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)

---

<table>
<thead>
<tr>
<th>Disease</th>
<th>Male:female ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASD</td>
<td>~4:1</td>
</tr>
<tr>
<td>Schizophrenia</td>
<td>2:1</td>
</tr>
<tr>
<td>Depresion</td>
<td>1:1</td>
</tr>
<tr>
<td>Alcohol</td>
<td>1:2</td>
</tr>
<tr>
<td>Gout</td>
<td>1:10</td>
</tr>
<tr>
<td>Prostate hypertrophy</td>
<td></td>
</tr>
<tr>
<td>Bladder/Esophageal cancer</td>
<td></td>
</tr>
<tr>
<td>Cancer</td>
<td></td>
</tr>
<tr>
<td>Perinatal/ Congenital conditions</td>
<td></td>
</tr>
<tr>
<td>Migraine</td>
<td></td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td></td>
</tr>
<tr>
<td>Chlamydia</td>
<td></td>
</tr>
<tr>
<td>Pregnancy related</td>
<td></td>
</tr>
<tr>
<td>Cervical, ovarian, breast and uterine cancer</td>
<td></td>
</tr>
</tbody>
</table>

---

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

---

**Diagram:**
- **X-axis:** Liability for ASD
- **Y-axis:** No. individuals in population
- **Population mean**
- **Diagnosed individuals**

**Legend:**
- *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

[Diagram showing the distribution of liability for ASD with a peak around the population mean, and a shaded area indicating differential risk factors for males and females.]

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

No. individuals in population

Liability for ASD →

Diagnosed individuals

i.e. exposure to risk factors such as genetic variants
FPE model predicts that diagnosed females carry greater risk than males.
FPE model predicts that diagnosed females carry greater risk than males.
FPE model predicts that diagnosed females carry greater risk than males
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, \textit{de novo} variants in ASD females

Copy number variants (CNVs)

Insertion/Deletions (Indels)

Single nucleotide variants (SNVs)

Siblings of female cases have higher ASD traits than siblings of male cases.
Autistic traits in siblings? 

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

Robinson et al, PNAS, 2013
FPE model predicts that females respond differently to liability that is sufficient for diagnosis in males.

Females with liability in this range are **not diagnosed**.
FPE model predicts that females respond differently to liability that is sufficient for diagnosis in males

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 1 | Periods of human development and adulthood as defined in this study

<table>
<thead>
<tr>
<th>Period</th>
<th>Description</th>
<th>Age</th>
</tr>
</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-conceptional weeks; Y, postnatal years.
There is no evidence of an autosomal gene with XY levels of sexual dimorphism in the brain.
There is no evidence of an autosomal gene with XY levels of sexual dimorphism in the brain.

Sex-DEX genes identified by permutation approach (Q ≤ 0.05; top-ranking sex-DEX in ≥ 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 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.
We observe a relationship between sex-DEX genes and ASD biology.
Enrichment evidence suggests a male sensitization model of ASD risk

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

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

- differential risk factors decrease liability threshold
- liability threshold

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

Large overlap leads to male sensitization

Small overlap leads to relative female protection

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
Acknowledgements

Stephan Sanders
UCSF

Matt State
UCSF

Nenad Sestan
Yale

Forrest Gulden

Sirisha Pochareddy

Xuming Xu

Mingfeng Li

Rob Kitchen

This work was supported by a grant to SS from the Simons Foundation (SFARI #307705), and a fellowship to DW from the Autism Science Foundation (ASF #16-009)