2022 MPA Poster Abstracts

1. Morphometric Similarity Network Alterations in Youth at Clinical High Risk for Psychosis

Gil Hoftman, Semel Institute for Neuroscience and Human Behavior at UCLA

Background: Given increasing recognition that psychosis onset is a late stage of a neurodevelopmental illness, and the importance of early intervention, efforts are focused on understanding the pathophysiology of early stages of psychosis. Here we characterize morphometric similarity networks (MSNs) using structural brain imaging measures in subjects at clinical high-risk (CHR) who converted (CHRc) or did not convert (CHRnc) to psychosis relative to healthy comparison (HC) subjects. We incorporate postmortem transcriptomic data from the Allen Human Brain Atlas (AHBA) to relate transcriptomic and MSN cortical spatial patterns.

Methods: MRI data were available for 757 subjects from the NAPLS2 cohort, including 71 CHRc, 467 CHRnc, and 219 HC subjects. MSNs were constructed from 7 structural MRI measures including volume, cortical thickness, surface area, and 4 curvature indices. Microarray data from the AHBA was related to MSNs using partial least squares (PLS) regression.

Results: Difference maps between CHRc-HC and CHRnc-HC both showed significant increases in MSNs in cingulate (FDR $p<0.04$) and decreases in posterior parietal areas (FDR $p<0.01$), but no significant differences between CHRnc-CHRc. PLS1 explained 33.6% of the variance between MSN and transcriptomic spatial maps in CHRc-HC. Key markers of GABA neurotransmission including parvalbumin and the GABAA receptor subunits alpha 1 and delta had loadings in the top 5%. Gene ontology (GO) pathway analyses included the terms metal ion transporter activity, cation channel complex, and main axon.

Discussion: These findings suggest that MSN topology differences between CHRc and HC subjects associate with markers of GABA neurotransmission and synaptic transmission-related GO pathways.

2. 12 H Rhythm Abnormalities in the Dorsolateral Prefrontal Cortex of Subjects With Schizophrenia

Madeline Scott, University of Pittsburgh

Introduction: Twelve-hour (12h) rhythms are a longstanding phenomenon observed in coastal marine organisms. While 12h cycles are indicated in certain human behavior, no study to date has characterized 12h rhythms in the human brain or in psychiatric illnesses. Previously we demonstrated circadian reprogramming in schizophrenia (SZ) dorsolateral prefrontal cortex (DLPFC). Specifically, mitochondria transcripts have 24 h rhythmic expression in SZ not observed in non-psychiatric subjects (NP). 12h rhythms are enriched for mitochondria-associated transcripts across studies, suggesting they may have a role in this temporal abnormality.

Methods: We utilize RNA-sequencing data from the DLPFC in both NP (n = 104) subjects and subjects with SZ (n = 46) and use a "time-of-death" rhythmicity analysis to determine 12 h rhythmicity of each transcript.

Results: 12h rhythms were enriched for mitochondrial function and protein translation genes that peak in expression at sleep/wake transitions (~9 AM/PM). In contrast, these pathways shift in timing to static periods (~3 PM/AM) in tissue from subjects with SZ. SZ subjects also lose 12h rhythms in genes associated with the unfolded protein response (UPR) and neuronal structural maintenance.

Conclusion: We observe 12h rhythms in the human brain in pathways essential for cellular function. Subjects with SZ have fewer 12h rhythms, likely due to lost UPR gene rhythmicity, a pathway implicated in 12h rhythm regulation. Mitochondria and translation pathways have altered timing in SZ, suggesting temporal differences in energy availability in the DLPFC, a brain region associated with the cognitive symptoms in SZ.

3. Striatal Thalamic Resting-State Network Dysconnectivity in Youth With 22q11.2 Deletion Syndrome

Charles Schleifer, David Geffen School of Medicine at UCLA

Background: 22q11.2 Deletion Syndrome (22q11DS) is a recurrent copy number variant (CNV) that results in greatly increased risk for developmental neuropsychiatric conditions. Studying individuals with 22q11DS can provide a powerful framework for linking genes to brain phenotypes and behaviors relevant to these conditions. The cortex, striatum, and thalamus are anatomically and functionally organized into a set of distributed circuits that support diverse brain functions. Dysconnectivity within these cortico-striatal-thalamic loops has been consistently implicated in idiopathic neuropsychiatric disorders, and in 22q11DS. Resting-state functional MRI has revealed cortical-thalamic and cortico-striatal disruptions in 22q11DS, but no study to date has explicitly investigated the functional

connectivity between sub-regions of the striatum and thalamus in relation to large-scale functional networks in 22q11DS.
Methods: We used an atlas-based approach to compute resting-state functional connectivity between a set of functionally-defined striatal and thalamic sub-regions in a sample of youth with 22q11DS (n=43, 58.1% female) and demographically matched healthy control subjects (n=40, 50% female).

Results: We report a novel finding of decreased functional connectivity between the components of the striatum related to the dorsal attention network, and a set of thalamic regions related to the auditory, default, and frontoparietal networks.

Conclusion: This pattern broadly aligns with findings in the 22q11 mouse model, and implicates dysfunction of circuits involved in auditory and executive function which are disrupted in 22q11DS and related conditions like Autism Spectrum Disorder and schizophrenia.

4. Schizophrenia and Cognitive Symptoms in 22q11.2 Deletion Syndrome
Paul Stümpges, Karolinska Institute

Background: 22q11.2 deletion syndrome (22q11.2DS) is the most commonly diagnosed microdeletion disorder in humans. Among the various symptoms, people with 22q11.2DS have markedly increased rates of schizophrenia (OR 67.7) and other neuropsychiatric diseases, as well as cognitive deficits. The prefrontal cortex (PFC) is crucial for cognitive function.
Methods: To investigate how this deletion affects the molecular composition of cells in the PFC, we studied a transgenic mouse that models the human 22q11.2 microdeletion (hemizygous for the murine homologous locus Df1). We performed single cell RNA sequencing using the 10X Genomics platform. Our study has a factorial design which includes pre- and post-adolescent mutant mice of both sexes and their littermate controls.

Results: We obtained single cell transcriptomes of ~90,000 cells from the PFC of 16 mice and identified all major classes of brain cell types. We will present an analysis of cell type abundance changes along with a differential gene expression analysis reflecting quantitative changes in gene expression on a cell type-specific level.

Discussion: Our approach allows us to detect and profile cell type-specific changes in the PFC that occur as a consequence of 22q11.2DS. By sampling mice of different ages, we will gain insight into the temporal course of these changes. In doing so, we hope to identify mechanisms that may play a crucial role in the development of the cognitive symptoms in schizophrenia and 22q11.2DS.

5. Alterations in the Expression of CircRNAs Derived From Schizophrenia GWAS Loci in iPSC-Derived Neuronal Cultures of Patients With Early Onset Schizophrenia
William Wylie, University of New Mexico Health Science Center

Circular RNAs (circRNAs) are a novel category of non-coding RNAs derived from the back-splicing and covalent joining of exons or introns. Recent studies have suggested that circRNAs are preferentially generated from synaptic plasticity-related genes and are particularly enriched in the brain. Although some circRNAs have been found to sequester microRNAs (miRNAs) and others to associate with RNA-binding proteins (RBPs), the mechanism of action of the majority of circRNAs remains poorly understood. Moreover, their relevance for psychiatric disorders still remains poorly understood. Using circRNA profiling in a large cohort of induced pluripotent stem cell (iPSC)-derived neuronal progenitors and differentiated neuronal cultures from childhood-onset schizophrenia (SCZ) and unaffected controls, we uncovered a significant enrichment for differentially-altered circRNAs generated from SCZ-associated GWAS genes. Furthermore, we uncovered a significant trend for upregulated expression for the differentially expressed GWAS-associated circRNAs in differentiated neurons but not neuronal progenitors of patients with childhood-onset SCZ. Validation using circRNA-specific qRT-PCR verified the altered developmental stage-specific expression of a subset of GWAS-associated circRNAs. Ongoing studies are focused on measuring the expression of such SCZ-altered circRNAs in postmortem brains from patients with SCZ and on examining the interactions between such circRNAs with miRNA and mRNA expression. Taken together our data present the first evidence of altered GWAS-associated circRNA expression in stem cell-derived neuronal cultures from patients with SCZ.

6. Electrophysiological Measures From Human IPSC-Derived Neurons are Associated With Schizophrenia Clinical Status and Predict Individual Cognitive Performance
Brady Maher, Lieber Institute for Brain Development / JHMI

Background: Here, we describe results of our investigation into the relationships between the electrophysiological properties of hiPSC-derived cortical neurons and the individual's clinical status, severity of symptoms, and cognitive performance.
Methods: We studied iPSCs from 13 SCZ patients with high polygenic risk scores (PRS) and 15 neurotypical individuals with low PRS. To identify phenotypes differing between SCZ cases and controls, a battery of assays were performed on cortical neurons in a double-blind manner. We correlated electrophysiology measures to the individual's rating on the Positive and Negative Syndrome Scale (PANSS), six cognitive domains (Verbal Memory, Nback, Visual Memory, Processing Speed, Card Sorting, and Digit Span), estimated IQ, and general cognitive ability composite.

Results: Five electrophysiological measures related to Na+ channel function were associated with diagnosis. Lines derived from SCZ donors showed an increase in membrane resistance, an increased number of Na+ current peaks, a decrease in the activation threshold of the second Na+ peak, and a significantly hyperpolarized shift in the half maximum voltage for activation and inactivation. In SCZ, the number of Na+ peaks showed directionally-consistent, positive associations with some positive symptom ratings. In striking contrast, sEPSC amplitude showed no correlation with positive symptoms, but showed a negative correlation with performance on the Wisconsin Card Sort Test.

Discussion: Our results demonstrate for the first time that common genetic variants associated with SCZ converge on electrophysiological mechanisms that relate to clinically relevant features of SCZ, and therefore underscore the potential of this approach for biomarker identification and perhaps downstream drug development.

7. Modulation of Arc to Regulate Synaptic Biology in Schizophrenia

Stephanie Santarriaga, Center for Genomic Medicine, Massachusetts General Hospital and Harvard Medical School

Background: Aberrant synaptic biology has been implicated in cognitive deficits in numerous neuropsychiatric disorders, including schizophrenia (SCZ). There is convergent genetic evidence for the involvement of the Arc (Activity-regulated cytoskeleton-associated; Arg3.1) protein in SCZ. Arc is an immediate early gene with a central role in synaptic plasticity and cognition. In animal studies, loss of Arc causes deficits in memory consolidation, whereas Arc overexpression increases dendritic spine density and cortical plasticity. Here, we utilized induced pluripotent stem cells (iPSCs) from SCZ and control (CTRL) subjects to identify antipsychotics that modulate Arc and regulate synaptic biology.
Methods: We screened a set of antipsychotics in clinical use for their ability to increase Arc in human cortical neurons. We analyzed the effects of our top hit, lurasidone, on Arc levels, dendritic spines, synapses and neuronal activity. We investigated whether lurasidone can modulate Arc levels in neurons from SCZ subjects and rescue synaptic deficits.

Results: In a small-scale screen of antipsychotics, we identified lurasidone as the strongest potentiator of Arc levels in human cortical neurons. We found that lurasidone increased Arc levels and increased dendritic spine density. We further found that SCZ neurons had reduced levels of Arc compared to CTRLs; however, lurasidone robustly increased Arc in SCZ neurons. SCZ neurons also had reduced dendritic spine density compared to CTRLs, which was rescued by lurasidone.

Conclusions: Lurasidone regulates both Arc levels and dendritic spines in human cortical neurons. Our studies suggest Arc is a potential tractable target for modulating synaptic plasticity in psychiatric disorders.

8. Schizophrenia-Associated SAP97 Mutations Increase Glutamatergic Synapse Strength in the Dentate Gyrus and Impair Contextual Episodic Memory in Rats
Yuni Kay, University of Southern California

Mutations in the putative glutamatergic synapse scaffolding protein SAP97 are associated with the development of schizophrenia in humans. However, SAP97's role in synaptic regulation is unclear. Here we discover that SAP97 is expressed in the dendrites of granule neurons in the dentate gyrus but not in the dendrites of other hippocampal neurons. Schizophrenia-related perturbations of SAP97 did not affect CA1 pyramidal neuron synapse function but produce dramatic augmentation of glutamatergic neurotransmission in granule neurons that can be attributed to a release of perisynaptic GluA1-containing AMPA receptors into the postsynaptic densities of perforant pathway synapses. Furthermore, inhibiting SAP97 function in the dentate gyrus was sufficient to impair contextual episodic memory, a commonly observed feature of schizophrenia. Together, our results identify a cell-type specific synaptic regulatory mechanism in the dentate gyrus that, when disrupted, may contribute to the development symptoms associated with schizophrenia.

9. Searching for Schizophrenia Biology: Overlap Analysis of Cell Type-Specific Phenotypes in CNV Mouse Models
Hayley French, Karolinska Institutet

Background: Recent advances in the understanding of schizophrenia genetics have revealed robust associations between several copy number variants (CNVs) and schizophrenia. Although rare in the population, these CNVs are the strongest identified individual risk factors for schizophrenia (OR 2-60). Several have been modelled in mice, allowing for the study of schizophrenia liability. By looking for converging expression patterns between multiple CNV models, we aim to identify underlying biology that is relevant to schizophrenia.
Methods: Using the 10x Genomics platform, we have performed a single-cell RNA-sequencing analysis on prefrontal cortex (PFC) sections from five schizophrenia-associated CNV mouse models: 15q13.3 del, 16p11.2 dup, 1q21.1 del, 22q11.2 del, and 3q29 del. Samples were collected from mice of both sexes at postnatal day 40 or 70.

Results: Our dataset profiles over 300,000 cells from 90 pre- and post-adolescent CNV mice and wildtype littermates. We find a high degree of concordance between our annotated transcriptomic cell types and those published by the Allen Institute. Using this

coverage, we will present differential gene expression analysis to uncover cell type-specific variation in expression levels between CNV and wildtype mice, and potential overlap between CNV models.

Discussion: Overall, our study aims to describe a schizophrenia-relevant temporal window of gene expression changes in the PFC. By investigating the effects of five unique genetic insults with a common association to schizophrenia, we hope this study will advance our understanding of biological mechanisms that can lead to schizophrenia.

10. The Development and Characterization of a New, Conditional Mouse Model Over-Expressing Cav1.2

Rachel Parent, Michigan Neuroscience Institute, University of Michigan

Background: CACNA1C encodes the alpha subunit of the L-type voltage-gated calcium channel (LVGCC), Cav1.2, which is known to play a role in controlling gene expression. Several SNPs have been identified within predicted enhancer regions of CACNA1C that significantly increase the risk of developing psychiatric disease, suggesting that changes in expression of CACNA1C may favor the development of mental illness.
Previous manipulations of expression levels of CACNA1C in the brain have resulted in decreases in expression or caused dominant mutations in the gene. To our knowledge, there been no studies done in transgenic animals that increase expression of wild-type(WT) Cav1.2 in the brain, therefore, we have developed a transgenic mouse model that conditionally (via cre recombinase) over-expresses Cav1.2.

Results: The Cav1.2HA transgene contains an HA tag that is easily visualized with anti-HA antibodies in mice with pan neuronal over-expression of Cav1.2 (Cav1.2Tg+), with Cav1.2Tg+ mice producing ~100% more Cav1.2 protein than their WT littermates. We evaluated somatosensory (S1) neuronal calcium network variables derived from GCaMP6 signals during tactile activation using a continuous wavelet transform approach. Cav1.2Tg+ mice exhibit changes in overall network activity, connection length and synchronicity.

An initial behavioral characterization revealed adult Cav1.2Tg+ mice show a decrease in anxiety-like behavior in elevated plus maze and zero maze, as well as a deficit in contextually conditioned fear consolidation (but no deficit in acquisition or tone conditioning). These mice show no gross motor or nociception deficits and express normal sociability. These results are consistent with previous studies associating CACNA1C with affective disease states.

11. Subcellular Localization of Schizophrenia Risk Genes Encoding Cav1.2 (CACNA1C) and VPAC2 (VIPR2) in Rhesus Macaque Dorsolateral Prefrontal Cortex

Dibyadeep Datta, Yale University

Background: The dorsolateral prefrontal cortex (dlPFC) mediates high-order cognition, and is profoundly afflicted in schizophrenia, including loss of dendritic spines from deep layer III, the microcircuits that generate persistent firing needed for working memory. Studies in monkeys show that these spines contain the molecular machinery for cAMP to magnify internal calcium release, where moderate levels sustain neuronal firing, but high levels weaken connectivity via opening potassium channels. Genetic studies have linked schizophrenia risk with gain-of-function alterations in the Cav1.2 calcium channel encoded by CACNA1C, and a duplication in VIPR2, which increases cAMP intracellular signaling. The current research examined the localization of these key signaling proteins in layer III of the rhesus monkey dlPFC.
Methods: Using high-spatial resolution immunoelectron microscopy, we interrogated the subcellular localization of 1) L-type voltage-gated calcium channel Cav1.2, and 2) Gs-protein coupled receptor VIPR2, in dlPFC layer III from aged monkeys with naturally occurring spine loss.

Results: We found significant labeling of VIPR2 and Cav1.2 in dlPFC layer III dendritic spines. VIPR2 and Cav1.2 were predominantly localized in perisynaptic or extrasynaptic compartments on the plasma membrane near glutamatergic asymmetric axospinous synapses, often in association with the calcium-storing SER spine apparatus.

Discussion: Cav1.2 and VIPR2 are positioned to drive calcium influx and cAMP signaling in layer III dlPFC spines, respectively. As both cAMP and Cav1.2 increase calcium efflux from internal SER stores, gain of function mutations in these signaling proteins in schizophrenia may lead to excessive calcium signaling, which may contribute to dendritic spine loss and cognitive deficits.

12. The Effects of Genetic Polymorphisms in Dopamine Signaling on Prefrontal Functional Connectivity and Working Memory Performance
Rye Young Kim, Ewha Brain Institute, Ewha W. University

Background: The effects of genetic variations in dopamine signaling on the dorsolateral prefrontal cortex (DLPFC)-related functional networks, which play a critical role in cognitive function, remain to be investigated. This study aimed to examine the effects of genetic variations in dopamine signaling on functional connections of the resting-state networks centering on the DLPFC as well as working memory performance.
Methods: Healthy individuals were genotyped for polymorphisms in the catechol-O-methyltransferase and dopamine D2 receptor genes. Participants were categorized into the high (high DS group) and low dopamine signaling groups (low DS group) according to their genetic composite scores. Resting-state functional neuroimaging and working memory performance were evaluated. Mean functional connectivity (FC) was measured

within the four major cognitive networks centering on the DLPFC including the dorsal attention network (DAN), bilateral frontoparietal networks (FPNs), and frontotemporal network (FTN).

Results: The mean FCs of the major cognitive networks were lower in the high DS group compared to the low DS group (left FPN, $P < 0.01$; right FPN, $P < 0.05$; FTN, $P < 0.01$ with appropriate statistical measures). The high DS group showed lower performance on the working memory than the low DS group ($P < 0.01$).

Discussion: These findings provide evidence that genetic modulation of dopamine signaling may contribute to the inter-individual differences in working memory performance through the modulation of DLPFC-related functional networks.

13. Alterations in Brain Metabolite Levels During Wakefulness and Sleep

Rye Young Kim, Ewha Brain Institute, Ewha W. University

Background: Sleep is critical for the maintenance of brain function by regulating synaptic activity. Considering the pivotal role of glutamatergic transmission in modulating high-level information processing and neurotoxicity, changes in glutamatergic signaling across the sleep-wake cycle are important to be investigated. We examined whether glutamatergic signaling of the human brain may exhibit any cyclicity by continuously monitoring brain glutamate and glutamine levels across the sleep-wake cycle.

Methods: Healthy participants with a regular sleep-wake schedule underwent two continuous proton-magnetic resonance spectroscopy (MRS) sessions including an afternoon wakefulness MRS session from 3:50 pm to 10:00 pm and a sleep MRS session from 0:00 am to 6:50 am. Brain glutamate and glutamine levels were obtained during afternoon wakefulness and night sleep MRS sessions. Between-session comparisons of metabolite levels were performed.

Results: Afternoon wakefulness MRS session consisted of 100-125 MRS spectra per subject, and sleep MRS session consisted of 140-175 MRS spectra per subject. There were significant differences in brain glutamate and glutamine levels between afternoon wakefulness and night sleep. Specifically, brain glutamate levels were higher during afternoon wakefulness than during night sleep ($P < 0.01$), while brain glutamine levels were lower during afternoon wakefulness than during night sleep ($P < 0.01$).

Discussion: In this study, we found that glutamate increased during wakefulness but decreased during night sleep, while glutamine changed in the opposite way. These results suggest that increased glutamine levels during sleep may reflect the homeostatic regulation of the glutamate-glutamine cycle to reduce extracellular glutamate levels in the human brain.

14. Bigenomic Examination of Accelerometry-Derived Sleep Measures in UK Biobank Using Two MDD Definitions

Lindsay Melhuish Beaupre, Center for Addiction and Mental Health

Introduction: Sleep disturbances occur in ~75% of depressed individuals. Oftentimes, they precede a depressive episode, and persist after remission, increasing risk for a subsequent episode. Mitochondria have recently sparked interest in sleep research because of Reimund's "Free Radical Flux Theory of Sleep" which posits sleep allows the body to scavenge excess free radicals produced mainly by mitochondria during the day.
Methods: We performed a genome-wide association study of eight accelerometry-derived sleep measures in a subset of UK Biobank participants. Depression was defined using ICD10 inpatient codes and questionnaire data. Haplogroup analyses were done using R. SNP heritability estimates were calculated using GREML. Transformations were performed on phenotype data.

Results: We were unable to detect any significant GWAS hits. Our top mtDNA finding was for m.5495T>C of MT-ND2 (z=2.98, p=0.003) in our broadly defined depression sample. There were no significant haplogroup associations. GREML analyses revealed moderate SNP heritability estimates for bedtime (0.2, p=4.75x10-7), sleep duration (0.17, p=8.58x10-6) and duration of longest sleep bout (0.23, p=4.64x10-4).

Discussion: We were unable to detect any genome-wide associations. We detected moderate SNP heritability for bedtime, sleep duration and duration of longest sleep bout, suggesting that genetics plays a role in these sleep measures. Like depression, sleep disturbances are also likely a polygenic disorder, with many genes involved, all of which have small effect sizes. Nevertheless, given the consequences associated with comorbid sleep problems, it is critical that we continue to uncover the biology of sleep disturbances to optimize treatment regimens.

15. Germline Mosaicism of a Missense Variant in KCNC2 in a Multiplex Family With Autism and Epilepsy

Tychele Turner, Washington University in St. Louis

Background: Currently, protein-coding de novo variants and large copy number variants have been identified as important for ~30% of individuals with autism. One approach to identify relevant variation in individuals who lack these types of events is by utilizing newer genomic technologies.
Methods: In this study, highly accurate PacBio HiFi long-read sequencing was applied to a family with autism, treatment-refractory epilepsy, cognitive impairment, and mild dysmorphic features (two affected female full siblings, an unaffected father, unaffected mother, and one unaffected brother) with no known clinical variant.

Results: From our long-read sequencing data, a de novo missense variant in the KCNC2 gene (Kv3.2 protein) was identified in both affected children. This variant was phased to the paternal chromosome of origin and is likely germline mosaic in the father. In silico assessment revealed the variant was not in controls, highly conserved, and predicted damaging. This specific missense mutation (Val473Ala) has been shown in both an ortholog and paralog of Kv3.2 to accelerate current decay, shift the voltage dependence of activation, and prevent the channel from entering a long-lasting open state. Seven additional missense mutations have been identified in other individuals with neurodevelopmental disorders ($p = 1.03\times10\text{-}5$). KCNC2 is most highly expressed in the brain; in particular, in the thalamus and is enriched in GABAergic neurons.

Discussion: Long-read sequencing was useful in discovering the relevant variant in this family with autism that had remained a mystery for several years and will potentially have great benefits in the clinic once it is widely available.

16. Identifying Cerebrospinal Fluid Protein Biomarkers of Autism Spectrum Disorder

Ozge Oztan, Stanford University

Background: Autism spectrum disorder (ASD) is a debilitating brain disorder. ASD is currently diagnosed using only behavioral criteria because no laboratory diagnostic test exists. However, we recently found that cerebrospinal fluid (CSF) arginine vasopressin (AVP) concentration is significantly lower in ASD cases versus controls. Given ASD's clinical heterogeneity, we hypothesized that other as yet unidentified biomarkers might contribute additional explanatory power and, thus, could be incorporated into a multiplex biomarker panel by which to detect ASD with even higher accuracy.
Methods: We evaluated CSF-based proteins in n=30 pediatric ASD cases and n=30 pediatric controls. We used Olink, a multiplex, high-throughput targeted protein discovery platform which collectively included 184 proteins.

Results: We found a highly significant difference in protein level profiles (P=0.0006), due to 16 proteins that differed at $P<0.05$, 5 of which passed a false discovery criterion of $q<0.05$. To test the ability of this 5-protein profile to improve prediction of ASD diagnosis over AVP alone, we confirmed that AVP predicted ASD diagnosis (P=0.0065; with 65% accuracy). Next, we added the 5-protein multiplex profile to AVP; this combined profile again predicted diagnosis ($P<0.0001$), but now with 90% accuracy. This improvement in prediction was highly significant (P=0.0000001446). Notably, this accuracy was achieved without having to account for age, sex, ethnicity, or other confounds.

Conclusion: These preliminary data indicate that a CSF-based multiplex protein profile differentiates ASD cases and controls accurately. Research is now required to test

whether this finding is specific to ASD, rather than atypical brain development more broadly.

17. MicroRNA-eQTLs in Developing Human Neocortex Identify MIR4707 as a Regulator of Human Brain Size

Michael Lafferty, University of North Carolina at Chapel Hill

Background: Expression quantitative trait loci (eQTL) data is often used to link the genomic risk for neuropsychiatric disorders and brain-related traits discovered through GWAS to putatively causal mechanisms via protein-coding genes. However, microRNAs (miRNAs) are poorly measured in standard eQTL studies despite their important influence on neurogenesis and known dysregulation in patients with neuropsychiatric disorders.
Methods: Here we used miRNA expression across 212 genetically distinct human fetal cortical tissue samples during mid-gestation to map cis-miRNA-eQTLs. We attempted co-localizations between cis-miRNA-eQTLs, mRNA-eQTLs on the same samples, and multiple neuropsychiatric disorders and brain traits. A lentiviral expression vector was used to increase miRNA expression in proliferating human neural progenitor cells (hNPCs) and their differentiated progeny.

Results: We identified 85 miRNA-eQTLs associated with expression of 70 miRNAs. We found enrichment of miRNA-eQTL signal within active transcription start sites and among chromatin associated with transcription. Co-localization of miRNA-eQTLs with GWAS summary statistics yielded one robust co-localization of miR-4707-3p expression to educational attainment and head size phenotypes. After increasing expression of miR-4707-3p in primary human neural progenitor cultures and their differentiated progeny, we detected an increase in both proliferative and neurogenic gene markers by qPCR and ICC assays, implying an early cell-cycle exit and an increase in neurogenic divisions.

Discussion: MiRNA-eQTLs in developing cortical tissue yield insight into the causal mechanisms by which genetic variants influence brain traits. In addition, genetic regulation of miR-4707-3p may alter human brain size through modulation of neural progenitor proliferation and differentiation.

18. Quantifying the Developmental Trajectory of Autism Associated Brain Overgrowth Using Magnetic Resonance and 3D Cellular Resolution Imaging

Felix Kyere, University of North Carolina at Chapel Hill

Disruptions in embryonic brain development can manifest as altered postnatal brain structure and function, leading to neuropsychiatric illness. One such disruption is mutation in Chromodomain helicase DNA binding protein 8 (Chd8); a gene that encodes an ATP-dependent chromatin remodeler. Heterozygous Chd8 loss of function mutations result in

macrocephaly in both humans and mouse models, and autism in humans. Our goal is to quantify neural progenitors and neuronal cell-types within annotated areas of the developing neocortex in both WT and Chd8+/- mice to determine the cell types that drive this ASD associated cortical hyper-expansion using MRI and lightsheet imaging.
Four pairs of mouse postnatal day 4(P4) littermate brains were prepared and imaged on a 9.4T MRI scanner at 60μmx60μmx60μm. Additionally, we generated 3D cellular high resolution (0.75μmx0.75μmx4μm) images from 6 littermate pairs at P4 labeled with upper- (Brn2) and lower-(Ctip2) layer neuronal markers following tissue clearing with iDISCO+.

We found increased cortical volume in Chd8+/- mice (~10%) relative to WT littermate controls in both males and females, replicating previous work. Additionally, we quantified all the nuclei in the cortex using computational tools and detected about 13.8 million cortical nuclei comprised of 21% each of upper- and lower-layer neurons.

These results demonstrate cortical volume increases in Chd8+/- mouse brains. Moving forward, we aim to perform similar analyses for 9 biological replicates per sex and per genotype at P4 and other early developmental timepoints to elucidate the cellular basis and spatial localization of brain overgrowth in our mouse model.

19. Relating Interindividual Differences in Cerebral Organoids to Longitudinal Infant Brain Growth

Madison Glass, University of North Carolina at Chapel Hill

Background: Cortical surface area hyper-expansion in infancy prior to diagnosis has been associated with increased risk for autism spectrum disorder (ASD) (Hazlett et al., 2017). Neurogenesis in fetal development influences cortical surface area (Rakic, 2009). Induced pluripotent stem cell (iPSC) derived brain organoids model neurogenesis on an individual's genetic background with limitations, but it remains unknown if inter-individual variability in organoids correlate with infant brain growth and clinical outcomes of the individual.
Methods: We generated 3 iPSC lines each from 18 participants in the Infant Brain Imaging Study (IBIS) at low familial risk for ASD, high familial risk without ASD, or high familial risk with ASD at 2 years old. IBIS participant-derived organoids were harvested at day 14 and 84 for imaging and scRNAseq. This study aims to identify in vitro-in vivo correlations regardless of diagnosis.

Results: A preliminary analysis of scRNA-seq data from 9 participants suggests that day 14 organoids contain predominately EMX2+ cortical neuronal progenitors and their DCX+ neuronal progeny. Using RNA velocity estimates of future cell states, progenitors were predicted to be undergoing self-renewal or neurogenic fate decisions (Lange et al., 2020). Neurogenic fate decisions were negatively correlated with cortical surface are a size at 24 months of age ($r=0.49$, when controlling for sex $p= 0.002$).

Discussion: Preliminary analyses suggests that organoid-derived early progenitor populations favor neurogenic fate decisions in participants with smaller cortical surface areas. Future unblinded analysis with 18 participants and orthogonal imaging measures will enable more rigorous hypothesis testing of in vitro-in vivo correlations.

20. Molecular and Circuit Mechanisms of Shared Neuropsychiatric Risk Caused by the 16p11.2 Microduplication

Marc Forrest, Northwestern University

Background: Neuropsychiatric disorders (NPDs) have shared symptoms and genetics suggesting convergent biology, but the mechanisms mediating shared risk are poorly understood. The 16p11.2 microduplication (16p11.2dup/+) is a highly pleiotropic copy number variant conferring risk of autism spectrum disorder, schizophrenia, intellectual disability, and epilepsy. This CNV therefore represents a key entry point to understand the biology of shared risk.

Methods: Here, we use the 16p11.2dup/+ mouse model to investigate the molecular, cellular and circuit basis for shared NPD risk. We used quantitative proteomics, to profile neocortical membranes of 16p11.2dup/+ mice. Molecular findings were followed up with synapse quantification, imaging of neuronal network activity and behavioural phenotyping. We used bioinformatics and interactome proteomics to identify candidate genes within the duplication for disease reversal. Mouse genetics was used to correct gene expression.

Results: Membrane proteomics revealed a dysregulation of synaptic networks and a disruption of proteins associated to diverse NPDs. Phenotypic characterization of 16p11.2dup/+ mice uncovered cellular and circuit alterations including increased synaptic connectivity, hypersynchrony of local cortical circuits, and increased seizure susceptibility. Using human co-expression data and interactome analysis, we find that PRRT2, a protein encoded within the microduplication, is a critical hub in the dysregulated disease-associated protein networks. Genetic correction of Prrt2 reversed key cellular and behavioural phenotypes associated with the 16p11.2 microduplication.

Conclusion: Together, our work suggests that PRRT2 is a nexus for disease reversal in the 16p11.2 microduplication and that increased cortical connectivity and synchrony in local cortical circuits may be predisposing factors for shared risk of neuropsychiatric disease.

21. Effects of Autism Risk Gene CHD8 Mutation on Zebrafish Neurodevelopment

Sarah Fitzpatrick, Yale School of Medicine

Background: Autism spectrum disorder (ASD) is highly prevalent (1 in 44 children) and clinically complex. Despite decades of research, an understanding of the specific

mechanisms underlying the abnormal neurodevelopment of ASD remains elusive. Whole exome sequencing studies implicate over 100 genes as “autism risk genes” – genes in which de novo rare variants in offspring are sufficient to cause ASD. Many of these genes play roles in gene expression, including chromatin modifiers. One of the most strongly associated ASD risk genes is chromodomain-helicase DNA-binding protein 8 (CHD8), a chromatin modifier gene.
Methods: To study the effects of CHD8 mutation on neurodevelopment, we generated a zebrafish line with a frameshift deletion in the zebrafish ortholog chd8, and performed behavioral, immunohistochemical, and molecular analyses to characterize the line.

Results: Heterozygous and homozygous mutants survive to adulthood, although homozygous mutants have decreased fertility and are grossly smaller as adults. Behavioral analyses show homozygous chd8 mutants have decreased daytime activity and decreased total sleep at night despite an increased number of sleep bouts compared to wild-type siblings. Brain activity mapping using phosphorylated extracellular signal-related kinase (pERK) and total ERK staining shows differences in baseline neural activity. RNA sequencing suggests homozygous chd8 mutants have alterations in several key neurodevelopmental pathways.

Conclusions: This work suggests disrupting mutations in a chromatin modifier gene has significant effects on zebrafish behavior and neurodevelopment. The chd8 mutant zebrafish line will be useful for dissecting the molecular mechanisms that underlie these changes and provide insight into the altered neurodevelopment of autism.

22. Investigating Functional Effects of scn1Lab Mutation in a Zebrafish Genetic Model of ASD

April Pruitt, Yale Child Study Center

Background: Previous exome-sequencing studies have identified over one hundred high confidence risk genes contributing to autism spectrum disorder (ASD). Variants in the SCN2A gene, which encodes voltage gated sodium channel Nav1.2, have been associated with infantile-onset epilepsy, intellectual disability, and ASD. Here we investigate functional effects of SCN2A mutations in larval zebrafish mutants of scn1Lab, an ortholog of the ASD risk genes SCN1A and SCN2A.
Methods: First, we characterized behavior of scn1Lab-mutant larval zebrafish using multiple paradigms: PTZ-induced seizure assay, rest-wake assay, and visual-startle response assay. To assess pan-neuronal activity, we then conducted whole-brain activity mapping. To understand how scn1Lab mutations affect GABAergic and glutamatergic neurodevelopment, we performed fluorescence-activated cell sorting of GABA and GLUT neurons in scn1Lab-mutant transgenic lines co-labeling these cell populations followed by RNA-seq.

Results: scn1Lab-mutant larvae show increased seizure activity, reduced daytime activity, and hyperactive startle response to light. scn1Lab-mutants also show decreased brain

activity as well as a deficit in GABAergic neuron populations. We have isolated GABAergic and glutamatergic neurons from the brains of 6 dpf larval zebrafish by FACS.

Conclusion and Discussion: These data show that scn1Lab mutants exhibit disruptions in visuomotor, rest-wake, and excitatory-inhibitory circuitry, leading to alterations in visual-startle responses, rest-wake activity, and seizure behavior. In future studies, we aim to assess associations between zebrafish and human SCN2A RNA-seq datasets by directly mapping across species and examining correlations between global t-statistic and FDR of differentially expressed genes.

23. Molecular Functions of ID/ASD-Associated MYT1L in CNS Development
Jiayang Chen, Washington University in St. Louis

Background: Human genetic studies have identified heterozygous mutations on Myelin Transcriptional Factor Like 1 (MYT1L) as the cause for ID/ASD-related MYT1L Syndrome. Given that most MYT1L studies have been performed in vitro and limited data were generated in vivo, MYT1L's targets and how it regulates target genes expression under physiological conditions are largely unknown. Furthermore, MYT1L's molecular functions across different developmental time points and the mechanisms underlying MYT1L Syndrome etiology have not been well established.
Methods: We adapted CUT and RUN to map MYT1L binding sites in mouse embryonic cortex, adult prefrontal cortex, and hypothalamus. Then, we compared MYT1L targets with published or newly generated multi-omics datasets on MYT1L knockout mouse from corresponding time points and brain regions to dissect MYT1L's direct and indirect impacts on chromatin structures and gene expression.

Results: We identified highly confident MYT1L DNA binding targets in the mouse embryonic cortex, adult prefrontal cortex, and hypothalamus. Although MYT1L appears to have many distinct targets at different developmental time points and brain regions, it shares some common ones related to neurodevelopment. Utilizing the MYT1L knockout mouse line, we also found MYT1L mainly functions as an activator on chromatin structures and genes expression. We further demonstrated such dysregulation affects cellular and circuit functions potentially associated with many disease phenotypes.

Conclusions: Our study revealed ID/ASD-associated MYT1L's molecular functions across development and brain regions in vivo. Meanwhile, we provided insights into the mechanisms by which MYT1L LoF leads to various phenotypes related to human MYT1L Syndrome.

24. Genome-Wide Association Study of Treatment Resistant Depression Defined Using EHR-Based Clinical Prediction Models
JooEun Kang, Vanderbilt University School of Medicine

Treatment-resistant depression (TRD) impacts about a third of individuals with major depressive disorder (MDD). Prior work suggests a significant genetic component of TRD however no genetic loci have been replicated.
Using electroconvulsive therapy (ECT) procedure code to ascertain TRD, we applied LASSO to electronic health record (EHR) data from Vanderbilt University Medical Center (VUMC, 106,565 patients, 225 cases) and Mass General Brigham (MGB, 78,378 patients, 242 cases) to derive predicted probabilities of receiving ECT on 152,113 genotyped MDD patients across four large biobanks (VUMC, MGB, Geisinger, and Million Veterans Program).

Both TRD models showed robust prediction performance internally (AUC 0.91-0.93) and externally (AUC 0.78-0.84). A meta-analysis GWAS of normalized TRD predicted probabilities yielded 1 genome-wide significant locus for the MGB model in an intergenic region on the FTO gene strongly implicated in obesity. We identified significant heritability estimates of TRD from both the VUMC (h2SNP =2.3%, p=4.5x10-9) and MGB model (h2SNP =4%, p=8.6x10-18). VUMC and MGB TRD GWAS were significantly correlated (rg=0.72, SE=0.05, P=6.8x10-44) and showed significant genetic correlations with ADHD, BMI, intelligence, alcohol dependence, and smoking. There was significant association of BMI polygenic risk score and TRD (MGB: p=6.9x10-31, VUMC: p=1.6x10-3) that remained significant among non-obese MDD patients (MGB: p=8.5x10-16, VUMC: p=0.002).

This work describes an approach to quantify TRD from EHR to improve power of genetic studies and demonstrates significant heritability. Significant genetic overlap of TRD and BMI independent of weight suggest a shared underlying biology between TRD and obesity.

25. The Role of Recent Chronic Stress in Overweight and Depression Among Adolescents and Young Adults From the General Population

Katja Beesdo-Baum, TU Dresden

Background: Overweight and depression are tremendous public health issues. Stress has been associated to both conditions, but scarce epidemiologic data exists on the link between overweight and depression and the role of neuroendocrine dysfunctions in relation to recent chronic stress.
Methods: In a random community sample of N=1,180 (response rate: 21.7%) 14-21 year olds from Dresden (Germany), Body Mass Index (BMI generated from measures of weight and height), current depression symptoms (PHQ-9), hair cortisol concentration (pg/mg; derived from 3-cm hair strands), and self-reported perceived stress (TICS) were assessed. All measures were available from N=953 participants.

Results: 18.4% were classified as overweight (BMI>25) and 7.7% as suffering depression (PHQ-score≥10). Higher BMI was associated with higher PHQ-score ($p<.05$), but the association between categorical overweight and depression failed significance. Using mutually exclusive groups of no overweight/no depression (76.1%), overweight/no depression (16.2%), depression/no overweight (5.6%), and overweight and depression (2.1%), regression analysis revealed that long-term hair cortisol secretion was associated with overweight with or without depression and subjective stress was associated with depression with or without overweight ($p<.05$). No associations or interactions were found between objective and subjective indicators of stress.

Conclusion: Findings suggest a considerable role of stress in the pathophysiology of both overweight and depression among adolescents and young adults, yet with stronger indication for a biologic mechanism via the neuroendocrine system for overweight and for a psychological mechanism via perceived stress for depression. Longitudinal research clarifying shared and distinct etiological pathways of overweight and depression may contribute to improved targeted interventions.

26. Investigating Microglial Remodeling of Synapses in Major Depressive Disorder-Associated Suicide Death

Elisa Gonçalves de Andrade, University of Victoria

Suicide is a major public health challenge that worldwide is responsible for one death every 40 seconds. Microglia, the resident immune cells of the central nervous system, present altered density, morphology, and gene expression in human brain tissue from individuals who died by suicide, particularly in regions involved in emotion and decision making, e.g. prefrontal cortex (PFC) and hippocampus (HIP). However, the consequences of these changes remain unknown. Microglial remodeling of synapses appears impaired in major depressive disorder (MDD), a strong risk factor for suicidal behaviours. Synapse remodeling can result from increased microglial contacts with synaptic elements, leading to phagocytosis, oxidative stress, or through decreased trophic support of neurons and synapses, all factors we hypothesize are exacerbated in individuals with MDD who died by suicide. To test this hypothesis we will investigate post-mortem PFC (n=4) and HIP (n=4) samples from individuals with MDD who died by suicide, as well as age-matched controls. Using a multiplex antibody technique, we will assess microglial (IBA1, TMEM119) contacts with neurons (NeuN), synapses (synaptophysin, PSD-95), and proxies for phagocytic activity (CD68), oxidative stress (iNOS, IDO), and neuronal proliferation (Ki67, PCNA). Next, microglial interactions with neurons and synapses will be further investigated at the ultrastructural level using focused ion-beam scanning electron microscopy. This analysis will provide the first nanoscale characterization of microglial activity at synapses in suicide. With the increasing development of therapies targeting microglial cells, our project represents a step of major importance towards using synapse remodeling to help reduce suicide behaviour.

27. In Vitro Modeling of Depression Neurobiology: Differentiating Blood-Derived Pluripotent Stem Cells Into Hippocampal-like Neurons

Anastasiya Nestsiarovich, University of California San Diego

Background: Our research team has previously implemented protocols developed by Salk Institute to differentiate induced pluripotent stem cells (iPSCs) derived from patients with bipolar disorder (BD) into hippocampal-like neurons via the neural progenitor cells (NPCs) stage. Further experimentation showed that lithium reversed hyperexcitability in neurons but only in patients with good clinical response to this mood stabilizer. The present study aimed to reproduce this protocol and generate dentate gyrus hippocampal-like neurons from iPSCs in patients with major depressive disorder (MDD) to further explore if lithium-modulated neural excitability can help distinguish MDD antidepressant responders vs. MDD non-responders vs. patients with BD.

Methods: The iPSC samples came from our collaborators' antidepressant trial in patients with MDD. The iPSCs were transformed into embryoid bodies which underwent a "rostral patterning" protocol resulting in NPCs, which were sequentially cultured in Expansion, Differentiation, and Maturation medias. Mature neurons were transfected with Prox-1 GFP+ lentiviral labeled cells, labeled with "Zombie" dye and APC antibody to NCAM, and underwent Fluorescence-activated cell sorting followed by 2-weeks recovery on a microelectrode plate. Cells electric signal recording is performed (optimization in progress).

Results: We received a small but relatively well-differentiated colony of hippocampal-like neurons (N=32,000) which are suitable for further in vitro experimentation to study lithium-modulated neural excitability.

Discussion: successful reproduction of the differentiation protocol validates the use of our iPSC/NPC cellular bank for further in vitro experimentation to answer clinical questions. Stem cell-derived neurons can serve as a model to determine cellular phenotypes associated with specific disease or medication response.

28. A Novel Cortico-Cortical Pathway Underlying Social Isolation-Induced Deficits in Postpartum Social Behaviors

Kyohei Kin, University of Alabama at Birmingham

Background: The biological mechanisms by which adolescent psychosocial stress influences postpartum behaviors later in life have not been well characterized. Previously, we have established a novel mouse model in which mild adolescent isolation stress leads to an aberrant reduction of extracellular glutamate levels in the prelimbic cortex (PrL) and subsequent social behavioral deficits at one week postpartum. Based on these findings along with human imaging studies from other groups, we examined whether adolescent

social isolation, in conjunction with the stressful events of pregnancy/delivery, leads to deficits in postpartum behaviors related to social cognition via functional alternations of the glutamatergic pathway from the anterior insula (AI) to PrL.
Methods and results: In vivo calcium image recordings revealed that the decreased preference for novel mice in stressed dams was accompanied by a decreased fraction of excited glutamatergic neurons and an increased fraction of suppressed glutamatergic neurons in PrL during interactions with a novel mouse. Optical activation of the AI-PrL pathway in stressed dams restored the preference for novel mice and increased the fraction of excited glutamatergic neurons and decreased the fraction of suppressed glutamatergic neurons in PrL during interactions with a novel mouse. In contrast, optical inhibition of AI-PrL pathway in unstressed dams led to the abnormal neuronal activity pattern and the subsequent behavioral deficits observed in stressed dams.

Conclusion: Our present study demonstrated that adolescent social isolation, in conjunction with the stressful events of pregnancy/delivery, induces hypofunction of the AI-PrL pathway which results in social behavioral deficits in the postpartum period.

29. Ketamine Activates Adult-Born Immature Granule Neurons to Rapidly Alleviate Depression-Like Behaviors
Radhika Rawat, Feinberg School of Medicine, Northwestern University, Chicago

Ketamine treatment decreases depressive symptoms within hours, but the mechanisms mediating these rapid antidepressant effects are unclear. We demonstrated that activity of adult-born immature granule neurons (ABINs) in the mouse hippocampal dentate gyrus is both necessary and sufficient for the rapid antidepressant effects of ketamine. Ketamine treatment activated ABINs in parallel with its behavioral effects in both stressed and unstressed mice. Chemogenetic inhibition of ABIN activity blocked the antidepressant effects of ketamine, indicating that this activity is necessary for the behavioral effects. Conversely, chemogenetic activation of ABINs without any change in immature neuron numbers mimicked both the cellular and the behavioral effects of ketamine, indicating that increased activity of ABINs is sufficient for rapid antidepressant effects. These findings thus identify a specific cell population that mediates the antidepressant actions of ketamine, indicating that ABINs can potentially be targeted to limit ketamine's side effects while preserving its therapeutic efficacy.

30. MiRNA Regulates Early Life Stress-Induced Depressive Behavior via Serotonin Signaling in a Sex-Dependent Manner in the Prefrontal Cortex of Rats
Yogesh Dwivedi, The University of Alabama At Birmingham

Background: The underlying neurobiology of early life stress (ELS)-induced major depressive disorder (MDD) is not clearly understood.
Methods: In this study, we used maternal separation (MS) as a rodent model of ELS and tested whether miRNAs target serotonin genes to regulate ELS-induced depression-like behavior and whether this effect is sex-dependent. We also examined whether environmental enrichment prevents susceptibility to depression- and anxiety-like behavior following MS and whether enrichment effects are mediated through serotonin genes and their corresponding miRNAs.

Results: MS decreased sucrose preference which was reversed by enrichment. Males also exhibited greater changes in forced swim climbing and escape latency tests, only following enrichment. Serotonin transporter (SLC6A4) and serotonin receptor 1A (HTR1A) were upregulated in the frontal cortex following MS. In male MS rats, enrichment slightly reversed HTR1A expression to levels similar to controls. miRNAs-200a-3p and -322-5p, targeting SLC6A4, were decreased by MS, but not significantly. HTR1A targeting miRNA, miR-320-5p, was also downregulated by MS and showed slight reversal by enrichment in male animals. miR-320-5p targeting of HTR1A was validated in vitro using SHSY neuroblastoma cell lines.

Conclusions: Altogether, this study implicates miRNA interaction with the serotonin pathway in ELS-induced susceptibility to depression-related reward deficits. Furthermore, because of its recovery by enrichment in males, miR-320 may represent a viable sex-specific target for reward-related deficits in MDD.

31. Sex-Specific Alteration of the Transcriptional Signatures in Corticoaccumbal and Corticotegmental Pathways in a Mouse Model of Depression.

Thibault P. Bittar, Université Laval

Background. The medial prefrontal cortex (mPFC) is part of a complex circuit controlling stress responses through its projections to limbic structures including the nucleus accumbens (NAc) and ventral tegmental area (VTA). While the sex-specific transcriptional alterations in the mPFC have been characterized, the transcriptional signatures of its heterogeneous projections have not been studied yet and the behavioral impact of these changes is still unknown.
Methods. We used chronic variable stress (CVS) to induce depressive-like behaviors in male and female RiboTag mice before collecting the transcriptional material. We performed RNAseq to screen pathway-specific transcriptional profiles. We identified key regulators of sex-specific gene networks as mediators of stress susceptibility in male and female mice and confirmed their behavioral contribution through viral-mediated gene transfer combined with a detailed behavioral assessment of stress susceptibility.

Results. Using RNAseq, we identified the unique transcriptional signatures of the corticoaccumbal and corticotegmental pathways in stressed males and females. Our analysis revealed pathway-specific transcriptional alterations in males and females induced by CVS, with minimal overlap between differentially expressed genes (DEGs) in males and females. The viral-mediated gene expression modifications of some DEGs recapitulated the sex- and pathway-specific behavioral alterations observed after CVS, for instance, Xlr4b in males for both projections and Nrn1 in NAc-projecting mPFC neurons of both sexes.

Conclusion. Our results suggest that chronic stress impacts the corticoaccumbal and corticotegmental pathways differently through sex-specific transcriptional alterations in mPFC neurons. Modification of the expression pattern of key regulator genes is sufficient to recapitulate the behavioral alterations observed after CVS.

32. Role of the Endocannabinoid System in Stress Resilience and Depression: A Master Regulator of Neurovascular Health

Katarzyna Anna Dudek, CERVO Brain Research Centre

Only 30 to 50% of major depressive disorder (MDD) patients completely remit, making it a leading cause of disability worldwide. This lack of efficacy suggests that current neuron-centric treatments do not address important biological factors. Chronic stress, the main environmental risk for MDD development, has been known to trigger a whole-body response including neuroimmune and neurovascular adaptations. We recently reported that chronic social stress causes a detrimental increase in blood-brain barrier (BBB) permeability, promoting infiltration of circulating inflammatory mediators and development of depressive-like behaviors in mice. Those pathological changes have been confirmed in brain samples of MDD patients. However, biological mechanisms underlying these molecular changes in response to stress remain elusive. Interestingly, the endocannabinoid system (ECS) is a crucial regulator of stress responses. Moreover, ECS was shown to regulate BBB permeability under homeostatic and pathological conditions. Here we combine molecular, cellular and morphological analyzes to behavioral studies and show that the ECS is actively involved in stress resilience to chronic social defeat stress, a mouse model of depression, in a sex- and brain region-specific manner.

Based on those results, we propose that stress-induced increased in BBB permeability could be due to pathological changes in the ECS system, enabling release of inflammatory signals into the circulation, vascular dysfunction and establishment of depressive behaviors.

33. Epigenome-Wide Association Study of Improvement in Depressive Symptoms Across Psychiatric Disorders

James Kennedy, CAMH, U Toronto

Background: Research is demonstrating that both genetic and environmental factors are involved in development and severity of psychiatric disorders, but epigenome-wide association studies (EWAS) examining medication treatment are limited. We conducted an EWAS to discover novel peripheral DNA methylation biomarkers associated with improvement in depressive symptoms across psychiatric disorders.
Methods: Patients (n=187) were derived from our naturalistic Toronto CAMH IMPACT study. DNA was collected at enrollment and methylation was interrogated using the Illumina 450K array. Quality control, normalization, and analyses were conducted using the ChAMP pipeline in R, adjusting for age, sex, batch effects, influential SNPs, and cellular composition. Change in BDI score was used to measure symptom improvement over eight weeks of treatment. The relationship between methylation levels and change in BDI score was investigated using linear modelling.

Results: The average percent change in BDI score after eight weeks of treatment was -23.05 ± 36.16. Quality control left 410,096 CpG probes for analyses. Our most significant results revealed methylation patterns of 10 probes located within 8 genes (C8orf71, SYT6, SLK, CNP, ZNF747, HSD17B4, TMEM50B, TMEM223) to be nominally associated with BDI score percent change. Increased methylation for our top hit, cg04315200, was nominally associated with impaired treatment response ($\beta = 17.43$, $p = 4.27x10\text{-}5$).

Conclusion: Our preliminary pharmaco-epigenetic EWAS suggests DNA methylation may be involved in change of depression symptoms during treatment across psychiatric disorders. Future work will include gene set enrichment and polygenic risk score analyses, in addition to a predictive machine-learning approach, in a larger data set.

34. Association Between the COMT Val158Met Polymorphism With the Latent Variable for Psychopathic Traits Changes Through Development in Youth

James Kennedy, CAMH, U Toronto

Background: Youth psychopathy is a serious public health concern, often leading to adult psychopathy. Catechol-o-methyltransferase(COMT) polymorphism of Val158Met(rs4680) has been reported to be associated with youth psychopathic traits. The dynamic nature of the COMT gene's contribution to executive functioning from childhood to adolescence suggests that there may be a differential effect of Val158Met on psychopathic traits through development. This study examines the developmental trajectory of the association between COMT-Val158Met and psychopathic traits in a unique sample of clinically aggressive youth.
Methods: 293 youth(142M:151F;age=12.41,SD=2.86) of European ancestry were recruited. COMT-rs4680 genotypes were obtained from the Illumina PsychArray Beadchip. A single factor score for youth psychopathy was extracted from the phenotype

data using CBCL and Psychopathic Screening Device. To examine the developmental change, the sample was split using >/< 13 years. Analysis was performed using PLINK and R with Wilcoxon Rank-Sum tests.

Results: In youth above age 13(n=139), Val carriers were significantly more susceptible to exhibiting psychopathic traits(p=0.03) than Met homozygous youth. In contrast, in below age 13 group(n=154), Met carriers were more prone to exhibiting psychopathic traits, close to significance(p=0.09), than Val homozygous youth. Results were similar for males and females when they were analyzed separately.

Conclusion: This is the first study to demonstrate change in the effects of the Val158Met polymorphism on psychopathic traits. Val158Met is consistently associated with psychopathy, but changes its effect during development. This demonstrates the importance of developmental stages in understanding the effects of Val158Met and may serve as a base for developing novel personalized interventions.

35. Anxiety-Related Target Genes and Heart Rate Variability in Europeans and East Asians

James Kennedy, CAMH, U Toronto

Background: Heart rate variability (HRV) is a heritable measure of the variation in milliseconds between consecutive heart beats, and reflects the ability of the parasympathetic nervous system (PNS) to deactivate the sympathetic nervous system (SNS). Anxiety disorder is associated with low HRV, which can indicate reactivity to, and recovery from, anxiety. Genetic variants related to HRV may provide insight on the underlying biology of anxiety.

Methods: We studied 95 individuals (21 anxiety disorder and 74 controls, age 20-59, 62 female, 73 European, 22 East Asian). Resting HRV was recorded via photoplethysmography for 5-minutes using the Empatica wristband. Pulse data was processed via Kubios HRV software. Global Screening Arrays captured genome-wide SNP data. We analyzed SNS/PNS-related SNPs in 16 genes (e.g. HPA-axis, serotonin, renin-angiotensin, norepinephrine, acetylcholine) using linear regression, and performed a meta-analysis across the ancestries.

Results: The average HRV was 46.6ms±24.6ms. In our targeted gene analysis in Europeans, TPH2 rs4570625 (β=13.1, p(unadj)=0.004), angiotensinogen (AGT) rs699 (β=9.4, p(unadj)=0.03), and CRHR1 rs110402 (β=8.2, p(unadj)=0.05) were nominally associated with HRV. TPH2 rs4570625 approached significance in East Asians (β=10.7, p(unadj)=0.06), and was significant upon meta-analysis of the ancestries (z=3.4, p=0.0006).

Conclusion: Our results support previous studies finding TPH2 rs4570625 and CRHR1 rs110402 associated with anxiety-related phenotypes. TPH2 appears to have a significant

effect on HRV across different ancestral backgrounds. Limitations include the small sample, which will increase with time. The SNPs of interest identified here, if replicated, may aid the identification of pathological anxiety risk and novel treatment targets.

36. Event-Related Oscillations to Emotional Faces are Related to a History of Internalizing Disorders and Suicidality

Cindy Ehlers, The Scripps Research Institute

Background: Event-related oscillations (EROs) to emotional stimuli may represent sensitive biomarkers or endophenotypes for internalizing disorders, as well as markers for suicidality. Methods: young adults of American Indian (AI) (n=479) and Mexican American (MA) (n=705) ancestry (age 18-30 yrs) were clinically assessed, and an internalizing scale was generated by extracting core diagnostic items from 6 lifetime DSM5-compatible diagnoses (social phobia, panic disorder, agoraphobia, obsessive compulsive disorder, post-traumatic stress disorder, major depressive episode) and symptoms of suicidality. EROs were generated to happy, sad and neutral faces. Results: An increase in delta ERO energy was found in the frontal lead (FZ) and in the parietal lead (PZ) following presentation of the sad facial expressions in those with a history of internalizing disorders compared to those with no symptoms. A history of internalizing symptoms was also associated with decreases in local ERO phase-locking in FZ. ERO delta was also associated with suicidality ((SA, suicide attempts, deaths) suicidal thoughts (ST, ideation, plans)). ST were more commonly reported among MA participants, whereas SA were more common among AI young adults. A lifetime history of ST was associated with increases in delta ERO energy, whereas SA were associated with decreases in both. A decrease in the percentage of correctly identified neutral faces also was seen among those with internalizing symptoms compared to those without internalizing symptoms. Conclusions: ERO measures may represent important potential biomarkers of internalizing disorders as well as risk indicators for suicidality.

37. Item Response Theory Analysis of Antisocial Personality Disorder Symptoms

David Gilder, The Scripps Research Institute

Background: Item Response Theory (IRT) was used to examine AntisocialPersonality Disorder (ASPD) symptoms and their psychometric in a Mexican American and American Indian community sample. Methods: Exploratory Factor Analysis (EFA) and IRT analysis was applied to the seven adult ASPD DSM-5 symptoms in 663 Mexican Americans and 920 American Indians. A two parameter IRT model was generated using the BILOG-MG statistical program for the seven adult ASPD criteria. The model yielded maximum likelihood estimates of discrimination (a) and threshold (b) parameters for each DSM-5 ASPD criterion. Gender and ethnicity were assessed for Differential Item Functioning (DIF). Results: EFA revealed six of seven criteria loaded well onto one factor, but the

seventh, remorse, loaded predominantly onto a second factor. Discrimination [a (S.E)] parameters ranged from a low for reckless disregard to a high for illegal activities. Severity parameters [b (S.E.)] ranged from the mildly severe criterion of illegal activities to the very severe criterion of lacking remorse. Females were more likely to endorse irresponsibility and impulsivity than males. Mexican Americans were more likely to endorse lying but less likely to endorse physical fights and irresponsibility than American Indians. Conclusions: In this sample, ASPD criteria appear to form a single underlying trait severity, except remorse. Gender and ethnicity exhibited some DIF, which should be taken into account when assessing clinical and institutional samples with ASPD.

38. Long-Range Recurrent Neural Circuits (RNNs) Mediating Emotion-Action Selections

Qian-Quan Sun, University of Wyoming

The medial prefrontal cortex (mPFC), a prominent brain region located within the frontal lobe, is primarily responsible for decision making and judgement, including the regulation of emotion and salience processing by integrating multimodal sensory inputs and facilitating behaviors based on context. However, However, the way in which the same mPFC canonical cortical circuits produce diverse responses that drive reward or aversion is unknown. Here report a long-range recurrent neural networks (RNNs), that links emotional hubs with brain regions controls voluntary movements. RNNs are designed to learn sequential patterns in silico. RNN is a circuit motif that can provide non-linear mixed selectivity and help consolidate this useful neural code scheme with the hardwiring of circuits. Here, we report an innate RNN, which is formed by the unidirectional connections from three basic units: input units arriving from emotional regions, a hidden unit in the mPFC neurons, and output units located at the somatic motor cortex (sMO). More specifically, the neurons from basal lateral amygdala (BLA) and insular cortex, project to the mPFC motor cortex projecting (MP) neurons. These MP neurons form a local self-feedback loop and target major projecting neurons of sMO, including mPFC projecting, CT, and pyramidal tract-corticospinal (PT-CSpi) neurons. Within sMO, the neurons in infragranular layers (L5a CT, L5b PT-CSpi, and L6 CT) receive stronger input than the neurons in supragranular layers (L2/3). Finally, we show in vivo evidence that the communications from emotion regions to the sMO is abolished when MP neurons are silenced with chemogenetics.

39. Parallel Non-Cpg Methylome and Hydroxymethylome Analysis in Cortical Neurons Identifies Novel Loci Associated With Post-Traumatic Stress Disorder

Diana Nunez, Yale Medical School

Background: DNA methylation (5mC) and hydroxymethylation (5hmC) are epigenomic modifications mainly investigated in the context of CpG dinucleotides and have been related with the etiology of psychiatric disorders. Non-CpG 5mC has been recently

observed in mammal and suggested to play an important regulatory role in the human neuronal epigenome; however, its role in psychiatric disorders remain poorly understood. 5hmC is another epigenetic modification highly enriched in brain, although the non-CpG hydroxymethylation has not been previously explored in this tissue. In this study, we examine differential non-CpG 5mC/5hmC in postmortem orbitofrontal neurons of individuals with PTSD and controls.

Methods: Reduced-representation bisulfite sequencing was conducted on 38 postmortem brain samples (25 PTSD, 13 controls) from the NPBB. Differential 5mC/5hmC of non-CpG sites (CHG and CHH) were analyzed using methylKit R package. Genome-wide significance was defined with FDR <0.05 and differential methylation >1.

Results: Comparing PTSD cases with controls, differential 5mC/5hmC were observed in both CHG and CHH. Differential 5mC was observed in 85 CHGs and 121 CHHs, while differential 5hmC was identified in 60 CHGs and 98 CHHs. ALOX12B, CAPN5, COL6A2, FBLIM1, QSOX1, RASAL3 and ULK1 genes with 5mC/5hmC in CHG/C sites located in promoter regions were identified as deregulated genes in previous transcriptomic PTSD study. Additional genes identified here are involved in neuronal processes, oxytocin signaling, and Wnt signaling.

Discussion: This study provides a novel characterization of neuronal non-CpG methylation and hydroxymethylation in PTSD. Genes epigenetically regulated in PTSD that were identified may be involved in the etiology of this disorder.

40. Response Inhibition in PTSD and ADHD

Zhewu Wang, Medical University of South Carolina

Background: Attention-deficit/hyperactivity disorder (ADHD) and posttraumatic stress disorder (PTSD) are common among military veterans, but the comorbidity of these two psychiatric disorders remains largely unstudied. Evaluating response inhibition and cue-dependent learning as behavioral and neurocognitive mechanisms underlying ADHD/PTSD can inform etiological models and development of tailored interventions.

Method: A cued go/no-go task evaluated response inhibition in 160 adult males. Participants were recruited from the community and a Veterans Administration medical center. Four diagnostic groups were identified: ADHD-only, PTSD-only, ADHD+PTSD, controls.

Results: Group differences were observed across most indices of inhibitory functioning, reaction time, and reaction time variability, whereby PTSD-only and ADHD+PTSD participants demonstrated deficits relative to controls. Significant group effect on mean reaction time ($F(3, 156) = 8.62$, $p < .001$), and rates of omission errors ($F(3, 156) = 5.19$, $p = .002$) were found. No cue dependency effects were observed.

Conclusion: Findings complement prior work on neurocognitive mechanisms underlying ADHD, PTSD, and ADHD+PTSD. Lack of expected group differences for the ADHD-only group may be due to limited power. Additional work is needed to better characterize distinctions among clinical groups, as well as to test effects among women and youth.

41. Astrocytic Protein Levels in Serum Before and After Transcranial Magnetic Stimulation for Pharmacoresistant Major Depressive Disorder

Andrew Fukuda, Alpert Medical School, Brown University

Background: Astrocytes and their contributions to the pathophysiology of depression have garnered attention recently. Peripheral astrocytic proteins show promise as biomarkers but have not been studied with TMS in humans.

Methods: Serum astrocytic protein concentrations pre- and post-TMS treatment for MDD were determined via ELISA. IDS-SR was used as a measure of depression symptom severity, clinical response, and remission.

Results: There was a positive correlation between % improvement in depressive symptom severity and % increase in concentrations of GFAP ($r = .561$, $p<0.05$), and VEGF ($r =.358$, $p<0.05$). Amongst those who remitted, VEGF increased from pre to post TMS whereas VEGF decreased in nonremitters (12.30±10.47 vs -9.24 ±5.78%; $p<0.05$). This same pattern was observed when comparing VEGF changes between responders and non-responders (+9.35 ±7.40% vs -12.42±6.82%; $p<0.05$). Similarly, GFAP also increased in responders but decreased in non-responders (+151.18 ± 29.28% vs -41.10 ±12.21%, $p<0.05$). S100B did not correlate with depression severity changes, nor did they differ between those with different clinical outcomes. AQP4 was also measured in a subset of the population (n=16, 25% male, 75% female; baseline AQP4 level had a positive correlation with the degree of depressive symptomatic improvement measured ($r = .410$, $p<0.05$).

Conclusions: This is the first study examining GFAP, S100B, and AQP4 levels in depressed patients receiving TMS and our data suggests their potential role in the mechanism of action of TMS for depression. Given these positive results, a larger study with more participants is being pursued.

42. Genetic Risk for Use of Hypnotics

Hanna Ollila, Institute for Molecular Medicine, University of Helsinki

Previous studies have estimated that approximately 5-10% of the European and US populations use hypnotics and sedatives. Here, we wanted to study the use patterns of the N05CF ICD10/ATC category Benzodiazepine related drugs including Zopiclone, Zolpidem and Zaleplon. We used the FinnGen Release 6 with registered N05CF drug

purchases and performed genome-wide association study (GWAS), genetic correlation and Mendelian randomization (MR). We identified 20 genome-wide significant lead SNPs. MR analysis exhibited significant causality for N05CF as an outcome with the exposures multisite chronic pain (Inverse-variance weighted (IVW) P = 6.5x10-5, Odds Ratio (OR)[95% Confidence Interval (CI)] = 1.13[1.06-1.2]), anxiety (IVW P = 8.6x10-12, OR[95%CI] = 1.03[1.02-1.04]), depression (IVW P = 4.5x10-5, OR[95%CI] = 2.13 [1.48-3.06]), insomnia (IVW P = 3.6x10-61, OR[95%CI] = 1.07[1.06-1.08]) and schizophrenia (IVW P = 2x10-4, OR[95%CI] = 1.02[1.01-1.03]), suggesting these conditions as risk factors for N05CF use. These results the growing literature between sleep medication use and sleep or psychiatric traits such as anxiety, depression, chronic pain and schizophrenia.

43. Acute Mania Following Ketamine Administration for Acute Pain in a Patient With Family History of Bipolar Disorder

Sarah Jackson, Dartmouth-Hitchcock Medical Center

Background: Ketamine, a noncompetitive antagonist of the N-methyl-D-aspartate (NMDA) receptor, has many clinical uses from treatment of psychiatric disorders to acute pain management. Ketamine's wide range of useful clinical applications is aided by its safety profile in terms of hemodynamic stability; however, variable responses to ketamine administration have been reported in populations with mood disorders.

Methods and Results: We present a case of a 53 year old male with a history of chronic pain on opioids at home who presented to the surgical ICU with concern for small bowel perforation. Given the severity of his pain and his history of high opioid requirement prior to presentation, a sub-anesthetic dose of ketamine was initiated via IV infusion. Within hours, he became euphoric, which was followed by labile mood, visual hallucinations and manic symptoms.

Conclusion: Previous case reports have documented transient mood elevation with sub-anesthetic doses of IV ketamine, but only a few of these cases have met criteria for acute mania. These previous cases were typically associated with individuals with bipolar disorder (BD) or unipolar depression. Our patient had no known history of bipolar disorder, though he does have a sibling with BD. Ketamine is thought to activate glutamate release and promote changes in cortical excitability, which may be a mechanism for mania in patients treated with ketamine. More research is needed to understand mania associated with ketamine and to better predict which patients may experience this adverse effect.

44. Psilocybin-Induced Plasticity and Behavioral Effects in Vivo

Clara Liao, Yale University

Background: Serotonergic psychedelics such as psilocybin have become an exciting new therapeutic avenue towards ameliorating neuropsychiatric diseases including major depressive disorder. Early clinical trials have shown evidence of psilocybin-induced antidepressant effects and yet we have a limited understanding of the biological actions of psilocybin in the frontal cortex.
Methods: We used two-photon microscopy to image longitudinally the apical dendritic spines of layer 5 pyramidal neurons in the mouse medial frontal cortex after a single dose of psilocybin. We also performed behavioral tests to assay head-twitch responses and learned helplessness to assess psilocybin-induced amelioration of depressive-like phenotypes. Conditional knockout animals, including Htr1a fl/fl, Htr2a fl/fl, and Htr2c fl/fl mice are used for pyramidal neuron- and region-specific knockout of 5-HT receptor subtypes in ongoing studies in similar two-photon microscopy and behavioral experiments.
Results: We found that a single dose of psilocybin affects structural plasticity of the frontal cortical circuit in vivo, specifically an increase in spine size and density. The structural remodeling occurred quickly within 24 hours and was persistent 1 month later. Psilocybin mitigates stress-related behavioral phenotypes in rodents. Ongoing studies are determining how Htr1a, Htr2a, and Htr2c knockout of frontal cortex pyramidal neurons affect these behavioral results.
Conclusion: Psilocybin-evoked synaptic rewiring in the cortex is fast, long-lasting, and may underlie therapeutic potential.

45. Psilocybin-Induced Synaptic Structural and Functional Alterations in Frontal Cortex

Lingxiao Shao, Yale University School of Medicine

Background: Psilocybin is a serotonergic psychedelic, which has been found to produce rapid and sustained antidepressant action in early clinical trials. However, the neurobiological mechanisms underlying psilocybin's potential therapeutic effects are still poorly understood.
Methods: We used chronic two-photon microscopy to image longitudinally the apical dendritic spines of layer 5 pyramidal neurons in the mouse medial frontal cortex. We tracked the same dendritic segments before and after administering a single dose of psilocybin (1 mg/kg, i.p.) or saline at 2-day intervals and then again ~1 month later for a total of 7 imaging sessions (n=12 mice). In a second group of mice pretreated with the 5-HT2A receptor antagonist ketanserin (1 mg/kg, i.p.), we repeated longitudinal two-photon imaging (n=8 mice). We also counted head-twitch responses after ketanserin and psilocybin treatment (n=10 mice). Using whole-cell recordings, we tested mEPSCs from putative layer 5 pyramidal neurons in Cg1/M2 region 24 hours after psilocybin or saline injection (n=17 mice).

Results: Psilocybin significantly increased dendritic spine density, spine head width, and spine formation rate within a day of treatment. A portion of the newly formed spines after psilocybin treatment persisted for 34 days. Ketanserin pretreatment abolished completely the psilocybin-induced head-twitch responses but did not eliminate the psilocybin-induced changes in spine density, spine head width, and spine formation rate. Psilocybin also increased mEPSC frequency.

Conclusions: A single dose of psilocybin acts through the 5-HT2A receptor, and promotes persistent structural plasticity the mouse medial frontal cortex. The psilocybin-induced structural remodeling is accompanied by enhanced excitatory neurotransmission.

46. A Common Signaling Pathway for Antidepressant Action

Elif Tunc-Ozcan, Feinberg School of Medicine, Northwestern University, Chicago

Background: Antidepressants affect a variety of neurotransmitter systems in different areas of the brain, and the mechanisms underlying their convergent effects on behavior have been unclear. Here we identify hippocampal bone morphogenetic protein (BMP) signaling as a common downstream pathway that mediates the behavioral effects of five different antidepressant classes and of electroconvulsive therapy.

Methods: We treated naïve or stressed mice with fluoxetine, bupropion, duloxetine, vilazodone, or trazodone for 14 days, or electroconvulsive therapy for 7 days, and performed the zero maze, tail suspension and open field tests to evaluate depression- and anxiety-like behaviors. Next, we examined the levels of primary BMP signaling molecules and neurogenesis in dentate gyrus of these mice. Further, we overexpressed hippocampal BMP4 and treated mice with antidepressants before scrutinizing the behavioral and molecular changes related to antidepressant treatment. Finally, we used chemogenetics to silence newborn hippocampal neurons generated after reduction of BMP signaling and tested the changes in antidepressant-related phenotypes.

Results: All of these therapies decreased BMP signaling and enhance neurogenesis in the hippocampus. Preventing the decrease in BMP signaling blocked the effect of antidepressant treatment on behavioral and molecular phenotypes. Conversely, inhibition of BMP signaling in hippocampal newborn neurons was sufficient to produce an antidepressant effect, while chemogenetic silencing of newborn neurons prevents the antidepressant effect.

Conclusion: Our results highlight that inhibition of hippocampal BMP signaling is both necessary and sufficient to mediate the effects of multiple classes of antidepressants.

47. Modifiers of High Genetic Risk for Methamphetamine Use

Tamara Phillips, VA Portland Health Care System

Little is known about genetic factors that impact risk for methamphetamine use. A mouse selective breeding project identified a SNP in the trace amine-associated receptor 1 (Taar1) gene that accounts for 60% of the genetic variance in voluntary methamphetamine intake. Though the identified spontaneous mutation increases overall methamphetamine intake in the population of homozygotes (Taar1m1J/m1J), some Taar1m1J/m1J individuals exhibit resistance. We performed a new selective breeding project to identify modifiers of the Taar1 mutation effect. Mice with a highly genetically heterogeneous background, but all Taar1m1J/m1J, were selectively bred for high (MAH) or low (MAL) methamphetamine intake. A population of Taar1+/+ mice was retained as a control line (MAC). At the end of selective breeding, average methamphetamine intake (±SEM) was 6.3±0.4 mg/kg and 3.0±0.3 mg/kg in MAH, MAL, respectively; MAC mice consumed only 0.5±0.1 mg/kg methamphetamine. Tissue samples from the nucleus accumbens, ventral midbrain and prefrontal cortex were submitted for RNA-Seq analysis. Principal component analysis of expression data supported significant clustering by tissue type, but not by sex or line. However, when the principal component analyses were performed on residuals, the MAH line data clustered separately from MAC and MAL for all brain regions, with MAC and MAL clustering together. This outcome suggests that selective breeding induced genetic changes in the MAL line that modified the impact of the high risk Taar1m1J/m1J genotype and reduced methamphetamine intake. Additional analyses will identify transcriptome changes differentiating the selected lines. This information could lead to novel therapeutics for methamphetamine addiction.

48. An International and Multi-Ancestral Genome-Wide Association Study Meta-Analysis of Cannabis Use Disorders

Daniel Levey, Yale University

Background: Cannabis use is becoming more pervasive as access has increased due to changes in legal status of the drug worldwide. It is crucially important to understand genetic risk factors underlying cannabis use disorder (CUD) that may have an impact on public health in light of the changing legal status of cannabis and treatment needs.

Methods: We performed a genome-wide association study in MVP and meta-analysis of CUD in more than 1 million individuals from four genetically-determined ancestries (European, EUR; African, AFR; Admixed-American, AMR; East Asian, EAS). Covariate-adjusted LD score regression (covLDSC) was applied to calculate heritability in each ancestry. Downstream in-silico analyses were performed to make causal inferences implied by genetic association, genetic correlations with related traits, and functional gene enrichments for cannabis use disorders. MultiXcan was used to integrate genetic association with information regarding expression quantitative trait loci in brain and blood tissues.

Results: We discovered genomewide-significant loci in all 4 ancestries: 22 in EUR (lead SNP rs56372821 p=7.24e-14, eQTL for CHRNA2), 2 each in AFR (lead SNP rs574008891

p=2.68e-8, in MCCC2) and EAS (lead SNP rs78561048 p=6.71e-9), and 1 in AMR (lead SNP rs9815757 p=4.36e-8). We show significant heritability: EUR h2=8.5% (SE=0.0045), AFR h2=12.7% (SE=0.0276), AMR h2=16.8% (SE=0.0653). MultiXcan implicated several genes including upregulation of bassoon presynaptic cytomatrix protein (BSN) expression with strongest evidence in cortex (p=9.10e-10).

Discussion: We extended previous CUD studies, greatly increasing the number of discovered risk loci in populations of European descent and uncovering the first genome-wide significant associations in other ancestry groups.

49. Runs of Homozygosity Predict the Severity of Alcohol Use Disorders and Potentially Link Inflammatory Response to AUD in an American Indian Population
Qian Peng, The Scripps Research Institute

Background. We have been investigating genetic and environmental risk factors for alcohol use disorder (AUD) in a group of American Indians (AI) that have elevated rates of AUD than the general U.S. population. One genetic characteristic, shared by many indigenous populations around the world who have experienced elevated risk of substance abuse, is an enrichment of long tracks of autozygosity in the genome, which can be estimated as runs of homozygosity (ROH). This study explored the potential link between ROH and AUD in the AI population.
Methods. We scanned the whole genome sequences of 742 admixed AI individuals to estimate overall levels of ROH and obtain consensus ROH segments.

Results. We have found that increased burden of ROH is likely a risk factor for severe AUD in the AI. ROH regions also harbor a higher proportion of immune genes as one's ROH burden increases; the relationship is most pronounced in highly admixed individuals but diminishes in individuals with high levels of AI ancestry, suggesting that the relationship between the immune genes and the AI ancestry within ROH was likely driven by population reduction and the subsequent bottleneck and admixture. We have identified an ROH island on chromosome 1p32.3 linked to AUD severity that contains genes involved in lipid metabolism, oxidative stress and inflammatory response. The region is enriched with genes regulated by TLR signaling in immune cells.

Conclusion. Our data suggest that ROH are associated with risk for AUD severity, potentially through inflammatory and immune responses, in this AI population.

50. Epigenome-Wide Association Study Identifies New Loci Associated With Cocaine Dependence in African Americans and European Americans
Sheila Nagamatsu, Yale University School of Medicine

Emerging evidence suggests that epigenetic mechanisms play an important role in the etiology of cocaine dependence. We examined genome-wide DNA methylation (DNAm) changes associated with cocaine dependence (CD) in African American (AA) and European American (EA) populations.
DNAm from whole blood was assessed in 1036 individuals (377 AAs; 514 EAs) from the Yale-Penn study of drug and alcohol dependence genetics using the Illumina HumanMethylation EPIC BeadChip. An epigenome-wide association study (EWAS) was conducted using the minfi R package. Meta-EWAS was performed using METAL software within and across populations. A differentially methylated region (DMR) analysis was performed in AAs and EAs, separately, using 'DMRcate'.

We identified three genome-wide significant (GWS) CpG sites in EAs, within the PCDHGA11 (cg24954895;p-value=1.565e-18), PTTG1IP (cg13438128;p-value=5.91e-10), and TAF4 (cg14080585;p-value=5.28E-08) genes; and one GWS site in AAs, within the COL18A1 gene (cg09706833;p-value=3.80E-08). Trans-ancestry meta-EWAS identified the same four sites as the ancestry-specific analyses. We identified one significant DMR associated with CD in AAs (chr20;36148767-36149022;12 CpGs;min_smoothed_fdr = 2.51567e-28;BLCAP); and none in EAs.

We found four novel loci that are differentially methylated in EAs or AAs with CD. One gene, PCDHGA11, was previously associated with cocaine withdrawal as being differentially expressed in the nucleus accumbens of mice. We also identified one DMR in the BLCAP gene that was associated with CD in AAs. These potential epigenetic biomarkers of CD, if replicated, can help to inform preventive and treatment interventions for CD.

51. Chromatin Architecture in Addiction Circuitry Elucidates Biological Mechanisms Underlying Cigarette Smoking and Alcohol Use Traits

Nancy Sey, University of North Carolina at Chapel Hill

Background - Alcohol and tobacco are prevalent substances used in the United States. Despite their health burden, there is a lapse in treatment options for individuals with substance use disorders (SUD), which can be attributed to insufficient understanding of their underlying neurobiology. Genome-wide association studies (GWAS) have identified over 400 genomic loci to be associated with alcohol use and cigarette smoking. However, given that the majority of risk variants reside in non-coding regions of the genome, deciphering their target genes and neurobiological processes remain a challenge.
Methods - To delineate the biological impact of substance use-associated genetic risk factors, we used Hi-C coupled MAGMA (H-MAGMA) developed from cortical neurons (CN) and midbrain dopaminergic neurons (DN). We applied neuronal subtype specific H-MAGMA to GWAS summary statistics of problematic alcohol use (PAU), drinks per week (DPW), nicotine dependence (ND), and cigarettes per day (CPD).

Results and Discussion - After the identification of risk genes, we found that pathways including ethanol metabolic process and alcohol catabolic process to be associated with PAU and DPW, while response to nicotine and acetylcholinergic pathways identified for ND and CPD. Lastly, we employed single-cell analyses to identify key cell types involved in substance use etiology. Using CN H-MAGMA-associated genes, we identified cigarette smoking and alcohol use traits to be enriched for cortical excitatory neurons. Moreover, we identified DN H-MAGMA-associated genes to be enriched for dopaminergic, GABAergic, and serotonergic neurons in the midbrain, suggesting them as relevant cell types that may contribute to substance use etiology.

52. Sex-Specific Reduction of Cortical Transcriptomic Aging in Individuals With Opioid Use Disorder

Jose Martinez-Magana, Yale University School of Medicine Department of Psychiatry

Background: Biological aging clocks are important for identifying genetic and environmental interactions on health risk. Studies suggest that substance use disorders can have an impact on biological clocks. Recent studies developed a transcriptome-based biological clock that may outperform other biological aging estimators. This study evaluated transcriptomic aging in four prefrontal cortex brain regions in tissue from groups differing in sex and opioid use disorder (OUD) status.
Methods: Tissue was collected from 138 individuals, including 24 females and 21 males with OUD. We generated transcriptome age (RNAage) based on a previously brain- and multi-tissue-trained transcriptomic clock (RNAAgeCalc) and calculated the RNA age acceleration (RNAageAcel).

Results: We found a higher correlation of the brain-trained chronological age and RNAage (r2 range = 0.67 to 0.72, p-value < 2.26e-16) compared to the multi-tissue trained clock (r2 range = 0.48 to 0.52, p-value range = 2.6e-09 to 3.4e-11) for all prefrontal cortex brain regions. In the sex-stratified analysis, no significant differences were found in RNAAgeAcel between males and females. In OUD, we found a reduction of RNAageAcel in OUD individuals compared to non-OUD in three (dACC, OFC, and sgPFC) of the four PFC regions.

Conclusion: Our findings indicate a decreased cortical transcriptomic age acceleration in males with OUD. These findings are consistent with a previous study that showed a lower epigenetic age in the orbitofrontal cortex of heroin users. However, more research is needed to understand better the implications of opioid use on biological aging.

53. Distinct Kappa-Opioid Receptor-Expressing Projections From the Ventral Tegmental Area Display Unique Molecular Identities and Contributions to Pain and Opioid Withdrawal

Ruby Holland, University of Pittsburgh School of Medicine

Background: Opioid withdrawal is a distressing flu-like illness resulting from the abrupt cessation of opioids. Opioid withdrawal frequently results in relapse and represents a significant barrier to recovery from opioid use disorder. Therefore, it is necessary to identify the circuits impacted by opioids which underly the development of withdrawal. Recruitment of the kappa-opioid receptor (KOR) system is critical in chronic pain and other aversive states. However, the mechanisms through which KOR-expressing neurons emanating from the ventral tegmental area (VTA-KOR neurons) contribute to opioid withdrawal remains elusive.
Methods: Here, we utilize genetic and behavioral approaches in the Oprk1-cre mouse to characterize VTA-KOR neural anatomy and elucidate their contributions to opioid withdrawal behaviors in vivo.

Results: We show through RNAScope FISH that the majority of Oprk1+ neurons in the VTA co-express markers for dopaminergic neurons, while a subset of Oprk1+ neurons express markers for glutamatergic neurons, or both. We also found that the relative expression of Th in VTA-KOR neurons is unique for distinct projection targets. In morphine-naïve mice, chemogenetic activation of VTA-KOR neurons increased pain thresholds, while inhibition of VTA-KOR neurons had no effect. In morphine-dependent mice, chemogenetic activation of VTA-KOR neurons reduced naloxone-precipitated withdrawal behaviors and abolished conditioned place aversion. Interestingly, morphine-dependent mice expressing channelrhodopsin in VTA-KOR neurons displayed a real-time place preference to light stimulation.

Conclusions: Taken together, this evidence identifies VTA-KOR neurons as a genetically diverse population overlapping with VTA dopamine neurons, and strongly implicates VTA-KOR neural downregulation in the development of opioid withdrawal.

54. Investigating the Role of Nitric Oxide Synthase 1 in the Interpeduncular Nucleus in Tolerance to Drugs of Abuse

Jessica Ables, Icahn School of Medicine At Mount Sinai

Background: Nitric oxide synthase 1 (NOS1) in the interpeduncular nucleus (IPN) is upregulated by chronic nicotine and disrupting Nos1 in the IPN abolishes place preference for nicotine. Systemic administration of a NOS1 inhibitor can prevent the development of analgesic tolerance to opioids, reverse established tolerance, and prevent and reverse withdrawal to chronic opioids. We are developing tools to visualize NO in behaving animals to investigate whether regulating Nos1 in the IPN affects the development of reward tolerance, similar to analgesic tolerance.
Methods: Mice underwent IVSA of escalating doses of nicotine or oxycodone and were subsequently perfused for IHC of Nos1. We acquired a plasmid containing the fluorescent

nitric oxide sensor geNOp and cloned it into a Cre-dependent adeno-associated viral vector. Mice were steroetaxically injected into the IPN (AP: -3.6, ML: 1.7, DV 5, 20 degree angle) and perfused 2-3 weeks later for verification of expression. Male mice were provided NOS inhibitors in the drinking water for 2 weeks and baseline behavior assessed to ensure no significant impairments of the doses tested. Mice were provided oxycodone in drinking water prior to CPP for oxycodone to establish an assay of tolerance.

Results: Both nicotine and oxycodone increase NOS1 expression in the IPN. While our virus expresses well in vivo, the fluorescence is not bright enough for fiber photometry. NOS inhibitors have no significant effect on locomotion or memory. Mice readily drink oxycodone water and 5d is sufficient to induce tolerance in CPP.

Conclusion: NOS1 may be a target to prevent reward tolerance.

55. Mechanisms for Brain- And Sex- Specific Alterations in Circular RNA Expression Following Prenatal Alcohol Exposure

Grigorios Papageorgiou, University of New Mexico School of Medicine

Circular RNAs (circRNAs) are a novel category of non-coding RNAs derived from the back-splicing and covalent joining of exons or introns. Recent studies have suggested that circRNAs are preferentially generated from synaptic plasticity-related genes and are particularly enriched in the brain. Although some circRNAs have been found to sequester microRNAs and others to associate with RNA-binding proteins (RBPs), the mechanism of action of the majority of circRNAs remains poorly understood. Moreover, little is known about the potential involvement of circRNAs in Fetal Alcohol Spectrum Disorders (FASD). Using circRNA-specific quantification, we have found that circHomer1, an activity-dependet circRNA derived from Homer protein homolog 1 (Homer1), is significantly downregulated in male hippocampus and frontal cortex of mice subjected to prenatal alcohol exposure (PAE). Our data suggest that the CREB-regulated RNA-binding protein Eukaryotic initiation factor 4A-III (Eif4a3) binds at the circHomer1 backspliced junction and can promote its biogenesis. Interestingly, levels of Eif4a3 are significantly reduced in the hippocampus of male PAE mice, whereas expression of an imprinted and sexually-dimorphic long non-coding RNA, capable of inhibiting Eif4a3 function, is significantly upregulated in the frontal cortex of male PAE mice. Furthermore, in vivo shRNA-mediated knockdown of circHomer1 in mouse frontal cortex suggests that is capable of modulating synaptic gene expression, neuronal function and coordinated cortical activity, as well as behavioral flexibility. Lastly, we show that circHomer1 and Eif4a3 are positively regulated by glutamate receptor activation via the PKA/MEK/Erk/CREB signalling. Taken together our work introduces novel molecular networks with potential improtance for FASD.

56. Neurobiological Mechanisms Underlying Vulnerability and Resilience to Cannabis Addiction

Elena Martin Garcia, Universitat Pompeu Fabra

Background: A hallmark of addiction is the loss of inhibitory control, leading to compulsive behavior in addicted individuals.
Methods: We used a mouse model of drug addiction using WIN 55,512-2 intravenous self-administration (0.0125 mg/kg/infusion) in C57Bl/6J, targeting the prelimbic medial prefrontal cortex (mPFC) to nucleus accumbens (NAc) pathway using chemogenetic approaches. We selectively expressed the inhibitory designer receptor exclusively activated by designer drug (hM4Di-DREADD) in mPFC projecting neurons by bilateral injections of a Cre-dependent AAV expressing hM4Di-DREADD into the prelimbic mPFC of mice and a retrograde AAV expressing Cre recombinase into the NAc core. Thus, the hM4Di receptor was expressed in prelimbic neurons that directly project to the NAc core. To activate the hM4Di-DREADD, clozapine N-oxide was administered using Alzet osmotic minipumps, implanted subcutaneously in the back of the mice, that delivered a constant flow rate of 0.25 µl/h during 28 days. In adolescent mice, we administered THC (5 mg/kg, i.p.) or vehicle during postnatal days 35 to 55, and we evaluated its impact on the vulnerability to develop cannabis addiction during adulthood.

Results: We found that the resilient or vulnerable phenotype can be obtained in the mouse model of cannabis addiction. Hypoactivity of mPFC to NAc projecting neurons promoted impulsivity-like behavior in C57Bl/6J mice. Finally, THC chronic administration during adolescence was a risk factor for impulsivity-like behavior in adulthood.

Conclusions: Understanding the neurobiological mechanisms underlying resilience versus vulnerability to cannabis addiction is expected to pave the way for novel and efficient interventions to battle this mental disorder.

57. Spinning out of Control: A Case of an Atypical Presentation of Cocaine Ingestion on Mirtazapine

Hajira Chaudhry, Henry Ford Hospital

Background: 13% of Americans started or increased substance use to cope with stress or emotions related to COVID-19. The rise in deaths involving both cocaine and fentanyl has tripled. Cocaine and fentanyl have a potential interaction via p-glycoprotein efflux, are metabolized in CYP3A4, and have other potential drug−drug interactions. To aid with timely initiation of treatment in atypical populations, a geriatric case presentation of likely ingestion of fentanyl or synthetic-opioid contaminated cocaine with concurrent mirtazapine will be presented.
Methods: We used search engines Pubmed, Scopus, and Web of Science using the keywords and search strategy {(cocaine+ fentanyl), (cocaine + bradycardia + hypotension), (cocaine + toxidromes), (fentanyl + toxidromes) (fentanyl + bradycardia + hypotension)} 383 articles found, (cocaine or fentanyl + mirtazapine) 17 articles found

Case/Results: A 72-year-old woman arrived with bradycardia, hypotension, respiratory alkalosis, metabolic alkalosis, GSC of 3, miosis, and urine toxicology positive for cocaine was intubated and then extubated after 4 hours. Electroencephalography, CT head, CTA head and neck, other laboratory studies were non-concerning. EKG showed Qtc of 520ms with a normal transthoracic echocardiogram. No evidence of intentional overdose or medication non-adherence. She was continued on duloxetine 60mg and mirtazapine 30mg QHS for depressive symptoms.

Discussion: Presentation was atypical for cocaine. Co-ingestion should be considered. Fentanyl-related respiratory depression peaks at 5 minutes, recovers in 4 hours, and can cause QTC-prolongation. Naloxone efficacy is inconsistent. Mirtazapine may have altered pharmacokinetics CYP3A4 with concurrent use of cocaine and fentanyl, but can potentially reduce anxiety and depressive-like behavior during cocaine withdrawal.

58. That's Snort a Good Idea: Possible Stroke-Like Symptoms in the Setting of Cocaine Use

Hajira Chaudhry, Henry Ford Hospital

Background: With a 13% increase in substance use during the pandemic, knowledge of substance toxicity presentations are vital. Cocaine remains a common cause of drug-related emergency department (ED) visits in the United States, and when combined with other substances the mortality rates are higher. In animal studies angiographic evidence shows that cocaine-induced vasospasm can cause vascular occlusion. Endothelin-1 may be an important mediator of cocaine-induced cerebral vasospasm. To help with timely assessment, an atypical case of cocaine intoxication in the setting of chronic cocaine use will be presented.

Methods: We searched Pubmed, Scopus, and Web of Science using the keywords {(cocaine + neurological), (cocaine + cerebral vasospasm)} 53 discussed cocaine and neurological complications which included 4 animal studies, 13 imaging or pathology studies.

Case/Results: A 25-year-old woman with polysubstance use and bipolar disorder was brought to the ED for altered mentation. Urine toxicology was positive for cocaine. Patient presents with hypertension, increased respiratory rate, dilated pupils, questionable muscle tremens and bradycardia. Patient's BMI was 17.74, and it was unclear if malnutrition was contributing. MRI brain and CT head were normal. Neurology was consulted.

Discussion: Patient's neurological symptoms including unilateral motor symptoms resolved within 24 hours and were thought to be due to vasospasms from cocaine intoxication. Her bradycardia could be due to chronic use. 73% of patients with cocaine-induced stroke or vasospasm have no cardiovascular risk factors. Due to the frequency of

cocaine use, providers should be aware of cocaine toxicity presentations to allow for timely management.

59. Mask but Not Least: A Case of an Intentional Lamotrigine Overdose and Methamphetamine Abuse With a Complicated Clinical Course

Hajira Chaudhry, Henry Ford Hospital

Background: With a 13% increase in substance use during the pandemic, knowledge of substances and their interactions, including methamphetamine is vital. Lamotrigine's (LTG's) anti-manic properties may be due to glutamatergic neurotransmission and voltage-gated sodium channel opening attenuating the release of excitatory neurotransmitters. In rodent studies, LTG prevented initiation of neuroplastic prepulse inhibition deficit induced via repeated administration of methamphetamine. LTG also blocked methamphetamine-induced increases in extracellular glutamate levels and the development of apoptosis in the medial prefrontal cortex. However, LTG ingestions of 525mg can produce seizures (55%), Glasgow Coma Scale ≤8 (20%), hypotension (12%), and cardiac arrest (6%). Methamphetamine lowers the seizure threshold, increases serotonin syndrome, worsens cardiac outcomes, and abrupt cessation results in CNS depression with changes on sleep EEG. Potential interactions may have complicated the clinical course for a case of LTG overdose with methamphetamine use.
Methods: We searched Pubmed, Scopus, and Web of Science using the keywords {(lamotrigine + amphetamine)} 31 articles were found

Case/Results: A 35-year-old female with epilepsy (lamotrigine 300mg BID), regular methamphetamine use, and bipolar type-2 (citalopram 40mg, buspirone 15mg TID, and aripiprazole 5mg) presented with hypoxia after an intentional overdose of 8,000mg of lamotrigine now status post tracheostomy and PEG tube placement following failed extubations, seizure per EEG, and prolonged ICU stay.

Discussion: Amphetamine withdrawal could have contributed to her agitation, seizure, and failed extubations. Mild hyperthermia, hypertension, agitation without concern for infection, etc. could be due to mild serotonin syndrome. Understanding interactions between lamotrigine ingestion and methamphetamine may have altered treatment.

60. A Fragile State: Seasonal Dysthymia in an Adult With Osteogenesis Imperfecta

Hajira Chaudhry, Henry Ford Hospital

Background: Osteogenesis Imperfecta (OI) is a rare congenital disorder that affects the COL1A1 or COL1A2 genes that code for type 1 collagen. Col1A1 gene is located on chromosome 17q 21.3 up to 22.1, while the serotonin transporter gene is between locations 17q11.1 and q12 which is associated with depression. possible overlap between genetic mutations has been purposed. Those with sleep apnea and CPAP compliance

continued to endorse depressive and sleepiness symptoms. To discuss possible mechanisms of dysthymia in this population a case will be presented.
Method: We searched Pubmed, Scopus, and Web of Science using Osteogenesis imperfecta with a combination of the following words: depression, psychiatry, psychology, dysthymia, mental health. A total of 24 articles were found 3 involved adult patients with 2 in English.

Case/Results: A 35-year-old female with Osteogenesis imperfecta presented after a fall and was placed on observation. She endorsed seasonal fatigue, a decrease in mood, and difficulty concentrating that did not cause significant functional impairment and did not meet the criteria for a depressive disorder. She agreed to follow up with her providers and declined other psychiatric management.

Discussion: Seasonal dysthymia symptoms were likely compounded by increased isolation due to Covid-19. However, people with OI are more likely to have low vitamin D, chronic pain, which can increase depression. One study also found elevated monoamine oxidase activity. Psychiatric complaints are also rare side-effects from bisphosphonates. Collagen disorders as also more likely to have gastrointestinal co-morbidities that may alter pharmacodynamics. All of which can inform or change treatment.

61. Genetic Determinants in Chronic Fatigue Syndrome

Anniina Tervi, Institute for Molecular Medicine, HiLIFE-Helsinki Institute of Life Science, University of Helsinki

Chronic fatigue syndrome (CFS) is severe chronic disease with unknown disease mechanism. A core characteristic of the disease is debilitating fatigue that worsens after physical or mental exercise and is not resolved by rest. Another key factor includes symptoms of dysautonomia. While mechanisms ranging from metabolic, immune and psychiatric symptoms have been suggested, the causal mechanisms behind CFS and dysautonomia are largely unknown. Therefore, it is crucial to identify mechanisms that contribute to risk and prognosis of CFS as this may provide tools to understand disease mechanisms and provide treatment options.
Our goal was to identify genetic risk factors that contribute to CFS in order to better understand biological mechanisms and causal factors behind the risk of developing CFS.

To capture the symptoms of dysautonomia and fatigue and to estimate their population prevalence, we defined a phenotype of fatigue and dysautonomia using 14 ICD codes of diseases that contained dysautonomia and fatigue. A total of 15 628 individuals in the UK biobank had ICD-code based fatigue and dysautonomia diagnosis. GWAS analysis identified genetic variant with statistically significant association with fatigue and dysautonomia at the rs7084501 in gene ADRA2A (P=2.1e-11), which codes for the Alpha-

2-adrenergic receptor. We then explored the effect on expression in GTEx. The same variant was an eQTL in arterial tissue for ADRA2A (P=3.0e-15).

Our findings indicate that ADRA2A may be involved in the aetiology of fatigue and dysautonomia. This finding points the possible biological mechanisms toward dysfunction of the autonomic nervous system in CFS, fatigue and dysautonomia.

62. International COVID-19 Host Genetics Initiative Untangling Genetic Risk Factors of Long COVID

Vilma Lammi, Institute for Molecular Medicine Finland (FIMM), University of Helsinki

Background: WHO has published a clinical definition of the post COVID-19 condition, "Long COVID", as a condition occurring "usually 3 months from the onset of COVID-19 with symptoms" "that cannot be explained by an alternative diagnosis". Debilitating fatigue, exercise intolerance, cognitive dysfunction, disturbed sleep, anxiety, and depression are among the most prevalent symptoms.

Methods: We have established an open global collaboration for elucidating genetic risk factors of Long COVID. The Long COVID Host Genetics Initiative comprises 46 studies across 23 countries with genotype data combined to questionnaire information of symptoms and/or electronic health record (EHR) data of diagnoses.

For our first data freeze, contributing studies (N=11) have performed genome-wide association analyses (GWAS) comparing Long COVID (N=1,588) to individuals who have recovered from COVID within 3 months (N=96,714), and to population controls (N=800,478).

Results: GWAS of individual cohorts have suggested potential variants associated with Long COVID but without genome-wide statistical significance. We are currently running meta-analyses combining data from these studies.

In FinnGen sample from Finland with 2,018 participants with a PCR-verified SARS-CoV-2 infection, and 50 (2.4%) with a Long COVID diagnosis, Long COVID associated with previous autoimmune diseases (p = 0.026, OR[se] = 2.15 [1.41]) but not with depression (p = 0.71, OR [se] = 1.20 [1.75]) or asthma (p = 0.43, OR[se] = 1.55 [1.74]).

Conclusion: Our findings indicate that Long COVID may be related to autoimmunity. The Long COVID Host Genetics Initiative allows us to identify genetic risk factors and test causality between autoimmunity and Long COVID.

63. Mitochondrial DNA Variants Associated With Bulimia Nervosa

Ana Silva, Centre for Addiction and Mental Health

Background: Bulimia nervosa (BN) is a type of eating disorder characterized by recurrent episodes of binge eating in combination with some form of inappropriate compensatory behavior. Studies have found increases in reactive oxygen species (ROS) production and revealed the presence of oxidative stress (OS) among patients with BN. Mitochondria are the primary source of ROS, being key players in the OS cascade. Conversely, increased ROS generation could result in the accumulation of further mitochondrial DNA (mtDNA) mutations. The underlying genetic mechanisms of BN are still poorly understood. This study aimed to examine the role of mtDNA variants in conferring risk for BN and verified whether mtDNA variants are associated with lower body mass index (BMI).
Methods: We analyzed mtDNA variants in a subset of 85 women diagnosed with BN, age range 17-45 years, using next-generation sequencing. mtDNA variant analysis from sequencing data was performed by using Mutect2 caller. Common mtDNA variants were tested with the phenotypes using linear regression in Plink.

Results: We identified 49 common mtDNA variants in our sample. In addition, we found variants nominally significant associated with BMI change and EDI body dissatisfaction subscales in our sample (MT-ND1 (g.3360 A>G, p=0.047), MT-ND2 (g.4824 A>G, p= 0.023), MT-ATP6 (g.8705 T>C, p=0.003), MT-CO2 (g.7819 C>A, p=0.025).

Conclusions: In summary, this study identified mtDNA variants associated with BMI change and body dissatisfaction subscales, suggesting that mtDNA play a role in BN. Replication in larger sample size is required to validate our initial report of mtDNA variants in BN.

64. Stability and Plasticity of Steady-State Visual-Evoked Potential Contrast-Response Functions

Ryan Ash, Stanford University School of Medicine, Dept. of Psychiatry and Behv. Sciences

Background: A repeated measure of neural activity that is stable over time when unperturbed is needed to be able to meaningfully measure neuroplastic changes in the brain. We assessed the repeated-measure within-day and across-day stability of the steady-state visual-evoked potential (ssVEP), an exceptionally high signal-to-noise electrophysiological readout of neural activity in human visual cortex, in preparation for studies of visual cortical neuroplasticity.
Methods: We re-analyzed data from Dmochowski et al Neuroimage 2015, in which ssVEP contrast-sweep responses (90 trials, 22 subjects) were measured daily for 4 days using a 128-channel EGI hydrocel EEG system and custom xDIVA software. Reliable components analysis was used for dimensionality reduction.

Results: Response amplitudes were stable in individual subjects, with measured within-day and across-day coefficients of variation (CV= SD/ Mean) of 10±1% and 17±2%,

respectively. The ssVEP protocol was powered to detect a 10% change with 10 subjects, a 20% change with 7 subjects, and a 40% change with 5 subjects (statistical power= 0.8).

Conclusions: Contrast-sweep steady-state VEPs are stable over time when unperturbed. Future work will partition the sources of response variability including brain state, learning/plasticity effects, physiological noise, and measurement. We are currently developing methods to induce plasticity in ssVEP contrast-response functions using repetitive transcranial magnetic stimulation, repetitive sensory stimulation, and transcranial focused ultrasound.

65. Implications of Psychological Comorbidities in Stuttering
Shelly Jo Kraft, Wayne State University

Background: The "Other Health Inventory" (OHI) was developed to examine general health conditions that present across the lifespan of those who have a developmental stutter. In a recent investigation of electronic medical records, over 80 conditions were statistically significant for this population. Of particular interest is the newly established presence of psychiatric disorders in people who stutter. The OHI examines health comorbidities via survey report in attempt to elucidate the underlying interconnectivity, genetic regulation, and pathophysiology between disorders; are they part of the sequela of stuttering or potentially linked through shared contributory genes?
Methods: A health inventory survey was posted online seeking individuals 18+ who identify as having a stutter to report on the health conditions they have experienced. A proportion analysis was conducted using the self-reported positive rate and comparing it to empirical prevalence data for that specific disorder.

Result: Conditions found to be statistically significant in this cohort included: motor tics, essential tremor, lack of coordination, mood disorder, anxiety disorder, trauma and stress-related disorder, suicidal ideation, respiratory disorders, abdominal pain, constipation, hemorrhoids.

Discussion: Multiple psychological disorders demonstrated significance in this study which lends support to the emerging body of literature reporting high levels of anxiety and depressed mood in individuals who stutter. Novel to this study is the finding of high levels of suicidal ideation which has not been previously identified the stuttering literature. Discussing this finding further is crucial to inform both clinical treatment approaches and scope of training to include competency in this area.

66. Key Discoveries of the Genetic Basis of Stuttering Hint at a Neurological Origin
Hannah Polikowsky, Vanderbilt Genetics Institute, Vanderbilt University Medical Center

Background: Recent population studies have provided evidence for both the heritability and polygenicity of developmental stuttering. Investigations leveraging large biorepositories and a clinically ascertained stuttering cohort have identified common population-based stuttering susceptibility variants with initial clues into a potential neurological basis for stuttering. This study utilizes the results of self-reported stuttering from a sample size of more than 1 million individuals to further elucidate the genetic basis of stuttering.
Methods: We performed a meta-analysis for 1,123,019 (99,776 cases) individuals with MR-MEGA using summary statistics provided by 23andme, Inc. Genes associated with stuttering were identified using MAGMA. We conducted an enrichment test for gene modules using genes associated with variant signals. Gene modules comprised groups of functionally related gene modules Gerring et al. identified from GTEx tissue gene expression data. We also performed genetic correlation analyses for various neurological traits.

Results: Meta-analysis identified fourteen loci reaching genome-wide significance. Genes associated with stuttering were enriched for expression in brain tissue. Gene module enrichment analysis identified enrichment for modules in the caudate basal ganglia, cerebellum, and amygdala. Gene modules included genes such as GRM5 and AUTS2 , both found in an enriched module with implicated biological processes including neurogenesis, anatomical structure, cell morphogenesis, and catabolic process. Genetic correlation analysis identified a significant inverse correlation with stuttering and rhythmicity (ie. The inability to clap to a beat correlated with stuttering).

Discussion: This large population study provides novel evidence for a neurological origin of stuttering, which may lead to improved treatment of disordered speech.

67. Sex-Stratified Genome-Wide Association Studies and Comorbidity Analysis of Stuttering Using DNA Biobank and 23andMe Data

Dillon Pruett, Vanderbilt University

Background: Large-scale heritability studies have established clear evidence that a genetic component for stuttering exists. Additionally, stuttering is more common in males and clinical evidence suggests a high incidence of comorbid disorders. This study examines sex-specific comorbidities and sex-specific genetic variants associated with stuttering by 1) utilizing electronic health records (EHRs) and DNA biobank data at Vanderbilt University Medical Center (VUMC) and 2) summary statistics of self-reported stuttering genotyped by 23andMe, Inc. to perform sex-specific comorbidity analyses and sex-stratified genome wide association studies (GWAS).
Methods: Confirmed stuttering cases identified via keyword search, text-mining, and manual review will be stratified by sex and matched to controls in a permutation-based, sex-stratified comorbidity analysis of phecodes (diagnostic groupings of EHR data derived

from ICD-9 codes). Enriched phecodes were used to create sex-specific phenome risk classifier models to highlight additional high-likelihood stuttering cases. These high-likelihood cases were then used to conduct sex-specific GWAS. Results were compared to sex-stratified GWAS on self-reported 23andMe stuttering cases.

Results: Preliminary results from a smaller, non-sex-stratified subset of 572 stuttering cases found 38 phecodes significantly associated with stuttering including codes for developmental delays, speech and language disorder, sleep disorders, and codes related to metabolism and the atopic triad. Results from a larger, sex-stratified dataset are expected to replicate and extend these findings.

Conclusions:Identifying stuttering comorbidities and genetic variants, especially variants that differ between the sexes, has the potential to reveal sex-specific gene involvement in this common, costly, heritable, and often debilitating disorder.

68. Leveraging Genetically-Regulated Expression and a Large Biobank to Phenome-Wide Investigate Late-Onset Alzheimer's Disease-Related Health Outcomes

Hung-Hsin Chen, Vanderbilt University Medical Center

Background: Late-onset Alzheimer's disease (LOAD) is a complex but highly heritable disease. Although previous studies have investigated and reported several comorbidities of LOAD, e.g., diabetes, dyslipidemia, and hypertension, the extent of shared genetic architecture between LOAD and its comorbidities is still unclear. Genetically regulated gene expression (GReX) is an emerging method to test the association between diseases and genes, and it has been used to identify more LOAD-related genes not previously detected through GWAS. Therefore, we leveraged GReX to investigate the shared genetic architecture between LOAD and other phenotypes in the large biobank at Vanderbilt University Medical Center, BioVU, which contains over 95,000 genotyped individuals with electronic health records (EHR).

Methods: PrediXcan was used to impute GReX for all individuals, using joint-tissue imputation models trained in GTEx.v8 data. Association between imputed GReX and each EHR code was tested using logistic regression. To represent known LOAD genes and identify associated health outcomes, we used a set of 216 genes from a previous LOAD GReX meta-analysis (N=58,713). We conducted an enrichment test for each outcome, and the number of associated genes ($p<0.01$) was compared with the empirical null distribution from 100,000 permutations.

Results: A total of 33 outcomes are phenome-wide significantly enriched for LOAD genes after Bonferroni correction, including Graves' disease (gene count=41, p-value<1x10-5), hyperlipemia (count=40, p-value<1x10-5), sleep apnea (count=35, p-value=1x10-5), and diabetes mellitus (count=41, p-value=2x10-5).

Conclusion: We used GReX association in a large biobank to investigate the genetic LOAD-related health outcomes, and identify both known and several potentially novel related traits.

69. Active Kinome Profiling of Disorders of Cognition

Robert McCullumsmith, University of Toledo

Background: We deployed the Pamgene kinome array platform for use with postmortem brain samples and iPSCs from schizophrenia (SZ) and Alzheimer's dementia (AD). The kinome array platform provides a read out of protein kinase activity across hundreds of peptide substrates, measuring global protein kinase activity across serine/threonine and tyrosine subkinomes. We used this omics-based platform for generating novel hypothesis for the pathophysiology of severe neuropsychiatric disorders with cognitive dysfunction.
Methods: We used the Pamgene kinome array platform to assess protein kinase activity in disease (AD, MDD, and SZ) and control (SZ) samples. We also assessed protein kinase activity in stem cell cultures for these disorders. We used R studio based programs (KRSA and UKA) to deconvolve the generated kinome array datasets to identify specific protein kinases altered across these disorders. Information for deconvolution of datasets was supplemented with recombinant kinase and kinase perturbation studies.

Results: A common hit for AD and SZ was adenosine monophosphate kinase (AMPK), a master regulator of insulin signaling pathways. Subsequent studies of AMPK in AD and SZ reveal subunit specific deficits in the frontal cortex in AD, with changes in the regulatory subunits for AMPK . Bioinformatics analyses revealed several novel pathways, as well as several candidate drugs that might be repurposed for the treatment of cognitive deficits in these disorders.

Conclusions: We used a hypothesis-free, kinome-based approach to extend understanding of the pathophysiology of SZ and AD, as well as to provide novel leads to advance the diagnosis and treatment of these often-devastating illnesses.

70. Synapsin-Caveolin-1 Gene Therapy Preserves Neuronal and Synaptic Morphology and Prevents Neurodegeneration in Mouse Model of AD

Shanshan Wang, UCSD

Alzheimer's disease (AD) is the most common form of neurodegeneration and cognitive dysfunction in the elderly. Identifying molecular signals that mitigate and reverse neurodegeneration in AD may be exploited therapeutically. Transgenic AD mice (PSAPP) exhibit learning and memory deficits at 9 and 11 months, respectively, with associated decreased expression of caveolin-1 (Cav-1), a membrane/lipid raft (MLR) scaffolding protein necessary for synaptic and neuroplasticity. Neuronal targeted gene therapy using

synapsin-Cav-1 cDNA (SynCav1) was delivered to the hippocampus of PSAPP mice at three months using adeno-associated virus serotype 9 (AAV9). Bilateral SynCav1 gene therapy preserved MLRs protein profile, learning and memory, hippocampal dendritic arbor, synaptic ultrastructure, and axonal myelin content in 9- and 11-month PSAPP mice, independent of reducing toxic amyloid deposits and astrogliosis. Since PSAPP mice overexpress APP that doesn't reflect human AD pathology, we have further tested SynCav1 in the clinically relevant APPNL-G-F/NL-G-F knock-in (APPKI) mouse model of AD. We have also observed preserved cognitive performance compared to APPKI mice that received the control vector. Our data indicate that SynCav1 can afford neuroprotective effect for the hippocampal neurons in the mouse model of AD independent of affecting amyloid deposits. We are currently testing the potential impact of SynCav1 on neurodegeneration using the PS19 tauopathy mouse.

71. Astrocytes Activities in the External Globus Pallidus Determine the Behavioral Flexibilty and Action Selection Strategy in Operant Conditioning

Doo-Sup Choi, Mayo Clinic

Background: Imbalance in goal-directed and habitual controls is a hallmark of decision-making-related disorders, such as obsessive-compulsive disorder and addiction. The role of external globus pallidus (GPe) in regulating action strategy has long been appreciated. However, it remains unknown how enriched astrocytes in the GPe involves in the action selection strategy.

Methods: We recorded the temporal dynamics of GPe astrocytic activity using calcium imaging during goal-directed and habitual operant conditioning. Then, we confirmed whether a support vector machine (SVM) with data of GPe astrocytic dynamics predicted the operant session type of mice learned. Moreover, we evaluated the effects of chemogenetic activation of the GPe astrocytes on GPe neuronal firing and operant behavioral manner. Furthermore, we examined whether the attentional stimuli recapitulates the effects of the activation of GPe astrocytes.

Results: We found silenced GPe astrocytes during habitual learning compared to goal-directed learning. In the timescale of action events, GPe astrocytic activities were increased immediately after termination of reward-taking behavior before the following action. However, during habitual learning, the increase of astrocytic activity was not evident. Moreover, SVM analysis predicted whether mice perform goal-directed or habitual behaviors. Interestingly, chemogenetic activation of the astrocytes dampened GPe neuronal firings and increased behavioral flexibility. Strikingly, attentional stimuli show similar behavioral consequences with chemogenetic activation of the GPe astrocytes.

Conclusions: Our findings provide a novel insight that the activation of GPe astrocytes improves behavioral flexibility, which may provide a potential therapeutic target for decision-making-related disorders.

72. Wnt Sensitive Regulatory Elements in Neural Progenitors Harbor Neuropsychiatric Disorder and Brain Structure Heritability

Nana Matoba, University of North Carolina at Chapel Hill

Background: GWAS for neuropsychiatric disorders and brain-related traits show enrichment of heritability in tissue-, and cell-type specific regulatory elements (RE)s. However, the function of brain trait associated variants within REs responsive to specific stimuli, like signaling factors, are likely missed at baseline or post-mortem conditions. Previous studies have found that genetic loci associated with risk for neuropsychiatric disorders and interindividual differences in brain structure are enriched near genes of the Wnt pathway.

Methods: Here, we performed ATAC-seq and RNA-seq on primary human neuronal progenitors from 84 genotyped donors treated with 5 nM Wnt3a or vehicle control to characterize Wnt-responsive elements and genes.

Results: 2.8% of peaks and 2.53% of genes were differentially regulated in cells treated by Wnt3a as compared to vehicle conditions (|LFC|>0.5; FDR < 0.05). We observed that LEF1, the known effector of the Wnt signaling pathway, was significantly upregulated (FDR = 7.01 x 10-108) and LEF1 motifs were strongly enriched in Wnt3a-responsive REs (FDR = 1.43 × 10-24). We also found that genetic variants associated with neuropsychiatric disorders and brain traits including schizophrenia and cortical surface are enriched in Wnt-responsive REs (FDR<0.05).

Discussion: We show that stimulus-responsive regulatory elements play important roles in neuropsychiatric disorders and brain traits. In the future, analyzing variants associated with chromatin accessibility or gene expression in response to stimuli may help explain stimulus-specific function of GWAS loci and inform pharmacogenomics approaches to optimize treatments based on genotype.

73. Common Genetic Variation Modulates Lithium-Induced Neural Progenitor Proliferation

Brandon Le, University of North Carolina at Chapel Hill

Background: Lithium stimulates proliferation of neural progenitor cells (NPCs) in the adult hippocampus, an effect required for the therapeutic effects of some antidepressant drugs and interventions. Only a subset of patients taking lithium for bipolar disorder respond to treatment, and an individual's genetic background may contribute to this variance. We explored common genetic effects on proliferative responses to lithium using a genetically diverse library of primary human NPCs.

Methods: We measured lithium-sensitive proliferation with an EdU-based flow cytometry assay in a panel of 80 genotyped primary human NPC lines. We performed genome-wide association tests using the change in the proportion of cells in S-phase between Li-treated and vehicle conditions. We measured Li-sensitive gene expression across time at the associated locus and manipulated GNL3 expression using CRISPRa/i to determine effects on lithium-induced proliferation.

Results: A study-wide significant ($p<1.67e-8$) locus associated with the proliferative response to 1.5mM Lithium on chr3p21.1 colocalized with GWAS signals for bipolar disorder, schizophrenia, and intelligence. Lithium-responsive gene expression at this locus and GTEx brain-eQTL data implicated GNL3 as a putatively causal gene mediating the GWAS result. Manipulation of GNL3 expression in hNPCs produced lithium-sensitive effects on proliferation.

Discussion: A cell culture-based GWAS approach identified a genetic locus and gene that alters human neural progenitor proliferation in response to lithium stimulation. Our results motivate exploration of GNL3's role in modulating lithium responses in adult NPCs and provide a pharmacogenomic map that may predict individualized therapeutic responses to lithium.

74. Modeling PTSD Differential Gene Expression in iPSC Derived Neural Cultures Treated With Glucocorticoids

Cameron Pernia, Harvard Medical School McLean Hospital

Background: Posttraumatic Stress Disorder (PTSD) is a common complex psychiatric disorder affecting 6.1% of Americans, and no efficacious cellular models exists for studying the disease. Our group recently generated single nucleus RNA-seq (snRNA-seq) data human DLPFC brain samples across PTSD, MDD, and healthy controls. In this work, we attempt to recapitulate our PTSD differential gene expression (DEG) results, and publicly available PTSD EWAS data, with in vitro iPSC derived cells, to better understand the role glucocorticoids play in PTSD associated molecular regulation in the brain.

Methods: Two healthy control iPSC lines were differentiated into 4 neurodevelopmental cell-types. At each stage, three samples were treated with dexamethasone (DEX), and harvested for RNA and DNA. RNA triplicates were pooled into 32 separate samples then RiboZero sequenced at ~70M read depths, while paired samples' DNA methylation was quantified with EPIC arrays at 850K CpG sites.

Results: After demonstrating iPSC derived neurons express functional glucocorticoid receptors, we found numerous DEGs (ex. FKBP5, PER1) and methylation sites altered by DEX. Excitingly, identified DEGs highly correlated with our PTSD snRNA findings, with more mature neural cells displaying more relevant PTSD-associated gene expression.

Conclusions: While many psychiatric disorders have been studies in recent years with advanced cell biology techniques, such as iPSC disease modeling, in vitro PTSD studies have yet to incorporate such approaches. iPSC derived neurons treated with DEX are an intriguing model for studying PTSD molecular pathology, as they may usefully model aspects of the neurobiology of stress other cell types don't possess.

75. Massively Parallel Reporter Assay on Schizophrenia Variants Reveals Allelic Regulatory Effects

Jessica McAfee, UNC Chapel Hill

Background: Genome-wide association studies (GWAS) have successfully identified ~6,000 variants that are statistically associated with schizophrenia. Nearly 90% of these risk variants lie within non-coding regions which are poorly characterized, leading to the need to functionally validate these variants in a relevant cell type. To investigate these variants, we employed a massively parallel reporter assay (MPRA) to functionally validate schizophrenia-associated non-coding variants.
Methods: We have taken ~6,000 finemapped schizophrenia GWAS variants provided by Pardiñas et. al. and inserted them into an AAV-based MPRA vector, which was subsequently introduced to human neural progenitors (HNPs).

Results: Previously, schizophrenia GWAS contained as many as 150+ variants per locus. Our MPRA results dramatically reduced the number of variants per locus; most functionally-validated loci contained a single regulatory variant, whereas others contained 2 to 16 regulatory variants. Furthermore, only 11 out of ~140 index SNPs (a SNP with the lowest P value within a GWAS significant locus) showed regulatory effects, suggesting that perfunctorily focusing on index SNPs purely based on statistical associations may not correctly pinpoint causal variants. We identified 440 out of 5,173 variants that exhibited allelic regulatory effects in HNPs (FDR < 0.1), covering 103 GWAS loci.

Conclusions: Our MPRA results exemplify a flaw in our previous assumption that the index variants will generally exert regulatory effects, and that there is only one significant variant per locus. This functional validation assay provides novel insights on causal mechanisms of schizophrenia-associated variants, and changes how we should interpret GWAS results.

76. Differential Vulnerability of CNS Cell Types to Diseases and Drugs

Rammohan Shukla, University of Toledo

In central nervous system (CNS) disorders, mismatches between disease states and therapeutic strategies are highly pronounced, largely because of unanswered questions regarding specific vulnerabilities of different cell types and therapeutic responses. Which

cellular events (housekeeping or salient) are most affected? Which cell types succumb first to challenges, and which exhibit the strongest response to drugs? Are these events coordinated between cell types? How does the disease state and drug affect this coordination? To address these questions, we analyzed single-nucleus-RNAseq (sn-RNAseq) data from the human anterior cingulate cortex—a region involved in many CNS disorders. Density index, a metric for quantifying similarities and dissimilarities across functional profiles, was employed to identify common (housekeeping) or salient functional themes across all cell types. Cell-specific signatures were integrated with existing disease and drug-specific signatures to determine cell-type-specific vulnerabilities, druggabilities, and responsiveness. Clustering of functional profiles revealed cell types jointly participating in these events. SST and VIP interneurons were found to be most vulnerable, whereas pyramidal neurons were least vulnerable. Overall, the disease state is superficial layer-centric, largely influences cell-specific salient themes, strongly impacts disinhibitory neurons, and influences astrocyte interaction with a subset of deep-layer pyramidal neurons. Drug activities, on the other hand, are deep layer-centric and involve activating a distinct subset of deep-layer pyramidal neurons to circumvent the disinhibitory circuit malfunctioning in the disease state. These findings demonstrate a novel application of sn-RNAseq data to explain drug and disease action at a systems level, suggests a targeted drug development and reevaluate various postmortem-based findings.

77. Sex, Age, and Cell-Type Impact the Function of Psychiatric-Associated Variants in Vitro and in Vivo

Din Selmanovic, Washington University School of Medicine

Background: Genome-wide association studies have revealed that non-coding regions of DNA harbor large blocks of common variants associated with neuropsychiatric disorders. Importantly, most psychiatric illnesses show sexual bias (i.e., female bias in MDD susceptibility, and males in ASD). The functional and biological impacts of common variants in linkage regions, as well as the biological effects that drive sexual divergence in psychiatric illnesses, remains fairly unknown.

Methods: We hypothesized that psychiatric risk-associated functional variants interact with sex and produce a greater impact in biased sexes of psychiatric illnesses. We sought to test this by assaying SNP effects on transcription in vitro and in vivo using Massively Parallel Reporter Assays (MPRAs). Given the known molecular effects that organizational testosterone surges have on masculinizing the brain, we characterized SNP effects between sexes in neonatal, young, and adult brains. Likewise, given the heterogeneity of brain cell types and intricacies of gene regulation, we assayed cell-type-specific MPRAs using novel cell-type-specific approaches.

Results: We identified rSNPs with effects consistent across sex and ages, with a subset displaying sex interacting effects. We also identified recurrent disruption of sex hormone

receptor binding sites by specific variants in neonatal brains undergoing hormonal surges and further cell-type-specific sex-by-allele effects.

Discussion: Our study provides novel insights into psychiatric genetics and how age, biological sex, and cell-type influences rSNPs functionality. Notably, this study provides a novel framework for in vivo MPRAs at various ages to assess functionality and define interactions between variables such as sex and disease variation.

78. Evaluating the Anti-Inflammatory Effect of Antidepressants on White Blood Cell Count Using Electronic Health Records

Julia Sealock, Vanderbilt University

Background: Antidepressant use consistently associates with decreases in pro-inflammatory biomarkers. It is unknown if all antidepressant classes exhibit anti-inflammatory effects and how long the effects last. We utilize electronic health records (EHRs) to examine the short and long-term effects of antidepressants on a clinical pro-inflammatory biomarker, white blood cell count (WBC).

Methods: Using Vanderbilt University Medical Center's de-identified EHR system, we extracted all medication mentions for SSRIs, SNRIs, TCAs, and Atypical antidepressants, and WBC measured in out-patient visits. Three separate longitudinal cohorts were constructed using individuals with WBC measured within 1 month, 6 months, or 1 year before and after the first mention of an antidepressant class. The effect of antidepressant use on WBC was modeled using a time-varying covariate in a linear mixed model controlled for fixed effects of sex, race, and age at WBC measurement, and the random effect of age at measurement.

Results: In the 1-month cohorts, SSRI and Atypical use associated with decreased WBC (pSSRI=5.28e-5, betaSSRI=-0.11; pAtypical=7.77e-8, betaAtypical=-0.21), while SNRI and TCA use did not associate with changes in WBC (pSNRI=0.05, betaSNRI=-0.13; pTCA=0.68, betaTCA=-0.02). All antidepressant classes associated with decreases in WBC in the 6-month cohort (pSSRI=4.43e-19, betaSSRI=-0.10; pSNRI=4.61e-5, betaSNRI=-0.09; pTCA=3.39e-5, betaTCA=-0.09; pAtypical=4.13e-12, betaAtypical=-0.11). Likewise, in the 1-year cohort all antidepressant classes associated with decreases in WBC (SSRI: pSSRI=2.19e-36, betaSSRI=-0.11; pSNRI=6.88e-7, betaSNRI=-0.08; pTCA=7.75e-6, betaTCA=-0.08; pAtypical=1.12e-17, betaAtypical=-0.10).

Discussion: Our results contribute to the immunomodulatory knowledge of antidepressants and suggest the anti-inflammatory effects of antidepressants are present in all classes and persist though up to 1-year of treatment.

79. Detection of Autism Spectrum Disorder-Related Pathogenic Variants by a Novel Structure-Based Approach

Sadhna Rao, University of Southern California

Background: Glutamatergic synapse dysfunction is believed to underlie the development of Autism Spectrum Disorder (ASD) and Intellectual Disability (ID). However, identification of genetic markers that contribute to synaptic dysfunction is notoriously difficult. We recently established the involvement of the TRIO-RAC1 pathway in ASD and ID and identified a pathological de novo missense mutation hotspot in TRIO's GEF1 domain. These ASD/ID-related missense mutations compromise glutamatergic synapse function and likely contribute to the development of ASD/ID. ASD/ID cases with mutations in TRIO's GEF1 domain are increasing, but tools for accurately predicting whether mutations are detrimental to protein function are lacking.

Methods: We deployed protein structural modeling to predict detrimental and benign mutations within TRIO's GEF1 domain. Mutant TRIO-9 constructs were expressed in CA1 pyramidal neurons of organotypic cultured hippocampal slices. AMPA receptor-mediated postsynaptic currents were examined in these neurons using dual whole-cell patch clamp electrophysiology.

Results: Missense mutations in TRIO's GEF1 domain that were predicted to disrupt TRIO-RAC1 binding or stability greatly impaired TRIO-9's influence on glutamatergic synapse function. In contrast, missense mutations in TRIO's GEF1 domain predicted to have no effect on TRIO-RAC1 binding or stability did not impair TRIO-9's influence on glutamatergic synapse function.

Conclusions: We show that structure-based computational predictions and experimental validation can be employed to predict whether missense mutations in the human TRIO gene impede TRIO protein function and role in glutamatergic synapse regulation. Along with genome sequencing, the use of such tools in the identification of pathological mutations will be instrumental in early diagnostics of ASD/ID.

80. Defining the Global Protein Interaction Landscape of High Confidence Autism Spectrum Disorder Risk Genes

Zun Zar Chi Naing, University of California, San Francisco

Autism Spectrum Disorder (ASD) is a highly heritable complex neurodevelopmental disorder. A recent large-scale whole exome sequencing study focusing on de novo and rare genetic variants identified 102 high confidence ASD (hcASD) risk genes. To unravel molecular pathways that might connect the hcASD risk genes, we systematically mapped the interaction landscape of proteins encoded by hcASD genes. To this end we individually expressed 93 affinity-tagged hcASD genes in HEK293T cells and performed affinity purifications coupled to mass spectrometry (AP-MS). The resulting protein-protein

interaction network (ASD-PPI) consisted of 1027 unique protein interactors ('preys'), of which 327 interacted with more than one hcASD gene. Upon integration of the ASD-PPI with data from ASD whole-exome sequencing studies we found that the preys are enriched for damaging variants from ASD individuals. Additionally, we found that preys mirror hcASD in several ASD-relevant characteristics, including enriched expression in prenatal brain tissue and high pLI scores, thus suggesting the relevance of ASD-PPI for ASD. Next, we applied the community detection method HiDeF (Hierarchical community Decoding Framework) to identify biological pathways and protein complexes within the ASD-PPI. This analysis revealed interactions of hcASD genes with stable protein complexes, including the Paf complex, eIF3 complex and BAF complex. Finally, to delineate how patient-derived genetic variants might alter protein interactions of hcASD, we have started to generate AP-MS data for missense mutations predicted to be highly deleterious. This data will be integrated with the ASD-PPI to identify molecular pathways and cellular processes dysregulated by ASD variants.

81. Genetic Architecture of Brain Gene and Isoform Expression
Minsoo Kim, David Geffen School of Medicine at UCLA

Background: The intricate molecular mechanisms occurring within the human brain are under tight genetic control. Existing work has examined genetic influences mainly at the gene-level and thus the extent to which isoform expression in the human brain is influenced by genetic factors is unexplored. This may be important as isoforms are significantly more diverse in the brain than in other tissues.
Methods: Here, we investigate the genetic architecture of 24,905 gene and 93,293 isoform-level expression by integrating the genotype array and frontal cortex bulk RNA-seq data from 855 unrelated European individuals.

Results/Conclusions: We find the average genome-wide SNP-based heritability (h2) estimates to be comparable between gene and isoform expression (0.21 and 0.18, respectively) with the average proportion of h2 from cis-SNPs being 17% and 12%. Multivariate variance components linear mixed model analyses reveal substantial shared genetic influences among isoforms and that genetic correlation typically mirrors phenotypic correlation. Importantly, many genes with increased transcript complexity are only heritable at the isoform-level, motivating the use of isoform-resolution analyses to fully uncover the genetic effects on gene expression.

82. Inducible Calling Cards: Developing Mouse Reagents for Temporally Controlled Recording of Molecular States and Neural Activity
Simona Sarafinovska, Washington University in St. Louis

Background: In psychiatric disorder models, genetically-identical rodents are classified as "susceptible" or "resilient" based on distinct behavioral responses to the same environmental manipulation. This individual-level vulnerability is hypothesized to be caused by epigenetic states prior to exposure and identifying the molecular and cellular circuits mediating such states may lead to new treatments. However, to link antecedent states to eventual classifications, the same animals need to be studied molecularly and behaviorally. Yet, most molecular techniques are destructive, precluding later behavioral analysis. Hence, there's an urgent need to capture molecular states nondestructively
Methods: Calling Cards (CC) records transcription factor (TF)-DNA interactions nondestructively in live mouse brain. CC utilizes a TF-fused piggyBac (PB) transposase which inserts self-reporting transposons (SRTs) near TF-binding sites thus continuously recording epigenetic states for later read-out by single-cell sequencing. Here, to record TF-DNA binding only before behavior, we're developing tamoxifen (TAM)-inducible CC (iCC) transgenic mice.

Results: We tested TFs SP1, which binds active promoters, and Jun, an immediate early gene mapping both circuit-activity and epigenetic states. We tested both via SRT-sequencing and fluorescent reporters to establish dependence of recording on TAM-induction. Furthermore, we also assessed Jun-iCC dependence on both TAM and concurrent neural-activity.

Conclusion: Our results indicate iCC may uniquely allow retroactive analysis of molecular state prior to behavioral manipulation for epigenetic and circuit mapping applications. We continue to benchmark these reagents, characterizing efficacy, general health, and behavior. We believe iCC could prove valuable for molecular psychiatry research broadly on topics including social development, early-life adversity, and stress resilience.

83. Calling Cards: A Platform that Enables Parallel Recording of Enhancer Usage and Gene Expression in Developing Mouse Tissues

Allen Yen, Washington University in St. Louis

Background: Brain development is complex, and how cell fate decisions are made within a seemingly homogenous population of progenitors to become specific neuronal subtypes is not well understood. Studies using ChIP-seq and RNA-seq have revealed epigenetic mechanisms driving such decisions; however, the destructive nature of these methodologies is a limitation which precludes our ability to directly associate the profiled epigenome to its eventual cell fate. Additionally, historical molecular events leading to the current cell state is lost.
Methods: We have recently developed viral transposon-based Calling Cards reagents to enable longitudinal recording of cell type-specific enhancer usage in healthy development and models of disease. We expanded upon this technology and developed transgenic CC mouse lines, aiming to record enhancer usage throughout embryonic development without

viral injections. Transgenic CC mouse lines were generated and crossbred with Cre-dependent piggyBac and Cre-expressing mice, which can be used to direct CC in genetically defined populations. New lines were validated by immunofluorescence and sequencing to recover and map CC insertions.

Results: Transgenic genomic CC insertion densities were found to be highly correlated with insertions derived using established viral CC reagents, demonstrating initial proof of principle. Crossbreeding with various Cre-expressing mice are ongoing to direct CC to the CNS and to specific genetically defined neuronal populations during embryonic development.

Discussion: The transgenic CC mouse lines can be a robust platform to record cell type-specific molecular events throughout development and associate them with eventual cell fate decisions, deepening our understanding of epigenetic states in the developing mouse brain.

84. Fine-Mapping Candidate Neuropsychiatric Regulatory Variants Using Cell Type-Aware Comparative Genomics Across Mammalian Genomes

BaDoi Phan, Carnegie Mellon University

Hundreds of loci with thousands of genetic variants have been associated with addiction-associated behaviors, which are largely thought to act at distal regulatory elements and in tissue- and cell type-specific contexts. Genome conservation has proven to be invaluable to prioritize candidate causal genetic variants. Although powerful, conversation does not account for the highly cell type-specific nature of regulatory elements.
We matched orthologous cell types in the caudate nucleus across human, monkey, and mouse with single-cell or cell type-targeted open chromatin assays. We developed machine learning models, the Cell Type-Activity Conservation Inference Toolkit (CellTACIT), to predict the open chromatin activity of orthologous conserved DNA of 240 mammals. We leverage large cross-mammalian genome constraint measurements and CellTACIT predictions of cell type-specific regulatory elements activity to improve the prioritization of genetic variants associated with addiction-associated and other neuropsychiatric traits.

We show that chromatin regions that can be mapped to increasingly distant mammals (e.g. primates, mouse, bats) and are predicted by CellTACIT to remain active show increasing heritability enrichment, up to 2- and 10-fold above human open chromatin and mammalian conservation, respectively. Furthermore, we fine-mapped loci dense with genetic variants for addiction- and sleep-associated traits to identify candidate causal variants overlapping human orthologs of D1 and D2 medium spiny open chromatin regions measured in macaque and mouse and predicted active across mammalia.

Mapping the conserved cell-type regulatory patterns in the human genome refines the list of candidate causal variants in regions associated with addiction-associated traits beyond conservation or human cell type-specific open chromatin alone.

85. Real-World Physiological Markers and Neural Correlates of Regulation Success and Failure in Remitted Major Depressive Disorder

Jonathan Stange, University of Southern California

Background: Affect regulation is disrupted in individuals with depression. Understanding processes that facilitate regulation success in ecologically valid contexts is critical to improving treatments and reducing risk for depression. To examine trait-like regulation phenotypes independent of active depression, we used ambulatory assessment to measure regulation engagement and success in everyday life, along with neural correlates, in a sample of 33 young adults with remitted major depressive disorder (rMDD) and 25 healthy comparisons (HCs).

Methods: Participants completed one week of ambulatory assessment, during which degree of adaptive and maladaptive regulation strategy engagement (reappraisal/distraction/acceptance and rumination/mind-wandering) over four hours was measured three times per day. Ambulatory electrocardiogram was measured with a biometric shirt and examined for 30 minutes after regulation windows. fMRI activation within emotion regulation networks was examined during a reappraisal task. Regulation success was operationalized as the slope of the relationship between degree of adaptive regulation strategy engagement and subsequent high-frequency heart rate variability (HRV), using multilevel modeling. Slopes were used as dimensional predictors of brain activation during reappraisal.

Results: Whereas HCs showed increased HRV following adaptive regulation engagement, individuals with rMDD did not, suggesting unsuccessful physiological regulation. Group differences appeared related to greater simultaneous use of rumination and mind-wandering in rMDD during moments when adaptive regulation strategies were employed. Greater regulation success slopes corresponded with greater activation in the left middle frontal gyrus, a region key to cognitive regulation of affect, during reappraisal.

Conclusion: These results have implications for refining behavioral, physiological, and neural interventions to improve regulation success.