorder. Findings that implicate biological hypotheses and that confer risk irrespective of disorders will be highlighted.

INDIVIDUAL ABSTRACT:
RARE VARIATION IN PSYCHIATRIC DISORDERS

Shaun M. Purcell

Mount Sinai School of Medicine

This talk will describe what we now know about the role of rare variation based on new data. Uncommon, rare, private, or *de novo* genetic variation is known to confer risk for schizophrenia or autism. This class of variation includes copy number variation and deleterious exonic mutations revealed by exome sequencing or exonic genotyping arrays. Such variation is of intense interest in human genetics as they may have more actionable implications for understanding the genetics and neurobiology of these disorders as well as for clinical utility. To date, exome sequencing of approximately 600 trios for both autism and schizophrenia have been conducted, along with 6,000 unrelated cases plus controls. These efforts are augmented by genotyping of ~250K uncommon exonic variants in over 11,000 schizophrenia cases plus controls and 5,000 autism cases plus controls. (All of these studies are now being analyzed and written up.) The purpose of this talk is to summarize the results of these studies with a particular focus on the genes and pathways implicated by greater burden of deleterious exonic variation.

INDIVIDUAL ABSTRACT:
INTEGRATION: THE GENETIC ARCHITECTURES OF PSYCHIATRIC DISORDERS

Peter M. Visscher

University of Queensland

This talk uses the conclusions from the first two presentations to answer several very old questions. The base nature of psychiatric illness has been debated for more than a century. This debate has flourished in the absence of data that directly and comprehensively address the key issues. With the results from the two talks above, we can now empirically address fundamental questions about genetic architecture with increasing confidence: for each disorder, how many loci are there? What are their frequencies and effect sizes? Taken together, how much of the heritability can be accounted for? What is the evidence for gene-gene interaction? How do the different disorders compare? To what extent do the genetic contributions to one disorder overlap with others? Using a new set of statistical tools, we have derived rigorous and well-grounded estimates that provide initial answers to these questions. In brief, we find that all of these disorders are highly polygenic and that most of the heritability can be explained by common genetic variation. The role of rare variation is probably lesser, but coverage is not yet optimal. We also find substantial overlap between disorders that suggest fundamental similarities between disorders (particularly schizophrenia-bipolar disorder and bipolar disorder-major depression).

INDIVIDUAL ABSTRACT:

THE GENOMICS OF AUTISM SPECTRUM DISORDERS

Benjamin Neale, Ph.D.

ATGU, Massachusetts General Hospital

The dominant paradigm in autism genomics has consisted of searched for rare variants of strong effect. Indeed, there has been considerable success using these strategies with the identification of rare copy number variants of small effect and several exonic variants. A number of critical questions remain incompletely understood - what fraction of ASD cases have a presumptively causal rare variant of strong effect? What, if any, is the role of common variation? This talk will review these issues, and highlight areas for which more research is urgently needed.

SYMPOSIA
2:30 PM – 4:30 PM

OVERALL ABSTRACT:
TRAUMA AND STRESS: EPIGENETIC PATHWAYS

Melanie Carless, Ph.D.[1], Jimmy Potash, M.D.[2], Kerry Ressler, M.D., Ph.D.[3], Douglas E. Williamson, Ph.D.[4], Elisabeth Binder, M.D., Ph.D.[5], Elisabeth Binder, M.D., Ph.D.[5] Akira Sawa[6]

[1]Texas Biomedical Research Institute, [2]University of Iowa, [3]Emory University, [4]University of Texas Health Science Center at San Antonio, [5]Max-Planck Institute of Psychiatry, [6]John Hopkins Schizophrenia Center

Exposure to some form of trauma or stress during the lifetime is almost inevitable, but in more extreme circumstances it can lead to psychological conditions such as anxiety, depression, post-traumatic stress disorder (PTSD) and substance abuse. The development of such disorders is not fully understood but in order to elucidate the pathology of trauma and stress, it will be necessary to study genetic, epigenetic, environmental and endophenotypic data. This symposium proposal will focus largely on the epigenetic factors contributing to stress and trauma, with an emphasis on PTSD, adolescent stressors and fear. Dr. Kerry Ressler will begin by examining the intergenerational transmission of learned olfactory fear, modulated by methylation-specific imprinted DNA marks. Dr. Ressler will present bisulfite sequencing findings showing that parental olfactory experience before conception can be inherited via changes in parental gametes. Dr. Akira Sawa will continue by discussing disturbances in epigenetic control on stress response and dopaminergic neurotransmission in human and animal models, at both the DNA and protein level. Dr. Sawa will present evidence showing that disturbances in epigenetic control on a specific projection of dopamine neurons underlie manifestations relevant to psychiatric depression proceeding genetic and environmental risk factors in adolescence. Dr Elisabeth Binder will present data on gene x environment interactions and their contribution to risk and resilience for stress related psychiatric disorders, focusing on DNA methylation and gene expression changes moderated by exposure to child abuse. Dr. Binder will present data demonstrating that early trauma x SNP interactions on gene expression could be modulated by changes in DNA methylation and that such data may be used to predict adult psychiatric disorders, including PTSD and depression. Finally, Dr. Doug Williamson will present data on genome-wide DNA methylation changes in post-mortem brain tissue from the posterior cingulate cortex and medial orbitfrontal cortex of individuals suffering from PTSD. Dr. Williamson will compare data from MBD-seq, microarray and pyrosequencing platforms for up to 28 PTSD cases and matched controls. Overall, this symposium will provide an overview of the epigenetic changes modulating fear, stress response and subsequent psychological outcomes.

INDIVIDUAL ABSTRACT:
INTERGENERATIONAL TRANSMISSION OF LEARNED OLFACTORY FEAR: EPIGENETIC, STRUCTURAL, AND BEHAVIORAL EVIDENCE

Kerry Ressler, M.D., Ph.D.

Emory University

In some instances, adult experience can result in information transfer from parents to generations of offspring. When and how such information transfer occurs is an important neurobiological question, particularly in mammals. Here we find that olfactory fear conditioning in adult mice causes subsequently conceived generations to display sensitivity to the paternally-conditioned odor. Odorant-receptor-specific neuroanatomical changes in the olfactory system of the F1 and F2 generations accompany this behavioral sensitivity. Epigenetic analyses in the olfactory epithelium indicate that these neuroanatomical changes may result from increased transcription of the specific receptor gene that detects the conditioned odor. Studies involving bisulfite sequencing of sperm DNA demonstrate methylation-specific imprinted DNA marks following olfactory conditioning. Furthermore, *in vitro fertilization* (IVF), analysis of the F2 generation, and cross-fostering experiments all suggest that the transgenerational effects can be inherited via changes in parental gametes. We conclude that parental olfactory experience before conception can be inherited at the level of structure and function in the nervous system in subsequent generations.

INDIVIDUAL ABSTRACT:
DISTURBANCE OF EPIGENETIC CONTROL ON STRESS RESPONSE AND DOPAMINERGIC NEUROTRANSMISSION IN MENTAL ILLNESS

Akira Sawa

John Hopkins Schizophrenia Center

Background Environmental stressors during childhood and adolescence influence postnatal brain maturation and human behavioral patterns in adulthood. Accordingly, excess stressors result in adult-onset neuropsychiatric disorders. Epigenetic modification in response to stressors is expected to be a promising molecular mechanism that accounts for the process. Methods To test this idea, we used olfactory neuronal cells derived from nasal biopsy to study epigenetic modifications in patients with major mental illness. According to the published protocol from our lab (Kano et al, Mol Psychiatry 2012; Tajinda et al, Mol Psychiatry 2010), we have obtained cells for the assay. In parallel, we built an animal model in which isolation stress is added to a genetic model in adolescence (5-8 weeks of age) to study how interaction of gene-environmental factors may result in a long-term behavioral changes possibly via epigenetic modifications. Results In human cell models (olfactory neuronal cells) obtained from patients with chronic schizophrenia, we observed changes of epigenetic hallmark on genes associated with response to oxidative stress. Meanwhile, in an animal model that is built in combination of genetic and environmental risk factors in adolescence, we obtained evidence that disturbance in epigenetic control on a specific projection of dopamine neurons (mesocortical projection) underlie manifestations that may be relevant to psychotic depression. Discussion These results suggest significant roles of epigenetic modifications associated with various stressors in major mental illnesses. In this presentation, by comparing data from the human and animal models, we will provide a further working hypothesis on more in-depth mechanisms. References (1) Niwa, M., Jaaro-Peled, H., Tankou, S., Seshadri, S., Hikida, T., Matsumoto, Y., Cascella, N., Kano, S.,

Ozaki, N., Nabeshima, T., Sawa, A. (2013). Adolescent stress-induced epigenetic control of dopaminergic neurons via glucocorticoids. Science, 339, 335-339. (2) Kano, S., Colantuoni, C., Han, F., Zhou, Z., Yuan, Q., Wilson, A., Takayanagi, Y., Lee, Y., Rapoport, J., Eaton, W., Cascella, N., Ji, H., Goldman, D., and Sawa, A. (2012). Genome-wide profiling of multiple histone methylations in olfactory cells: further implications for cellular susceptibility to oxidative stress in schizophrenia. Mol Psychiatry AOP. (3) Tajinda K, Ishizuka K, Colantuoni C, Morita M, Winicki J, Le C, Lin S, Schretlen D, Sawa A, Cascella NG. (2010). Neuronal biomarkers from patients with mental illnesses: a novel method through nasal biopsy combined with laser-captured microdissection. Mol Psychiatry, 15, 231-2.

INDIVIDUAL ABSTRACT:
IDENTIFYING GENETICS VARIANTS FOR GENE X ENVIROMENT INTERACTIONS USING EARLY TRAUMA MODERATED METHYLATION AND EXPRESSION QUANTITATIVE TRAIT LOCUS ANALYSIS.

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Gene x environment (G x E) interactions are likely important contributors to shape risk and resilience for stress-related psychiatric disorders and these might be mediated by allele-specific epigenetic changes. In fact, we used data from a cohort of 344 adult individuals with genome-wide gene expression and DNA methylation data in peripheral blood and SNP genotype data to identify expression and methylation quantitative trait loci (eQTLs and mQTLs) that were moderated by exposure to child abuse. We identified 598 cis eQTLs (580 eSNPs and 445 unique transcripts) showing significant SNP x early trauma interaction on gene expression after Bonferroni correction. Of these eSNPs, 93% were also associated with mQTLs in the same cis window, suggesting that the early trauma x SNP interaction on gene expression could be mediated by changes in DNA methylation. In an independent sample of 2041 individuals, we could show that 43% of these eSNPs (262 of 580) also showed an interaction with exposure to early trauma to predict adult psychatric disorders, including depression, PTSD, suicide attempts and substance abuse. In addition, up to 40% of the transcpripts within these moderated eQTLs in peripheral blood were also regulated by glucocorticoid receptor agonists in mouse brain, with the maximal overlap in the prefrontal cortex. These results suggest that trauma moderated eQTLs and mQTLs in peripheral blood could be used to identify genetic variants that moderate the effects of child abuse on adult psychiatric phenotypes and inform on new pathways relevant for the pathophysiology of these disorders.

INDIVIDUAL ABSTRACT:
PATTERNS OF DNA METHYLATION AND GENE EXPRESSION IN PTSD: FINDINGS FROM POST-MORTEM TISSUE, PRE-CLINICAL, AND CLINICAL SAMPLES

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Post-traumatic stress disorder (PTSD) is a chronic and disabling anxiety disorder. Recent research has identified several genetic loci and alterations in their expression associated with PTSD. However, little is known about the role DNA methylation plays in the development of PTSD after exposure to a traumatic event. As part of our larger effort to examine genomic

predictors of PTSD, we chose as a starting point the interrogation of post-mortem tissue in PTSD cases/controls and selected two brain areas; the posterior cingulate cortex (pCC) and the medial orbital frontal cortex (mOFC). Whole genome DNA methylation patterns of PTSD and control post-mortem tissue were surveyed using methyl-CpG binding domain-based capture sequencing (MBDCap-Seq). MBDCap-seq libraries were sequenced using the Illumina HiSeq2000. A total of 25 genes were differentially methylated in the pCC, 4 hypermethylated in PTSD cases. Conversely, 355 genes were differentially methylated in the mOFC, 344 hypermethylated in PTSD cases. In the mOFC, the glutamate receptor, ionotropic, AMPA 2 (GRIA2) and the lysine (K)-specific demethylase 6B (KDM6B) were identified as two of the top genes hypermethylated in PTSD cases (fold changes of 3.39 and 4.53 respectively). In a subsequent study, we ran the Illumina Human Methylation450 array in DNA samples from the mOFC in 8 PTSD cases and 8 controls and identified additional genes and CpG sites uniquely methylated in PTSD. Interestingly, we observed two novel genes the arachidonate 5-lipoxygenase (ALOX5) and the ataxin 7-like 1 (ATXN7L1) genes that were hypo-methylated and accompanied by increased mRNA expression in PTSD. Studies are currently underway by our group using pre-clinical models to examine the specific effect of stress, shown to result in fear conditioning, on changes in DNA methylation in the PCC and mOFC. In addition, we will present data on whole genome microarray data describing DNA methylation patterns in peripheral blood of Soldiers with PTSD (n=90) and controls (n-90). This is one of the first reports using NextGen sequencing and microarray strategies to identify DNA methylation and expression patterns associated with PTSD in post-mortem tissue. The relevance of these findings as they inform our ongoing research in combat-related PTSD as they are associated with the presence of PTSD, change during the course of treatment, and are altered due to combat-related stress will be discussed.