• Listening to our Daughters: Girls & Women with Autism will Inform Novel Treatments

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Autism and Neurodevelopmental Disorders Institute

October 26, 2016
Brain Systems for Social Cognition

Pelphrey et al. (2003) *Journal of Neuroscience*
Can we see autism’s signature in the individual brain?
Classification Analysis

Discovery

Replication

?
A weak response to biological motion is a marker of autism in boys (but not girls!)

Björnsdotter et al., *JAMA: Psychiatry*, 2016
Autism Center of Excellence: Girls Network

125 Boys with ASD
75 TD Boys
125 Girls with ASD
75 TD Girls
Unaffected siblings:
50 boys & 50 girls
<table>
<thead>
<tr>
<th>Milestones</th>
<th>April 1 2016</th>
</tr>
</thead>
<tbody>
<tr>
<td>Target: Total Recruitment</td>
<td>374</td>
</tr>
<tr>
<td>Actual: Total Recruitment</td>
<td>454</td>
</tr>
<tr>
<td>Actual/Target Ratio: Total Recruitment</td>
<td><strong>121%</strong></td>
</tr>
<tr>
<td>Target: Racial Minority Recruitment</td>
<td>65</td>
</tr>
<tr>
<td>Actual: Racial Minority Recruitment</td>
<td>110</td>
</tr>
<tr>
<td>Actual/Target Ratio: Racial Minority Recruitment</td>
<td><strong>169%</strong></td>
</tr>
<tr>
<td>Target: Hispanic Ethnicity Recruitment</td>
<td>33</td>
</tr>
<tr>
<td>Actual: Hispanic Ethnicity Recruitment</td>
<td>60</td>
</tr>
<tr>
<td>Actual/Target Ratio: Hispanic Ethnicity Recruitment</td>
<td><strong>182%</strong></td>
</tr>
</tbody>
</table>
Sex differences in brain response to coherent versus scrambled biological motion  ASD ♀ > ASD ♂

1.3% ASD prevalence

4:1 sex bias

Female ASD liability threshold: Mean + 2.7SD

Male ASD liability threshold: Mean + 2.1SD

Population Frequency

ASD genetic liability

♀: female; ♂: male; dmPFC: dorsomedial prefrontal cortex; FFG: fusiform gyrus; ITG: inferior temporal gyrus; pSTS: posterior superior temporal sulcus; vmPFC: ventromedial prefrontal cortex

ASD ♀ > ASD ♂
How do we translate basic science into practicable treatments aimed at target engagement?
Imprecision Medicine

For every person they do help (blue), the ten highest-grossing drugs in the United States fail to improve the conditions of between 3 and 24 people (red).

1. **ABILIFY** (aripiprazole)
   - Schizophrenia

2. **NEXIUM** (esomeprazole)
   - Heartburn

3. **HUMIRA** (adalimumab)
   - Arthritis

4. **CRESTOR** (rosuvastatin)
   - High cholesterol

5. **CYMBALTA** (duloxetine)
   - Depression

6. **ADVAIR DISKUS** (fluticasone propionate)
   - Asthma

7. **ENBREL** (etanercept)
   - Psoriasis

8. **REMICADE** (infliximab)
   - Crohn’s disease

9. **COPAXONE** (glatiramer acetate)
   - Multiple sclerosis

10. **NEULASTA** (pegfilgrastim)
    - Neutropenia

Based on published number needed to treat (NNT) figures. For a full list of references, see Supplementary Information at goo.nature.com/4dr78L.

Pivotal Response Training (PRT)
Change in Behavior: Social Responsiveness Scale (SRS)

Yang et al. (in press) Nature: Translational Psychiatry
Neuro-prediction of treatment response

Yang et al. (in press)
*Nature: Translational Psychiatry*
Change in brain, driving change in behavior

Yang et al. (in press)
*Nature: Translational Psychiatry*
R inferior frontal gyrus
R precentral gyrus

MASD > F ASD

Total SFS change - PRT vs. Waitlist Comparison

-15.67
-3.98

Boys
Girls

Vineland - Socialization Change: PRT vs. Waitlist

WLC Boys: -1.20
WLC Girls: 3.50
Boys: 1.82
Girls: 7.25
Can we boost brain responses before treatment, to make treatment work?
Intranasal Oxytocin – Social Judgments

Gordon et al. (2013) *Proceedings of the National Academy of Sciences*
Linking neural signatures, genes, and behavior in to shape developmental trajectories
Results: fNIRS (LR and HR 3-Month-Old Infants)

Low Risk

BIOLOGICAL

Hemoglobin change (micromolar)

Time (in tenths of seconds)

Low Risk

BIOLOGICAL

Hemoglobin change (micromolar)

Time (in tenths of seconds)

High Risk

BIOLOGICAL

Hemoglobin change (micromolar)

Time (in tenths of seconds)

High Risk

BIOLOGICAL

Hemoglobin change (micromolar)

Time (in tenths of seconds)
Acknowledgments

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NICHD
NINDS
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Autism Speaks
Hilibrand Foundation
John Merck Scholars Fund
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I thank my colleagues who make this work so much fun.
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The role of genetics and sex-differential biology in risk for autism

Donna Werling, PhD
Sanders & State Labs, UCSF
October 26, 2016
Autism prevalence is sex-biased

• ~4:1 males:females have a diagnosis of autism spectrum disorder (ASD)

Autism prevalence is sex-biased

- ~4:1 males:females have a diagnosis of autism spectrum disorder (ASD)\(^1\)

- 8 males and 3 females in the 11 cases originally reported by Leo Kanner, 1943\(^2\)

- Male bias consistent over time and across countries\(^1\)

Why study sex bias in ASD from a biological perspective?

Sex appears to be a potent modulator of ASD risk

Males: 1 in 42 diagnosed

Females: 1 in 189 diagnosed
Why study sex bias in ASD from a biological perspective?

Sex appears to be a potent modulator of ASD risk.

Males: 1 in 42 diagnosed

Females: 1 in 189 diagnosed
Why study sex bias in ASD from a biological perspective?

Sex appears to be a potent modulator of ASD risk

Treatment development

Novel/key insights into fundamental biology of ASD
Female Protective Effect (FPE) Model for ASD = Liability model

No. individuals in population

Population mean

 Liability for ASD

i.e. exposure to risk factors such as genetic variants
Female Protective Effect (FPE) Model for ASD = Liability model

Population mean

Liability for ASD → Diagnosed individuals

i.e. exposure to risk factors such as genetic variants
Female Protective Effect (FPE) Model for ASD = *Multiple threshold* liability model

- differential risk factors decrease liability threshold
- differential protective factors increase liability threshold

i.e. exposure to risk factors such as genetic variants
Female Protective Effect (FPE) Model for ASD = *Multiple threshold* liability model

![Graph showing the Female Protective Effect (FPE) Model for ASD. The graph illustrates a bell curve representing the distribution of liability for ASD in the population. The x-axis represents the liability for ASD, defined as exposure to risk factors such as genetic variants. The y-axis represents the number of individuals in the population. The model highlights differential risk factors that decrease liability threshold and protective factors that increase liability threshold, leading to a lower proportion of diagnosed individuals among females compared to males.](image-url)
FPE model predicts that diagnosed females carry greater risk than males.
FPE model predicts that diagnosed females carry greater risk than males
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FPE model predicts that diagnosed females carry greater risk than males.
FPE model predicts that diagnosed females carry greater risk than males.
Higher incidence of disruptive, de novo variants in ASD females

Copy number variants (CNVs)

<table>
<thead>
<tr>
<th></th>
<th>Duplications</th>
<th>Deletions</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>0.06</td>
<td>0.03</td>
</tr>
<tr>
<td>Female</td>
<td>0.07</td>
<td>0.04</td>
</tr>
<tr>
<td>Male</td>
<td>0.05</td>
<td>0.02</td>
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</table>

Insertion/Deletions (Indels)

<table>
<thead>
<tr>
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<tbody>
<tr>
<td>All</td>
<td>0.15</td>
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<tr>
<td>Brain</td>
<td>0.10</td>
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Single nucleotide variants (SNVs)

<table>
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<tr>
<th></th>
<th>p&lt;0.01</th>
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<tbody>
<tr>
<td>All</td>
<td>0.05</td>
</tr>
<tr>
<td>LoF</td>
<td>0.02</td>
</tr>
<tr>
<td>Missense</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Siblings of female cases have higher ASD traits than siblings of male cases

Autistic traits in siblings?

Robinson et al, PNAS, 2013
Siblings of female cases have higher ASD traits than siblings of male cases

Autistic traits in siblings?

### Increase in risk to siblings of female probands

<table>
<thead>
<tr>
<th>Group</th>
<th>TEDS</th>
<th>CATSS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Siblings of All Non-Probands</td>
<td>-0.06</td>
<td>-0.05</td>
</tr>
<tr>
<td>N(TEDS) = 3,444 N(CATSS) = 5,340</td>
<td>(p&lt;0.0001 *$)</td>
<td>(p&lt;0.0001 *$)</td>
</tr>
<tr>
<td>Siblings of Male Probands</td>
<td>0.42</td>
<td>0.34</td>
</tr>
<tr>
<td>N(TEDS) = 262 N(CATSS) = 470</td>
<td>(p=0.002 $)</td>
<td>(p=0.02 $)</td>
</tr>
<tr>
<td>Siblings of Female Probands</td>
<td>0.78</td>
<td>0.55</td>
</tr>
<tr>
<td>N(TEDS) = 136 N(CATSS) = 230</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Robinson et al, PNAS, 2013
FPE model predicts that females respond differently to liability that is sufficient for diagnosis in males.
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1. Quantitative:
   Females are protected and have neurotypical phenotype.

2. Qualitative:
   Females present symptoms differently than males, and are not diagnosed.
FPE model predicts that females respond differently to liability that is sufficient for diagnosis in males.

Hypothesis:
Sex-differential biology contributes to male and female differences in ASD risk and/or symptom presentation.

1. Quantitative:
   Females are protected and have neurotypical phenotype.

2. Qualitative:
   Females present symptoms differently than males, and are not diagnosed.
We can use gene expression analysis to identify sex differences that contribute to the FPE

1. Identify genes with sex-differential expression levels in the human brain

2. Characterize the relationship between sex-DEX genes and ASD biology
<table>
<thead>
<tr>
<th>Period</th>
<th>Description</th>
<th>Age</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>Embryonic</td>
<td>4 PCW ≤ Age &lt; 8 PCW</td>
</tr>
<tr>
<td>2</td>
<td>Early fetal</td>
<td>8 PCW ≤ Age &lt; 10 PCW</td>
</tr>
<tr>
<td>3</td>
<td>Early fetal</td>
<td>10 PCW ≤ Age &lt; 13 PCW</td>
</tr>
<tr>
<td>4</td>
<td>Early mid-fetal</td>
<td>13 PCW ≤ Age &lt; 16 PCW</td>
</tr>
<tr>
<td>5</td>
<td>Early mid-fetal</td>
<td>16 PCW ≤ Age &lt; 19 PCW</td>
</tr>
<tr>
<td>6</td>
<td>Late mid-fetal</td>
<td>19 PCW ≤ Age &lt; 24 PCW</td>
</tr>
<tr>
<td>7</td>
<td>Late fetal</td>
<td>24 PCW ≤ Age &lt; 38 PCW</td>
</tr>
<tr>
<td>8</td>
<td>Neonatal and early infancy</td>
<td>0 M (birth) ≤ Age &lt; 6 M</td>
</tr>
<tr>
<td>9</td>
<td>Late infancy</td>
<td>6 M ≤ Age &lt; 12 M</td>
</tr>
<tr>
<td>10</td>
<td>Early childhood</td>
<td>1 Y ≤ Age &lt; 6 Y</td>
</tr>
<tr>
<td>11</td>
<td>Middle and late childhood</td>
<td>6 Y ≤ Age &lt; 12 Y</td>
</tr>
<tr>
<td>12</td>
<td>Adolescence</td>
<td>12 Y ≤ Age &lt; 20 Y</td>
</tr>
<tr>
<td>13</td>
<td>Young adulthood</td>
<td>20 Y ≤ Age &lt; 40 Y</td>
</tr>
<tr>
<td>14</td>
<td>Middle adulthood</td>
<td>40 Y ≤ Age &lt; 60 Y</td>
</tr>
<tr>
<td>15</td>
<td>Late adulthood</td>
<td>60 Y ≤ Age</td>
</tr>
</tbody>
</table>

M, postnatal months; PCW, post-conception weeks; Y, postnatal years.
There is no evidence of an autosomal gene with XY levels of sexual dimorphism in the brain.
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Sex-DEX genes identified by permutation approach ($Q \leq 0.05$; top-ranking sex-DEX in $\geq 2$ consecutive developmental periods from same brain region):

- Higher expression in males:
  - 505 protein-coding genes, 129 noncoding transcripts

- Higher expression in females:
  - 442 protein-coding genes, 466 noncoding transcripts
Sex-DEX genes are not enriched for neuronal markers

- Male-DEX
  - Neurons: 0.006
  - Male-DX: 923, 504, 203, 113, 111, 57

- Female-DEX
  - Neurons: 0.013
  - Female-DX: 363, 145, 81, 2612

- Striatal Neurons
  - Module: M36, M38, M40
  - N genes: 131, 119, 107

- Pyramidal Neurons
  - Module: M18, M37, M65, M2
  - N genes: 363, 145, 81, 2612

- Interneurons
  - Module: M10, M32
  - N genes: 715, 129

Odds ratio for enrichment
Male-DEX genes show enrichment for microglial and endothelial cell markers.
Male-DEX genes show enrichment for microglial and endothelial cell markers.
Male-DEX genes show enrichment for microglial and endothelial cell markers
We observe a relationship between sex-DEX genes and ASD biology.

Protein-coding sex-DEX genes:
- 488 male-DEX genes
- 415 female-DEX genes

* $p<0.05$ (adj.)
** $p<0.01$ (adj.)
*** $p<0.001$ (adj.)

Dataset:
- Darnell
- Cotney
- Sugathan
- Voineagu
- Gupta
- Gupta
- Gupta
- Voineagu
- Gupta
- Gupta

Gene count:
- M12: 411
- Mod1: 1,468
- Mod2: 1,235
- Mod6: 585
- M16: 359
- Mod5: 700
- Mod7: 562

Data type:
- FMRP targets
- CHD8-bound promoters
- Neuronal
- Microglial & Astrocytic
- Post-mortem ASD brain
- WGCNA modules
We observe a relationship between sex-DEX genes and ASD biology

Convergence between male-typical and ASD neurobiology
Enrichment evidence suggests a male sensitization model of ASD risk
Enrichment evidence suggests a male sensitization model of ASD risk

- Differential risk factors decrease liability threshold
- Liability threshold

Population mean

No. individuals in population

Liability for ASD → Diagnosed individuals

i.e. exposure to risk factors such as genetic variants
Enrichment evidence suggests a male sensitization model of ASD risk

Large overlap leads to male sensitization

Small overlap leads to relative female protection

ASD neurobiology
Enrichment evidence suggests a male sensitization model of ASD risk

To understand ASD sex bias, we must characterize the intersection of typical male neurobiology and ASD neurobiology.
Summary

• Intersection of ASD neurobiology and sex-differential neurobiology provides an approach to understand sex bias

• Male-biased expression:
  – Microglial genes
  – Collagen genes and endothelial cell markers
  – Glial genes dysregulated in ASD brain, suggesting a male-sensitization effect

• Validation in independent samples is needed
  – Results are preliminary and based on analysis of a single data set
Looking forward

• Well powered, foundational data sets comparing males and females will be required for:
  • Rigorous validation of sex-differential patterns
  • Thorough investigation of relationships between sex-differential and ASD biology

Data types
• RNA sequencing for gene expression
• ChIP-seq for identifying gene targets of the estrogen and androgen receptors

2x2 design

Developmental stages
• Fetal
• Perinatal
• Early postnatal/childhood
• Puberty
• Adulthood

Cell types
• Neurons
• Microglia
• Astrocytes

Brain regions
• Neocortex
• Thalamus
• Striatum
• Cerebellum

Organisms
• Human
• Primate
• Mouse