Autism Spectrum Disorder Part III: Genetics and Autism
No conflicts of interest to declare

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AUTISM RESEARCH INITIATIVE

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AUTISM SCIENCE FOUNDATION
SEARCHING SOLUTIONS

BRAIN & BEHAVIOR
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Awarding NARSAD Grants

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FOUNDATION
Learning objectives

- Discuss the role of genetic and environmental factors that contribute to ASD
- Identify the wide range of genetic variation that is involved in ASD including gene-gene and gene-environment interactions
- Review the current status and key findings of genetics research in ASD
Autism Spectrum Disorder (ASD) is a developmental neurological impairment.

- CDC estimates incidence of 1 in 60 (1.7%)
  - More common in males (2:1 to 4:1)
- Individuals with ASD have fewer children – Power et al. 2013
Genes vs. “The Environment”

- Trait: A characteristic of individuals in a population
  - E.g. milk production in cows
  - Height in humans
  - Sociability in humans
  - Autism spectrum disorder diagnosis
Genes vs. “The Environment”

• Trait: A characteristic of individuals in a population
  • E.g. milk production in cows
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  • Autism spectrum disorder diagnosis

2% diagnosed With ASD
Genes vs. “The Environment”

• Trait: A characteristic of individuals in a population
  • E.g. milk production in cows
  • Height in humans
  • Sociability in humans
  • Autism spectrum disorder diagnosis

• Genes: The variance in a trait explained by heritable factors

• Environment: The variance in a trait explained by everything else
  • "Unaccounted" would be a better phrase
  • Environmental factors are one contributor to this term

2% diagnosed With ASD
What are researchers trying to achieve?

• Genetics
  - Find genetic variants that “cause” ASD
  - Understand how they cause symptoms
  - Develop treatments

• Environmental factors
  - Find genetic variants that “cause” ASD
  - Improve health
  - Understand how they cause symptoms
  - Develop treatments
Genetics and heritability analyses assess the flow of information in biological systems.

Genotype → Mendel’s laws of inheritance → Phenotype

Mendel’s laws

Genotype

Darwin’s theory of evolution by natural selection

Phenotype

Darwin’s theory

Genotype
Genotypes are amplified to produce observable phenotypes.

Sanders SJ, Curr Opin Genet Dev, 2015
Genotypes are amplified to produce observable phenotypes.
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Genotypes are amplified to produce observable phenotypes

DNA remains constant through life; this makes it easier to establish causality

Sanders SJ, Curr Opin Genet Dev, 2015
Twin studies compare identical (monozygotic) to non-identical (dizygotic) twins

Twin study

100% genetic sharing
50% genetic sharing
Twin study: Combining data across 7 twin studies

Additive genetic effects

- Steffenburg et al., 1989 (n = 21)
- Le Couteur et al., 1996 (n = 48)
- Taniai et al., 2008, TH 2% (n = 45)
- Taniai et al., 2008, TH 5% (n = 45)
- Lichtenstein et al., 2010 (n = 7982)
- Hallmayer et al., 2011, TH .6% (n = 192)
- Hallmayer et al., 2011, TH 5% (n = 192)
- Nordenbaek et al., 2014 (n = 36)
- Colvert, Tick et al., 2015 (n = 127)
- Meta-analysis 1 (N = 586)

~75% “genetic”

Twin study: Combining data across 7 twin studies

~25% “environment”

Family/sibling studies compare relatives (e.g. siblings) to the general population.

Diagram:
- Family tree showing genetic sharing
  - 50% genetic sharing
- Population frequency
Analysis of heritability across ~130 complex human disorders; ASD is the most heritable

ASD: 92% “genetic”

Many other family studies of ASD alone; most estimate ~75% heritability

Wang et al. Nature Genetics 2017
Neuropsychiatric disorders, like ASD, are frequently genetic

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Clinical utility</th>
<th>Heritability</th>
</tr>
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<tbody>
<tr>
<td>ASD</td>
<td>Parental counseling</td>
<td>70 - 95%</td>
</tr>
<tr>
<td>Schizophrenia</td>
<td>None at present</td>
<td>60 - 90%</td>
</tr>
<tr>
<td>Alzheimer's</td>
<td>APOE4 testing?</td>
<td>60 - 80%</td>
</tr>
<tr>
<td>Height</td>
<td>Mid-parental height used to estimate expectation</td>
<td>55 - 81%</td>
</tr>
<tr>
<td>Multiple Sclerosis</td>
<td>None at present</td>
<td>64%</td>
</tr>
<tr>
<td>Migraine</td>
<td>Commonly assessed in the evaluation of headaches</td>
<td>53%</td>
</tr>
<tr>
<td>IQ</td>
<td>None at present</td>
<td>50%</td>
</tr>
<tr>
<td>Personality</td>
<td>None at present</td>
<td>50%</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>Family history guides genetic testing and counseling</td>
<td>25 - 56%</td>
</tr>
<tr>
<td>Coronary Heart Disease</td>
<td>Used to inform models of cardiac risk</td>
<td>49%</td>
</tr>
<tr>
<td>Type 2 Diabetes</td>
<td>None at present</td>
<td>26%</td>
</tr>
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</table>

Source: SNPedia
What are we trying to achieve?

- **Genetics** – strong contributor, causality can be established; 20,000 genes
  - Find genetic variants that “cause” ASD
  - Understand how they cause symptoms
  - Develop treatments

- **Environmental factors** – weaker contributor; causality is hard to establish; 100,000s of factors to assess
  - Find genetic variants that “cause” ASD
  - Improve health
  - Understand how they cause symptoms
  - Develop treatments
Environmental factors associated with ASD

- Maternal valproate use for epilepsy during pregnancy
  - 2-fold increase in risk Christensen et al. JAMA 2013
  - Does epilepsy carry genetic risk for ASD, or valproate carry environmental risk?
    - 1,623 mothers with epilepsy (22 had children with ASD, 1.4%)
    - 388 mothers on valproate (12 had children with ASD, 3.1%)
    - But was there an additional reason the mothers were on valproate vs. other treatments?
    - Causality is hard to establish

- Many other factors considered, but few thoroughly assessed:
  - Congenital infection, pollutants, pesticides, heavy metals

- Very hard to “guess” what to study without knowing the biology
The prevalence of ASD has risen over the last few decades.
Comparison of Growth Areas and Emissions, 1980-2015

Gross Domestic Product: 153%
Vehicle Miles Traveled: 106%
Population: 41%
Energy Consumption: 25%
CO₂ Emissions: 18%
Aggregate Emissions (Six Common Pollutants): -65%
There are many explanations for rising prevalence; simply looking for “new” factors is a risky strategy.

- Changing diagnostic methods
- Replacing other diagnoses
  - Developmental delay
  - Intellectual disability
- Increased surveillance
- Rising parental age
- New ways to find a partner
- Environmental factors
Genetic factors associated with ASD

- Several genetic syndromes have ASD as a feature
  - Fragile X, Rett’s, TSC1, TSC2, NF1, NF2, PTEN, CACNA1C
Example of a family with neurofibromatosis (NF1) and autosomal dominant dominant inheritance
Genetic factors associated with ASD

• Several genetic syndromes have ASD as a feature
  • Fragile X, Rett’s, TSC1, TSC2, NF1, NF2, PTEN, CACNA1C

• Using exome sequencing, ASD-associated de novo loss of function mutations are found in ~7% of children with ASD (>10-fold increase in risk)
Large cohorts, new technologies, and new statistical approaches have revolutionized genetics

~2,500 ASD families in Simons Simplex Collection

2,500 2,500

2,500 2,100

Microarray

Exome sequencing

SFARI
SIMONS FOUNDATION
AUTISM RESEARCH INITIATIVE

illumina®
The human genome has 3.2 billion base pairs and 3.3 million variants.

Common inherited
Fathers
Mothers
Children

Variants in the human genome

3,000,000
Common inherited
The human genome has 3.2 billion base pairs and 3.3 million variants.

- Common inherited variants:
  - Fathers
  - Mothers
  - Children
  - 3,000,000 Common inherited

- Rare inherited variants:
  - Fathers
  - Mothers
  - Children
  - 300,000 Rare inherited
The human genome has 3.2 billion base pairs and 3.3 million variants.
Rarer variants have greater potential to transmit ASD risk due to natural selection.
Missense variants alter one copy of a protein, LoFs disrupt one copy of a protein

**Missense variants**
- Gene from mother
- Gene from father
  - e.g. c.2558G>A
  - e.g. p.R853Q

**Protein truncating variant (PTV)**
- Gene from mother
- Gene from father
  - e.g. c.796G>T

PTV includes nonsense, splice site, and frameshift
An excess of *de novo* protein truncating variants in ASD cases shows they contribute to ASD risk

Sanders et al, Neuron, 2015
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• Several genetic syndromes have ASD as a feature
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• Using exome sequencing, ASD-associated *de novo* loss of function mutations are found in ~7% of children with ASD (>10-fold increase in risk)

• Using gene microarrays, ASD-associated *de novo* CNVs are found in ~3% of children with ASD (>10-fold increase in risk)
Missense variants alter one copy of a protein, LoFs disrupt one copy of a protein, copy number variants (CNVs) disrupt one copy of multiple proteins.

**Missense variants**

- Gene from mother
- Gene from father

  - e.g. c.2558G>A

  - Protein from mother
  - Protein from father

  - e.g. p.R853Q

**Protein truncating variant (PTV)**

- Gene from mother
- Gene from father

  - e.g. c.796G>T

  - Protein from mother

  - PTV includes nonsense, splice site, and frameshift

**Copy Number Variant (CNV)**

- e.g. 22q11.2
Individuals with a diagnosis of ASD have more de novo CNVs than unaffected controls.

1,991 ASD cases and 1,991 unaffected sibling controls

De novo mutation rate

Sanders et al. Neuron 2015
ASD is a frequently a combination of *de novo*, rare, and common variants.

Gaulger et al. 2014
Finding genes linked to ASD

Nav1.2 structure from AJ Campbell, Broad
To distinguish risk mutations from neutral mutations, we identify genes with clusters of mutations.
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Assessment of ~8,000 ASD cases has identified 65 ASD risk genes: a constellation of cryptic syndromes

Table 4. Integrating Small De Novo Deletions in TADA Identified 65 ASD Genes

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Genes with a small de novo deletion are in bold. FDR, false discovery rate.

Sanders et al. Neuron, 2015
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Progress in Understanding and Treating SCN2A-Mediated Disorders

SCN2A/Na\textsubscript{v} 1.2 initiates the action potential at the axon initial segment
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Along with ASD, genetic variants in SCN2A are associated with three disorders

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<th>Disorder name</th>
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<td>BIS</td>
<td>Y</td>
<td>N</td>
<td>N</td>
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<td>Y</td>
<td>Y</td>
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**Sodium conductance**

- *De novo* GoF missense: Increased
- *De novo* or inherited GoF missense: *SCN8A/Na\(_v\)1.6* excitability
- *De novo* truncating or *de novo* LoF missense: Reduced

**Disorder name**
- BIS: Benign (familial) infantile seizures
- IEE: Infantile epileptic encephalopathy
- ASD/ID: Autism Spectrum Disorder/ID
Loss of function in one copy of SCN2A makes excitatory neurons less excitable during development.

Before 1yr of age

- WT (+/+)
- PTV (+/-)
- D12N
- D82G (Vα)
- T1420M

After 1yr of age

- WT (+/+)
- PTV (+/-)
- D12N
- D82G (Vα)
- T1420M
Understanding the role of one gene provides some insight into when and how ASD occurs

• A 50% reduction in SCN2A function leads to ASD
• This implicates excitatory neurons in ASD
• It suggests that a reduction in neuron excitability may be involved
• It suggests that the “cause” of ASD occurs before 1yr of age
Since each gene has multiple functions, how do we know which functions lead to specific symptoms?

- Cerebellar dysfunction
- Increased excitability
- Back-propagation
- Reduced developmental excitability

SCN2A
Since each gene has multiple functions, how do we know which functions lead to specific symptoms?
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<th>SSC</th>
<th>ASC/ARRA</th>
<th>AGP</th>
</tr>
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<tbody>
<tr>
<td>N=2,591</td>
<td>N=1,445</td>
<td>N=2,096</td>
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Sanders et al. Neuron, 2015
The 65 ASD risk genes converge on chromatin and synaptic networks

Gene expression regulators

Neuronal communication

Sanders et al. Neuron, 2015
By considering the ASD-associated genes alongside other datasets, we can start to understand the 20,000ft view.
Considering multiple genes in together also provides insight into when and how ASD occurs

- Two main groups of ASD-associated genes involved in:
  - Gene expression regulation
  - Neuronal communication
- Implicates prefrontal cortex in mid-fetal development
- Enriched for excitatory neurons and striatal neurons
Summary

• There is strong evidence that genetic factors play a role in ASD
  • Twin studies
  • Family studies
  • Syndromes
  • De novo mutations
• De novo mutations have identified ~65 genes associated with ASD
• These genes are providing insight into ASD etiology
  • Gene expression and neuronal communication
  • Early development (<1yr)
  • Excitatory neurons in the cortex
• Environmental factors are likely to be involved
  • Harder to search for than genetic factors
Useful information sources of ASD information

- Spectrum: https://www.spectrumnews.org
- Autism Science Foundation: https://autismsciencefoundation.org
- International Society for Autism Research: https://www.autism-insar.org