ADVANCES IN GENETICS AND AUTISM SPECTRUM DISORDERS

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  Consultation, PI - Research study

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  PI Clinical trials in Down Syndrome individuals

• SIMON’S FOUNDATION – SPARK
  co-PI - Research study
OBJECTIVES

• Explain the DSM-5 diagnostic criteria for Autism Spectrum Disorders
• Discuss the current genetic testing recommendations for children with Autism Spectrum Disorders
• Describe recent advances in the understanding of the genetics of Autism Spectrum Disorders
• Discuss the future impact of genetic testing in the diagnosis and treatment of individuals with ASD
History of autism as a diagnosis

1943-44 Kanner and Asperger case series published

1952 DSM-1, autistic symptoms under schizophrenia

1968 DSM-2, autistic symptoms under childhood schizophrenia

1979 Wing and Gould- broader autism spectrum

1980 DSM-3, infantile autism and childhood onset pervasive developmental disorder

1987 DSM-IIIR, broadened criteria for autism

1994 DSM-IV, five pervasive developmental disorders
Advantages of DSM-IV classification

- Allowed for individuals with milder symptoms to be diagnosed
- Provided for a broader spectrum of symptoms
- Brought agreement to who is “on the spectrum”
- Asperger identity
<table>
<thead>
<tr>
<th>Need for criteria applicable to adults and adolescents</th>
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<tbody>
<tr>
<td>• Unlikely to know language level at age three years</td>
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<td>• Age of onset may be unclear</td>
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<td>Individual change in autism spectrum diagnosis over time</td>
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<td>• Some initially diagnosed with autism or PDD-NOS, may look like Asperger syndrome later</td>
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<td>Asperger criteria- normal intelligence</td>
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<td>• Other DSM diagnoses do not have a specific criteria for level of intelligence</td>
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<td>Rett syndrome</td>
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<td>• Individuals autistic like for short period</td>
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<td>• Specific genetic etiology (MECP2)</td>
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<td>ADHD and autism</td>
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<td>• Data now shows that individuals can have both a PDD and ADHD</td>
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**Problems with DSM IV classification**
Autism Spectrum Disorder

- Autistic Disorder
- Pervasive Developmental Disorder NOS
- Asperger Disorder
- Childhood Disintegrative Disorder

*American Psychiatric Association
www.dsm5.org/ProposedRevisions/Pages/proposedrevision.aspx?rid=94
**DSM IV symptom domains**

- Communication
- Social Interaction
- Restricted and Repetitive Behaviors

**Proposed DSM 5 symptoms domains**

- Social Communication
- Fixated interests/repetitive behaviors
Deficits in communication and social interaction are not separable

- DSM IV gave too much weight to certain symptoms

Delays in language are not unique to ASD and not always present

- Language delay occur in many other conditions
- Children with ASD may not have language delay

Use of new criteria increases specificity without decreasing sensitivity

- Based on review of literature, expert discussion and secondary analysis of data set

Fit the present scientific understanding of ASD

- Genetic data
- Neurophysiologic and functional neuroimaging studies

Rationale for new criteria
AUTOPSY STUDIES IN INDIVIDUALS WITH ASD

• Most replicated neuropathological finding: Decreased Purkinje cells (neurons) in cerebellum

• Increased cell packing density (immaturity)
  - hippocampus, medial septal nuclei
  - amygdala - cortical, medial, central nuclei

• Decreased secondary and tertiary (more distant) dendritic branching and connections – problem with brain connectivity
BRAIN WIRING IN ASD

- FMRI findings indicate reduced long-distance and increased short-distance connectivity in ASD.
- Diffusion-weighted MRI has enabled the investigation of in vivo structural brain connectivity, which has led to a significant amount of evidence concerning abnormalities in structural connectivity.
- The integration of structural brain networks is decreased in individuals with ASD and there are abnormalities in the connectivity of the right caudate and right superior temporal pole.
Brain Wiring in Autism and Developmental Disorders

Normal

Mental Retardation

Autism
Chen JA, et al. 2015.
CLINICAL HERITABILITY IN ASD

• Studies in idiopathic ASD suggest a significant heritable component of risk.

• In the last decade, over 30 twin studies of autism spectrum disorders have been published.

• The median values for MZ and DZ concordances, were 76% and 0%, respectively, from the four original studies of narrowly defined autism, and 88% and 31% from the three new studies of the broader ASD group.

• In large prospective cohort studies of infants with older siblings with ASD, the rate of developing ASD has been reported in 18% of infants.
However,

- Pinpointing the responsible genetic variants has been difficult
- Two decades of linkage, candidate gene, and common variant association studies have revealed daunting genetic heterogeneity with multiple modes of inheritance but few clear solid findings, mostly due to small sample sizes and lack of power
The broad phenotype spectrum of ASD is also reflected in the underlying genetic etiology, which ranges from identifiable monogenic syndromes to large chromosome imbalances.

Molecular genetic studies have identified both de novo and inherited copy number variants (CNV) as the most well replicated ASD risk factors, including deletion and duplication events.
MECHANISMS OF GENETIC TRANSMISSION IN ASD

• **SINGLE GENE DISORDER**
  - Chromosomal deletion/duplication involving key gene in affected interval (other genes affected but not key phenotype determinants)
  - Intragenic mutation

• **CONTIGUOUS GENE DISORDER**
  - Chromosomal deletion/duplication – numerous genes in affected interval combine to give phenotype
  - Imprinting center mutation affecting multiple contiguous genes

• **MULTIFACTORIAL (majority)**
  - Several or multiple risk genes inherited together
  - No one gene would be causative alone
SINGLE GENE DISORDERS ASSOCIATED WITH ASD

• INTRAGENIC MUTATIONS
  - Tuberous Sclerosis (TSC1, TSC2)
  - Rett Syndrome (MeCP2, CDKL5) – MeCP2 dup special case
  - Fragile X Syndrome (FMR1)

• CHROMOSOMAL DELETION/DUPLICATION INVOLVING KEY GENE THAT DETERMINES SPECIFIC PHENOTYPE
  - Angelman Syndrome (UBR3A)
  - Smith-Magenis Syndrome (RAI1)
  - Sotos syndrome (NSD1)
  - Phelan McDermid Syndrome (SHANK3)
ANGELMAN SYNDROME

- Developmental delay
- Very delayed motor skills ataxia
- Laughing spells
- Seizures
- Severe ID
- Behavior problems
- Dysmorphic features

70% deletion
10% UPD
20% UB3A mutation
PHELAN MCDERMID SYNDROME

- 22q13 microdeletion, ring chromosome, balanced translocation deleting SHANK3 or intragenic mutation in SHANK3
- global developmental delay
- intellectual disability
- Severely delayed speech
- hypotonia
- dysmorphic features
CONTIGUOUS GENE SYNDROME DELETIONS WITH ASD/NDD

• Wolf-Hirschhorn Syndrome (4p16)
• Prader–Willi Syndrome (15q11-13)
• Williams Syndrome (7q11)
• Chromosome 15q11-q13 Duplication Syndrome – Isodicentric 15, Partial tetrasomy 15q

most frequently identified chromosome problem in individuals with ASD
GDD, Motor Delays, ASD features
1:30,000 - De novo mutation
MULTIFACTORIAL

- Only a small percent of genetic risks/causes of ASD is known

- High functioning ASD – multiple common genetic influences on population background
  - Many genes – each one responsible only for a small amount of risk
  - High frequency/low risk (relatively) – Common
  - May need up to 5 “hits” of risk genes to get ASD

- Low functioning autism with cognitive impairment – more likely single gene disorders
  - Many genes – each one only responsible for a small fraction of autism spectrum disorders
  - Low frequency/high risk - Rare
AUTISM IS NOT A FINAL MEDICAL DIAGNOSIS

- ASD is a heterogeneous group of disorders
- ASD has many causes
- YOU ARE NOT DONE WITH A DIAGNOSIS OF AUTISM. DON’T STOP HERE
- The clinical presentation and outcome vary substantially in ASD
- MUST ASK: WHAT CAUSED THE AUTISM?

- ORDER: MICROARRAY, FRAGILE X ANALYSIS
GENETIC TESTING YIELD IN ASD

• GENETIC TESTING IS THE **ONLY** STANDARD MEDICAL WORK UP RECOMMENDED FOR ALL CHILDREN DIAGNOSED WITH ASD

• Cytogenetic studies, such as karyotype and fluorescence in situ hybridization (FISH), each with diagnostic yields in the range of 2–3%, have historically been the evaluations of choice for patients with neurodevelopmental disabilities (NDD).

• Chromosomal microarray analysis has a higher detection rate (ranging from 7%-20%) for ASD.
Scale of Genomic Variation

- Chromosome: 100 million bases
- CNV: 30,000 to 3 million bases
- Single gene: 3,000 bases
- Sequence change: 1 base
SYNDROMIC VS NON SYNDROMICS ASD

- simple or "essential" ASD - no dysmorphology or physical malformations
- complex ASD no identified syndrome
- complex ASD specific syndrome (e.g. TSC, FXS)
All children with ASD

1. Three generation family history
2. Detailed physical examination to identify known syndromes
3. Chromosomal Microarray
   Oligonucleotide Array-Comparative Hybridization
   OR
   Single-Nucleotide Polymorphism Microarray

Specific testing

MALES: DNA testing for Fragile X

FEMALES: MECP2 sequencing

MACROCEPHALY (>2.5 SD’s above the mean): PTEN gene sequence analysis

Genetic counseling for all families

Negative Test (no etiology identified):
Counseling about recurrence risk (up to 20% based on infant sib studies)

Positive Test (etiology identified):
Counseling about specific mutations and associated clinical features, including comorbidities, treatment, prognosis

Fig. 1. Recommendations for clinical genetic testing in children with ASD. (Data from [1,2])
WHY GENETIC TESTING?

- Diagnostic clarity
- Genetic counseling about future risk
- To identify CNVs with specific medical vulnerabilities. Allows tailored health monitoring
- Appropriate allocation of supports and services
For some children with positive genetics test results, treatment plans targeting ASD-associated medical conditions can be offered. Examples include:

- screening for cardiac defects in patients with 1q21.1
- maturity-onset diabetes in patients with 17q12 deletion syndromes
- obesity in those with 16p11.2 microdeletions
What is Chromosomal Microarray (CMA)?

- CMA is a technology used to determine if there are small extra (micro-duplication) or missing (micro-deletion) pieces of genetic information. These gains and losses are called copy number variants (CNVs).
- A CNV can be: of no medical consequence; pathogenic, resulting in physical and/or intellectual consequences; or protective against disease (e.g. HIV infection).
What does CMA detect?

• Chromosomal microarray (CMA) testing looks for extra (duplicated) or missing (deleted) chromosomal segments, sometimes called copy number variants (CNVs). These include:
  • **Microdeletions and microduplications** of chromosome segments, which are too small to see under a microscope, but may contain multiple genes. **Most abnormalities of chromosome number** (trisomy, monosomy, etc.), including Down syndrome
  • **Most unbalanced rearrangements** of chromosome structure (translocations, etc.)
  • As with traditional karyotype, mosaicism (a mixture of normal and abnormal cells) of greater than 20-25% can be detected by CMA testing.
WHAT IS NOT DETECTED BY CMA?

• No test can rule out all genetic diseases. Some types of variants require a different test, and some regions are technically difficult to isolate and analyze.

• CMA does NOT detect:
  – Small changes in the sequence of single genes (point mutations)
  – Tiny duplications and deletions of DNA segments within a single gene (Fragile X syndrome, for example)
  – Balanced chromosomal rearrangements (balanced translocations, inversions)
In an extensive literature review of 33 studies including 22,698 patients with idiopathic ASD or intellectual disability. (Miller et al 2010):

- CMA diagnostic yield of 15-20% (relevant CNVs)
- Karyotype diagnostic yield of 3%

This and other many other similar studies clearly suggest an important role of CNVs in the genetic etiology of ASD.

For several loci, the over-representation of CNVs in autistic individuals vs controls has been replicated in multiple studies.

The clinical phenotypes and genotype-phenotype correlations are being characterized.
What do microarray results mean?

**Normal**
No copy number variant (microdeletion/microduplication) detected. *Does not exclude a syndrome caused by a mutation within a single gene or detect a balanced translocation.*

**Pathogenic**
Copy Number Variant has been previously described and associated with a known phenotype.

**Incidental finding**
Results that are not apparently relevant to indication for which test was ordered.

**Variant of uncertain significance (VOUS)**
Not yet described in the literature, is challenging to interpret and benefits from knowledge of parental status.
VOUS identified in patient → Test parents

Neither parent has the VOUS (both have a normal result)

- This finding is new, de novo, and likely pathogenic

One parent has the same CMA result as child

- Finding in the patient is pathogenic/risk, and the parent displays reduced penetrance (not everyone with the CNV will have symptoms), variable expressivity (individuals with this CNV have varied presentation)

- Finding in patient is a normal familial variant and not pathogenic
CNV Interpretation

• Pathogenic CNVs are mostly:
  – Bigger >500kb
  – Deletions or amplifications (multiple copies)
  – Gene rich
  – Contain genes expressed in CNS
  – May contain genes known to be mutated in CNS disorder

• Benign CNVs are mostly:
  – Smaller
  – Gene poor
  – Present in healthy relative
  – Duplications > deletions
CNV Interpretation

• Risk CNVs are:
  – Inherited
  – Have variable penetrance but occur in disease state more often than in population
  – Genetic counseling is difficult
  – Deletions or duplications
  – Contain genes expressed in CNS
  – May contain genes known to be risk genes for CNS disorder with variable penetrance

• Known risk CNVs: 1q21, 1q41q42, 3q29, 15q11.2, 15q13.2q13.3, 16p11.2, 16p13.1

• As experience with arrays increases – will have better databases and can categorize pathogenic, risk, benign CNVs better
Databases to Search for VOUS

- **Database of Chromosomal Imbalance and Phenotype in Humans using Ensemble Resources (DECIPHER)**, https://decipher.sanger.ac.uk/application/
- **Database of Genomic Variants (DGV)**, http://projects.tcag.ca/variation/
- **Database of Structural Variation, dbVAR**; http://www.ncbi.nlm.nih.gov/dbvar/
- **International Standard Cytogenomic Array Consortium**, https://isca.genetics.emory.edu
- **UCSC Genome Bioinformatics Site**, http://genome.ucsc.edu/
• **WHOLE GENOME SEQUENCING (WGS)** identifies the order of individual bases in the DNA chain, rather than extra or missing regions of chromosomes.  
• Like CMA, whole genome sequencing uses array technology to analyze many regions of the genome at once.  
• However, CMA detects changes in gene dosage caused by microdeletions and microduplications, while sequencing identifies smaller variants in the “spelling” of genes.  
• **WHOLE EXOME SEQUENCING (WES)** targets only protein-coding regions of DNA that are most likely to have a functional role.
Whole Exome Sequencing (WES) and Microarray Analysis (CAM) Limitations

- WES does not provide equal coverage for all the coding sequence regions and lacks sensitivity and specificity for detection of structural variants.
- CMA has resolution limitations
- CMA and WES are unable to detect smaller CNVs (<20KB)
- WES will become the primary genetic test for ASD because all classes of genetic variation can be detected in one experiment
- Genetic counseling for ASD-related genomic mutations, especially for rare-sequence variants, is often challenging because of their variable and incomplete penetrance and expressivity.
24/258 (9.3%) had a molecular ASD microarray diagnosis
- Complex ASD group 13/53 (24.5%)
- Essential ASD group 7/168 (4.2%)
- 12/21 (57%) de novo mutations in the cases were parental testing was available
- 11/12 cases with de novo mutations had a complex type ASD

8/95 (8.4%) trios that a molecular ASD WES diagnosis
- Complex ASD group 4/24 (16.7%)
- Essential ASD group 2/64 (3.1%)

Combined Yield of CMA & WES
- Two participants had abnormal results in both tests
- Total combined yield of CMA & WES 15.8% (15/95)
- Essential ASD: Combined yield 6.3% (4/64)
- Complex ASD: 37.5% (9/24)
DE NOVO MUTATIONS

• Every human genome is replete with new (de novo) mutations

• There are various types of de novo mutations, involving changes both large and small. The most common class involves a change in a single DNA nucleotide, whereas others delete or duplicate entire chromosomal regions and encompass tens of thousands of nucleotides.

• Since 2007, Whole Exome Sequencing (WES) studies have shown a strong source of causality for ASDs, namely de novo mutations (that is, new mutations) that originate in the parental germ line.
The Simons Foundation Autism Research Initiative assembled a set of thousands of autistic families, the Simons Simplex Collection, and performed whole exome sequencing testing looking for new mutations.

From the work done so far, it has been found that these de novo mutations underlie at least 30% of autism cases, and there are about 400 genes and regions involved (out of about 25,000 in humans).

There is still no definitive genetic test for autism. Of the 400 genes, only 20% have been conclusively identified.
FUNCTIONAL CONSIDERATIONS

- **Bioinformatic approaches** are beginning to put genetic findings into a more functional context.

- Gilman et al 2011, used **network-based analysis of genetic associations** and identified a large biological network of genes affected by rare de novo CNVs in autism.

- The genes forming the network were not functionally independent, but related to certain biological functions and pathways, such as **synapse development, axon guidance, and neuron motility**.

- This result provides further evidence for the involvement of specific neurodevelopmental pathways in the pathogenesis of ASDs that had also been found in previous studies.
Network analyses of the functioning of the potentially causative agents has been finding genes implicated in synaptic formation and integrity and in chromatic modulation.
As the cost of next generation sequencing techniques continues to drop, whole genome sequencing will become affordable and is expected to replace other techniques within the next few years.

Variants that were too small or too rare to be identified by prior technologies will be identified.
Advances in genetic testing will lead to earlier detection of autism spectrum disorders and targeted brain treatments.
The next decade will likely witness tremendous advances in pharmacologic treatments.

Mechanism-based treatments targeting the underlying pathology of specific genetic disorders associated with ASD and NDD are being studied with the goal of expanding targeted treatments to non-syndromic forms of NDD and ASD.
- National Autism Cohort Network study
- Rush University Medical Center – site
  - Individuals of any age with a professional diagnosis of ASD living in the United States and their parents can participate.
  - Participation can be entirely from home: online registration process, shipping home saliva sample kits and returning saliva samples to center research lab for genetic studies.
  - Positive genetic reports will be sent to health providers according to participants’ choice.
  - Future research opportunities...

Research coordinator email: Hai_Li@rush.edu
CONCLUSIONS

• Patients with Autism Spectrum and other Neurodevelopmental Disorders should undergo genetic testing to make a genetic diagnosis if possible.

• The percentage of patients who can be given a diagnosis is increasing based on newer testing tools (CMA, single gene sequencing and WES).

• The association of genes and pathways with ASD and neurodevelopmental disorders through diagnostic testing will lead to increased understanding of brain wiring mechanisms, earlier diagnosis and ultimately to pathway-based pharmacological treatments.
TARGETED BRAIN TREATMENT IN DOWN SYNDROME
• Attractive mechanism to treat cognitive impairment in DS given mouse results

• BUT….non-selective GABA-A antagonists (e.g. picrotoxin or PTZ) can cause SEIZURES and, therefore, can’t be used in people with DS.

GABA-A Antagonists and DS
GABA<sub>A</sub> Benzodiazepine (BDZ)-Sensitive Subtypes

**In vivo effects**

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<thead>
<tr>
<th>Agonism</th>
<th>Inverse Agonism</th>
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<tr>
<td>Cognitive impairment</td>
<td>Cognitive enhancement</td>
</tr>
<tr>
<td>Sedation</td>
<td>Pro-convulsant</td>
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<tr>
<td>Anti-convulsant</td>
<td>Anxiogenic</td>
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<tr>
<td>Anxiolytic</td>
<td>Anxiogenic</td>
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THE STUDY DRUG

• RO5186582 is a molecule combining both binding and functional selectivity at the GABAA α5 subunit-containing receptors that improves cognition in rats and monkeys, as well as in Ts65Dn mice.

• RO5186582 lacks anxiogenic or convulsant effects in rodents and dogs at the exposures tested in GLP toxicology studies.
Normal

GABA-A

GABA

Glutamate
DS + GABA-A Inhibitor

GABA

GABA-A

Glutamate

GABA