Advances in Autism Spectrum Disorders and Intellectual Disability

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Advances in sequencing the entire human genome and large-scale genetic analyses have led to dramatically increased insights into neurodevelopmental disorders, including autism spectrum disorder (ASD) and intellectual disability (ID). Efforts are underway to use these insights to characterize the underlying molecular processes and functional pathways, with the goal of developing mechanism-based therapies for these disorders.

In this session, Anna Szekely, MD, Yale University School of Medicine, New Haven, Connecticut, USA, provided an update on current approaches for the clinical and molecular genetic evaluation of individuals with ASD and ID. Francois V. Bolduc, MD, PhD, FRCPC, University of Alberta, Edmonton, Alberta, Canada, focused on emerging treatments for neurodevelopmental disorders that have been made possible by extensive characterization of the involved biological pathways.

ADVANCES IN GENOMIC TESTING ENHANCE UNDERSTANDING OF ASD

ASD represents a wide range of complex neurodevelopmental disorders resulting from multiple etiological factors. Dr. Szekely presented the new *Diagnostic and Statistical Manual of Mental Disorders* (*DSM-5*) definition, which emphasizes that ASD is primarily characterized by deficits in social communication and interaction and restricted, repetitive patterns of behaviors, interests, or activities beginning in the early developmental period [American Psychiatric Association. ASD *DSM-5*. 2013]. ID is common among children with ASD, with severe ID in about 50%, mild to moderate ID in 35%, and normal intellectual ability in 25% of affected children [Fombonne E. *J Clin Psychiatry* 2005].

The increasing incidence of ASD has spurred research on the wide phenotypic variability and highly heterogeneous genetics of these disorders. ASD is highly heritable, as shown in classical twin studies demonstrating 70% to 90% concordance in monozygotic twins [Bailey A et al. *Psychol Med* 1995]. Other risk factors include male gender and advanced maternal or paternal age.

Large-scale genomic studies have yielded a wealth of data on ASD and ID. Novel genome- wide approaches (ie, high resolution arrays using single nucleotide polymorphism [SNP] and copy-number variations [CNV] markers) have identified significantly enriched rare CNVs, including CNVs harboring new susceptibility genes encoding neuronal cell adhesion molecules and ubiquitin pathways [Sanders SJ et al. *Neuron* 2011; Pinto D et al. *Nature* 2010; Glessner JT et al. *Nature* 2009]. Analysis of the protein-coding portion of the genome with whole exome sequencing (WES) has detected many *de novo* and inherited single nucleotide mutations and rare variants in ASD. Using these and other sequencing technologies, genetic mutations have been identified in ~20% of ASD cases, but none of the mutations individually accounts for >1% of cases.

Several single-gene syndromes are associated with a high rate of patients with ASD symptoms, including Fragile X and Rett syndromes. Many of these disorders are also associated with clinical abnormalities, including dysmorphic features, microcephaly, macrocephaly, and seizures.

Increased numbers of rare CNVs have been found in individuals with ID, many of which overlap with those found in ASD, epilepsy, schizophrenia, attention deficit disorder, and language impairment, suggesting, at least in part, common abnormalities in early brain development. More than 100 X-linked gene mutations have been associated with single-gene disorders in ID.

Evaluation of individuals with ASD and ID using advanced genomic methods can provide a diagnosis and etiology for the disorder, helping to determine prognosis and guide medical care while avoiding unnecessary testing. Genomic testing allows early intervention, counseling, and family planning, providing empowerment and closure to the family. According to the American College of Medical Genetics and Genomics (ACMG), advances in technology have increased the diagnostic yield from 6% to 10% to in the 30% to 40% range [Schaefer GB et al. *Genet Med* 2013].

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Table 1. American College of Medical Genetics and Genomics Practice Guidelines for ASD Genetic Evaluation

First Tier Evaluation	Second Tier Evaluation
3-generation family history	*MECP2** sequencing in all females with ASD
Identify known syndromes or associated conditions	*MECP2* duplication testing in males if suggestive phenotype
Examination with special attention to dysmorphisms	*PTEN* testing if head circumference >2.5 SDs above average
Detailed neurological examination	Brain MRI for specific indicators
Targeted testing if specific syndrome suspected	Microcephaly
Metabolic/mitochondrial testing if clinical indicators present	Regression Intractable seizures
CMA testing	History or stupor or coma
Fragile X DNA testing	
Routinely in males	
If indicators present in females	

CMA=chromosomal microarray; *MECP2*=methyl CpG binding protein 2 gene; MRI=magnetic resonance imaging; *PTEN*=phosphatase and tensin homolog gene; SDs=standard deviations.
*Mutations associated with Rett syndrome.

The ACMG Practice Guidelines for ASD evaluation are summarized in Table 1. A similar diagnostic strategy is emerging for evaluating ID of unknown etiology, starting with chromosomal microarray (CMA) for first-line genetic testing [Schaefer GB et al. *ACMG* 2013].

Projected diagnostic yields expected in genetic testing for ASD are shown in Table 2 [Schaefer GB et al. *ACMG* 2013].

Table 2. Projected Diagnostic Yields in Genetic Evaluation of ASD

Genetic Test	Diagnostic Yield in ASD
CMA	10% (unselected population)
Fragile X	1% to 5%
MECP2	4% of all females
PTEN	5% if head circumference >2.5 SDs
Karyotype	3%
Other	10%

CMA=chromosomal microarray; *MECP2*=methyl CpG binding protein 2 gene; *PTEN*=phosphatase and tensin homolog gene; SDs=standard deviations.

The clinical goals of ASD and ID evaluation are to provide affected individuals with tools to achieve their best potential and to provide the best care for the families. Further research on the phenotypic characteristics and their underlying biological mechanisms is needed to develop therapies based on the key vulnerabilities of the pathological mechanisms.

TARGETING GENE CLUSTERS IN ASD AND ID

Developing potential treatments for ASD and ID is a challenge due to the difficulty in defining clinical phenotypes, severity of the disorders, and the presence of comorbid conditions that can affect the response to treatment, according to Dr. Bolduc. Although there is significant phenotypic and genetic overlap between the 2 disorders, ASD is characterized primarily by restricted behavior and deficits in social communication, while ID is characterized by deficits in intellectual and adaptive functions. In fact, 50% to 85% of ASD patients have ID and 40% of ID patients have ASD [Brereton AV et al. *J Autism Dev Disord* 2006; Matson JL, Shoemaker M. *Res Dev Disabil* 2009]. Additionally, 20% of novel ASD genes identified with exome sequencing were Fragile X targets [Darnell JC et al. *Cell* 2011].

The overlapping nature of biological pathways in these disorders suggests that the genes could potentially be clustered into groups to be then targeted by similar drug therapy. Among other ways, ASD and ID genes can be grouped according to their biological function—control of gene expression; regulation of protein synthesis; structural modification of the synapse and neuron; and functional modification of the synapse and neuron. Table 3 lists the

Table 3. Summary of Gene Clusters and Targeted Treatments for ASD and ID

Functional Category	Genetic Pathways	Potential Treatments
Control of gene expression	Transcription factors	
	Zinc finger protein mutations in ID	
	Epigenetic modification DNA modification in Rett syndrome	Naltrexone targeting *MECP2* regulated genes improved breathing, caused motor deterioration in Rett patients [Percy AK et al. *Ann Neurol* 1994] AMPAkine to target BDNF in Rett syndrome IGF-1 affects dendritic spines and breathing, safe and potentially some clinical improvement in Phase 1 trial [Khwaja OS et al. *PNAS* 2014]
	Histone modification, CBP mutation in RTS	PDE inhibitors rescue memory in RTS mouse model
Regulation of protein synthesis	Translational control FXS caused by absence of FMRP	mGluR5 inhibition for FXS: phase 2/3 trials of Mavoglurant [Gomez-Mancilla B et al. *Expert Opin Investig Drugs* 2014]
	TSC gene mutations in ASD/ID; TSC proteins upstream of mTOR	Inhibition of mTOR with everolimus for TSC approved for SEGA but ongoing trials for epilepsy and cognitive improvement.
	Ubiquitin dependent protein degradation	
Structural synapse modification	Modifiers of actin configuration (profilin, filamin, cofilin) can also be affected in FXS	Lovastatin decreases cofilin activity in mouse models [Samuel F et al. *Mol Neurobiol* 2014]
	Microtubule, MAP1B most reproduced target of FMRP	
	Extracellular matrix	MMP9 inhibitor, minocycline improves behavior in FXS [Leigh MJS et al. *J Dev Behav Pediatr* 2013; Paribello C et al. *BMC Neurol* 2010]
Functional synapse modification	Presynaptic proteins, STXBP1, synapsin involved in neurotransmitter vesicle release identified in ASD, ID, epilepsy	Ketogenic diet for epilepsy
	Postsynaptic proteins, NMDA and AMPA receptors in ID, metabotropic glutamate receptors	Open-label trial of mGluR inhibitor, fenobam showed some benefit in FXS [Berry-Kravis E et al. *J Med Genet* 2009]
	GABA (imbalance of excitatory/inhibitory)	FXS trial of $GABA_B$ agonist, arbaclofen showed no benefit in FXS [Berry-Kravis E et al. *Sci Transl Med* 2012] Oxytocin mediated shift in GABA signaling and enhanced brain function in ASD [Gordon I et al. *Proc Natl Acad Sci* 2013]

ASD=autism spectrum disorders; BDNF=brain-derived neurotrophic factor; CBP=CREB binding protein; FMRP=FX mental retardation protein; FXS=Fragile X syndrome; GABA=gamma-aminobutyric acid; ID=intellectual disability; IGF-1=insulin-like growth factor-1; MAP1B=microtubule associated protein-1B; *MECP2*=methyl CpG binding protein; mGluR=metabotropic glutamate receptor; MMP9=matrix metalloproteinase inhibitor-9; mTOR=mammalian target of rapamycin; NMDA=N-Methyl-D-aspartate; PDE=phosphodiesterase; RTS=Rubenstein-Taybi syndrome; TSC=tuberous sclerosis complex; SEGA=subependymal giant cell astrocytoma.

genes and proteins in each of these pathways that are associated with ASD, ID, and related disorders. A summary of therapies targeting these pathways is also included in the table.

Large-scale genetic studies have shown that ASD and ID may have overlapping phenotypic and genetic features. ASD and ID genes may express different phenotypes if they are expressed in different cortical regions [Parishak. *Cell* 2013; Willsey AJ et al. *Cell* 2013]. Clustering ASD and ID genes by function into networks helps in understanding the disorders and in developing treatments targeting these genes. Dr. Bolduc presented a list of multiple online resources that can help clinicians and researchers stay up-to-date on these genetic disorders and related clinical trials: clinical description, OMIM.org; gene function, geneontology.org; where to test, Genetests.org; and treatments, ClinicalTrials.gov.