An Overview of the Neurobiology of Autism

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What is the Cause of Autism?

• Background
• Genetic Contributions
• Where is the Lesion?
• Neurochemistry
• Immune Factors

Diverse Autistic Symptoms Makes Search for Biomarkers Difficult

Core Symptoms
• Poor Eye Contact
• Impaired Social Reciprocity
• Impaired Communication
• Echolalia
• Need for Sameness
• Stereotypies

Associated Symptoms
• ADHD symptoms
• Irritability
• Anxiety
• Insomnia
• Intellectual Disability
Heterogeneity Complicates Research

• Range of Autistic Severity
• Range of Intellectual Ability
• Possible differences among phenotypic subtypes
  – Autistic disorder (autism)
  – Asperger’s disorder
  – PDD NOS (atypical autism)
• Might heterogeneity lead to missed findings?

Autistic Regression

• 25-30% of children with autism
• Loss of language and/or social at 15-30 months
• Doesn’t necessarily imply environmental “hit”
• Possible links
  – Gastrointestinal symptoms
  – Autoimmunity
  – Sleep
  – Epileptiform activity on EEG

Werner & Dawson (2005) Arch Gen Psychiatry
Autism is Genetic

- Autism is one of the most heritable disorders in neuropsychiatry
- Twin Studies: monozygotic concordance rates as high as 60 to 90%
  - Compared to 5% risk in dizygotic twins/siblings
  - Compared to 1.0% in general population
- Family Studies: increased risk of social and communication problems in family members (broader autism phenotype)


Genetic Syndromes Associated with Autism

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Genetic 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>
</tr>
</thead>
<tbody>
<tr>
<td>15q duplication—Angelman syndrome</td>
<td>UBE3A (and others)</td>
<td>&gt;10%</td>
<td>1–2%</td>
</tr>
<tr>
<td>16p11 deletion</td>
<td>Unknown</td>
<td>High</td>
<td>-1%</td>
</tr>
<tr>
<td>22q deletion</td>
<td>SHANK1</td>
<td>High</td>
<td>1%</td>
</tr>
<tr>
<td>Cortical dysplasia-focal epilepsy</td>
<td>CNTNAP2</td>
<td>~70%</td>
<td>Rare</td>
</tr>
<tr>
<td>Fragile X syndrome</td>
<td>FMR1</td>
<td>25% of males; 6% of females</td>
<td>1–2%</td>
</tr>
<tr>
<td>Joubert syndrome</td>
<td>Several loci</td>
<td>25%</td>
<td>Rare</td>
</tr>
<tr>
<td>Potocki–Lupski syndrome</td>
<td>Chromosome position 17p11</td>
<td>~10%</td>
<td>Unknown</td>
</tr>
<tr>
<td>Smith–Lemli–Opitz syndrome</td>
<td>DHLA1</td>
<td>50%</td>
<td>Rare</td>
</tr>
<tr>
<td>Rett syndrome</td>
<td>MECP2</td>
<td>All individuals have Rett syndrome</td>
<td>~0.5%</td>
</tr>
<tr>
<td>Timothy syndrome</td>
<td>CACNA1C</td>
<td>60–80%</td>
<td>Unknown</td>
</tr>
<tr>
<td>Tuberosis sclerosis</td>
<td>TSC1 and TSC2</td>
<td>20%</td>
<td>-1%</td>
</tr>
</tbody>
</table>

Abrahams & Geschwind (1997) Am J Psychiatry
Copy Number Variation (CNV)

- The deletion or insertion of a DNA segment
- More or less than 2 copies of a particular gene may result
- Can be inherited or de novo
- Distinct from concept of single nucleotide polymorphisms (SNPs)
- Can identify rare variants that through CNV either
  - greatly modify risk of autism
  - cause specific syndrome ("an autism")


CNV Findings in Autism

- Comparative genomic hybridization (CGH) on subjects with autism and controls
- Confirmed de novo CNVs
  - 10% from simplex families
  - 3% from multiplex families
  - 1% from controls
- Microdeletions of 16p11
  - May occur in 1% of persons with autism
  - Exceedingly rare in controls
  - ? Specificity since also found in intellectual disability without autism

Where is the Lesion?

Meta-Analysis of Head Circumference and MRI Studies
Autism-Neuroimaging

- Increase in cerebral grey and white matter (9-15% increase in children ages 2 and 3 years)
- Inconsistent results regarding localization
- Possible white matter abnormalities using diffusion tensor imaging (DTI)

Courchesne et al (2001) *Neurol*

Autism and the Cerebellum

- Cerebellum enlarged in 2- and 3-year olds, but decreased in older children
- 30% reduction in number of purkinje cells in postmortem cerebellum (ages 4-67 y)
- Conflicting views regarding contribution of neurodegeneration and neuroimmune factors in loss of purkinje cells

Courchesne et al (2001) *Neurol*
Autism and Temporal Lobes

• Increase in autism in tuberous sclerosis when tubers are present in temporal lobes
• Multiple case reports of acquired autism secondary to herpes simplex encephalitis which primarily affects temporal lobes


Autism and Fusiform Gyrus

• Fusiform Face Area is hypoactive in fMRI studies involving face identification

fMRI Studies

• Amygdala hypoactive in fMRI studies involving social perception and cognition


Mirror Neurons and Autism

• Neurons that activate while doing or observing doing
• Studied in macaque monkeys (inferior frontal gyrus and inferior parietal lobule)
• EEG differences (lack of mu suppression) over sensorimotor cortex while watching in autism
• Decreased inferior frontal gyrus activity by fMRI during observing and imitating facial expressions

Neurochemistry of Autism

• 5-HT
• GLU
• GABA
• OXYTOCIN
• MELATONIN

Serotonin
(5-Hydroxytryptamine; 5-HT)

• 5-HT neurons widely distributed in brain
• 5-HT one of the earliest systems to develop
• Turnover of 5-HT highest in immature brain
• Directs proliferation and maturation of brain
Blood measurements

- Consistent finding of hyperserotonemia in approximately 1/3 of prepubertal autistic children (Schain & Freedman, 1961)
  - Age and race factors are important
- Replicated in numerous studies
- Meaning of the result remains uncertain - ? Compensatory change related to reduced brain 5-HT function; abnormal maturation
5-HT challenge studies

- Acute Tryptophan Depletion
- TRYP is essential AA for 5-HT production
- Administration of TRYP-free AA mixture results in significant reduction of 5-HT in plasma and 5-HIAA in CSF in 5 hours
- 11/17 adults with autism worse with TRYP depletion vs. 0/17 worse with sham depletion

McDougle et al (1996) Arch Gen Psychiatry

Imaging 5-HT

- PET Studies have demonstrated age-related differences in 5-HT synthesis
  - Controls: 5-HT synthesis 2X higher in preschool children compared to adults with subsequent decline past age 5 years
  - Autism: 5-HT synthesis gradually increases throughout childhood (2-15 years) and only reaches 1.5X adult values

Serotonin Transporter Gene (SLC6A4)

- Encodes 5-HT Transporter
- Conflicting findings regarding association with autism
  - Long variant
  - Short variant
  - No association
- Possible association between autism subtypes (rigid compulsive behavior)
- Possible association with cortical gray matter volume

Glutamate and GABA

Glutamate
Excitatory neurotransmitter

GABA
Inhibitory neurotransmitter

GAD
Glutamate Abnormalities

- Elevated peripheral glutamate levels in majority of studies
- Elevated glutamate/glutamine in amygdala-hippocampal region by MRS


GRIK2

- Glutamate Receptor 6 (GRIK2) shows linkage in many, but not all studies
- Homozygous mutation linked to autosomal recessive mental retardation in one family

GABA Abnormalities

- Decreased number of GABA receptors in postmortem hippocampus
- Decreased GABA subunit expression in cerebellum, superior frontal cortex, and parietal cortex
- GABA-A Receptor Beta 3 (GABRB3) gene (encodes subunit of GABA-A receptor) linked to autism in some, but not all studies
- GABRB3 Found in 15q11-13 region
  - Autism (linkage)
  - Prader-Willi Syndrome (paternal copy deletion)
  - Angelman Syndrome (maternal copy deletion)
  - Isodicentric chromosome 15 (duplicated genetic material)


Glutamic Acid Decarboxylase (GAD)

- GAD polymorphisms not associated with autism
- Decreased GAD mRNA and GAD protein levels in postmortem cerebellum and parietal cortex

Oxytocin

- Importance in formation of monogamous pair bonds (Voles)
- Importance in maternal attachment
- Increases trust in others
- Decreases amygdala activation and fear response

Nair & Young (2006) Physiology

Oxytocin in Autism

- Plasma oxytocin levels low in children with autism
- Intravenous (IV) oxytocin led to reductions in repetitive behavior in adults with autism
- IV oxytocin led to possible improvement in affective speech comprehension in adults with autism
- Intranasal oxytocin in autism studies have begun

Melatonin in Autism

- Synthesized in the pineal gland
- $5\text{-HT} \rightarrow \text{N-acetyl}(5\text{-HT}) \rightarrow \text{Melatonin}$
- Decreased melatonin secretion during dark phase
- Decreased urinary 6-sulphatoxymelatonin
- Widespread clinical use of melatonin for sleep disturbance
- Ongoing clinical trials of melatonin for insomnia in autism

Immune Factors in Autism

- Inconsistent findings regarding exposure to various viruses as risk factor for autism
- No vaccine-autism link
  - No link to MMR vaccine
  - No link to Thimerosal
  - Evidence against alternative vaccine theories
    - Evidence against theory that vaccines overwhelm or weaken immune system

Neuroimmune Studies

- Neuroinflammation and neuroglial activation in postmortem samples
- Inconsistent findings regarding antibodies to specific brain proteins


Mean Number of Family Members with Autoimmune Disease per Family

PDD > Autoimmune (p=.03)
PDD > Healthy (p=.000003)
### Number of Families in Each Group with a Specific Autoimmune Disease

<table>
<thead>
<tr>
<th></th>
<th>PDD (n=101)</th>
<th>Autoimmune (n=101)</th>
<th>Healthy (n=101)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rheumatic Fever</td>
<td>23</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td>Hypothyroidism/Hashimoto’s</td>
<td>36</td>
<td>11</td>
<td>14</td>
</tr>
</tbody>
</table>

PDD > Controls (p < .05)


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### NEOPTERIN

- Metabolite produced in high amounts by monocytes and macrophages
- Marker for cell-mediated immune activation
- Blood and urine levels are increased in:
  - Autoimmune disease
  - Infectious disease
  - Cancer

![Diagram of Neopterin Metabolism](image)

- IFN
- GTP
- (GTPCH)
- Neopterin
- Monocyte/Macrophage
Increased Monocyte Count in Children with Autism


p = .049

Increased Plasma Neopterin Levels by ELISA in Children with Autism


p<0.00008
What is the Underlying Cause of Autism?

• Autism is clearly genetic
  – Multiple genes acting together
  – Epigenetics (changes in gene expression)
  – Increased identification of specific genetic syndrome associated with autism

• Environmental contributions may also be important
  – Complex interactions with genes and neurodevelopment

• fMRI reveals abnormalities in fusiform face area and amygdala

What is the Underlying Cause of Autism?

• 5-HT abnormalities
  – Peripheral blood
  – PET
  – Challenge Studies

• Emerging evidence of glutamatergic and oxytocin abnormalities

• Immune system activation may also play a role
Lack of Singular Neurobiology

- Heterogeneity of “the autisms”
- Complexity of neurodevelopmental disorders
- Innate challenges of research
  - Low prevalence of ASDs
  - Age of onset
  - Communication and cognitive limitations
  - Importance of choosing suitable controls to account for specificity of findings

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http://www.massgeneral.org/children/services/treatmentprograms
(781)-860-1700

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