Modeling Neurodevelopmental Disorders: From Mouse Mutants to Human Induced Neural Stem Cells

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Autism Science Foundation: Scientific Advisory Board
International Meeting for Autism Research
International Rett Syndrome Foundation: Scientific Review Panel
Autistica, United Kingdom: Scientific Advisory Board
Simons Foundation
Society for Neuroscience
Dana Foundation

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National Alliance for Autism Research (NAAR)
Autism Science Foundation
New Jersey Governor’s Council for Medical Research and Treatment of Autism
Hallmark Feature - Abnormal Social Interaction

Figure 5. Example fixation patterns from study 1 using the iView tracking system. RT Schultz et al, 2000
Functional MRI studies in typical and autistic individuals – Altered long distance connectivity!

Allen and Courchesne, 2003
Autism Spectrum Disorders (ASD)- DSM-5

Diagnostic Criteria

A. Persistent deficits in social communication and social interaction across multiple contexts

B. Restricted, repetitive patterns of behavior, interests, or activities

C. Present in early developmental period (Recognized 12–24 months)

D. Significant impairment in social, occupational, or other functioning

E. Not better explained by intellectual disability or global delay

Specifiers:
+/- intellectual impairment
+/- language impairment
+/- genetic, medical, environmental factor-FXMR, Tuberous Sclerosis, Rett, Epilepsy, VPA (Depakene), Very Low Birth Weight

Co-Morbidity: 70% with one and 40% with two!
Anxiety & Mood(>60%), Epilepsy (30%), ID (65%), ADHD, OCD
Etiological Factors in Neurodevelopmental Disorders

“The Autisms”

Many genes (500-1000) but fewer common pathways
developmental genes, synaptic molecules, chromatin modifiers

**Genetic Factors**- Family studies, GWAS, CNV

X

**Environmental factors**- Maternal and external

Causes and symptoms may overlap with many disorders
Schizophrenia, bipolar depression, intellectual disability
Synthesis of Results of Genetic Architecture of ASD

- Rare variants - large effects
- Common variants - small effects
- Genetic background on which other factors act?

Gaugler, Buxbaum and colleagues, 2014
Our Approach to Study Autism Etiologies

Using animal and human systems

**Genes**
- Common variant: Engrailed-2
- Rare variant
- **Idiopathic**
  - CNV 16.p11.2 (1%)

**Environment**
- **Developmental**
  - Growth Factors
  - Inflammation
  - ROS/Mitochondria
  - H$_2$O$_2$, MeHg

- **Chemical**
  - Drugs
  - VPA
  - Air Pollution

Define molecular pathways to design therapeutic interventions
**DEVELOPMENTAL Brain Abnormalities in Autism**

**Developmental Genes?**

**Brain Growth – Proliferation, Survival**
- accelerated brain growth rate in first years, then stabilizes
- increased head circumference
- enlarged cerebral cortex, amygdala and cerebellum; grey & white matter

**Cerebral Cortex – Proliferation, Differentiation and Migration**
- disorganized neuronal layers and misdirected pyramidal neurons
- increase in neuron number and density/ others show decreases!
- neuron columns are smaller in size (mini-columns)
- ectopic neurons and dysplastic regions
- increases in dendritic spines

**Cerebellum – Proliferation, Survival**
- reduced numbers of Purkinje neurons
  
  ![Engrailed-2](image_url)
- decreased vermis
- increased size of hemispheres

Approximately 15-20% of individuals with ASD have macrocephaly

**Neural Systems:** Abnormalities in transmitters, synapses and connectivity
Cerebellar Growth In Vivo

Mouse

Wild Type (WT)  Engrailed-2 Knockout (KO)

Millen et al. 1994

Human

Normal

Autism

Courchesne et al.
**ENGRAILED 2 (EN2)** is a transcription factor that can repress gene expression.

*En2* functions in pre- and post-natal brainstem and cerebellar patterning when circuits develop.

*En2 deletion* as well as *over-expression* reduce Purkinje neurons and granule cells.

**ENGRAILED 2 gene structure**

![Gene Structure Diagram]
*Engrailed 2* gene is expressed in the back of the embryonic brain where monoamine neurons originate.

Monoamine neurotransmitters
Serotonin, Dopamine, Norepinephrine

(Sgaier et al., 2007)
Roles of monoamine neurotransmitters in forebrain behaviors

Norepinephrine – Locus Coeruleus
- modulates attention, behavioral performance, sleep-wake states and mood

Serotonin – Raphe
- roles in mood, obsessive-compulsive signs and aggression

Dopamine – Mesolimbic
- reward, repetitive & obsessive behaviors, attention movement

Roles of Monoamines in Forebrain Development?
Proliferation, survival, differentiation
Social behavior in mice – preference for another mouse. *Engrailed-2* mutants do not prefer another mouse!
Learning and remembering where things are in space!
Multiple behaviors are abnormal in En2 mutants

Many behaviors reflect forebrain circuits

Depression-like behavior
  Monoamines

Social interactions in adolescence and adulthood
  Same sex juvenile dyads, opposite sex adults
  Hippocampus, hypothalamus, amygdala, striatum

Spatial learning
  Novel object recognition
  Fear learning in different environments
    Hippocampus, thalamus, amygdala, cerebellum

Sensory gating
  Sensory-motor cortex, thalamus

Grip strength, rotarod performance
  Cerebellum, thalamus, cortex
  Brielmaier et al., 2012, 2014
Model: Can changes in hindbrain monoamine systems impact forebrain development?

Forebrain Development - Growth Function

Engrailed-2

Norepinephrine
Serotonin
Dopamine

Hindbrain Genes – Neurons
Norepinephrine levels are abnormally distributed in the En2 mutant mouse at adolescence!

Genestine et al, Hum Mol Genet, 2015
Brain growth abnormalities in *En2* mutants

### Brain region weight (mg)

<table>
<thead>
<tr>
<th></th>
<th>Hippo</th>
<th>Striatum</th>
<th>Forebrain</th>
<th>Hindbrain</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Decrease</td>
<td>12</td>
<td>13</td>
<td>4</td>
<td>8</td>
</tr>
</tbody>
</table>

Jacob Ellegood
Hippocampal dentate gyrus has 16% fewer neurons in *En2 KO*

Genestine et al, Hum Mol Genet, 2015
Tyrosine hydroxylase (TH) levels are altered in brain regions in parallel with NE.

TH is the rate limiting enzyme in NE biosynthesis.

Genestine et al, Hum Mol Genet, 2015
Norepinephrine (TH) nerve fibers from Locus Coeruleus are reduced in hippocampus CA1-3 DG GCL
Cell death and proliferation are increased in the hippocampus of En2 mutants

Genestine et al, Hum Mol Genet, 2015
Intra-hippocampal injections of NE agonists at P21

Genestine et al, Hum Mol Genet, 2015
Desipramine (NE enhancer) repairs adult social interactions

“Taking these drugs caused me to look at myself in a whole new light,”

Brielmaier et al, *Genes, Brain and Behavior* 2014
Desipramine (NE enhancer) repairs depression-like behavior

“Taking these drugs caused me to look at myself in a whole new light,”

Tail Suspension

Also Forced Swim

Brielmaier et al, Genes, Brain and Behavior 2014
Insights from study of common variant Engrailed-2

Changes in Neuronal Connections between brain regions

Changes in Synapses and Neurotransmitter Systems

Hindbrain developmental gene effects on the forebrain - common path?
Altered connectivity of hindbrain to forebrain- Rett (LC), Tuberous Sclerosis, FXS

Monoamine neurotransmitter systems
Monoamine metabolism- MAO A/B, COMT, VMAT, 5HTT
Depression, schizophrenia, Disc1, Rett, Timothy syndrome, ASD, VPA

Abnormal neurogenesis- pathological mechanism vs biomarker
Depression, schizophrenia, epilepsy, Tsc, FMR1, Rett, PTEN; ASD?

Question: Do common variants that have small effect provide a background vulnerability on which other rare variants or environmental factors act?
Human induced pluripotent stem cells (iPSC)

Proliferation
Differentiation
Neurite Outgrowth

Patient
Blood cells

Personalized Medicine
Effective
Drug screen
Mechanistic study

Novel Therapy

NPCs

iPS cells

WBCs

neurons

disease modeling

Personalized Medicine
New Jersey Autism Center of Excellence (NJACE) Grant
Jim Millonig, PI and colleagues

- Population created by Linda Brzustowicz
- Criteria
  - 1 autism child and 1 unaffected sibling
  - 1 family member w/ Specific Language Impairment (SLI)
- 8 Families
  - 16 patients total
Converting T Cells to iPSCs
Growing NSCs as a monolayer in Neural Induction Media---Gibco/Invitrogen

*We receive cells at passage 3 and begin our analysis at this time*
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