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Recent Advances in Understanding and Managing Autism Spectrum Disorders

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Abstract

Autism spectrum disorder in children is a group of neurodevelopmental disorders characterized by difficulties with social communication and behavior. Growing scientific evidence in addition to clinical practice has led the *Diagnostic and Statistical Manual of Mental Disorders* (*DSM-5*) to categorize several disorders into the broader category of autism spectrum disorder. As more is learned about how autism spectrum disorder manifests, progress has been made toward better clinical management including earlier diagnosis, care, and when specific interventions are required. The 2014 Neurobiology of Disease in Children symposium, held in conjunction with the 43rd annual meeting of the Child Neurology Society, aimed to (1) describe the clinical concerns involving diagnosis and treatment, (2) review the current status of understanding in the pathogenesis of autism spectrum disorder, (3) discuss clinical management and therapies for autism spectrum disorder, and (4) define future directions of research. The article summarizes the presentations and includes an edited transcript of question-and-answer sessions.

Keywords

autism; developmental delay; neurobiology; language; social communication

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Clinical Aspects

Advances and Changes With DSM-5

Sarah Spence, MD, PHD, Boston Children's Hospital—Dr Spence began her discussion on advances and changes with *DSM-5* by providing an introduction to autism. Autism is a behaviorally defined disorder according to the American Psychiatric Association *DSM* or ICD. It was previously thought to be one of the groups of pervasive developmental disorders. However, it is now considered a neurodevelopmental condition with symptoms appearing early in life; earlier than the age of 3 as previously believed. Autism occurs 4 times more frequently in males than females, however females seem to be more severely affected. There is no single etiology. Both treatment approaches and outcomes vary widely.

The *DSM-5*, which came out in May 2013, takes a more dimensional approach to autism. The number 5 opposed to the previous Roman numeral was used with the intention to make this a living document with place holders for important biological advances.

Previously, in *DSM-IV*, autism was described as a group of disorders including autistic disorder, Asperger's, Rett, childhood disintegrative disorder, and pervasive developmental disorder–not otherwise specified. There were several challenges to the diagnostic criteria in *DSM-IV*. One challenge was that it worked well for the classic school age or younger children (Kanner type) but not for younger children such as toddlers or adults. Also, pervasive developmental disorder–not otherwise specified was not very specific and overuse of this category led to diagnostic confusion and possibly contributed to the autism "epidemic." There was also substantial overlap of all of the categories (with the exception of Rett syndrome).

The *DSM-5* Neurodevelopmental Disorders Workgroup consisted of a diverse group including pediatric neurologists, child psychiatrists, child psychologists (clinical and experimental), developmental behavioral pediatricians, and a speech and language pathologist. The process involved biweekly teleconferences, semiannual in-person meetings and additional web conferences totaling over 2500 hours of work over the course of 5 years. The process was vetted through public comments, presentations at scientific and advocacy meetings, reviewed by leading experts and advocacy group members and is supported by almost all autism groups.

One major changes in the *DSM-5* criteria for autism included changing the name from pervasive developmental disorder to autistic spectrum disorder. This name change was done because symptoms are not pervasive but rather specific to social communication and behavior. Also the term autism spectrum disorder had already been in common use. Another change was that the previous 3 domains of social reciprocity deficits, communication deficits and repetitive-restrictive behaviors became 2 domains of social communication deficits and restrictive interests/repetitive behaviors. This change was statistically supported, namely by Frazier et al 2012 in the United States and Mandy et al 2012 in the United Kingdom, both of which supported the 2- versus 3-factor model.

The *DSM-5* also takes autism, Asperger's, and pervasive developmental disorder–not otherwise specified and combines them into the single diagnosis of autism spectrum disorder. The justification of merging the autism spectrum disorders into a single diagnosis stems from scientific evidence and clinical practice showing that a single spectrum better reflects the symptom presentation, time-course and response to treatment. It is thought that while separation of autism spectrum disorder from typical development is reliable and valid, separation of disorders within the spectrum is not. This decision was supported by a study by Lord et al 2012 in the *Archives of General Psychiatry* that included a large collaboration across 12 sites chosen for their autism expertise whose best estimate clinical diagnoses of the 3 aforementioned categories was not consistent across sites.

Under the *DSM-5*, Rett syndrome and childhood disintegrative disorder are subsumed under autism spectrum disorder only if they meet diagnostic criteria. In Rett syndrome, autism spectrum disorder behaviors are not particularly salient. Those who meet criteria are diagnosed and the specifier "with known genetic or medical condition" is included. Similarly, with childhood disintegrative disorder, *DSM-IV* criteria overlapped with autism with a regressive onset. Regression in autism spectrum disorder is a continuous variable, with wide range in the timing and nature of the loss of skills, however rarity made systematic evaluation difficult.

The new criteria in *DSM-5* for autism spectrum disorder is as follows: (A) Persistent deficits in social communication and social interaction across multiple contexts, as manifested by the following, currently or by history: (1) deficits in social-emotional reciprocity, (2) deficits in nonverbal communicative behaviors used for social interaction, and (3) deficits in developing, maintaining and understanding relationships. (B) Restricted, repetitive patterns of behavior, interests, or activities, as manifested by at least 2 of the following, currently or by history: (1) stereotyped or repetitive motor movements, use of objects, or speech, (2) insistence on sameness, inflexible adherence to routines, or ritualized patterns of verbal or nonverbal behavior, (3) highly restricted, fixated interests that are abnormal in intensity or focus, and (4) hyper- or hyporeactivity to sensory input or unusual interest in sensory aspects of the environment. (C) Symptoms must be present in the early developmental period (but may not become fully manifest until social demands exceed limited capacities, or may be masked by learned strategies in later life). (D) Symptoms cause clinically significant impairment in social, occupational, or other important areas of current functioning. (E) These disturbances are not better explained by intellectual disability (intellectual developmental disorder) or global developmental delay. Intellectual disability and autism spectrum disorder frequently co-occur; to make comorbid diagnoses of autism spectrum disorder and intellectual disability, social communication should be below that expected for general developmental level.

Examples are included in the new criteria, to allow a better description of the whole range of behaviors seen across the spectrum. Examples include descriptions of higher order social communication skills which allows for mapping of many symptoms onto a given criterion rather than having to see a specific behavior. It also allows for more clinician judgment. These examples are described as illustrative and not exhaustive so as to describe a range rather than reify them.

In the second criterion, requirement of meeting 2 of the 4 criteria was necessary for specificity. Addition of sensory abnormalities and allowance for symptom presence "by history" is thought to help capture most patients.

As a way to not lose specificity, specifiers were added to better describe each individual as well as severity ratings. Specifiers include with or without accompanying intellectual impairment, with or without accompanying language impairment, associated with a known medical or genetic condition or environmental factor, associated with another neurodevelopmental, mental, or behavioral disorder, and with catatonia.

Severity ratings across each domain were included in the *DSM-5* with the recognition that severity in 1 domain is independent of the other domain. The *DSM-5* avoids the terms "mild," "moderate," and "severe" and instead uses "requires support," "requires substantial support," and "requires very substantial support."

In summary, the advances include individual criterion which allows higher order symptom description and allows many different behaviors to qualify. The text description includes symptoms unique to various ages/developmental stages and verbal abilities. And last, the breadth of symptom description better captures the variability and allows for better subtyping.

There is controversy on the effect of the *DSM-5* on autism prevalence. However, most of the studies expressing concern about "missing cases" were done before the criteria were published. The main concerns are toddlers and the higher-functioning individuals and whether these cases are being missed.

Dr Spence concluded her discussion by referencing an article by Isabelle Rapin in 2014 discussing biology versus the *DSM* when classifying behaviorally defined disorders. All behaviorally defined disorders, including autism, involve 3 levels of investigation. These include a pseudo-categorical classification of mostly dimensional descriptions of behaviors/disorders (ie *DSM*) which is where most treatments are aimed, pathophysiologic biological mechanisms underlying these aforementioned "diagnoses" that compose hierarchically interacting molecular, cellular, and neural networks and categorical classification of genetic and environmental causes of these disorders.

Epidemiology—Prevalence, Environment, Risk Factors

Craig Newschaffer, PhD, Drexel University—Dr Newschaffer discussed the epidemiology of autism. He began by speaking on the rising prevalence of autism. The Autism and Developmental Disability surveillance system (ADDM), funded by the Child Health Act and started by the Centers for Disease Control and Prevention (CDC), is a records-based public health surveillance system. The ADDM works by defining a catchment area and partnering with the clinical and educational sources providing diagnostic and interventional services to the children in that catchment area. They pull records from kids with a variety of diagnostic classifications, including but not limited to children with an existing classification of autism and all children with any kind of special education exceptionality. These records are reviewed for a single age group, 8-year-olds, in the study

year. Information is abstracted from those records, which is then reviewed by expert clinicians who have been trained on a standardized review protocol. Based on the comprehensive review, the decision is made on whether it is autism. ADDM found that in the 2010, approximately 80% of the identified cases had an existing classification of autism in the community.

For 2010, the prevalence of autism among 8-year-olds in the United States was found to be 1 in 68 by the ADDM. Variability in their data is found among those areas where they did not have access to school data and prevalence is significantly lower. Otherwise, the prevalence estimates are in a fairly tight range with the exception of New Jersey which is an outlier on the higher end.

Although the 4-to-1 gender ratio has been maintained in all the years, there has been an increasing overall trend found in ADDM data (each 2-year interval has been between 14% and 29% over the 5 estimates that have been done). One factor believed to have contributed to this increasing trend is increased case ascertainment among children who have normal or above average intellectual functioning. In 2002, approximately one-third of the ADDM cases had normal or above average intellectual functioning. In 2010, it's approaching 50%. This raises the question of whether this is due to an increase in real risk in those cases or an improvement in recognition and assessment of those children, with the latter believed to be more likely. This question also comes into play with the variation of prevalence found among the different socioeconomic statuses, with the higher prevalence found in high socioeconomic status. Dr Newschaffer referenced a study by ADDM in 2002 and 2004, highlighting the prevalence of autism spectrum disorder by ecologic socioeconomic status and individual-level race/ethnicity and posed the question of whether this could reflect underlying risk or that diagnostic factors and diagnostic-seeking and service-seeking behaviors moves with socioeconomic status which influenced ADDM prevalence estimates.

There have been attempts in different data sources to try to quantify the impact of diagnostic tendency on prevalence estimation. There have been several studies that have looked at individual factors such as the recognition of autism at earlier ages and diagnostic substitution, particularly on the lower-functioning end (children who have acquired an autism spectrum disorder label that would have previously been labeled as cognitively impaired). However, it is virtually impossible to quantify the total effect of all these changing diagnostic tendency factors retrospectively.

Aside from ADDM and their records-based approach to assessing prevalence of autism, other approaches may be taken. In a Korean study in 2010, a community-based screening was done attempting to screen every child in a defined geographical catchment area followed by direct clinical assessments on all the screen-identified children. However, this approach faced problems relating to differential nonparticipation. Among those children who were in some specialized special education service programs, only about 30% actually consented all the way through to get an evaluation. Of those who were screened in the general education population, about 60% consented. Those who agreed to participate were more likely to have issues related to autism spectrum disorder which introduced bias and therefore an increased prevalence was found.

Another approach, done in the National Survey of Child Health, is to ask parents whether or not their child has been diagnosed. Approximately one-third of the children whose parents reported them ever being diagnosed with autism spectrum disorder no longer had a current diagnosis. This approach published prevalence estimates that tended to be higher than ADDM and is believed to be due to overreporting by parents.

In regard to global prevalence, the estimates of prevalence of autism in the developed world are fairly consistent (Asian prevalence estimates are slightly higher but still consistent). However, there is a lack of data available on prevalence in the developing world and therefore there is not much known on global variation.

Risk factors of autism spectrum disorder include both genetic and environmental factors. Earlier twin studies suggested a higher heritability of autism spectrum disorders, but in the most recent twin studies the heritability estimates for autism are around only 50%. In regard to environmental exposures as being important in autism spectrum disorder etiology, it is uncertain if there is a critical window during which these may occur with evidence suggesting prenatally. For example, studies have shown that findings like the disrupted microcolumn architecture would have to initiate in utero, suggesting that pathogenesis begins then. In an article published by Dan Geschwind and colleagues, it was reported that a characteristic gene expression pattern of normal development, differential gene expression across 2 different regions of the cortex that’s known to be established by midgestation was not seen in virtually all the brains of children with autism that they reviewed. This further implies that this is initiated prenatally.

In regard to the epidemiologic evidence, Dr Newschaffer presented meta-analyses from 2009 and 2011 which revealed that although the evidence was deemed as insufficient by the standards used in formal meta-analysis, factors that were implicated included older parental age, preterm/low birth weight birth, maternal medication use, pregnancy complications (ie bleeding and gestational diabetes) and perinatal/neonatal health. The effect of any exogenous environmental chemicals on autism and folic acid exposure protecting against autism are areas where some studies are being done but more epidemiologic work is needed.

There are 2 major challenges that arise when developing epidemiologic evidence. One challenge is exposure assessment. For example, prenatal environmental exposures are difficult to measure and present with chance of error which can lead to misleading results. Sensitivity analyses can be done to address this challenge. Dr Newschaffer uses an article he published looking at maternal prenatal SSRI exposure and autism risk as an example. The challenge with studying SSRI exposure and autism risk in mothers who take SSRIs in pregnancy is that these women had indications such as depression which might be independently related to autism through genetic pathways. This work was done using the Denmark registry consisting of 5000 cases and 50 000 controls. However, it was found that both the prevalence of SSRI exposure prenatally and prevalence of maternal depression during pregnancy in the registries were below that of published estimates in Scandinavian countries and therefore these results had to be corrected for statistically. Another strategy to face this challenge of exposure assessment is to work on exposure biomarkers. In the EARLI cohort study done by Dr Newschaffer et al, mothers who have a child with autism are

followed from pregnancy. Biological samples are collected from both the pregnant mothers and fathers during the window when it is believed that environmental factors are most likely to influence outcome and then the babies are followed until 36 months of age.

There is currently and analysis being done on exposure to PBDEs, the endocrine-disrupting compound, the flame retardants that are commonly used and that bio-accumulate in the environment. The only previous data on PBDEs and autism is from a case control study where blood levels of PBDEs were measured in children with autism at the time diagnosis which showed no differences seen across the range of PBDEs. However, if PBDE exposure is going to have an etiologic effect, it will likely be during the prenatal period. It is hypothesized that the mechanisms of action might be that PBDE influences DNA methylation, epigenetic regulation. PBDEs are also known to affect thyroid hormone levels, which are known to have developmental effects.

The second challenge that arises when developing epidemiologic evidence is focusing on gene-environment interaction. However, to get the scale to detect gene-environmental interactions with the current approaches available are challenging and should be a continuing focus in autism spectrum disorder research.

Early Infant Development and Intervention

Lonnie Zwaigenbaum, MD., University of Alberta—Dr Zwaigenbaum discussed early infant developments and intervention in autism spectrum disorder. He stressed the importance of early diagnosis of autism. There is a delay between parents' first concerns and confirmation of diagnosis with parents being aware of early differences in their children in the first year or 18 months of life and the average age of diagnosis, even currently, remaining around 4 years in the United States and in Canada. Early diagnosis would allow children to enter autism-specific interventions at an earlier stage. Also, there is broader scientific relevance to studying autism spectrum disorder early in life with the opportunity to understand the evolving neurobiology in real time rather than only in retrospect after the point of diagnosis.

As more is learned about how autism manifests, even in the first year of life, it can help to guide both clinical decision making and opportunities to develop specific interventions. However, there are broader systems at hand that influence the timing of diagnosis such as the ability for parents to make themselves heard, the current uptake of autism spectrum disorder screening in community practice and availability of specialized assessments and intervention.

The remainder of Dr Zwaigenbaum's discussion focused on 3 objectives: early behavioral and neurological features of autism spectrum disorder, implications for early detection and diagnosis, and recent advances in early intervention.

Many of the advances over the past decade have been through prospective longitudinal studies of at-risk infants, typically, younger siblings of children with autism. Currently, this high-risk design is being applied in other areas of neurodevelopmental disability and neuropsychiatric disorders, most notably, schizophrenia; studies of parents or offspring of

schizophrenia looking at some of the underlying endophenotypes. This high-risk design is particularly well suited to learning about autism in terms of the early onset of autism, as well as the early diagnosis. By working with younger siblings of children with autism, it would be possible to differentiate which siblings will go on to develop autism versus those who aren't, and actually map the early behavioral and neurobiological features onto diagnosis.

Over the years, early detection and screening approaches have been guided by evidence learned from other study designs, from speaking to parents and from review of early home videos. However, it is becoming evident that being able to work with infants in real time provides unique opportunities to both directly study the neurobiology and develop and evaluate interventions specifically for that infant age group.

While it is known that younger siblings are at a higher risk of developing autism, there is some debate regarding the recurrence risk. Previously, the best recurrence risk estimates came from studies that were done in the 1980s from the *DSM-III* era (with a more restrictive definition of autism and at a time when the prevalence of autism was roughly 1 in 1000 and this recurrence risk estimate was about 8%. A few years ago a collaboration of research groups studying these at-risk infants came together and looked at the recurrence risks and the pooled recurrence risk estimate was about 18.7% which coincided with the increasing prevalence of autism. However, 3 recent large population studies done between 2013–2014 in Denmark, Sweden, and California placed the recurrence risk, again, closer to 8 to 10%. While it can be postulated that these population-based studies rely on passive ascertainment and perhaps there are missing cases, it can be said that the recurrence risk is somewhere between 10 and 20% and there is further work to be done to further narrow down this risk.

In regard to what is being learned about early development by following these at-risk infants, there is accumulating evidence that the defining features (symptoms that map onto the *DSM-5* framework) are present in most children by the age of 12 to 18 months. In addition, there are a number of features which a number of groups have described as prodromal symptoms because they involve developmental domains that are not necessarily specifically viewed as part of the autism spectrum disorder phenotype where they involve behavioral correlates that map onto social communication symptoms, and a number of groups have found, again, evidence of difference, even if it's not autism symptoms, in the first year of life.

Given the diversity among children with autism, it shouldn't be surprising that there's variability in onset and early developmental course. Similarly to the identification of prodromal symptoms behaviorally, there's evidence from a number of groups suggesting that there are differences in brain development and function that are detectable in the first year of life that in a way identify children with specific neurobiological vulnerability perhaps even before the onset of cardinal symptoms and creating an important window for intervention that might even prevent some of the disability of autism.

A number of the behavioral findings in autism, even by 12 months of age, very clearly map onto the symptoms seen in older preschoolers. One is example is deficits in social reciprocity. Children with autism consistently by the age of 1 are not responding when their

name is called; they show a reduction in social smiling; a general reduction of sharing a positive effect in interactions. There are a number of studies showing deficits in nonverbal communication: reduced eye contact, reduced initiation of joint attention, reduced use of gestures. Deficits in developing relationships in some respects are more difficult to assess because the primary relationship, of course, is with the parents early in life. In fact, a number of studies have shown typical attachment behaviors in high-risk siblings, but clearly interactions with the parents and other caregivers are impaired.

In addition to deficits in social reciprocity, restricted, repetitive patterns of interests and behavior may be seen. The use of objects tends to be of 2 types. One is of actual repetitive actions, such as spinning and rotating and actually using the objects in a repetitive rather than in an imaginative way. The second broad type is the unusual, visual inspection of objects. This is a form of sensory exploration, particularly of using the visual modality; looking at the toy from different angles; rotating it. With increasing interest in studying early sensory regulation and responsivity in infants, it has been found that younger infants (infant siblings of children with autism) do have atypical auditory processing and underreactivity to sensory input.

A number of groups have also looked at the temperament as a way of getting at differences early in autism. As such, infants who later develop autism have either underreactivity or over-reactivity and irritability. This is consistent with what parents report in terms of among the first features that they become aware of is just that their child is just not responding to the environment like other children.

The prodromal symptoms previously mentioned, occur in the first 12 months of life and include motor and atypical visual orienting. Based on standardized measures such as the Mullen, groups that have looked at developmental trajectories over time find that by the age of 12 months there are deficits in both fine and gross motor skills. These deficits were not detected at 6 months, but by 12 months showed clear motor lags that on an individual basis wouldn’t necessarily identify this child as having autism, but there was definitely a shift in the distribution of those skills to later development.

In addition, there are a number of studies that suggest that the quality of motor skills even earlier in life (around 6 months of age), are different in at least children who go on to develop autism versus infants who don’t have a family history of autism. Dr Zwaigenbaum references 2 studies from Rebecca Landa, one looking at the quality of movement using the Alberta infant motor skills, and the second looking at head lag in 6-month-olds during a general developmental assessment, suggesting that infants with autism have reduced tone, reduced quality of movement, and persistent head lag.

Interestingly, though, infant siblings who do not go on to develop a diagnosis of autism have a pattern of motor development that’s somewhat intermediate between the high-risk infants that develop autism versus infants who don’t have a family history. Because these studies have been relatively small and perhaps underpowered, the difference hasn’t been between the high-risk infants who develop autism versus the high-risk infants that don’t. As such, it is still undetermined whether this is an endophenotype that affects high-risk infants more

generally or specific to autism. However, it is interesting that motor development, which does not tend to be something often thought about in early diagnosis of autism, is among the earliest emerging signs.

In addition, there's been a lot of interest in looking at patterns of visual orienting in response to social scenes, and whether those kinds of differences might identify infants at risk for autism before more overt impairments in social communication skills. For example, in a study done by Katarzyna Chawarska in 2013, which involved infants sitting on their parent's lap in front of videos of dynamic social scenes and using eye tracking as a way of looking at where the children were looking. Essentially, it was found that infants within the high-risk group who went on to develop a diagnosis of autism spent less time looking at the person, and, in particular, spent less time looking at her face. Now, interestingly, in this particular study, there was no difference in the time spent looking at the eyes versus the mouth, but rather just a general reduction of time looking at the face at 6 months in those infants who went on to develop autism.

In a subsequent study, Warren Jones and Ami Klin used a similar paradigm. They sat infants in front of an engaging person in a video and examined where they were looking using eye tracking and they added the innovation of assessing eye tracking at multiple points in time in the first year of life. They essentially found that the typically developing infants over time spent more and more time looking at the woman's eyes. By the time that the child was 6 months and beyond, there had been a real shift from examining simple visual contrast in the scene and also examining the mouth to really focusing on the eyes, which is expected in typical social interaction. In contrast, the infants who went on to develop autism, actually started by spending a reasonable amount of time at looking at the eyes, and then there was actually a decline over time. In conclusion, what they found was not that there were significant cross-sectional differences in the first 6 months, but rather that if one looked at the change in the relative amount of time that the infants were spending looking at the eyes versus the mouth, the infants with autism showed a reduced looking at the eyes; the infants who didn't have autism showed an increased time looking at the eyes; and the difference in slope between 2 and 6 months was highly predictive of which infants went on to develop autism.

Dr Zwaigenbaum and his group developed an observational scale to help track early signs of autism in the first 18 months of life. In this study, they assessed children at multiple intervals between 6 months and 18 months, and tracked the total score on an observational scale where higher scores indicated higher levels of symptoms. The data were analyzed using semiparametric group-based analysis. There were essentially 3 groups within the high-risk infants including ones that have a progressive increase in symptoms, and then 2 groups who have a steady level of symptoms (1 with a moderate level of symptoms and 1 with a low level of symptoms). Interestingly, when the outcomes of these 3 groups were mapped onto trajectory patterns, the children with autism were roughly evenly distributed among those 3 trajectories. Therefore, while there is a minority of children who really declare themselves in the first year, there is a majority of children with autism who have a relatively stable pattern of symptoms in the first 18 months. Not surprisingly, it's the children with stable or lower symptoms that tend to be diagnosed later, and who even will be missed with use of

conventional screening and diagnostic assessment tools. In conclusion, while the progressive symptoms are quite specific to autism and can help us identify a subgroup, there is still work to be done in identifying the children across the spectrum.

In regard to brain development and brain functioning, evoked response profiles and neuroimaging have been studied. A study by Mayada Elsabbagh in 2013, looked at brain responses in response to looking at faces, and specifically the ability to differentiate looking at faces with eyes looking at versus eyes looking away. She found that the children with autism stood out in terms of the amplitude difference. This was seen as early as 8 months and would differentiate the children who went on to develop autism.

From a study of infant brain imaging by Joe Piven, differences in brain volume were found to emerge after 6 months of age and progress between 6 months and 24 months. There was also evidence suggesting atypical connectivity, both from Diffusion Tensor Imaging (DTI) studies, as well as from network analyses. The major caveat is that although head growth is correlated with brain growth, in fact, in this infant sib research differences in head growth relative to community controls were not seen. Therefore, being that previous head circumference literature in autism comes from comparing to CDC norms rather than community controls, the evidence on the whole is actually much more mixed for head growth than is currently thought. Another study done by the Infant Brain Imaging Study Network, found evidence of atypical connectivity with blunted white matter tract trajectories in children between 6 and 24 months of age and a reduced network efficiency at 24 months.

There have been exciting advances in the intervention world and by working with infants directly early in life, there's been the opportunity to evaluate and establish an evidence base for intervention as early as the first year. Dr Zwaigenbaum discussed several specific intervention models. The first was the Social ABCs which was done by his group and was adapted from pivotal response training, a specific form of applied behavioral analysis. Both a case series, as well as an initial randomized control trial have been completed. This included in-home, parent-mediated, in-vivo coaching with a 12-week parent-training (tapered) and a 24-week follow-up with generalization probe. They found improvement in language skills, social responsiveness, and sharing of affect. Another intervention model, called the First Start, studied by Sally Rogers and Geraldine Dawson, which was adapted from the Early Start Denver model, which had already shown benefit for children with autism as young as 18 months. In the First Start they are now targeting infants with early symptoms from as early as 7 to 15 months. Symptoms that are identified based on an observational scale, the autism observational scale for infants. Based on a relatively brief period of parent-directed intervention, they're finding long-lasting effects on reducing autism spectrum disorder symptoms and improving overall developmental health.

Comorbid Epilepsy

Roberto Tuchman, MD., Miami Children's Hospital Dan Marino Center—Dr Tuchman discussed the relationship between autism spectrum disorders and epilepsy focusing on the impact of epilepsy on autism, the impact of autism on epilepsy and the shared mechanisms of both of these disorders. He began with a conceptual model showing the clinical overlap between epilepsies and autisms. Being that both of these disorders are

heterogeneous, there is not one etiology that includes autism and not one etiology that includes epilepsies, and both have variable outcomes. In addition, there is intellectual disability which is a big part of this relationship and what strongly unites this relationship. Other risk factors include age, gender, language function, and regression.

In a study done by Suren in 2012, he found that 11.2% of children with autism had epilepsy. This was a study looked at children up until the age of 11 years old, with the thought that this number could have potentially been higher had the children been followed until later in life. In addition, 6.1% of children with epilepsy also had a coexisting diagnosis of autism.

In a meta-analyses done by Amiet in 2008, 24 reports on autism and epilepsy published between 1963 to 2006 were looked at and the pooled prevalence of epilepsy found that 21.4% of the 1485 individuals with epilepsy had autism and intellectual disability with an IQ less than 70. In the children with epilepsy, 8% of the 627 individuals had autism without intellectual disability (IQ > 70). Of note, in the articles included in the meta-analysis, intellectual function was tested in a standardized manner. It was also found that gender might also be 1 of the factors related. It is known that there is a 4-to-1 ratio of male to female, however, females seem to be more likely to have intellectual disability when identified, and that might be 1 of the reasons why a higher level of epilepsy is seen in females.

Another meta-analysis done by Woolfenden in 2012 looked at 16 studies measuring the rates of epilepsy in autism spectrum disorder. There was only 1 study included that overlapped with the Amiet meta-analysis. In these studies an IQ of 70 was used as a cutoff as well. In 4 of the studies, if the IQ was less than 70 which was seen in less than 70% of the children and if the age of follow-up was greater than 12 years old the pooled estimate of epilepsy at follow up was 8.9%. In 1 study where less than 70% of the children had an IQ of less than 70 and the age of follow-up was less than 12 years old, the estimate of epilepsy was 1.8%. In 9 studies where greater than 70% of the children had an IQ of less than 70 and age of follow-up was after 12 years old, the pooled estimate of epilepsy at follow-up was 23.7% and in 2 studies where greater than 70% of the children had an IQ of less than 70 and follow-up at less than 12 years old, the estimate of epilepsy was 6.1%. Therefore, the rate of epilepsy was higher in those with intellectual disability and with age of follow-up. The other outcome measure that Woolfenden looked at was also mortality and found that the mortality is higher in those children with autism and epilepsy than it is just with autism concluding that epilepsy does impact autism spectrum disorders, and therefore is important identify.

Dr Tuchman also referenced a study he did in 1991 which looked at the risk of epilepsy in children with autism when all other risk factors are eliminated. The cumulative risk was 67% by age 10 with both severe intellectual disability and motor deficit, and it was 27% by age 10 in children with ID and severe ID. It was 6% by age 10 in those with no intellectual disability and no other risk factor (ie, no family history of epilepsy). The control group was children with language impairment and they had 8% cumulative risk of developing epilepsy in this language-impaired nonautism group. Interestingly, it was found that there was a very early peak of epilepsy at around age 5 and then a later peak during adolescence. However, when looking at the early autism spectrum disorder group, there were children with epileptic

encephalopathies who started out with epilepsy very early on and were placed in the group with children with autism and epilepsy. While it is known that children with autism regress, the question was raised on whether children who have regression in autism and have an abnormal EEG are similar to some of the other epileptic encephalopathies that are defined by the epileptic abnormalities contributing to the progressive disturbance in cerebral function.

Dr Tuchman emphasized the point that autism spectrum disorders, even with regression and an abnormal EEG, are a very different group of disorders than epileptic encephalopathy. While many of the genes that are being identified that lead to epileptic encephalopathies are the same genes being linked to autism spectrum disorders, there are several differences. One difference is that in children with autism spectrum disorders, the age of regression is usually below the age of 2 where they lose about 5 words while in epileptic encephalopathies such as Landau-Kleffner syndrome, the children will have developed much more language and their age of regression is usually after age 3. Also, the EEG that is usually seen in autism spectrum disorders show spikes that come out on and off which is very different from what is seen in epileptic encephalopathies. In addition, there's a difference in terms of the behavioral patterns seen in these kids with Landau-Kleffner syndrome and autism spectrum disorders.

In regard to the children with autism spectrum disorder who start presenting with seizures later in life, a study done in 2005 by McDermott et al, showed that there is an increase of prevalence of epilepsy in Down syndrome and in autism spectrum disorders beginning in early adolescence. When looking at children who had intellectual disability and cerebral palsy, the prevalence declined in the 20s and 30s.

In a study by Bolton et al in 2011, a genetic cohort of 150 children excluding those with epileptic encephalopathies, such as infantile spasms, were studied and epilepsy was present in 22% of individuals with the mean age of seizure onset being 13.3 years, and the majority of children were over 10 years old. Epilepsy was more common in those with intellectual disability, low levels of language, and in females, and the presence of epilepsy in the proband was a significant predictor of a relative having a broader autism phenotype which suggested shared genetic components.

Dr Tuchman referenced another study which was done by Viscidi et al in 2013 which included 3 genetics groups including the Simon Simplex group, the AGRE group, the Autism Consortium, and then also the National Survey of Children's Health, which was not a genetic group. Again, this cross-sectional study showed the average prevalence of epilepsy was 12% and reached 26% by adolescence. In a multivariate regression model looking at language, gender, and other factors, only age and cognitive ability were independently associated with epilepsy. Children age 10 or older had 2.35 times the odds of being diagnosed with epilepsy and for a 1 standard deviation increase in IQ, the odds of having epilepsy decreased by 47%. They also looked at other behaviors, such as attention-deficit/hyperactivity disorder in a subsequent study and they didn't find that after controlling for IQ that there was any major difference, other than in the children who had normal IQ and had

epilepsy and autism, they were more likely to be somewhat more irritable and somewhat more hyperactive than the general population.

Cuccaro and colleagues in 2012 used a latent class cluster analysis to explore the relationship between autism spectrum disorder and epilepsy. Of the 5 clusters, the cluster with the highest prevalence of epilepsy at 29% was those with an earlier age of autism spectrum disorder recognition, greatest rate of repetitive object use and unusual sensory interests, late onset of first words and high frequency of gross motor coordination problems. The lowest prevalence (8%) was seen in those with the least cognitive impairment.

Research questions regarding epilepsy in autism spectrum disorder include what can be done for the early identification of children with autism spectrum disorder at risk to develop epilepsy: While autism spectrum disorder is not an epileptic encephalopathy, why does it frequently travel along with epileptic encephalopathies? Are there genetics reasons for this? And what is the contribution, added effect, of epilepsy or interictal epileptiform discharges on the development of autism spectrum disorder?

Dr Tuchman then discussed the risk of having autism spectrum disorder after first presenting with epilepsy. Several studies have shown that a lot of children with epilepsy also have autism spectrum disorder. In a prospective study done by Berg et al in 2011 using the Connecticut Epilepsy Cohort, 5% of the overall group met criteria for autism spectrum disorder. Of those whose seizures started in the first 2 years, 10% met criteria; 13.8% in those with IQ less than 80 met criteria and 2.2% with normal cognitive abilities met criteria for autism spectrum disorder. West syndrome/infantile spasms accounted for 30% of those children with autism spectrum disorder. Intellectual impairment and male sex all were independently associated with autism spectrum disorders.

In a study by Saemundsen et al in 2007, 7% of 84 children with seizures in the first year of life were diagnosed with autism spectrum disorder and all had intellectual disability. Of 17 children with infantile spasms, 35% were diagnosed with autism spectrum disorder. In a nationally representative large population-based study done in 2012 by Rai et al in the United Kingdom, autism spectrum disorder occurred in 8.1% of patients with epilepsy above the age of 16 years old. After adjusting for verbal IQ, an individual with epilepsy still had a 7-fold increase in the odds of having an autism spectrum disorder.

It is evident that the early identification of children with epilepsy at risk of autism spectrum disorders is important. One big group at risk are those with epileptic encephalopathies and in addition to recognition, intervention is of great importance.

Also, while epilepsy and intellectual disability in epileptic encephalopathies are associated with autism spectrum disorders in 30% of the population, there are subsequently 70% that do not have it and therefore it is important to identify these protective factors.

In an article done by Jeste and Geschwind in 2014 looking at the genetic component in autism and epilepsy, there were many syndromes/genes that are seen in both autism and epilepsy.

From a pathophysiology viewpoint, there are shared mechanisms between epilepsy and autism including altered neuronal networks, structural and molecular connectivity, and altered neuronal excitation/inhibition. Ultimately, abnormal excitability in the developing brain disrupts the synaptic plasticity and can lead to cognitive and behavioral problems, such as autism. This is also the mechanism of epilepsy. The question here still remains to what degree the epilepsy then further impacts these global cognitive effects seen in autism.

The discussion concluded with the idea of not only treating children with epilepsy with pharmacological antiepileptic drugs, but also to think about behavioral intervention that can be done in children with early onset epilepsies at risk for autism spectrum disorders. Also, with beginning to understand the important pathways in both autism and epilepsy, specific molecular treatments may be developed.

Q&A Session

DR MOSTOFSKY: I'm going to invite the 4 speakers from this morning's session to come back up. We've got 3 and here comes the fourth. So we're happy to take questions.

MALE VOICE: Hospital Philadelphia. A question for Dr Spence. As a clinician I know the developmental pediatrician who cannot spend a lot of time doing 3 hours evaluation, obviously, we use *DSM-IV* and other short tools. How good are we in using these tools? And my specific question is are there any studies correlating the diagnosis with *DSM-IV*, *DSM-5* in the future and other specific diagnostic tests for autism like ADOS or others?

DR SPENCE: So I think that the answer is in terms of—so I think the question was are there any studies that are looking at how good clinician evaluation is compared to those using—

MALE VOICE: [Interposing] Using *DSM-IV* versus a specific confirmatory testing of autism in patients.

DR SPENCE: I think to my knowledge there aren't in terms of taking a big cohort that was clinically diagnosed and then applying the gold standard diagnostic tools. I guess what I would say to you is the gold standard diagnostic tools were developed for research. They were not developed for clinical purposes. And we talked a lot about this in *DSM-5*. It is a book for clinicians. It's a book of diagnostic criteria to be used in the clinic.

And everybody said you do not need an ADOS, you do not need any of these tools that have been developed, which I think are lovely tools, but as you say, most of us can't afford to do a 4-hour assessment. And, in fact, the insurance companies won't pay for that 4-hour assessment. So we very specifically said you don't have to use them. You have to use your judgment. You ought to do a good developmental history and you ought to do a good behavioral observation. And for those of us who are doing it in an hour, which many of us are, you have to get some corroborating information. So I spend a lot of time calling school or calling the daycare. I mean, an hour is probably not enough to really know that child, but there are a lot of people out there that do know that child and then you use the diagnostic criteria.

MALE VOICE: But confirmation would be the gold standard to see how good what you are doing in the clinic is, yes?

DR SPENCE: Sure. You know anybody who wants to fund that study?

MALE VOICE: I don't know, but we are spending all this time doing *DSM-IV*, *-IIIs*, *-IVs*, *-5s*, and the question is how good are we doing this. We are thinking that these are better. Are they? So I think it would be an interesting study.

DR SPENCE: Well, I would say to you that all those diagnostic tools were based on the *DSM-III*, *-IV*, and *-5*, so it's a little bit of a circular argument of saying are we better as clinicians than the tools are when the tools were based on the diagnostic criteria.

DR MOSTOFSKY: I mean, I guess it's worth quickly mentioning that the gold standard is still experienced clinician diagnosis. And I'll say from my own clinical experience, and I think everybody here could probably confirm this or most people could confirm this, that there's certainly been times where I've been in the clinic, we've had an ADOS done, and we've disagreed with the ADOS results. Most commonly that the ADOS on the side where the ADOS will say that the child—it will come back with a score consistent with autism and spectrum and we'll disagree with that.

But I think we need to move on to the next question. So number 2.

FEMALE VOICE: This is for Dr Tuchman. Two parts to the question. One, what do you do with the epileptogenic EEGs in children with autistic spectrum and they haven't had clinical seizures, do you treat them? And, 2, with the treatment do you go for the standard antiepileptics or do you also give immunomodulators IVIG and so on? Want to get your thoughts on it.

DR TUCHMAN: So the question is do you treat an abnormal EEG in a child with autism spectrum disorder. And I think that is a discussion that comes down to what I was discussing in terms of epileptic encephalopathy. The answer to the question is it depends on what we see on the EEG. If it's a very dramatic EEG that shows continuous spike and waves that are ongoing for greater than 50%, especially during sleep and it's disrupting that or if they're having discharges that last more than 3 seconds where it is a seizure, if you will, then I would treat it.

But if not, for the regular EEG where there's nothing else, the answer is no, I don't, because there's no evidence right now that's standard antiepileptics or frankly even the immunomodulators make a difference in terms of that.

I think that the point that I was trying to make is that one of the interesting things is we have to go away, move away from the fact that the EEG, it may be the cause of the behaviors when there's nothing else, and start thinking about what are the shared mechanisms there and begin to use some of the new tools of neurobiology and molecular understanding to begin to develop new treatments. I think this is actually a really good model to look at that.

But from a clinical perspective, I think that we still need to use clinical judgment in these kids and you usually wouldn't treat a child just who has an abnormal EEG with a few spikes in practice and nothing else going on.

DR MOSTOFSKY: Let's move to number 3.

MALE VOICE: Amat Kadura [phonetic] from Flint, Michigan. It's follow up on the same question. So if a patient with autism present with a history of regression, do you routinely order an EEG and you order routine EEG or long-term? This is something that—

DR TUCHMAN: So from that perspective, I would say to you that if a child has autism and regression it, again, depends on the clinical history; how bad was their regression; when did the regression occur; did he subsequently continue to develop. So, again, it's you're using your clinical skills to determine what's going on; is the regression more than just a couple of words or did it include motor regression. So that all becomes very important and I'm assuming that all child neurologists would be looking at all of those factors.

My own experience is if I believe that this child is having regression that is really significant and has other behaviors that are suspected of seizures, I do an EEG. And, yes, an overnight EEG is better because you want to capture sleep because what you're looking for it's the same workup that you would do if you're suspecting that a child has an epileptic encephalopathy.

I think that in general, my experience and I think what the studies have shown is that when you see a routine child with autism and regression whom is doing well at that time, that it's unlikely that you're going to find an EEG that has ESES or continuous spike and wave during slow-wave sleep. If you see a child who has autism regression and frankly a regression was more than just 5 words and was a little bit more complicated and has some other behaviors that are significant to suggest seizures, then in that child I would do an overnight EEG and look to see if we are looking for—if that child has ESES, and then I would use the treatments that are appropriate for more of an epileptic encephalopathy.

DR SPENCE: Can I just add? We just did a review for epilepsy and behavior that will be coming out a little bit later. And the regression piece either for epileptiform EEG or for epilepsy itself, whether it predicts is actually the data are all over the place. You can find just as many studies that say it predicts that it doesn't predict. So I think it's a hard thing. But as neurologists, when we see it, we think about an epileptic encephalopathy or Landau-Kleffner and that's a treatable disorder and we don't want to miss it. So I think we all look for it, but I'm not sure that it is actually as predictive as some people have said.

DR MOSTOFSKY: Dr Dresher [phonetic].

DR DRESHER: This is for Dr Newschaffer. When I looked at your ADDM data, when you looked at the SES, there was quite a spread between recognition of autism amongst different groups. I don't know if that's important, but is there anything that you interpret from that? So among non-Hispanic Whites there was a relatively low difference whether they were high

SES or low SES as opposed to, say, the Asian population where there was a fairly high difference.

DR NEWSCHAFFER: So, first of all, it's a little deceiving to make that inference from that graphic because what you have to realize is that the racial ethnic differences are confounded by the SES differences. So, for example, although in the non-Hispanic White group some of the prevalence in the higher SES group was actually lower than in the high SES group in the other race groups. But in the non-Hispanic White group there are obviously a disproportionate amount of families in the higher SES category.

So, overall, in the ADDM data, the highest prevalence by racial ethnic group is in the non-Hispanic Whites. And in the other racial ethnic groups the overall prevalence is still lower, and it's been fairly stable since 2002 to 2010. There's been a little narrowing in the African-American groups, the prevalence coming a little closer to the Whites, but it's been fairly stable.

What that is? Some of it is certainly explained by the SES confounding. Are there other confounders going on as well? Hard to say. Could there be some real risk differences? Difficult to say.

MALE VOICE: For Dr Zwaigenbaum. What is the hypothesis of how therapies, ABA, behavioral therapy, and others work in children with autism are improving their symptoms?

DR ZWAIGENBAUM: In terms of the theory, I suppose that one could think about it both —the question is what's the theory about how therapists, particularly using ABA, can improve behavior and well being in children with autism.

So I think that one can think about it at 2 levels. One if actual direct skill building and reinforcing positive social behavior, and using behavioral teaching strategies in order to implement skill development. But I do think the study from Geraldine Dawson that Roberto just cited was also quite interesting, because the same children who showed benefit in terms of developmental progress in improving symptoms based on the Early Start Denver model also showed normalization of brain responses to exposure to faces, which suggests that it's occurring both at a behavioral level, as well as a brain processing, at least, if not brain function level.

DR MOSTOFSKY: I'll say one comment about ABA. Thoughts that I've had that sort of tie into notions that we've had in our lab that we've been pursuing with regard to motor dysfunction in autism, and the idea that there's maybe some basic dysfunction in procedural or skill-based learning. Is that ABA tends to use a more sort of explicit declarative kind of approach to teach children with autism things that we learn, tend to learn more naturally and implicitly and procedurally. So there may be some notion to the benefit of ABA in providing that kind of scaffolding for incorporating other learning approaches more declarative explicit-based learning approaches to teach skills that are normally gained implicitly or procedurally.

Number 3.

MALE VOICE: This question for Dr Lonnie. Have you looked into the sensory symptoms as the predominant features in autistic spectrum disorder? Like oral-motor dysphagia or reflux. And the kid is coming in 3 or 4 months' time they have a lot of problem with feeding difficulties, they have a lot of refluxes, and they have problem with solid food. That can be an early sign for autistic spectrum disorder later on.

DR ZWAIGENBAUM: I apologize because I'm actually having problem hearing.

DR MOSTOFSKY: I actually did not understand the question at all.

MALE VOICE: My question is regarding the sensory symptoms as a prodromal sign for the autistic spectrum disorder.

DR ZWAIGENBAUM: Sensory. Absolutely. I think some of the earliest key studies reported early differences in basic biological functions, so feeding and sleeping and those kinds of self-regulation procedures that have a sensory component. And certainly in the infant studies we are seeing parents report even in the first year sensory regulation differences.

Now, when you first asked the question I think you asked about refluxes and—

MALE VOICE: [Interposing] Yeah, the refluxes like a lot of children come with like spitting or reflux problem. They are oral-motor dysphagia.

DR ZWAIGENBAUM: So, again, I think a lot of this has been reported in retrospect so we do know that infants who go on to develop autism often have feeding problems. I don't know that they've been investigated that systematically in terms of the source, in terms of dysphagia or discoordinated movements around feeding and swallowing. But I think in general, there's certainly interest in emerging evidence around sensory and regulatory processes being abnormal very early on, even before some of the social communication symptoms.

DR MOSTOFSKY: And one of the changes in *DSM-5* is actually including sensory dysfunction as a specific criteria. So it's certainly increasingly recognized and I should say as well that our group and several other groups around the world are actually starting to pursue actual physiologic studies of tactile, visual, auditory, sensory dysfunction that are providing some key insights into potentially into the pathophysiology of autism.

The ability to look at these more basic aspects of brain functioning and neurologic functioning more generally may provide some important windows, for instance, relationship to GABAergic processes and other pathophysiologic mechanisms.

MALE VOICE: Thank you.

DR MOSTOFSKY: If no other questions then we're going to take a break.

Session II: Pathogenesis

Genetics

Jonathan Sebat, PhD, University of California, San Diego—Dr Sebat discussion covered the genetic basis of autism from studies of rare genetic variation, copy number variants and exome sequencing and new discoveries from comprehensive analysis of de novo mutation in autism. The genetic architecture of autism consists of a wide spectrum of different alleles, and although previously the full spectrum has not been captured, a subset of it has been captured reasonably well.

Along this spectrum, there is frequency and effect size. There are some rare variants of very large effect, but invariably these are variants that have very low frequencies in the patient sample. In addition, there are common variants of small effect that contribute risk to the disorder. These are variants that are common enough to be observed at 20%, 30%, 40% frequencies in patients, but they're also observed in practically the same frequency in healthy individuals, so there's a very modest effect on risk coming from the common variants. There's very substantial effect on an individual's risk coming from the rare variants.

What is known about the rare contributing genetic variants came mostly from copy number variation studies performed over the last decade. Information regarding the common variants is currently beginning to be learned from the genome-wide association studies.

In 2004, Dr Sebat and colleagues published the first study of copy number variation in the human genome. When it was found that there was a tremendous amount of structural variation contributing to human genetic variants, they hypothesized that these might actually be an important contributor to human disease. These are potentially a very strong foothold into understanding the biology of disease, because once there is a genetic glitch in the genome that carried high risk for an individual's autism, then there is potentially a causal variant; variants that have predictable effects on gene function.

Therefore, identifying a copy number variant associated with disease can provide a quick hypothesis about the actual underlying genetic mechanism. If the gene involved is something which the biochemical pathway is understood, a hypothesis can be generated about the biochemical mechanism. Also, this is a mutation which could be created in an animal model and it will have a large effect. There is a chance of getting a real effect of the mutation on behavior in animal models. These variants can have significant effects on clinical phenotypes in humans, and therefore understanding the relationship of that variant to clinical phenotype is tractable.

Dr Sebat emphasized that genetic contributions extend to many different types of autism and not only the ones that occur in families with multiple-affected siblings. In fact, it is the sporadic cases which have made the most progress. In 2007, Dr Sebat and colleagues led a study looking specifically at sporadic cases and the contribution of de novo mutations to disease. They performed genome-wide strands of copy number variation in a family and compared the genotypes of mother, father, and the affected offspring, and their typically

developing siblings. They looked at the rates of copy number mutation in the affected offspring and compared it to their typically developing sibling. This is an example of a de novo mutation that can be found by copy number analysis. This is a loss of a region of the genome that is seen based on the loss of signal across the scan in the child. In a similar scan of the mother and the father, that deletion is not seen. In this study, only relatively gross submicroscopic rearrangements that impacted multiple genes were seen. However, it was found that there was a 10-fold difference in the rate. Approximately 10% of cases of autism, sporadic cases, had a genetic glitch like this compared to 1% of the typically developing siblings which was a highly significant result. This was one of the first robust genetic findings in the genetics of autism. It's now been widely replicated. Any subsequent autism cohort will have a 5 to 10% rate of submicroscopic copy number variants that are contributing.

These copy number variants as contributors to disease is not unique to autism. In fact, these have been found to be a factor in a whole variety of different disorders, including schizophrenia and bipolar disorder. A common theme throughout is de novo mutation as a factor in all of these disorders. There have been specific copy number variants that are contributing to risk and can be related to clinical phenotypes. Whenever there is a strong association with a single spot in the genome, it always turns out to be a mutation hotspot; a site in the genome that tends to mutate at much higher rates than other sites in the genome.

Now that there is clear evidence for specific variants contributing to risk for autism, there's also another important fact is that they're never autism specific. Once a copy number variant is found to be contributing to autism, it can also be found in other disorders such as intellectual disability, epilepsy, and schizophrenia. Thus, there's a wide spectrum of clinical outcomes that can arise due to a particular rare variant of large effect. These are, therefore, risk factors that don't produce a clear syndrome but that carry risk for a wide range of potential clinical outcomes.

From the information previously obtained from copy number variants, next gen sequencing technologies have arisen and have become the routine type of analysis. The first example were 4 back-to-back-to-back exome sequencing studies that took exactly the same design where they sequenced all the protein coding regions of the genome, compared genotypes of the both parents to the genotypes in the children, and compared the typically developing siblings to the affected offspring. When looking at sequence variants, they found point mutations and endos that disrupted genes were twice as frequent in the affected individuals compared to their typically developing siblings. Currently, these studies have reached a larger sample size, allowing identification of specific genes. Because point mutations can only hit 1 gene at a time, these types of studies have been particularly good at delineating specific genes. Dr Sebat presented a list ranking the genes in terms of the number of times it's been observed as a de novo mutation in a family. The genes at the top of the list were neurodevelopmental genes such as SCN2A which has been found to be unequivocally a gene contributing to autism and intellectual disability. It's been observed 13 different times in 13 different families, but additional sequencing, unpublished sequencing studies have found even more mutations. Interestingly, SCN1A, is also one of the top genes seen in autism and intellectual disability. The list consisted of a range of different types of neurodevelopmental

synaptic proteins or genes involved in cell signaling, but heavily enriched in genes that are expressed in the brain.

While exome sequencing studies is revealing substantial information about the specific genes, still only a fraction of the variation in the genome is being captured. About 99% of the genome is not sequenced by exome sequencing studies or by copy number variant studies. Dr Sebat's lab has been attempting to do whole genome sequencing in an effort to comprehensively characterize the patterns of mutation in both coding and noncoding regions of the genome. They've recently completed phase one of the study where they performed 30 × whole genome sequencing using Illumina HiSeq platform and using the exact same study design; mother, father, affected offspring, and unaffected offspring. They've processed all of the data obtained through a custom next gen sequencing pipeline and have developed custom tools that have high accuracy for calling de novo mutations genome wide with about 90% accuracy which is then validated. They also included a set of identical twin pairs to tell the difference between the somatic mutations and the germline mutations. As it turns out, 99.9% of the de novo mutations found in a 30 × whole genome sequence from a blood sample are germline. There are very few somatic mutations found in a 30 × whole genome sequence. There probably are somatic mutations in blood, but they're at lower frequency than can be detected by 30 × coverage. There was a lot of variation found in the global rate of mutation. They found no variation between cases and controls. Autism genomes are not fragile or hypermutable. When looking at all mutations genome-wide, they found exactly the same rate of mutation in autism genomes compared to controls. In the control they saw about 50 point mutations genome-wide and didn't see any difference between cases and controls. The influencing factor for the variation was the father's age with roughly half of the global mutation rate explained by father's age. This amounts to about one and a half additional mutations every year. Those with higher rates of mutations were individuals whose fathers were between 50–60 years old and those who had very low rates of mutation were those whose father was 20–30 years old when they had their children. The effect of maternal age was essentially undetectable.

In addition to the global variation in mutation rates, a model was developed by Michaelson and colleagues in 2012 to predict mutation rates around the genome. They looked to identify the occurrence of specific hypermutable hotspots in the genome and studied the genes in those hotspots. They found that there was a nonrandom assortment of genes. The genes that are expressed in the brain are enriched inside of these mutation hot-spots, and genes that have been implicated in autism are also enriched within these mutation hotspots. Therefore, while the genome is evolving, it doesn't seem to be evolving to protect its genes and the hypermutability persists.

While this was a small study of only 90 individuals, they integrated their data with large exome sequencing studies and found a significant amount of overlap; 24 of 46 mutations overlapped with a gene that was hit in one of the autism, intellectual disability or epilepsy studies, which was a significant enrichment over expected.

Whole genome sequencing provides a rich amount of data that allow for the detection of copy number variants with a better sensitivity than could be found with arrays. It also allows

for the assembly of sequence data in ways that allows for assembly of break points and fine sequence level characterization of mutations which provides additional information including the ability to determine the parent of origin.

De novo and rare mutations contribute to approximately 20% of autism spectrum disorders. Specifically, de novo copy number variants are contributing in approximately 6% of cases, de novo loss of function mutations in the exome are contributing to about 10% of cases, rare homozygous point mutations are contributing to about 2.5%, and X-linked variants contribute to approximately 1.5%. The rare variants and de novo mutation in the other 99% of the genome (the noncoding region) remains largely unexplored and may explain some of the missing heritability of autism.

Tim Roberts has been a real leader in looking at the neuropathophysiology of autism using imaging methodologies, including magnetoencephalography, and he's here to update us on that, so welcome him.

Magnetoencephalography Insights Into Neural Signaling and Connectivity

Tim Roberts, PhD, Children's Hospital of Philadelphia—Dr Roberts began by discussing the importance of biomarkers in autism which can be used for diagnostic and prognostic purposes as well as a way to bridge to experimental models and to monitor response to therapy.

Magnetoencephalography, 5D spectro-spatio-temporal imaging, can essentially be thought of as high-density magnetic EEG. It is used clinically in the identification of the ictal onset zone in patients with epilepsy. Distinct from how EEG is used, with magnetoencephalography, data can be routinely analyzed in the brain source space and "virtual depth electrodes" can be constructed. With magnetoencephalography, there is a color map which identifies spiky electrophysiology in both a time and frequency domain.

Dr Roberts then discussed the application of magnetoencephalography to children with autism. He discussed the event-related potential–type responses to auditory sensation, the gamma-band activity and the timing of these responses which seems to be of significance to the clinical phenotype. He focused mainly on auditory processing in relation to language function, however, this could be applied to other domains of relevance as well.

When studying children with magnetoencephalography, there was a cortical evoked response delay in those with autism spectrum disorder by a few milliseconds with 2 possible explanations for this delay being conduction velocity and synaptic transmission.

Examining the auditory pathway from ear to cortex, it is clear that it is mediated by white matter acoustic radiations, especially from thalamus to cortex and this can be imaged using diffusion MRI.

By using diffusion MRI, these white matter fiber pathways cans be constructed and interrogated in terms of their microstructural quality. The microstructural quality changes with age and it increases in typically developing children from school age to adolescence while the event-related potential latency shortens toward the adult value of about 100

milliseconds. Being that white matter has myelin, as white matter matures it can be hypothesized that it would cause an increased conduction velocity and thus cause a shorter latency. It can then be hypothesized that the development trajectory of white matter is different in autism, and that's perhaps in part subserving the very clear difference in auditory evoked response component latency.

In addition to these white matter findings, a large multicenter trial conducted by the Simons Foundation found that a copy number variant, a deletion of approximately 600 KBs on chromosome 16, which is a common predictor of autism, was associated with profound event-related potential prolongation while a duplication was not associated with a prolongation. It can be concluded that the deletion does not allow the M100 to form properly and is unaffected when duplicated.

The event-related potential can be broken down into its various spectral components. The gamma band, which can be defined as anything between about 30 and 80 hertz varies among the cortices and varies with age. The phase-locking intertrial coherence is a sensitive indicator of the regional neuronal circuitry and a decrease in the phase-locking intertrial coherence is seen in autism. This distinction can be looked at in terms of excitation inhibition and neurotransmitters in general wherein with an atypical level of GABA and glutamate, there can be loss of phase coherence.

With edited magnetic resonance spectroscopy, specifically MEGA-PRESS, GABA can be closely studied. A previous study studied the association between GABA and gamma in the visual cortex in healthy people showing that the concentration of GABA measured by spectroscopy predicted the gamma band oscillations in visual cortex. Subsequently, Dr Roberts conducted a study looking at the motor cortex and also found that GABA predicts the gamma band oscillation.

In applying this to autism, there is preliminary support for GABA variation in children with autism. When measuring GABA in children with autism spectrum disorder, GABA levels were diminished by about 20% in auditory cortex, 10% in most cortices and not at all diminished in the visual cortex.

Dr Roberts concludes with the translatability to clinical practice by discussing work done by Steve Siegel et al involving in utero valproate exposure of mice. The valproate mouse was somewhat used as a model of autism. While humans show a delay in the M100, the valproate mice showed a delay in the N1 component measured with real electrodes rather than with magnetoencephalography. Also, the gamma-band phase synchrony that is diminished in humans was also diminished in the valproate mice.

CNS Structure and Neuropathology

Dr Cyndi Schumann, PhD, UC Davis MIND Institute—Dr Schumann focused on what is known on the neuropathology of autism. When mapping the brain with MRI from a global perspective, in typical development there is a steady increase over time throughout early childhood that tapers off at about 10 years of age and then potentially has a slight decline as those connections are being groomed and shaped. In the development of a brain

with autism, some children appear to have that initial overgrowth followed by a tapering off that's happening earlier than seen in typical development. While there isn't a difference in size overall, it is apparent that the developmental process varies in typical development and those with autism. Also, there are some cases in adulthood where the brains of those with autism are smaller than those of typical development and may be attributed either to lack of growth of that developmental trajectory or a possible degenerative process.

Regional differences in brain development were studied and it was found that areas that contribute to behavioral symptoms were the areas that underwent an abnormal growth trajectory such as the frontal and temporal cortex.

A study done by Melissa Bauman et al in 2013 found that approximately 12% of mothers who have a child with autism have autoantibodies that likely crossed the placenta and can impact the development of the brain in the offspring.

This study took these autoantibodies from the mothers and put them into gestating female Rhesus monkeys and then looked at the social and brain structural development of the offspring. These offspring did not show species-typical social behavior. Specifically, when observing reciprocal social interactions, social impairments were incredibly obvious thus concluding that maternal antibodies from mothers of children with autism alter brain development of rhesus monkey offspring.

They also looked at the MRI longitudinal development of the brain starting at birth and through late adolescence into adulthood in the Rhesus monkey offspring. Their findings were remarkably similar to that of what would be seen in a child. There was no difference at birth. At around 6 months of age, which is roughly equivalent to about a 2-year-old child, there was a peak in enlargement of the offspring that were exposed to these autism-specific antibodies. This difference continued in the later ages up to about 24 months which is the equivalent of an 8-year-old child. When looking at the individual lobes, they saw these differences seemed to be in the frontal cortex.

Over the past 15 years, there has also been a lot of focus on the amygdala and the role that it plays in autism and the aberrant structural development. The amygdala is involved in modulating social behavior and detecting what might be danger in the environment. In humans, that would also include looking to the eye region of the face to indicate where there might be danger. If that system goes awry then increased anxiety and aberrant social behavior can be observed.

It has been found that there is amygdala enlargement early on in development. It is seen at around 3 years of age, later than what is seen in the more global brain development changes. Interestingly, from 3 to 4 years of age that enlargement appears to increase. When looking at the amygdala in a typical child through adulthood, the amygdala increases up until around 10 years of age as is seen in the rest of the brain, but then it continues to increase up through adolescence. When looking at the developmental trajectory of a child with autism, this enlargement is seen later on, around 8 years old and is about a 10% enlargement. As the amygdala in the typical child continues to increase, around 12 years of age, it overtakes the size of the amygdala in the child with autism so that later on in adulthood there is either no

difference in size or a decrease in size of those with autism which raises the question again of either a lack of growth or a degenerative process.

While MRI is beneficial at answering questions of when in development and where in the brain to look, the next step is to go to the level of brain tissue to the microscopic level to be able to explain the cellular and molecular contributions. In a study done in 2006 by Dr Schumann and colleagues, they took brain tissue and defined the amygdala and studied what was happening at the cellular level in adolescence and adulthood (ages 11–44 years old). They found no changes in the number of neurons early on but a reduced neuron number in aberrant cellular development in the amygdala in those with autism compared to those with typical development.

In an attempt to figure out what is contributing to this reduction in neuron numbers, they looked at cells related to aberrant immune responses starting with microglia. When microglia are activated as a normal part of the immune system, they multiply and grow in size. While some cases of autism had aberrant microglial activation, many of them did not.

They also looked at other cells in the amygdala including astrocytes, oligodendrocytes, and endothelial cells and looked in early adolescents. They found that there really wasn't a difference of any of the cells. In the older ages, they found that there was a reduction in the number of oligodendrocytes and are still attempting to figure out why this is the case.

Interestingly, while there are very few brain studies to date, it appears that of the studies done, the areas that appear to be enlarged early in development on MRI are those showing reductions in the number of neurons later on in development.

Dr Schumann concluded her talk by stressing the importance of having brain tissue to make progress in understanding the neurobiology and neuropathology of autism. They have had fewer than 150 brains in the 30 years of autism research.

Q&A Session

DR MOSTOFSKY: If we can get the panelists up and I'll in the meantime reiterate certainly how important that was. In fact, I think it was about a year ago I helped connect a family to Dr Schumann and that was successful. We are certainly a community that is able to contribute to this important work, so please keep that in mind.

Questions for our last 3 speakers. Number one.

FEMALE VOICE: Hi. I'm Christy…from the University of Wisconsin. That was a very nice symposium. I have a question for Dr Schumann. I was very impressed by your nonhuman primate studies. If I'm not mistaken, you had 4 nonhuman primates that you treated with maternal IgG from mothers that had offspring with autism spectrum disorders. What were your controls? What did they receive?

DR SCHUMANN: Just a saline. So they underwent—

FEMALE VOICE: [Interposing] So they did not receive human IgG?

DR SCHUMANN: Actually, there were 2 different control groups. So there was a saline group and there was just from typical mothers that—

FEMALE VOICE: [Interposing] So we don't really know what human IgG in general. Not from mothers who had children with ASD what that—whether that has an impact on the development of the brain?

DR SCHUMANN: Yes. So we did actually have from mothers who did not have a child with autism. It just happens that these specific autoantibodies at 37 and 73 kilodaltons are only present in mothers of children with autism. They haven't been found yet in mothers of typical children.

DR MOSTOFSKY: Dr Dresher. Number 2.

DR DRESHER: Question for Dr Schumann. So with regards to that autoantibody and I don't know, maybe this is not an…question. But is there any role for a nongenetic environmental maternal factor preconception contributing to autism? I don't know if that's clear. But so are there things that happened to the mother before—and I'm not talking about just before they decide to conceive, but are there environmental things that happened to mothers who then developed autism—maybe not completely separate from genetic but related to environmental factors? Is that clear?

DR SCHUMANN: I think so and I wish I had an answer. It's kind of that's one of the questions that we would love to answer. Looking at the animal models, though, it's a fairly controlled environment, so there isn't anything specific of why that would be the case, but then we're getting the autoantibodies from the moms of the kids with autism, and I have no idea, but we'd love to know that.

DR SEBAT: If you're talking about preconception, then you're talking about constitutional components of the mom that are transmitted to the child, among them are genes, correct? And there are environmental factors that can, in principle, influence your genes. So it's been shown that epigenetic smoking influences the epigenetic program of your white blood cells. And it's also known that if you irradiate sperm—gonadal cells you can introduce mutations. So in principle, there are model-systems studies that can show environmental effects that can then be transmitted to kids.

DR MOSTOFSKY: We're going to go back to number 1 and then we'll go to number 4. So number one.

MALE VOICE: This is regarding, again, autoantibodies for Dr Schumann.

DR MOSTOFSKY: Can you speak up a little bit?

MALE VOICE: This is regarding, again, autoantibodies for Dr Schumann. These autoantibodies, were they specific for any receptors to the brain-related or viral or what kind of autoantibodies you had and came across?

DR SCHUMANN: I'm sorry. I couldn't hear him very well.

DR MOSTOFSKY: Can you repeat the question?

MALE VOICE: These autoantibodies were they specific for neuronal receptors or they were viral markers? What kind of autoantibodies you had?

DR SCHUMANN: What kind of?

MALE VOICE: Maternal autoantibodies.

DR MOSTOFSKY: Asking whether—what is the protein. That's what you're asking.

MALE VOICE: Right. Were there neuronal-specific markers…directly to the neuronal receptors or they were postviral something?

DR SCHUMANN: I'm not understanding the question. But, honestly, I actually don't know. So I'm going to go ahead and defer to my colleague who actually runs those studies. I don't know, but it's a great question.

DR MOSTOFSKY: So then number 4.

MS KRISTIN LINGREN: So then number 4.

My name's Kristin Lingren [phonetic]. I'm a pediatric neurology resident Mass General. This question is for Dr Sebat. Based on the studies that you're showing, where do you think this is going in routine genetic testing for kids being diagnosed with autism?

DR SEBAT: So a handful of the copy number variants that we identified in the early studies are already being reported in routine clinical genetic testing. And you've essentially defined a genetic disorder in a child and they get referred to clinical genetics from there. Then that actually has a pretty significant influence on their trajectory from that point on. They end up getting additional referrals for indications that are known; things that occur in that genetic disorder.

So there's a subset of kids who are already having their treatment course influenced by the genetic testing, but that's only a small fraction of cases where you have success. But there's now, in addition to the handful of copy number variants, there's now a handful of genes where exome sequencing has unequivocally linked autism to these genes. The chromo domain 8 gene, SCN2A, P10, DYRK1A. We've already known about Rett syndrome and Fragile X for a long time, so there's now additional new genes that are easily sequenced and ascertained in a clinical genetic setting. It's really only a matter of a few years before those get validated tests that are then offered.

So the real trick right now is still a minority of kids with autism ever get referred for clinical genetic testing. It's really just a matter of referring them. The diagnostic yield will improve as new genes are identified, but the bottom line is we have to refer kids for genetic testing.

DR MOSTOFSKY: Number 2.

FEMALE VOICE: Hi. My question is for Dr Schumann. Do you have access to typically developing brains or brains of children with developmental delays so that you can compare the pathological findings that you're seeing in the brains of individuals with autism?

DR SCHUMANN: Oh, I wish we had a better sample. You obviously can't look at a brain most of the time and say, oh, that child had autism. It just doesn't work that way. So we have to have those typical cases in order to be able to compare. And, remarkably, they are actually harder to get than the autism cases because oftentimes the individual with autism is also already involved in research or associated with a center. But I have 2 kids that are not on the spectrum. I don't want to think about that. And, of course, parents don't want to think about it. So it's a lot more difficult to get. We're doing okay, but if you definitely have any referrals let me know.

DR MOSTOFSKY: Dr Maria.

DR MARIA: Bernie Maria. This is for Dr Roberts. It's on sort of the role of standard structural imaging hasn't been spoken to and I'd love to hear you say something about that.

And then are we beginning to blend MEG into clinical practice, and if not yet, how do you envision that working?

DR ROBERTS: So it's a 2-part question. I didn't talk about the structural MRI findings. There have been a number of reports of gross developmental trajectory differences in ASD. I think that the reason that we don't focus—and there also have been some studies where you can combine different features from the structural MRI and come up with some sort of amalgam that you can use, I suppose, to support a diagnosis.

I think that the reason we take a slightly more biological or physiological approach is because we're not addressing maybe a diagnostic question, but rather a mechanistic one because I think it's mechanism that's going to guide therapy.

And so we have taken sort of the next level of what some people call advanced imaging because it's pointing us to targets which are actually useful to the pharmaceutical industry. Maybe not at the level of genetics and molecules, but we are at least pointing to systems and perhaps suggesting approaches of the, let's say, neurochemical level of GABA and glutamate.

In fact, as I'm sure you know, there have been exploratory trials of drugs essentially borrowed from Fragile X, but GABA-agonists, for example, even have very, very anecdotal data showing that these are that physiological responses that we call biomarkers are indeed sensitive to the action of a synaptically targeted drug, which is exactly what you would want in a biomarker. First, select, stratify those patients who have a synaptic problem, then use a similar synaptically sensitive imaging modality to demonstrate, let's say, an early sign of efficacy.

So I think the trouble with structural MRI is that it's slow to respond and I think the yield is not as rich as you can get with perhaps more biological techniques.

The second question was about MEG in general. Dr Brown is sitting 2 seats behind you. We use MEG routinely in our clinical work in pediatric epilepsy for 2 purposes. Essentially, to identify or to source localize spontaneous epileptiform activity, particularly in cases that are nonlesional—where there's no lesion on MRI or if the EEG is nonnaturalizing or equivocal, essentially to either suggest somebody is a surgical candidate or to rule out that in the case of maybe multifocal disease.

We can also, of course, use MEG in the same way that we use FMRI for identifying—I don't like to call it elegant cortex because what's the rest of the cortex if it's not—but those areas of the brain subserving functions that you would not want to interrupt by surgery. Networks of function. Unfortunately, that's more and more.

So I would say that at those centers that have MEG, which is maybe 150 worldwide now, it's adopted in clinical practice. It's reimbursed in the United States and—

MALE VOICE: —

DR ROBERTS: Well, we get quite a lot of calls, especially after events like this and public speaking engagements. We get quite a lot of calls from parents saying can I get that test; will my insurance pay for that test. And the answer is no and no.

But the fact is that there's public awareness that this has a role to play. We're not completely sure about the extent of the role, but there is a role, and it starts with the public. I believe that this will become something that—I don't think we'll need a new CPT code. We already have CPT codes. I think we'll need a new indication suggesting that autism is, in fact—autism is indicated for MEG or MEG is indicated for autism. That will happen. Parents are already demanding it.

DR MOSTOFSKY: I'm going to indulge myself for a second and ask a question then in follow up. First of all, I'll come to the defense of structural imaging to some degree. Dr Schumann presented some data on that, but there certainly is a place and a role for structural imaging. It can reveal regional abnormalities that may be—and, in fact, more diffuse abnormalities that may be relevant.

The main question I want to ask you, Dr Roberts, is so you had some very elegant data combing DTI and MEG findings, and its association with clinical features of autism, specifically performance on the cell 4, which is a language-based task, but not necessarily association with the core features of autism.

So I wonder if this examination of the auditory pathways may be epiphenomenologic to some degree to other abnormalities and disconnectivity that may be more directly contributing to some of the more global, social and community features. In our lab, we've had a particular interest in visual-motor integration given its importance in imitation and the key role that visual-motor integration plays in social skill acquisition.

And so I'm wondering what are your thoughts about applying some of the methodologies that you showed to sort of look at other connections within neural systems.

DR ROBERTS: So I think, again, there's going to be sort of 2 answers. The question centers around why do we study auditory processing, why do we study auditory cortex, and, frankly, why do we study a sensory system and not something like the social brain or some higher order cognitive function.

The reality is the sensory system is earlier and simpler, and already is showing clear evidence for atypicalities in both timing and, let's say, these oscillopathies. The question is are we specifically interested in the auditory pathway or is this, let's say, a convenient probe for a more generalized dysfunction.

And the answer, I think, is there's increasing support for the notion that a little bit of both. That is to say that—again, from whole brain studies and from the diffusion studies there are plenty of regions in the brain which are showing atypical maturation, atypical development, and probably subserve other functions.

Yet, it is interesting that the temporal lobe seems to be particularly impacted. If you look at the GABA glutamate results, it is interesting that there are widespread deficits, but they vary in extent, and, again, the temporal lobe seems to be particularly hit.

So I think the answer is yes, it's a probe of a more general dysfunction, and, yes, it's specific to something particularly odd about the auditory system.

DR SCHUMANN: Can I add to that?

DR MOSTOFSKY: Yes, sure.

DR SCHUMANN: And I would defend structural imaging, too, for a few reasons, but I actually want to defend—

DR ROBERTS: [Interposing] I'm not opposed to structural imaging. I do it every day.

DR SCHUMANN: —but—wait, wait, wait. I want to defend looking at the auditory system, too. One of the things that I didn't talk about was our genomic studies. We started with looking at micro RNA, but also looking at MRNA, too, and moving into that with brain tissue.

And, actually, what we've looked at are differences between regions within the temporal lobe, and what we're seeing is there appears to be more of a difference in the auditory cortex than in primary auditory cortex than there is even in the adjacent superior temporal sulcus area. So I'm defending your auditory cortex, too.

DR ROBERTS: Thank you. And we love structural MRI, really.

DR MOSTOFSKY: Number 3.

MALE VOICE: I wonder whether there are recommendations for clinical trial neurologists because we see lots of cases with autism and you wonder what type of testing you need to order, especially regarding the genetic testing. So frequently the microarray, for example, is not authorized by the insurance. So what are we supposed to do? If there's no clinical—clear

clinical diagnosis for a syndrome, shall we refer all these kids to geneticists, for example, or are we going to get hopefully by the end of the day some statement, consensus statement this is what child neurology or the panel recommends so that we really have baseline to support our recommendation to families?

DR SEBAT: So in terms of the statement that you're looking for as to whether genetic testing is applicable, that statement exists in terms of the American College of Medical Genetics. It has clearly stated that for autism clinical genetics is a standard of care. So that exists.

That doesn't change the fact that some—that there's variation in the reimbursability of the tests and it varies from provider to provider. Again, you're at the mercy of the provider as to whether you'll get it reimbursed or not, but it is reimbursable. The large clinical genetics centers, like Baylor, are getting routine reimbursement from insurance for clinical arrays.

DR MOSTOFSKY: We're going to take number 4 and then we're going to wrap up.

FEMALE VOICE: This is a question for Dr Roberts. You had talked about or you alluded to the differences in latency of the M100 could be related to language impairment. Those may be to really compared to results you talked about. But has there been anything looking at treatments improving the M100 latency has shown in improvement in either ASD function or language function?

DR ROBERTS: Yeah, I think that that's pointing to a direction that we certainly need to go in. And I think 1 of the claims for a biomarker is that it would be responsive to effective therapy and perhaps even an early indicator of that.

I do have just the 1 pharmaceutical data point suggesting that these latencies can be—normalized is the wrong word. Reshortened by a drug which subsequently appeared to have in that— again, it's an anecdotal case—a clinical benefit.

I think it's a little bit tricky to go with generally the idea of improving language performance necessarily having a physiological correlate that's as simple as what I'm talking about. I think it's one thing to have a deficit in your neural system that leads to a clinical impairment, and then it's sort of like integration. There are many ways of fixing the language impairment that perhaps are work around that may not directly impact the underlying physiology that was—so it's one way of getting the problem, but then fixing it may not necessarily act that way.

I think the drugs will. I think that the pharmaceutical approaches will be motivated by the physiology. In terms of behavioral therapy and improved language performance, it remains to be seen, but I would be very interested in studying it. I think your first hypothesis would be yes, indeed, this is working so it must be fixing the brain, but I don't think it's unequivocal that it would work out like that. But, certainly, it really should be tested and we just don't have the data at the moment.

FEMALE VOICE: Is there a spectrum of the M100 across children who are, per se, nonverbal versus those who are more higher functioning?

DR ROBERTS: Yes, there is a spectrum of results that tie coupling to a clinical measure like the core language index on the cell 4 or cell 5 isn't there. The mismatched latency correlates quite well with some gross measure. We are, of course, looking at more domain-specific things and looking at phonological processing of the —, and perhaps there's tighter coupling there, but.

So, yes, there's a spectrum and there's a spectrum, of course, of phenotypes, but there isn't —it's not that this is an index of— this physiology is an index of a clinical measure. And some of that well, if that were true you wouldn't really need the physiology because you have the clinical measure. And, anyway, it seems like that's not true. It's part of the story. It's maybe sort of necessary but not sufficient to protect the clinical outcome. But it's a very, very important line of investigation; how do we have functions. That's a big question, right? What does it mean to have a clinical performance? We're interrogating it sort of bottom up, millisecond by millisecond quite literally. But it remains to be seen.

DR MOSTOFSKY: Dr Maria has a few housekeeping points and then we'll break for lunch.

DR MARIA: Many thanks to our panelists and all our speakers this morning.

III: Therapeutic Targets and Translational Opportunities

TSC and mTOR Inhibitors

Dr Mustafa Sahin, MD, PhD, Harvard University—Dr Sahin discussed the translational work currently being done using both mouse models and patients with tuberous sclerosis. Approximately half of the patients affected with tuberous sclerosis are also affected with autism spectrum disorder. Unique to other diseases that have a high risk for developing autism spectrum disorder, tuberous sclerosis is usually diagnosed prenatally or at the time of birth because of the cardiac rhabdomyomas that develop.

Also, importantly, the cellular mechanisms aberrant in tuberous sclerosis, in particular, the mammalian target of rapamycin pathway are beginning to be understood both in nonneurological systems, as well as in neurons. Currently, there are FDA-approved specific inhibitors of the mammalian target of rapamycin pathway that can be repurposed and used in proof-of-concept studies to test the role of tuberous sclerosis in autism.

Tuberous sclerosis causes benign tumors or hamartomas in the brain, eyes, skin, kidneys, and the heart. Of patients, 90% will develop seizures. Approximately half of them will have intellectual disability; half of them will have autism spectrum disorder. The incidence of people affected with tuberous sclerosis is about 1 in 6000. The expected prevalence is 50 000 in the United States and about 1 million people worldwide. The casual genes have been known for about 2 decades: TSC1 and TSC2. Loss of function of either one of these genes results in the disease.

Tuberous sclerosis, at the cellular level, is a disease of cell size dysregulation. When looking at epilepsy tissue taken at the time of resection of an epileptogenic focus in a patient with tuberous sclerosis, pathognomonic giant cells, approximately 150 microns in diameter and about 10 times the size of a normal neuron can be seen.

In all organisms in which TSC1 and TSC2 genes are inactive, there is enlargement of the cell size and organ size. This occurs secondary to an evolutionary-conserved pathway in all cells that are regulated by a GTPase called Rheb and a kinase called mammalian target of rapamycin, which stands for mammalian target of rapamycin. Together they turn on a machinery of protein synthesis, which increases protein synthesis and cell growth. TSC1 and TSC2 proteins form a heterodimeric complex that regulates this pathway thus regulating protein synthesis and cell growth. Without either of these genes, this pathway becomes overactive and an excess of cell growth and protein synthesis occurs.

Identifying this relationship between tuberous sclerosis and mammalian target of rapamycin has allowed for the development of an inhibitor of this kinase, called rapamycin. The first clinical application of rapamycin on patients with tuberous sclerosis was done by David Franz in 2006 on about 5 or 6 patients. In one of the patients who had a subependymal giant-cell astrocytoma (occurring in about 5 to 10% of patients with tuberous sclerosis) which was causing hydrocephalus, rapamycin was given for 6 weeks with subsequent shrinking of the tumor and resolution of the hydrocephalus. Within 4 months after the medication was stopped, the SEGA and resultant hydrocephalus returned and when she was placed back on the rapamycin, it resolved again. A second study, a phase II trial with 28 patients, led to a *New England Journal of Medicine* article, which in turn led to the FDA approval of this drug for subependymal giant-cell astrocytomas. There is also another small molecule, everolimus, which is another mammalian target of rapamycin inhibitor that is FDA approved for this indication.

Given that about half of patients with tuberous sclerosis also have autism, an important question is why these patients develop autism. One study suggested that bilateral temporal lobe tubers were necessary but not sufficient for developing autism while several other studies show that other parts of the brain, such as the cerebellum might be implicated in different cohorts. Due to variation among patients with similar tuber load and locations, it has been concluded that performing structural MRIs with attention to cortical tubers is not efficient in predicting who’s going to be affected with autism.

Therefore, instead of focusing on the cortical tubers, Dr Sahin and colleagues have focused on the nontuber pathology of the brain. By using mouse models of tuberous sclerosis, they tested the hypothesis of miswiring of neural connectivity contributing to the pathogenesis of tuberous sclerosis.

They obtained antibodies in the TSC1 and TSC2 protein synthesis pathway and stained hippocampal neurons in culture as they were developing to determine whether these proteins were present in axonal growth cones or dendritic growth cones. Most of the staining for the TSC2 phosphorylated form of this protein was in the axon and very little staining was seen in the dendrites. To determine the significance of the presence of the tuberous sclerosis

protein in the axons, they knocked down tuberous sclerosis using interferons and looked at the morphology of the neurons. In the control neuron, there was a single axon with multiple dendrites. In the same experiment in a neuron that's missing tuberous sclerosis, there were multiple axonal processes coming out of the cell body with a very robust phenotype. As expected, in neurons with more than 1 axon, there are issues in terms of the directionality of flow of information in the central nervous system. They showed that when these tuberous sclerosis–deficient neurons are treated with rapamycin, this reduces the multiple axon phenotype.

Axons navigate to their final destination in the central nervous system by interacting with either positive or negative cues. One of the best-studied axon pathways in the central nervous system are the projections from the retina to the lateral geniculate nucleus in the thalamus. Dr Sahin and colleagues tested the results of mice missing 1 copy of TSC2. They placed a dyad crystal, a small ligand in the retina, which was taken up by the axons, and they studied where these axons were projecting in the thalamus and found that these axons had abnormal projections in the central nervous system when compared to the controls.

Another aspect of axonal development that has been found to have deficits in the tuberous sclerosis mouse brains was myelination. Dr Sahin and colleagues made a knockout of TSC1 gene in the neurons under a promoter called a Synapsin-cre promoter. These mice were born at the expected frequency, but they developed seizures around 3 weeks of life and they all died from seizures by about 60 days of life. These mice did not develop tubers but they had a marked hypomyelination phenotype.

Normally, myelination occurs in mice within the first 2 to 3 weeks of life. In the mice that were missing the tuberous sclerosis gene just in neurons, there was a marked deficit of myelination both in the gray matter and in the white matter. They used 2 different inhibitors-rapamycin and everolimus (RAD001) and found that untreated mice died from seizures by 60 days of life while the treated mice didn't develop any seizures and they survived. Of them, 90% survived while kept on the medication. If the medication was stopped at 30 days, they developed seizures around 60 days and died soon thereafter.

Last, Dr Sahin addressed the circuitry and localization of lesions in the brain and how they can contribute to clinical abnormalities observed in patients. In the lab, Dr Sahin and his colleagues made region-specific knockouts of the TSC1 and TSC2 genes in the brain starting with a Purkinje cell specific knockout of TSC1 in the cerebellum. These mice were born at the expected frequency, were healthy and weighed as much as their litter mates. When looking at the Purkinje cells in the cerebellum of the heterozygous mutants that were missing just 1 copy of the gene, they looked normal and did not have any cell death. However, in those that were missing both copies of the TSC1 gene, they were enlarged and started to die between 6 and 8 weeks of life. When investigating the behavioral output of this deficit of TSC1 gene in just the Purkinje cells in these mice they used a 3-chambers social interaction apparatus developed by Jackie Crowley at the NIH. They put each mouse in the middle chamber and allowed it to interact with a live mouse versus an object. The controls, typically developing mice, explored the mouse more than the object. In the second phase of the experiment they introduced a novel mouse in the third chamber, and the typically

developing wild type mice explored the novel mouse more than the familiar mouse. The heterozygous and the null mutants did not show preference of either the live mouse over the object or the novel mouse over the familiar mouse. They therefore concluded that TSC1 mutation in just the Purkinje cell results in a deficit in social interaction.

They also looked at repetitive behaviors or cognitive inflexibility in the mice. The mutant mice have more self-grooming as 1 repetitive behavior. They also performed a T-maze experiment where they taught the mouse the location of a T-maze and then reversed it to the other side. The mice had no trouble learning the location of the T-maze, which is a hippocampal test. However, when they switched the location of the platform they got stuck in the original location and had a hard time learning the new locations, suggestive of mental or cognitive inflexibility in this mouse model.

When studying the physiology in the Purkinje cells they found that they are less active than the control Purkinje cells. Also, their axons showed varicosities and abnormal branching that are not seen in the wild type animals. The dendritic spines were increased in these mice compared to the wild type litter mates. They found that treating these mice with rapamycin can prevent the cell death that would typically occur and improve the neurological and social deficits.

One advantage in tuberous sclerosis is that many patients with tuberous sclerosis are diagnosed either prenatally or at the time of birth because of the cardiac tumors that they develop. If a patient has multiple cardiac tumors perinatally, they have a 95% chance of having tuberous sclerosis diagnosis. Given that 50% of these patients will develop autism, Dr Sahin and colleagues are using a multimodal approach to determine this using imaging, electrophysiology, and neurocognitive assessment.

Using diffusion MRI, they looked at the corpus callosum in 3 sets of patients: typically developing controls, tuberous sclerosis patients that are not on the spectrum, and tuberous sclerosis patients with the diagnosis of autism spectrum. Tuberous sclerosis patients that are on the spectrum had much less organized microstructure in the corpus callosum, and in other experiments, also in the arcuate fasciculus in the brain.

Once is can be determined that a child is high risk or low risk for developing autism, it would be important to be able to do something about the development of symptoms. The mammalian target of rapamycin inhibitors looked very promising in mouse models and along those lines, Dr Sahin and colleagues recently started a Phase II trial of an mammalian target of rapamycin inhibitor looking at neurocognition as the primary endpoint in tuberous sclerosis patients which is currently an ongoing study.

Dr Sahin concludes his talk by discussing that 1 challenge being faced in autism is that there are 400 to 1000 genes that gives susceptibility to autism which makes finding a treatment challenging. There will unlikely be 1 broad-spectrum treatment for all of these genes and the hope is that some of these genes will fall into convergent pathways that can intervened with using similar drugs or interventions.

Sinai Shank3 and IGF1

Joseph Buxbaum, PhD, Seaver Center, Mt. Sinai—After a brief introduction describing the Seaver Autism Center and the importance of finding a genetic etiology of autism for both counseling families as well as for developing new treatments, Dr Buxbaum began with a general discussion of the genetics of autism. There are both common variants of low impact and rare gene variants which have high effects including rare inherited, nonadditive, and de novo variants. These same genes can present with autism, intellectual disability and schizophrenia. One possible explanation for these occurrences is that the common variation sculpts the manifestation of the phenotype. Furthermore, there is a risk architecture in the family and then there's a de novo mutation that manifests as autism or schizophrenic or intellectual disability depending on the background risk. Therefore, many cases of neurodevelopmental disorders are an interaction between common and rare variants.

The Autism Sequencing Consortium was created as a way for people to share genetic data prospectively. Collectively, 15 000 individuals including families with autism, cases, and controls, had whole exome sequencing. They found about 107 likely autism genes. Many of the genes mapped to synaptic sites as well as to chromatin remodeling. They also found that the top mutations were de novo loss of function or de novo missense 3 mutations which had odds ratios of above 20. Also helpful was discovering if these mutations were de novo and therefore not as likely to recur in the family or inherited.

One of the top hundred genes they found in the consortium was Shank3. Shank3 was the most disrupted gene in the study with the most copy number variants. They also found other genes that are known as Shank3 interactors (calcium channel and all mutations in the region that binds Shank3) all of which are found to contribute to autism.

Shank3 is the cause of Phelan-McDermid syndrome which is also known as haploinsufficiency of Shank3. It accounts for at least 0.5% to 0.6% of autism, about the same as Rett syndrome, and can either be point mutations or deletions. In previous studies, in individuals with lower intellectual function and more cognitive impairment, the rates of Shank3 mutations or deletions were upward of 2% making it a major risk factor for developmental delay syndromes. There are either deletions on the terminal part of chromosome 22 that knock out the Shank3 gene or small deletions or point mutations that only target the chain.

In the first prospective study of Phelan-McDermid syndrome, Dr Buxbaum and colleagues studied patients with Phelan-McDermid syndrome and found 75% met criteria for autism. Very significant proportions had profound or severe intellectual disability, severe problems in receptive and expressive language, and motor abnormalities, both gross and fine. These findings are all very nonspecific. With the exception of dysplastic toenails which are seen in some of the patients with Phelan-McDermid syndrome, there is nothing that's pathognomonic and therefore this diagnosis is unknown unless a genetic test is performed.

Because Phelan-McDermid syndrome is a haploinsufficiency syndrome, to best capture the human disorder, Dr Buxbaum and colleagues focused on heterozygotes in their mice studies of Phelan-McDermid syndrome. When missing even 1 copy of the gene, the mice showed

behavioral, motor and social deficits. They had AMPA receptor deficits which affected the synapses. Similarly, when looking at IGF1 in Rett syndrome, Dr Khwaja et al discovered that administering IGF1 seemed to promote neuronal development, specifically synaptic development, in the CNS even when administered peripherally. Conducting a similar study, Dr Buxbaum and colleagues first administered IGF1 into mice missing 1 copy of Shank3 and found that after 2 weeks they looked clinically the same as the wild type mice. They then performed a pilot placebo-controlled crossover design where they administered IGF1 to 9 children with Phelan-McDermid syndrome and found that they improved on both the social withdrawal scale and the restricted behavior subscale when compared to the placebo.

Rett's and BDNF

David Katz, PhD, Case Western Reserve University School of Medicine

Pharmacologic Interventions

Q&A Session

DR DECICCO-BLOOM: Thank you. If I could ask the speakers to join us up here we'll have a series of questions.

[Housekeeping]

DR DECICCO-BLOOM: Microphone number 3, please.

MALE VOICE: One of my questions with Phelan-McDermid syndrome. I was telling me that she is aware of some person in the states doing studies with intranasal insulin and I wonder whether we have any information and I wanted just to guide her about that for the treatment of Phelan-McDermid syndrome.

DR BUXBAUM: So there have been some publications about it. It's actually in Europe. There are some ongoing studies that we collaborated with around trying to give intranasal insulin to mouse models to see if we could see changes in the biology, electrophysiology. Like I said, those studies are ongoing and if you find me afterward I can give you a brief update. I'd have to check. I don't remember. I don't have the status of it in my head right now. I just know that that group just wrote an abstract that I think I can share some of the high points.

DR DECICCO-BLOOM: Thank you. Microphone number one.

MALE VOICE: Dr —. This is regarding a question to Dr Chugani regarding your experience with buspirone and these children with autistic behavior problems. How long do you usually recommend to give buspirone?

DR CHUGANI: So we did a pilot trial before we did the current trial, and during that study we did 3 months of treatment. And for the new trial, we actually had 6 months that was placebo-controlled portion of the trial and then another 6 months of open label so that all comers could have the drug. I would ask you to wait until we have the paper for publication to comment further on how long to use the drug.

DR DECICCO-BLOOM: Another question at number one. Please.

DR ROSE-MARY BOUSTANY: Rose-Mary Boustany from Beirut. Beautiful session this afternoon. My question is for Mustafa. And are there any ongoing trials with rapamycin or rapamycin-like drugs in autistic kids?

DR SAHIN: Idiopathic autism you mean, Rose-Mary? Not that I know of. And we haven't planned on doing those trials till we get a signal from the trials with more mechanistic understanding, such as the ones on tuberous sclerosis. We are actually planning as a part of the Developmental Synaptopathies Consortium with Joseph Buxbaum there is a pilot study that's going to focus on children with PTEN mutations and we're going to test the efficacy and safety or mTOR inhibitors in that group. If those look promising I think then we can see if there's a subset of children with idiopathic autism that might be candidates.

The question there is, is there a predictive biomarker or some way of categorizing all comers with autism into those that might respond to the mTOR pathway. And I'm not sure if we have that understanding or that specificity at this point. Does that answer your question?

DR BOUSTANY: It does. And 1 more question. Let's say it works. Do you have to begin very early on for it to work? Meaning like you have to target siblings of autistics or—

DR SAHIN: [Interposing] Yeah. That's a very good question. So the question there I think gets into is there a sensitive or a critical period during which you need to initiate the treatment. And I don't think any of the studies that have been done to date have addressed that question. We're ensuring to address that question in the mouse models to the best of our knowledge. We have this mouse model that looks autistic like after we delete TSC in the Purkinje cells. And as I showed you in my talk, we can prevent development of those symptoms if we start treatment early in life. However, more recently, we have done studies where we can treat those mice later in life and we can still prevent some of the symptoms; not all of them.

DR DECICCO-BLOOM: Maybe we could go to microphone number 4.

DR MARK MINTZ: For Dr Chugani. Some of the commercially available—Mark Mintz from New Jersey. Some of the commercially available pharmacogenomic panels report gene variations, for example, in serotonin transporter genes. What's your experience or opinion on the clinical utility of that in determining maybe serotonin reuptake and resistance in the autism population, for example?

DR CHUGANI: I couldn't hear you very clearly, but I think you were asking the genetic testing available for—in particular for serotonin genes.

DR MINTZ: Right. There's a lot of commercially available pharmacogenomic panels for different drug categories. And some of them report, for example, genetic variations in serotonin transporter genes that would suggest maybe resistance to SSRI-category drugs for example. And in the autism population have you had any experience in that in terms of clinical applications or utility?

DR CHUGANI: So I think that that's one thing that I was suggesting is that going forward that one might consider what particular mutations that a person had and kind of deciding whether a particular treatment would be useful or not. But I think at this point we don't know that.

DR MINTZ: Thank you.

DR DECICCO-BLOOM: Microphone number one.

MALE VOICE: So each of you have made a compelling argument that perhaps bundling several different disorders that have autism as an ultimate phenotype by targeting pathways that are shared by these disorders might make a lot of sense. So 2 related questions. One is can we link serotonin into the TSC and the Shank3? I mean, what do we know about serotonin in those model systems? And question number 2, Mustafa, the sort of axonal phenotype that you demonstrated, that sort of very impressive phenotype, is that shared across other mutations in the pathway? Well, would Shank3 share the same kind of axonal phenotype? And would that then serve as, if you will, the way to screen for compounds or across a range of disorders? Is this axonal chaos a potential target for therapy?

DR SAHIN: You want to take the serotonin question?

DR CHUGANI: Yeah, I'll take the serotonin part of it and then I'll turn it over to you. So in tuberous sclerosis there's very clearly activation of the kynurenine pathway in lesions, so in a subset of cortical lesions, but also in subependymal nodules and in lesion of cerebellum, those near the thalamus. And a number of studies from psychiatry have been relating activation of the kynurenine pathway to a stealing of tryptophan for the serotonin pathway. So tryptophan metabolism by these divergent pathways would impact each other. So activation of kynurenine pathway may lead to a deficit of serotonin, so I definitely think that serotonin has an impact on tuberous sclerosis.

And I wouldn't want to suggest that serotonin is the only thing to treat autism. And another thing that I've kind of thought about with regard to the cancer analogy is that we may come to a time where there would be several different targets in a particular individual and that perhaps a cocktail of agents may be useful for different aspects of the deficit.

I thought it was interesting that—of course with the BDNF examples, it kind of like affected 2 things in 2 different ways, but that might not always be the case. It might be that you might want to treat 1 part of the signaling here because you're not replicating the whole—especially if you're going for something that's a transcription factor, you may be hitting a couple of different pathways or a couple of different targets and that might be useful. I'll turn it to Mustafa now before I dig myself in any deeper.

DR SAHIN: So going back to the second question; the axonal phenotype that we see in culture with TSC. We believe that it probably will not hold for all of the genes that we're talking about. It might be true for some of the genes. The genes in which you have an upregulation of mTOR may present in that way.

I think what we are now able to do or we are proposing to do is to compare those genes that we just talked about, the sort of bona fide autism genes using both cell models, as well as animal models and, hopefully, patient models as well, and looking at that comparative auto physiology. My guess is that when we look back on this in 5 years is that some of the genes will have an external phenotype, some of them may have a serotonin phenotype, other's may have a postsynaptic density phenotype. There might be different ways of catching autism, in a way, but they all will affect the circuitry, whatever the circuitry it ends up being in different ways.

So the question you're asking I think is very interesting from a translational perspective now that we have access to…stem cell derived neurons in culture. We can actually start to answer that question in a very—I mean, it used to be a dream, but we can actually ask that question now in culture and see if different autism genes—different patients skin cells will allow us to test whether they have the phenotype or not.

DR KATZ: I'll take a slightly different spin on your question. I think it's—

DR DECICCO-BLOOM: [Interposing] Could you have that microphone on, please, here in the front?

DR KATZ: If you noticed, Mustafa and I are in many ways looking at mirror images and mirror phenotypes. So in TSC you're trying to inhibit the mTOR pathways with therapeutic strategy. In Rett we're trying to activate it as a therapeutic strategy. So the biochemistry of the 2 disorders mirrors each other, but also the phenotypes, the neuronal phenotypes. So we have in Rett reduced dendritic growth, reduced density of dendritic spines, which we're trying to reverse by activating mTOR. So I think there may be more to be learned by looking at these as phenotypically mirror images of one another with some common molecular endpoints.

DR BUXBAUM: Since you asked the panel, let me just throw in something. So we've kind of ignored axonal development in Phelan-McDermid syndrome or in Shank3 knockouts because it was kind of thought to be a postsynaptic density, synaptic pathology thing. And we were just talking, Al and I, that we should do more on it based on Mustafa's work, but also Karen Dahlman [phonetic] at the University of Miami has been working with zebra fish. We collaborated a little bit and it's quite profound. There is an early neurodevelopmental phenotype when you knock down Shank3 in the fish long before there are synapses. So it's not impossible that there's actually developmental effect and then there is a synaptic effect.

DR DECICCO-BLOOM: Are there additional questions? I might field just one. David, you want to say something about IGF1 in Rett? Because we're hearing about it in Shank3 and maybe we could just have a little discussion about the utility of that or not or what the data are for that.

DR KATZ: Well, I'm not directly involved in the IGF1 work. You saw some of the data in Joe's talk from the Boston study. I think we have to wait until the current trial ends and we see in more depth what the data show. I think the published data show some modest effects. I think what's interesting is that some of the rationale for the IGF1 is that it targets similar

downstream signaling partners as BDNF. And that since we have in IGF1 an approved treatment, why not begin with that in the hope that we would be activating some of the downstream partners that are common to the pathways?

DR DECICCO-BLOOM: Repurposing something that's safe and already being used in children.

DR KATZ: Right. But I think we have to wait and see how the data come out.

DR DECICCO-BLOOM: Other questions or any comments the panelists might want to ask one another?

DR BUXBAUM: It's a good time to be a child neurologist.

MALE VOICE: One of the lessons from cancer is that very effective targeting of the given pathway that's directly linked to invasiveness and drug resistance and recurrence has largely been ineffective with exceptions because of upregulation of other pathways around the block, right, or around the inhibition. That is the cells have the ability to use multiple pathways. So why wouldn't that be the case here?

DR SAHIN: I think one advantage we have in this particular disorder that the genome seems to be relatively stable, as Jonathan Sebat was showing earlier. There doesn't seem to be a lot of genomic instability as it occurs in cancer.

So the disadvantage we have in autism is that we don't have access to the samples of the brain tissue that we really want to have, but if we could we would find that other pathways are not getting secondhand, so you're not getting—if you started drug you are not necessarily getting resistance to that drug, I think. At least in the mouse model studies that many of us have been involved in, we haven't seen that sort of homeostatic change that prevents that first treatment to fail.

So in that sense I'm hopeful. But I think having more human tissue like the iPSC-derived neurons and access to autism brain specimens like was mentioned in the morning session will be incredibly helpful to answer those kind of questions that you're asking.

MALE VOICE: [Off mic]

DR SAHIN: Well, unless we find a critical period in which the treatment is necessary and sufficient to prevent the symptoms, so far the therapies seem to be lifelong, at least in the mouse models.

DR DECICCO-BLOOM: Just to build off on the idea of cancer and autism. I think Jonathan Sebat earlier just referred to the chromatin remodeling, and then Joe, you brought it up as well. So there are a whole host of genes that have recently been identified as a large important network regulating histone and transcription, methylases and transferases, and deacetylases and demethylases, which were all well known in cancer and they're now coming up as very significant.

Maybe up to a half of the really—well, maybe 30% of the really exciting genes that have come out in the last 6 months seem to be in this very complex network in regulation. And it's an opportunity, but it's also somewhat overwhelming. I also think that cancer is different. As we said, it's a continuously adapting process, whereas, stages of development when these genes might be working may be very, very discreet.

However, we also know that the nervous system is constantly undergoing experience=dependent change. To some degree that is epigenetic changes of gene readout, which then engages those pathways, so there may be other times and it may be some of these pathways that allowed adult mice to be revised by turning genes back on later. I don't know if others have more specific comments on that.

DR BUXBAUM: Clearly, Dan showed some of the slides that when you treat adult mice with some of these genetic mutations you can reverse some things but not everything. And many of the cases they kind of gloss over that it was imperfect that some of the core neural behavioral things didn't get better. And I think we all think from behavioral interventions that earlier is better, so there probably is something to be done. You can affect trajectory over life. But we also know and there's beautiful work by Takayla Hench [phonetic] that critical periods can be reversed. He's doing it with vision and you can actually reactivate the critical period in adults.

So I worry less about—I think for our trials to be successful we have to start young so that they have the best chance of being successful, but I think that once we have something that's working we can think about how to give it to older individuals. I'm not sure if that's what you were getting at.

The other thing that's interesting about the transferases and the methylases is they're opposing enzymes and mutations in either one produce the same phenotype. And this is back to what David was saying about all of biology is a U-shaped curve. Too much or too little. And so with the chromatin-remodeling genes it's probably the same thing. So we'll have to then, if that's true, treat those that are too high with reducing compounds and those that are too low with upregulating compounds. So it will not be one size fit all.

DR KATZ: It's clear from the work on MeCP2 that the protein is required throughout life. That no matter when during development you knock it out you get disease phenotypes. It doesn't completely recapitulate Rett, but they're severe phenotypes. So the flip side of that is that if you can reverse the resulting signaling deficit at any stage of life, you have the potential to improve function.

So I think the kinds of data you've seen today are very, very hopeful in that regard that even —there are many adult women today with Rett syndrome, for example. And we're by no means excluding the possibility that they are candidates for these kinds of therapies because of the ongoing requirement for MeCP2 and neuronal signaling.

DR DECICCO-BLOOM: There's one point that you made, Joe, about all the biology is a U-shaped curve. Too little or too much of the same thing in a pathway gives a common phenotype.

When I went to medical school there was this dose-response curve; more drug more outcome. And so a lot of us have had to retool the way we think, but probably both principles are true depending upon what you're looking at.

I think the other thing that David you're referring to is Rett has been extensively analyzed. Not only has there been a constitutive knockout in the whole animal or just in the brain, they've actually removed it from neurons only, astrocytes only, or microglia only, or even oligodendrocytes only and produced much of the same phenotype, and, in some cases, reversed by reexpressing only in one cell group.

So I suddenly become very cautious and say the mouse gestates for 19 days, it's an adult by a month and a half. People might be a bit more complicated and we do want to have a little caution about quickly going from mice to people, and so there is a little fear in me about these rapid movements, but. I think I'll stop there on that one.

DR MARIA: Before we thank our terrific group of speakers here, I want to point out we're going to take a short break.

DR MARIA: At 3:15 we're going to get a really nice executive summary of what we've covered today from Deborah Hirtz, and then we're going to empanel a very distinguished panel of people who haven't been speakers, but have been listening today and each have something more to contribute so that we can walk away with a really comprehensive view of where the field is and where we think things are going. So please thank our terrific speakers.

Executive Summary of the Day

Welcome to the final session. I'm going to try to do a quick summary of what we have heard today. Just hitting some of the highlights. Then I'd like to introduce our very distinguished panel, and we'll have a good panel discussion focused on future directions.

First, I'll go ahead and do my best to summarize some of the things that you've heard today. First, we heard from Sarah Spence who gave us a very good explanation of the new *DSM-5* and how it differed from the *DSM-IV*. What she mentioned was the new categorizations so that autistic disorder, Asperger's Syndrome and pervasive developmental disorder– not otherwise specified are now all considered under autism spectrum disorder.

The most important change is that the domains, which were previously 3 separate domains are now 2, and that the domains also have additional specifiers and particularly that they have severity ratings. She said that the symptoms must be present early, although they may not be fully manifest as early and that they cannot be explained by simply developmental delay.

I won't read this, but she showed us the categorization of the deficits in social communication as opposed to social and communication, and the second category of restricted repetitive behaviors. It's not intended that anybody should be rediagnosed because of this new categorization, and the question remains as to whether it will really make any difference. Probably not. It probably won't make any difference in the prevalence, but we don't know yet and the only study that's been done may report just a very small decrease.

Next, we heard from Craig Newschaffer who gave us a very good thorough explanation of what has been done so far in the epidemiologic studies and where we need to go. As you know, the prevalence has been increasing greatly over the past years and the current best estimate is 1 in 68 among 8-year-old children. And, of course, the prevalence in gender hasn't changed with it being much more frequent in boys than girls. But the data suggest that the prevalence may possibly even be still increasing, and we don't know if a major contributor to this could be the increased diagnosis among children who are otherwise intellectually normal.

He also mentioned that the geographic variability in the prevalence of autism spectrum disorder is not—that there is a great deal of geographic variability, particularly if you're looking at the children who don't have intellectual impairment. So we don't really know, the question always comes up as to why the prevalence is increasing, and the answer is we don't know, and we do know that there may be some contribution to the diagnostic practice and community awareness to this increase.

Craig also noted that the autism spectrum disorder prevalence is generally the same in developed countries where it has been ascertained, but that the prevalence in undeveloped countries has not really yet been determined.

In terms of looking toward the future, he stressed how we need to look not at genes versus environmental interaction, but studies that look at genes and environmental interaction and environment. We know that the epidemiologic studies that deal with environmental exposure must have very careful measurements of that exposure and that is sometimes an issue. We need to work on that.

And we need to work on study design so that the analysis is more complex and more specified so that we can really dissect out the factors that are involved in the genes versus environmental interaction. And we need to use biomarkers that are easily available, that are inexpensive, and that don't use up all of the samples so that we can determine the specific exposure not just at any given time but over various periods of time and the cumulative exposures.

Next, we heard from Lonnie Zwaigenbaum who talked about early diagnosis. Although classically the defining features are apparent at 12 to 18 months, we know that there are many features that can be determined even before the age of 12 months, primarily between 6 and 12 months. And these prodromal features involve deficits in social reciprocity and nonverbal communication, such as joint attention and eye tracking. And these children tend to have atypical motor development, as well as atypical tone.

It has been shown that early intervention, particularly models such as the Denver model, but also others can really, really benefit children especially when they're begun very early, even before 1 year. And so because the current mean age of diagnosis in the country is still 4 or 4.5 years of age, this is something that clearly needs to be worked on, both from a public health point of view and a research point of view, and we need to address the goal of earlier diagnosis.

Roberto Tuchman talked about the coexistence of epilepsy and autism, which is very interesting and a very important comorbidity; one of the major comorbidities. He gave us numbers of prevalence that included studies of children who did and did not have intellectual impairment, so 6 to 24% of those with intellectual impairment and autism have epilepsy. But if the children don't have the intellectual impairment, the figure is lower, around 8%. And there are various studies that give us different numbers in terms of age and IQ. But we do know that the mobility and mortality are increased when both conditions coexist.

So we need to think about how we can identify early those children with autism spectrum disorder who are at risk for epilepsy and how epilepsy may affect the symptoms and course of the autism spectrum disorder. One interesting question is why is it that some children with epilepsy and intellectual deficiency or cognitive impairment and an epileptic encephalopathy don't develop autism. And he noted the differences in regressive autism and epileptic encephalopathy in terms of age and the EEG factors. But the question remains what are the protective factors.

The area of shared mechanisms of these 2 conditions is a very rich one for further research. We know that both may involve altered neuronal networks, including structural and molecular connectivity, as well as altered neuronal excitation and inhibition.

Jonathan Sebat talked about mutations that are known in autism and how important contributions they are to risk. In those with the de novo copy number mutations contribute about 10%, but those that are sporadic contribute about 10% to the autism spectrum disorder risk. And he described his studies of whole genome, as well as exome sequencing, which have been very fruitful. The copy number variations and the single nucleotide variations in other mutations in coding regions have been shown to contribute up to 20% of cases. The genes that are implicated in brain development are seen especially in the mutation hot spots.

Now, there are rare variants and de novo mutations in the other 99% of the noncoding genome that they have that still remain largely unexplored. Rare mutations in the regulatory elements of genes may be actually very important in explaining some of the heritability factors in autism.

Tim Roberts showed us the magnetoencephalography correlates of studies that he's found in children with autism which revealed timing abnormalities, deficits in synchronicity, and they reveal specific cortical-evoked response delays and M100 latencies and Magnetic Mismatch Field (MMF) latencies in autism spectrum disorder, and these, very importantly, correlate with abnormalities in auditory processing clinically, as well as structural evidence of white matter abnormalities using diffusion imaging. The diffusion imaging also he showed to support conduction velocity abnormalities.

A promising area is studying the gamma frequency coherences and including magnetic resonance spectroscopy, and these support the mechanisms of imbalance of excitation and inhibition, and in the glutamate GABA pathways. These biomarkers may be very helpful for both diagnosis and for studies that look at treatment effects and responses to treatment, and probably those combinations that are multimodal will be the most fruitful.

Cindy Schumann gave us a talk about some of the neuroanatomy and neuropathologic findings. We know that very early in most children with autism you see brain overgrowth which then gradually normalizes. She noted especially that there were larger frontal and temporal gray matter areas that you can see up to age about 2, 2.5, and that later these normalize whether they are due to decreased growth in part in the children with autism, as well as catching up in terms of the normal children.

Also, the amygdala has been noted to overgrow early, be larger than in typically developing children, and then later have either a degeneration or lack of growth. And she has seen an increase in the number and size of the microglia in the amygdala of some children with autism spectrum disorder, as well as neuronal cell loss and aberrant cellular development.

The differential expression of small noncoding RNAs can be very important and these need to be looked at in terms of both their regional expression and their age-related differences as these may have profound effects on brain development, as well as brain function.

Now, she also emphasized to us that fewer than 150 autism brains have been studied, which seems like a—considering all the time that there has been research on autism and autism brains, it seems like an awfully small number, and it's very, very important for all of us to encourage the donation of autism brains to scientific research. And I just want to mention there's another Web site other than what Cindy showed that is called takesbrains.org and that gives some information to prospective donors or the families of prospective donors.

Mustafa Sahin talked about tuberous sclerosis, his work in tuberous sclerosis patients. Since at least half of these children do have autism it's very useful and important area for study. The cell mechanisms that are beginning to be understood in tuberous sclerosis may contribute to our knowledge about the mechanisms of autism.

With increased mammalian target of rapamycin activity and its association with issues in axon guidance, myelination and circuitry, this is an important area to pursue, particularly because there are specific inhibitors available and treatments. So the mammalian target of rapamycin inhibition in the tuberous sclerosis mouse model shows an improvement in myelination, learning, seizures, and decrease in autistic features. There is a currently randomized Phase II trial looking at children using mammalian target of rapamycin in tuberous sclerosis with outcomes of cognition, development of autism, seizures, and sleep problems.

So one of the issues is in tuberous sclerosis how can we—if the diagnosis is made early in many children because of the cardiac features, can we detect which of these children will develop autism. There is an ongoing ACE, Autism Centers of Excellence, study with Mustafa and 5 or 6 other centers that are looking at trying to determine which children will be the ones that develop autism.

And he noted that there are many genes, probably 500 to 1000 that are related to both autism spectrum disorder and intellectual disability. And we are not likely to have or be able to develop different treatment for each of those genes, and, hopefully, they will be grouped

together so that we will have phenotypically and biologically groups of children responding to specific drugs.

Next, David Katz talked about Rett syndrome and brain-derived neurotrophic factor (BDNF) and how the loss of MeCP2 disrupts microcircuitry and synaptic function and connectivity, and this may be either increased or decreased. He showed how BDNF and TrkB signaling caused decreased fore-brain activity and brain stem hyperexcitability, and how TrkB receptors may, therefore, possibly be therapeutic targets. Treatments directed at these receptors may restore the imbalances in excitation and inhibition. Increases in mammalian target of rapamycin C1, as well as other related pathways may permit repair of structural synaptic deficits.

Phelan-McDermid syndrome, which is a 22Q13 deletion is also another fruitful condition to study to determine more about pathophysiology and treatment of autism. It causes the loss of 1 copy of the Shank3 gene, which is among the top 100 genes listed as relevant to autism spectrum disorder and the pathways involved in multiple areas of—he described a clinical pilot trial in Phelan-McDermid, which is of IGF1, which may be specifically targeted. The very preliminary results are promising in that there was a benefit in social withdrawal and repetitive behaviors.

Most recently, Diane Chugani talked about the promise and work that's currently being done in pharmacologic treatments for autism. Previously, it's been thought that there's really no treatment for autism. You can only treat the symptoms. There have been studies such as Citalopram and risperidone for repetitive behaviors and anxiety, and specific symptoms of children with autism, and some of those have been fairly successful and others have not. But there hasn't been any study that has been aimed—any clinical trial that has been aimed at core features of autism and really changing the autism.

But now, there's promise that that could happen in the future because studies of mouse models and related genetic disorders do show the potential for reversibility of primary symptoms. And our understanding of how genetic variability can affect the synapse is going to be very promising and may lead to different and new treatment options, as well as the biomarkers and what we learn further when we do genetic testing.

And she gave us 1 example. The serotonin, which is increased in the blood of many children with autism and has been shown to have altered function and enhanced brain clearance in animal models, which is potentially reversible. Her earlier PET scan studies showed serotonin synthesis capacity was lower in young children with autism and so she has actually recently completed a multicenter clinical trial of buspirone, which is a serotonin agonist and that trial, hopefully, we'll be able to see the results of that very soon. But it's just one of the most promising recent approaches for the fundamental deficits, the basic core deficits in autism.

So I just want to put up this last slide of what I thought were some of the common themes that ran through these presentations.

We hope that *DSM-5* will lead to both clearer diagnosis in a clinical setting, as well as better research and that it will work better for research purposes.

We need to continue to study the gene and environmental interactions, but we need to use biomarkers, sophisticated designs and modern techniques, and really improve the quality of these and the quantity of these studies.

We need from a clinical sense to work hard to address earlier diagnosis in the community for these children and, therefore, the ability to give them earlier intervention and treatments, behavioral treatments. And when we finally get them, earlier pharmacologic treatments.

We need to better understand comorbidities. For example, the pathophysiology that underlies the intersection of epilepsy and autism. We are making progress in genetics. This continues to show us specific mutations that are associated with autism spectrum disorder and is probably the most promising lead we have for developing specific treatment targets. And so we should try very hard to refer for genetic testing and learn as much as we can about the genetic variants in these children.

We definitely need more pathologic studies of brains and need to encourage brain donation.

It will be very useful to continue to develop better, and more accurate, and easy to use, and inexpensive, and sample conserving biomarkers. These can be used in multimodal combinations with imaging and will help to advance the characterization of children, as well as help us do studies of intervention, both behavioral and pharmacologic, actually, if we have these good biomarkers for before and after studies and who will respond to what specific treatments.

We need to continue to learn from associated conditions and genetic disorders, such as tuberous sclerosis and Phelan-McDermid syndrome and others like this.

Session IV: Future Directions Panel Discussion

DR HIRTZ: So now I would like to introduce the panel that we have here. We have a wonderful, illustrious panel to end the day of people with various expertise and each one is going to talk just for a few minutes, and then entertain discussion and questions.

So just to introduce, this is Bob Schultz in the far end from University of Pennsylvania. Nancy Minshew from University of Pittsburgh. Marshalyn Yeargin-Allsopp, who actually is from the CDC. It is Atlanta, but not Emory. It is the CDC. And the next is Omar Khwaja. Did I say it right? Okay. Who is from Roche Pharmaceutical Research. Mike Johnston from Hopkins. And Ashura Buckley who is from NIH, both NIMH and NINDS. I think we'll start maybe with Ashura and go that way. And we'll have just a few minutes of what future directions she thinks are important.

DR ASHURA BUCKLEY: Good afternoon. Can everybody hear me okay? Okay. So I'm just going to take a couple of minutes to highlight take-home future directions for research; highlight some of the current NIH efforts that'll help us get there; and just briefly outline a new approach that Sarah Spence alluded to at the end of her first talk.

So just very quickly, I think the models to identify and correct neural dysfunction are right up there as the most important things that we need to be looking at, and to include people with intellectual disability in those trials. We need to put newly identified ASD genes in context with what we already know about molecular pathways and brain circuitry, and we heard some great talks today really focusing on those.

And we need to continue to explore—I know Deb said it and we've talked about it today. I'm just going to underline it again. The co-occurring conditions that we see in ASD. So for those of you who are clinicians, this is really what's bringing patients into your office. Whether it's seizures, it's the monogenetic disorder that comes with other things. And something we didn't talk about today, the incredible prevalence of sleep abnormalities in this population, whether it's sleep-wake transitions, the rhythms that are off. There has been some work in melatonin abnormalities, things like that that will help us walk backward to where these abnormalities in the disorder might be coming from.

Just to switch gears very quickly, current NIH efforts. The NIH is, obviously, very involved and very interested in ASD and helping us get to some solutions. We heard from Dr Chugani who is NINDS funded and we'll be analyzing her PET studies with buspirone looking at some of the very early biomarkers in serotonin, and that was great. We're looking forward to that.

I'd like to mention the Norway Autism Birth Cohort Study funded by the NINDS and Norway, obviously. Very large case-control study integrating prospective health data—integrating that with the broad-based profiling of immune molecules, analysis of infection, fever, medication use, genetics, really looking at genetic and environmental interaction in ASD. It's a great study; longitudinal. Looking forward to that.

We heard from Dr Sahin and Dr Buxbaum who are recent recipients of NINDS, NIMH, and NCATS funding for the rare diseases clinical research network developmental synaptopathies associated with TSC, PTEN, and Shank3 mutations.

And one last thing. Just very recently, last week, actually, the biomarker consortium RFA was just announced, and this is actually very exciting. It's a multisite trial really intended to establish standards for ASD biomarkers. What's really important here is that we all use the same language when we're talking about ASD. This is an effort to do that. So it'll standardize both the collection and the analysis of data. It's supposed to qualify biomarkers for specific application in clinical trials, diagnostics, therapeutics. So the general areas that we're going to be looking at are focused on eye tracking and electrophysiologic paradigms.

It's supposed to make results broadly available to the scientific community, and this is also really important. The data that's collected will be deposited in NDAR, which is the National Database for Autism Research, as well as NIMH's repository for genomics research database. And that should help us really further our research and all get on the same page about what we're talking about and what populations of autism we're talking about.

And, last, I just want to mention the research domain criteria or RDOC. If any of you have heard of that, that is spearheaded by Dr Tom Insel who is the head of NIMH. It started in

2009. And it's a work in progress. But really—and Sarah talked about this a little bit when she put Isabelle Rapin's recent paper.

Really, it's a project to redefine the research agenda for behaviorally defined disorders. So it should be complementary to *DSM-5* and not replace it. But the major goal is to accelerate the pace of new discoveries by fostering research that translates findings from basic science into new treatments, addressing fundamental mechanisms across diagnostic categories. So sort of getting away from just the behavioral self-reports diagnosis and really looking at the mechanism, the abnormality across very many diagnoses. So you would be looking at maybe patients who had anxiety who also had a diagnosis of ASD and schizophrenia, or someone who had anxiety because they had OCD, and what does that look like; are those the same or different constructs in behaviorally based syndromes.

So I invite you to actually look at the NIMH Web site and RDOC and you can just Google that and it'll go through the matrices with you and what that approach looks like. I think it's sort of a formalization of what a lot of the researchers have been talking about already today. Just sort of a different conceptual approach that may help us walk these back and, hopefully, come up with some new categories that will be generated from this basic research. That's it.

DR MICHAEL JOHNSTON: I really liked this symposium and I'd like to hear it again tomorrow, so I hope to watch all the recordings. I thought it was terrific. There wasn't a dull moment. I came away with 3 clinical pearls.

The first was that someone in the audience asked how does ABA therapy work. After listening to the speakers today, I think it works through synapses. The synapses are inefficient, they're aberrant, they're distorted and this therapy somehow shapes the synapses and maybe you have to turn up the volume and give it over and over again, but somehow I think that's probably where the ABA therapy works. You're giving sustenance to synapses that are having a hard time on their own creating memories and networks.

The second thing that I found most interesting was the work on mTOR and mTOR being increased in tuberous sclerosis brain. And I have some interest in that myself. I think someone asked about whether this had been found in brains of children with autism. I read before I came here a paper by Tang in *Neuron*, September 2014. It's a great paper. And, basically, they used postmortem brain of individuals with autism and say that they did find increased elevation of mTOR enzyme. And they linked this to the increased size of dendritic spines through a process called macroautophagy and a disruption.

I found this so interesting because we all know that the brains of children with autism are bigger when they're younger and that's a characteristic that's been documented. This paper would suggest that that's because of increased mTOR, and this mTOR reduces the amount of autophagy that's reducing synapses and makes them too big. So this could link the mTOR with the size of the brain and the synapses. I think that it's a beautiful paper. I would urge you to read it. It really ties together what we've been talking. Especially the link between autism and—idiopathic autism and tuberous sclerosis. Tuberous sclerosis may be a model for autism.

Finally—oh, and I wanted to mention in addition to the mTOR that Tanjala Gipson, who is a young investigator, pediatric neurologist at Kennedy Krieger at Hopkins, and has an… award, presented award yesterday is studying patients with tuberous sclerosis that function at a lower level and have self-injurious behavior. These patients can have very severe seizures and self-injurious behavior, and she has preliminary data that mTOR inhibitors can reduce that behavior and can reduce seizures. I think that's worthy that there may be some hope that patients, even the lower-level patients, may benefit by this therapy in the future.

And, finally, Rett syndrome, I loved David Katz's talk. Every time I hear him I love it because he talks about excitation; glutamate. That's a subject that I love. And he says that the problem in Rett syndrome is either too little inhibition or too much glutamate. We think so, too. The Rett syndrome brain, both in humans and in mice, has too much glutamate. So not only are the receptors increased, but there's too much glutamate. They have about 50 to 100% more glutamate than they should, which is bizarre. You would think the receptors would go down.

The reason I mention this also is that SakkuBai Naidu, who has done research in Rett syndrome for years has an FDA RO1 grant to study dextromethorphan, a very simple, over-the-counter inhibitor of the NMDA receptor, and she has 30 subjects that she studied. What she's studying is the ability of the dextromethorphan to enhance the performance. Preliminary work suggests that indeed dextromethorphan does help performance. She has 30 subjects. She needs 30 more. If any of you know of subjects, I would make that appeal to contact Sakku.

I thought it was a terrific symposium and I'm going to be dreaming about it.

DR OMAR KHWAJA: Thanks. So, certainly, too, I want to echo that. I want to thank the organizers and congratulate them. I think it's been a really fantastic survey of where autism research is.

So I guess for me the highlights, what I'd like to do is sort of pick 2 things that I saw. One I saw and the second I heard today.

The first one where I think really is where the future lies is in a slide that Mustafa Sahin presented where he showed that at one end we have the genetic pathologies that I think Jonathan Sebat and others very elegantly alluded to. These converging genetic pathways and mechanisms that relate at least to part of the etiology of autism.

And at the other hand, this simplification of the behavioral criteria that are necessary to diagnose autism that Sarah Spence presented and others have talked about.

But I think the sweet spot, if you like, for the future of developmental autism therapies, at least in the near and medium term are that point in between. In other words, not necessarily single treatments for single genetic disorders and not necessarily behavioral treatments for a constellation of behavioral complaints. But, essentially, this middle area that I would talk about as perhaps circuitry. I think that Dr Buckley very wisely—I think this has been a great initiative of the NIH to introduce the research domain criteria, and I would think that this is

something that we as child neurologists should certainly start to look at and try to look at disorders that we encounter, not just autism, but other developmental disorders through the lens of.

The other thing was that Joe Buxbaum said it's a great time to be a child neurologist, and I think that's certainly true. I think we are at this—a number of people have used the term—revolution. I think we're at this really incredible point where we're child neurologist with a grounding in neurobiology and neuroanatomy, but also an understanding of the developmental tempo of what's happening in the brain. I think we're really ideally placed to try and make the next push in the treatment of the disorders.

So want to spend 1 minute just expanding a bit on that. Just to level set a bit, though. I look at things now through the lens of industry and biotech and knowing that neuroscience products they take essentially—for 1 new molecular entity to launch is an investment of somewhere now north of $1.8 billion for 1 molecule. And the likelihood of success, the probably of launch is around 6%. In pediatric neurological disorders, much lower than that, and, essentially, it's incalculable because there really haven't been any. I think that we're often looking at medications which are being repurposed.

We heard about the epidemiology this morning. I mean, this is a prevalent disorder with an incidence for reasons we don't fully understand that's increasing. Probably more common than schizophrenia, at least 1.5 to 2 times more common than schizophrenia. If you look at the industry pipeline for new molecular entities for autism, it's vanishingly small. If you look at it for schizophrenia, it's dramatically larger. We have to ask about why.

I think where can we go, really, with this? I think what I've heard today where I would say that we could best put our resources as a community would be to try and understand these domains of behavior that relate to social impairment and repetitive behaviors. In other words, these, I think, are highly targetable. I think they're tractable from a drug development standpoint.

And the presentations by Tim Roberts, by David Katz, for example, I think one of the things that we really urgently need to look at is to try to understand the excitation imbalanced circuitry in the brain, because these are ideally targetable by compounds that are already available, at least in screening libraries or in already-existent, safe compounds. So those that modulate GABAergic and glutamatergic systems.

The other that we haven't heard about today, but I think is important, are the social neuropeptides, so vasopressin and the oxytocin system. I think in the near term these are highly targetable.

I think one of the other things that the NIH has done, which is a great beginning, is the development of Fast-Fail Trials using biomarkers such as electrophysiology, which can be easily applied to children.

So there is a challenge for us, I think, from a drug development standpoint. In industry, this is something which is high risk and that's why not many people are going into it. But I think

what this is really a peak time for us to seize the initiative and go forward. I think that this sweet spot between behavior and between genetic pathology is really, from my standpoint, where the highest likelihood of progress is to be made in the coming 2 to 5 years.

DR MARSHALYN YEARGIN ALLSOPP: As Deborah said, I'm at the Centers for Disease Control and Prevention. I'm a medical epidemiologist. Our discipline is epidemiology and we use that within a public health framework. I feel that we are doing a number of studies and activities now that allow us to be well poised to contribute to some of these really important questions about autism. I'm just going to point a few of them right now.

The first is in the presentation about *DSM-5*. As Craig News-chaffer explained how we collect data on children to determine the prevalence of ASD in 8-year-olds in the United States, known as the ADDM Network, we are now going to be able to continue to collect data in such a way that we will be able to look at the prevalence of ASD in children diagnosed or at least, for our surveillance purposes, determined to be cases under *DSM-5* and *DSM-IV* criteria. So we will be able to tell you what the difference is.

We've already done a preliminary study that has shown that the prevalence was about 20% lower. That was using the methods that we have established now and, of course, *DSM-5* was not in the field, and so we don't know how this is going to play out from the standpoint of clinical practice. But as that occurs with our next study year we will be able to report both. So stay tuned for that. We're looking forward to being able to inform this conversation about what happens when *DSM-5* criteria are applied at a community level compared to *DSM-IV*.

The second area that I wanted to highlight was from Craig's presentation where, of course, everyone is continuing to ask the question why is the prevalence of autism increasing. And it is the ADDM Network from CDC that has reported, as Craig said, 5 surveillance years and our most recent report in March of this year was the 1 in 68 children.

Why? We don't really know. But we do know that there's a lot of geographic variability. We also know that this group of children who do not have intellectual disability seems to be a growing group and seems to be contributing more to the increased prevalence than previously.

One of the questions that we are trying to answer, of course, is whether there is an increase in risk factors that are associated with ASD. Not just the recognition, increased recognition, but is there an increase due to an increase in risk factors.

Some of the scientists at CDC, I was included, did a literature search and came up with a number of perinatal factors that from the literature are important or have been associated with an increased prevalence of autism, and we selected 3. One was being small for gestational age, being low birth weight, and having had a C-section. And when we looked at these factors, we found that all 3 of these factors, one or more of these, only contributed about 12 to 13% of the overall prevalence of ASD. So we do feel that there are multiple factors that contribute small amounts and that there's no one big factor that is responsible for this huge increase, although, we are continuing to study that.

We have a case control study that we're doing on young children. It's called the Study to Explore Early Development. In addition to a range of medical information from records, questionnaires of the parents, clinical examinations of their children, we are also collecting biospecimens. And as we begin to analyze those data, we hope that we will be able to answer some of the questions related to what are risk factors and potential causes of autism.

I was also interested in Lonnie's presentation where he pointed out that the median age of diagnosis of ASD has not changed. From our ADDM data, the first report was in 2002, it still remains at about 4.5. We have a program at CDC called Learn the Signs Early, in addition to the work with the American Academy of Pediatrics, and many of the work that you and others are doing to identify children earlier. We haven't really moved that needle very much over time. What we found that there are more children being diagnosed at every age, but, again, the median age has not changed, and we know that there are disparities, racial and ethnic disparities in terms of recognition of autism. So we are still doing a lot of work in that area and there's a lot more to be done.

The last area that I wanted to emphasize is this overlap between autism and epilepsy. This is an area that we're very interested in. We're not doing a lot in this area now. We are looking at the co-occurrence of epilepsy with cerebral palsy and we do surveillance for cerebral palsy as a primary disability in children with autism as well. But we are starting to look at this and to see if we can incorporate more about the co-occurrence of epilepsy in the children that we have identified with autism.

Overall, we have a lot of cohorts now. We have cohorts that we have identified from our surveillance beginning in the year 2000. We also have cohorts of children from our case control study. So I think it's going to be important for us to use the cohorts that we have already assembled, whether it's longitudinal studies or other special studies, to try to answer some of these really important questions related to autism.

DR NANCY MINSHEW: Thank you. I'm Nancy Minshew from the University of Pittsburgh. It's getting late, so I only want to make a few comments. I'll try to be a little briefer.

Just to be provocative, I think we could say the prevalence ranges from 1.5 to 3%. The 3% being if you take the Korean study seriously. And then as Marshalyn just said, a lot of the increase has been in the improved recognition of those individuals with ASD who have intact formal language and average or above IQ scores. There is a wide variability, as you know, in expression. If you're the practicing neurologist and you have children come in—and remember they can be adults. There's a lot of adults out there now.

Are you comfortable recognizing autism across the spectrum? If you're not, you have to see a lot of kids to get really comfortable with the full spectrum, and that may not be something that's achievable in your particular setting, but you could at least watch the movie about Temple Grandin. John Robison who's as smart or smarter than, has written some books that are just hilarious. You can take a walk in a couple hours you've listened to them. His last book was Raising Cubby. Fascinating. Makes me laugh out loud. He's writing a book called Switched On about the impact of RTMS on his capacity to recognize face emotion, which is

really interesting and provocative in terms of the potential for future treatments. So that's that.

With regard to the switch to *DSM-5*, there was 1 large study and we did a smaller study, but covered the whole spectrum of age and IQ. Those who received a diagnosis of autism under *DSM-IV* also received a diagnosis under *DSM-5*. We were about 93-plus%. And the few percent that didn't make it on *DSM-5*, we just didn't have the right information in the records as we corrected them.

But I think the gentleman that asked the question about how well are the community clinicians doing when they use these criteria to make a diagnosis how likely is that to be accurate. I think that's really an excellent question and one that we do somehow need to look into, which suggests to me also the possibility that if clinicians had a questionnaire or 2 that's kind of backup support that they're on the right track that would be very helpful.

I think generally, neurologists haven't focused on the kinds of complex behavior issues that characterize autism. So we're not as familiar with the intricacies of social interactions or pragmatic language, perhaps, although, Isabelle Rapin certainly educated us on that, or repetitive behavior or concreteness or whatnot. That was not our training. Most of us didn't get any training on autism, and I'm not sure that's changed very much, which is very unfortunate.

You might try to see if your center would have at least 1 speaker a year who's more clinically oriented to come in and help you to understand what are the current treatments, what are the ones that are available in your area; what are the target manifestations across the age and severity spectrum.

Aside from that, I would say that neurologists, when you receive a referral you need a specific question. And the question, Does my child have autism or not, may not be the best question for them to be addressing to a neurologist. But there are questions that only neurologists can answer and they have to do with where in the brain does this live; and what might have caused it; and, by the way, my child is one of the extreme preemies and he had this periventricular stroke and now he had that right hemiparesis, but he also has autism, and did it all come from that right periventricular stroke. You can answer those questions, but nobody else they see can answer that.

So questions about cause are ones that you can certainly address. I think with confidence you can address issues about vaccines. That may be also last year for a lot of people, but it's not out in the community. There are still people afraid of that. But if you look at the neuropathology, and you look at the under-connectivity of the brain, and all of the genetics, you're in a position to confidently say none of the evidence fits that. I think certainly the studies of infants identifying findings by 5 to 6 months and epidemiology implicating in utero is helpful, but I think families need to understand that there are things going on in the brain in the first year of life, and it's not until you get to the second year when all that scaffolding for higher-order functions is coming online. That's when you're' going to see the majority of the symptoms. We wouldn't expect them to see in the first year, just like with dyslexia. We know that that's highly familial, but do we expect to make that diagnosis in the

first 2 or 3 years? No. If it shows up at 5 does that mean there was some event? So at any rate, you can do that.

Now, I think what I'd like to know from the geneticists is we need to know which genetic test to order that will give families the latest information knowing that a year or 2 years from now there will be more information. I know Bernie Devlin at our site is not happy with the policy that's been put up by the organization. So my challenge to the geneticists is you tell us what should be done and then the societies need to get behind it in order to advocate to insurance companies to insist that they do this.

And then last, I would say that if we're going to look for more brain donations, most of these kids live a normal life span and we're going to have to turn to the adults, many of whom are very popular, Temple Grandin or, as I said, John Robison, and ask them to get behind this. The adults, when they do pass, many times their parents are already deceased and nobody is around who even knows that they have autism, let alone is in a position to advocate. So I think if we're going to look for more donations we're going to have to go at that level as well.

DR ROBERT SCHULTZ: Hi. I'm Bob Schultz. I'm from the Children's Hospital of Philadelphia. The privilege of going last, which means I get to be the shortest. Oh, I need to be closer according to Manny.

I want to reiterate that I go to a lot of conferences and a lot of symposiums. This was really a terrific symposium. I want to really congratulate the organizers. I especially liked the emphasis on biomarkers and genetics.

I was thrilled with Joe Buxbaum using the word that we have a revolution going on in our approach and thinking about treatment. So genetically informed treatment. I think I'll use that in my next grant application.

I also liked Sarah Spence's explaining to me why we used a numeral 5 rather than a Roman numeral, so that we can get the extensions of point 1 and point 2. That was always puzzling to me.

But as we think about treatments for autism and we think about these wonderful treatments that are being done in animal models, I just want to throw out a caveat that in animal model research we always have a common genetic background. In people with autism, we don't have a common genetic background. So someone who has a Shank3 mutation is not going to be against the same background.

And so the task as we move forward with these trials into clinical practice, let's say in the near future, that would be optimistic, is that we have to treat individuals. And, ultimately, what we have to understand is how to make predictions at the individual level. I believe that it's going to be quite a multivariant prediction problem. We may have signals for MEG; we may actually know what genes are involved but only some of them, because a lot of the variance is going to be due to common genetic variants, and I'm not sure how we'll ever be

able to kind of accurately or articulate what that common genetic variance is, but we may have biomarkers, we may have genetic markers.

One of the things that seems to get short shrift and I'm a neuorimager, but I always like to proclaim this point, is actually behavioral markers. If we're treating kids with autism we don't really want to treat a biomarker because that's not what brought the kid into the office. We want to treat the child's behavior and the behavior that they or their family finds troubling.

And there is another revolution going on, I think, in measuring behavior that I want you to be aware of. The people in the computer science community will call it the quantified self. So how do we actually get big data on individuals? We want to measure their movement throughout time. We want to measure and sample language continuously and there's recording devices for this. We are deploying gaming systems in the home like the Connect video game, which can really accurately quantify movement in 3 dimensions. And we want to apply machine learning, big-data approaches to understanding the behavior of individuals with autism. This is in addition to the gold standard and in many cases can precede the gold standard kind of characterization because it may be done more cheaply because it can be done remotely.

As many of you know, it's very expensive to bring kids into the lab to do research. It costs between 5 and probably $10 000 per person once you add up all the costs associated with that. So the idea of reaching out into the family and into the community to start the measurement process ahead of time is probably a really cheap way to do research, and will allow us to get more quantified on the behavioral end.

The other thing that's a shame that I want you all to recognize is that even though the people who prescribe a big-data approach often say we're going to use the electronic medical record. For autism that really doesn't work that well because autism—hospital systems is not the medical home for kids with autism. So that, again, gets back to the point we have to find ways to reach families in their local environment to do these kinds of measurements.

One of the most difficult things for clinical researchers, such as myself, is actually acquiring samples. We get a grant, we say we're going to see 100 kids, and then we spend most of our time talking about how we're going to increase recruitment. It would be nice if there were supports at local and national levels for research registries, and if we could get the word out to families about how important research is so that they could participate at higher levels.

Finally, I think a lot about treatment and when we eventually get to the point where we have these wonderful treatments that target genetic mutations and biological pathways. It's probably likely that these treatments are not going to be magic bullets; that they're going to need to be done in combination because kids, at whatever age we give the treatment to, are going to be deprived of certain experience-dependent learning opportunities. So it's probably going to be combing behavioral treatment with a medication. That medication will unlock a biological impediment to their profiting from experience the same way that typically developing kids do.

So I think we need to think behavioral treatments that will be matched with different biological pathways now, because I, like Joe, I love this idea that it's a revolution. I'm optimistic that in the near future we may be actually using these and we'll have to be responsible about how we combine them with existing therapies. So I'll stop there.

DR MINSHEW: And I think I would add to that. One of the concerns is that we've had quite a few advances in interventions, but unless you live within an hour of the few sites where that was developed, you're out of luck. So there's a real obstacle in how do we disseminate the treatments that we do have that are effective and we need to really do that. There are more treatments in development designed to be combined with whether it's a brain stimulation approach or guided pharmacologic approach, but we're going to hit the same wall if we don't figure out how to support dissemination of treatments that we've demonstrated do have efficacy.

DR HIRTZ: I want to thank the panel very much. I think those were excellent comments. I would also like to open up the floor if anybody has any questions for the panel. Bernie, you start.

DR MARIA: I would like you to address the pragmatics of evaluating a new child. So you have high clinical confidence that the child has autism. Say the child is 2 years of age. It's a young family. They're thinking perhaps of having other children and they have a lot of questions about genetics and also about what kind of additional diagnostic steps need to be taken.

So we heard earlier that MEG, for example, is not ready for prime time, but I haven't heard a clear answer about whether MRI is really standard of care. So I'd like that question to be answered. In the absence of any kind of seizure history, should EEG be included or not included; or video EEG be included or not included. I'd like to hear that.

And then on the genetic testing, for counseling reasons are obviously good reasons to be getting that kind of data, but is there a case to be made to the families that if a specific mutation was identified that that wouldn't just be useful from a counseling perspective with additional children, but it might also be predictive of how that child would do over time by virtue of families interacting around the world using the Internet, linking up based on what mutation they have.

So if they have an SCN mutation, is that likely to be predictive of the clinical course? They ask about mental retardation, for example. That's one of the things that drives their concern. Is that likely to be predictive or not?

DR HIRTZ: Nancy, do you want to start?

DR MINSHEW: Yes. I would say that routine imaging is not indicated. If there's an argument, we'll go ahead and argue that. But for the most part, we don't expect to see some gross brain abnormality or anything treatable on these scans, and there's been enough done to know that. So in the absence of some focal findings or something else going on, no on the MRI scan.

I think the EEG is another where unless you have some good reason to think it's an epileptic encephalopathy or strong clinical suspicion that there are seizures, then I wouldn't do that either. And the same for the blood and urine amino acids unless there's a specific suggestion that one of those syndromes is occurring, then I wouldn't just send those out either.

The only thing that I think is really valuable is the genetic studies. My question is I don't think we do have the most sophisticated recommendation yet, and we don't have the insurance companies reimbursing, but that would probably be the only thing. I think there will be growing value in parents knowing.

I think first of all, there's always a relief when parents can say it's that, there is that right there. They do need this new understanding of what genetic means. It can be a de novo mutation. And many parents I see they'll say oh, that comes from my side or oh, that comes from my side. So that's what I would say.

DR MARIA: Is there predictive validity to given mutations in terms of prognosis or not?

DR MINSHEW: I think there's some. I think we have to gather enough of them together to be able to consider the variation. I think if you look at the Simon's experience with 16P11, they thought the genetic homogeneity would give them great phenotypical homogeneity and it didn't, but that's because it's just too course at this point.

DR HIRTZ: Thank you, Nancy. Number 3.

DR AMY GOLDSTEIN: Hi. Amy Goldstein from Pittsburgh. Nancy, if last year's concern was vaccines and autism, I would say this year's concern that we're seeing in our clinic is MTHFR in autism. There are a number of parents coming in where MTHFR testing has been done and the parents want to have explained to them how the methylation defect is causing autism. Could you please give us some guidance as to how to answer this question?

DR MINSHEW: Parents know it before we do, don't they? They know that that thought is out there that there be an alteration in methylation, especially during pregnancy that may result in the expression. I think we need others that know more than I do to speak up, but I think they're too soon on that and they're at risk of doing the wrong thing and making it worse would be my concern. But who knows—

DR HIRTZ: [Interposing] Anyone else on the panel have any comments about that? No. No takers.

DR MINSHEW: We must have a methylation person here, isn't there? No?

DR HIRTZ: All right.

DR MINSHEW: Sorry, Amy.

DR HIRTZ: Let's go back to number one, please. Your next question.

MALE VOICE: It's regard to the prodromal symptoms and I'm interested in the predictive value of seeking out these patients with recurrences in families. Now, for some of the white-haired fellows over here, white-haired fellows, there was a German, Brazelton, who, in Boston, about 45 years ago did some visual preference studies or visual orientation studies in newborns. In fact, in the first 48 hours comparing normal newborns and those—I believe the other control was the anoxic encephalopathies. And then these 2 groups very, very definitively identified the preferences, the normal preferences shown by these normal kids.

My question is, looking at recurrences, and we're still a few years off with genetic studies, would there be any value in these visual boxes in looking at potential patients in the recurrent families?

DR HIRTZ: Who would like to answer? Bob?

DR MINSHEW: I think they're doing that.

DR SCHULTZ: We are, and Lonnie mentioned it this morning, so that's the whole baby sibs kind of approach. You take a child that there's already an index case and you have the opportunity from early in life to be able to observe the unfolding of potential autism. If the recurrence risk is 15%, you get a sample of 100 and you have 15 cases where you can watch it carefully and measure it carefully.

So Ami Klin is doing it down in Emory where he's using eye tracking every month for the first 6 months, and I think every 3 months after that. Our infant brain imaging study, which is headed by Joe Piven, but there's 4 data collection sites is actually doing brain imaging at 3 to 6 months of age at the first time point, and repeating it 4 to 5 times up through age 24 months, and then following the kids behaviorally out to 48 months.

I think there's a huge value and it'll tell you about multiplex autism. It might not tell you about simplex autism, which is only occurring in 1 family member. But huge value in understanding it because the retrospective bias that it's ordinarily there by trying to figure out what happened from history.

DR HIRTZ: Thank you. Number 3.

MALE VOICE: I have a question for Omar. So the billion dollar price tag for developing a drug is an incredibly daunting prospect. I wonder is there ways around that? Did the Orphan Drug Act that the US passed in 1983—was intended to try to provide incentives to encourage the development of drugs for small populations. Are there strategies, such as the ones encouraged by that legislation that you can apply?

DR KHWAJA: Yes. So there are incentives which have been made, particularly here in the US and similar ones in Europe. The Orphan Drug Act is one. The more recent revision of the FDASIA, the PDUFA V and the pediatric legislation here in the US, for example, also incentivizes drug development in terms of extending patent life and intellectual property protection for drug developers. And that certainly helps incentivize, but given the paucity of

drugs in the pipeline at the moment it certainly hasn't been enough to really incentivize it sufficiently.

The biggest cause of failure—I mean, the biggest point of failure in drug development in neuroscience is in Phase II. So we have drugs which have some efficacy in animal models and I think there's a whole discussion about what animal models potentially contribute in the drug development process in autism or, I guess, orders of higher hold or of human functioning, social functioning. And a lot of drugs make it through the safety barrier through the GLP Toxicology in Phase I type of studies.

Where they really fail is in Phase II and that, I think, is where the initiative like EU-AIMS and others that Paul Wang talked about have the biggest role to play. In other words, how do we gate the drug development process so that we discontinue development of drugs which are unlikely to show benefit in larger expensive Phase III trials or how do we progress drugs so that we can build a sufficient evidence of efficacy to get them through that Phase II hurdle.

I think that's the approach that we and other companies are taking, which is to really build these experimental medicine studies, proof-of-mechanism and proof-of-concept studies, to essentially gate the Phase II process before taking very expensive investments in Phase III. I think it's a combination of external regulatory incentives, advocacy, and patient groups demanding. I think a recognition of the economic costs, I mean, now the paper that came out in *JAMA* suggesting lifetime—societal costs per annum of $127 billion that autism costs in the US; 60 billion to 90 billion in the U.K. These are really very large figures.

But for industry, I think it's about addressing the risk of taking drugs into expensive Phase III trials is how do we actually increase the potential for success or discontinue development in Phase II early enough that we haven't unnecessarily invested large amounts of money.

MALE VOICE: It sounds to me, based on what you say, that we need more drugs in the pipeline at Phase I.

DR KHWAJA: We do and that's where the pipeline is really empty. There are a lot of discovery initiatives based on genetic pathologies, on synaptic pathologies, on potentially extrapolation from other mechanisms. But where there really is, particularly for pediatric drug development, there is a real paucity of drugs. Entry into GLP toxicology, entry into human, entry into Phase I, Entry into Phase II, there's essentially nothing really at the moment, and it's how do we fill that.

And what is the process of filling it? It isn't that there's an absence of molecules to go into that pipeline, its' an absence of evidence to actually progress them along that. This is where I think the role of biomarkers, particularly, has for understanding that a drug is going to be efficacious in the human brain that is penetrant to the brain. There was a discussion about the IGF1 studies in rats about understanding dosing.

I think Manny talked about this idea of there being this old concept if a drug a dose-response relationship that's essentially linear or geographic or whatever, but, essentially, it's likely

more U-shaped or some version of U-shaped, and how do we actually address dosing, how do we address dosing in the developing brain. I think these are all the issues that we struggle with in industry the most is how to actually progress molecules of which there are multiple, but how do we get them effectively through Phase I and Phase II.

DR JOHNSTON: I'd like to point out one low-cost…that was reported in Proceedings in National Academy of Science last week. Andy Zimmerman and his colleagues from Hopkins and Mass General reported the use of broccoli sprouts as a cure for autism. It's an interesting paper. The active compound has been purified and it's got a strong effect, but I don't know how far it'll go.

DR KHWAJA: Yeah, I mean, there's an interesting…

MALE VOICE: You'll need to teach kids how to eat their broccoli.

DR KHWAJA: I think mothers everywhere probably are going to be happy about it. I won't comment on the efficacy of that particular compound. But I think what that paper very usefully does is give some important information, which is one of the big killers of autism trials is placebo effect and expectation and the bias and trying to understand that. I think that study actually very interestingly showed with the outcome that they chose as one of their primaries, the SRS, was actually the lower than anticipated placebo rate in that, and I think that's very interesting compared to more traditional measures, such as the ABC.

I think the other thing about it is that it is realistic to actually do these trials in children and that's often been a big barrier is that actually doing studies in children with autism is difficult. There are parents that are willing to enroll their children into these studies as long as they're safe, and that they're well controlled, and that they're well designed, and that they weed out early enough.

The other thing I did want to say is that we also, with this revolution, in industry we're looking a little bit now at a number of failures in Fragile X particularly, which is whether the GABA-B and the mGluR5 hypothesis has really failed. There have been now a large Phase II and Phase III failures in Fragile X. People are saying well, you guys came to us and said this is a clear mechanism, we have a nice animal model, we have safe compounds. You put it into your patient population and it doesn't work, so why should we continue to invest in this company and in this idea that we can mechanistically address autism.

And we also have to back translate now and really try and understand why those studies failed. Did they fail at the endpoint? Did they fail because they were the wrong populations; wrong duration of treatment; or was there something about the mechanistic hypothesis that was wrong as well?

DR HIRTZ: Paul.

DR PAUL ROSS: Paul Ross from Boston. First of all, Brussels sprouts taste a lot worse than a ketogenic diet.

DR JOHNSTON: No. You got to be careful. It's not Brussels sprouts. It's broccoli sprouts.

DR ROSS: That's even worse. I really liked Dr Minshew's answer to the question as to what you do about working up these patients. I see a lot of children with autism and most of the times I think no testing is indicated. But I think it depends on your examination. If a child, for example, has microcephaly and is 2 years old, you do want to get an MRI. There's no point in getting amino acids if they've been checked in the newborn period. I think if a child undergoes a really impressive autistic regression, you have to think about the possibility of an epileptic encephalopathy and I'd get an EEG then.

With regard to the genetic testing, the microarray most of the time shows abnormalities that are innocent and have nothing to do with the autism, and you're really obliged then to get a microarray on both parents to see if this is a benign genetic aberrant. This costs a lot of money and many of the families can't afford it. And if the family is older and don't plan to have additional children, there's no real compelling reason, as far as I'm concerned, to get genetic testing.

So I think most of the time, a careful neurological examination will determine whether or not any additional testing needs to be done.

DR HIRTZ: Thank you, Paul. Number 3.

MALE VOICE: I think we have an excellent day of presentation. I wish and I understand the limitation of time. I wish there was room for brief presentation from the clinical point of view to help us out as far as what's available for the intervention that all these parents come —hyperbaric oxygen therapy recently and NMDA or anything like that. So I really think it would have been a very helpful thing for us in clinical practice how to answer. You understand? They bring me an article about hyperbaric oxygen therapy or other therapies, how shall we respond? I think that would be very helpful.

DR HIRTZ: I think you're right. I think there just wasn't time to fit everything in about autism. But, clearly, that's something that would be very useful at the CNS meeting in some form. Maybe we can have a breakfast symposium or something like that. Mary.

DR DECICCO-BLOOM: Number 2? Marshalyn, since I don't do epidemiology, as you well know, and we think about environmental factors, many of which are ambient, whether it's flame retardants, it used to be lead in the gasoline, it's certainly pesticides.

So I always use as a teaching model how lead levels in the environment, in the blood have an impact on IQ, and then we cleaned it up and all those things improved. And so environmental factors clearly can be detected by population studies. Can you, the CDC, are there mechanisms in place to ask a similar question about the flame retardants, the pesticides or do we no longer have these structures that allow us to do that kind of a study or am I thinking about it wrong?

DR ALLSOPP: So Manny's question pertains to what we can use to measure environmental exposures, if I understand you correctly. To be honest, I don't think we've made much progress in that area. So all of the environmental studies that I'm aware of that look at autism are looking at very gross environmental measures. They're looking at the

HAPs System, which is by the Environmental Protection Agency, which may look at a geographic area and may show that there are elevated rates within a geographic area, but it doesn't get to the level of the person.

So with the National Children's Study the original proposal was to really try to measure environmental toxins within individuals so that you would get more to the person level in understanding what the impact would be of an environmental exposure. But we really don't have data and so there are suggestions. There are a couple of studies out there where they've looked at these—what we call ecological studies that are looking at measures of the environment overall and are showing some elevations, and then looking at, say, an outcome, such as autism, and trying to draw some correlation.

But we still have a lot of work to do in that area and I think that, again, that there are still—it's an area of interest, but I think that we really don't have good measures yet that allow us to say whether specific environmental exposures are related to autism or other developmental outcomes for that matter.

DR DECICCO-BLOOM: And I had a second I might—because of this—and it's not for you. It's for everybody. Families come to us as neurologists to be the advocates for the child. So now if you come in the door with autism and you leave with that diagnosis we are entitled and feel obligated and pleased to support a child with 25 hours a week or more of therapy because we've done this. But we all know that this has been societally driven, advocacy organization driven. Since the genetics and the environmental factors contribute to multiple disorders, I know you and I have had conversations of this offline, it is likely to be true that this will have the same consequences for intellectual disability.

So we've been seeing twice as many of those children over the years and they would likely benefit from those 25 hours or more of directed. It would likely be true that if the families and society and the health care profession consider that those interventions are valuable and are deserved, and we're the small community that actually kind of knows the secret. I was just wondering when and how and whose mission it is to say that; that we could have a tremendous impact in intellectual disability, which we kind of say oh, intellectual disability never going to get better, go home. But no, put them in the autism classroom. So I don't know who wants to discuss that.

DR ALLSOPP: Well, I'll just start and I think that Manny knows that we've been studying developmental disabilities for more than 30 years, and have been studying intellectual disability, cerebral palsy, and sensory abnormalities, hearing loss, vision impairment. And we've only been looking at autism in the last 15 or so.

So I think it's extremely important that we not forget about the other disabilities and not that they're comorbid with autism, but these are primary disabilities of great concern with serious consequences and prevalence, for example, of intellectual disability, which, historically, has been higher than with autism and now may be equal. So I do think that some of these interventions would be effective for children with other disabilities as well, and I continue to ask where's the advocacy for the other disabilities that we see with autism.

DR KHWAJA: Just to add to that, I fully agree. I think this has been a personal bugbear of mine for a long time is that children with other types of developmental disability, particularly intellectual disability have been…that I think children with autism were seen at once, which this is a static and there's nothing you could do about it other than provide some type of schooling, etc.

You look at prevalence overall of neurodevelopmental disabilities; that's 13% of the population. When you take children with cerebral palsy and nonsyndromic intellectual disability into account it's a magnitude greater than autism alone.

I think one of the things that is moving, at least within, I would say in the pharmaceutical and biotech industry, is the understanding that autism and intellectual disability and schizophrenia and the intellectual disabilities associated, for example, in Down syndrome in cerebral palsy that these are half of biological and depending that's addressable through medication together with behavioral therapy. So, for example, in our company we have a program in autism. We also have a program with a new molecular entity for intellectual disability in Down syndrome.

I think there is a move in industry to expand beyond autism because commercially, obviously, it's a bigger case, but I think Marshalyn is right. I think it's who owns the advocacy for those children outside of autism, which I think has been extraordinarily successful in advocating for itself. But I think it's that broader community of individuals with intellectual disability that still are neglected from just the day-to-day clinical care, intervention, schooling, additional therapy standpoint.

DR HIRTZ: Bob, do you have a last word to add?

DR SCHULTZ: Very briefly. What's interesting is that you mentioned ABA and 25 hours a week of intensive treatment. If you look at the treatment trials, the outcome measure that changes is IQ. So you go back to Lovaas, it's IQ. Go back to Early Start Denver, it's IQ. So you might say well, it's going to work in intellectual disability, but that's actually a test to the specificity of the treatment as well. So even an autism researcher should be really interested in trying this in kids with developmental disabilities to understand this issue.

DR HIRTZ: The last 2 questions. I think that Joe was up there first.

MALE VOICE: Yeah, he was up there first. I was watching. You're so polite, Joe. So I'm going to sort of make a point and a question that's—since one of the organizers of this—I'll sort of criticize my own organization.

I think that this symposium was really exciting in that amongst other things it demonstrated the enormous potential for novel therapeutic approaches that leverage recognition of specific biological mechanisms that could be affecting subsets or in some ways, perhaps, as Dr Johnston pointed out with mTOR, perhaps the broader autism spectrum.

I think what this symposium lacked and I want to sort of see— Dr Schultz started to—I have to stop myself from calling him Bob in these formal settings, but Dr Schultz sort of referred

to some of this, but I think it would be good to get commentary from the panel about this about leveraging some of the findings from neuroimaging and other studies to really develop novel behavioral therapeutic approaches and other sort of more neural circuitry approaches potentially with TMS, TDCS, other kinds of brain stimulatory approaches that could possibly combine— of course, be combined with these medication pharmacologic approaches.

Dr Schultz alluded to this. We are ourselves very interested in visual-motor disconnectivity in autism and its relation to autism. We are ourselves thinking about connect system models for therapeutic intervention leveraging—knowing and having identified these abnormalities in connectivity and wanting to use that and potentially brain stimulatory approaches. So I would be curious if the panel had any other thoughts about the opportunities for novel behavioral interventions.

DR MINSHEW: I think that's another half day or a couple of hours. There are certainly ones in development.

DR SCHULTZ: I'll just mention one of the new ones that we're doing now in the last couple years is constraint-based therapy for cerebral palsy. That would be kind of an example. And there it's clear that in order to get results you need a behaviorally salient task for the kids to do. You just can't have the arm constraints. So it's a real plasticity therapy.

Also the evidence that you can inhibit the good hemisphere in a hemiparetic child and that will prevent that from inhibiting the bad hemisphere, and with TMS, so that becomes more —so there's a lot of objective evidence now that on which to base that.

DR KHWAJA: Just a short comment on that. I think from a drug development standpoint that's also a very critical issue because the bottom line is that pills are not going to teach skills. So that development of behavioral paradigms are all going to be—treatment paradigms are going to be the foundation of treatments of children with not just with autism but intellectual disability and other forms of developmental disability.

I think that Dr Minshew alluded to the real challenge now and also related to Dr Buckley's comments on the RDOCs, which is for neurologists and child neurologists in particular to become a lot more sensitive to the specific neuropsychological underpinnings of the behaviors that we're seeing in the clinic, and being able to understand those. Because development of treatments is going to be really dependent on fully understanding those individual components, I guess, of the spectrum of behavior that we see in children.

MALE VOICE: So I guess I just want to mention that obviously…with use of TMS, for example, in depression and just identifying specific neural circuitry and neural pathways at this sort of more larger scale level that may be—as opposed to micro-structural level that may be impaired may be really, really crucial for more specific highly targeted behavioral interventions, as well as potentially other right-brain stimulatory efforts.

DR HIRTZ: Okay. Thank you. One last comment/question from Joe.

DR BUXBAUM: And I'll try not to make it too long winded. But I thought there's been a lot of statements about genetics and I'm a research geneticist and not a clinical geneticist, but maybe I'd like to kind of answer—

DR HIRTZ: Is the mic on?

DR BUXBAUM: It's not on? I hear it.

DR MINSHEW: Bend over so we can hear you.

DR BUXBAUM: So the question as to the positive predictive value of genetic testing depends on the genetic variant. There are some where you're pretty much guaranteed a severe neurodevelopmental disorder and there are some where the risk is 3-fold. So there's no one answer to that.

It's also important to recognize that most of them, as Nancy mentioned, with 16P they don't track perfectly with autism and they could have broad phenotypes. And so it has really been shown very clearly when you look at families with, for example, an…mutation in multiple generations that you really can't say for sure it's going to be intellectual disability or autism or both, and you can't say either whether there's going to be dysmorphologies or not.

So the idea that we have a perfect neurological or genetic test that will say—clinical genetic test that will say this person should go forward for genetic testing, that's been excluded in almost every study done to date. So the reason to do genetic testing is because there is a developmental disability. And the benefits are subtle. I put up the slide of the 3 benefits; patient, family, and society. That's kind of the bioethical frame. It's going to be rare that you're going to look at a child and say this child will have Long QT syndrome because of this deletion, but you will make those claims sometimes.

But for the family who then can call or who can Google and can advocate, that's game changing. It's also the source of a lot of our funding and drive. Also, the genetic testing gives us the data about the odds ratios and about the PPV. If nobody tested, we would know nothing.

So I love this idea that Nancy mentioned that it's up to this group to make the insurance companies—give them the baseline that they should be funding or should be paying for and then have it driven that way rather than people attempt to get genetic testing done and have it turned down by insurance. I think that the benefits are subtle, but they're so critical. And if you think about Fragile X, how much good can we do to Fragile X and yet we test all the time. It's because testing benefits the larger community.

Closing Comments and Thanks

DR MARIA: Thank you. So I'd like to just recognize the terrific leadership of our codirectors, Dr Mostofsky, DiCicco-Bloom, and Hirtz. Give them a hand. They put on a great agenda. And our speakers and panelists, I think in 1 day we've covered a lot of ground.

For those of you who don’t need transcranial magnetic stimulation at this point you can stay where you are to hear about EV68 and flaccid paralysis that I mentioned that there’s been a lot of discussions with CDC. That’s going to take place in this room in a few minutes.

I wanted to thank all of you for staying for the full day and for enjoying NDC, for participating, supporting it, and I hope I’ll see you next year. Thank you so much.

[END RECORDING]

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