Autism: Diagnosis and Underlying Neurobiology

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Guiding Questions

• Why are prevalence estimates of autism spectrum disorders (ASDs) much higher than 20 years ago?
• What is important in terms of screening, diagnosis, and evaluating the individual with suspected ASD?
• What is the underlying cause of ASD?

Autism Spectrum Disorders (ASDs)

• Roughly equivalent to DSM-IV pervasive developmental disorders (PDDs)
  - Autistic disorder (autism)
  - Asperger's disorder
  - Pervasive developmental disorder not otherwise specified (PDD NOS)
  - Childhood disintegrative disorder
  - Rett's disorder
• Sometimes excludes the latter two PDDs which are rare and often excluded in studies

Autism Affects Three Core Domains

• Social
  - Nonverbal behavior
  - Relationships
  - Seeking interaction
  - Reciprocal interaction
• Communication
  - Repetitive
• Behavior
  - Obsessive
  - Inflexible
  - Repetitive

Asperger’s Disorder

• Social impairment and narrow interests similar to autism
• Lack history of significant language or cognitive delay
• Verbal skills often a strength
• Often identified later (e.g., school age)

Childhood Disintegrative Disorder

• Marked regression occurring after age of two
• Often associated with regression in other areas (e.g., toileting, cognition)
• Not diagnosed if child meets criteria for autism (i.e., onset prior to age three)
• Rare; not well-studied
Rett’s Disorder
- Occurs primarily in females (in contrast to autism); Rare
- Normal early development thru 5 months
- Followed by:
  - deceleration of head growth
  - loss of hand skills
  - social impairment
  - poorly coordinated gait/trunk movements
  - severely impaired language
- MECP2 mutations on X chromosome discovered in 1999

PDD NOS
- A disorder characterized by a core problem with social relatedness and either communication impairment or narrow interests/repetitive behaviors, but not meeting specific DSM-IV criteria for another PDD
- “Atypical autism”

Autism Prevalence (1943)
- Leo Kanner, a child psychiatrist at Johns Hopkins describes 11 children
- All had an “innate inability to form the usual, biologically provided affective contact with people”
- Thought to be rare

Early PDD Prevalence Studies
- Review of studies 1966-1998
  - 0.052 % for autism (1 in 2000)
  - 0.072 % for autism (1 in 1400)
  - 0.187 % for all PDDs (1 in 500)
- U.K. Study in 2001
  - 0.168 % for autism (1 in 500)
  - 0.626 % for all PDDs (1 in 160)


Early CDC Case Finding Study
- Active case ascertainment through CDC program in 5 Atlanta counties
- Children aged 3-10 years
- Review of records only
- 0.34 % rate of ASDs (1 in 300)
  - 40% identified only in school records
  - School records most important in children of black mothers, younger mothers, and non-HS graduates


Pervasive developmental disorders in preschool children: confirmation of high prevalence
- Replication of study done 4 years earlier that found rates of 1 in 500 for autism and 1 in 160 for all PDDs.
- Screened 10,903 children (ages 4-6) in U.K.
- 659 had developmental or behavioral concern and had stepwise evaluation as indicated beginning with consultation by a developmental specialist and eventually autism-specific diagnostic interviews in some (n=83)

Pervasive developmental disorders in preschool children: confirmation of high prevalence

- 64 confirmed cases (1 in 170)
  - 0.248 % prevalence for PDD NOS
  - 0.22 % prevalence for autism
  - 0.11 % prevalence for Aspergers
  - 0.009 % prevalence for CDD
- Similar prevalence to study done 3-4 years ago suggesting stable incidence over this short time span
- Only 30% of all PDD had MR; 67 % of group with autism had MR


More Recent ASD Prevalence Estimates

- CDC National Study
  - 1 in 150
  - Case enumeration only (i.e., no direct examination)
- National Children’s Health Survey Study
  - 1 in 100 (1 in 91)
  - Based on telephone survey of parents only
  - 40% lose diagnosis
  - Number may be confirmed by CDC Study

The Debate and Implications

- The increase in diagnosis of autism is due to increased awareness, better recognition, diagnosis of milder or atypical cases, and other social pressures
  - Increased funding for research into causes and treatment
  - Increased funding for treatment
- There is a real increase in the incidence of autism
  - All of the above plus proportionally more research into environmental causes

Autism: Red Flags

- No babbling or pointing or other gesture by 12 months
- No single words by 16 months
- No 2-word spontaneous (not echolalic) phrases by 24 months
- Loss of language or social skills at any age

Filipek et al 2000

Pediatricians Urge Autism Screening

- New recommendation that all children be screened for autism at 18 and 24 months
- Autism Surveillance
- Autism Screening

Johnston, Myers, and the Council on Children With Disabilities 2007

Autism Surveillance
Autism Screening

• Does your child take an interest in other children?
• Does your child ever use his/her index finger to point, to indicate interest in something?
• Does your child ever bring objects over to you (parent) to show you something?
• Does your child imitate you? (e.g., you make a face—will your child imitate it?)
• Does your child respond to his/her name when you call?
• If you point at a toy across the room, does your child look at it?

Robins et al 2001

M-CHAT Critical Items

• Does your child take an interest in other children?
• Does your child ever use his/her index finger to point, to indicate interest in something?
• Does your child ever bring objects over to you (parent) to show you something?
• Does your child imitate you? (e.g., you make a face—will your child imitate it?)
• Does your child respond to his/her name when you call?
• If you point at a toy across the room, does your child look at it?

M-CHAT scoring

• For ages 18-60 months
• 23 items answered yes/no
• Each item scored as pass/fail
• Failing two critical items OR failing three items should prompt referral

Early Signs: Internet Resources

• First Signs
  - http://www.firstsigns.org
  - Downloadable screening instruments (e.g., M-CHAT)
• Autism Speaks
  - http://www.autismspeaks.org
  - Video Glossary of autism spectrum disorder (ASD) symptoms
  - Links to Research opportunities
  - Contribute to Research

Autism: Evaluation

• Evaluation by experienced clinician
• Use of certain psychological instruments can be helpful if available
  - Autism Diagnostic Interview-Revised (2 hours)
  - Autism Diagnostic Observation Schedule (1 hour)
  - Social Communication Questionnaire (10 minutes)
  - Social Responsiveness Scale (15 minutes)
• Medical evaluation to consider further work-up for other conditions that may mimic or co-occur with autism

Internet Resources

• http://handsinautism.org
  - Choose “physicians and nurses” link
  - http://handsinautism.org/qstart-healthcare.html
  - Many tools and resources
• Comprehensive Review of Evaluation (2007)
  - http://pediatrics.aappublications.org/cgi/content/full/120/5/1183
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


Autism is Genetic

- Autism is one of the most heritable disorders in psychiatry
- Twin Studies: monozygotic concordance rates as high as 90%
  - Compared to 5% risk in dizygotic twins/siblings
  - Compared to 0.6% 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 defect</th>
<th>Proportion of patients with the syndrome that have an ASD</th>
<th>Proportion of patients with an ASD that have the syndromes</th>
</tr>
</thead>
<tbody>
<tr>
<td>22q deletion</td>
<td>Angelman syndrome</td>
<td>UBE3A (and others)</td>
<td>&gt;90%</td>
</tr>
<tr>
<td>15p deletion</td>
<td>Autism</td>
<td>Unspecified</td>
<td>5%</td>
</tr>
<tr>
<td>1q42 deletion</td>
<td>Williams syndrome</td>
<td>Nebulin</td>
<td>High</td>
</tr>
<tr>
<td>15q13</td>
<td>Sodium channel</td>
<td>Low</td>
<td>10%</td>
</tr>
<tr>
<td>Fragile X syndrome</td>
<td>FXD</td>
<td>Uniparental disomy</td>
<td>3%</td>
</tr>
<tr>
<td>Klinefelter syndrome</td>
<td>Substance</td>
<td>Unspecified</td>
<td>1-50%</td>
</tr>
<tr>
<td>Smith-Lemli-Opitz syndrome</td>
<td>DMR</td>
<td>Unspecified</td>
<td>1-50%</td>
</tr>
<tr>
<td>Patau syndrome</td>
<td>Unspecified</td>
<td>Unspecified</td>
<td>Unspecified</td>
</tr>
<tr>
<td>Klinefelter syndrome</td>
<td>MECP2</td>
<td>Unspecified</td>
<td>1-50%</td>
</tr>
<tr>
<td>Timothy syndrome</td>
<td>CASPA2</td>
<td>Unspecified</td>
<td>1-50%</td>
</tr>
</tbody>
</table>

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) or mutation within gene
- 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


Recent Genome-wide CNV Study

- Independent ASD samples of 859 and 1336, respectively, and controls
- CNV deletions or duplications replicated
  - UBE3A 15q11-13
  - NRXN1
  - CNTN4
  - 22q11.21
- CNVs not replicated
  - 16p11.2
  - AUTS2
  - NLGN3
  - SHANK3

Glessner et al Nature 2009 Epub

Additional CNV Findings Implicate Two Pathways

- Ubiquitin Pathway
  - UBE3A, PARK2, RFWD2, FBXO40
  - Post-translational modification
  - Regulation of synaptic attributes
    - Neurotransmitter release
    - Synaptic vesicle recycling
    - Dynamic changes in dendritic spines and postsynaptic density
- Neuronal Cell-Adhesion Molecules
  - NRXN1, CNTN4
  - CNS development
  - Axonal guidance
  - Synaptic formation and plasticity
  - Neuronal-glial interaction

Glessner et al Nature 2009 Epub

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


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

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-HT $\rightarrow$ N-acetyl(5-HT) $\rightarrow$ 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
- Clinical observations
- Inconsistent findings regarding exposure to various viruses as risk factor for autism
- No vaccine-autism link
  - No link to MMR or Thimerosol
  - 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


Number of Families in Each Group with a Specific Autoimmune Disease

<table>
<thead>
<tr>
<th>Autoimmune Disease</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></td>
<td>23</td>
<td>10</td>
</tr>
<tr>
<td>Hypothyroidism/Hashimoto's</td>
<td></td>
<td>36</td>
<td>11</td>
</tr>
</tbody>
</table>


PDD > Controls (p < .05)
Oral Human Immunoglobulin (IGOH)

- Possible increase in gastrointestinal symptoms in children with autism
- Oral immunoglobulin possibly effective in certain gastrointestinal infections
- Open-label study in autism found improvement in autistic and gastrointestinal symptoms


IGOH Study in Pediatric Autism (n=125)


What is the Underlying Cause of Autism?

- Autism is clearly genetic
  - ? Multiple common gene variants acting together in individual to increase risk of autism
  - ? Multiple rare genetic mutations that each lead to syndrome of autism
- Environmental contributions are also likely important
  - Complex interactions with genes and neurodevelopment

What is the Underlying Cause of Autism?

- Multiple brain areas implicated
- Emerging evidence of underconnectivity between various cortical and subcortical areas
- Emerging evidence of disturbance in synaptic development and function
- Neurochemical abnormalities (5-HT, Glutamate/GABA, Oxytocin)
- Immune system activation may also play a role

Lack of Singular Neurobiology

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

Acknowledgments

- U. S. National Institute of Mental Health
- National Alliance for Research on Schizophrenia and Depression
- Autism Speaks
Christian Sarkine Autism Treatment Center

- Christopher J. McDougle
- David J. Posey
- Naomi B. Swiezy
- Kimberly A. Stigler
- Noha Minshawi
- Craig A. Erickson
- Iryna Ashby
- Heather Coates
- J.T. Diener
- Doug Gaebler
- Cheryl Habenicht
- Elizabeth Kiefert
- Arlene Kohn
- Patricia Korzekwa
- Lauren Mathieu-Frasier
- Jennifer Mullet
- Stacie Paudel
- Melodie Rose
- Amy Tennant

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Autism Resources

- Helping Answer Needs by Developing Specialists (HANDS) in Autism program
- http://handsinautism.org
  - Choose “physicians and nurses” link http://handsinautism.org/qstart-healthcare.html
  - Many tools and resources (including attached)