Understanding the female protective effect and role of sex-differential biology in risk for autism

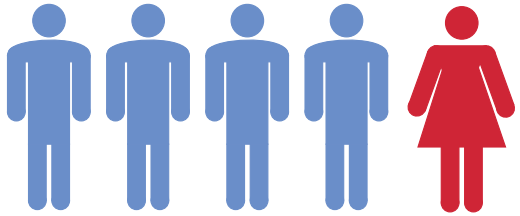
Donna Werling, PhD
Sanders & State Labs, UCSF
February 2, 2018

Insights into Autism

UCSF
University of California San Francisco

Outline

- Autism prevalence and risk in males vs. females



- How scientists think about sex-differential risk: The Female Protective Effect (FPE) model

- Research in progress: Relationship between autism biology and sex-differential biology

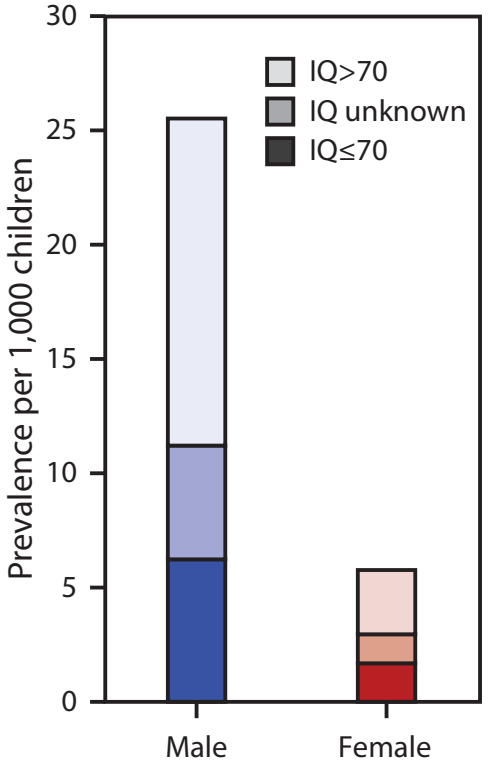
Autism spectrum disorders (ASDs)

- Pervasive neurodevelopmental disorders
  - Deficits in **social communication** and interaction
  - Restricted, **repetitive behavior**, interests, or activities[1]
- Prevalence in US is **1/68 children** = 1.5%[2]
- Costs of ASD
  - Monetary: $236-262 billion per year in US[3]
  - Quality of life: Poor engagement in society, poor health outcomes
- Best current treatments are **behavioral interventions**
- **Genetic factors** contribute to ASD risk

[1]APA, 2013, *DSM-5.* [2]Christensen et al., 2016, *MMWR Surveil. Summ.* [3]Buescher et al., 2014, *JAMA Pediatr.*

ASDs have sex-biased prevalence

- 4.5:1 males:females have an ASD diagnosis in US[1]



[1]Christensen et al., 2016, *MMWR Surveil. Summ.*

ASDs have sex-biased prevalence

- 4.5:1 males:females have an ASD diagnosis in US[1]
- 8:3 M:F in Leo Kanner's original cases, 1943[2]

PATHOLOGY

To understand and measure emotional qualities is very difficult. Psychologists and educators have been struggling with that problem for years but we are still unable to measure emotional and personality traits with the exactness with which we can measure intelligence.
—Rose Zeligs in *Glimpses into Child Life**

AUTISTIC DISTURBANCES OF AFFECTIVE CONTACT

By Leo Kanner

SINCE 1938, there have come to our attention a number of children whose condition differs so markedly and uniquely from anything reported so far, that each case merits—and, I hope, will eventually receive—a detailed consideration of its fascinating peculiarities. In this place, the limitations necessarily imposed by space call for a condensed presentation of the case material. For the same reason, photographs have also been omitted. Since none of the children of this group has as yet attained an age beyond 11 years, this must be considered a preliminary report, to be en
and further observation of their develop

Case 1. Donald T. was first seen in C
month. Before the family's arrival fron
thirty-three-page typewritten history th
detail, gave an excellent account of Don
full term on September 8, 1933. He weig
breast fed, with supplementary feeding, u
were frequent changes of formulas. "Eati
a problem with him. He has never shov
eating candy and ice cream has never h
proceeded satisfactorily. He walked at 1

At the age of 1 year "he could hum an
he was 2 years old, he had "an unusual r
names of a great number of houses" in h
the family in learning and reciting short
third Psalm and twenty-five questions a
chism." The parents observed that "he v
answer questions unless they pertained t
often then he would ask no question exc
was clear. He became interested in pictu

From the Henry Phipps Psychiatric Clini
Children, the Johns Hopkins Hospital, and (
Maryland.

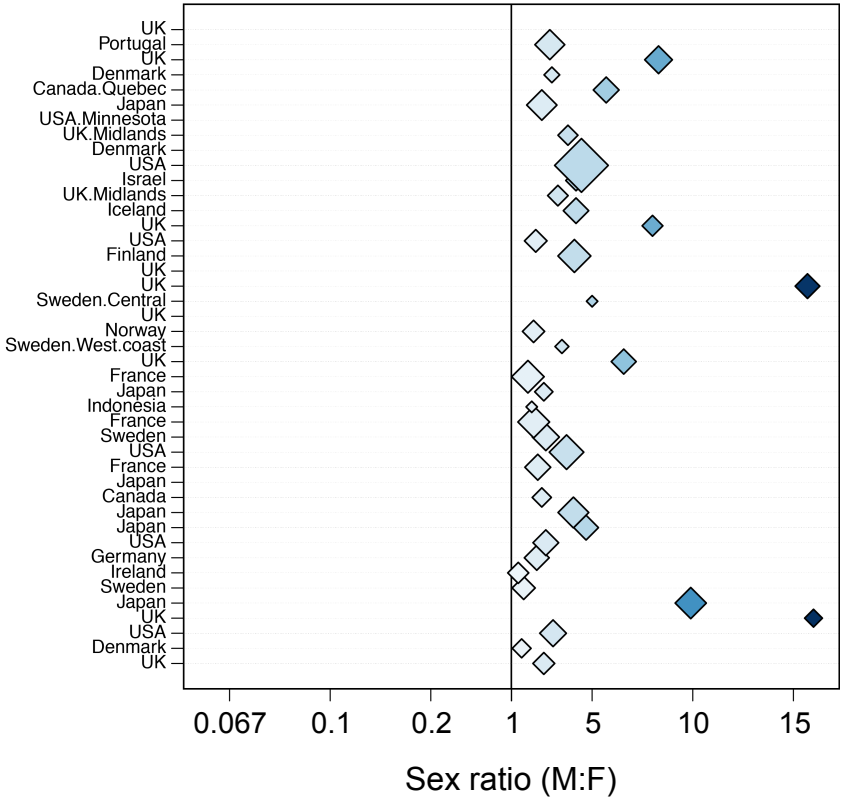
* See The Recent Books.

217

[1]Christensen et al., 2016, *MMWR Surveil. Summ.*, [2]Kanner, 1943, *Nervous Child.*

ASDs have sex-biased prevalence

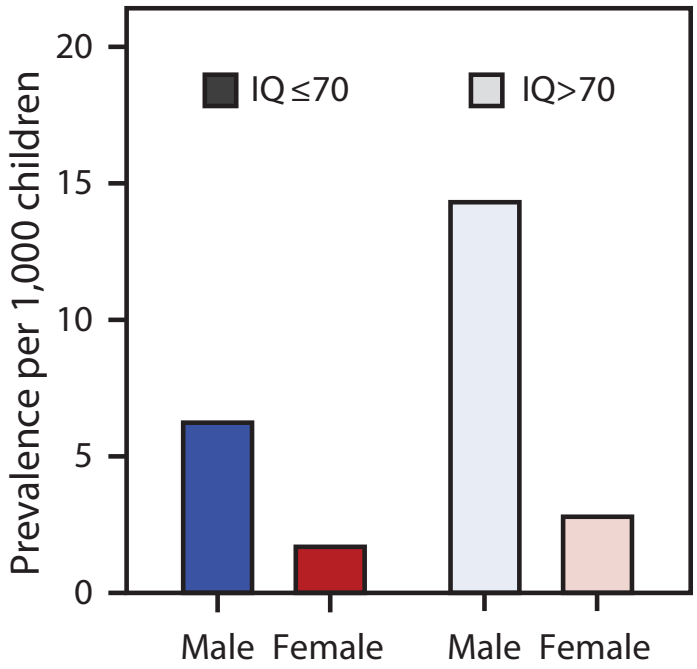
- 4.5:1 males:females have an ASD diagnosis in US[1]
- 8:3 M:F in Leo Kanner's original cases, 1943[2]
- Male bias consistent over time and across countries[3]



[1]Christensen et al., 2016, *MMWR Surveil. Summ.*, [2]Kanner, 1943, *Nervous Child.*, [3]Fombonne, 2009, *Pediatr Res.*

ASDs have sex-biased prevalence

- 4.5:1 males:females have an ASD diagnosis in US[1]
- 8:3 M:F in Leo Kanner's original cases, 1943[2]
- Male bias consistent over time and across countries[3]
- Male bias varies by intellectual ability[1]
  - IQ>70, M:F 5.1:1
  - IQ≤70, M:F 3.7:1



[1]Christensen et al., 2016, *MMWR Surveil. Summ.*, [2]Kanner, 1943, *Nervous Child.*, [3]Fombonne, 2009, *Pediatr Res.*

SPECTRUM

DEEP DIVE

In-depth analysis of important topics in autism.

SEE ALL

ILLUSTRATIONS BY
PEP BOATELLA

The lost girls

Misdiagnosed, misunderstood or missed altogether, many women with autism struggle to get the help they need.

BY **APOORVA MANDAVILLI**
/ 19 OCTOBER 2015

34 COMMENTS

DIAGNOSIS, SCIENCE & SOCIETY, THE BRAIN

DOWNLOAD PDF

SCIENTIFIC AMERICAN

SUBSCRIBE

SHARE LATEST

SCIENTIFIC AMERICAN MIND

MENTAL HEALTH

Autism—It's Different in Girls

New research suggests the disorder often looks different in females, many of whom are being misdiagnosed and missing out on the support they need

By Maia Szalavitz on March 1, 2016 اعرض هذا باللغة العربية

Credit: PAMELA N. MARTIN Getty Images

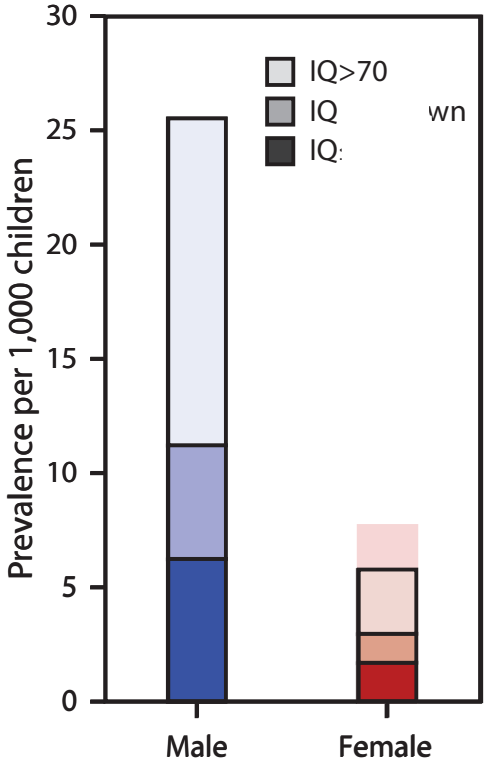
ASD females show different social abilities and strategies than males

- Female children with ASD:
  - Show greater **desire to liked by peers**[1]
  - More likely to use **mimicking as a social strategy**[1]
- Adult, autistic females with normal-to-high IQ:
  - Present with **fewer social communication difficulties** than males (clinician observation)[2]
  - **Self report more ASD traits** than males[2]
- Females might "camouflage" their social difficulties
  - "Conscious, observational learning of how to act in a social setting by adopting social roles and following social scripts"
  - **Requires substantial effort** to maintain this strategy on a prolonged basis

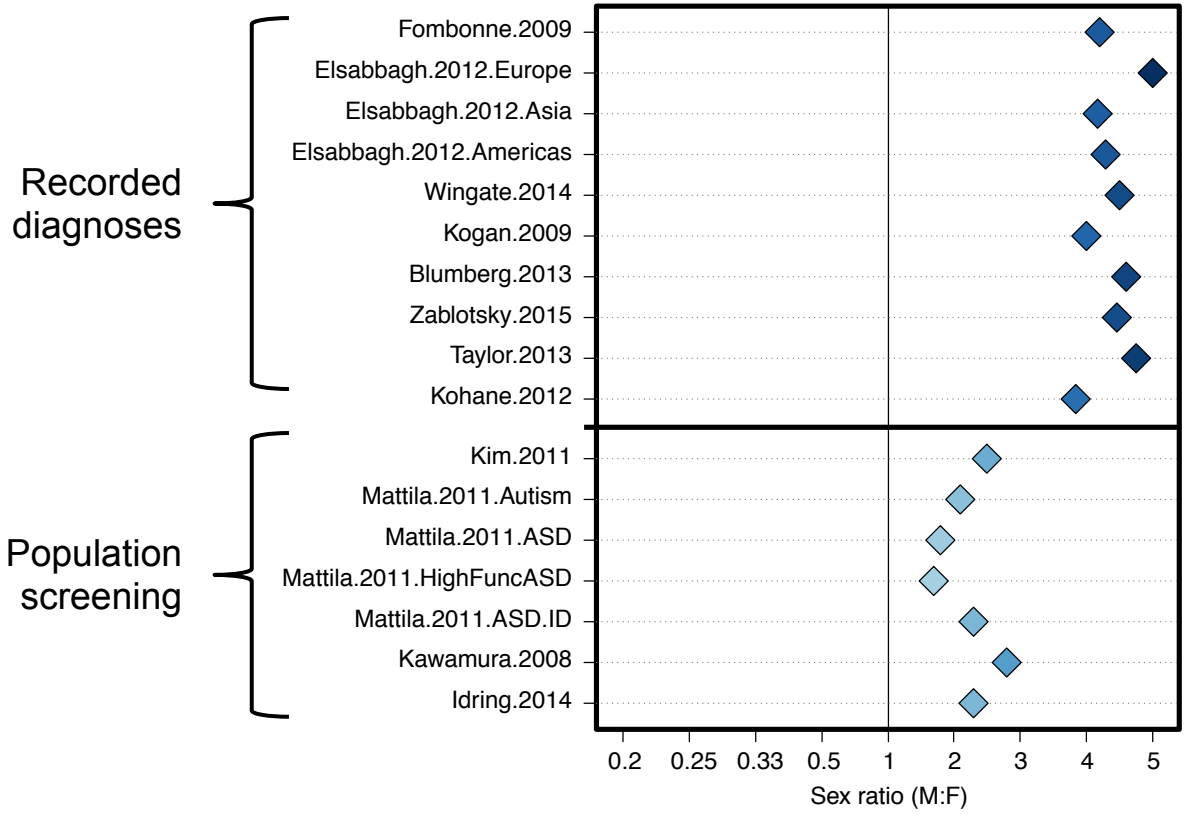
[1]Hiiller et al, 2015, *Autism;* [2]Lai et al, 2011, *PLoS One.*

Questions about ASD diagnoses

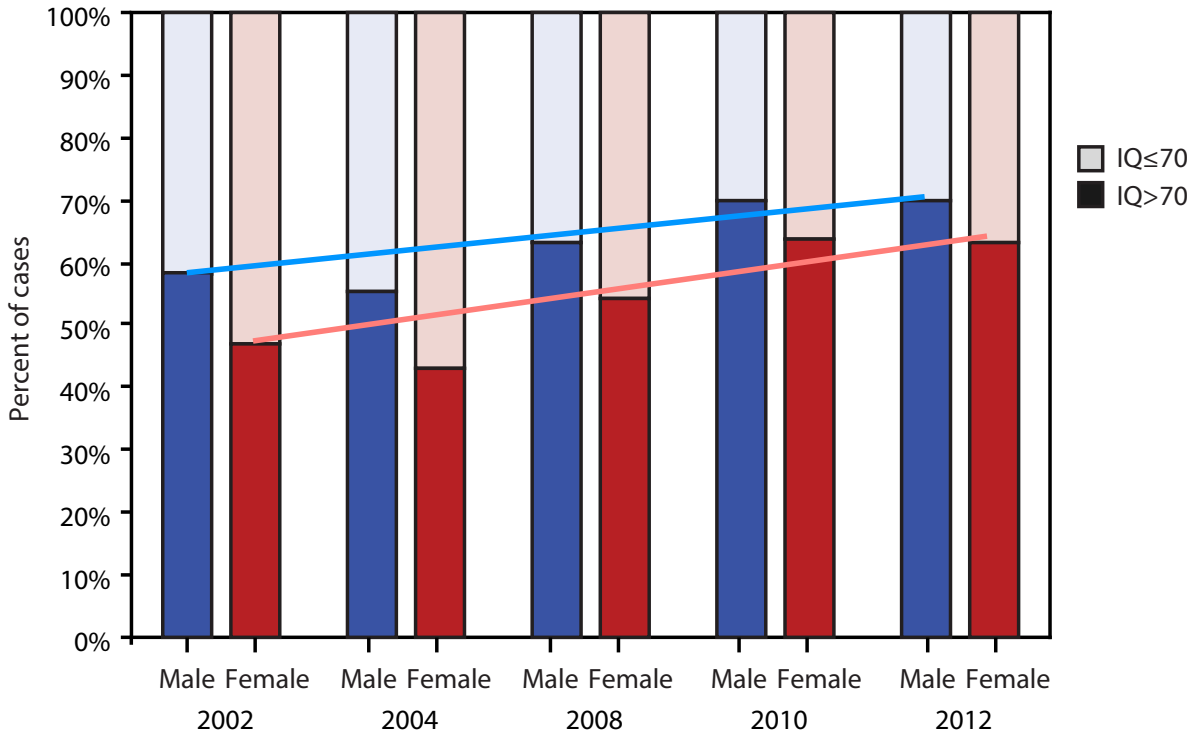
- Are the diagnostic criteria for ASD biased toward a male presentation?
- Are autistic females not being diagnosed?



When we look harder, we find *more* ASD females …but still fewer than males



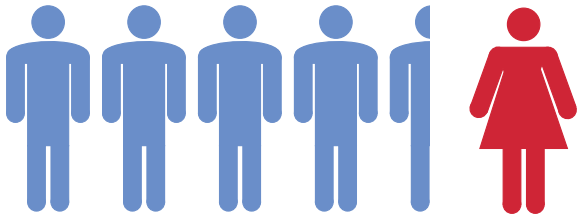
More females, including those with normal-to-high IQ, are being diagnosed as the years go on

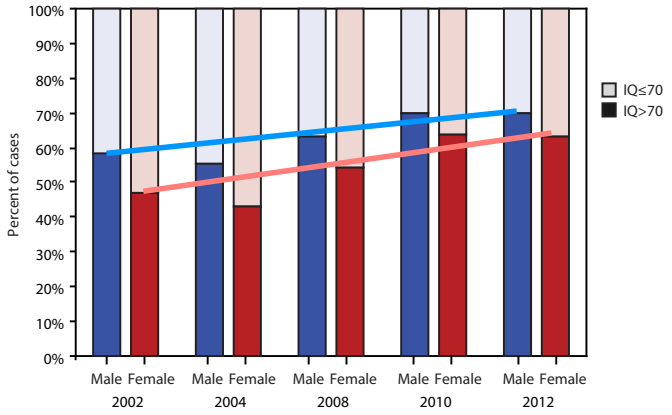


Adapted from CDC ADDM, 2007-2016, *MMWR Surveil. Summ.*

Summary:
Sex differences in ASD prevalence

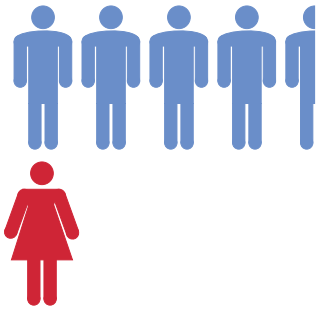
- 4.5 times as many males have an ASD diagnosis than females in the US
- ASD may present differently in females
- Population screens are better at identifying ASD females
- Over time, we are diagnosing more ASD females, especially those with normal-to-high IQ

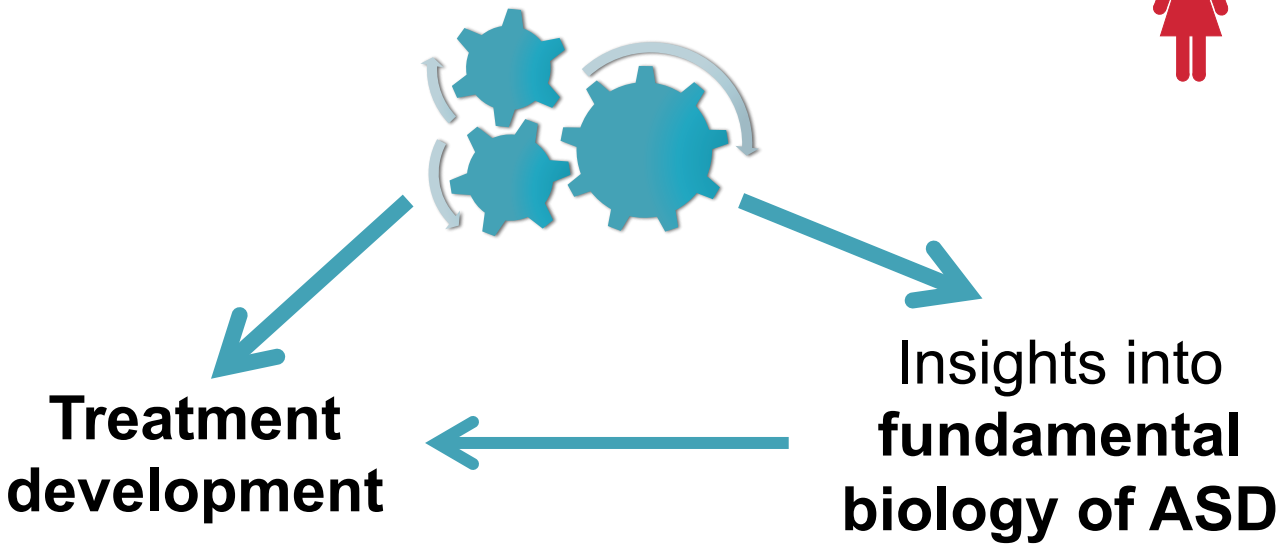




Why study sex bias in ASD from a biological perspective?

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





Outline

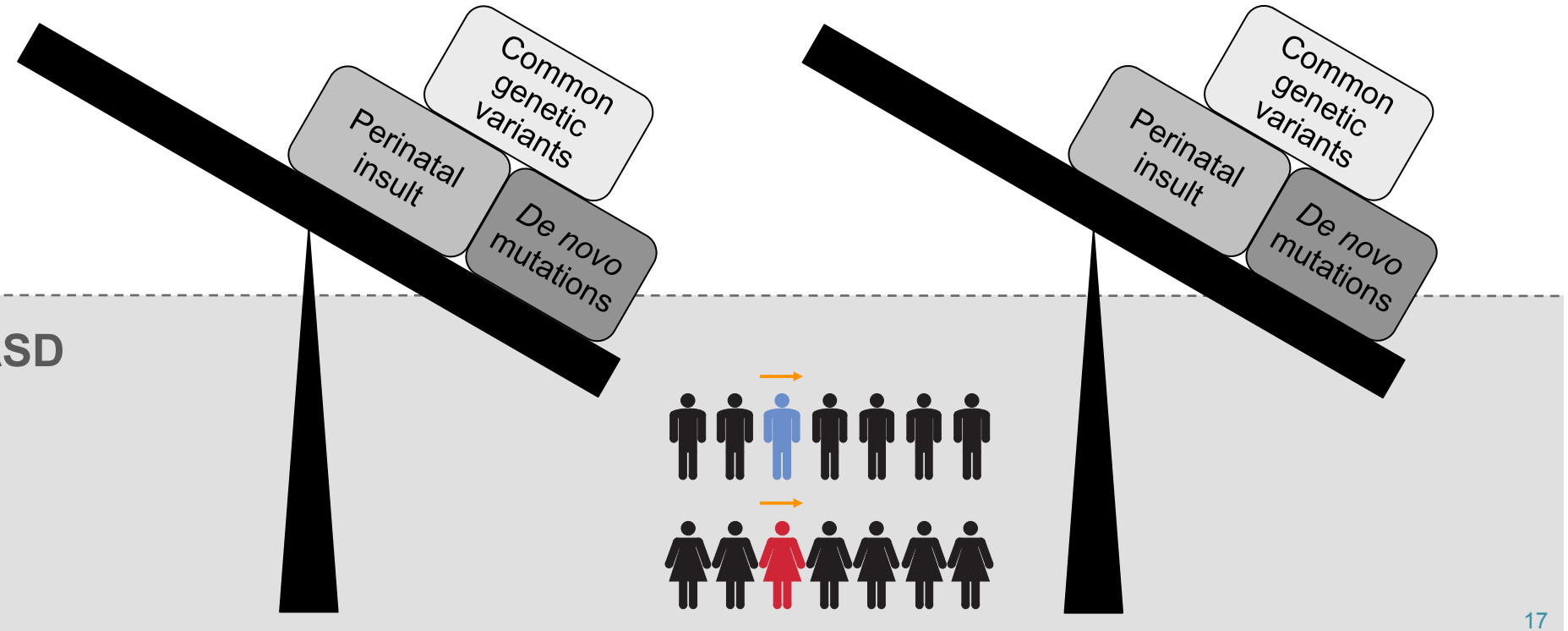
- Autism prevalence and risk in males vs. females

- How scientists think about sex-differential risk: The Female Protective Effect (FPE) model

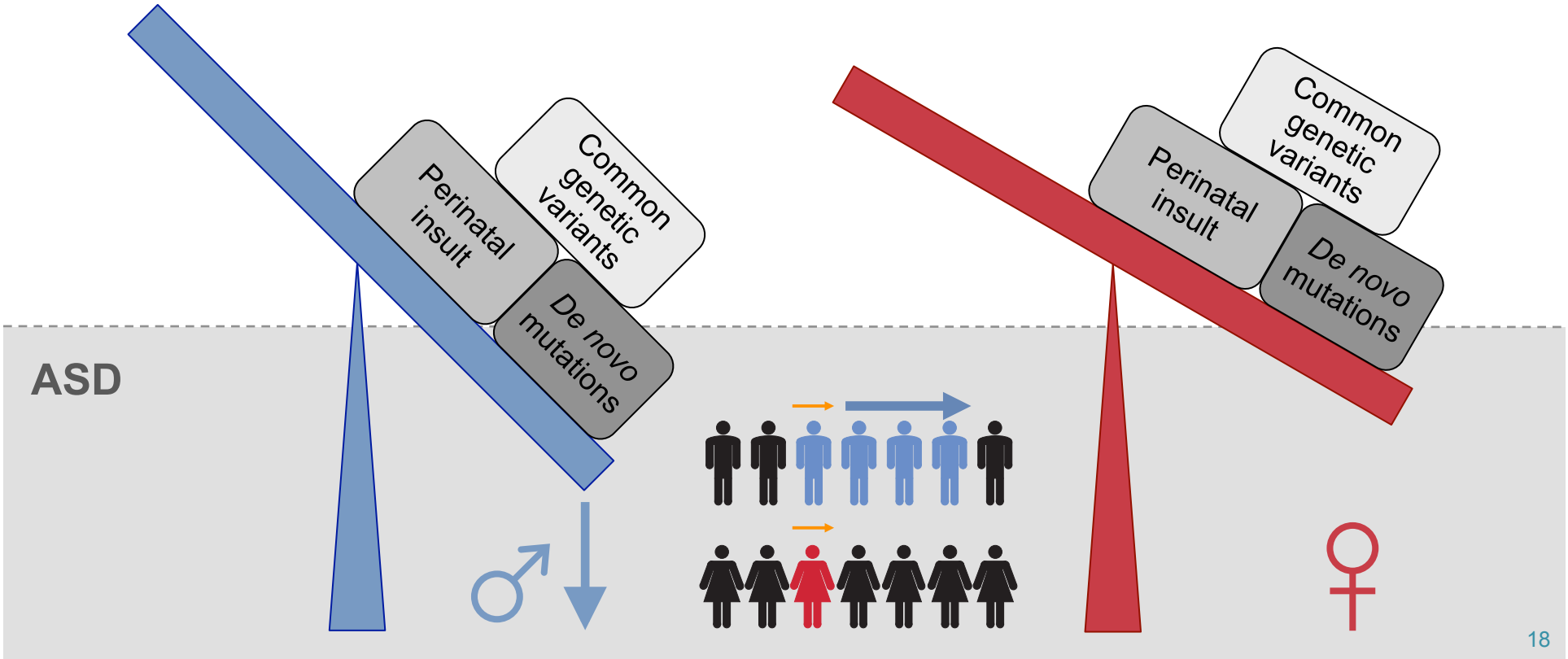
- Research in progress: Relationship between autism biology and sex-differential biology

Male **risk** for, or female **protection** against, ASD?

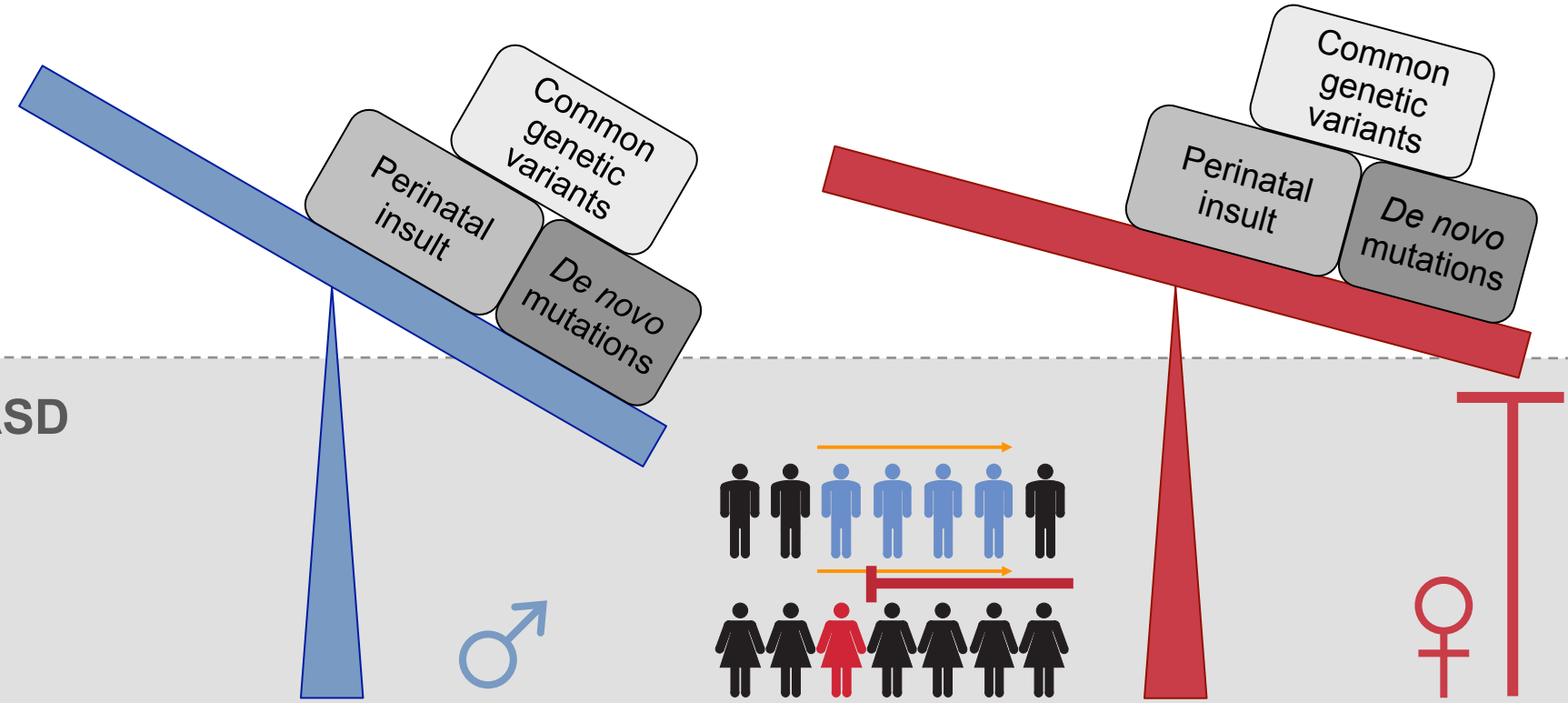
Male **risk** for, or female **protection** against, ASD?



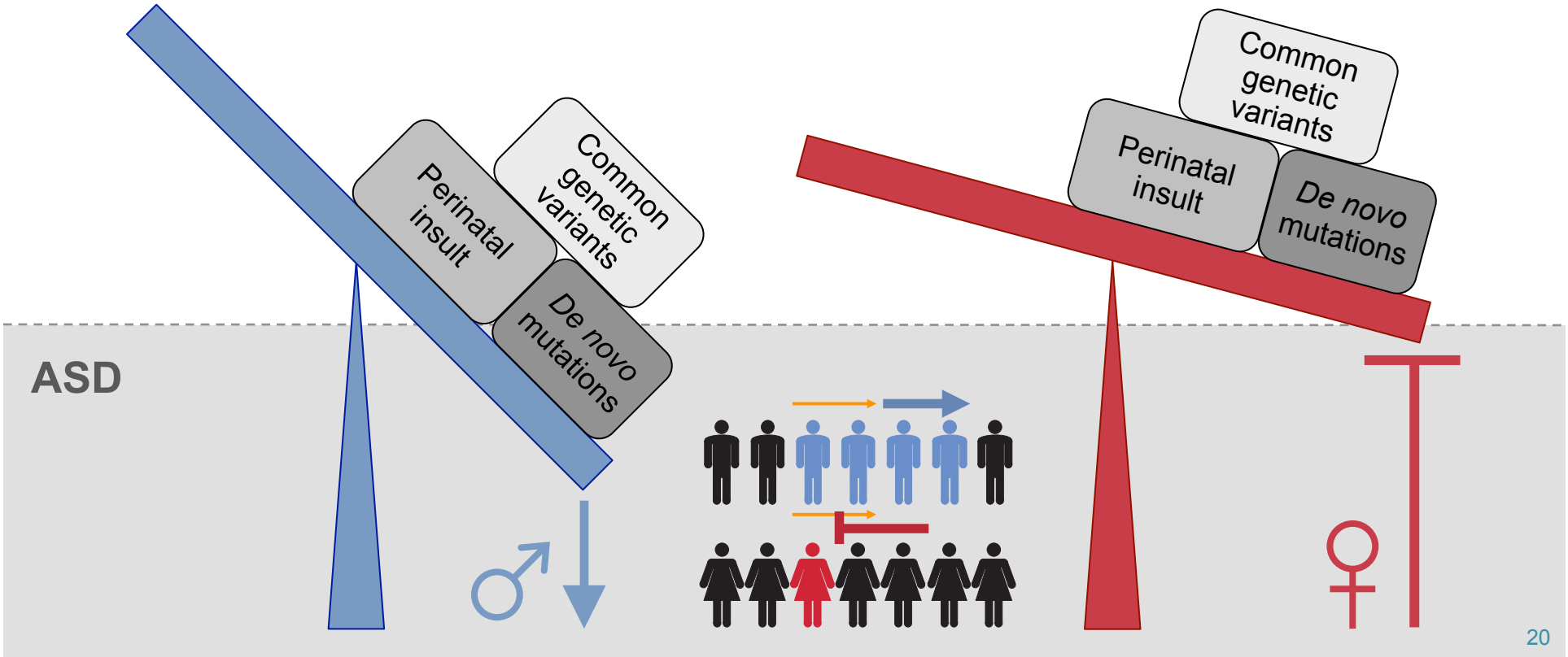
Male **risk** for ASD



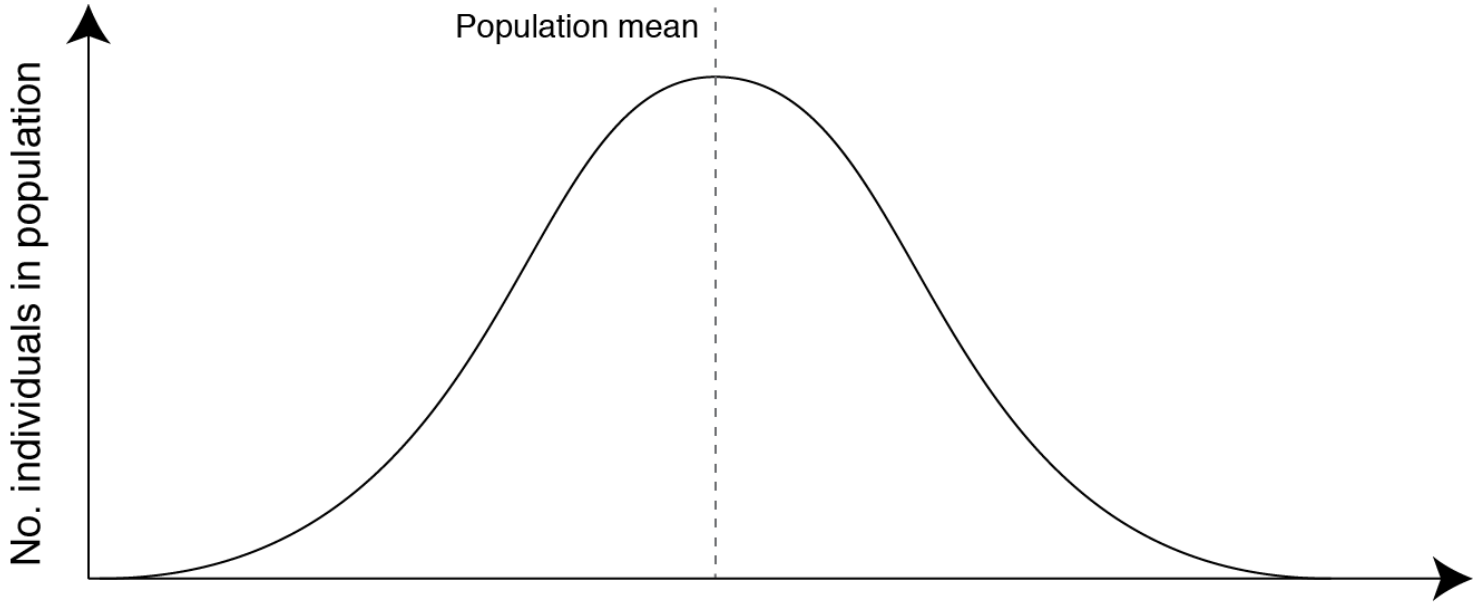
Female **protection** against ASD



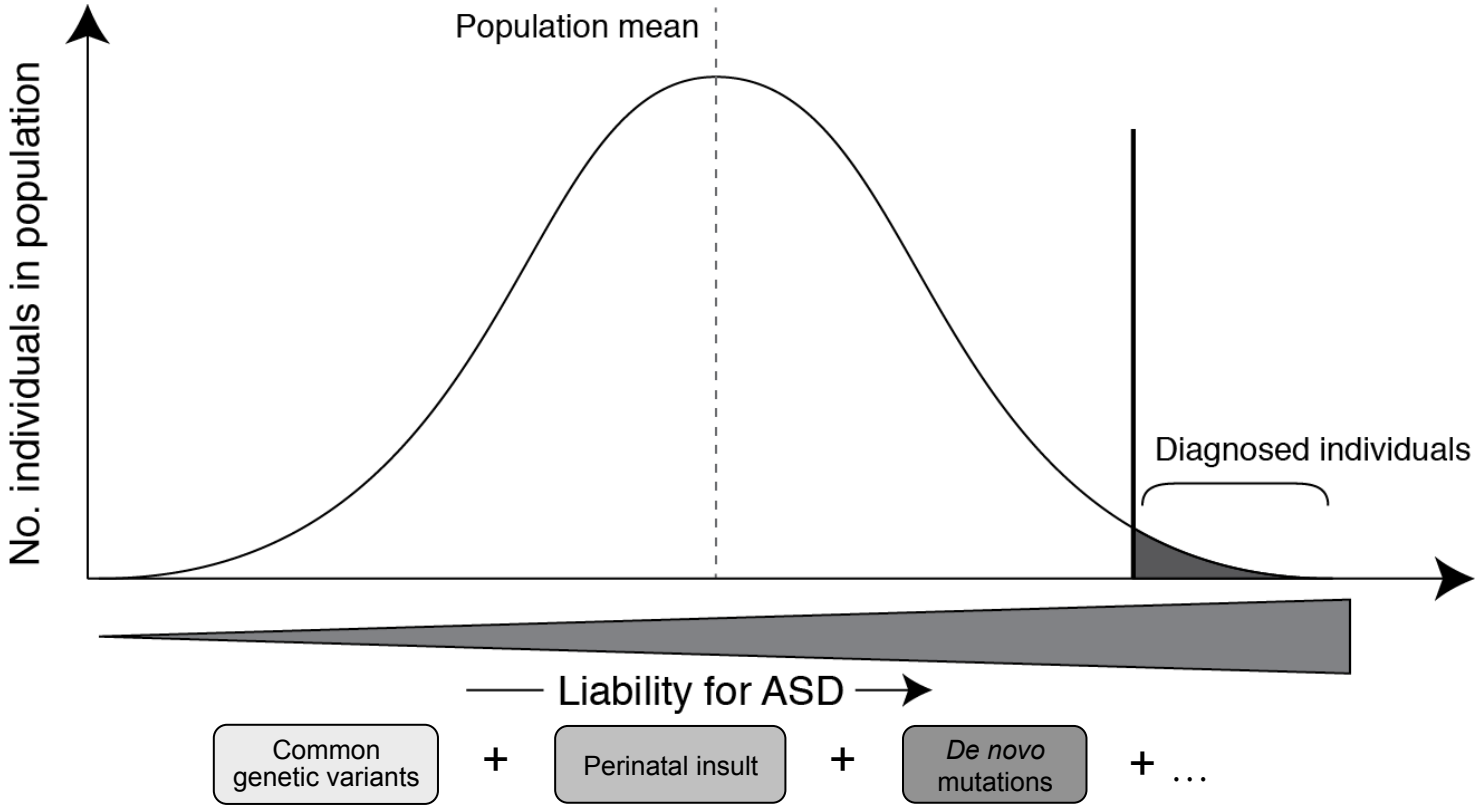
Protection, risk, or both mechanisms may be acting in ASD



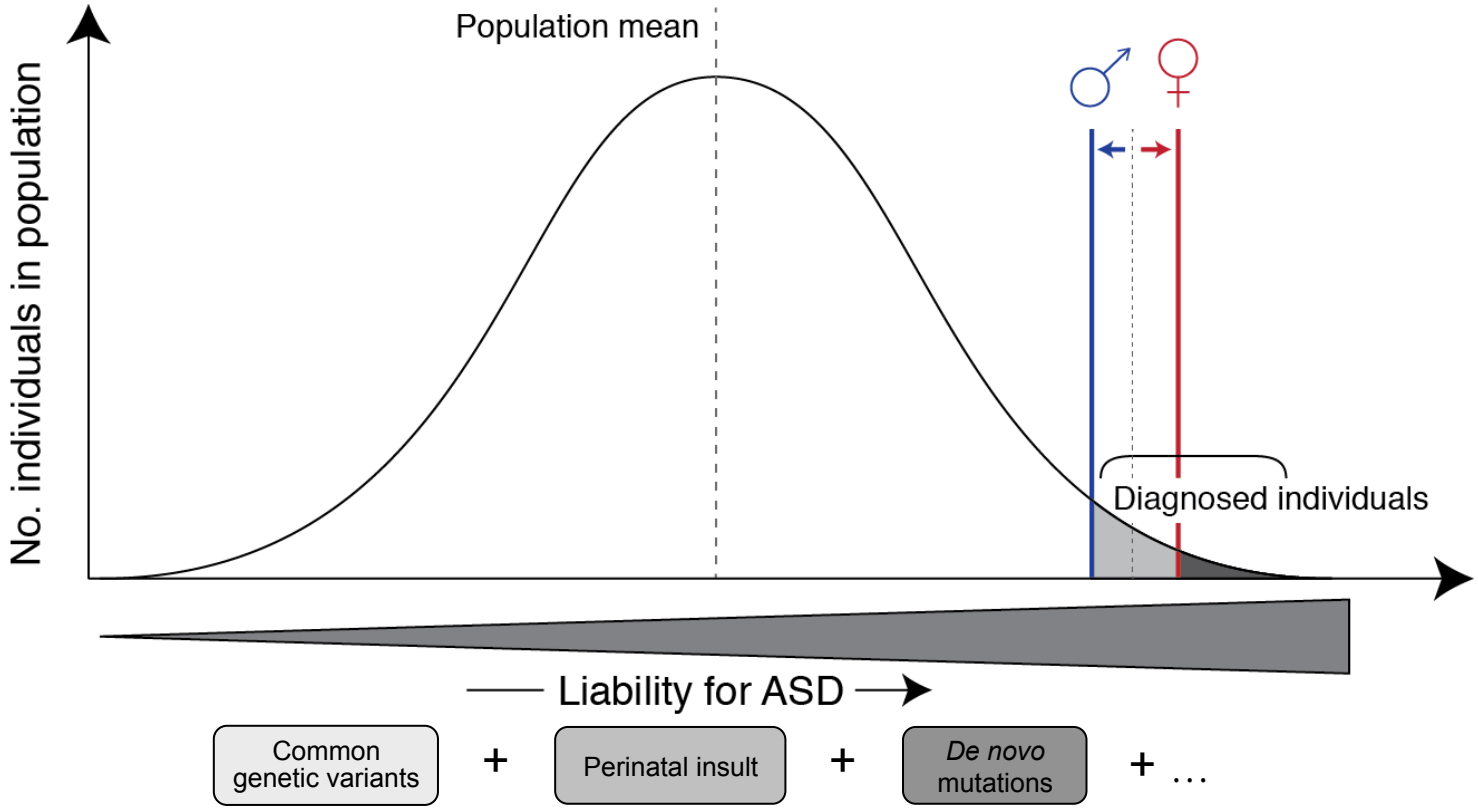
ASD risk is normally distributed in the general population



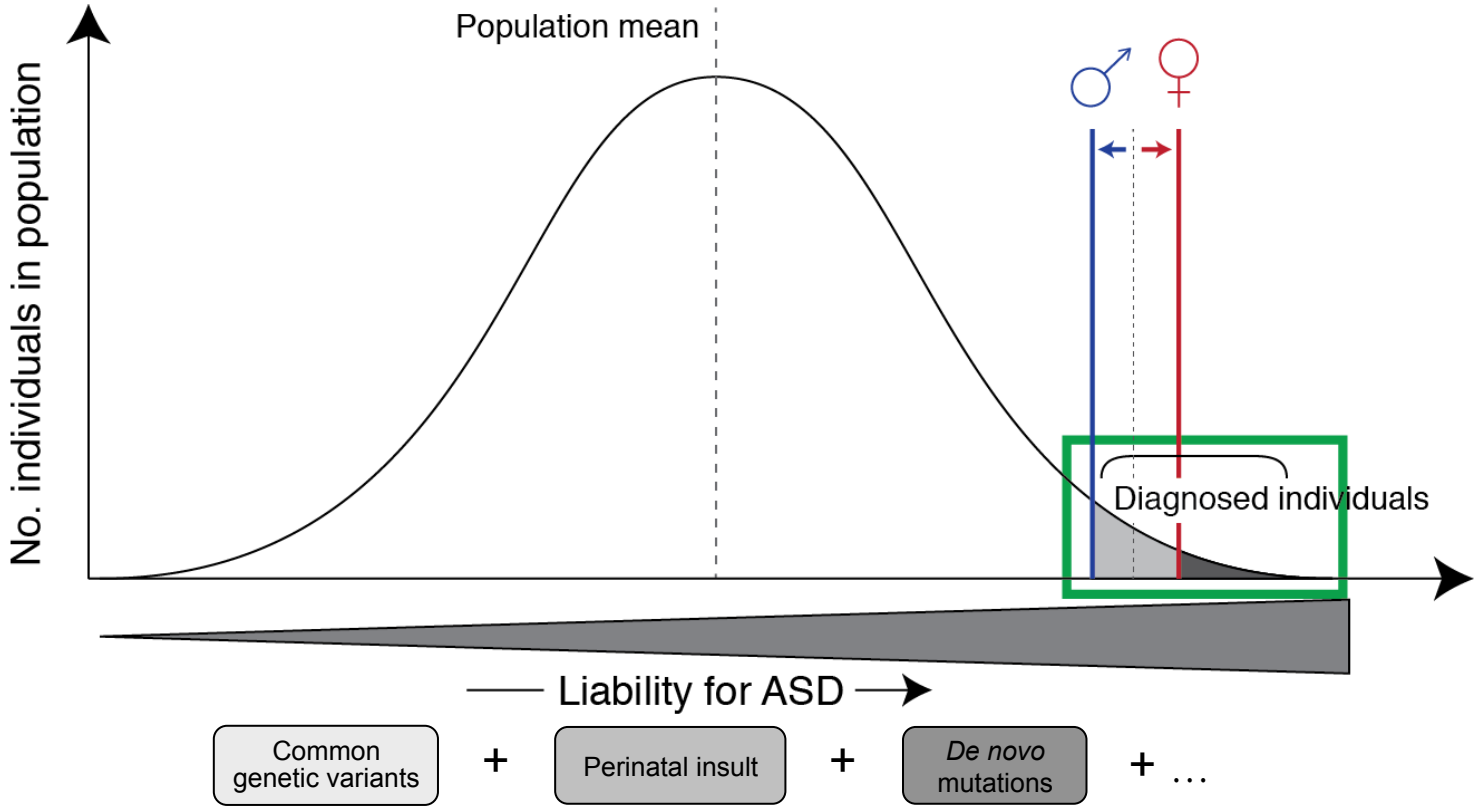
Individuals who have been exposed to high levels of liability present ASD symptoms



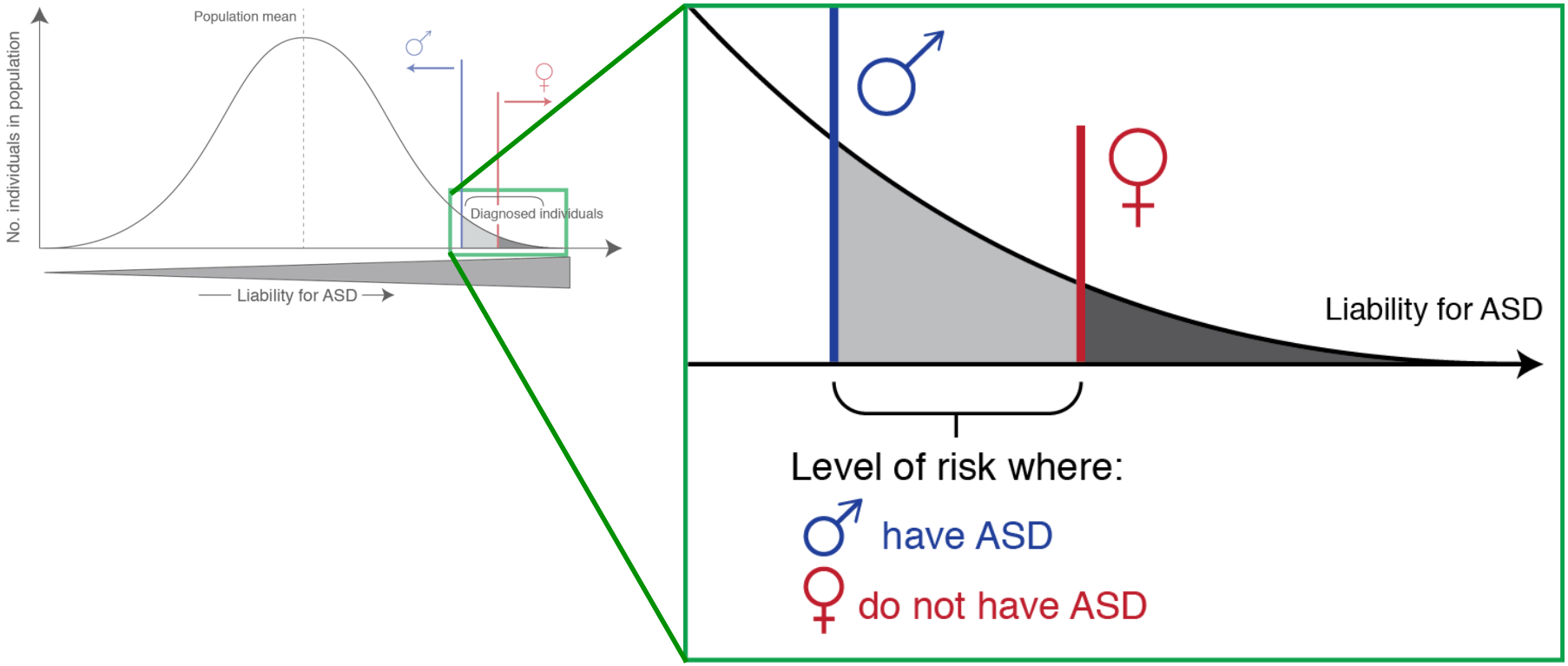
Female Protective Effect Model:
Females have a higher liability threshold than males



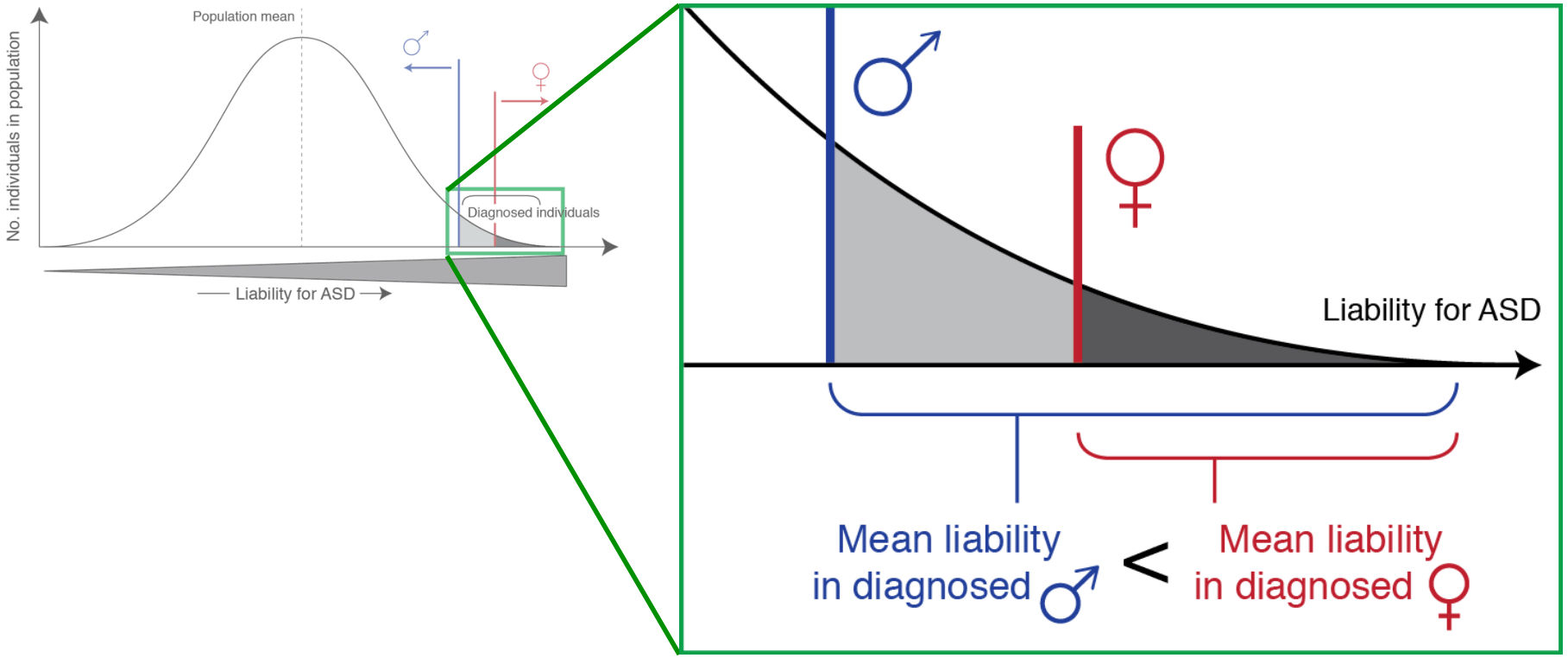
Female Protective Effect Model:
Females have a higher liability threshold than males



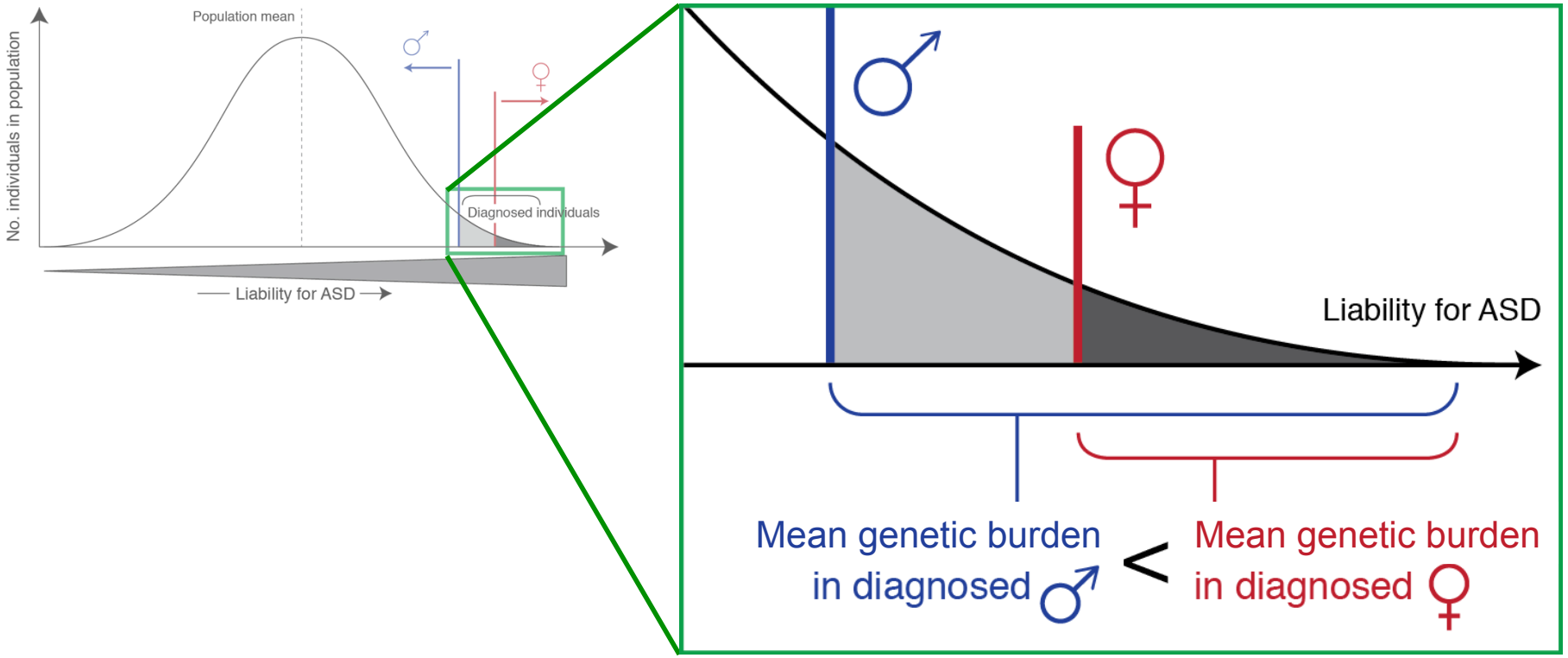
Prediction 1: Females can tolerate/are **protected** from a higher level of risk than males



Prediction 2: Females with ASD have greater burden of risk factors than males

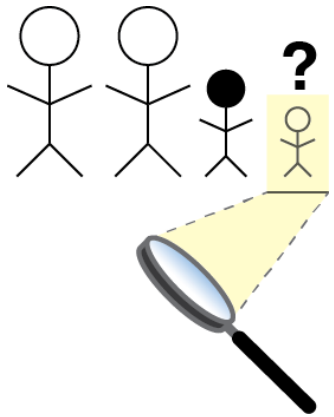


Prediction 2: Females with ASD have greater burden of risk factors than males

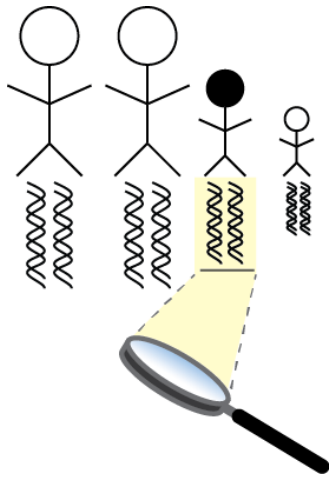


There are 2 ways to test for greater genetic burden in females with ASD

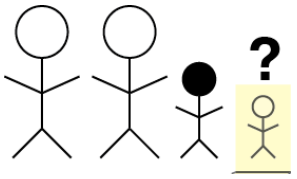
- Indirect:
  - Compare recurrence rates in siblings of ASD females vs. males = “Carter Effect”



- Direct:
  - Compare genetic variants in the genomes of ASD females vs. males

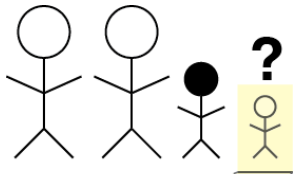


Evidence for higher ASD recurrence in siblings of females is inconsistent



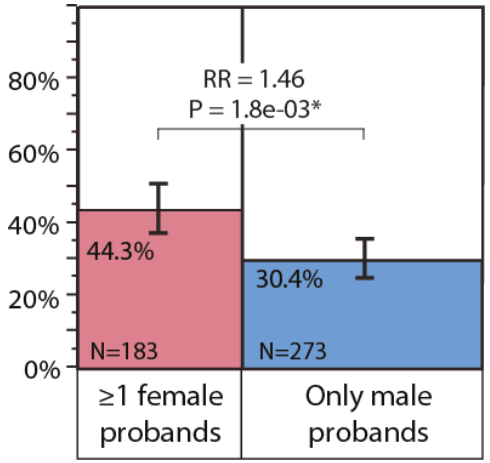
- The sex of the older, affected sibling does not affect recurrence

Evidence for higher ASD recurrence in siblings of females is inconsistent



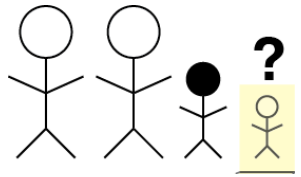
- The sex of the older, affected sibling does not affect recurrence

- Siblings of females with ASD have a higher rate of ASD diagnoses and traits



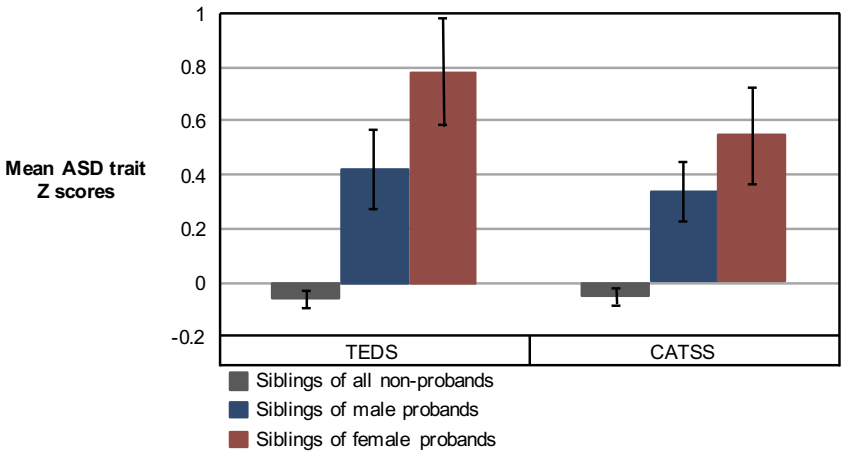
Werling & Geschwind, 2015, *Mol Autism.*

Evidence for higher ASD recurrence in siblings of females is inconsistent



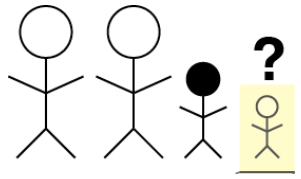
- The sex of the older, affected sibling does not affect recurrence

- Siblings of females with ASD have a higher rate of ASD diagnoses and traits



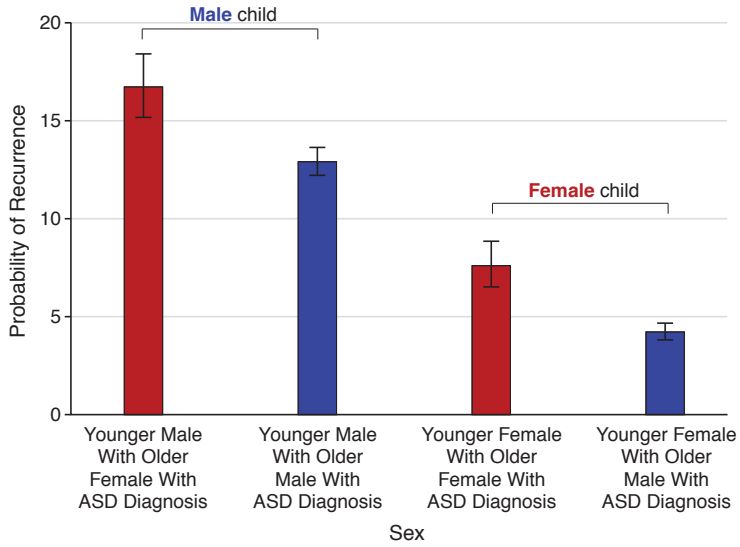
Werling & Geschwind, 2015, *Mol Autism*; Adapted from Robinson et al., 2013, *PNAS*

Evidence for higher ASD recurrence in siblings of females is inconsistent



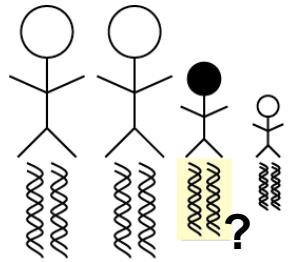
- The sex of the older, affected sibling does not affect recurrence

- Siblings - especially males of females with ASD have a higher rate of ASD diagnoses and traits

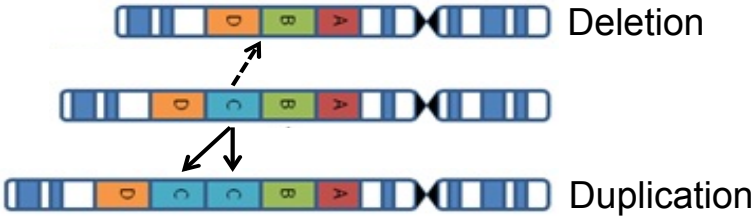


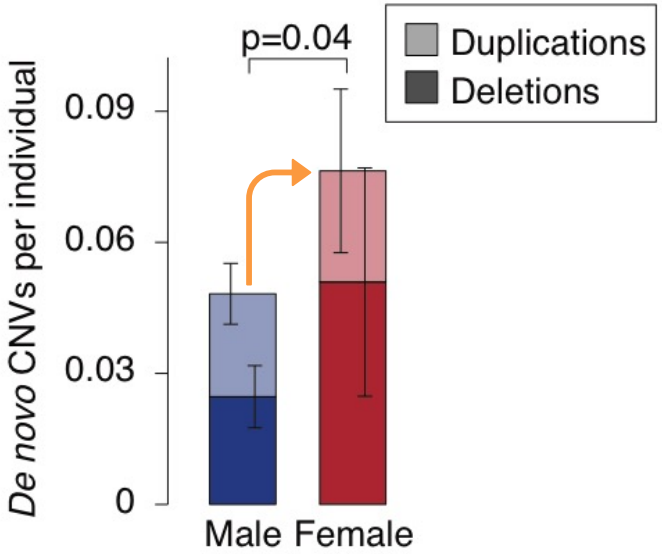
Werling & Geschwind, 2015, *Mol Autism*; Robinson et al., 2013, *PNAS*; Adapted from Palmer et al., 2017, *JAMA Pediatr*

ASD females have a higher incidence of disruptive, *de novo* variants than males



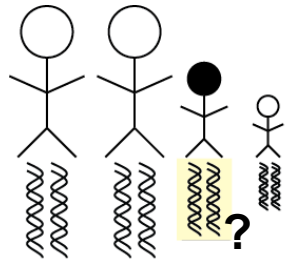
Copy number variants (CNVs):





Adapted from Sanders et al, 2015, *Neuron*.

ASD females have a higher incidence of disruptive, *de novo* variants than males



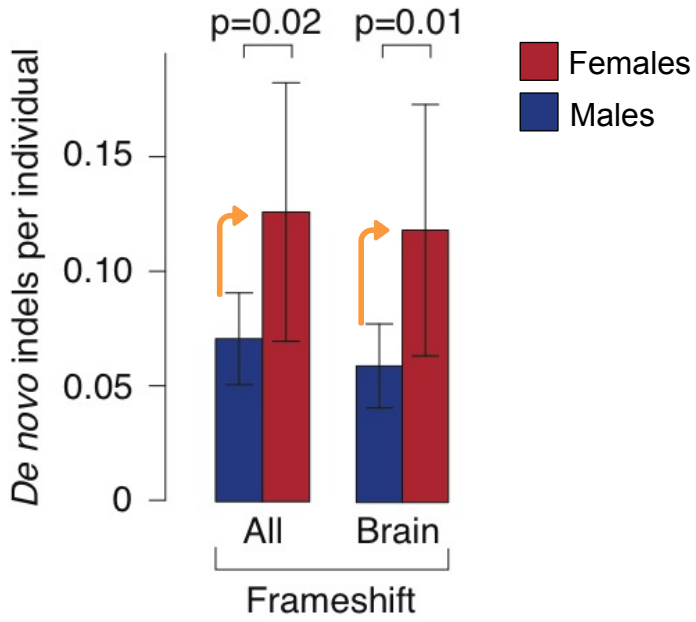
Insertion/Deletions (Indels):

AGGCTACCGTAAGTCG Insertion

AGGCTAAAGTCG

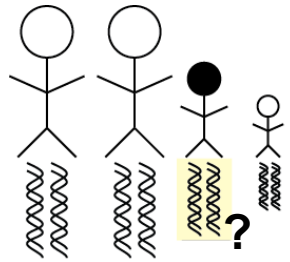
AGGCAAGTCG Deletion

~~TA~~



Adapted from Dong et al, 2014, *Cell Rep.*

ASD females have a higher incidence of disruptive, *de novo* variants than males



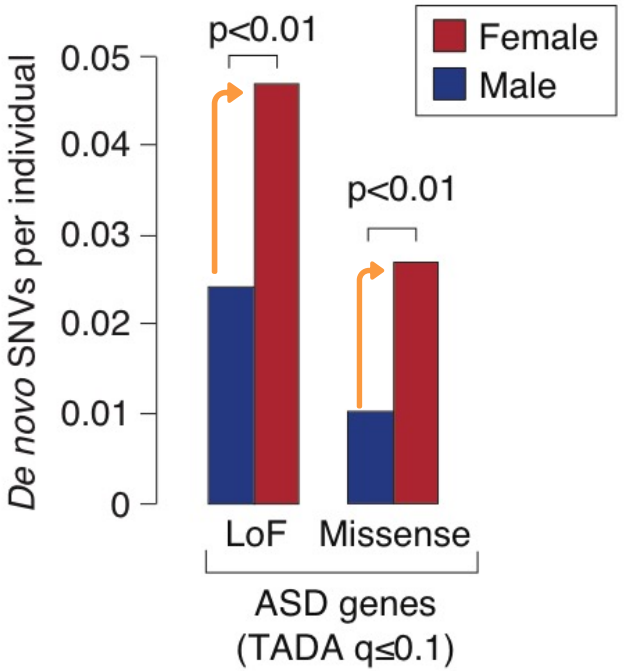
Single nucleotide variants (SNVs):

1ytslpre90heatjkopscna
spovenmbxsd844pojalqtu
tooiewclnviowa56850oab
snedegreesskbinalcbeat
sda44ggkwbutterasandie
urnasugarnncippefmnbl8
9lmcdinoomfmosalargeSTOP

Missense variant

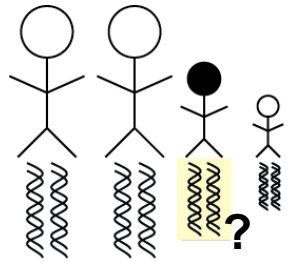
Nonsense variant (Loss-of-function, LoF)

Silent variant

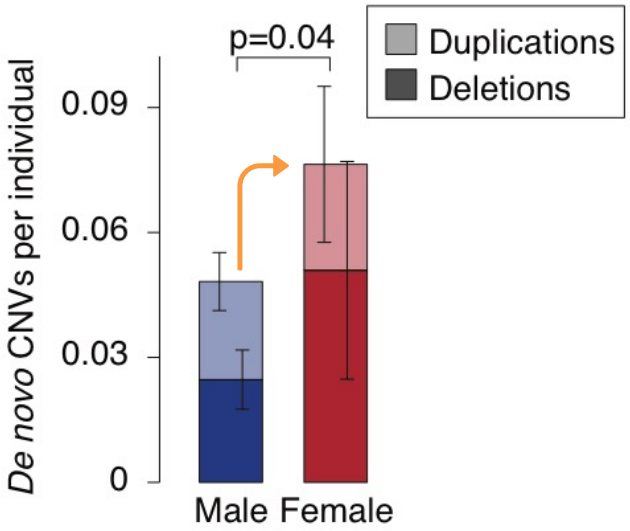


Adapted from De Rubeis et al, 2014, *Nature*.

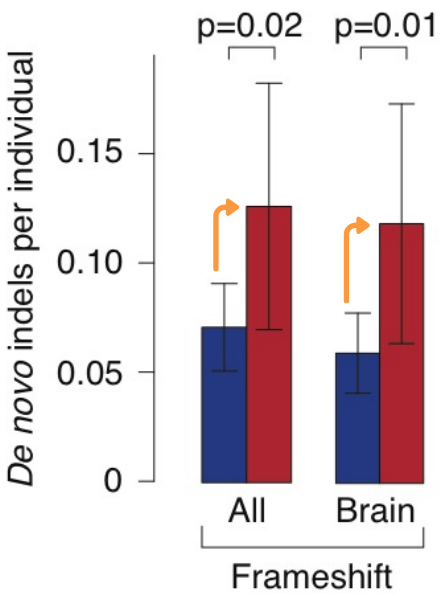
ASD females have a higher incidence of disruptive, *de novo* variants than males



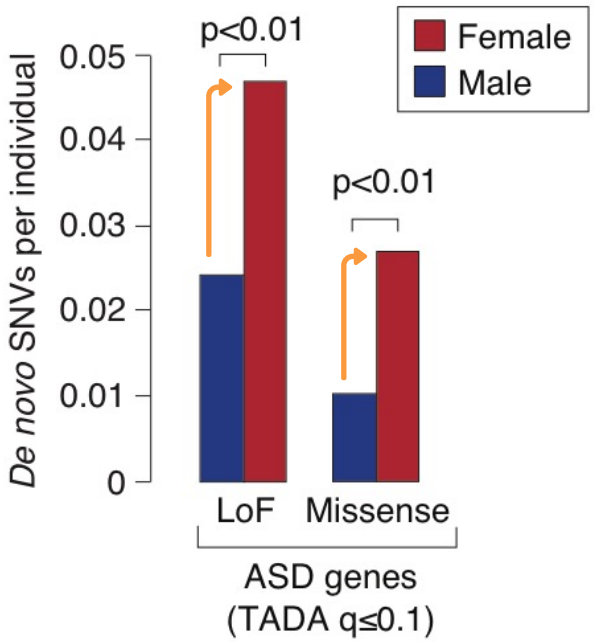
Copy number variants (CNVs)[1]



Insertion/Deletions (Indels)[2]



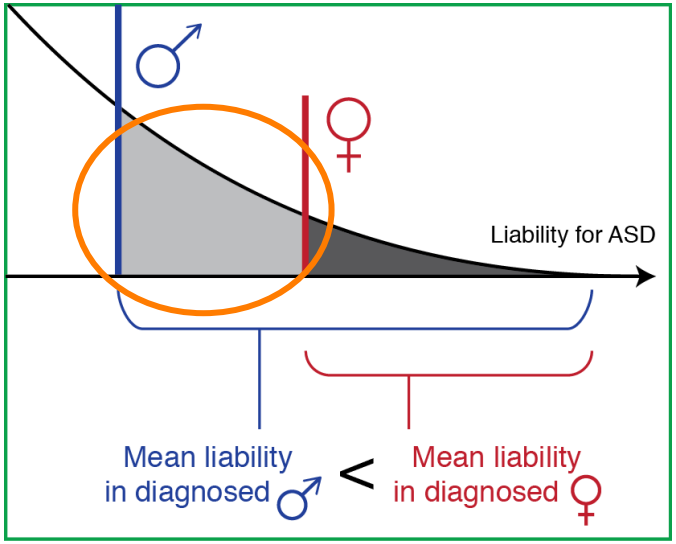
Single nucleotide variants (SNVs)[3]



Adapted from: [1]Sanders et al, 2015, *Neuron.* [2]Dong et al, 2014, *Cell Rep.* [3]De Rubeis et al, 2014, *Nature.*

Summary:
Female Protective Effect (FPE) model

- Females with diagnosed ASD show *greater genetic liability* than diagnosed males
  - More frequent, larger, more deleterious genetic variants
  - Genetic liability might be shared with siblings, in some cases
- Pattern suggests that females are protected from less deleterious variants/risk factors



What is responsible for this protective effect?

Outline

- Autism prevalence and risk in males vs. females

- How scientists think about sex-differential risk: The Female Protective Effect (FPE) model

- Research in progress: Relationship between autism biology and sex-differential biology

Do known sexual dimorphisms contribute to ASD risk?

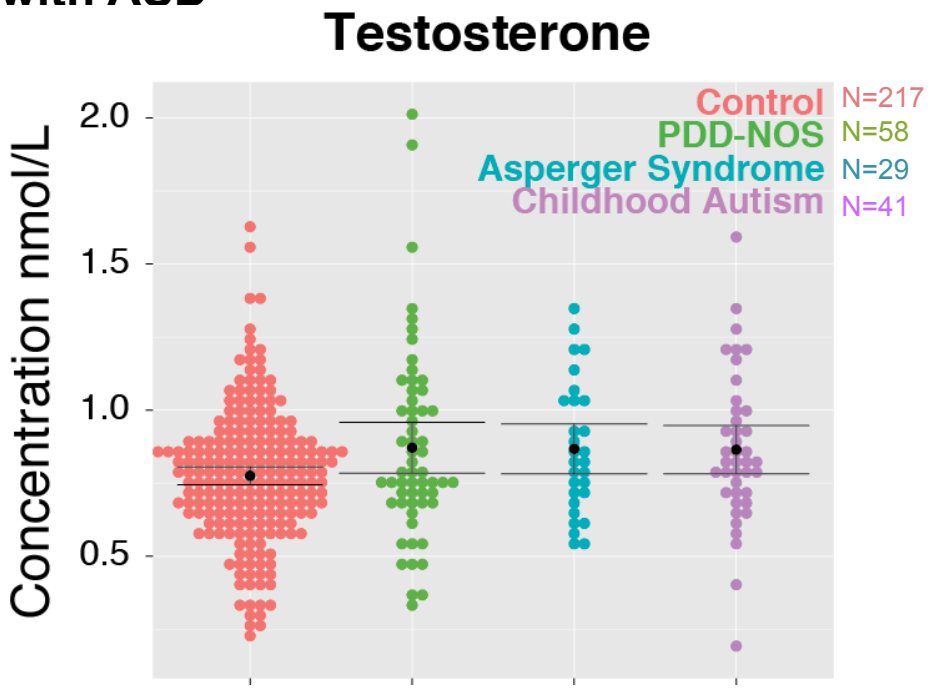
Proposed mechanisms

Hypothesis	Males	Females
X-linked condition	X_mY = 1 X	X_pX_m = 2 Xs
X chr carries protective genes, imprinted & paternally expressed[1]	X_mY = NO paternal X	X_pX_m = has paternal X
Fetal testosterone exposure increases risk (aka Extreme Male Brain theory[2])	T exposure, prenatally	Little/no T exposure prenatally

[1]Skuse, 2000, *Pediatr Res.* [2]Baron-Cohen, 2002, *Trends Cogn Sci.*

Elevated prenatal testosterone exposure is associated with ASD diagnoses

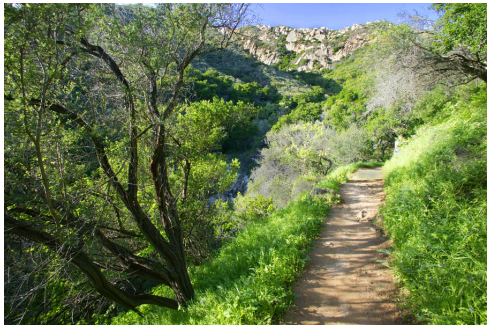
Fetal testosterone levels are elevated in males who are later diagnosed with ASD



Baron-Cohen et al, 2014, *Mol Psychiatry.*

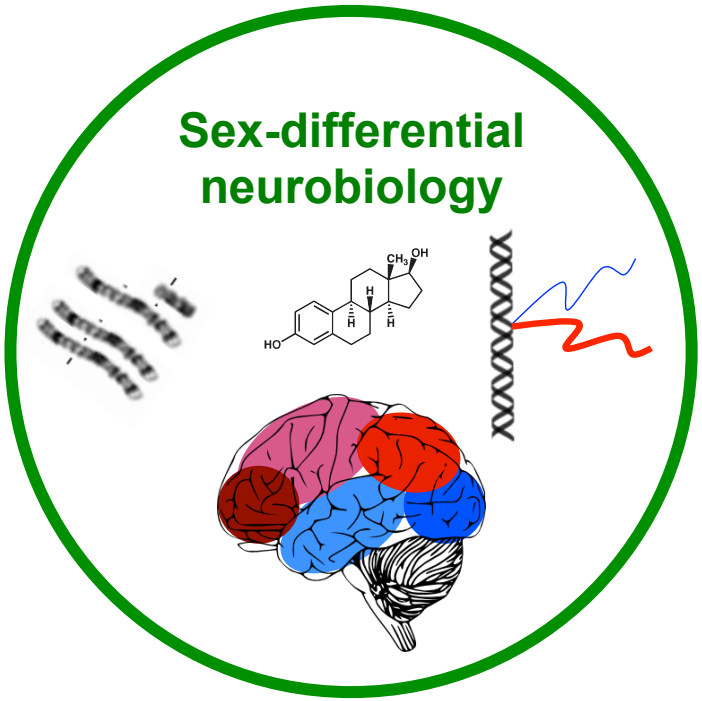
Genomic analyses provide an alternate approach for identifying key mechanisms

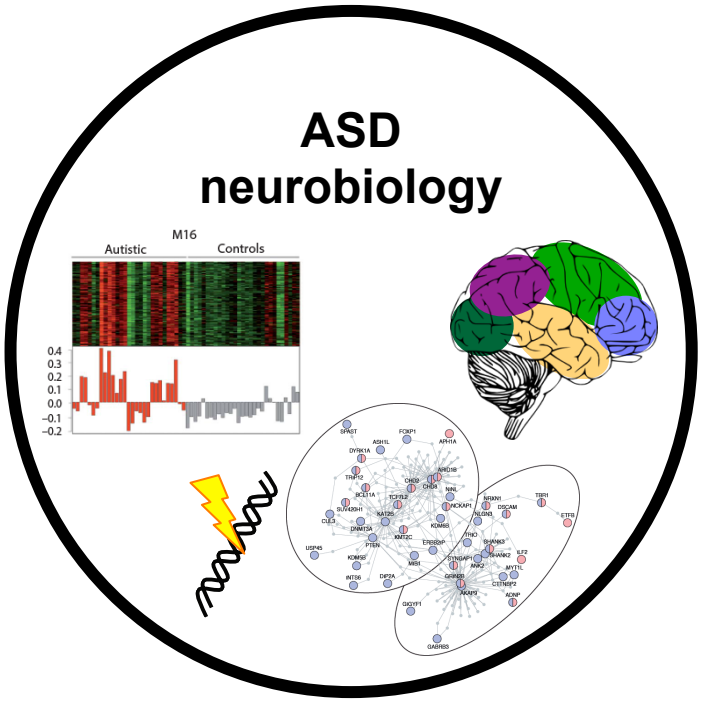
- Candidate mechanism approach
  - Sex chromosomes
  - Sex hormones
  - Sexually dimorphic neural circuitry (e.g. hypothalamic nuclei)



- Genome-wide survey approach
  - Characterize sex differences in the brain
  - Characterize ASD differences in the brain
  - Look for common processes

How can we find the factors that protect females, or increase males' risk?

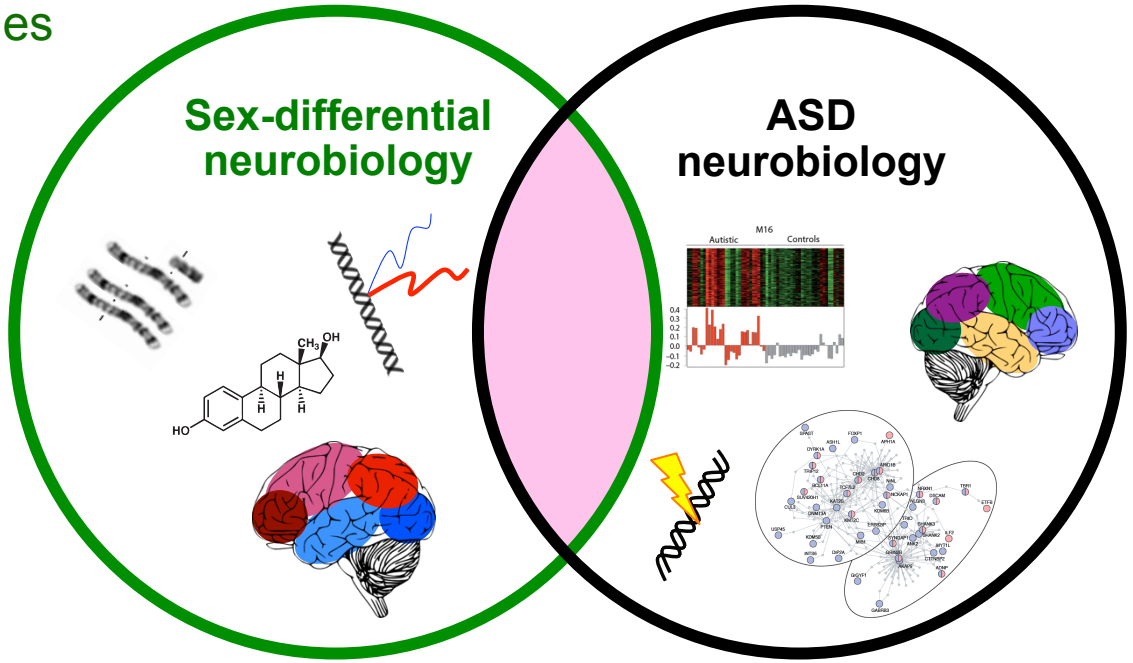




How can we find the factors that protect females, or increase males' risk?

- Identify biological processes that:
  - Differ between typical males and females

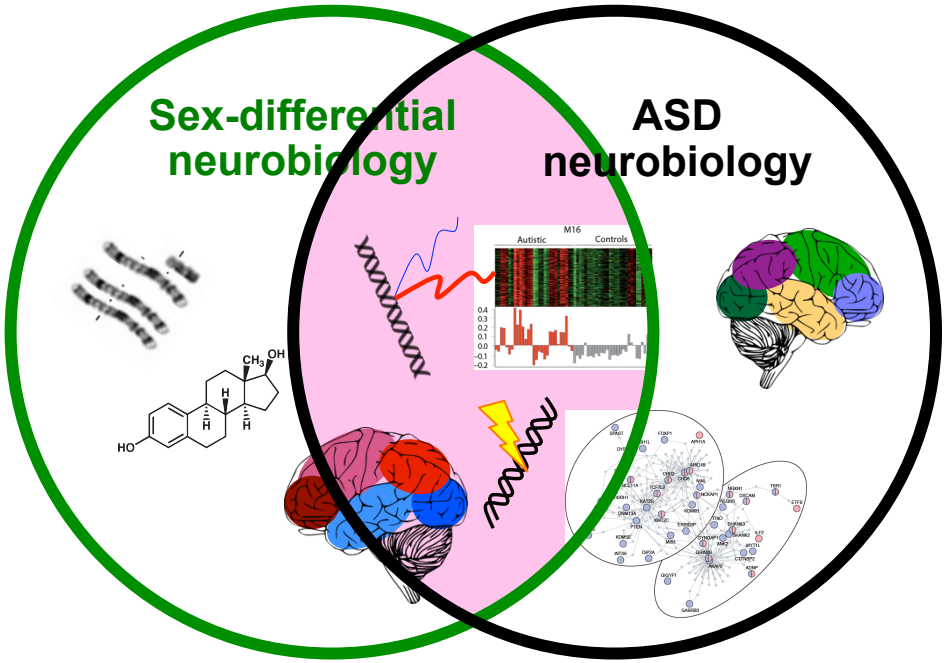
    and
  - Are affected in ASD



How can we find the factors that protect females, or increase males' risk?

- Identify biological processes that:
  - Differ between typical males and females

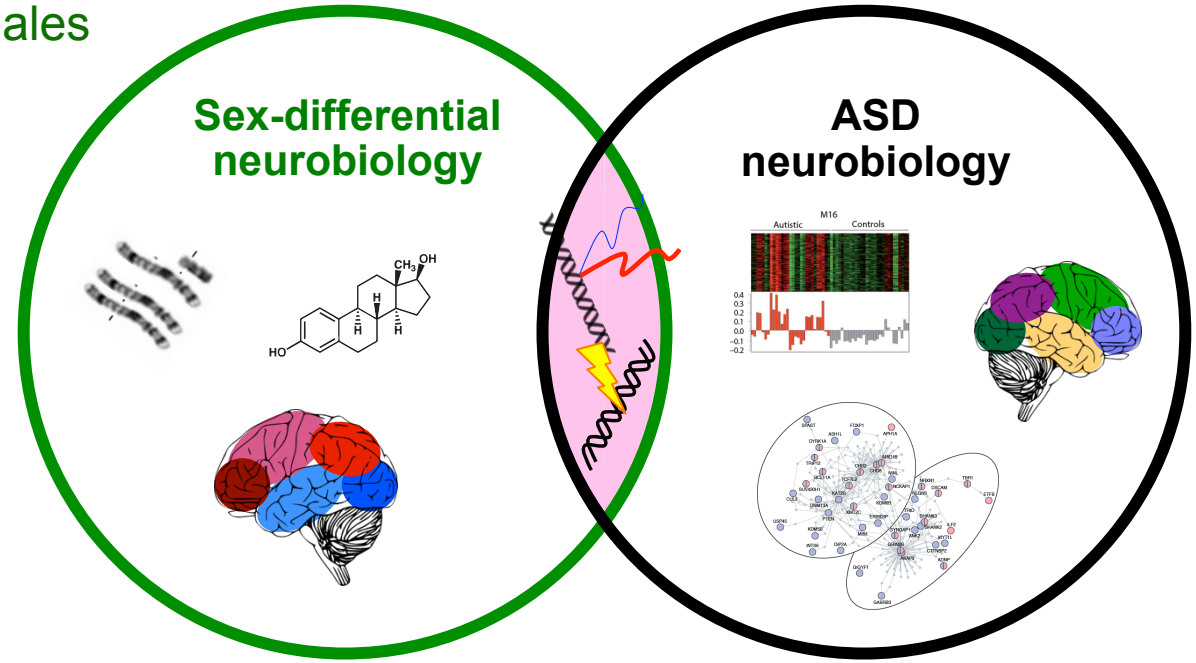
    and
  - Are affected in ASD



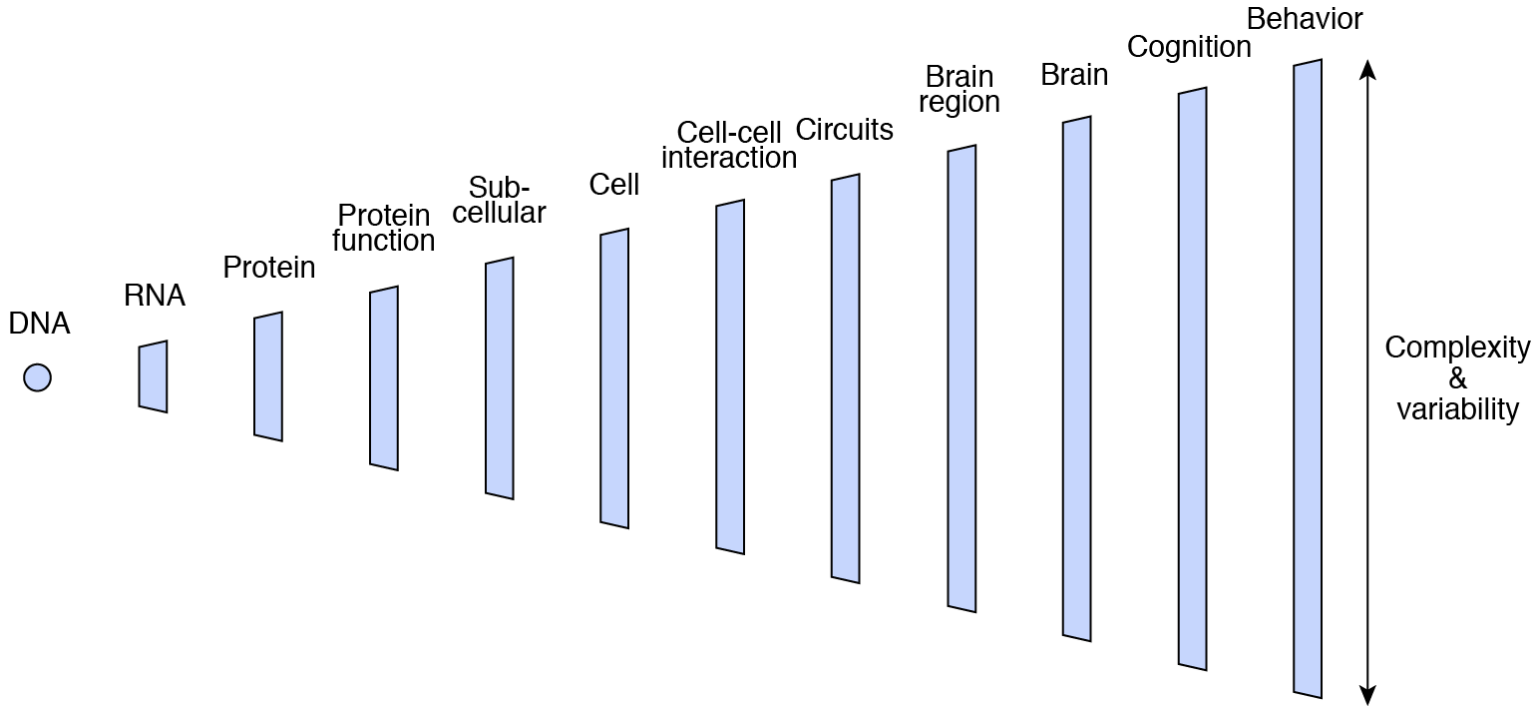
How can we find the factors that protect females, or increase males' risk?

- Identify biological processes that:
  - Differ between typical males and females

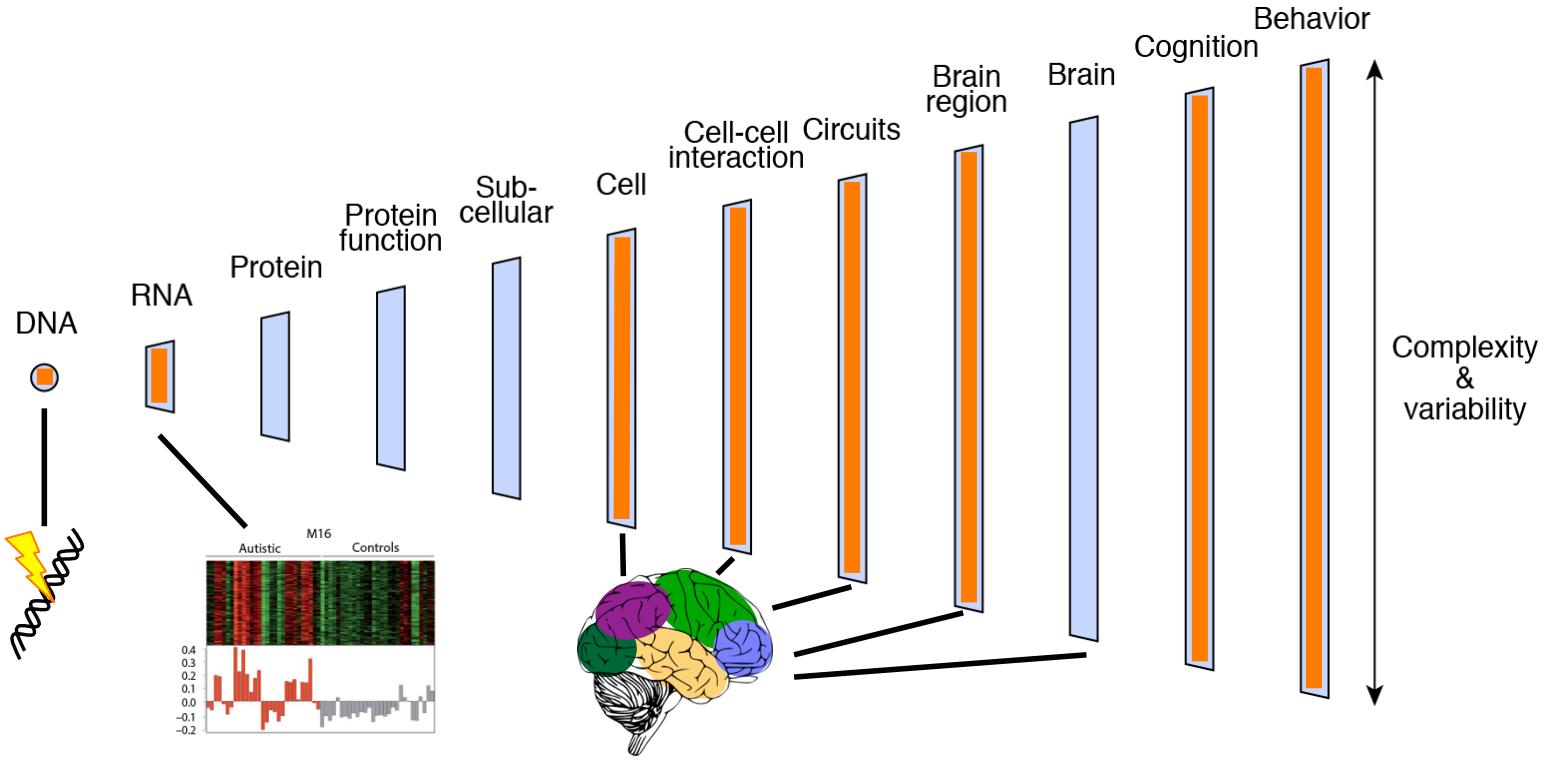
    and
  - Are affected in ASD



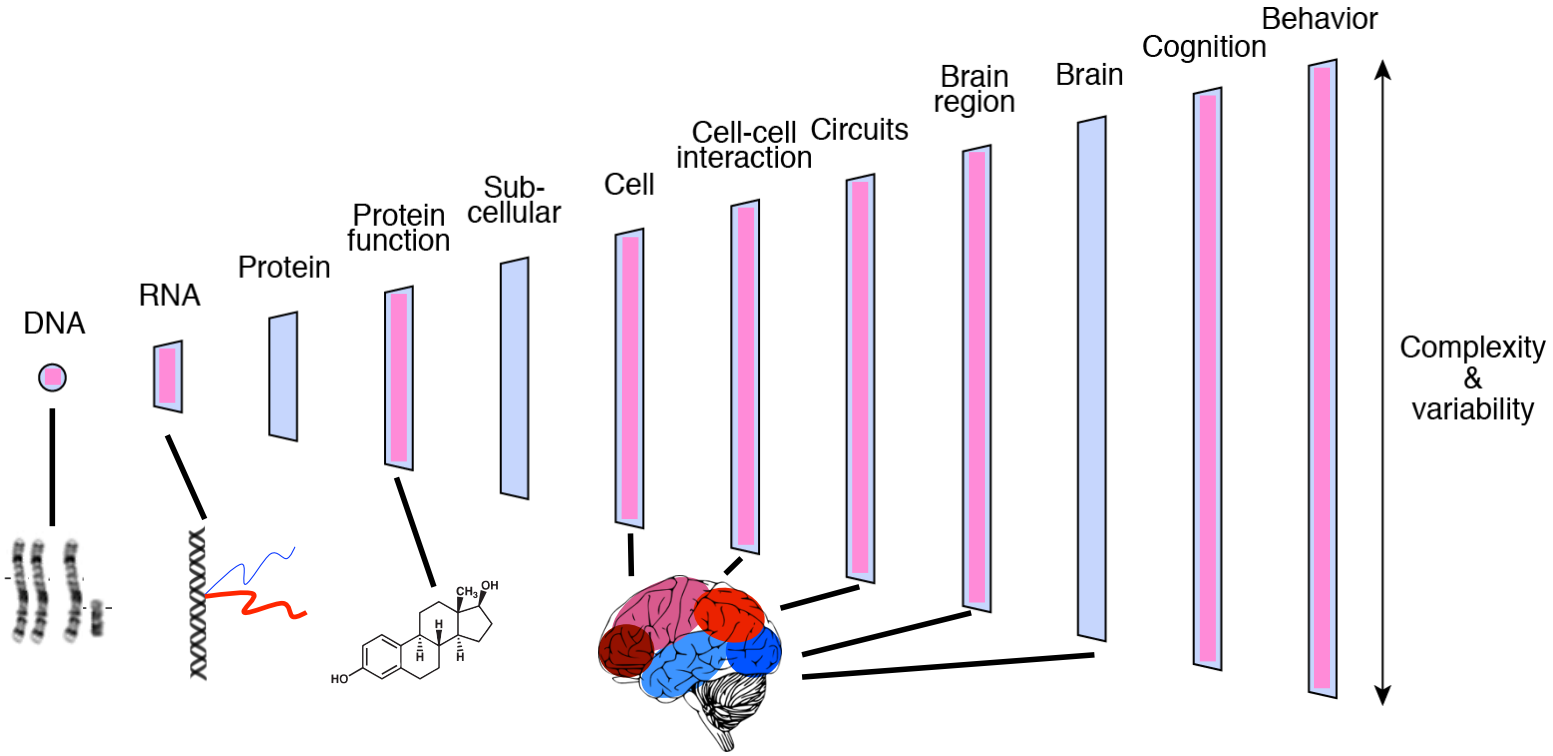
ASD cognition and behavior are the end result of changes across multiple levels of biology



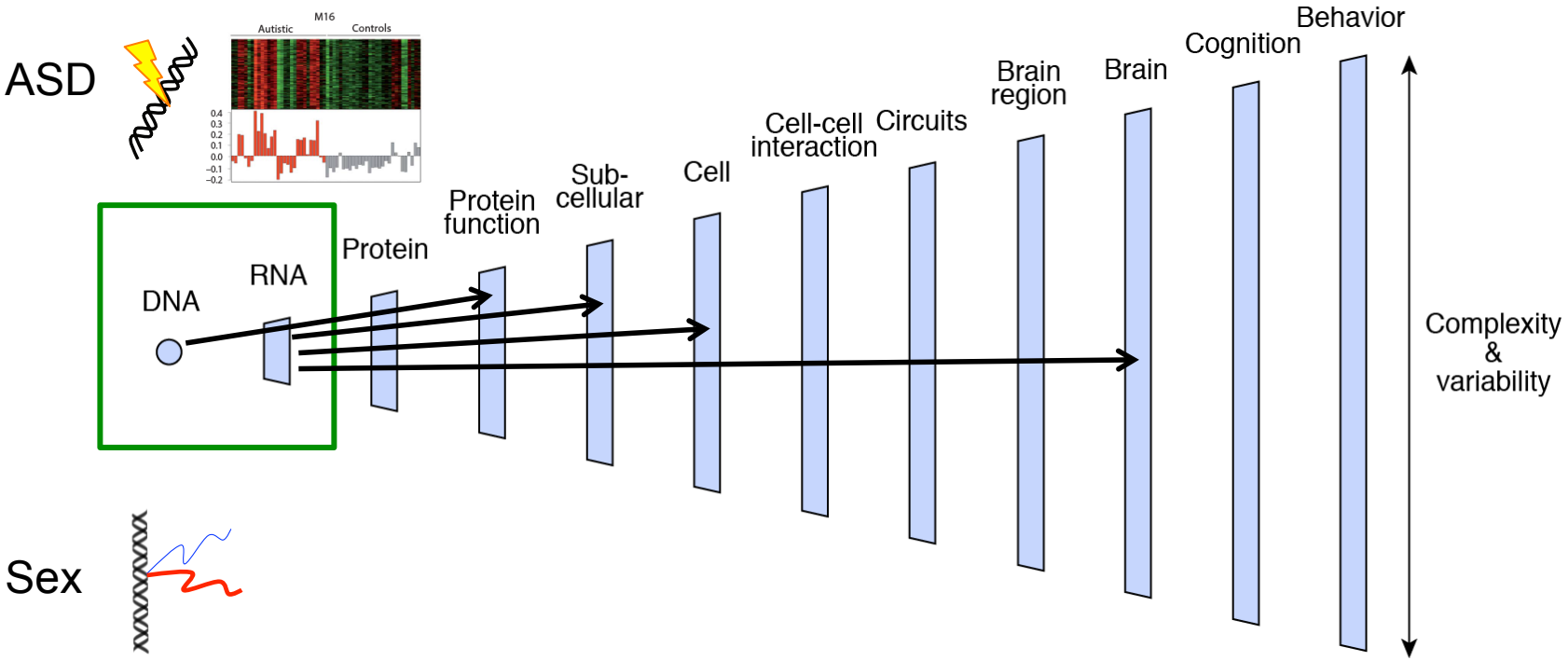
ASD cognition and behavior are the end result of changes across multiple levels of biology



We can also observe sex differences at multiple levels of biology

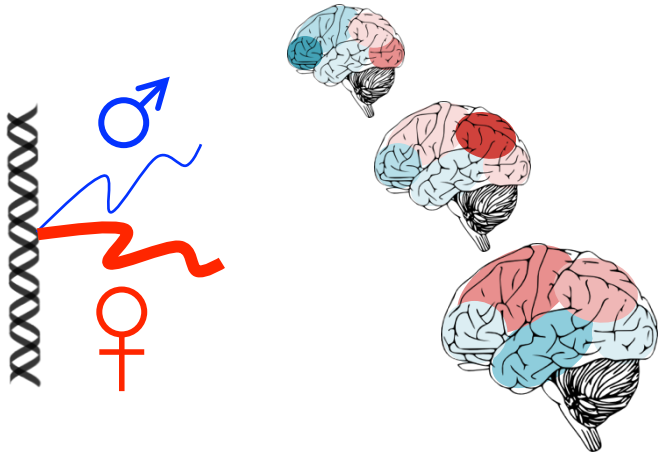


DNA and RNA are easy to measure and informative about downstream biological processes

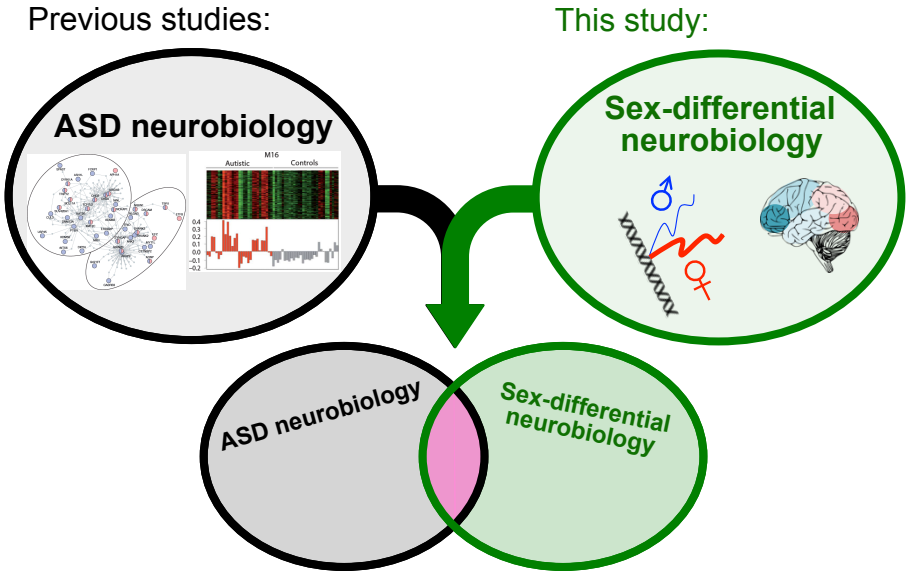


We can use gene expression to identify sex differences in the brain

1. Determine whether sex-biased gene expression is apparent in the human brain
2. Identify genes with sex-differential expression levels in the human brain



3. Characterize the relationship between sex-DEX genes and ASD biology



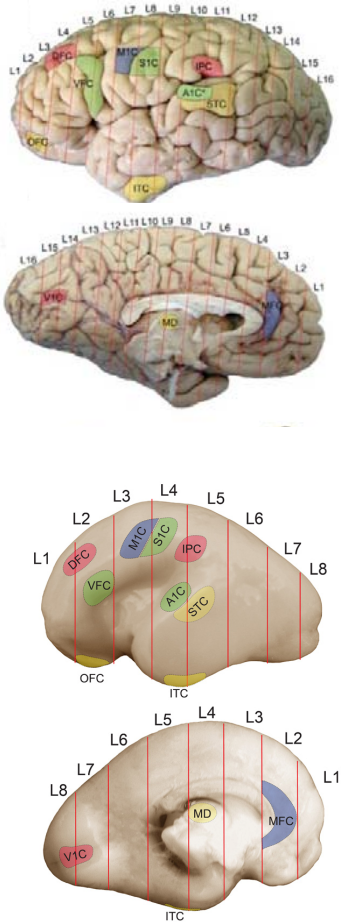
BRAINSPAN

ATLAS OF THE DEVELOPING HUMAN BRAIN

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

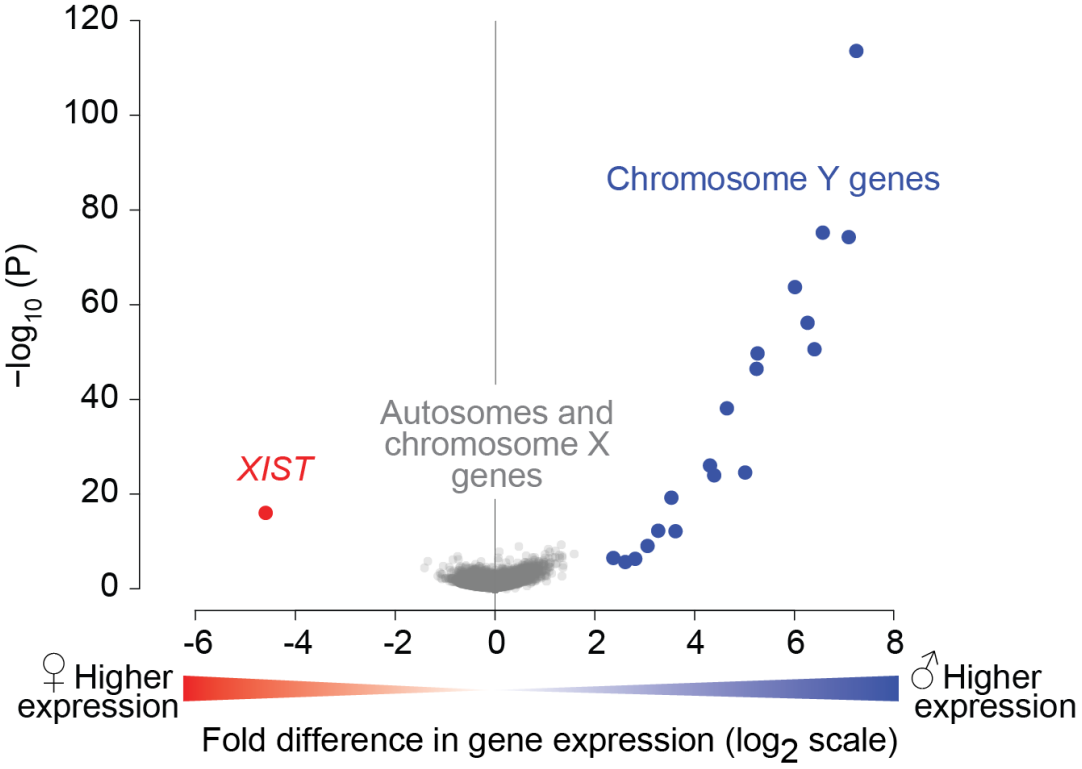
Period	Description	Age
1	Embryonic	4 PCW ≤ Age < 8 PCW
2	Early fetal	8 PCW ≤ Age < 10 PCW
3	Early fetal	10 PCW ≤ Age < 13 PCW
4	Early mid-fetal	13 PCW ≤ Age < 16 PCW
5	Early mid-fetal	16 PCW ≤ Age < 19 PCW
6	Late mid-fetal	19 PCW ≤ Age < 24 PCW
7	Late fetal	24 PCW ≤ Age <38 PCW
8	Neonatal and early infancy	0 M (birth) ≤ Age <6 M
9	Late infancy	6 M ≤ Age < 12 M
10	Early childhood	1 Y ≤ Age <6 Y
11	Middle and late childhood	6 Y ≤ Age < 12 Y
12	Adolescence	12 Y ≤ Age < 20 Y
13	Young adulthood	20 Y ≤ Age < 40 Y
14	Middle adulthood	40 Y ≤ Age < 60 Y
15	Late adulthood	60 Y ≤ Age

M, postnatal months; PCW, post-conceptional weeks; Y, postnatal years.



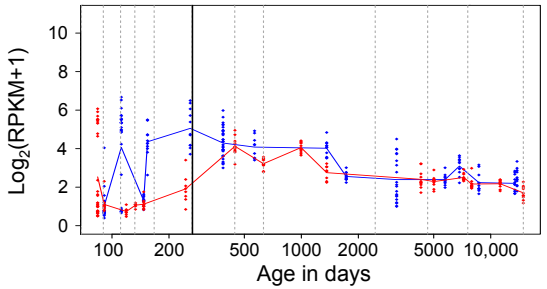
Kang, *et al.*, *Nature*, 2011; www.brainspan.org

There is no evidence of an autosomal gene with XY levels of sexual dimorphism

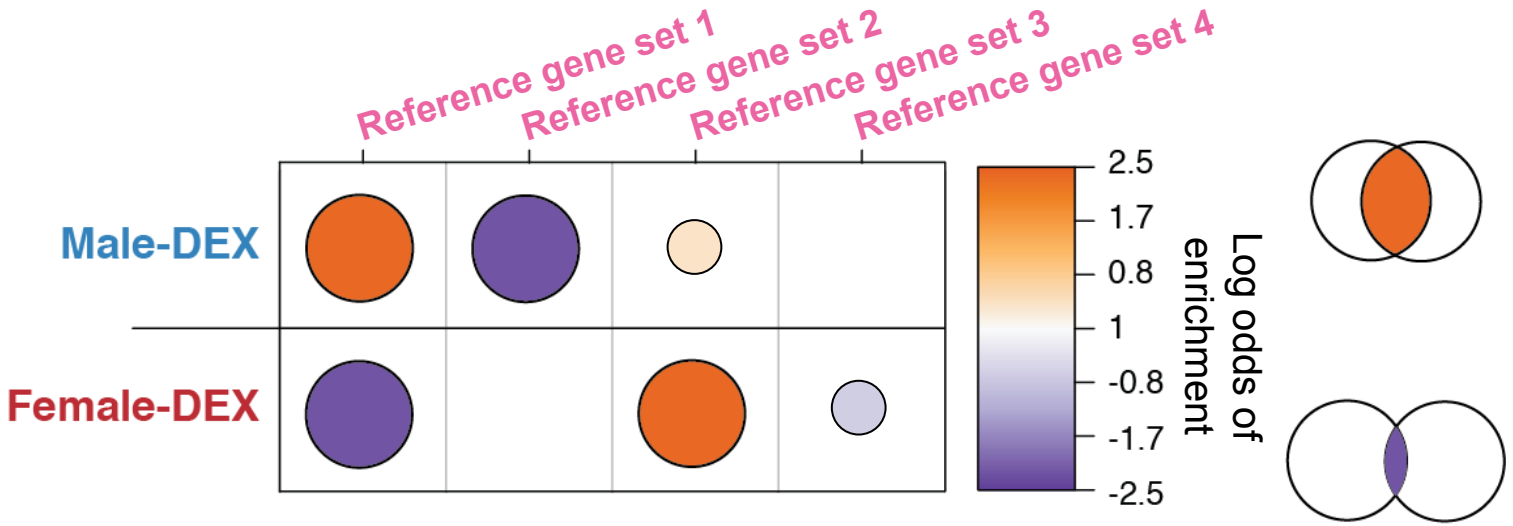


Sex-DEX genes identified by permutation approach (Q≤0.05; top-ranking sex-DEX in ≥2 consecutive developmental periods from same brain region):

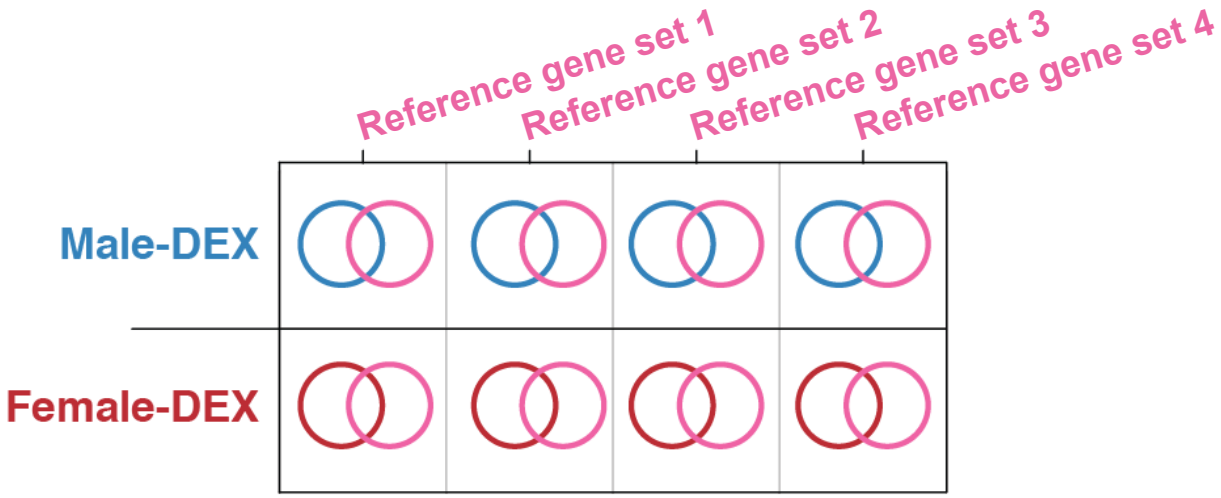
- Higher expression in males:
  - 343 protein-coding genes, 54 noncoding transcripts
- Higher expression in females:
  - 244 protein-coding genes, 176 noncoding transcripts



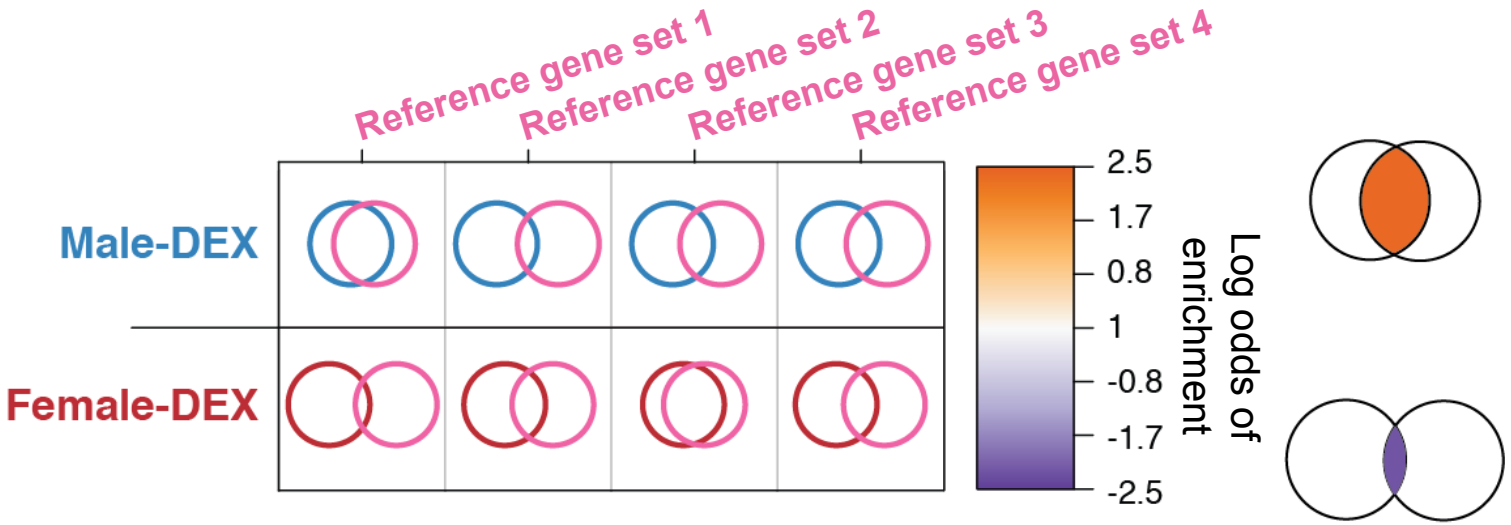
We use enrichment analysis to find biological processes that differ by sex



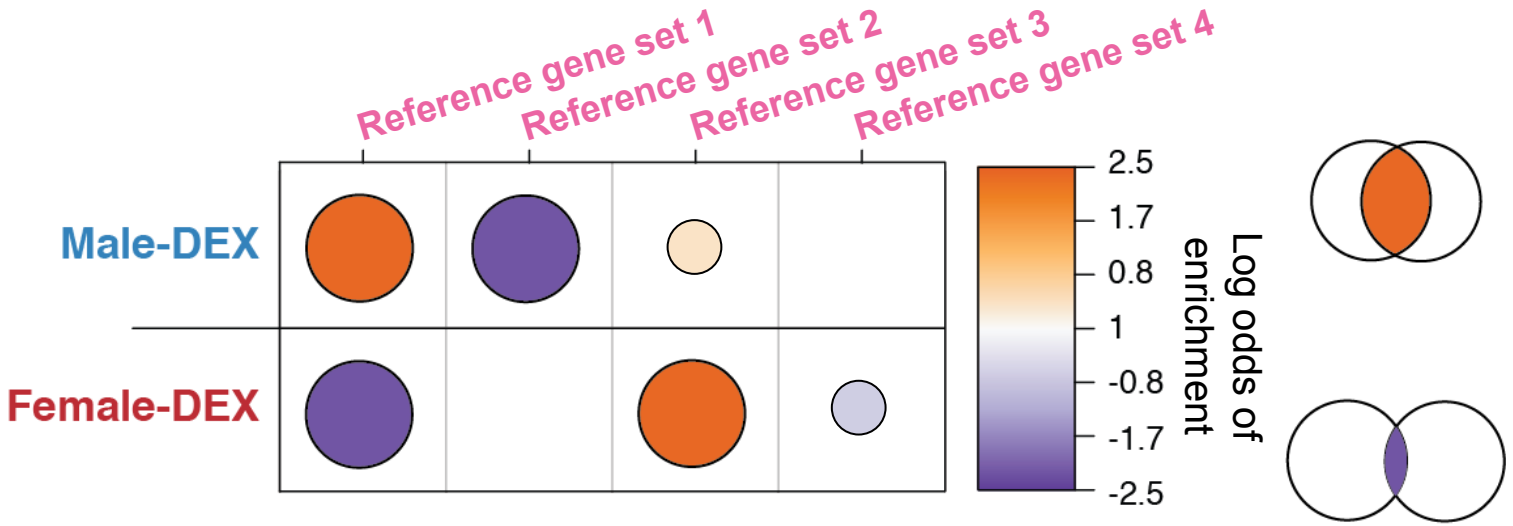
We use enrichment analysis to find biological processes that differ by sex



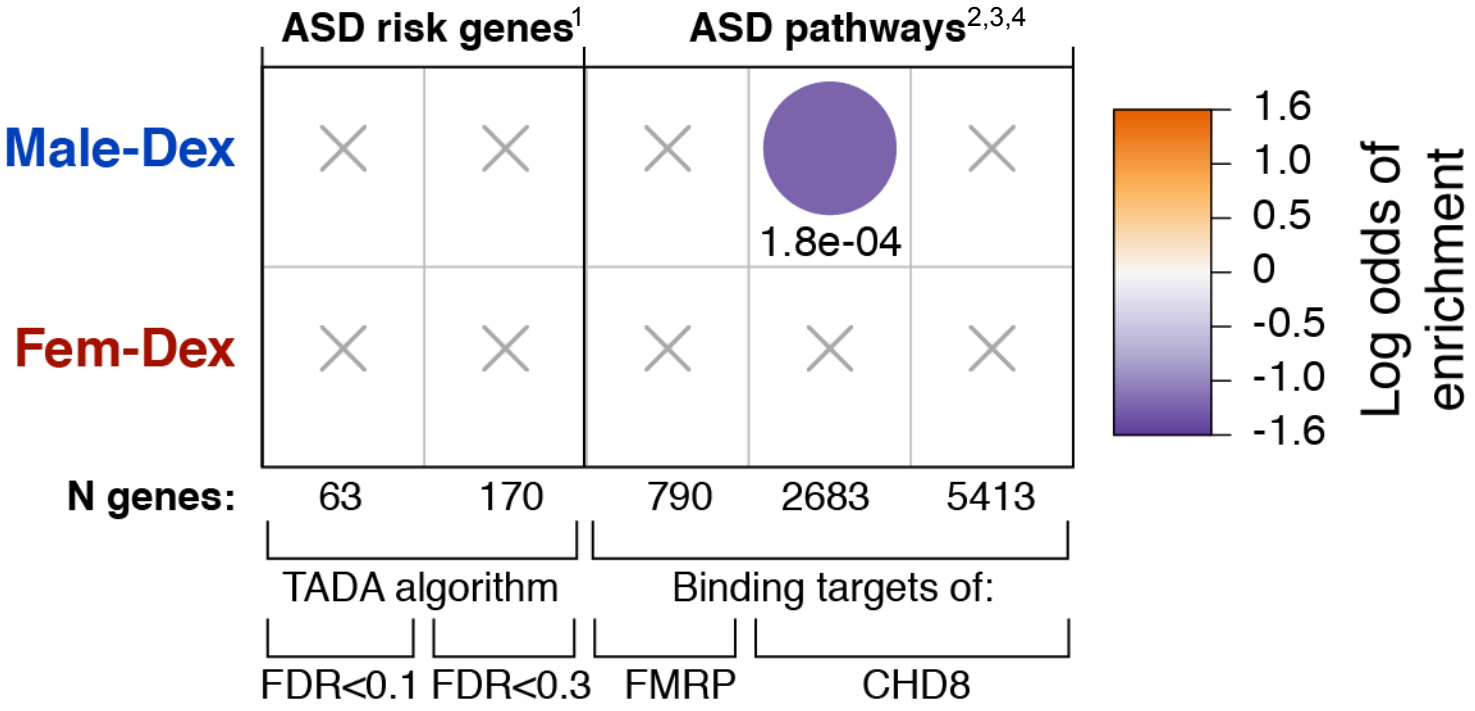
We use enrichment analysis to find biological processes that differ by sex



We use enrichment analysis to find biological processes that differ by sex

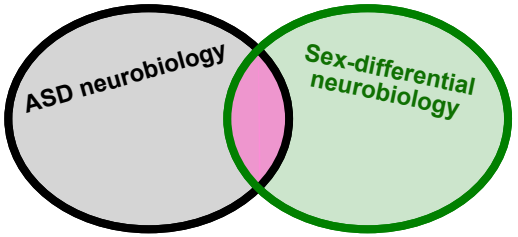


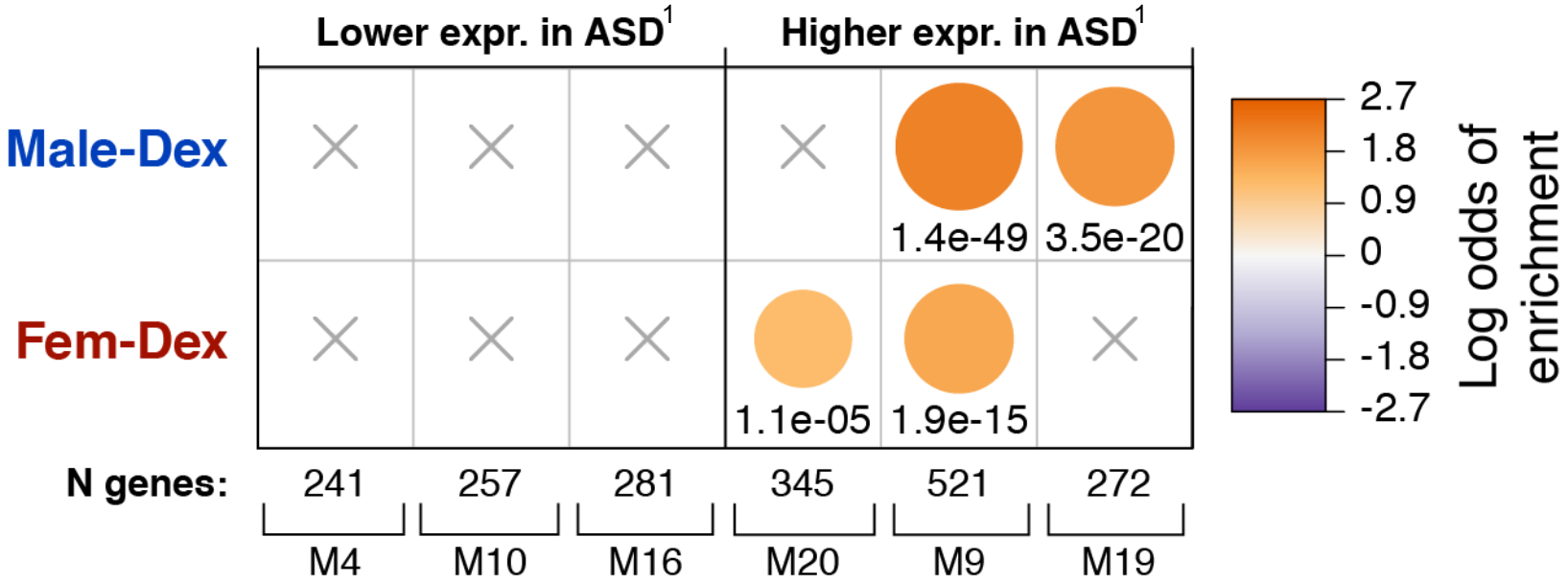
ASD risk genes and associated pathways do not show sex-differential expression



[1]Sanders et al, *Neuron*, 2015; [2]Darnell et al, *Cell*, 2011; [3]Cotney et al, *Nat Comm*, 2015; [4]Sugathan et al, *PNAS*, 2014

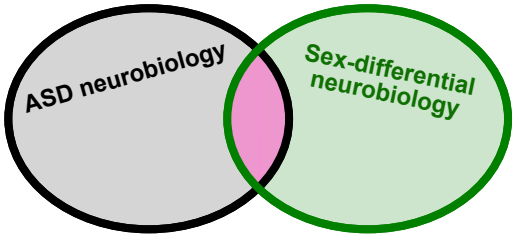
Genes up-regulated in the ASD brain are also sex-differentially expressed

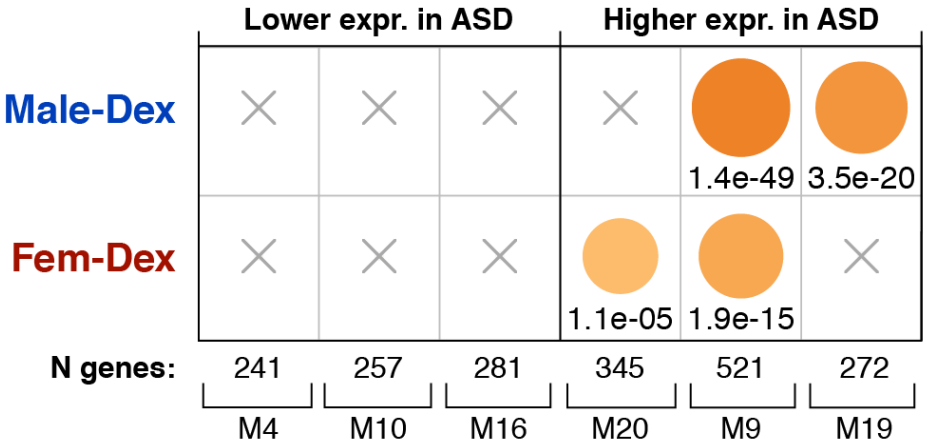


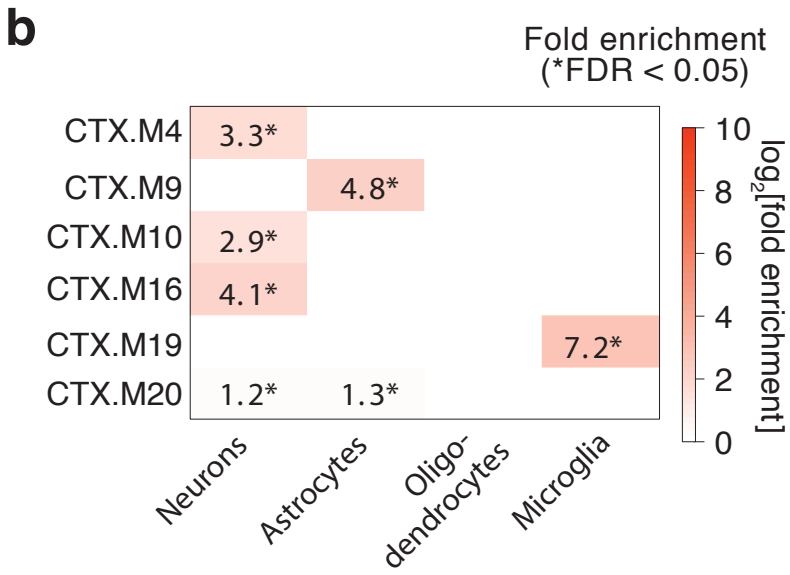


[1]Parikshak, Swarup, Belgard et al, *Nature*, 2016.

Genes up-regulated in the ASD brain are also sex-differentially expressed

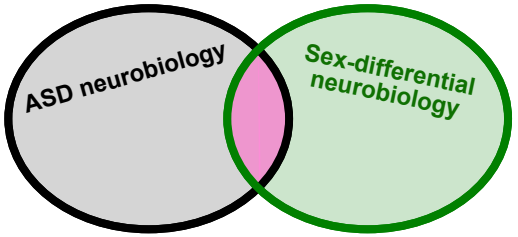


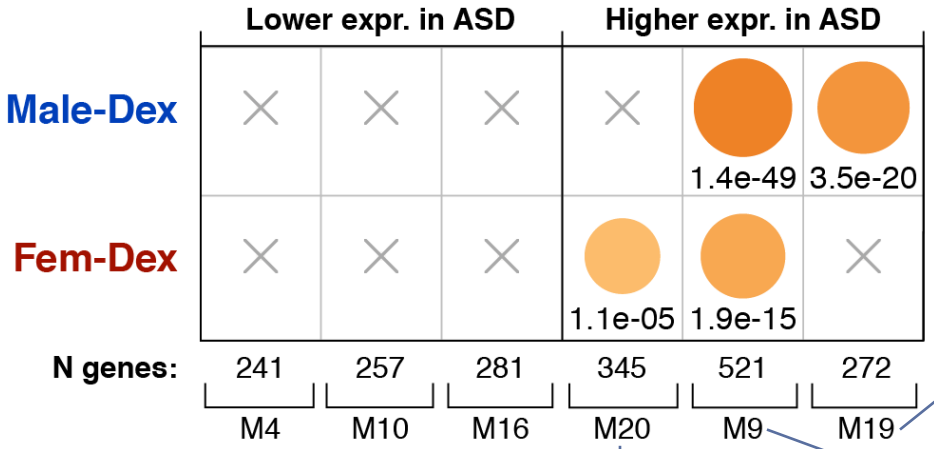


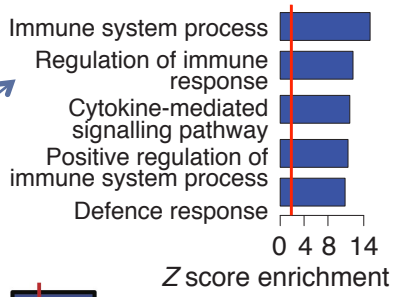


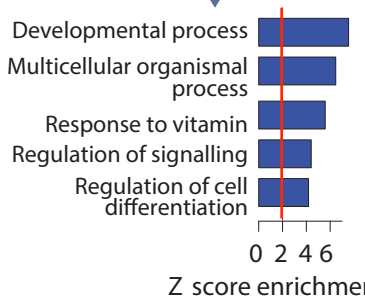
[1]Parikshak, Swarup, Belgard et al, *Nature*, 2016.

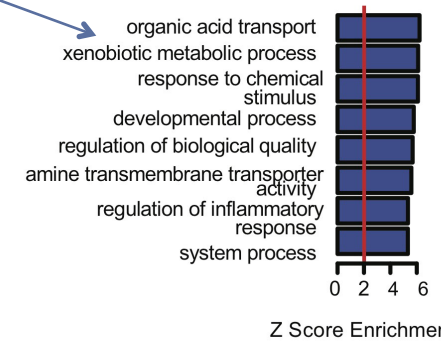
Genes up-regulated in the ASD brain are also sex-differentially expressed





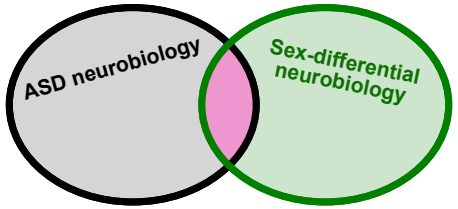


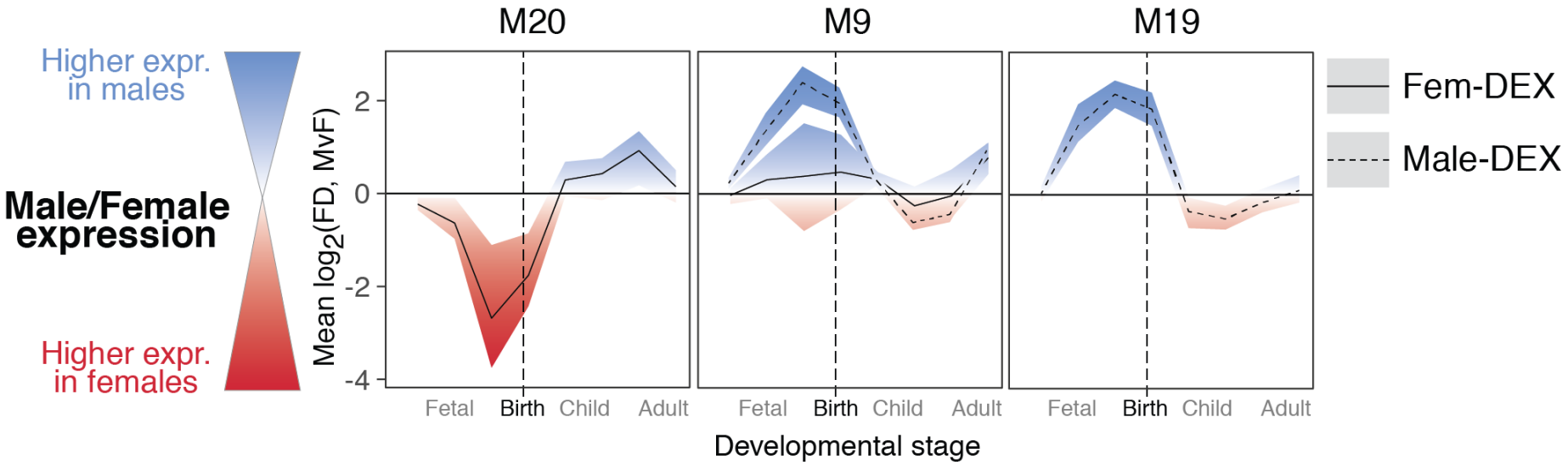




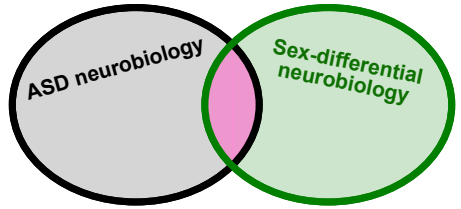
[1]Parikshak, Swarup, Belgard et al, *Nature*, 2016.

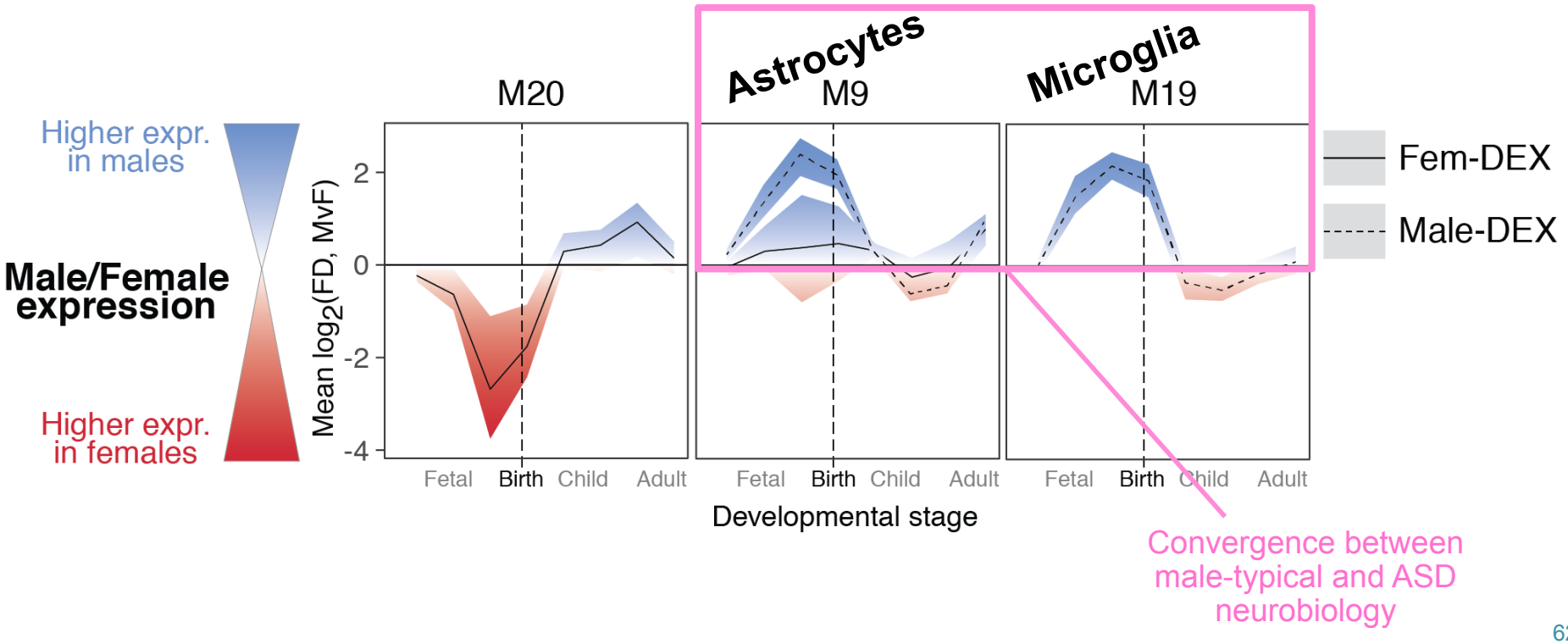
ASD-up-regulated genes show largest sex differences in prenatal cortex



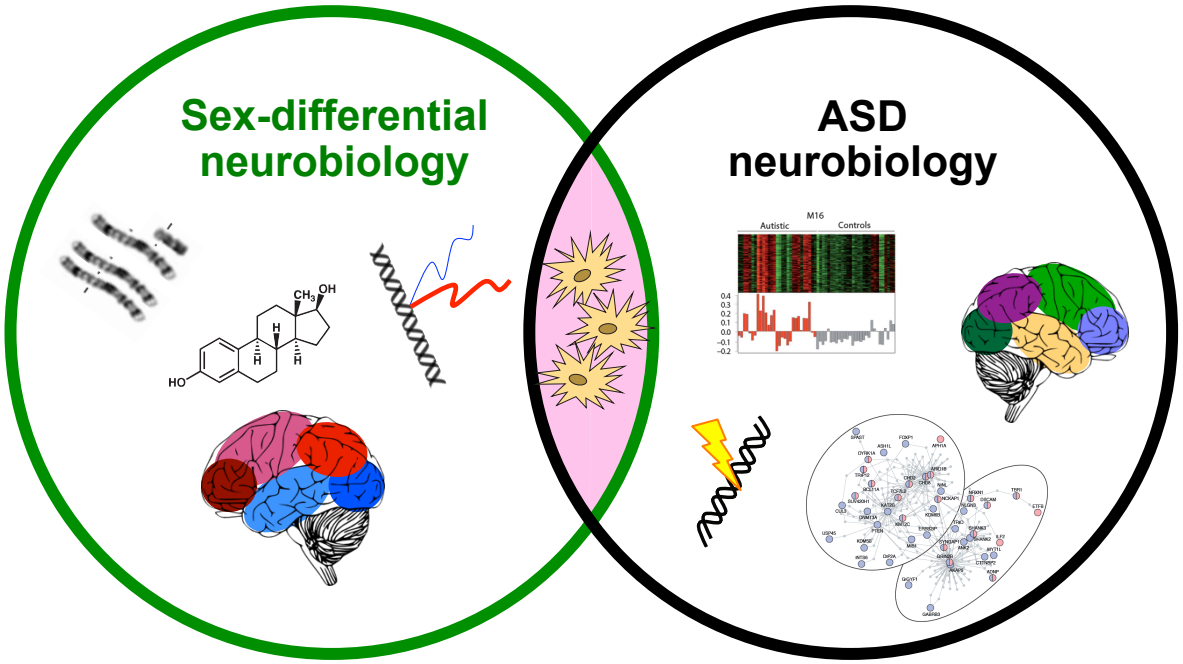


ASD-up-regulated genes show largest sex differences in prenatal cortex

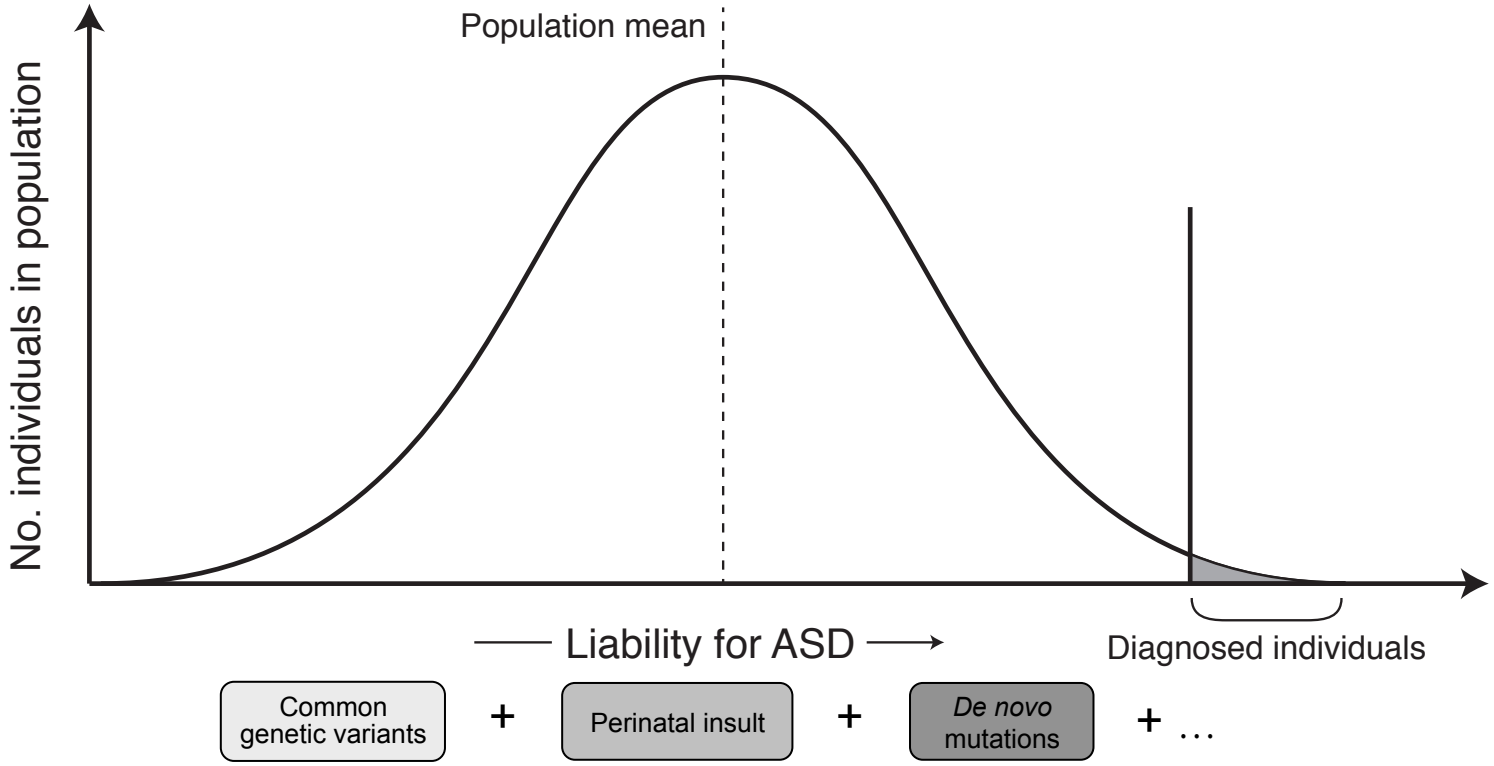




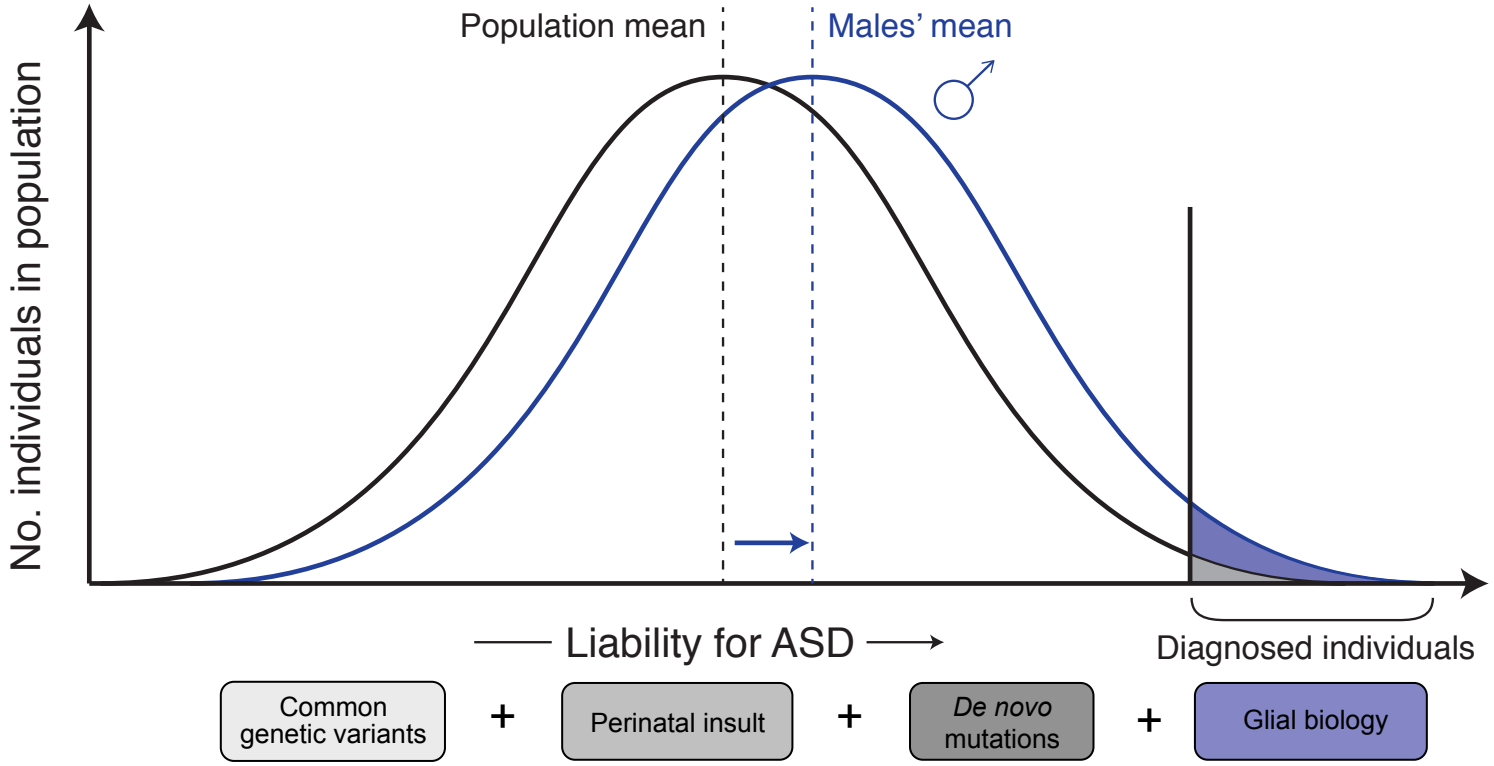
Males' glial biology may be similar to glial biology in ASD



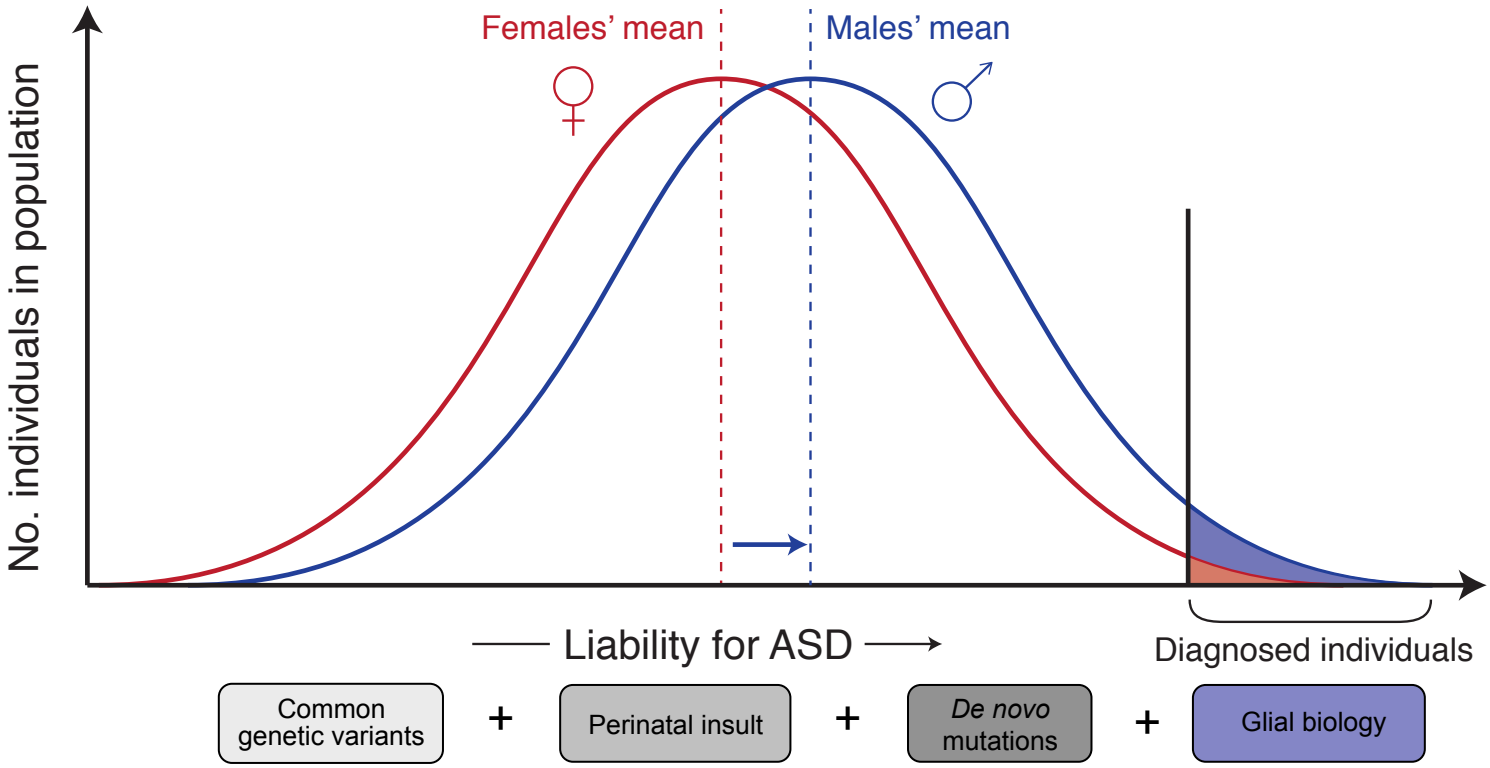
Males' glial biology may put them one step closer to an ASD phenotype than females



Males' glial biology may put them one step closer to an ASD phenotype than females

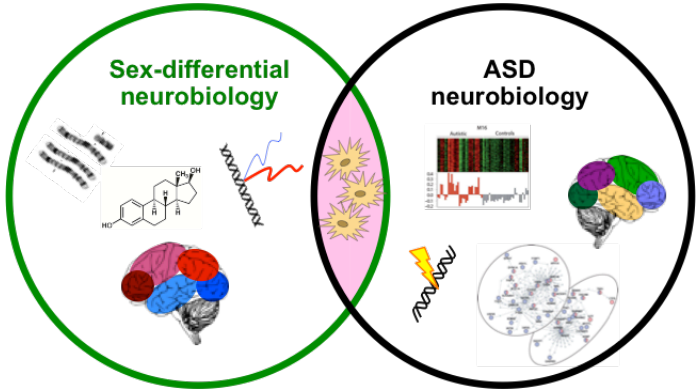


Males' glial biology may put them one step closer to an ASD phenotype than females



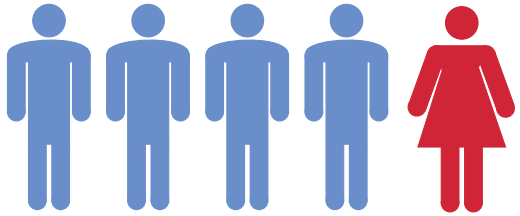
Summary:
Sex-differential and ASD biology

- Intersection of ASD neurobiology and sex-differential neurobiology provides an approach to understand sex bias
- ASD risk genes are not sex-differentially expressed
- Genes up-regulated in ASD brain do differ by sex:
  - Males: Glial-associated genes (M9, M19)
  - Females: “Cortical patterning” genes (M20)

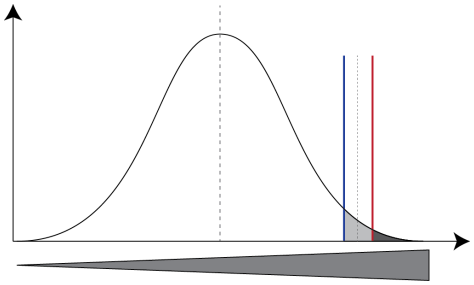


Review

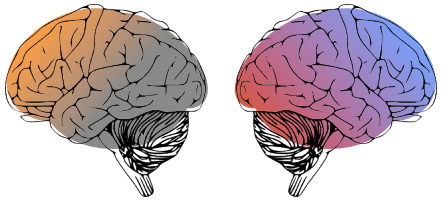
- Autism prevalence and risk in males vs. females



- How scientists think about sex-differential risk: The Female Protective Effect (FPE) model

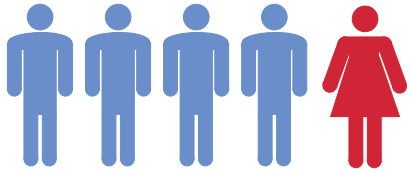


- Research in progress: Relationship between autism biology and sex-differential biology

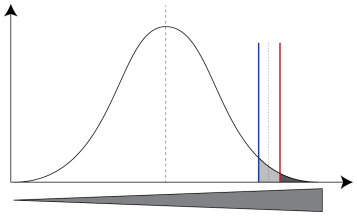


Review

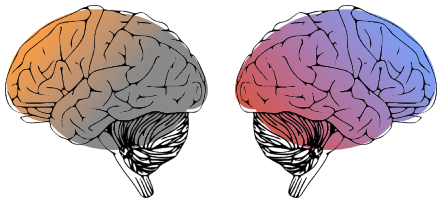
- Autism diagnoses remain more prevalent in males
- Knowledge and awareness of the female ASD phenotype is growing



- Female Protective Effect model is a useful tool for scientists to formulate and test hypotheses about ASD risk
- Genetic evidence supports the hypothesis that females are protected from ASD risk



- Gene expression from brain suggests that glial biology differs by sex and is altered in ASD
- Working hypothesis: Males' glial biology may push them closer to ASD threshold than females



Acknowledgements

Stephan Sanders
UCSF

Matt State
UCSF

Nenad Sestan
Yale

Insights into Autism

BRAINSPAN
ATLAS OF THE DEVELOPING HUMAN BRAIN

Yale
- Forrest Gulden
- Sirisha Pochareddy
- Xuming Xu
- Mingfeng Li
- Rob Kitchen

Allen Brain Institute
- Ed Lein
- Jeremy Miller
- Trygve Bakken

USC
- Jim Knowles

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)