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Autism Research: Prenatal Beginnings to Early Childhood Clinical Outcome

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APA Hotel Woodbridge, Iselin, NJ

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WHAT ARE THE EARLY BRAIN BASES OF AUTISM?
Early Brain Overgrowth in Majority of Autism Toddlers
Courchesne et al., Neurology 2001, JAMA 2003

Overgrowth

Hypothesized Due to an Excess of Neurons


80% of ASD 2-16 Year Old Males Have Brain Weight Above Normative Mean for Age

Redcay & Courchesne, Biological Psychiatry, 2005

80% of ASD 2-16 Year Old Males Have Brain Weight Above Normative Mean for Age

Redcay & Courchesne, Biological Psychiatry, 2005

**Brain Growth Pathology in ASD**

**Overgrowth**

![Brain Diagram]

**SIZE**

**NORMAL**

**AGE**

LARGEST META-ANALYSIS STUDY OF BRAIN SIZE IN ASD
Sacco, Gabriele, Persico 2015

- 44 MRI brain size studies, 3,085 subjects
- Significant brain overgrowth in ASD, \( P = 1.21 \times 10^{-21} \)
- 27 head circumference studies, 5,225 subjects
- Significant enlargement in ASD, \( P = 5.20 \times 10^{-50} \)
- and 15.7% macrencephaly

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2) **Non-Uniform Gray Matter Overgrowth:**

**Frontal > Posterior**

1. Carper et al. 2002  Age 3.4 years
2. Bloss & Courchesne, 2007 3.8 years
3. Kates et al. 2004  7.6 years
4. Palmen et al. 2005  11.1 years
5. Hazlett et al. 2005  19.1 years

(Left cerebrum only)

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**Excess Frontal Connections But Small Axons**

FA  VOLUME


- Forceps Minor
- IFSF
- Uncinate
- fSCS
- Arcuate
- IFOF
- White = Tracts not different in ASD
### Differences in Age-Related FA Changes in ASD and TD

- **Forcipps Minor**
  - FA Value vs Age in Months
  - p < 0.05

#### Abnormal Laterality to Language in ASD Infants & Toddlers

- **Typical**
- **ASD**
- Effect Size (Exp)
- L > R
- R > L
- Mean Amplitude of Response vs Age in Months

#### BRAIN SIZE AND GENE EXPRESSION IN CELL CYCLE NETWORK

- **CONTROL**
- **ASD**
- Smaller brains
- Bigger brains
- Absence of normal hub-gene expression
- Gene expression patterns
WHEN DOES AUTISM BEGIN?

Schematic of Dorsolateral and Mesial Prefrontal Cortex

Courchesne, E. et al. JAMA 2011;306:2001-2010

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Overgrowth: PRENATAL CAUSE

Autistic vs Control Males
Ages 2 to 16 years

70% more neurons

29% more neurons

Courchesne et al., JAMA, 2011
Abnormally Small Neurons at Young Ages in Autism

Table 3. % Difference in Neuron Size in Autism vs Control Across Different Regions, Studies, and Cases

<table>
<thead>
<tr>
<th>Brain Region</th>
<th>% Different Neuron Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prefrontal *</td>
<td>-11%</td>
</tr>
<tr>
<td>Fusiform **</td>
<td>-13%</td>
</tr>
<tr>
<td>Occipital **</td>
<td>-4%</td>
</tr>
<tr>
<td>BA44-45 ***</td>
<td>-19.5%</td>
</tr>
</tbody>
</table>

All are averages of layers measured
*Courchesne et al 2011b
**van Kooten et al 2008 online data
***Hof et al, work in progress

ASD and Typical Child or Adult

IPSC → NPC → Neurons → Astrocytes

Graphs showing differences in neuron size between Control and ASD.
79% more DL-PFC neurons

What is the Cause of Excess Cells?

What are the Resulting Cortical Defects?

Gene expression and CNV analyses of DNA

Dispersion of the neurons expressing layer specific markers in the reeler mouse brain

Dekimoto et al., Development, Growth and Differentiation, 2010

Patches of Disorganization in the Neocortex of Children with Autism
Rich Stoner and Eric Courchesne at UCSD and Ed Lein at the Allen Institute
New England Journal of Medicine, 2014
Focal Patches of Disorganized Cortical Layers, Abnormal Migration, and Clusters of Disoriented Neurons

Stoner et al
**NEJM**, 2014

79% more DL-PFC neurons

What is the Cause of Excess Cells?

What are the Resulting Cortical Defects?

Gene expression

Anatomic Microstructure

Dysregulation of Pathways Governing Cell Numbers and Functional Integrity in Frontal Cortex at Young Ages

- Cell cycle regulation
- DNA damage responses
- Apoptosis and survival
- Cell differentiation
- Immune

Abnormal Down-Regulation of Several Neural Patterning Genes

FGF1, HOXD1, NDE1, NODAL, PCSK6

Chow, Pramparo et al
*PLoS Genetics*, 2012
Activated Microglia in ASD
See RED ARROWS

Morgan, Courchesne
Biological Psychiatry, 2010

High Confidence ASD Genes Active in Neurodevelopment in PreFrontal Cortex in 2nd Trimester

Willsey et al
Cell 2013

Intrauterine

Cell Birth & Proliferation (Neurogenesis and Glialogenesis)
Cell Migration
Initiation of teratogenesis
Axonal/Neuronal Outgrowth
Synaptogenesis
Migration of immune stromal cells and expression of progenitor cells
Colonization of immune cells

1st Trimester 2nd Trimester 3rd Trimester
ASD PATHOLOGY:
Overabundance of Neurons
in Fetal Development

Number of Neurons
0.5 1.7
0 3 6 9
Mid 2nd Trimester Birth 30 yrs

Prenatal Life

Perinatal

Kathleen Campbell
Eric Courchesne
WHAT CAUSES THESE EARLY BRAIN ABNORMALITIES IN AUTISM?

ANY INCREASE IN NEURON NUMBERS SEEMS TO BE SUFFICIENT TO CAUSE ASD

NO MUTATION OR ENVIRONMENTAL TRIGGER REQUIRED
Overproduction of Upper-Layer Neurons in the Neocortex Leads to Autism-like Features in Mice

Fang et al., Cell Reports 2014

Gene-disrupting de novo mutations:
Can cause increase neuron numbers and brain overgrowth as well as other ASD-known abnormalities

Evidence of Genetic Causes of Fetal Brain Maldevelopment and Early Brain Overgrowth: Mutation of WDFY3 Gene in Mouse Model of Autism

1) Early Brain Overgrowth
2) Greatest Growth Abnormality is Frontal, Least Occipital
3) Abnormal Cell Cycle Function
4) Abnormal Cell Proliferation
5) Patches of Laminar Disorganization And Nearby Clusters of Abnormal Neuron Migration

Orosco et al, Nature Communications, 2014
**Patches of Laminar Disorganization & Abnormal Neuron Migration**

![Image of experimental results](image)

Figure 4 | Homozygous disc mutants exhibit neuronal migration defects. Immunofluorescent analysis of cortical lamination markers Tbr1 (layer V) and Chx10 (layer V) reveals abnormalities in layer formation of disc/disc mutants at P0. Arrowheads point to individual focal heterotopias of displaced cells for either marker in the mutant. Asterisks highlight smaller scale lamination anomalies. All sections shown are in the coronal plane of the somatosensory cortex. Scale bar, 200 µm.

**Recurrent Gene Mutations in Small Subset of ASD**

![Graphs showing genetic data](image)

O’Roark, Eichler and colleagues, Science 2012

**Common CHD8 targets enrichment**

![Bar chart](image)

From Sugathan et al 2014
NON-GENETIC:
MATERNAL IMMUNE ACTIVATION
IN PRENATAL LIFE

Increased brain size
Upregulation of cell cycle gene expression
Shortening of cell cycle
Excess neurons
Increased cortical thickness
Disruption of genes involved in neuronal migration
Focal patches of disorganized cortex
Microglia abnormalities and enhanced microglia priming
Cerebellar vermis defects
Defects of prefrontal dendritic morphology

Making “autistic” mice
The Maternal Immune Activation (MIA) Mouse
For years researchers have known that by simulating a severe infection in a pregnant mouse, the MIA (maternal immune activation) model produces offspring with overtly autistic behavior.

Abnormalities Observed in MIA Mice AND Autism

<table>
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<th>Behavioral</th>
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Prenatal Maternal Immune Activation (MIA) Via LPS Amplifies Effects of Genetic Mutations in Mouse Models of Autism

Le Belle et al 2014, Stem Cell Reports
HOW CAN THIS KNOWLEDGE IMPACT CLINICAL PRACTICE AND CARE?

- Autism Is Present Already By Birth
- Early Clinical BioMarkers Must Exist
- Vastly Different BioTypes Exist
- Different BioTypes Need Different Tx-Types
- Some BioTypes May be Preventable; Others Not
- Normal Outcomes Might Occur in Some BioTypes
- Early Tx Is Essential
The Need: New Approaches to Universal Screening for Autism in 1-2 Year Olds:

Example #1: The 1 Year Well-Baby Check-Up Approach and the Get S.E.T. Early Model
The Get S.E.T. Early Model for Autism

1 Yr Well-Baby Check-Up Approach Using CSBS

- Fast • Easy • Inexpensive
- Can be done by anyone, anywhere NOW in community pediatric settings
- Results in early risk detection at ages 1-2 years, and early diagnosis & early treatment in community clinical settings
- Detects risk for other developmental delays as well as autism
- Improves standard-of-care for all babies

Karen Pierce et al., 2011
J. Pediatrics

Benefit to Research on Autism

- Enables prospective research on autism as it occurs in general pediatric population
- Babies who do not end up with autism serve as important contrast groups showing how autism looks as compared to other disorders during early development

Karen Pierce et al., 2011
J. Pediatrics
UCSD ACE PEDIATRICIAN NETWORK  N=170
Dr. Robert Bjork, Dr. Michael Nelson, Dr. Cheryl Jenett
Dr. Dr. John Kale, Dr. Douglas Wilson, Dr. Crystal De Pretis
Dr. Martin Gilbre, Dr. Patricia Juarez, Dr. George Madany,
Dr. Sean Brody, Dr. Ingrid Martinez-Andrea, Dr. Irene Chang
Dr. Stephanie Powell, Dr. Adam Breiner, Dr. Patricia Flasing
Dr. Isabel Baratta, Dr. Sheila Carson, Dr. Thomas Neglia
Dr. Stephen Batch, Dr. Randall Metoch, Dr. David Schmottlach
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Dr. Marshall Litman, Dr. Leslie McCormick, Dr. Sharon Sternfeld
Dr. Cara Cohen, Dr. Nicholas Toukias, Dr. Elena Fishman
Dr. Hilary Bowers, Dr. Albert Martinez, Dr. Genevieve Minka
Dr. Wendy Chester, Dr. Leon Kelley, Dr. Victor Liggs, Dr. Jeffrey Sabov, Dr. Lynn Herling, Dr. Teresa O’Lea, Dr.
Richard Wallis, Dr. Vyvan Tang, Dr. Christian Archambault, Dr. Veronique James, Dr. Stuart Cohen, Dr. Nancy
Steen, Dr. Leon Kelley, Dr. Aita Martry
Guido, Dr. Rose
James Morasmar, Dr. Dinyal Bailey, Dr. Leo
Warren, Dr. Sheetal C. 
Jonghans, Dr. Richard G.
Siegel, Dr. Lori Gourley
Dr. Maltesa Rambula, C.
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Dr. Hilary Bowers, Dr. Albert Martinez, Dr. Genevieve Minka
To Date
> 60,000 babies screened!

Karen Pierce

Improve Screening Process

Triple Screen

12 months
18 months
24 months

Screening and referral App

Automatic Score  Automatic Referral

Track pediatrician reason if non referral

Track pediatrician clinical impression for later dx tracking

Karen Pierce

HIGHLIGHTS OF 2011 STUDY

• Autism can be detected by 12-24 months in most cases.

• Treatment can start by 14-16 months.

• Pediatricians from different groups can work together to form a network to detect ASD at the earliest possible ages

• The mean age of ASD detection can drop considerably in any city with systematic effort
THE NEED:
NEW APPROACHES TO UNIVERAL SCREENING FOR AUTISM IN 1-2 YEAR OLDS:

EXAMPLE #2:
RNA Gene Expression Classifier

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THE NEED:
NEW APPROACHES TO EARLY DIAGNOSIS & PROGNOSIS IN AUTISM AT AGES 1-2 YEARS:

EXAMPLE:
GEO-PREFERENCE:
A DIAGNOSTIC & PROGNOSTIC MARKER OF A SUBTYPE OF ASD IN 1 TO 2 YEAR OLDS
Karen Pierce et al., Biological Psychiatry, 2016
ASD 15-Month Old

THE GeoPref TEST FOR AUTISM
Detects 23% of ASD Cases with
98% Specificity

Karen Pierce
Archives of General Psychiatry
2011
Biological Psychiatry
2016
In preparation

Sample Size = 917
Ages 1-3 years

Mullen Scales of Early Learning

Geometric Responders  Social Responders

Receptive Language T

Expressive Language T

Early Learning Composite

Autism Diagnostic Observation Schedule (ADOS)

Geometric Responders  Social Responders

Social Affect / Comm. Social

Total Score
THE NEED:
TREATMENT-RELEVANT BIOLOGICAL SUBTYPES OF AUTISM IN 1-2 YEAR OLDS:
EXAMPLE: fMRI Language Activation
Lombardo et al Neuron 2016

WHY DO SOME INFANTS WITH ASD GET BETTER?
ARE THERE BIOMARKERS OF PROGNOSIS?
Brain Activation in the Story Language Paradigm
Reveal ASD Outcome Subtypes

Lombardo et al
Neuron, 2015

THE NEXT MAJOR ADVANCE:
BIOTYPE SPECIFIC TREATMENTS
FOR AUTISM
AT AGES 1 TO 2 YEARS


