The Amazing Brain Webinar Series: Select Topics in Neuroscience and Child Development for the Clinician

Part VII – Recent Advances in the Genetics of Autism Spectrum Disorders

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Jointly sponsored by the Association of University Centers on Disabilities, the Maternal and Child Health Bureau/Health Resources and Services Administration and Yale School of Medicine, Section of Developmental-Behavioral Pediatrics
Webinar Overview

• Webinar Recording
  – Visit www.aucd.org/webinars

• Q & A
  – Please submit your questions throughout the webinar via the “question box” on your webinar dashboard. Questions will be answered following the presentation.

• Survey
  – Please complete the short survey at the end of the webinar!
Introductions

Carol Weitzman, MD, Professor of Pediatrics and the Child Study Center, Yale School of Medicine

Abha R. Gupta, MD, PhD, Associate Research Scientist in Pediatrics and the Child Study Center, Yale School of Medicine
The Genetics of Autism Spectrum Disorders

Abha R. Gupta, MD, PhD
Developmental-Behavioral Pediatrics
Department of Pediatrics and Child Study Center
Genes contain instructions for making proteins.

Proteins act alone or in complexes to perform many cellular functions.

U.S. Department of Energy
DNA sequence changes
DNA sequence changes

**Missense mutation**

Original DNA code for an amino acid sequence.

DNA bases

C A T C A T C A T C A T C A T C A T C A T

Amino acid

Replacement of a single nucleotide.

C A T C A T C A T C C T C A T C A T C A T

Incorrect amino acid, which may produce a malfunctioning protein.

**Nonsense mutation**

Original DNA code for an amino acid sequence.

DNA bases

C A G C A G C A G C A G C A G C A G C A G

Amino acid

Replacement of a single nucleotide.

C A G C A G C A G C A G T A G C A G C A G C A G

Incorrect sequence causes shortening of protein.

U.S. National Library of Medicine
DNA copy number variation (CNV)
Evidence for genetic basis of ASD

Sibling recurrence risk: 18.7% (Ozonoff et al 2011)

Twin studies (Bailey et al 1995, Hallmayer et al 2011)
• Identical twins share 100% of their genetic info
• Fraternal twins share 50% of their genetic info
• Identical twins share an ASD diagnosis more frequently than fraternal twins
• Identical twins are not always concordant

10% of cases also have a genetic syndrome
<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Gene(s) associated with the syndrome</th>
<th>Proportion of patients with the syndrome that have an ASD</th>
<th>Proportion of patients with an ASD that have the syndrome</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>15q duplication</td>
<td>Unknown</td>
<td>High</td>
<td>1–2%</td>
<td>101</td>
</tr>
<tr>
<td>Angelman syndrome</td>
<td>UBE3A (and others)</td>
<td>&gt;40%</td>
<td>Rare</td>
<td>102, 103</td>
</tr>
<tr>
<td>16p11 deletion</td>
<td>Unknown</td>
<td>High</td>
<td>~1%</td>
<td>20, 35, 44</td>
</tr>
<tr>
<td>22q deletion</td>
<td>SHANK3</td>
<td>High</td>
<td>~1%</td>
<td>21, 22, 104</td>
</tr>
<tr>
<td>Cortical dysplasia-focal epilepsy syndrome</td>
<td>CNTNAP2</td>
<td>~70%</td>
<td>Rare</td>
<td>37</td>
</tr>
<tr>
<td>Fragile X syndrome</td>
<td>FMR1</td>
<td>25% of males; 6% of females</td>
<td>1–2%</td>
<td>105</td>
</tr>
<tr>
<td>Joubert syndrome</td>
<td>Several loci</td>
<td>25%</td>
<td>Rare</td>
<td>106</td>
</tr>
<tr>
<td>Potocki–Lupski syndrome</td>
<td>Chromosome position 17p11</td>
<td>~90%</td>
<td>Unknown</td>
<td>107</td>
</tr>
<tr>
<td>Smith–Lemli–Optiz syndrome</td>
<td>DHCR7</td>
<td>50%</td>
<td>Rare</td>
<td>108</td>
</tr>
<tr>
<td>Rett syndrome</td>
<td>MECP2</td>
<td>All individuals have Rett syndrome</td>
<td>~0.5%</td>
<td>109</td>
</tr>
<tr>
<td>Timothy syndrome</td>
<td>CACNA1C</td>
<td>60–80%</td>
<td>Unknown</td>
<td>24</td>
</tr>
<tr>
<td>Tuberous sclerosis</td>
<td>TSC1 and TSC2</td>
<td>20%</td>
<td>~1%</td>
<td>110</td>
</tr>
</tbody>
</table>
Genetics of ASDs

ASDs are complex disorders with:

• Wide phenotypic variability
• Multiple etiological factors
• A great deal of genetic heterogeneity

There is no one gene for autism, not even a few. There are likely hundreds.
Chromosomal regions implicated in ASD etiology
Disease gene identification

Positional: genome-wide, unbiased

Candidate genes

Mutation screening, functional analysis

Disease genes

Functional: hypothesis driven
Cytogenetic analyses

Chromosomal abnormalities

- Balanced: inversion, translocation
- Unbalanced: duplication, deletion, aneuploidy
Glessner et al 2009: CNVs are significantly enriched in:
  • Neuronal cell adhesion molecules (NRXN1, CNTN4, NLGN1, ASTN2)
  • Ubiquitin pathway (UBE3A, PARK2, RFWD2, FBXO40)

Pinto et al 2010: Higher rate of rare, genic CNVs
  • SHANK2, SYNGAP1, DLGAP2, DDX53-PTCHD1

Sanders et al 2011: Rare de novo CNVs
  • chr7q11.23, chr16p13.2 (USP7, c16orf72), CDH13, chr15q11.2-13.1, chr16p11.2, NRXN1

Individually rare but implicate important gene networks
CNVs in ASD and schizophrenia

Red: ASD
Blue: schizophrenia
Black: both

Merikangas et al. 2009
Genome-wide association studies (GWAS)

• **Wang et al 2009**
  - 5p14.1, between *CDH9* and *CDH10*, which mediate neuronal cell adhesion
  - Perhaps contributes to cortical underconnectivity in ASD

• **Weiss et al 2009**
  - 5p15, between *SEMA5A* and *TAS2R1*
  - *SEMA5A* has been implicated in axonal guidance
  - Reduced *SEMA5A* expression in postmortem brains from autism patients
Whole-exome sequencing

Obtain the DNA sequence for the entire coding region (exome), which is 1% of the human genome.
Whole-exome sequencing
Illumina HiSeq

Sample preparation DNA (5 μg)
Cluster growth

Template dNTPs and polymerase

Bridge amplification

100–200 million molecular clusters

Metzker 2010
Whole-exome sequencing
Experimental design

DNA studied for 200 quartets from the Simons Simplex Collection to investigate the rate of \textit{de novo} variants in patients versus unaffected siblings

Whole-exome capture: 32 million base pair target
Sequencing yields 100 million reads, each 75 base pairs long
Analysis on high performance cluster
Whole-exome sequencing

Results

Brain-expressed, protein-changing *de novo* mutations
- 114 in 200 patients vs. 67 in 200 siblings
- $p=0.001$, OR 2.22 (95% CI 1.19-4.13)

**SCN2A (Sodium channel, voltage gated, type II, alpha)**
- Previously reported in epilepsy
- 2 *de novo* nonsense mutations in patients, neither has seizures

Including 3 other studies: *CHD8, DYRK1A, GRIN2B, KATNAL2, POGZ*
Developmental neurobiology...

Hawrylycz et al 2012

...will inform ASD pathophysiology

Kang et al 2011
The neural synapse
Candidate genes for ASD

State and Sestan 2012
Gene expression analysis in postmortem brain

Differential gene expression in frontal and temporal cortices is attenuated in autism.

Discrete set of co-expressed genes in autism is:

- Down-regulated compared to control tissue
- Enriched for synaptic function and known autism risk genes

Voineagu et al 2011
Whole-genome sequencing is here!

Ion Proton
Cost per human genome

Moore's Law

National Human Genome Research Institute
genome.gov/sequencingcosts
Clinical genetics evaluation in ASD

AACAP practice parameters 1999
- FraX DNA test and Wood’s lamp for TS is typically indicated

American Academy of Neurology 2000
- Karyotype and FraX if ID, family history of ID, or dysmorphisms

American Academy of Pediatrics clinical report 2007
- Karyotype and FraX if ID

American College of Medical Genetics practice guidelines 2013
- Tiered evaluation...
Clinical genetics evaluation in ASD

First tier

• 3 generation family history
• Identify known syndromes or associated conditions
  • Examination with special attention to dysmorphisms
  • If specific syndrome suspected, do targeted testing
  • If clinical indicators present, do metabolic/mitochondrial testing
• Chromosomal microarray
• DNA testing for Fragile X
  • Males: routinely
  • Females: if indicators present (family history, phenotype)
Clinical genetics evaluation in ASD

Second tier

• *MECP2* sequencing for all females with ASD
• *MECP2* duplication testing in males if phenotype suggestive
• *PTEN* testing if head circumference >2.5 SDs above average
• Brain MRI for specific indicators (microcephaly, regression, seizures, history of stupor/coma)

Schaefer *et al.* 2013
Clinical genetics evaluation

Projected diagnostic yields:

- CMA: 10%
- Fragile X: 1-5%
- *MECP2*: 4% of females
- *PTEN*: 5% if head circumference >2.5 SDs
- Karyotype: 3%
- Other: 10%

Estimated to result in an identified etiology in 30-40% of individuals

Schaefer et al. 2013
Clinical genetics evaluation will become increasingly routine in the medical assessment of ASD, regardless of specific diagnosis:

Abnormal genetics test:

- Autism: 3.1% (2/65)
- PDD-NOS: 5.1% (6/117)

(Challman et al. 2003)
Rett syndrome and MECP2

- Mutations in MECP2 cause most cases of Rett syndrome.
- MECP2 controls the expression of other genes.
- Patients show abnormal neurons but not neuronal death: Can viable but defective neurons be repaired?
- Mutant mice lacking MECP2 develop neurological symptoms.
- Reactivation of MECP2 in adult mutant mice reversed symptoms, including deficits in neuronal signaling in the hippocampus (Guy et al 2007).
- Absence of MECP2 doesn’t irreversibly damage neurons.
Fragile X syndrome and \textit{FMR1}

- Loss of \textit{FMR1} causes FXS.
- \textit{FMR1} controls the expression of other genes.
- Pathophysiology involves hyperactivity of the glutamate receptor, mGluR5.
- Mutant mice lacking \textit{FMR1} develop neurological symptoms.
- Reducing expression of mGluR5 by 50\% showed rescue of symptoms, including alterations in dendritic density and hippocampal protein synthesis (Dolen \textit{et al} 2007).
- GABA agonist R-baclofen corrects increased synaptic protein synthesis and spine density (Henderson \textit{et al} 2012).
Tuberous sclerosis and TSC

- Mutations in TSC1 and TSC2 cause TS.
- Pathophysiology involves the mammalian target of rapamycin (mTOR) signaling pathway in the hippocampus.
- Mutant mice lacking 1 copy of TSC2 show cognitive deficits. Treatment of adult mutant mice with rapamycin improved synaptic plasticity and behavioral deficits (Ehninger et al. 2008).
- Mutant mice lacking 1 or 2 copies of TSC1 in cerebellum show decreased neuronal activity, abnormal social interaction, and repetitive behavior. Treatment with rapamycin prevented pathological and behavioral deficits (Tsai et al. 2012).
Future directions

- Increase study populations by ten-fold
- Whole-genome sequencing: regulatory elements
- Pathway analysis
- Epigenetics
- Biomarkers: neuroimaging, eye tracking, ERPs
- Genetic overlap between neuropsychiatric disorders
- Functional analysis of variants: bridging genetics and neuroscience
  - *In vitro* and *in vivo* studies
  - Postmortem brain tissue, induced pluripotent stem cells, animal systems
Thank you for your attention!