New insights into genetic causes of autism and other childhood brain disorders
Autism and genetics

• Inherited, and non-inherited, genetic causes of autism spectrum disorders (ASD)
• Mechanisms of ASD genes
• New genetic variability of human brain cells
How does autism relate to other developmental brain disorders?

✓ **Features:**
  ✓ impaired social interactions
  ✓ impaired language
  ✓ repetitive or stereotypic behaviors
  ✓ typically diagnosed ages 3-4

✓ **Epidemiology**
  ✓ Autism: 1-2 in 1,000
  ✓ Autism spectrum: ~6 in 1,000

✓ **Comorbidities:**
  ✓ cognitive impairment in 50-60%
  ✓ regression in 10-25%
  ✓ seizures/epilepsy in 30%

Problems with the term ‘autism’

• An observational diagnosis
• Not always clinically stable
  – Children will move into and out of the diagnosis
• No clear correlation with genetics
• No clear correlation with prognosis
• No clear correlation to pathogenesis
• Substantial causative and treatment overlap with other conditions
Do genes play a role in autism?

Twin studies

✓ **Twin studies** argue that autism is one of the most highly heritable neuropsychiatric conditions

✓ Look at concordance rates of autism among **identical twins** (who share 100% of their same DNA)

✓ Compare this to concordance rates of autism among **non-identical twins** (who share 50% of their same DNA)
Do genes play a role in autism?  
Twin studies

<table>
<thead>
<tr>
<th></th>
<th>DZ = dizygotic</th>
<th>MZ = monozygotic</th>
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<tbody>
<tr>
<td>Non-identical</td>
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<td>twins share</td>
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<td>...50% of their</td>
<td>...100% of their genes +50%</td>
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<td>10-30% concordance for autism traits</td>
<td>70-90% concordance for autism traits</td>
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Compared to non-identical twins, identical twins share 50% more DNA, and are 3-9X more likely to share autistic traits

Genetic disease may be recessive, dominant, or complex

✓ Single-gene recessive:
  ✓ Inactivating both copies of a single gene are necessary to exhibit the trait
  ✓ Examples: sickle cell, cystic fibrosis, albinism, blue eyes

✓ Single-gene dominant:
  ✓ Inactivating one copy of a single gene is sufficient to exhibit the trait
  ✓ Examples: Huntington’s, neurofibromatosis, tuberous sclerosis

✓ Complex:
  ✓ No one gene is critical for the trait, but many genes contribute
  ✓ Examples: type 2 diabetes, hypertension, heart disease, height

X = gene mutation
| = chromosome
Inheritance patterns provide clues about whether a trait is recessive or dominant
...but not all genetic traits are inherited!

error in copying DNA causes a “de novo” mutation

Sometimes, genetic illness is heritable, but not inherited
What is the genetic architecture of autism spectrum disorders?

✓ Severe pediatric disease often reflects “rare” diseases
✓ Strong ‘negative evolutionary selection’ against pediatric disease
✓ “Complex traits”, like height, weight, etc., typically reflect effects of many common alleles

Role for ‘common alleles’ predisposing to ASD has not been widely replicated

Wang et al, 2009:
✓ 3,101 + 1,204 subjects
✓ one SNP associated with autism on 5p14.1
✓ rs4307059
✓ $p = 3.4 \times 10^{-8}$, odds ratio=1.19

Weiss, Arking, et al, 2009
✓ Weiss et al:
✓ 1,553 + 1,755 subjects
✓ one SNP associated with autism on 5p15
✓ rs10513025
✓ $(p = 9.58 \times 10^{-6}$, odds ratio=0.55)

Sample size too small?
Greater role for common variation in milder forms of autism?
“Copy number variants (CNV)” are an important cause of Autism

Small chromosome 14 deletion: only 1 copy of part of chr. 14
Array CGH: The Complete Process

Steps 1-3 Patient and control DNA are labeled with fluorescent dyes and applied to the microarray.

Step 4 Patient and control DNA compete to attach, or hybridize, to the microarray.

Step 5 The microarray scanner measures the fluorescent signals.

Step 6 Computer software analyzes the data and generates a plot.
Interpreting CNVs

✓ Is it a CNV known to cause autism? (16p11.2, 15q11, 22q11, etc)
✓ Do the patient’s parents have the same CNV?

✓ More likely to significant if it:
  ✓ disrupts the function of one or more genes
  ✓ is large (e.g., >1,000,000 bases)
  ✓ is not found in the “normal” population
  ✓ is not found in the patient’s parents, proving it is de novo

✓ Less likely to be significant if it:
  ✓ lands outside of a gene
  ✓ is small
  ✓ is present in normal controls and/or inherited from patient’s parents

✓ Not always a “smoking gun”
Copy number variants are more common in autistic children than in their normal sibs

Sanders et al, Neuron, 2011
Certain CNVs are seen repeatedly in ASD

- 7q11.23
- 16p11.2
- 22q11.2
- 15q11.2-13
- 1q21.1
- 22qter
- NRXN1
Autism CNV’s can cause other conditions

- 16p11.2 deletion/duplication
  - also associated with Schizophrenia, ADHD, ID, epilepsy, normal cognition, obesity (deletion), anorexia (duplication)
- NRXN1 deletion
  - also associated with Schizophrenia, ID, ADHD, epilepsy
  - Recessive mutations associated with severe MR, dysmorphic features
- 22q11.2 deletion/duplication
  - Schizophrenia, ID, epilepsy
- 17q12 deletion
  - Schizophrenia, ID, epilepsy
- 15q13.2-15q13.3
  - Schizophrenia, ID, epilepsy
- The same holds for known syndromic autism genes

Why many genes, one disorder, as well as one gene, many disorders??
Many different mutations can cause autism

Clinically recognizable syndromes
✓ Fragile X (FMR1), Rett’s (MeCP2), Tuberous Sclerosis (TSC1/TSC2), Angelman (UBE3A), certain metabolic disorders

Recurrent chromosomal abnormalities
✓ 16p11.2, 15q11-13, 22q11

Other monogenic disorders
✓ Neurexin 1, NLGN3/4, SHANK3, CNTNAP2, ...

Private de novo CNVs

Unknown

adapted from data from David Miller, Autism Consortium
Rapid decrease in DNA sequencing costs has fueled a revolution

<table>
<thead>
<tr>
<th>Year</th>
<th>Event</th>
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<tbody>
<tr>
<td>2000</td>
<td>Cost of a human genome in 2009: $20,000</td>
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<tr>
<td>2002</td>
<td>Cost of a human genome in 2013: $4,000</td>
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<tr>
<td>2004</td>
<td>Cost of whole exome in 2013: $1,000</td>
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**Cost of DNA sequencing (per million bases)**

- **1998**: $10,000
- **2000**: $1,000
- **2002**: $0.10
- **2004**: $0.01
- **2006**: $0.01
- **2008**: $0.01
- **2010**: $0.01
- **2012**: $0.01

Data: Chad Nusbaum
What is “whole exome” sequence?

Focuses on the 1% of the genome that codes for proteins

Based on “purifying” the DNA of exomes from the noncoding “junk” DNA

Dense coverage of exons

Tim Yu
How do we interpret whole exome or whole genome sequence?

• How many variants (i.e., differences from the reference sequence) are there in one person’s genome?
  – average of 35,000 variants per exome sequence
  – average of 3,500,000 variants per genome

• How do we interpret these variants?
How to sort through 3.5M variants?

- Is the DNA sequence reliable?
- Is the DNA change novel?
- Is the DNA change predicted to alter function?
- Does the DNA change segregate with disease?

Candidate mutations

Databases of common variation:
dbSNP, 1000Genomes project

Databases of disease
HGMD, etc…

Prediction of mutation effects
Coding and splicing changes…
Amino acid conservation…

Progressive filtering is necessary to discard artifacts, filter out benign variants, and identify pathogenic lesions.
If *de novo* CNV’s are important, is there a role for *de novo* point mutations in autism?

- De novo mutations occur about 1 per exome per generation
- Overall rates of de novo mutation are comparable in cases versus controls
- Types of mutation differ somewhat

Certain de novo point mutations are more common in autistic children than normals

Exome sequencing in sporadic autism spectrum disorders identifies severe de novo mutations

Brian J O’Roak\textsuperscript{1}, Pelagia Deriziotis\textsuperscript{2}, Choli Lee\textsuperscript{1}, Laura Vives\textsuperscript{1}, Jerrold J Schwartz\textsuperscript{1}, Santhosh Girirajan\textsuperscript{1}, Emre Karakoc\textsuperscript{1}, Alexandra P MacKenzie\textsuperscript{1}, Sarah B Ng\textsuperscript{1}, Carl Baker\textsuperscript{1}, Mark J Rieder\textsuperscript{1}, Deborah A Nickerson\textsuperscript{1}, Raahel Bernier\textsuperscript{1}, Simon E Fisher\textsuperscript{3,4}, Iav Shendure\textsuperscript{1} & Evan E Eichler\textsuperscript{1,5}

Sporadic autism exomes reveal a highly interconnected protein network of de novo mutations

Brian J. O’Roak\textsuperscript{1}, Laura Vives\textsuperscript{1}, Santhosh Girirajan\textsuperscript{1}, Emre Karakoc\textsuperscript{1}, Niklas Krumm\textsuperscript{1}, Bradley P. Coe\textsuperscript{1}, Roie Levy\textsuperscript{1}, Arthur Ko\textsuperscript{1}, Choli Lee\textsuperscript{1}, Joshua D. Smith\textsuperscript{1}, Emily H. Turner\textsuperscript{1}, Ian B. Stanaway\textsuperscript{1}, Benjamin Verno\textsuperscript{1}, Malika Malig\textsuperscript{1}, Carl Baker\textsuperscript{1}, Beau Reilly\textsuperscript{2}, Joshua M. Alcoy\textsuperscript{3}, Bhiman Boreinstein\textsuperscript{2,3,4}, Mark J. Rieder\textsuperscript{1}, Deborah A. Nickerson\textsuperscript{1}, Raphael Bernier\textsuperscript{1}, Jay Shendure\textsuperscript{1} & Evan E. Eichler\textsuperscript{1,5}

De novo mutations revealed by whole-exome sequencing are strongly associated with autism

Stephan J. Sanders\textsuperscript{1}, Michael T. Muntha\textsuperscript{1}, Abha R. Gupta\textsuperscript{4}, John D. Murdoch\textsuperscript{4}, Melanie J. Rubez\textsuperscript{1}, A. Jeremy Wilsey\textsuperscript{4}, A. Gulhan Ercan-Sencicek\textsuperscript{4}, Nicholas M. DiLullo\textsuperscript{4}, Neelroop N. Parkhshkale\textsuperscript{3}, Jason L. Stein\textsuperscript{4}, Michael F. Walker\textsuperscript{1}, Gordon T. Ober\textsuperscript{1}, Nicole A. Teran\textsuperscript{1}, Youun Song\textsuperscript{2}, Paul El-Fishawy\textsuperscript{2}, Ryan C. Muntha\textsuperscript{1}, Murun Choi\textsuperscript{1}, John D. Overton\textsuperscript{1}, Robert D. Bjornson\textsuperscript{2}, Nicholas J. Carriero\textsuperscript{2}, Kyle A. Meyer\textsuperscript{2}, Kaya Bilgic\textsuperscript{2}, Shrikant M. Mane\textsuperscript{2}, Nemad Susan\textsuperscript{2}, Richard P. Lipton\textsuperscript{4}, Murat Gunel\textsuperscript{1}, Kathryn Roeder\textsuperscript{4,5}, Daniel H. Geschwind\textsuperscript{1}, Bernie Devlin\textsuperscript{5} & Matthew W. State\textsuperscript{1}

Patterns and rates of exonic de novo mutations in autism spectrum disorders

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- Slight excess of de novo mutations n cases (Rouleau et al, 2010)
- Cases have largest excess of the most damaging mutations
- Tremendous heterogeneity of the genes that are mutated
Sequencing thousands (2,246) of children identifies genes recurrently mutated in autism

Multiplex Targeted Sequencing Identifies Recurrently Mutated Genes in Autism Spectrum Disorders

Many autism mutations show frequent *de novo* mutation

All of these mutations are typically *de novo*

Recessive mutations

Syndromic single gene disorders

Nonsyndromic single gene disorders

Recurrent chromosomal abnormalities

Non-recurrent, ‘private’ CNVs

*De novo point mutations*

Neele et al, 2012
Sanders et al, 2012

De novo mutations do not account for the heritability of autism--what are the inherited causes?
Suggested explanations of heritable autism

Dominant-like transmission from a normal or mildly affected parent

parent carries a de novo mutation
Mutations can occur in any dividing cell
Suggested explanations of heritable autism II.

Autosomal recessive
How can we efficiently identify ‘two hit’ (i.e., recessive) gene mutations?
Consanguinity raises the risk of neurodevelopmental illness

Cousin marriages

Birth defects
& mental retardation

March of Dimes Annual Report, 2008
Homozygosity Mapping Collaborative for Autism (HMCA)

✓ Homozygosity Mapping Collaborative for Autism (HMCA)
  ✓ Tim Yu, Maria Chahrour, Eric Morrow, Seung-Yun Yoo, Sean Hill, Jen Partlow, Brenda Barry, Muna al-Saffar

✓ Referral centers:
  ✓ Saudi Arabia, Kuwait, Turkey, UAE, Egypt, Oman, Jordan, Pakistan

✓ >210 autism pedigrees with parental consanguinity
  ✓ 39 multiplex
  ✓ 177 simplex

✓ Genotyped ≥500K SNP’s

Research Support:
Simons Foundation, NLM Family Foundation, HHMI, NIMH
Known autism genes often relate to the synapse.
Abnormal spine shape in intellectual disability

Huttenlocher, 1974

Purpura, 1974
Known autism genes often relate to the synapse.
Are there other types of autism mutations?

De novo point mutations

- Neele et al, 2012
- Sanders et al, 2012
Mutations can occur in any dividing cell

Alberts, Mol Biol of the Cell
Hemimegalencephaly (abnormal enlargement of one cerebral hemisphere) reflects somatic, “brain-only” mutation.
Hemimegalencephaly defined as abnormal, highly epileptic, enlargement of half of the cerebral cortex

Ann Poduri, Gilad Evrony, Xuyu Cai
Mosaic, “clonal” mutations (limited to brain) in hemimegalencephaly

- Spontaneous chromosome 1q duplication (in brain only)
- Spontaneous AKT3 activating point mutation (in brain only)

$\text{AKT3 } \text{c.49G}\rightarrow\text{A} , \text{E17K}$

E17K mutation is activating (Davies et al., Br J Cancer, 2008)

Ann Poduri
Proteus syndrome also caused by mosaic mutations (in \textit{AKT1}) in some cells but not others - the mutation was seen in 3.6-51\% of cells is various lesions throughout the body.
Single cell sequencing shows the Hemimegalencephaly-causing AKT3 E17K mutation in neurons and non-neuronal cells

- Both gray and white matter affected radiographically
- Mutant cells cover much of the hemisphere
- Both neurons and glia carry the mutation
  - 30–40% of neurons, ≈30% of non-neuronal cells contain the mutation
- despite prevalence of mutation over the entire hemisphere
Hemimegalencephaly is a spontaneous mutation limited to brain

zygote
What else can be attributed to somatic mosaicism?

— Smaller focal malformations?
— Broader category of focal epilepsy without visible lesions?
— Beyond epilepsy—intellectual disability, autism

Hemimegalencephaly

Focal cortical dysplasia

Normal cortex
All autism mutations--except recessive ones--show frequent *de novo* mutation: can some occur in brain-only?

Most of these mutations, if mosaic in brain, would not be evident radiographically.

- Syndromic single gene disorders
- Nonsyndromic single gene disorders
- Recurrent chromosomal abnormalities
- Non-recurrent, ‘private’ CNVs

*De novo point mutations*

- Neele et al, 2012
- Sanders et al, 2012
ASD is clinically and causally highly heterogeneous

• Chromosomal rearrangements, 0-5%
• Smaller, *de novo* CNV’s about 5%
• *De novo* ‘point’ mutations, 5-15% (?)
• Inherited recessive mutations, ≈5%
• Genes implicate synapses
• Many different genetic causes look very similar
  — extreme genetic heterogeneity
• Genetics is not the only cause
Causes of Autism can cause other conditions

- 16p11.2 deletion/duplication
  - also associated with Schizophrenia, ADHD, ID, epilepsy, normal cognition, obesity (deletion), anorexia (duplication)
- NRXN1 deletion
  - also associated with Schizophrenia, ID, ADHD, epilepsy
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  - Schizophrenia, ID, epilepsy
- The same holds for known syndromic autism genes

Why many genes, one disorder, as well as one gene, many disorders??
Developmental brain disorders

• Fact: mutations seem not to directly cause a specific disease phenotype
  – no specific “autism” mutations that cause autism and nothing else
  – no specific “schizophrenia” mutations so far

• Fact: mutations impair the normal plasticity, “learning,” of brain

• Inference: manifestations of this abnormal plasticity appear to vary in different people
  – normal development
  – autism
  – normal development, then schizophrenia
  – obesity of anorexia

• What are the features that modulate this normal and abnormal plasticity?
  – other genetic differences (‘common alleles’; ‘genetic background’)
  – “environment”
  – teaching
  – ‘epigenetics’
  – random differences in the way the brain wires itself?