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# Harnessing rare variants in neuropsychiatric and neurodevelopment disorders—a Keystone Symposia report

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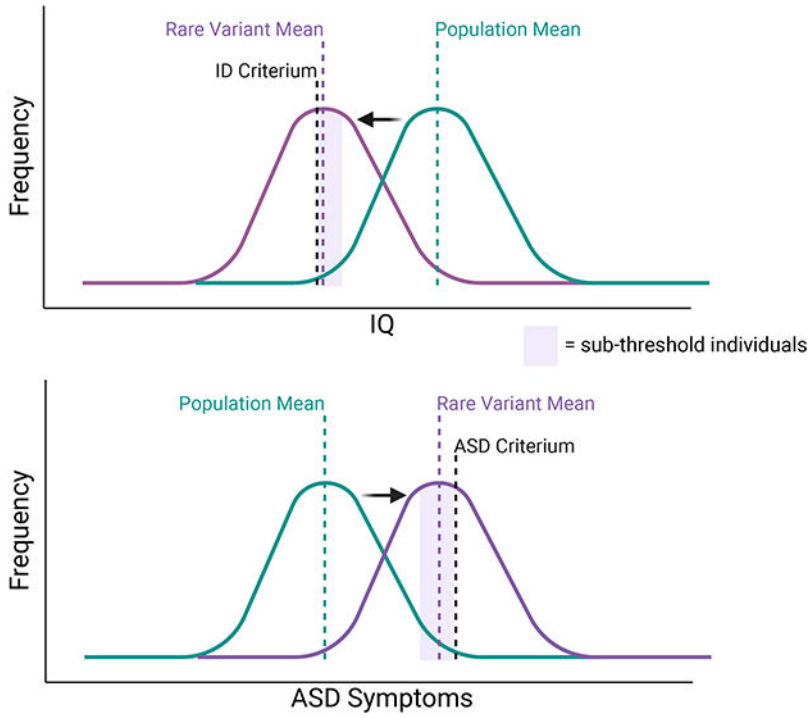
## Abstract

Neurodevelopmental and neuropsychiatric disorders, such as autism spectrum disorder and schizophrenia, have strong genetic risk components, but the underlying mechanisms have proven difficult to decipher. Rare, high-risk variants may offer an opportunity to delineate the biological mechanisms responsible more clearly for more common idiopathic diseases. Indeed, different rare variants can cause the same behavioral phenotype, demonstrating genetic heterogeneity, while the same rare variant can cause different behavioral phenotypes, demonstrating variable expressivity. These observations suggest convergent underlying biological and neurological mechanisms; identification of these mechanisms may ultimately reveal new therapeutic targets. At the 2021 Keystone Symposium “Neuropsychiatric and Neurodevelopmental Disorders: Harnessing Rare Variants”, a panel of experts in this field of research described significant progress in genomic discovery and human phenotyping and raised several consistent issues, including the need for detailed natural history studies of rare disorders, the challenges in cohort recruitment, and the importance of viewing phenotypes as quantitative traits that are impacted by rare variants.

## Graphical abstract

Neurodevelopmental and neuropsychiatric disorders such as autism spectrum disorder and schizophrenia have strong genetic risk components, but the specific, underlying mechanisms have proven difficult to decipher. Rare, high-risk variants may offer an opportunity to delineate the biological mechanisms responsible more clearly for more common idiopathic diseases.



**Keywords**

autism; autism heterogeneity; autism spectrum disorder; copy number variant; intellectual disability; neurodevelopmental disorders; neuropsychiatric disorders; polygenic risk score; rare variants; schizophrenia; 3q29 deletion; TSC; 16p11.2 deletion; 22q11.2 deletion

## Introduction

Genetic factors account for a substantial proportion of the risk for neurodevelopmental psychiatric disorders (NPD) such as intellectual disability (ID), autism spectrum disorder (ASD), and schizophrenia (SCZ) and are often broadly categorized into common and rare genomic variants. Common genomic variants are typically single nucleotide polymorphisms that are prevalent in the general population and each individual variant confers a small amount of risk for a NPD. In contrast, some rare genomic variants, including copy number and single gene variants, have been found to confer high risk for NPDs.[1] Copy number variants (CNVs) are deletions or duplications of DNA segments that can span up to several dozen genes. Hundreds of rare genomic variants have been associated with neuropsychiatric traits and psychiatric conditions, indicating the considerable progress of sequencing efforts and also the staggering complexity of neuropsychiatric genetics.

While rare variants of large effect size increase the risk of clinical phenotypes more than common variants of small effect size, current evidence does not support a highly specific genotype to phenotype relationship. Instead, most rare variants demonstrate variable expressivity for a given phenotype, i.e., while the presence of a rare variant greatly increases the risk of a neurodevelopmental or psychiatric condition, the severity of the phenotype can vary among individuals and often includes some not displaying the phenotype (see Box 1).[2] Rare variants also display pleiotropy, i.e., one variant can be associated with more than one phenotype, and affects both neurological and non-neurological phenotypes. For example, pathogenic variants in the gene *SETD1A* are associated with an increased risk of schizophrenia, epilepsy, ASD, and sleep disturbance, as well as joint hypermobility, gastrointestinal issues, cranio-facial dysmorphology, and recurrent infections.[3,4] Heterozygous individuals typically present with one or several of these features, but the phenotypic pattern is variable between individuals. This type of phenotypic variability is even observed within families, as has been described for recurrent, inherited copy number variants, such as deletions at 1q21.1 and 15q13.3, where the clinical phenotypes and diagnoses of family members with the deletion is variable.[5] However, while it may appear that the clinical impact of rare variants is broad and nonspecific, accumulating evidence shows that the overall phenotypic presentation of rare variants has some specificity.[6-10] Broader genetic screening and an improved understanding of the natural history of rare variant associated syndromes will further clarify the overlapping and differential phenotypic profiles of NPD-associated rare variants.

For translational researchers, rare variants of large effect size are attractive targets because they may offer a better opportunity to identify the underlying molecular and neurological mechanisms of neuropsychiatric disorders. Moreover, the recognition that different genomic variants can result in similar clinical and behavioral phenotypes suggests evidence for

convergent molecular mechanisms of pathogenesis. For example, rare variants that increase risk for ASD are primarily involved in functions like regulation of gene expression, neuronal communication, and cytoskeleton function.[11] This convergence in gene function suggests both causality at a genetic level and a common underlying biology between seemingly disparate genetic disorders with distinct, yet overlapping phenotype profiles. Ultimately, there is hope that convergent biological mechanisms could be targeted by a small number of common therapies and treatment strategies to address a large group or subset of disorders.[12,13] Indeed, genetic etiology has already had an impact on clinical research. Genetically informed clinical trials in ASD increased from around 10 in 2001–2003 to almost 60 in 2013–2015.[14] Notwithstanding the success of neuropsychiatric genetics in identifying disease-associated loci, the pleiotropy and variable expressivity of rare variants suggests an important role for environmental, epigenetic, and complex polygenic factors in modifying an individual's phenotype.

Often, rare genetic variants and disorders are viewed through a specific lens. For example, researchers interested in ASD may focus only on the ASD-related phenotypes associated with a rare variant and not on other symptoms.[15] Convening researchers from different fields and specialties is key to appreciating and eventually understanding the full complexity apparent in rare genomic variants and neurodevelopmental disorders. On February 11, 2021, experts in genomics, neuroscience, neurodevelopmental and neuropsychiatric disorders, and clinical trials met virtually for the Keystone eSymposium "Neuropsychiatric and Neurodevelopmental Disorders: Harnessing Rare Variants". Co-organizer **Jennifer Mulle** (Emory University) gave opening remarks and emphasized the importance of harmonizing methodologies and comparing across disorders. This concept of cross-disorder comparison is particularly relevant for rare genetic variants, because the phenotypic spectrum associated with these variants is often not confined to specific diagnostic categories. This theme would be addressed by several speakers. **Christa Martin** (Geisinger) and **Audrey Thurm** (NIH), co-organizers of the eSymposium, served as moderators for the presentations where invited speakers described different hypotheses and models for how to explain the variable expressivity seen with rare variants, large collaborative efforts to propel genomic research, and therapeutic approaches to address NPDs.

## Genetic clues to phenotypic heterogeneity in autism

**Elise Robinson** from the Harvard T.H. Chan School of Public Health and the Broad Institute of MIT and Harvard presented an overview of the genomic landscape in ASD. She discussed how the differential impacts of rare and common variants, as well as overlapping phenotypes with other neurodevelopmental disorders, can lead to heterogenous phenotypes in individuals with ASD.

One way to understand the differential impact of rare and common variants is to assess overlap with other neurodevelopmental disorders. While there is a large overlap in the rare variants associated with ID and those associated with ASD, there is little overlap in common variants associated with these disorders.[12] Furthermore, common variants associated with ASD are associated with higher intelligence quotient (IQ) in the general population while ASD-associated rare variants are associated with a lower IQ. These differential effects of

common and rare variants on IQ may contribute to the highly expanded phenotypic variance of IQ among people with ASD.[16]

While many rare variants have been associated with ASD, it can be difficult to delineate whether a given variant is more likely to be observed in individuals ascertained for ASD or ID. Some genomic variants are typically found in individuals who have both autism and ID, while others appear typically found in individuals with autism without ID. This heterogeneity can complicate genetic association studies.[17] New, larger cohorts like SPARK (Simons Foundation Powering Autism Research for Knowledge) (https://sparkforautism.org/)[18] may help to tease out the impact of rare variants on neurodevelopment.

Large-scale genomic studies to identify common variants associated with ASD have faced a number of challenges. A genome-wide association study (GWAS) of approximately 19,000 ASD cases identified 5 single-nucleotide polymorphisms (SNPs) associated with autism.[11] Unfortunately, the addition of new cases to the dataset, which would be expected to increase statistical power, can actually decrease the statistical significance of previous findings due to increases in sample heterogeneity. Therefore, it is important to consider possible ascertainment bias in cohorts and how the phenotypic heterogeneity of ASD has changed over time given changing diagnostic definitions. In addition, phenotypic similarities between ASD and ID could lead to misdiagnosis and the inclusion of cases in a dataset with a phenotype that has no genetic correlation to autism can be detrimental to GWAS analyses. More stringently defining inclusion criteria for ASD GWAS could alleviate these complications.

An important unresolved issue in ASD is the strong sex bias toward males. In people with ASD, males outnumber females by approximately 3-4 to 1.[19] The idea of a female protective effect is supported from studies of rare variants where girls with ASD have double the rate of strong acting *de novo* events, suggesting that girls, on average, must accumulate more risk factors to manifest the same phenotype as boys.[20,21] However, the influence of common variants is more complicated. Data from large cohorts like SPARK and the Simons Simplex Collection[22] are shedding light on their effects.

Moving forward, studies should include both genotype-first designs and phenotypically-defined ascertainment strategies. Beginning with a defined pathogenic variant provides a valuable vantage point to observe the heterogeneous phenotypes that can result. However, a genetics-first approach skews ascertainment towards people who manifest clinical characteristics that elicit testing. Genetic syndromes are often less simple than commonly believed as individual genes have many functions and it can be difficult to determine which is most important for a given phenotype. A phenotype-first approach could address this source of bias by starting with the processes and pathways most commonly disrupted to cause a certain phenotype and then identifying genomic variants that may be responsible.

## Understanding penetrance and pleiotropy in ASD through 22q11.2DS

**Jacob Vorstman**, from the University of Toronto, presented work from the International Brain and Behavior 22q11.2DS Consortium (IBBC) aimed at understanding variable expressivity and pleiotropy in ASD using the rare copy number variant (CNV) 22q11.2 deletion as a model.

22q11.2 deletion syndrome (22q11.2DS) is the most common recurrent CNV and is typically diagnosed early in life due to congenital abnormalities that trigger genetic testing.[23] In addition to a range of congenital conditions, individuals with 22q11.2DS are also at an increased risk for neurodevelopment disorders, such as ASD, attention deficit hyperactivity disorder (ADHD), anxiety, and ID and for developing schizophrenia as an adult.[24] For 22q11DS, and similarly for other rare pathogenic variants, studies have examined the risk for various neurodevelopmental outcomes.[25,26] While this information helps to improve our understanding of the phenotypic profile of the disorder, these risk estimates are insufficient when used for individual outcome prediction. In this context, both variable expressivity and pleiotropy, discussed above, are important challenges for clinicians and researchers alike.

Interestingly, the impacts of 22q11.2 deletion on cognitive impairment and psychiatric disorders appear to be largely independent. IQ differences are only weakly correlated to a diagnosis of ASD or ADHD.[27-29] However, a diagnosis of schizophrenia in 22q11DS is associated with lower IQ in childhood as well as a decline of mostly verbal IQ prior to the first psychotic episode, which mirrors findings in the general population.[30]

Further complicating matters is the fact when considering phenotypes such as ASD, ADHD and ID in a binary way, the thresholds for a categorical diagnosis are somewhat arbitrary. For example, someone with an IQ of 68 could be considered as having ID, whereas someone with an IQ of 72 would not meet formal diagnostic criteria. Similarly, many individuals with 22q11.2 deletion have symptoms associated with ADHD, anxiety disorders and ASD, but often not severe enough to qualify for a formal diagnosis, even though such “subthreshold” symptoms can have substantial impact on their overall wellbeing and functioning, in particular when symptoms in more than one domain co-occur.[26,31] A categorical, binary approach disregards important complexities and the constellation of an individual’s symptoms. Rather than speaking in terms of the Mendelian concept of penetrance, it may be more accurate to consider diagnoses in dimensional rather than binary terms and refer to variable expressivity (see Box 1). In fact, it has been suggested that when considering phenotypic measures as quantitative traits (rather than diagnostic categories) most neurodevelopmental variants would be nearly completely penetrant.[32] In other words, it may be more informative to consider the effect of rare variants as “shifting” a given trait or phenotype from a baseline value determined by an individual’s genetic background than to state that a variant causes a given disorder in a certain percentage of individuals with that variant.[33] A logical inference from this view would be to take the genomic context (“the rest of the genome”), in which a rare variant exists, into account.

The IQ distribution in people with 22q11DS follows a normal distribution shifted approximately 30 points lower than the distribution for the general population.[32,34] A recent study by the IBBC found that common genetic variation, summarized as a polygenic risk score, influences the expressivity of the variant for complex traits such as ID and SCZ, [35,36] . These findings highlight the need to recognize that rare variants exist within the context of the rest of the genome, which can determine the apparent penetrance of a rare high impact variant. There was broad support among speakers, organizers, and panelists at the symposium for moving away from Mendelian terms like penetrance as a binary concept when referring to rare variants. There was a broad consensus for the need to move towards a more precise and descriptive language of quantitative trait distributions and the inclusion of other genomic factors, including common variation.

Vorstman suggested a conceptual model in which there is a gradient from normal to abnormal brain function, with a threshold beyond which neuropsychiatric disorders manifest. The cumulative impact of common genetic variants follows a normal distribution. For the general population, only the tail end of this distribution falls above the threshold of phenotypic manifestations; the majority of people are well below the threshold for clinically manifesting a neuropsychiatric disorder. Subsets of people who carry rare high impact variants are shifted closer to that threshold, such that a larger part of the tail end of their polygenic risk distribution falls above the threshold of phenotypic expression (Fig. 1).[31]

Given the rarity of neuropsychiatric disorders in the general population, polygenic risk scores alone are not sensitive enough to assess an individual's risk. However, in individuals with high-risk variants, the power of polygenic risk scores to assess individual risk is increased due to the increased prevalence of the disorders.[36] While polygenic risk scores currently have limited utility in the general population[37], they could improve individual risk prediction for rare variants.

## SPARK: Accelerating genotype-driven research in ASD

**Wendy Chung**, from Columbia University, described several initiatives spearheaded by the Simons Foundation to understand the natural history and biology of rare genetic conditions and eventually move toward therapies. She first described SPARK, a US research cohort to accelerate genotype-driven research in ASD at scale.[18] SPARK has a goal of collecting genomic, behavioral, and other medical data for at least 50,000 families with a child affected by ASD. In this initiative, participants have an active role in the research process and provide input on study designs; over 100 studies have been approved by the access committee to date. Participants also receive information and data collected by the study. Researchers help participants understand the research opportunities available to them and connect them with researchers and communities. All SPARK data are freely accessible to researchers at base.sfari.org.

Echoing previous speakers' concerns about ascertainment bias, Chung noted that the inclusion criteria of SPARK were designed to be broad, where participants only needed have had a professional diagnosis of autism. These broad inclusion criteria initially raised concerns that sample heterogeneity would compromise statistical power. However, analysis

of the first 500 families in SPARK revealed genetic characteristics similar to that of other cohorts. While some new risk genes were identified in the SPARK cohort, they largely represented similar biology to known genes.[38] We estimate that approximately 2/3 of the highly penetrant genes with *de novo* variants responsible for autism have already been identified and that the newly discovered genes with *de novo* variants will largely have functional similarities to what is already known. There is now hope that focusing on inherited rare variants and multi-variant interactions and polygenic risk scores can reveal new biological mechanisms involved in ASD, though these analyses will require even larger sample sizes.

In SPARK, individuals with autism as well as their unaffected family members are assessed on measures of social communication, repetitive behavior, motor coordination, and other quantitative and qualitative measures. The participants are asked to provide annual updates so that these measures can be tracked longitudinally. While the study uses gold-standard, validated tools to the extent possible, these tools are not always feasible to implement at scale. This tradeoff highlights the need for better quantitative, validated measures that can scale to large cohorts.[18]

SPARK is designed to minimize burden and intrusion in participant's lives by assessing quantitative and qualitative measures largely online, from a participant's home. Improved remote phenotyping technology may allow for more quantitative data to be collected in an unobtrusive way that prioritizes a participant's privacy—for example, phone recordings to analyze speech patterns, actigraphy to monitor sleep, behavior-tracking apps to glean information about social interactions and analyzing eye tracking via web cams or camera apps. SPARK is an attractive cohort to test the feasibility and scalability of these approaches.

SPARK participants with a genetic diagnosis can also participate in Simons Searchlight, an international online registry that contains genetic data, phenotypic information, and medical history on participants as well as biospecimens, such as whole blood DNA, saliva DNA, fibroblasts, and induced pluripotent stem cells (iPSC) to facilitate preclinical research (https://www.simonssearchlight.org). The study also provides an online community for participants, connecting them with individuals with similar diagnoses.

Simons Searchlight began in 2010 and initially focused on families living with 16p11.2 copy number variants. Since then, it has expanded to include over 150 different genetic diagnoses. Participants, including affected individuals, their parents, and siblings, provide information via phone interviews and online surveys. Annual updates are requested to provide longitudinal information. Simons Searchlight has already provided several insights on the effects of 16p11.2 CNVs, including its effect on brain volumetrics and anatomical differences, IQ, and social cognition.[39,40]

Chung ended by listing some of the key challenges that remain in autism research, including (1) defining the relevant genetic and non-genetic contributions to ASD, (2) accurately quantifying behavior, (3) understanding the natural history of individuals with rare conditions, (4) sharing best practices and identifying affected individuals early to

positively impact change the trajectory of their disorder, and (5) understanding how to safely move from clinical observation to intervention at scale.

## Genotype-phenotype relationships in heritable autism

**John N. Constantino** from Washington University in St Louis discussed the association between genotype and phenotype in heritable autism. While *de novo* mutations can help to reveal the biology of autism, most cases of autism result from inherited polygenic factors. Moreover, determining the phenotypic traits that co-occur with heritable autism may provide a better understanding of the genetic etiology of the disorder. A recent population-based cohort study of over 500,000 individuals showed that a family history of any of several neuropsychiatric conditions is associated with substantially increased risk for ASD.[41] For example, a family history of ID, ADHD, depression, bipolar disorder, personality disorder, schizophrenia or other nonaffective psychotic disorder among others in first-degree relatives significantly increases risk for ASD. In other words, a family history of a large number of seemingly disparate conditions can significantly increase risk for ASD.[41]

Discussion of the heritability of ASD included four key concepts. First, while autism is clearly heritable, the severity of the disorder in an individual may not be. Studies in identical twins show little correlation in symptom burden.[42,43] Autism severity may be strongly influenced by stochastic effects and non-shared environmental effects above the clinical threshold.

Second, Constantino and colleagues have put forward a model for a developmental sub-structure for autism.[44] In this model, inheritance exerts its effects via independent, separate components of additive genetic liability, most of which are not specific to autism. For example, poor eye contact is often associated with autism and eye-tracking experiments in twins show that eye and mouth gazing is highly heritable.[45] While most children with ASD show eye gaze abnormalities, some children with abnormalities do not develop ASD. Thus, abnormalities in a trait like early eye gaze may be necessary but not sufficient to cause ASD. Other behavioral traits associated with autism, such as attention and motor coordination, may also be heritable; however, each of these traits is genetically independent.[46] In this way, risk for ASD may be likened to risk for hypertension. While high blood pressure is a highly heritable trait, it is controlled by several independent functions. Sometimes these functions are correlated or can compensate for one other functionally, but they are inherited separately.[44] Moreover, heterogeneity in autism may be a function of different combinations of these liability factors.[47] Importantly, because these polymorphisms are also present in neurotypical control subjects, interpretating the contributions of common variants to overall ASD risk is complicated. If the family genetic liability to autism operates via multiple independent liabilities, permutations of which give rise to autism, this would mean that autism is fractionable before it develops, but not after. Therefore, treatment strategies that focus on normalizing these liabilities, such as social visual disengagement, inattention, motor coordination deficits, and tactile sensitivity before autism develops may provide a strategy for early intervention.

Third, there is evidence that the sex ratio in autism may be the result of male sensitivity rather than female protection. Based on the female protective effect, one would predict that unaffected sisters of males with autism have a higher burden and would be more likely to have offspring with autism than unaffected brothers. However, data from a large cohort in Sweden contradict this notion: the risk to children with a family history of ASD in the maternal lineage is only slightly higher than those with a history in the paternal lineage and not significantly different from what would be expected for a second-degree relative.[48] Framing the ASD sex bias as a function of male sensitivity could have implications for studying the effects of sex on ASD.

Finally, ASD should perhaps be considered the result of multiple quantitative traits that are each at the extreme end of the distribution. This re-framing could also improve polygenic risk scores, which are currently based on binary diagnostics and could expand to include a larger risk landscape by focusing on genes associated with quantitative neurodevelopmental liabilities.[44]

## *SCN2A* mutations: a model for clinical development based on genotype-phenotype relationships

For many genes associated with neurodevelopmental disorders, there are not yet sufficient data to define a genetic syndrome and develop functional assays or preclinical models to investigate molecular pathogenesis. The genotype-phenotype relationship for *SCN2A* mutations, however, is more clearly defined.

**Stephan Sanders** of the University of California, San Francisco presented the latest in preclinical and translational studies on *SCN2A* mutations. *SCN2A* encodes a neuronal sodium channel $Na_v1.2$ that has been associated with ASD, ID, epilepsy and other neurodevelopmental syndromes.[49] Functional studies have compellingly demonstrated a direct correlation between *de novo* variants in the *SCN2A* gene, sodium channel activity, and phenotype: gain-of-function *de novo* variants alter channel activity leading to neuronal hyperexcitability and are associated with early-onset seizure disorders whereas loss-of-function *de novo* variants are associated with ASD and/or ID, and sometimes late-onset seizures.[49] Interestingly, the degree of functional gain correlates with increased seizure severity; a similar correlation with severity for loss-of-function variants is also emerging.[50] While *SCN2A* loss-of-function variants are expected to be more frequent than gain-of-function variants, existing databases contain similar numbers of each. This apparent discrepancy probably reflects ascertainment bias due to clinicians being more likely to order genetic testing in a case of infantile seizures than developmental delays. Indeed, genetic testing in cases of ASD and developmental delay is still vastly under-utilized.[51,52] Moving forward, in order to generate robust data from which to draw genotype-phenotype relationships, it will be imperative to address ascertainment bias and expand genetic testing.[53]

The expression levels and splicing patterns of genes involved in neurodevelopment can change during the course of development, which can be missed by cross-sectional studies. For example, *SCN2A* has two isoforms, a neonatal version (5N) expressed predominantly

*in utero* through 1 year of age and an adult version (5A) expressed predominantly after 1 year.[49,54] In addition, the subcellular location and function of $Na_v1.2$ changes across development. These developmental changes are reflected in function. In infants, $Na_v1.2$ is critical in excitatory neurons for action potential initiation; however, around 1 year of age, $Na_v1.6$ (encoded by *SCN8A*) becomes important for the forward propagation of the action potential down the axon to other neurons, while $Na_v1.2$ remains important for back propagation back to the dendrites of the same neuron. In mice, heterozygous deletion of *Scn2a* decreased neuronal excitability early in development and excitability normalized at around the time $Na_v1.2$ is replaced by $Na_v1.6$.[49] Conversely, back propagation of the action potential, a key mediator of synaptic plasticity, was normal early in development but became abnormal later in development.[55] In this way, *SCN2A* may serve as an archetypal neurodevelopmental risk gene in terms of the differential effects of loss- and gain-of-function mutations, and also the importance of developmental timing in assessing genotype-phenotype relationships.

From a translational perspective, the evidence suggests that decreasing $Na_v1.2$ levels may improve symptoms in the face of gain-of-function variants while restoring $Na_v1.2$ function in patients with loss-of-function mutations may present a therapeutic strategy.[56] CRISPR-based technologies can be used to upregulate specific genes and rescue haploinsufficiency phenotypes,[57] and future studies could test the activation of the functional *SCN2A* allele in this paradigm.

Looking ahead, Sanders proposed three priorities for the field. First, there is an urgent need to delineate the critical window for intervention. The *SCN2A* story illustrates the importance of timing in the dynamic process of neurodevelopment. In addition, while some phenotypes may be treatable or even reversible, many others may not be. Determining the optimal point in development for intervention will be critical to the success of any clinical trials. Second, there is a need to identify biomarkers associated with specific clinical phenotypes. While hundreds of biomarkers have been identified, current studies lack the statistical power to detect all but the most dramatic effects. Larger studies will be required to tease out which biomarkers may have clinical significance. Finally, geneticists should work to address ascertainment bias and promote interdisciplinary approaches to investigating etiologies. In practice, research and medicine tend to divide neurological, developmental, and psychiatric disorders, however these are somewhat artificial categorizations that are not based in biology. For example, for *SCN2A* mutations, researchers focused on the seizure-related aspects of the disorder often neglect to investigate autism-related traits and vice versa.[15] Approaching these complex disorders with a narrow focus likely limits our ability to understand the complicated relationships between genotype and phenotype.

## Leveraging convergent pathways for therapeutics

As the number of ASD susceptibility genes increases into the hundreds, developing a unique treatment for each genetic disorder is unlikely. Therefore, most of the focus in therapeutic development is on grouping disorders into subcategories that converge at the cellular level and could potentially be targeted via similar therapeutic strategies.

Toward this end, **Mustafa Sahin** of Boston Children's Hospital presented investigations of the use of the mechanistic target of rapamycin (mTOR) inhibitors in neurodevelopmental disorders. The mTOR inhibitor strategy stems from treatment for tuberous sclerosis complex (TSC), a neurodevelopmental disorder caused by a loss-of-function mutation in *TSC1* or *TSC2*. These loss-of-function mutations result in hyperactivity of the mTOR complex, which leads to increased protein synthesis and cell growth.[58,59] Individuals present with benign tumors and can develop seizures, ID, and ASD. Approximately 50% of individuals with TSC develop ASD that phenotypically resembles non-syndromic ASD.[60,61] At this point, TSC is unique among neurodevelopmental disorders in that many individuals are diagnosed before or at birth,[62] which presents an opportunity for early intervention and to understand the natural history of the disorder.

Intriguingly, evidence is emerging that abnormal mTOR activation may be a convergent, targetable pathway in multiple neurodevelopmental disorders.[63] Several mTOR inhibitors have been approved in cancer and other disease states, and thus there may be a way to repurpose these approved agents for ASD.

From a neuroanatomical standpoint, investigations into the neuropathology and neuroimaging have highlighted the role of the cerebellum in ASD symptomatology. Several compelling lines of evidence link cerebellar dysfunction to autism, in both non-syndromic forms of ASD as well as in TSC.[64,65] In children, cerebellar injury at birth increases the risk of ASD.[66-68] Likewise, in individuals with TSC, cerebellar pathology correlates with ASD.[69-71] Sahin and colleagues have investigated the role of cerebellar dysfunction in the pathogenesis of ASD in TSC in a mouse model in which *Tsc1* is deleted only from Purkinje cells of the cerebellum.[65] While the mutant animals were relatively healthy, they displayed increased Purkinje cell size and cell loss by approximately 2 months of age, reminiscent of Purkinje loss observed in TSC patients.[72] Mutant mice also showed several behavioral deficits, including impaired social interaction, increased repetitive behaviors, and impairment in tasks requiring cerebellum-based learning (i.e., reverse learning), but not in hippocampal-based learning tasks.[65] Treatment with the mTOR inhibitor rapamycin abrogated Purkinje cell death and improved behavioral deficits. However, the window for therapeutic intervention varied for different phenotypes. If administered soon after cerebellum pathology began, treatment rescued only the social interaction phenotype and not the repetitive behavior phenotype. Later treatment failed to rescue either phenotype.[65,73]

Building on this line of evidence, placebo-controlled Phase 2 clinical trial have been conducted in individuals with TSC to test the effects of the mTOR inhibitor everolimus on neurocognition.[74] However, after 6 months of treatment, everolimus failed to demonstrate an improvement in neurocognitive measures, which could be the result of advanced disease beyond the critical treatment window, or insufficient duration of treatment.

## Panel discussion

The meeting concluded with a panel discussion moderated by meeting co-chairs **Carrie E. Bearden** from the University of California, Los Angeles and **Jennifer G. Mulle** from Emory University, **Ricardo E. Dolmetsch** from UniQure, **Bina Maniar Shah** from Project

8p and the Commission on Novel Technologies for Neurodevelopmental CNVs, and the eSymposium presenters.

A key, recurring topic of the meeting was the enigma of variable expressivity at a phenotypic level. Panelists were asked how variable expressivity and phenotypic heterogeneity can be understood at a mechanistic level. Dolmetsch noted the importance of stochasticity in variance. During development, cells make a series of decisions that are not preordained but instead rely on statistical probabilities. Human development is likely buffered against that noise, with mechanisms built in to ensure proper development within a range of environmental effects. However, in individuals with a pathogenic CNV, where a copy of many genes is lost or gained, the process may be noisier and less robust to withstand external influences. In theory, this noise may manifest as a variety of NPD. If part of the phenotypic variability is a result of stochastic noise, understanding the developmental steps most susceptible to environmental noise could help to narrow the etiologic time course. A more quantitative, probabilistic description of cellular differentiation may help to test this hypothesis.

Another possibility is that mutations loosen the constraints of development, i.e., instead of changing the mean, they expand the standard deviation for a given process and therefore phenotypic variability. In this case, searching for compensatory mechanisms within the polygenic background of individuals with a pathogenic rare variant who do not develop a phenotype, or develop a milder phenotype may lead to clues about what processes may be involved in stabilizing individuals with variants.

Admittedly, the road to effective therapies is long and arduous. Most therapies rely on molecular strategies that interfere with one or a limited number of targets. The hope is that several of these disparate genetic disorders mechanistically converge at either the cellular or circuit level. For genetic conditions that converge at the phenotype level, it stands to reason that there must be some level of convergence at a mechanistic level. However, as Robinson noted, it remains to be seen how narrowly behaviors must be defined to result from a common mechanism. Expanding diagnostic criteria has also complicated mechanistic investigation, as disorders may be grouped that are not strongly biologically related.

The panel also discussed the need for more robust human studies and clinical trials, including addressing ascertainment bias in cohorts. A much better understanding of the natural history of rare variant conditions will be needed to determine (1) the critical windows for intervention, (2) the true and developmentally-based phenotypic spectrum of a condition, and (3) optimal clinical trial endpoint measures. Without this information, potentially effective treatments will stand little chance of success in trials. Understanding the true extent of convergent biology at the mechanistic level will help to determine whether human cohorts should be recruited in a genetics-first or phenotype-first manner, especially for clinical trials.

The expanded availability of large datasets with genomic data, like the UK Biobank[75] and the All of Us Research Program[76] may substantially help to eliminate the ascertainment bias that plagues many cohorts. Researchers will have access to a more complete distribution

of phenotypes for a rare variant and will be able to investigate risk and resiliency and protective mechanisms in individuals who escape a clinical phenotype. Understanding these mechanisms could also inform effective therapeutic strategies.

The discussion concluded with panel members calling for a more global, holistic approach to genetic testing and interventions. While highly-effective pharmaceutical therapeutics are still lacking, Chung stressed that early intervention can still change the trajectory for better outcomes in individuals. For example, if a person has a high risk of ID, parents, pediatricians, and social workers can ensure that they are in an appropriate early intervention plan or school environment. Part of the problem, of course, lies in the lack of genetic diagnosis for many individuals.[51] This not only impacts the individual or caretaker's ability to understand potential medical risks and find appropriate resources, but it also leads to a reluctance from pharma to invest in rare disorders. In addition to the value for families in receiving a genetic diagnosis,[77] it should be emphasized to parents and clinicians alike that the development of treatments depends on the ascertainment of individuals carrying risk variants. Thus, broader genetic testing is a key step in the journey toward effective therapeutics. A mandate to comprehensively genetically test all individuals with NPD could simultaneously solve ascertainment bias, improve access to interventions, address socioeconomic disparities, and justify additional funding for specific disorders. The panelists were hopeful that finding an effective therapy for even a few conditions associated with NPD will provide an impetus for wide-scale testing and lead to further interest in therapeutic development.

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Competing interests

JASV serves as a consultant for NoBias Therapeutics Inc.

CEB serves on the Scientific Advisory Board for Novartis Neuroscience.

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**Box 1.**

## Key Concepts

***Penetrance –***

The strength of the relationship between genotype and phenotype where highly penetrant alleles elicit a phenotype in most individuals.

***Variable Expressivity –***

The relative severity of a specific phenotype varies across a population of individuals with the same genotype at a given locus.

***Pleiotropy –***

A specific genotype is associated with multiple phenotypic outcomes, often across multiple organ systems.

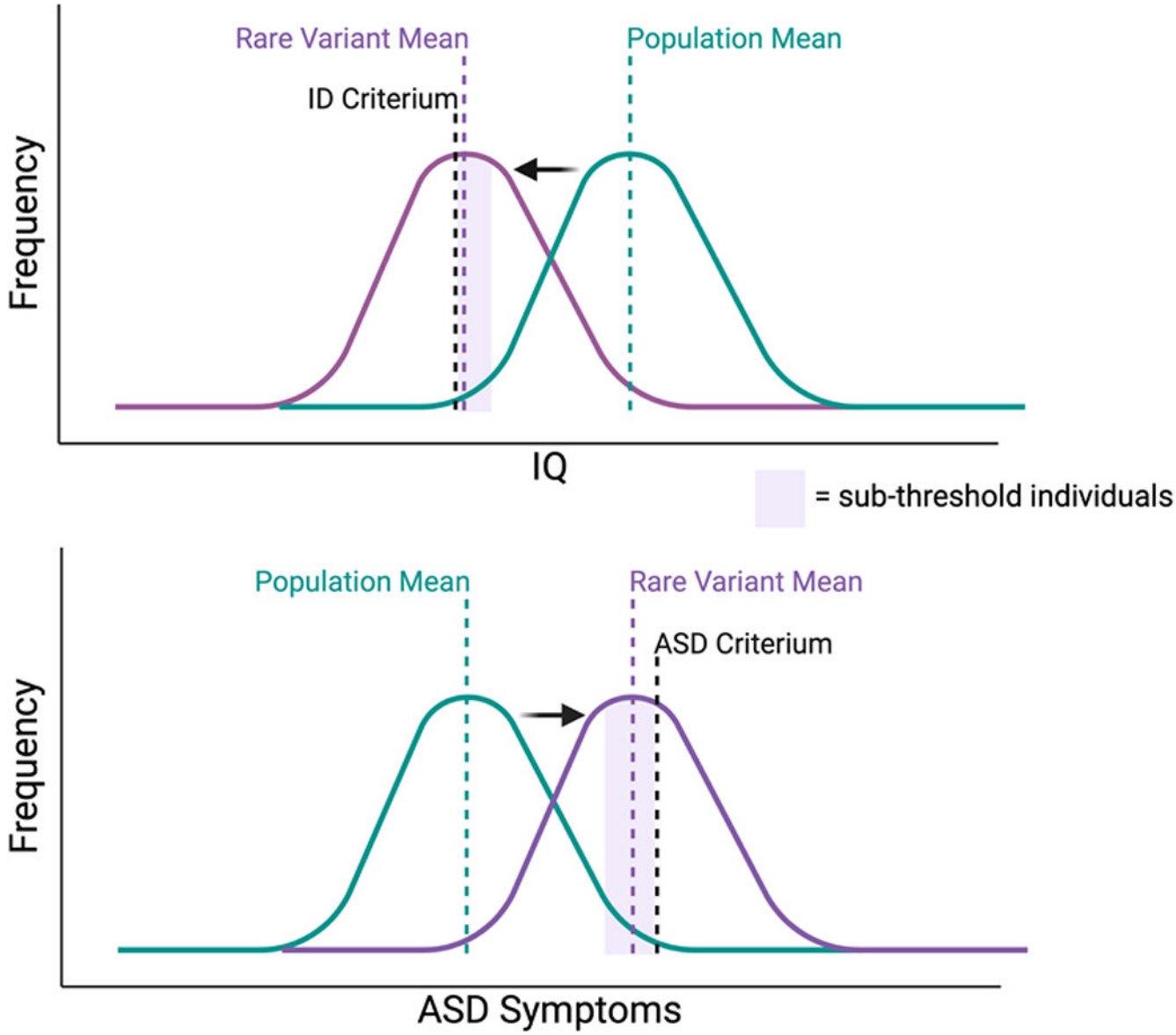


**Figure 1.**

Rare, large effect variants like the 22q11.2 deletion shift the distribution of quantitative traits such as IQ (top) or ASD symptoms (bottom). A much higher percentage of 22q11.2DS individuals qualify for diagnoses. However, many of those who do not meet diagnostic criteria still experience significant symptoms. Therefore, a concept of penetrance based on binary diagnostics does not capture the true effect of the variant as well as a shift in the distribution of a quantitative trait.