Symposium Title: Uncovering Genetic Subtypes of Neurodevelopmental Disorders

Chair: Caitlin M. Hudac

Discussant: (None selected)

Overview: The significant etiologic and phenotypic heterogeneity of neurodevelopmental disorders, such as autism spectrum disorder (ASD), has made it challenging to target underlying mechanisms of pathology and identify replicable subtypes. A burgeoning “genetics-first” approach has been proposed to reduce heterogeneity and enable identification of subtypes of individuals with neurodevelopmental disorders across multiple domains. In this panel, we will focus on recent discoveries spanning multiple genetic subtypes of neurodevelopmental disorders associated with disruptive genetic variants.

The first presentation (Hudac) introduces the concept of a genetics-first approach and uses the SCN2A subtype as a case study to illustrate the current progress and challenges associated with integrating across multiple biological and clinical levels. In the second presentation (Beighley), we look broadly at the utility of a genetics-first approach to evaluate shared genetic mechanisms across disruptive variants by describing the relationship between the CHD8 subtype and children with heterogeneous mutations within a shared molecular pathway. We then inspect two genetic subtypes that are distinct from CHD8, emphasizing the associated clinical, behavioral, and medical patterns within each subtype (Arnett describing ADNP; Peterson describing GRIN2B).

Clinical relevance: Uncovering genetic and molecular subtypes of neurodevelopmental disorders creates an avenue for precision medicine, such that identification, treatments, and interventions can be tailored to specific clinical needs. In addition, with decreasing costs for genetic sequencing, more families are becoming aware of their children’s genetic subtypes and subsequently connecting with other families with common disruptive variants via research and social media networks. The current panel promotes multiple approaches to defining these genetic subtypes in order to promote relevant, effective medical and behavioral care for affected individuals.

Paper 1 of 4

Paper Title: The Current Progress and Challenges of the Genetic-First Approach: An Example Using the SCN2A Subtype

Authors: Caitlin M. Hudac, Brianna Cairney, Trent D. DesChamps, Anne B. Arnett, Monique M. Mahony, Sara Jane Webb, & Raphael Bernier

Introduction: To make progress in identification of biomarkers, we must establish connections across multiple levels of assessment, including genetics, behavior, and neurophysiology. In this talk, we will focus on how genetics-first approaches (Stessman et al., 2014) have led to recent discoveries spanning multiple units of analysis to describe neurodevelopmental disorder phenotypes of children and individuals with genetic variants. This introduction will use the SCN2A subtype as an example to describe clinical phenotype connections between molecular and physiological levels. Specifically, we will review research connecting animal and human models of SCN2A (Ben-Shalom et al., 2017; Schwarz et al., 2016) and present current data describing the neural patterns associated with SCN2A.

Methods: As part of our ongoing genetics-first characterization studies, targeted recruitment focused on children with a severe disruptive genetic variants associated with ASD. In this study, we focus on a subset of children with disruptive SCN2A variants (n = 7, to date; age 4-16 years) who completed laboratory clinical and behavioral assessments. SCN2A brain phenotypes were
compared to IQ-matched children with a disruptive mutation to a different gene (Other Variant, n = 9) or without a known genetic etiology (Idiopathic ASD, n = 10). A passive auditory oddball EEG experiment measured attention and speed of habituation as reflected by the central P3a component (180-350 ms). Multilevel analyses tested group effects on condition (i.e., novel sounds vs. repeated tones) and habituation (i.e., the rate of decreasing P3a amplitude).

**Results:** An interaction between condition and group, $F(1, 2364) = 4.02, p = .018$, indicated the SCN2A group showed reduced auditory discrimination relative to Idiopathic ASD, but increased discrimination relative to the Other Variant group. The SCN2A profile was characterized by rapid habituation to both frequent and novel conditions (see Figure below) that was faster than that of the Idiopathic ASD ($p = .09$) and Other Variant ($p = .04$) groups. Pearson correlations indicated that an increased rate of novelty habituation in the SCN2A group was related to better adaptive skills ($r = .97, p = .07$), particularly in the communication domain ($r = .93, p = .03$).

**Discussion:** Compared to other children with similarly low cognitive abilities, children with SCN2A mutations demonstrated an intermediate level of attention relative to children with and without other variants. However, the SCN2A group exhibited a unique pattern of rapid habituation, indicating a diminishing response to both novel and repeated auditory information. We will discuss the implications and the potential for this distinct SCN2A attention phenotype and the relationship to clinical symptomatology to serve as a biomarker.

**References/Citations:**

Paper Title: The CHD8 phenotype: Relationship to Shared Molecular Subtypes

Authors: Jennifer S. Beighley, Caitlin M. Hudac, Anne B. Arnett, Jessica Peterson, Jennifer Gerdts, & Raphael Bernier

Introduction: In families where only one child is affected with autism spectrum disorder (ASD; simplex families), an estimated 30% of ASD is thought to be caused by the presence of a de novo disruptive variant to one of 353 currently identified candidate genes (Iossifov et al, 2014). However, specific gene events are still quite rare and subsequently, recent efforts have begun to consider subtypes based on gene function or common molecular pathways. For instance, mutations to CHD8 (a chromatin modifier) are strongly linked to ASD via disruption of a regulatory network during neurodevelopment (Cotney et al., 2014). Further, CHD8 appears to have regulatory effects to many other genes, which suggests a commonality of pathogenesis for genes grouped based on shared mechanisms (Sugathan et al., 2014).

Methods: Participants (N=99) were recruited due to the presence of a confirmed disruptive variant thought to be associated with ASD. Thirteen individuals had a mutation to CHD8, 39 had a mutation to a gene that is considered to be a binder to CHD8 (e.g., ADNP), and 47 had a mutation to a gene that is considered to be a non-binder to CHD8 (e.g., GRIN2B). Measurements included standardized behavioral assessments of functioning level, ASD symptoms, psychiatric symptoms, and medical history, as well as caregiver interview and questionnaire responses and a review of records.

Results: Among the three groups, non-binders had the highest prevalence of intellectual disability (ID; 85.1%) compared to binders (71%) and the CHD8 group (53.8%) with a similar pattern of relative adaptive impairments among groups. A trend in the opposite direction was found for the prevalence of ASD among the three groups: CHD8 group (100%), binders (79.5%) and non-binders (71.7%). The CHD8 group had significantly higher ratings of repetitive and restrictive behavior compared to the non-binders (p=.019) and binders (p=.001) There were also differences in seizures among groups with infantile spasms and atonic or drop attacks occurring only in individuals from the non-binders group (p=.047). Finally, head circumference differed among groups with microcephaly occurring disproportionately in the non-binders, whose mean head circumference was significantly lower than the binders as well as the CHD8 group (p=.001).

Discussion: We will discuss the clinical utility in parsing disruptive variant groups based on common molecular mechanisms. Here, the CHD8 group was found to have more similarities to the CHD8 binders group as compared to the non-binders group. Specifically, the CHD8 group was more similar to the binders group related to prevalence of ID and adaptive deficits, seizure type, and head circumference. Because there is such a high likelihood of ASD in individuals with mutations to CHD8, and because CHD8 regulates many other genes that are critical in early development, this mechanism may be a good target for deeper phenotypic examination. Gaining a better understanding of the relationship among CHD8 and gene targets may elucidate the nature of ASD pathogenesis.

References/Citations:


**Paper Title:** A Unique Autism Spectrum Phenotype in ADNP Syndrome

**Authors:** Anne B. Arnett, Candace Rhoads, Ruqian Ma, Jennifer Gerdts, Arianne S. Wallace, & Raphael A. Bernier

**Introduction:** One of the most common genetic variants associated with autism spectrum disorder (ASD) involves heterozygous, *de novo* mutations affecting Activity Dependent Neuroprotector Protein (*ADNP*), sometimes referred to as Helsmoortel-Van der Aa Syndrome (Vandeweyer et al., 2014) or ADNP syndrome (NIH, 2017). Reports of individuals with *ADNP* mutations indicate frequent ASD diagnoses; however, these studies have suffered from ascertainment bias due to targeted recruitment from ASD samples. To address this confound, we employed a “genetics-first” recruitment approach, resulting in an *ADNP* sample with a broader behavioral phenotype than previously described.

**Methods:** We report on a cohort of 11 participants (10 previously unreported) with a mutation to *ADNP* as well as three comparison samples: individuals with a disruptive mutation to *CHD8* (*n*=12), other likely gene disrupting mutations associated with ASD (*n*=72), or idiopathic ASD (*n*=42). Genetic mutations were confirmed by whole family-based exome sequencing or molecular inversion probe based targeted sequencing. Individuals and caregivers participated in a comprehensive behavioral and cognitive evaluation.

**Results:** Among the *ADNP* group, ASD was diagnosed in 73% of cases and intellectual disability (ID) was present in 100%. Relative to comparison groups, *ADNP* showed milder social communication impairments (*p*=.004) but comparable severity of restricted and repetitive behaviors (RRBs; *p*=.057). Variance in social communication difficulties was explained by verbal intelligence to a greater extent in the *ADNP* group (57%) than in the comparison groups (0.3-45%). The profile of RRBs in the *ADNP* group was also significantly different than that of the idiopathic ASD group, with the former characterized by severe stereotyped and repetitive motor behaviors and the latter showing greater severity of restricted interests.

**Discussion:** Although *ADNP* syndrome has previously been strongly associated with ASD, ascertainment bias has likely inflated the rates of ASD in these studies. The profile of ASD symptoms in our unbiased cohort is consistent with that seen in other ID syndromes and social impairment is statistically explained by verbal cognition. Moreover, despite the fact that *ADNP* is considered a binding target of *CHD8* (see Paper 2), our results highlight a phenotypic contrast between mutations to *ADNP* versus *CHD8*, which may reflect differences in timing of genetic expression during development (Iossifov et al., 2014). Altogether, disruption to *ADNP* appears critical for broader cognitive and adaptive functioning and may have non-specific effects on ASD-related symptoms.

**References/Citations:**
Introduction: Disruptive de novo mutations in GRIN2B have been implicated as an important risk factor for ASD and ID although detailed characterizations of the ASD phenotype associated with this genetic variant are limited (Hu et al., 2016; O’Roak et al., 2012b). Patients with de novo GRIN2B mutations described in extant reports have presented with epilepsy, hypotonia, cortical visual impairment, and behavioral characteristics such as increased rates of hyperactivity, impulsivity, and social exuberance and disinhibition (Freunsch et al., 2013; Platzer et al., 2017). To further delineate the phenotypic spectrum associated with de novo GRIN2B variants and better understand the unique features of this group, we compared quantitatively assessed clinical and behavioral characteristics to patients with other ASD candidate genes and idiopathic autism.

Methods: Participants include 27 individuals with de novo GRIN2B variants (ages 1-16.3 years) and two comparison groups: Idiopathic autism defined as individuals without identified de novo mutations to ASD associated genes from the Simons Simplex Collection (n = 2,387) and individuals with de novo protein truncating variants to CHD8 (n = 16). Groups were compared on key demographic variables, medical comorbidities, and standardized measures of social and behavioral functioning.

Results: The GRIN2B group was characterized by ID ranging from mild to profound (100% present) and ASD (75% received diagnosis by a licensed psychologist using DSM-5 criteria). Higher rates of hypotonia, feeding problems, gastrointestinal problems, and seizures were found in the GRIN2B group relative to the idiopathic autism group. All differences were significant following Bonferroni-correction of p = 0.007 for multiple comparisons. The GRIN2B group evidenced a significantly higher rate of growth problems (i.e., underweight) (p=.006) and significantly lower rate of macrocephaly compared to CHD8 (p=.006). Clinical observations and psychiatric history suggested elevated hyperactivity and impulsivity symptoms in the GRIN2B-VAR sample, with 35% of patients carrying an ADHD diagnosis. Critically, differences in the social functioning profile of GRIN2B relative to comparison groups were found, including significantly lower difficulties with social motivation in the GRIN2B group versus Idiopathic ASD (p=.002) and CHD8 (p=.0004).

Discussion: The neurobehavioral phenotype of de novo GRIN2B variants is characterized by intellectual disability, feeding difficulties, growth problems, vision impairments, motor impairments including abnormal muscle tone, intact social interest and social disinhibition despite high ASD risk and presence of ASD traits, and possible elevated externalizing symptoms (e.g., inattention, impulsivity). Despite some heterogeneity in the GRIN2B presentation, comparisons with samples of idiopathic ASD and CHD8 cases suggests the GRIN2B phenotype may represent a distinct ASD/ID subtype with a unique social presentation suggesting less severe impairments in specific social domains.

References