WHAT ARE THE EARLY BRAIN BASES OF AUTISM?
Early Brain Overgrowth in Majority of Autism Toddlers


Overgrowth

Hypothesized Due to an Excess of Neurons


CONB 2005
80% of ASD 2-16 Year Old Males Have Brain Weight Above Normative Mean for Age

Redcay & Courchesne, Biological Psychiatry, 2005
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, 5225 subjects
significant enlargement in ASD $P = 5.20 \times 10^{-50}$
and 15.7% macrencephaly
2) Non-Uniform Gray Matter Overgrowth: Frontal > Posterior

* p<0.05

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)

Courchesne, Pierce et al

NEURON, 2007
Excess Frontal Connections But Small Axons

Solso et al
*Biological Psych*, 2016

FA

VOLUME

Forceps Minor
IFSF
Uncinate

fSCS
Arcuate

IFOF
ILF

White = Tracts not different in ASD
Abnormal Laterality to Language in ASD Infants & Toddlers

Eyler, Pierce and Courchesne

BRAIN, 2012

Typical

13 to 45 months

ASD

14 to 47 months

Temporal cortex

Left - Right Difference in Mean Amplitude of Response

Age in Months

Effect Size (Eta²)

0.0

0.01

0.02

0.03

-0.04

-0.03

-0.02

-0.01

0.00

0.01

0.02

0.03

0.04

Fw

Bw

Typical

L > R

ASD

R > L
WHEN DOES AUTISM BEGIN?
Schematic of Dorsolateral and Mesial Prefrontal Cortex

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

Copyright restrictions may apply.
Overgrowth

Autistic (n=7) vs Control (n=6)
Males
Ages 2 to 16 years

79% 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>
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<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 Infants & Toddlers

iPS Cells

Neuroprogenitor Cells

Neurons

Astrocytes
Marchetto et al. Molecular Psychiatry, 2016
ALTERATION OF CO-EXPRESSED HUB-GENES UNDERLIES PATHOLOGICAL BRAIN SIZE AND IS MOST SEVERE IN BIGGER BRAINS

Pramparo Courchesne et al
Molecular Systems Biology 2015
What is the Cause of Excess Cells?

What are the Resulting Cortical Defects?

Gene expression and CNV analyses of DNA

Anatomic Microstructure

79% more DL-PFC neurons
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
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 2$^{nd}$ Trimester

Willsey et al

Cell. 2013
WHAT CAUSES THESE EARLY BRAIN ABNORMALITIES IN AUTISM?

Genetic, Animal, Cellular and Maternal Immune Activation Studies
LARGE INCREASES IN NEURON NUMBERS SEEM 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

Single in utero injection of XAV939 elevated the Axin protein level in neuronal progenitors

More neurons in L2/3
Neocortex enlarged
Imbalance of excitatory/inhibitory neurons

Fang et al., *Cell Reports* 2014

Deficits in social interaction
More self-grooming
Buried more marbles

Autism-like behaviors
GENE-DISRUPTING DE NOVO MUTATIONS
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
Figure 4 | Homozygous disc mutants exhibit neuronal migration defects. Immunofluorescent analysis of cortical lamination markers Tbr1 (layer VI) and Ctip2 (layer V) reveals abnormalities in layer formation of disc/disc mutants at P0. Arrowheads point to individual focal heterotopia 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.
NON-GENETIC:
MATERNAL IMMUNE ACTIVATION IN PRENATAL LIFE
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 MALE offspring will exhibit behavioral, biochemical, and physiological characteristics that highly resemble autism.

Viral Mimic

Abnormalities Observed in MIA Mice AND Autism

Behavioral

- Abnormal social preferences (Lego vs. mouse)¹
- Increased anxiety-like behavior (open field exploration)²
- Deficits and delays in vocalizations²
- Impaired motor coordination³

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

¹Naviaux (2013); ²Hsiao (2013)
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
Autism Patch of Cortical Malformation and Neural Dysplasia

MIA Animal Model of ASD

<table>
<thead>
<tr>
<th>Poly(I:C)</th>
<th>anti-IL-17a</th>
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</thead>
<tbody>
<tr>
<td>Cortical Phenotype</td>
<td></td>
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</tbody>
</table>

Stoner et al., *NEJM* 2014

Choi et al., *Science* 2016
THE NEED:
NEW APPROACHES TO AUTISM RISK DETECTION IN 1-2 YEAR OLDS

EXAMPLE

RNA Gene Expression Classifier

Pramparo et al
_JAMA Psychiatry_, 2015
BLOOD RNAseq Autism Diagnostic Classifier in Male Infants and Toddlers

ACCURACY = 87%
SPECIFICITY = 90%
SENSITIVITY = 82%

Metacore Pathway Enrichment of Classifier Genes

- Translation_Translation...
- Translation_Elongation...
- Translation_Regulation of...
- Reproduction_Feeding and...
- Cell cycle_G1-S Growth...
- Cell adhesion_Amyloid...
- Cell adhesion_Cell junctions
- Protein folding_Protein...
- Cytoskeleton_Regulation of...
- Cell cycle_Mitosis
- Inflammation_Protein C...
- Immune response_TCR...
- Inflammation_IL-2 signaling
- Inflammation_MIF signaling
- Cell adhesion_Leucocyte...
- Signal...
- Inflammation_IL-6 signaling
- Cell cycle_G1-S Interleukin...
- DNA damage_DBS repair
- Immune response_BCR...
EXAMPLE:

GE0-PREF TEST OF AUTISM:

A DIAGNOSTIC AND PROGNOSTIC MARKER OF A SUBTYPE OF ASD IN 1 TO 2 YEAR OLDS

Karen Pierce et al

Archives of General Psychiatry 2011

Biological Psychiatry 2016
ASD 15-Month Old

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

Karen Pierce
Archives of General Psychiatry 2011
Biological Psychiatry 2016

Sample Size = 917
Ages 1-3 years
Mullen Scales of Early Learning

Receptive Language T

Expressive Language T

Early Learning Composite

Geometric Responders

Social Responders
THE NEED:
TREATMENT-RELEVANT BIOLOGICAL SUBTYPES OF AUTISM IN 1-2 YEAR OLDS:

EXAMPLE:
fMRI Language Activation

Lombardo et al
*Neuron* 2016
Change in Verbal IQ

- ASD Good Language Outcome
- ASD Poor Language Outcome

Lord et al, 2015
WHY DO SOME INFANTS WITH ASD GET BETTER?

ARE THERE BIOMARKERS OF PROGNOSIS?
IT'S TIME FOR BED LITTLE ...
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
Common CHD8 targets enrichment

brain

- Transcription_mRNA processing
- Proteolysis_Ubiquitin
- Cell cycle_Mitosis
- Cell cycle_G1-S
- Transcription_Chromatin
- Immune response_BCR
- Inflammation_IL-2 signaling
- Signal transduction_NOTCH
- DNA damage_Checkpoint
- Immune response_Phasosome

-log(pValue)