Sex Bias and the Genetics of Autism

Tychele N. Turner, Ph.D.

Assistant Professor

Washington University School of Medicine

Department of Genetics

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@tycheleturner

Outline

- Background of autism epidemiology and genetics
- Sex bias in autism and the Diagnostic and Statistical Manual of Mental Disorders, 5th Edition (DSM-5)
- Potential biological reasons for a sex bias
- Evidence for a female-protective effect in autism
- Study of *de novo* variants by sex
- Gene regulatory networks in autism

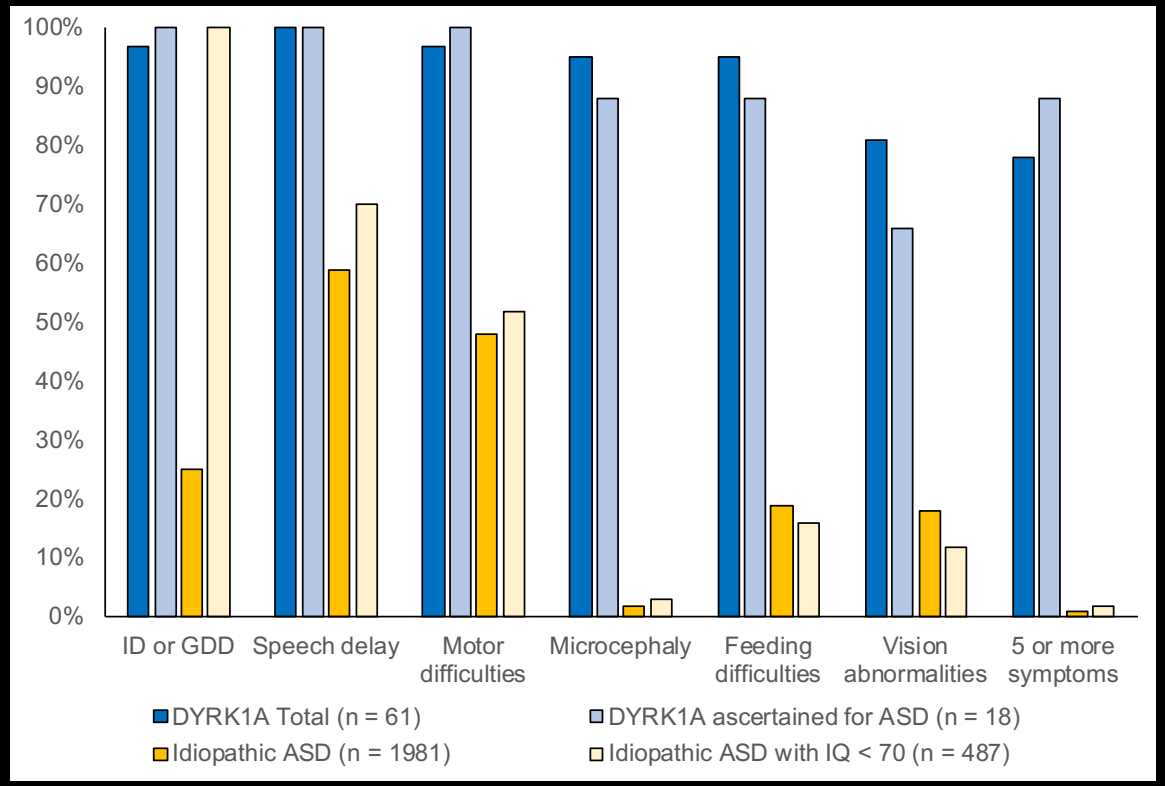
Autism

- Childhood neurodevelopmental disorder
- Heritability: ~80%
  Sandin et al. 2017, Steffenburg et al. 1989, Bailey et al. 1995
- Prevalence: 1/59
  Centers for Disease Control and Prevention
- Sex ratio of 4 males : 1 female
  Palmer et al. 2017, Jorde et al. 1991

Photo credit: Dr. John Constantino, MD
Washington University School of Medicine

Genetic features of autism

- Single gene syndromic causes
  - *ADSL, CDKL5, FMR1, MECP2, PTEN, SHANK3, TSC1*
    - Fragile X syndrome
    - Expansion of repeat sequence on the *X chromosome* and affecting the *FMR1* gene
    - Primarily affects boys
- Common variation: genome-wide association studies and polygenic risk scores
  - Overall contribution up to 50%
  - Five genome-wide significant loci
- Rare variation: copy number variants and *de novo* gene-disrupting mutations
  - 15q11-13 maternal duplications, 16p11.2, many others, most are recurrent
  - 124 genes (*e.g., CHD8, DYRK1A, FOXP1, GRIN2B, PTEN, SCN2A, TBR1*)



Earl et al. 2017, *Molecular Autism*

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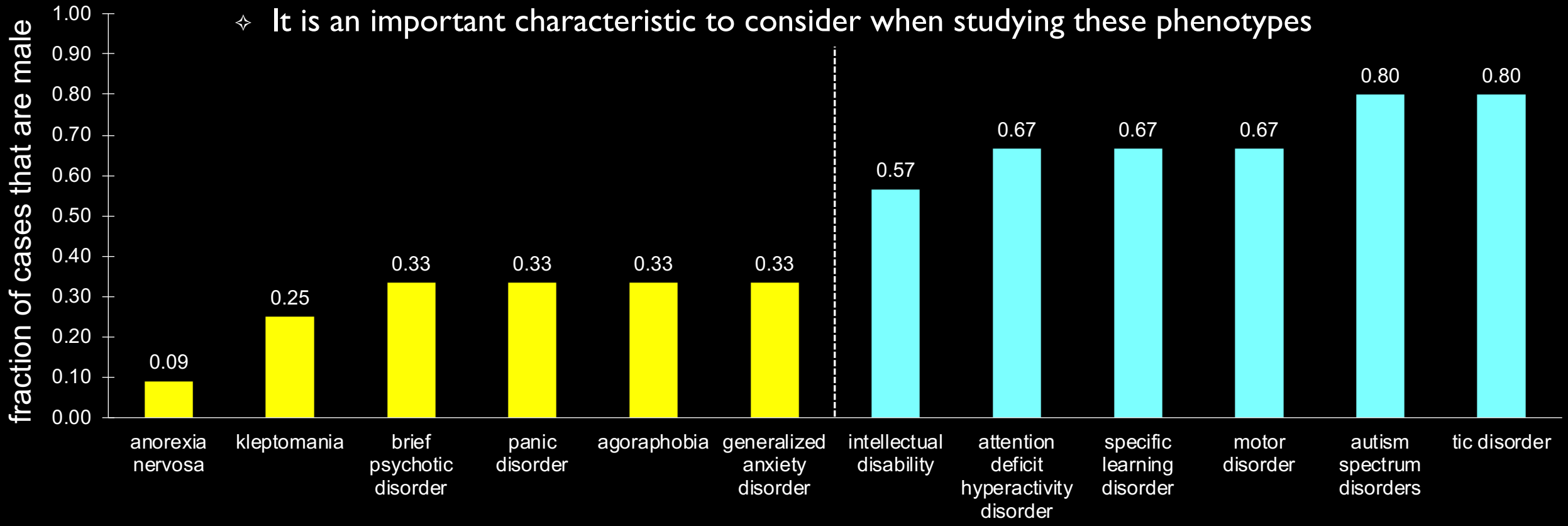
Disorders in the DSM-5 with sex biases

- Sex bias is seen in many neuropsychiatric and neurodevelopmental disorders
- It is an important characteristic to consider when studying these phenotypes



Aggregated from "Understanding Mental Disorders, Your Guide to DSM-5" by the *American Psychiatric Association*
A comprehensive review on sex bias in human disease is Ober et al. 2008, *Nature Reviews Genetics*

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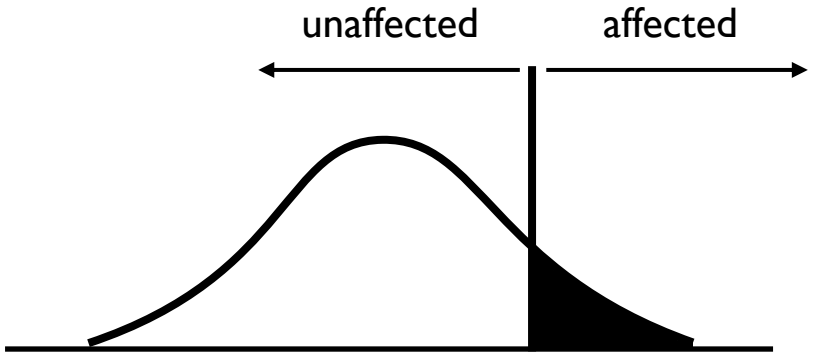
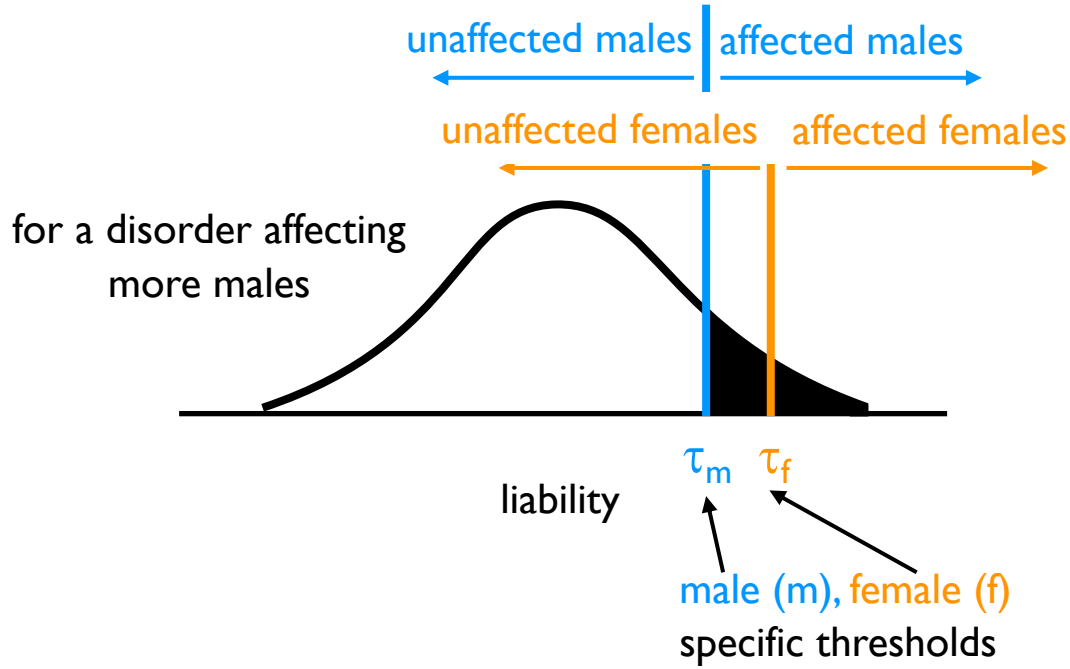
Liability distribution in multifactorial disorder



liability = genetic and environmental factors



Reasons for sex bias

- **S**ex chromosomes (females have two X chromosomes and males have one X, males have a Y chromosome and females do not, some X chromosome genes can escape X inactivation, …)
- **H**ormones like estrogen and androgen
- **I**mprinting
- **P**rotective effect (greater load of mutation in severity or number)

Falconer 1965, *Ann. Hum. Genet.* ; Strachan and Read, *Human Molecular Genetics*; Chakravarti and Turner 2016, *Bioessays;* Lady Washington, *Vancouver Family Magazine*

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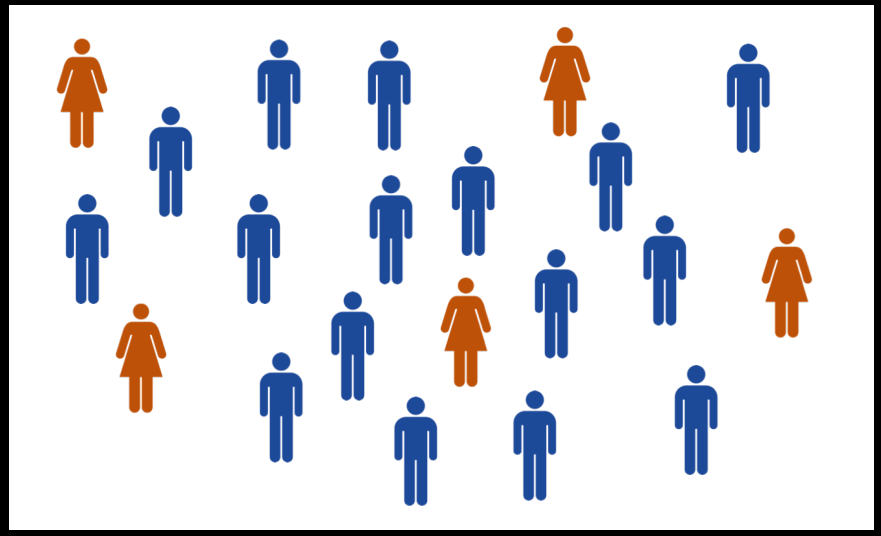
Evidence for the female-protective effect in neurodevelopmental disorders (NDDs)

2011	Higher frequency of *de novo* CNVs in females with autism (Levy et al., *Neuron*)
2012	Increased rate of *de novo* variants in females with autism (Neale et al., *Nature*)
2013	More autistic impairments in siblings of females with autism than males with autism (Robinson et al., *PNAS*)
2014	Excess of deleterious autosomal CNVs in females with NDDs (Jacquemont et al., *AJHG*)
2015	Gene discovery in female-enriched multiplex families (Turner et al., *Nature*)
2015	Higher frequency of *de novo* CNVs in females with autism (Sanders et al., *Neuron*)
2019	Excess of females with recessive gene disruptions (Doan et al., *Nature Genetics*)

Consistent with more severe mutations (rare variants of large effect) in females to develop autism



We (and others) have hypothesized that gene discovery would be greatest in the rarer class (females in NDDs)

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Three main questions

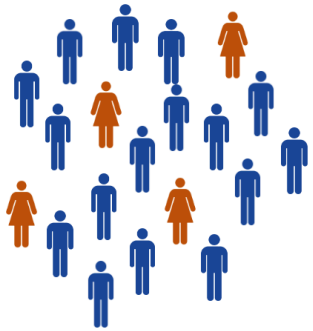
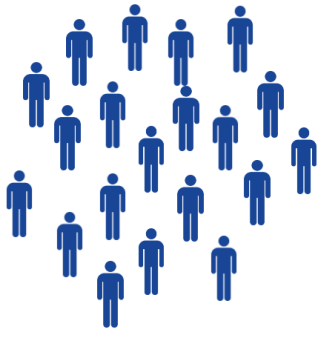
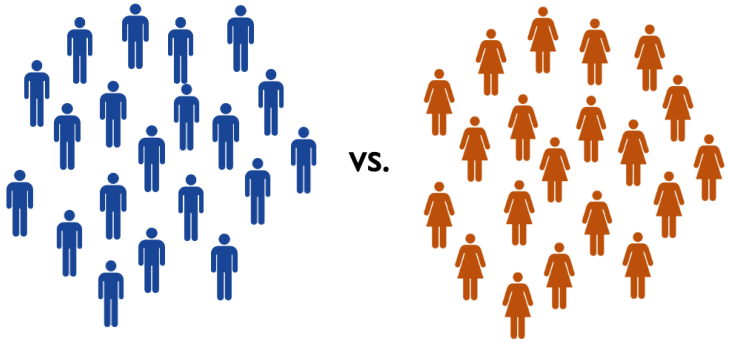
1. Are there any genes significant in only one sex and not the other?

2. Is there a differential gene discovery rate in males and females? (i.e., is gene discovery greater in females as the female-protective effect would suggest?)

3. Are there genes with differential counts of *de novo* variants in a direct comparison of males and females?

Statistical framework for assessment of *de novo* variants in neurodevelopmental disorders

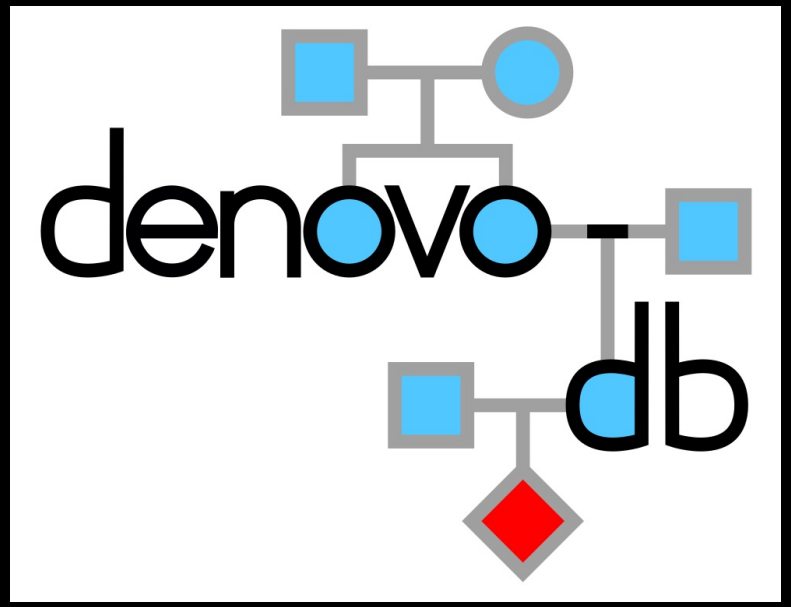
Turner et al. 2019, *American Journal of Human Genetics*

	standard approach	current study	
	pool all samples together and assess for *de novo* enrichment	separate by sex and assess for *de novo* enrichment	direct comparison of males and females
		males only / females only	
			
statistical test	<u>Assessment of *de novo* likely gene-disrupting and missense variants</u> chimpanzee-human (CH) model (Coe et al. 2019, *Nature Genetics*) denovolyzeR (Ware et al. 2015, *Current Protocols in Human Genetics*)		Fisher's exact test

de novo mutations from whole-exome and whole-genome sequencing in NDDs

discovery cohort

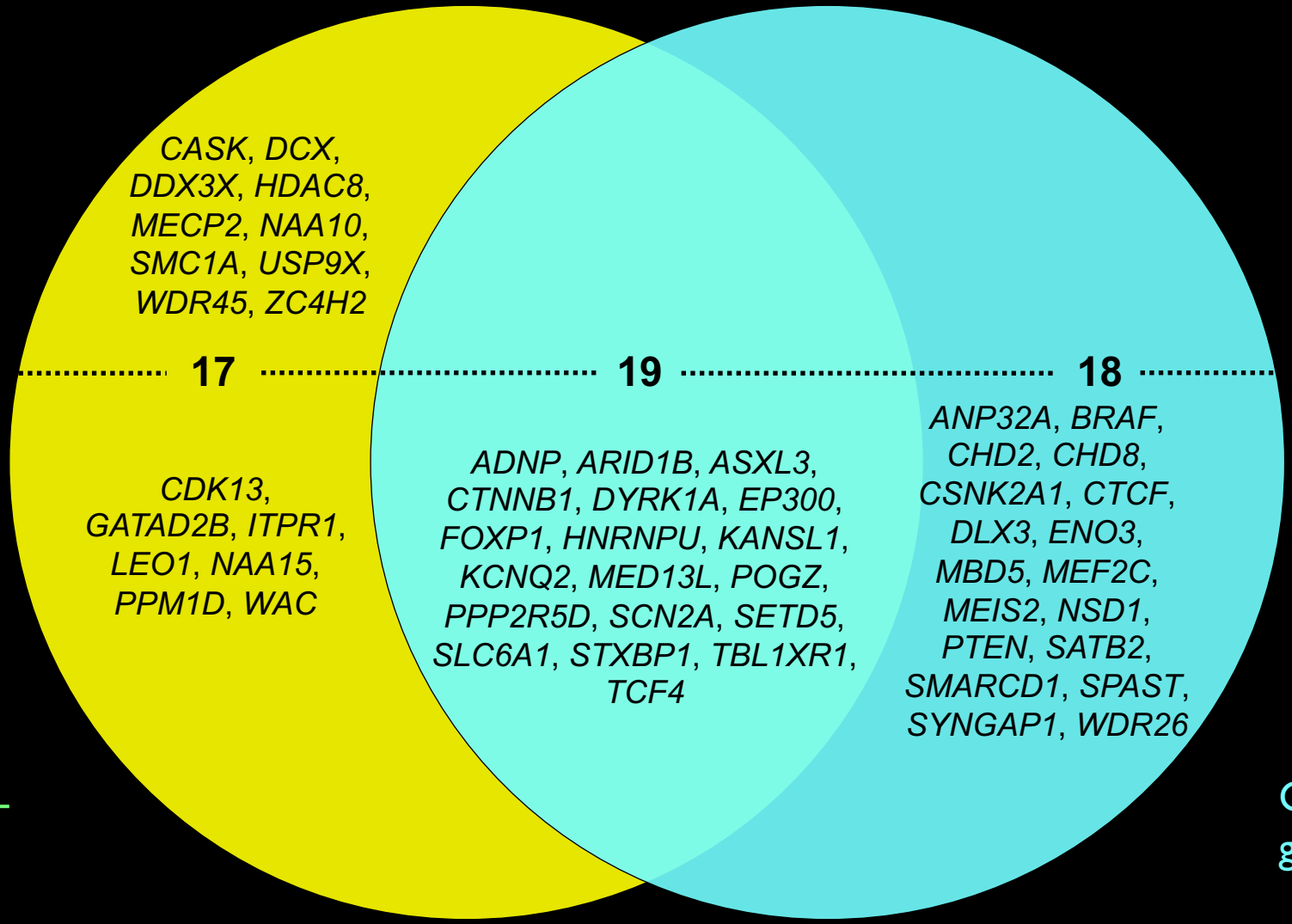
- 8,825 sequenced parent-child trios (n = 26,475 individuals)
- *de novo* variants
- denovo-db v.1.5
- sex ratio = 2.2



http://denovo-db.gs.washington.edu/

Turner et al. 2016, *Nucleic Acids Research*

Twice as many males are needed to reach similar numbers of significant genes in the discovery cohort



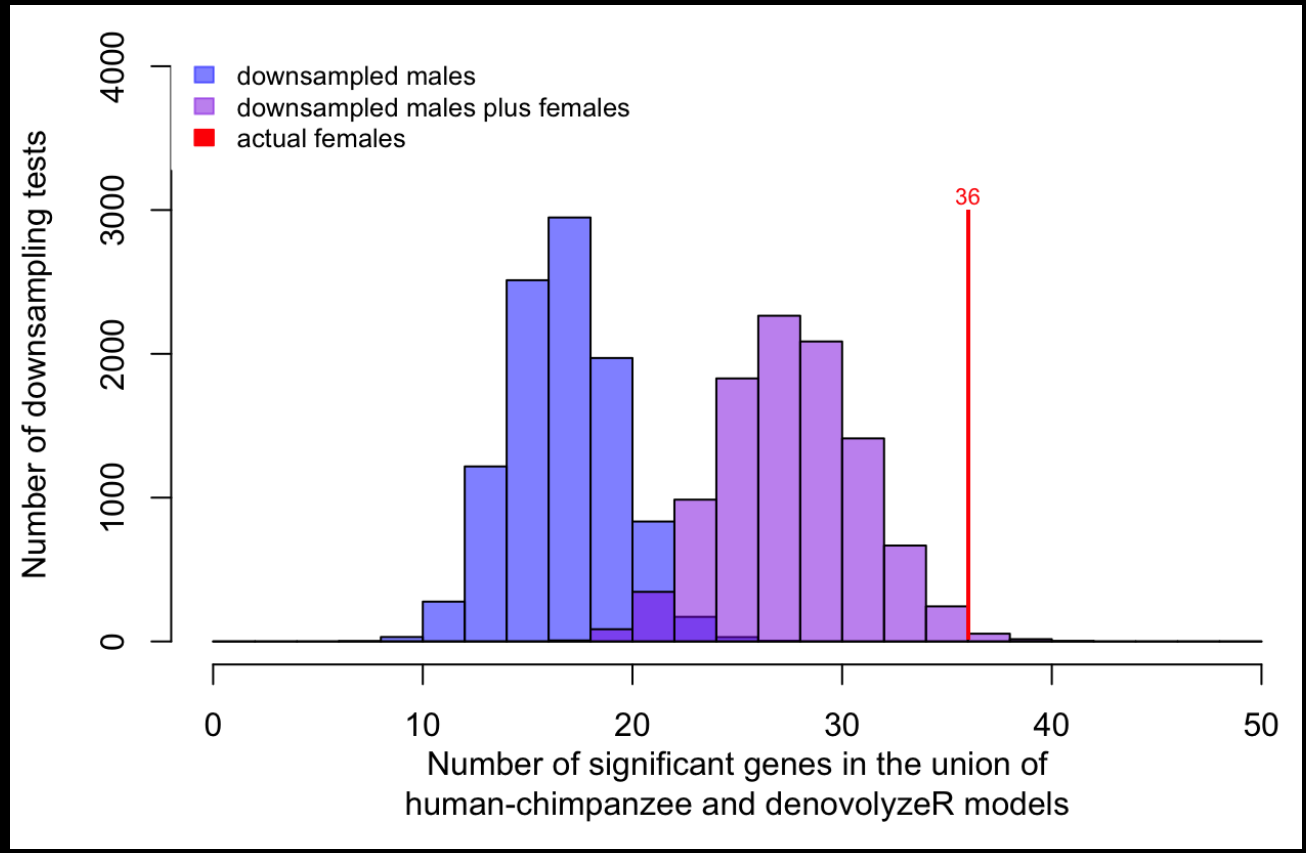
Exome-wide significance at $p < 4.3 \times 10^{-7}$

- 36 genes in females
- 37 genes in males
- 54 in the union

Gene discovery is greater in females

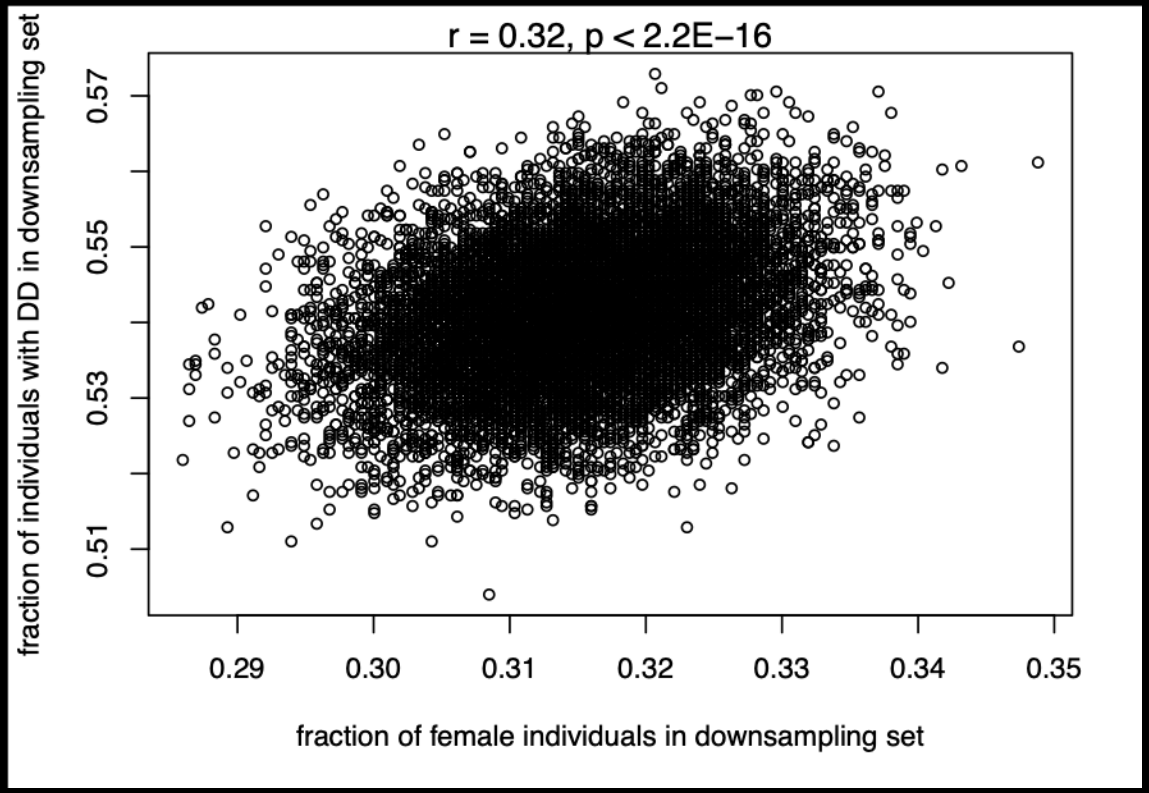
Greater significant gene discovery in females

- Downsampling analysis to the same number as the original NDD females (n = 2,779 females, 36 significant genes)
- <u>10,000 sets of only NDD males</u>
  Significantly more genes discovered in females than male-only ($p < 1x10^{-4}$)
- <u>10,000 sets of NDD males and females</u>
  Significantly more genes discovered in females than males and females combined ($p = 0.02$)



Potential NDD-related phenotypic differences in females

- Correlation between fraction of individuals with developmental disorder (DD) and fraction that are female in downsampling sets
- For individuals with autism where IQ data are available, IQ is lower in females than in males ($p = 1.8 \times 10^{-6}$)
  - female mean = 75.0
  - male mean = 82.1



de novo mutations from whole-exome and whole-genome sequencing in NDDs

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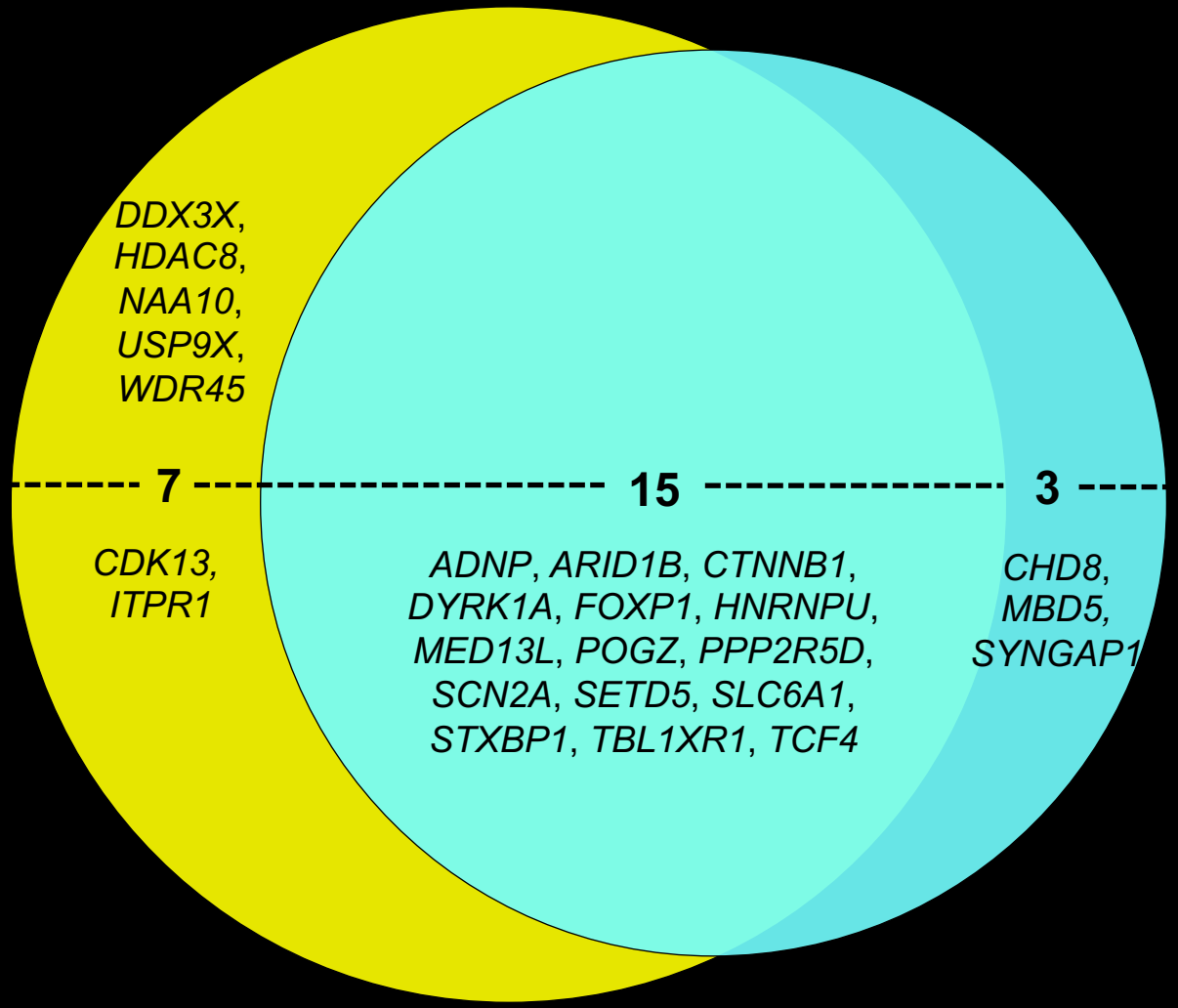
- sex ratio = 2.2

replication cohort

- 18,778 sequenced parent-child trios (n = 56,334 individuals)
- *de novo* count data for genes significant in discovery cohort
- GeneDx
- sex ratio = 1.2

After replication, proportionally there are still more genes enriched in females

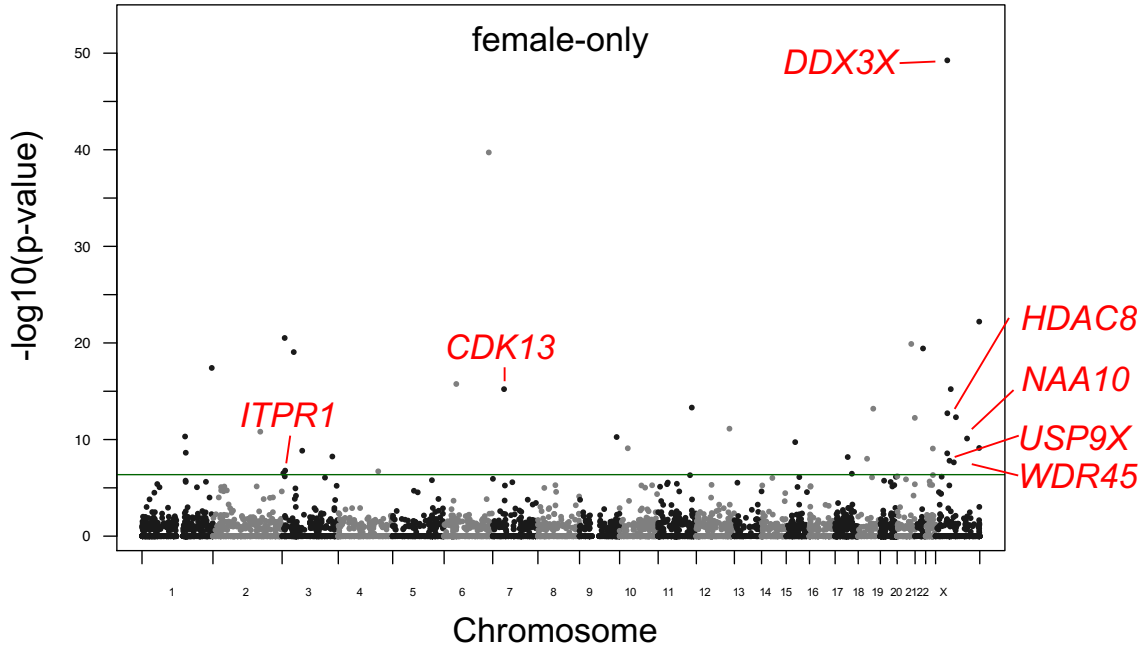


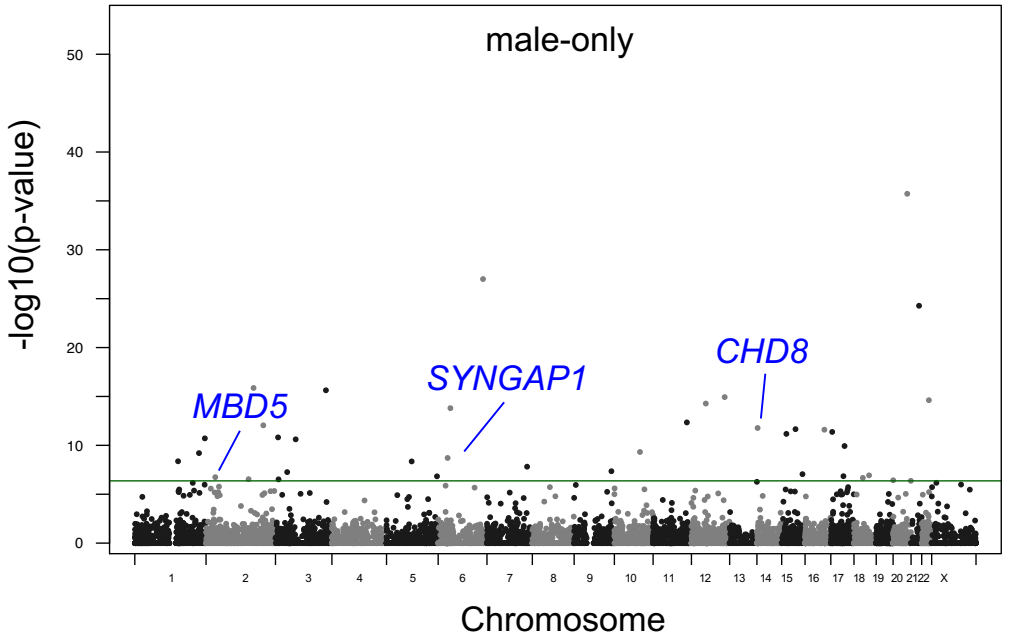
Exome-wide significance at $p < 4.3 \times 10^{-7}$

- 22 genes in females
- 18 genes in males
- 25 in the union

Gene discovery is greater in females

Replicated genes significant in only one sex

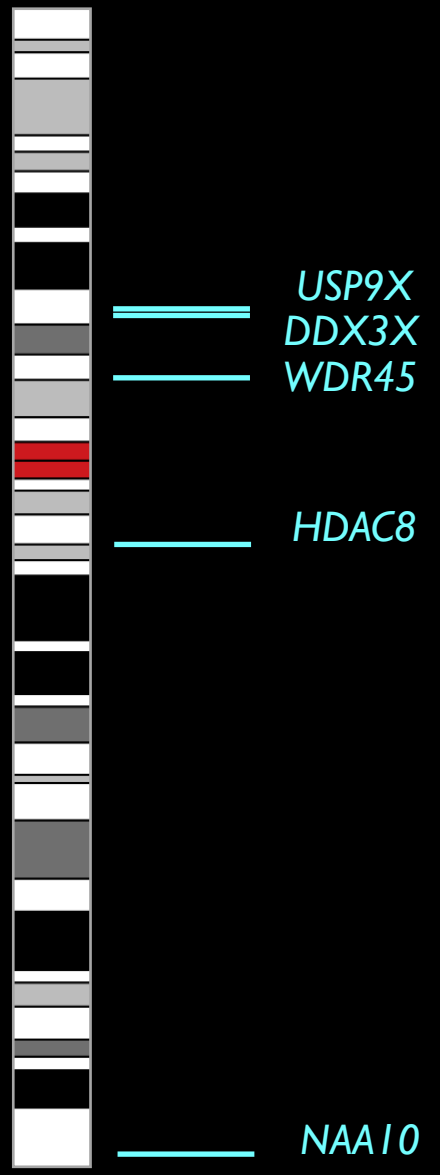




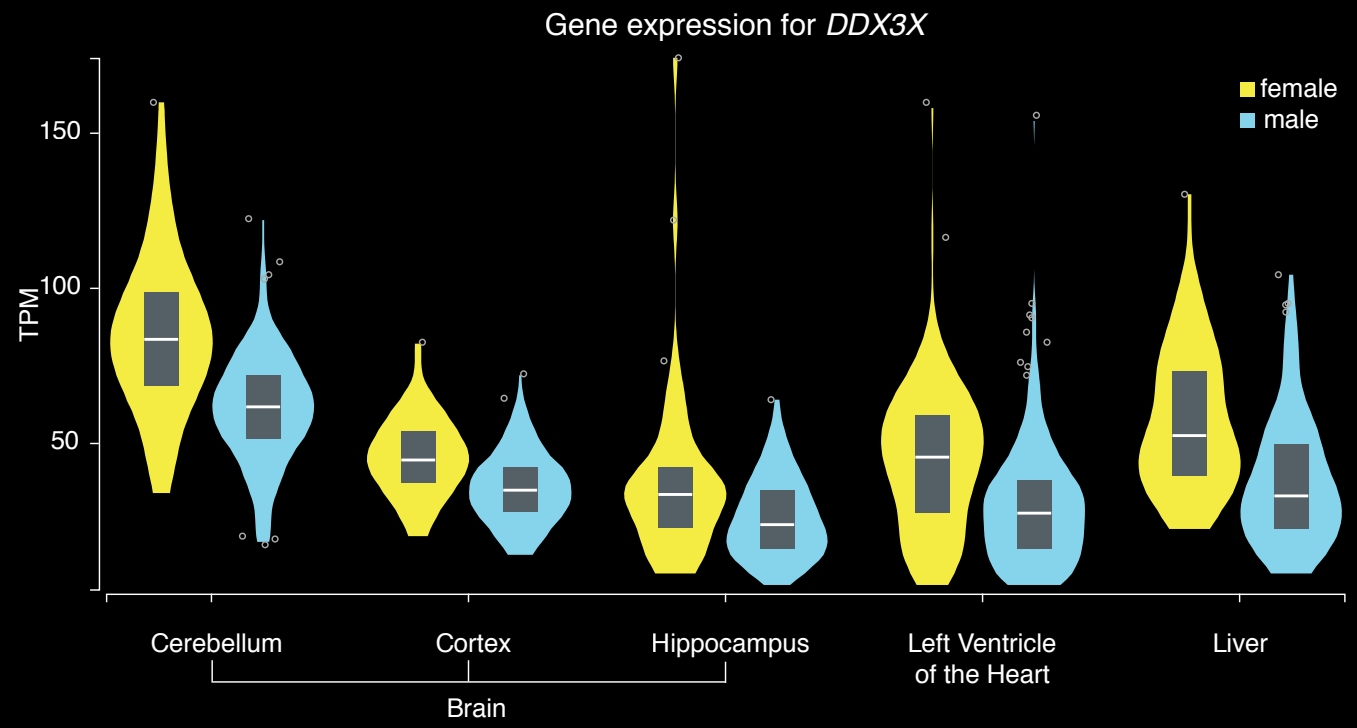
		Discovery		Replication	
gene	chromosome	male minimum p-value	female minimum p-value	male minimum p-value	female minimum p-value
CDK13	chr7	0.0623	6.05E-16*	7.00E-07	1.45E-07*
DDX3X	chrX	NA	5.54E-50*	7.51E-02	4.41E-117*
HDAC8	chrX	0.109	4.88E-13*	NA	1.32E-16*
ITPR1	chr3	0.0139	3.07E-07*	1.19E-05	1.03E-07*
NAA10	chrX	NA	7.47E-10*	NA	1.45E-18*
USP9X	chrX	0.182	2.67E-09*	3.83E-01	6.54E-12*
WDR45	chrX	0.0025	1.56E-08*	2.99E-04	8.83E-41*
CHD8	chr14	2E-12*	5.64E-06	1.08E-12*	1.48E-04
MBD5	chr2	3E-07*	2.35E-02	4.32E-08*	6.95E-02
SYNGAP1	chr6	2E-09*	2.15E-04	3.39E-18*	2.09E-03

* multiple-test correction significant

Female-only enriched genes on the X chromosome



- Three of the five genes are either constitutive (*DDX3X*, *USP9X*) or facultative (*NAA10*) escapers of X inactivation

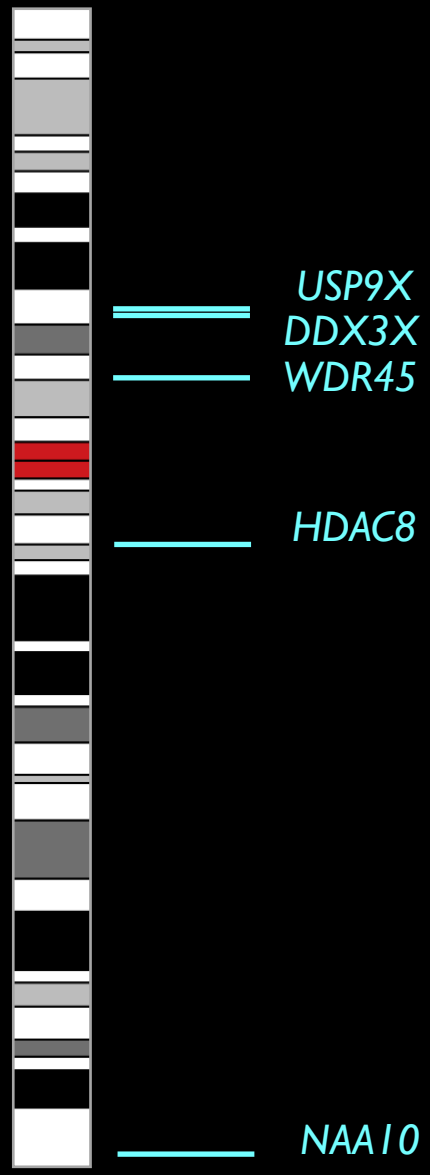


- Consistent with a model where females have buffered expression and develop NDD whereas male with same mutation is lethal
- Different than phenotypes like Fragile X Syndrome where females more often do not have the syndrome and males do

Generally, 13% of X chromosome genes constitutively escape inactivation (Tukiainen et al. 2017)

https://gtexportal.org/home/gene/DDX3X

Female-only enriched genes on the X chromosome



Gene	Known phenotype
USP9X	Intellectual disability in females (Reijnders et al. 2016, *AJHG*)
DDX3X	Intellectual disability in females (Snijders Blok et al. 2015, *AJHG*)
WDR45	Neurodegeneration with brain iron accumulation mostly affecting females (Haack et al. 2012, *AJHG*)
HDAC8	Cornelia de Lange syndrome with more severe intellectual disability observed in males than females (Kaiser et al. 2014, *HMG*)
NAA10	Ogden syndrome which affects both males and females (Rope et al. 2011, *AJHG*)

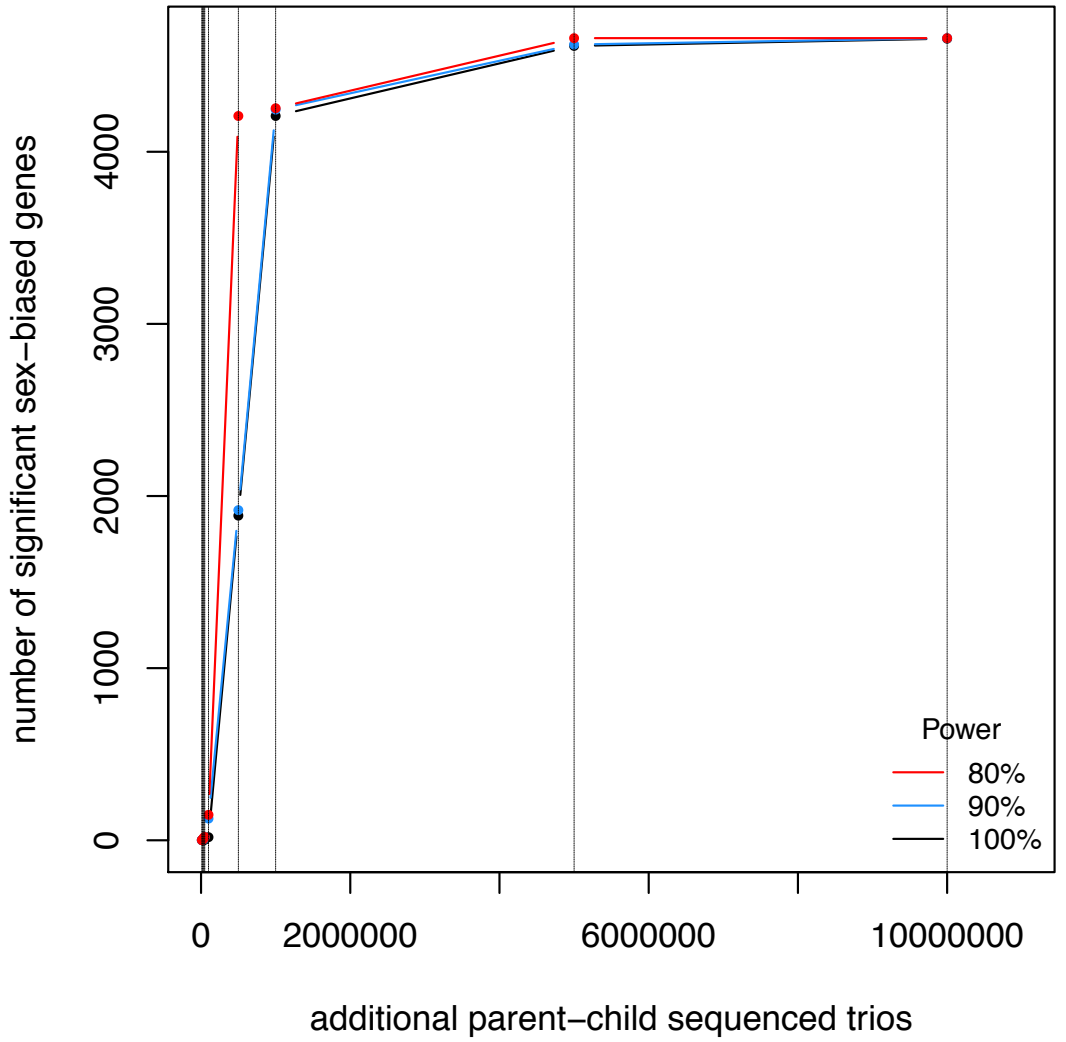
Direct comparison of males and females reveals *DDX3X* as the one genome-wide significant gene

		Discovery				*Replication*					
Gene	Chromosome	male counts	female counts	Fisher's exact test p-value	Fisher's exact test odds ratio	male counts	female counts	Fisher's exact test p-value	Fisher's exact test odds ratio	genome-wide significant in both	nominal significance in both
DDX3X	X	0	26	4.73×10^{-9}	Inf	2	65	1.76×10^{-11}	20.15	yes	yes
MECP2	X	1	12	1.26×10^{-3}	13.08	8	54	1.77×10^{-5}	4.18	no	yes
NAA10	X	0	5	2.52×10^{-2}	Inf	0	11	9.12×10^{-3}	Inf	no	yes
HDAC8	X	1	7	3.23×10^{-2}	7.62	0	11	9.12×10^{-3}	Inf	no	yes

The other genes reaching nominal significance are also on the X chromosome

Power for the direct comparison of males and females

Additional parent-child sequenced trios	Genes at 80% power	Genes at 90% power	Genes at 100% power
10,000	1	1	1
20,000	4	4	1
30,000	7	4	1
40,000	17	8	2
50,000	19	18	4
100,000	148	126	18
500,000	4,208	1,918	1,886
1,000,000	4,253	4,245	4,208
5,000,000	4,660	4,623	4,615
10,000,000	4,660	4,660	4,657



Conclusions for "Study of *de novo* variants by sex"

- Sex bias is an important consideration when assessing a phenotype
- Gene discovery, based on rare variants of large effect, is greatest in the rare class (females with NDDs) and can also reveal important genes on the X chromosome
- While studies of NDDs have focused on a pooled (male and female) approach, other researchers studying new phenotypes may want to focus on the rarer class to detect genes with variants of large effect
- Genes with rare variants of large effect provide key insight into the biology of the disorder (next part of talk)

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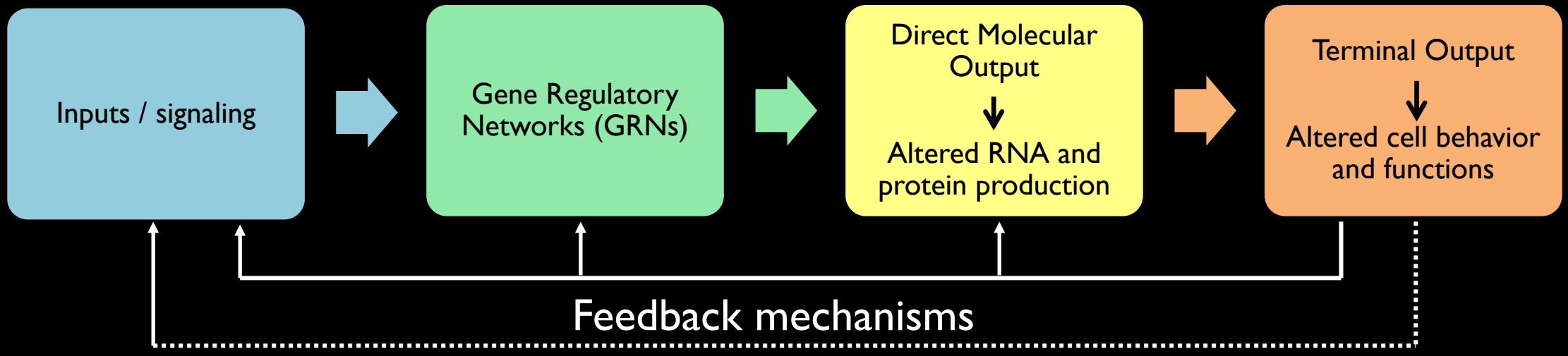
Gene regulatory networks (GRNs) and their effect on biological activities

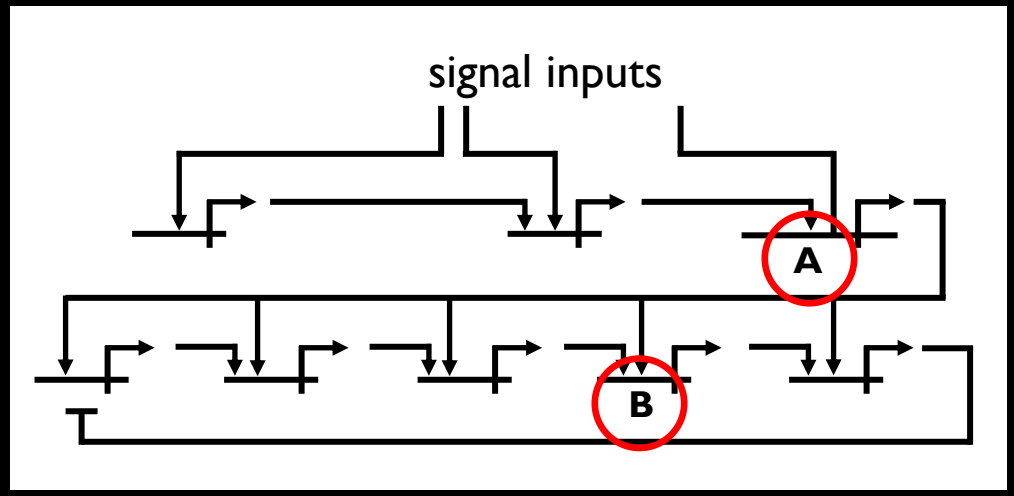


Gene regulatory networks are composed of a set of regulators, working together, that control output of RNA and protein levels

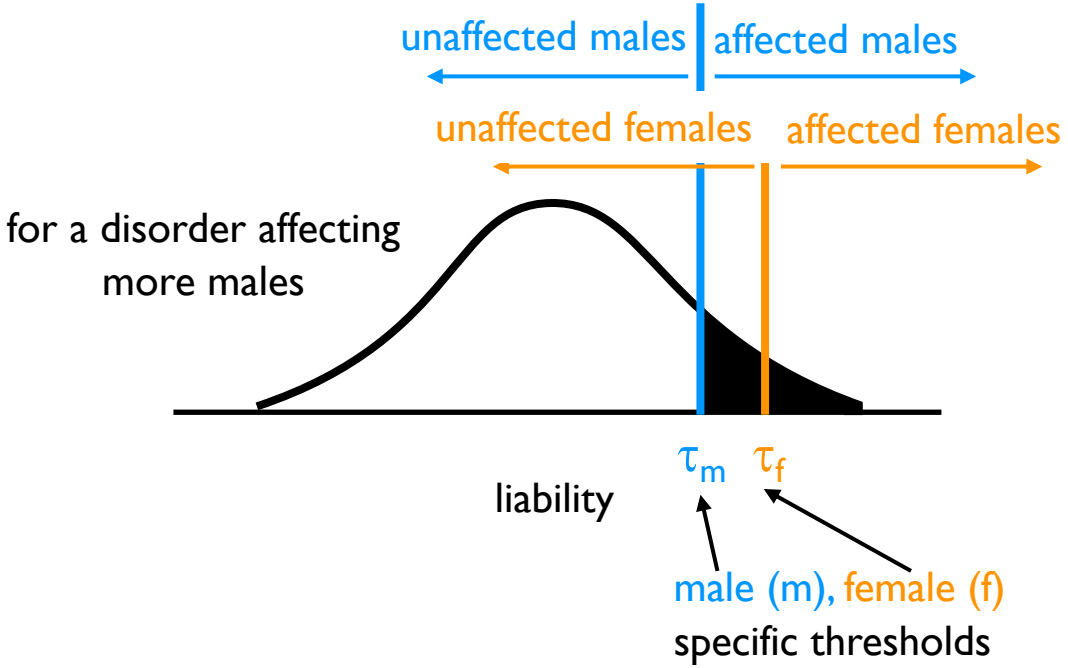
Davidson 2006, *The Regulatory Genome: Gene Regulatory Networks in Development and Evolution*

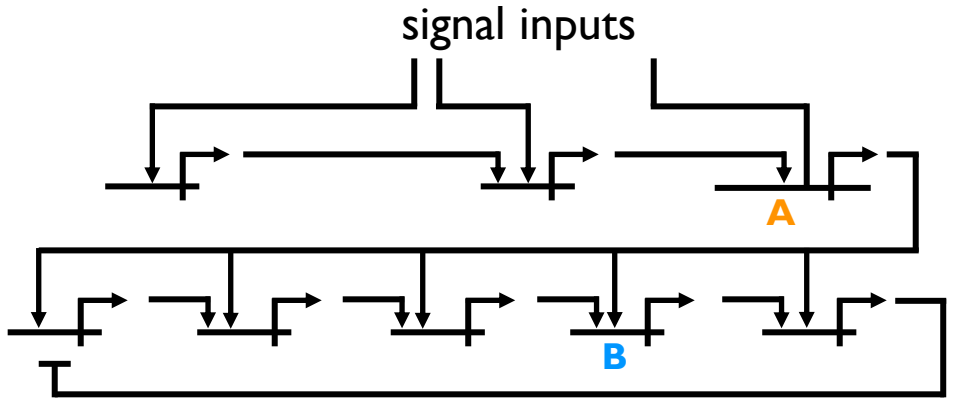
An example of a transcriptional GRN

- Input signals use cis-regulatory elements to regulate multiple genes
- Expression of these genes can regulate other genes and provide + or – feedback on the network
- Genes **A** and **B** have different effects because of their position in the network
- Deletion of **A** abrogates all downstream effects (is rate-limiting in the GRN)
- Deletion of **B** only has local effects



Individuals at the extreme for discovery of key rate-limiting genes

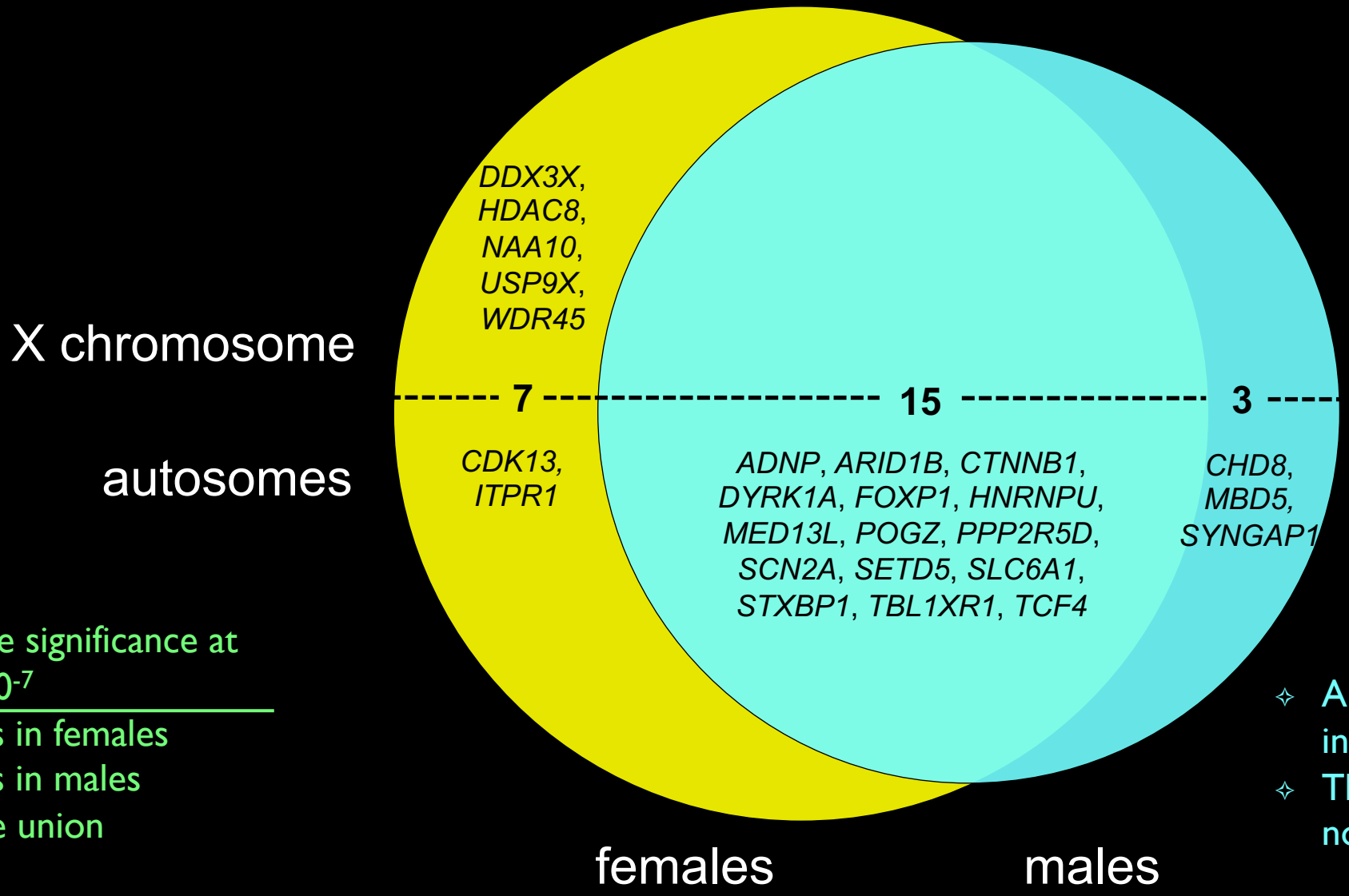




Females with NDDs (rarer class) may have rare variants of large effect in genes that are rate-limiting to GRN (e.g., they may regulate many other genes)

Chakravarti and Turner 2016, *Bioessays*

Replicated, significant genes in NDDs

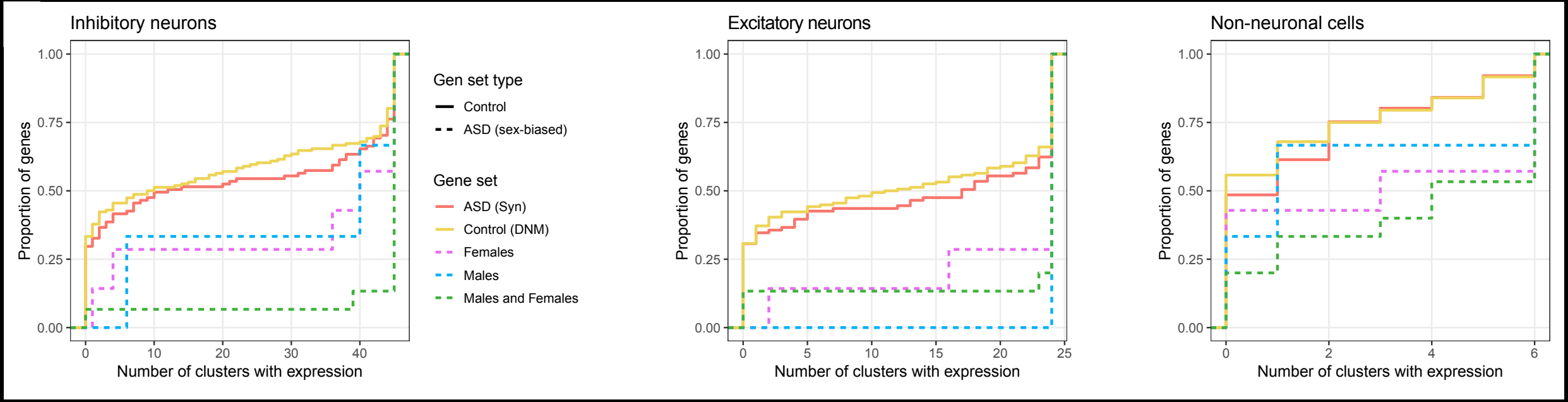


Exome-wide significance at $p < 4.3 \times 10^{-7}$

- 22 genes in females
- 18 genes in males
- 25 in the union

- Almost all genes are significant in females
- The 3 male-only are also nominally significant in females

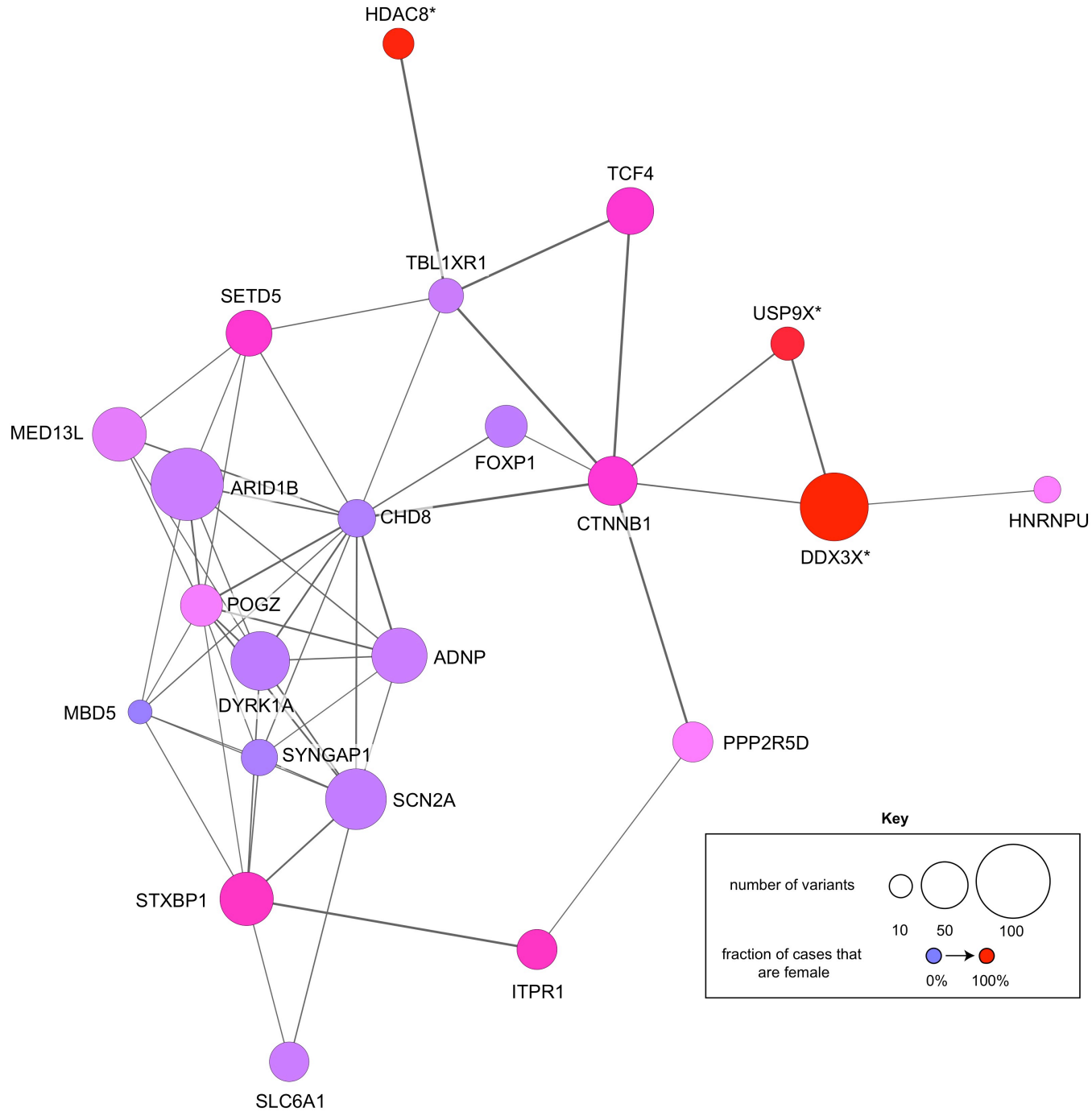
Expression of significant gene sets



- Sex-biased genes show broad expression across adult human cortical cell types
- Shared risk genes
  - are expressed in significantly more cell types than control genes
  - are enriched in significantly more neuronal cell types than non-neuronal cell types

Replicated significant genes are highly connected and are localized to the nucleus

- 22 of the 25 genes code for proteins within the same PPI network ($p < 1.0 \times 10^{-16}$)
- Associated with transcriptional processes ($p = 5.4 \times 10^{-4}$) and chromatin binding (4.7×10^{-3})
- 90.9% localize to the nucleus ($p = 9.3 \times 10^{-4}$)
- Very consistent with one of the core modules identified previously by many other groups
- These are key regulators of other genes



Conclusions for "Gene regulatory networks in autism"

- GRNs are important for our understanding of translation of gene discovery to molecular impact
- Study of the rarer class (females in autism) can identify genes that are rate-limiting to GRNs thereby revealing the pathways of focus
- When looking at NDDs, significant genes are highly connected and related to one another
- These genes are responsible for regulating many other genes

Conclusions and Future Directions

- Sex bias is an important consideration when assessing a phenotype
- Gene discovery, based on rare variants of large effect, is greatest in the rare class (females with NDDs) and can also reveal important genes on the X chromosome
  - However, it is important to note that most significant genes are not exclusively discovered in males or females
- Work remains in understanding the sex difference in autism with regards to both genetic (e.g., inherited variation) and non-genetic factors

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http://turnerlab.wustl.edu/

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