The genetic landscape of autism spectrum disorders

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ABBREVIATIONS

ASD	Autism spectrum disorders
CGH	Comparative genomic hybridization
CNV	Copy number variants

Autism spectrum disorders (ASDs) are a group of heterogeneous neurodevelopmental disorders that show impaired communication and socialization, restricted interests, and stereotypical behavioral patterns. Recent advances in molecular medicine and high throughput screenings, such as array comparative genomic hybridization (CGH) and exome and whole genome sequencing, have revealed both novel insights and new questions about the nature of this spectrum of disorders. What has emerged is a better understanding about the genetic architecture of various genetic subtypes of ASD and correlations of genetic mutations with specific autism subtypes. Based on this new information, we outline a strategy for advancing diagnosis, prognosis, and counseling for patients and families.

Autism spectrum disorders (ASDs) are a group of complex neurodevelopmental disabilities that affect social interaction and communication skills. The prevalence of ASDs appears to be constantly and gradually increasing, but it is not clear if this is because of clarification of diagnostic criteria or an actual increase in the number of cases. Most recent estimates find that the median of prevalence estimates of ASD is 62 out of 10 000.[1] Those with molecularly defined causes make up roughly 20% of the cases, but the heritability has been estimated to be 90%, suggesting as yet undiscovered causes. However, there are new reports suggesting that the previous estimate of heritability was too high and may need to be adjusted downwards.[2]

The diagnostic criteria have evolved with increasing clinical and molecular understanding of this umbrella term. This aspect makes the diagnosis more challenging as the clinical spectrum is highly variable and the etiological subgrouping tends to change with the ever-growing molecular data now fed by high throughput techniques such as array comparative genomic hybridization (CGH), whole exome and whole genome sequencing. The judgment of the physician, critical in achieving accurate prognosis and genetic counseling, requires a systematic approach. By incorporating these techniques along with careful clinical and neuropsychological assessment, a more accurate diagnosis of an ASD disorder can be achieved.

Distinguishing between essential autism and complex (syndromic) autism might be considered the starting point of this systematic approach.[3] Of all individuals meeting criteria for autism, essential autism makes up approximately 75% of the cases. Although essentially a diagnosis of exclusion, the main characteristics are the lack of dysmorphic features, higher male to female ratio (6:1), higher sibling recurrence risk (up to 35%), and positive family history (up to 20%). Syndromic autism, on the other hand, is characterized by accompanying recognizable patterns of dysmorphology, a reduced male to female ratio (3.5:1), lower recurrence risk (4%–6%), and family history to a lesser extent (up to 9%).[1]

Clinical recognition of well-known phenotypes leading to a targeted molecular testing approach can strengthen the hand of the clinician in answering additional questions about the recurrence risk and prognosis according to the molecular basis identified by targeted testing. However, for most forms of essential autism, there is no familiar phenotype that points to one particular genetic cause or another. In the absence of a specific genetic test option after clinical evaluation, array CGH testing is recommended as it has been reported to provide the highest diagnostic yield in cases with ASD – ranging from 10%[4] to 18%.[5] Once such a genetic alteration is identified in an affected individual, more specific information about diagnosis, prognosis, recurrence risks, and possible targeted therapy options can be conveyed to the family.

ASD phenotypes caused by genetic alterations can be divided into three subgroups: cytogenetic alterations that can be detected by conventional karyotyping (not the primary focus of this paper). These comprise classic chromosomal syndromes such as Turner and Down syndrome, and large segmental cytogenetic alterations that have been associated with autism.[6] Other subgroups are single gene disorders (detected in <5%) and copy number variants (detected in 10%–35% of the cases[1]).

NON-SYNDROMIC AND POSSIBLE TREATABLE FORMS OF ASD: SINGLE GENE DISORDERS

Single gene disorders are mainly composed of metabolic conditions in which accompanying ASD features are

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reported. Among these, phenylketonuria is common enough to be included in newborn screening programs in most countries. Comorbidity of ASD and phenylketonuria was consistently described in the literature in up to 6% of patients.[7] The fact that none of the 62 patients with phenylketonuria who were treated with the phenylketonuria diet early in life met ASD diagnostic criteria, whereas 5.7% (2 out of 35) of individuals with late diagnosis met diagnostic criteria, underlines the importance of early diagnosis in successful treatment in such metabolic conditions.[8] Conditions such as mitochondrial disorders, adenylsuccinate lyase and creatine deficiencies may also phenocopy ASD. Accompanying autistic features in these disorders range from 0.4% to 80%.[1,9,10] Although mitochondrial disorders can present with autistic features, atypical findings of hypotonia, fatigue with activity, failure to thrive, intermittent episodes of regression, especially those after fever and elevated plasma lactate concentrations, make diagnosis of the condition more straightforward. Non-specific features such as epilepsy and intellectual disability that accompany autism in the setting of mitochondrial disorders on the other hand, can make the diagnosis more challenging.

A recent addition to this group of disorders was made by Novarino et al.[11] who reported mutations in the *BCKDK* gene in the affected individuals from three consanguineous families who had epilepsy, autistic features, and intellectual disability. The encoded protein is responsible for phosphorylation-mediated inactivation of the E1α subunit of branched-chain ketoacid dehydrogenase enzyme. Patients with *BCKDK* mutations displayed reductions in *BCKDK* messenger RNA and protein, E1α phosphorylation, and plasma branched-chain amino acids. *Bckdk* knockout mice showed abnormal brain amino acid profiles and neurobehavioral deficits that respond to dietary supplementation. By supplementing the diet of humans with branched-chain amino acids, the authors were able to normalize plasma branched-chain amino acids levels, but the degree to which neurocognitive changes are treatable or reversible remains to be determined.

ASD ASSOCIATED WITH RECOGNIZABLE PATTERNS OF MALFORMATIONS CAUSED BY SINGLE GENE DISORDERS

Clues to recognizable or syndromic forms of autism may be apparent in the clinical evaluation (Table I). Many patients with ASD show unique additional findings (Table II), which will enable the clinician to evoke more targeted testing strategies. A proposed diagnostic work-up for patients with ASD is shown in Figure 1.

Fragile X

When 'ASD' and 'syndrome' are pronounced in the same sentence, fragile X pops into every clinician's mind. This syndrome is so enmeshed with ASD that general practice is to test almost every patient with ASD for *FMR1* mutations, which yield positive results only in 1% to 3%.[12] Whether it is wise to continue the practice of testing all

What this paper adds

- Genetic origins of ASDs and a diagnostic strategy for clinicians at a glance.
- Summary of the most common ASD-associated phenotypes that display unique additional findings.

patients with ASD with such a low diagnostic yield remains a subject of debate, especially as no treatments for fragile X exist, and as other testing strategies result in a much higher yield. The behavioral overlap between fragile X and ASD is so common that 30% to 72% of patients with fragile X were reported to exhibit ASD symptoms in various reports.[13,14] In our own practice, we reserve testing for those displaying one or more of the fragile X traits, but recognize that some patients will be missed as CGH and whole exome/whole genome sequencing cannot identify the common repeat expansion mutation.

MECP2-related

Another syndrome that overlaps with autism phenotype is Rett syndrome. *MECP2* mutations, responsible for approximately 96% of classic Rett syndrome, have been reported in approximately 1% of patients diagnosed with essential ASD.[15] The most important diagnostic handle in differentiating Rett syndrome from autism must be the acquired microcephaly seen in Rett syndrome patients. Nevertheless, early periods can be confusing for definitive diagnosis as the study of Young et al.[16] shows, in which analyses of multiple databases determined 17.6% of patients eventually proven to have *MECP2* positive Rett syndrome had an initial diagnosis of autism. As most *MECP2* mutations will be identified on whole exome/whole genome sequencing, future strategies might relegate the need for specific testing.

Tuberous sclerosis

Many children with tuberous sclerosis complex exhibit autistic behaviors. This rate decreases from 66% at age 1.5 years to 50% at age 2.5 years, probably because of other tuberous sclerosis complex features becoming more prominent later in life.[17] It has been reported that 1.1% to 1.3% of patients with an initial diagnosis of ASD have been found to test positive for either *TSC1* or *TSC2* mutations.[18,19] Autosomal dominant inheritance pattern and characteristic skin lesions such as Shagreen patches,

Table I: Clues to certain clinical scenarios and unique findings

Presence of epilepsy, dysmorphism, and intellectual disability might suggest a syndrome
Schizophrenia: 1q21.1 del and dup syndromes, 3q29 del syndromes, 17q12 del syndromes, *NRXN1*
Gilles De La Tourette features: *CNTNAP2*
Obesity: 16p11.2 del syndrome
MODY: 17q12 del syndrome
Macrocephaly: 1q21.1 dup syndrome, *PTEN*
Microcephaly: 16p11.2 dup syndrome, 17q21.31 dup syndrome
Limb/hand abnormalities: 1q21.1 del syndrome, 15q13.3 del syndrome, 17q21.31 dup syndrome

MODY, maturity onset diabetes of the young.

Table II: Autism spectrum disorder genotypes with clinically unique relevant associated findings

ASD-related genotype	Epilepsy	Dysmorphism	Intellectual disability	Other
1q21.1 deletion syndrome	–	Mild facial	Mild to moderate	Schizophrenia, CHA, cataracts, limb/hand abnormalities
1q21.1 duplication syndrome	–	Mild facial	Mild to moderate	Macrocephaly, schizophrenia
2q37 deletion syndrome	–	Mild facial	Moderate to severe	Pain insensitivity, brachydactyly
3q29 deletion syndrome	–	Mild facial	Mild to moderate	Severe schizophrenia
3q29 duplication syndrome	–	Mild facial	Mild to moderate	Excessive hand creases, pes planus
13q14 deletion syndrome	–	Facial	Mild to severe	Retinoblastoma
15q13.3 deletion syndrome	Idiopathic generalized	Mild facial	Mild to severe	Dyspraxia and dysarticulation, skeletal/hand abnormalities
15q11–q13 duplication syndrome	+	–	Moderate to severe	Ataxia, hypotonia, sudden unexpected death
16p11.2 deletion syndrome	+	Variable, systemic	Moderate to severe	Obesity
16p11.2 duplication syndrome	–	Mild facial	Mild to moderate	Microcephaly, attention-deficit–hyperactivity disorder
16p12.1 deletion syndrome	+	Facial	Mild to moderate	CHA and skeletal abnormalities
17q12 deletion syndrome	Complex partial	Variable, systemic	Mild to moderate	Schizophrenia, MODY
17q21.31 deletion syndrome	+	Variable, systemic	Mild to severe	Severe hypotonia, friendly demeanor
17q21.31 duplication syndrome	–	Mild facial	Mild to moderate	Microcephaly, hirsuitism, limb/hand abnormalities, atopic dermatitis
BCKDK	+	–	Mild to moderate	Low plasma BCAA
CACNA1C	–	Webbing of fingers and toes, no hair at birth	Mild to moderate	Long QT syndrome
CNTNAP2	Focal	–	Mild to moderate	GTS features
FOXP2	–	–	Mild to moderate	Verbal dyspraxia
MECP2 dup (males)	–	–	Severe	Recurrent respiratory infections
NLGN4 (males)	–	–	Moderate to none	Depression, anxiety and intellectual disability in females
NRXN1	+	Facial	Mild to moderate	Schizophrenia
PTEN	–	Facial	Mild to moderate	Macrocephaly
SHANK1 (males)	–	–	None	Reduced penetrance in females
SHANK2	–	–	Mild to moderate	
SHANK3	–	Mild facial	Moderate to severe	Essentially non–verbal
SLC6A8 (males)	+	Facial	Severe	Megacolon, ileus
TMLHE (males)	–/+	–	None	

ASD, autism spectrum disorder; BCAA, branched chain amino acids; BCKDK, branched chain alpha keto acid dehydrogenase kinase; CACNA1C, calcium channel voltage dependent Alpha 1 C subunit; CHA, congenital heart abnormalities; CNTNAP2, contactin associated protein like-2; FOXP2, forkhead box P2; GTS, Gilles de La Tourette syndrome; MECP2, methyl-CpG binding protein 2; MODY, maturity onset diabetes of the young; NLGN4, neuroligin 4; NRXN1, neurexin 1; PTEN, phosphatase and tensin homolog; SHANK1, SH3 and multiple ankyrin repeat domains 1; SHANK2, SH3 and multiple ankyrin repeat domains 2; SHANK3, SH3 and multiple ankyrin repeat domains 3; SLC6A8, solute carrier family 6, member 8; TMLHE, epsilon-trimethyllysine hydroxylase.

adenoma sebaceum, and hypopigmented macules that can flourish in late childhood or early adolescence should be indicators for the clinician to differentiate these disorders.

PTEN-related

PTEN gene mutations, known to cause a group of disorders collectively called PTEN hamartoma tumor syndromes, were found in a subset of patients with the autistic phenotype and extreme macrocephaly of average +5 SD.[20] Frequency of *PTEN* mutations in cohorts of children with autism and macrocephaly are repeatedly between 1% and 17%.[20,21] As *PTEN* gene mutations are strongly associated with tumor syndromes (Cowden, Bannayan–Riley–Ruvalcaba, and proteus-like syndromes), it is important that patients and their families are referred to a surveillance program after molecular diagnosis.

Phelan–McDermid syndrome

Another syndrome associated with ASD, Phelan–McDermid syndrome, is caused by contiguous gene deletion of the 22q13.3 region. *SHANK3*, a gene within this region, is reported to be mutated as the cause in about 1% to 5% of patients with ASD, epilepsy, overgrowth, hypotonia, and absent language.[22] Patients presenting with these symptoms as well as hearing impairment, tendency to overheat, lack of perspiration, increased tolerance to pain,

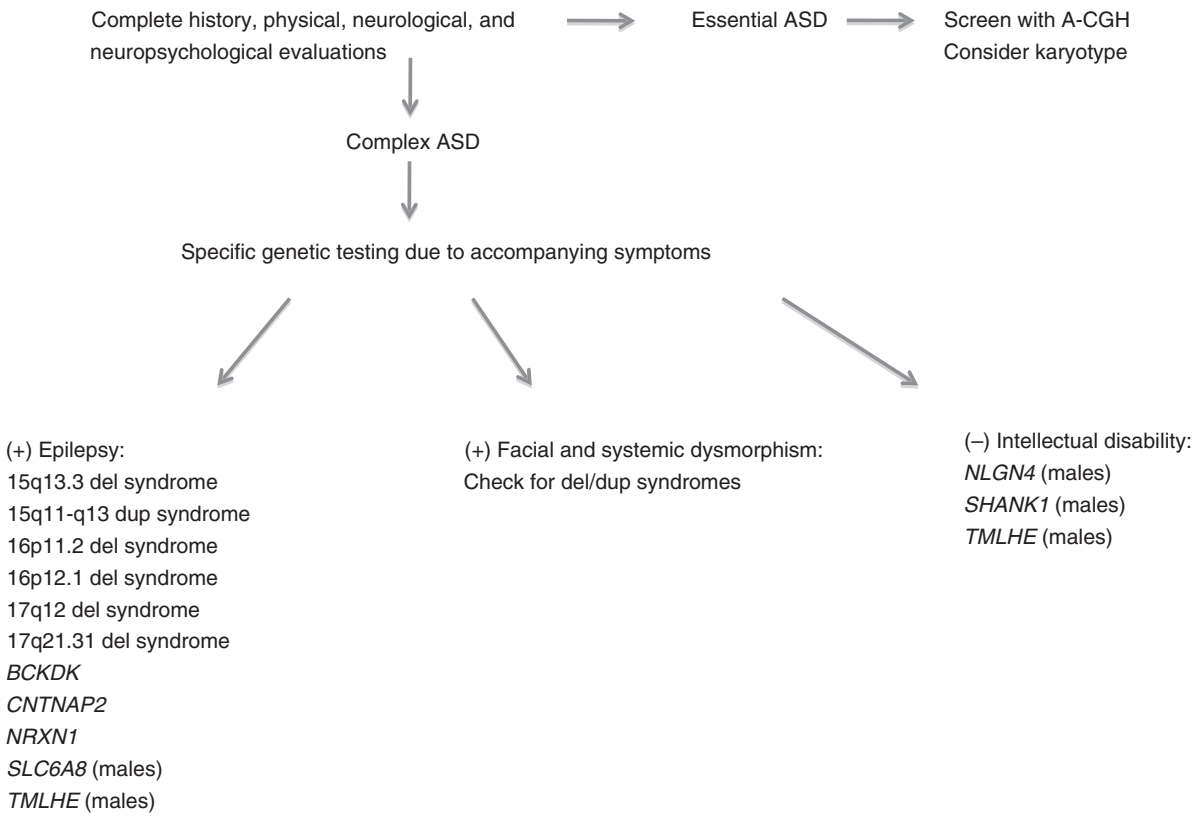

Figure 1: Proposed genetic diagnostic strategy work-up for a child with autism spectrum disorders (ASD). A-CGH, array comparative genomic hybridization; del, deletion; dup, duplication.

inappropriate chewing behavior, and associated craniofacial dysmorphisms should especially be considered for this contiguous deletion syndrome.

Duchenne/Becker muscular dystrophy

Recent studies have also shown that the incidence of ASD in Duchenne/Becker muscular dystrophy cohorts is higher than observed in the general population and these rates vary from 3.1% to as high as 19%.[23] These findings indicate that in patients presenting with ASD, clinicians should be alert for signs that can be observed in muscular dystrophy such as mild intellectual disability, hypotonia, positive Gower's sign, and calf muscle pseudohypertrophy.

Lastly, clinicians should be aware that mild forms of Mendelian diseases that do not have the expected usual presentation may be diagnosed as and included in ASD populations referred to clinic offices. Confirming this point is a recent publication by Yu et al. in which a surprisingly high frequency of biallelic hypomorphic (i.e. mild or partially inactivating of both chromosomal copies) mutations is found in an ASD cohort, which cause conditions that are dramatically different from the null mutations in the same gene. Hypomorphic mutations in genes *AMT*, *PEX7*, and *SYNE1* normally causing non-ketotic hyperglycinemia, rhizomelic chondrodysplasia punctata and cerebellar ataxia respectively, were found in patients who have some but not all of the features of the classic syndrome. In all, about 7% of their large ASD cohort had mutations on such genes. The authors argue the interpretation that biallelic mutations in some settings can cause a spectrum of clinical phenotypes, which at one extreme cause a Mendelian disorder, but at the other extreme represent risk alleles or causes for ASD.[24]

COPY NUMBER VARIANTS LANDSCAPE OF ASD

Recent advances in analysis techniques, such as detection of copy number variants (CNVs) and its application to various cohorts, have led to some surprising findings. Certain CNVs disrupt developmental homeostasis of neuronal development resulting in a range of disorders as part of a neurodevelopmental continuum.[25] Most recent data support an oligogenic model in which severity of the neurodevelopmental disease increases with the increasing CNV burden (i.e. as the number of genes altered increases).[26] ASD is in the middle of this neurodevelopmental disease pyramid along with epilepsy and schizophrenia, whereas bipolar disease marks the less severe end and intellectual disability and developmental delay constitute the more severe end. In such cases, there is often one major CNV that is found to accompany smaller CNVs resulting in different combinations of CNVs and different phenotypes

along the neurodevelopmental spectrum. Whether there can be many CNVs piling up to contribute to a specific phenotype is still controversial. This has been called the 'one to many and many to one' phenomenon.[27]

16p11.2

16p11.2 has been highlighted in a number of recent publications. The deletion is associated with more severe clinical phenotypes with dysmorphic features, intellectual disability, autism, and a strong correlation with accompanying obesity.[28] The reciprocal duplication has a somewhat milder phenotype, and patients are usually underweight in contrary to the obesity seen in 16p11.2 deletion.[29,30]

16p12.1

Deletion of a nearby locus, 16p12.1, was found to be associated with autism phenotype in 42 probands who also had developmental delay, craniofacial dysmorphology, congenital heart defects, and skeletal abnormalities in varying degrees.[31]

1q21.1

1q21.1 deletions and duplications exhibit some of the most varied phenotypic spectrum in the CNV landscape of ASD. Autism is more prevalent in patients with higher rates of duplication, whereas those with deletions may be more recognizable,[29,32] several authors have reported findings of microcephaly, intellectual disability, cardiac abnormalities, cataracts, schizophrenia, and extremity abnormalities associated with this deletion.[33,34]

15q13.3

15q13.3 deletion is also observed across multiple phenotypes with strong associations to idiopathic generalized epilepsy, dyspraxia, and disarticulation.[29,32,35] Frequency of idiopathic generalized epilepsy reaches approximately 1% in the deletion of this locus, which makes it a diagnostic possibility to consider in patients with ASD together with idiopathic generalized epilepsy and an oro-buccal speech disorder.

3q29

Although rare, 3q29 microdeletion is strongly associated with severe childhood-onset schizophrenia.[36] These patients also exhibit intellectual disabilities and mild dysmorphism.[37]

17q12

17q12 deletions are also intriguing from a clinical perspective as patients can exhibit features such as schizophrenia, complex partial epilepsy, and maturity onset diabetes of the young type 5 (MODY5). Thus, these patients frequently stand out clinically from other ASD presentations.[38]

CONVERGENT LOCALIZATION AND MOLECULAR PATHWAYS OF SUSPECTED ASD GENES

As the genetic variability of ASDs mirrors the phenotypic variability, there have emerged a few recurring themes for genes mutated in ASD that are expressed in specific cellular populations and where their associated proteins play an important functions in the nervous system. These potential points of molecular convergence are especially important as they may serve as targets for potential therapeutic efforts in the future.

Postsynaptic translational regulation

The postsynaptic density is a protein rich specialization at the postsynaptic membrane critical for effective neural transmission and synaptic maturation.[39] This complex was one of the first candidates to emerge in the etiology of ASD. The first clue was the localization of the protein mutated in fragile X, FMRP, which regulates activity-dependent protein-synthesis at the postsynaptic density.[40,41]

High throughput techniques have revealed that the encoded proteins of many suspected ASD genes are associated with FMRP and are located in the postsynaptic density, making this region a hotspot for ASD-causing mutations. Synaptic scaffolding proteins SHANK2 and SHANK3, key players in ASD pathophysiology, are located in the postsynaptic density as well.[42,43] Others include autism candidate genes such as *CYFIP1* – located in the 15q11–13 duplication region, as well as *MET*, *PTEN*, *TSC1*, *TSC2*, and *NF1*.[44–48]

Ubiquitination pathways that modulate protein metabolism at the synapse are also implicated by mutations in genes in ASD. A well known example is the gene for Angelman syndrome, *UBE3A*, which has been found to play a role in this pathway as well as genes such as *PARK2*, *RFWD2*, *FBXO40*, and *USP7*.[49,50] Clearly the assembly, maintenance as well as the remodeling of the synapse as a result of neuronal activity, are major determinants of nervous system function and play into ASD pathology.

Neuronal cell adhesion

Another molecular convergence in ASD pathophysiology is the finding of mutations in neurexin and neuroligin families. Neurexins, which have a presynaptic localization, bind to postsynaptically localized neuroligins and together they modulate inhibitory and excitatory synaptic function.[51] Expression of neuroligin genes 1 and 2 even in non-neural cells is sufficient to drive neurons to develop synaptic contacts on these cells, indicating a potent role in neural development.[52] Genes of these superfamilies that were identified to play a role in ASD are *CNTNAP2*, *CNTN4*, *CNTN6*, *NLGN1*, *NLGN3*, and *NRXN1*.[39]

Neuronal activity modulation

Recent studies have implicated the protein products of many suspected ASD genes displaying activity dependent

expression and known to modulate neuronal activity. Some of these genes, such as *SCN2A*, *SCN1A*, and *GRIN2B*, encode for ion channels and help mediate synaptic plasticity,[53,54] whereas others, *UBE3A*, *PCDH10*, *DIA1*, and *NHE9*, are regulated by transcription factors that are themselves regulated by neuronal activity.[49,50,55,56]

Excitatory and inhibitory function imbalance

Yet another commonly encountered defect in ASD models comes from the results of functional studies in mice. Knockout mouse models of genes such as *Nrxn1*, *Cntnap2*, *Fmr1*, and *Shank3* have all resulted in imbalances of excitation and inhibition in different regions of the brain. All these knockout models had overlapping behavioral endophenotypes relevant to ASD, such as repetitive self-grooming, impairments in social interactions, or reduced ultrasonic vocalizations. It is interesting to note that some antipsychotic drugs such as risperidone and gaboxadol were shown to rescue behavioral abnormalities including hyperactivity and perseveration in these mouse models, validating them as useful surrogates of human disease.[57–61]

FUTURE GOALS

High throughput techniques such as array CGH and whole exome and genome sequencing are yielding new regions and genes of interest in ASD phenotypes every day. As these techniques become more accessible to researchers and clinicians alike around the world, these approaches in future ASD cohorts will only grow. As clinical sequencing enters the mainstream, it will take a concerted effort to build knowledge from an ever-expanding clinical/research infrastructure of data.

Multicenter efforts are under way to tackle the problem of impaired pathways therapeutically. One such trial was on the selective GABA(B) receptor agonist STX209 (arbaclofen, R-baclofen), which corrected the increased basal protein synthesis in the hippocampus, and the increased spine density and synaptic abnormalities in *Fmr1* knockout mice. This approach is paving the way for other therapy trials in ASD.[62] Until such results are available, it is essential to correlate these data with a meticulous examination of the ASD phenotypes in order to provide accurate diagnosis, prognosis, and counseling to our patients.

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