Autism spectrum disorders:
Transcriptomic characterization and implications for sex-differential risk

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Workshop on Sex Differences in Brain Disorders: Emerging Transcriptomic Evidence
National Academies' Forum on Neuroscience and Nervous System Disorders

September 23, 2020

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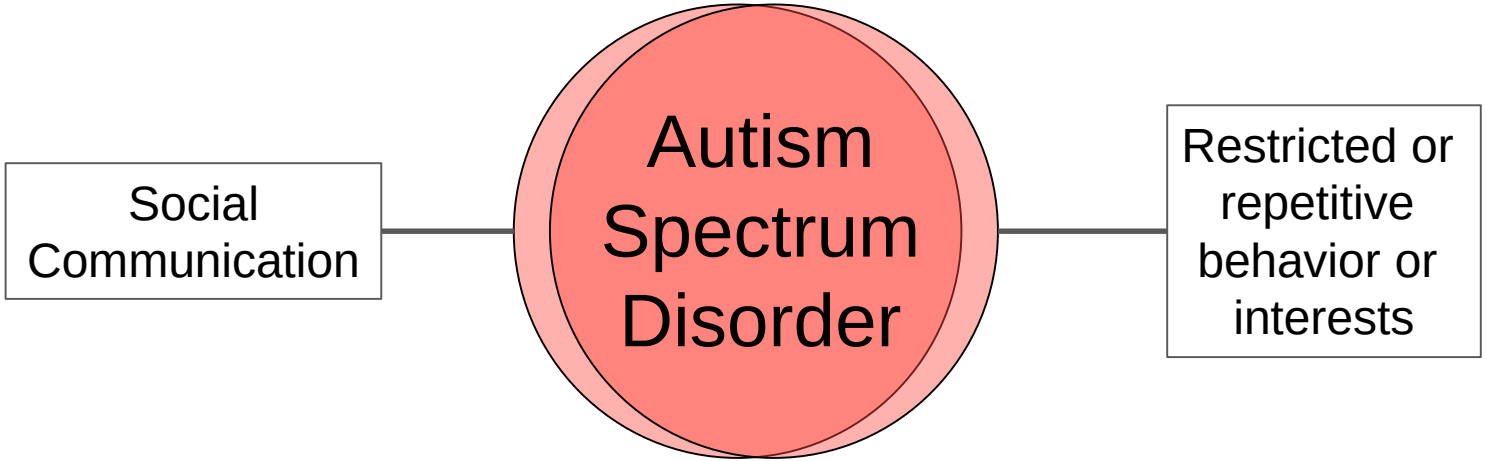
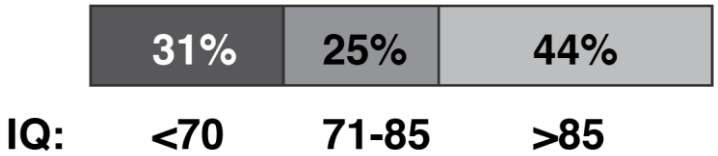
Autism spectrum disorders (ASDs)

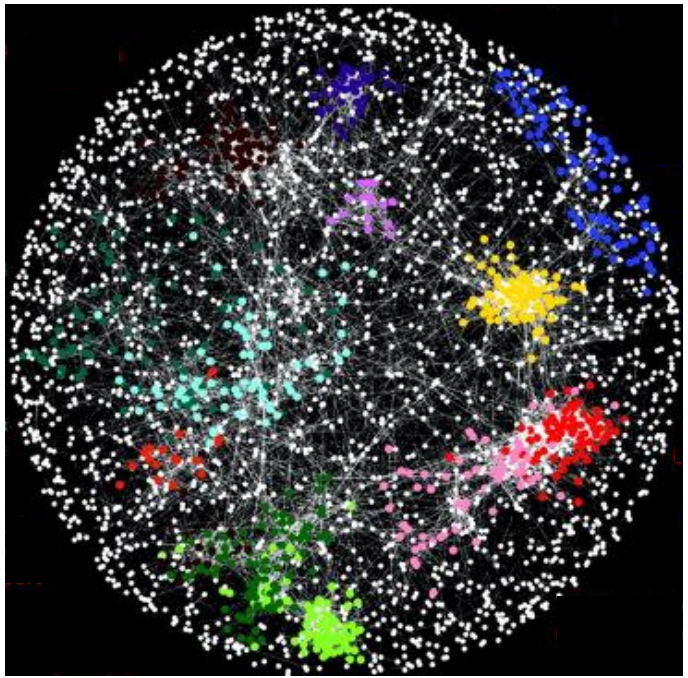


- Pervasive neurodevelopmental disorders, clinical impairment[1]
- Prevalence in US is **1/59 children** (1.7%)[2]
- Wide range of abilities/disabilities[2]
- Genetic variants associated with ASD risk



[1]APA, 2013, *DSM-5.* [2]Baio et al., 2018, *MMWR Surveil. Summ.*

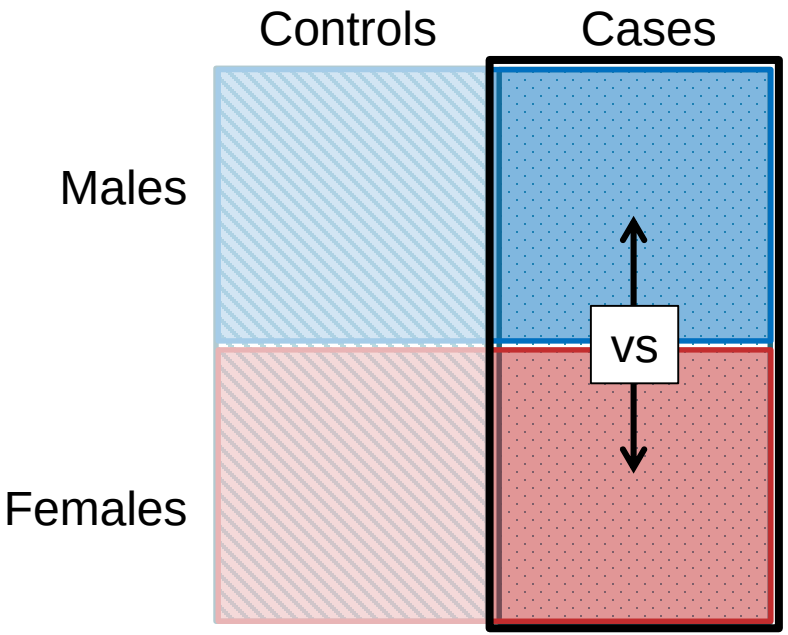
Transcriptomics can facilitate unbiased discovery of biological processes involved in a disorder or trait



Costanzo et al, *Science,* 2010

Genomics + sex differences: Transcriptome analyses can be used to characterize sex differences

1) In affected cases:



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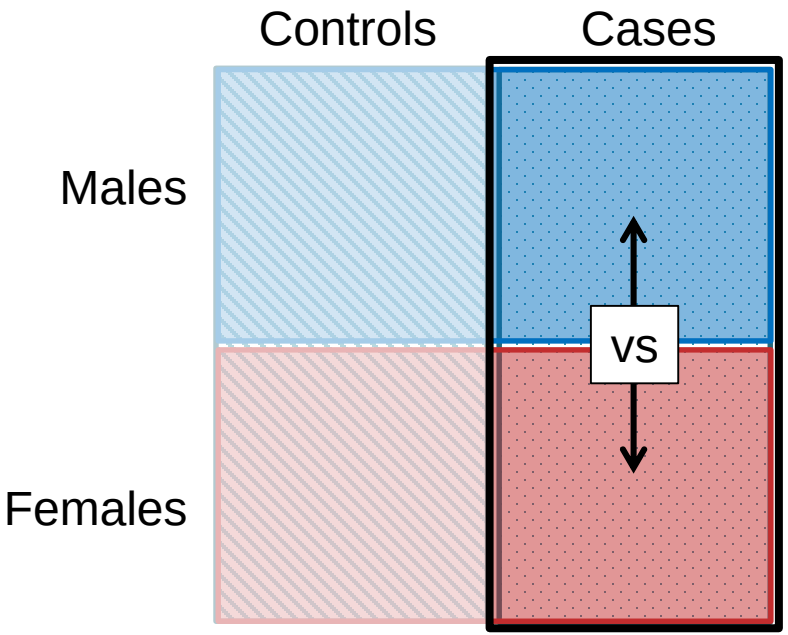
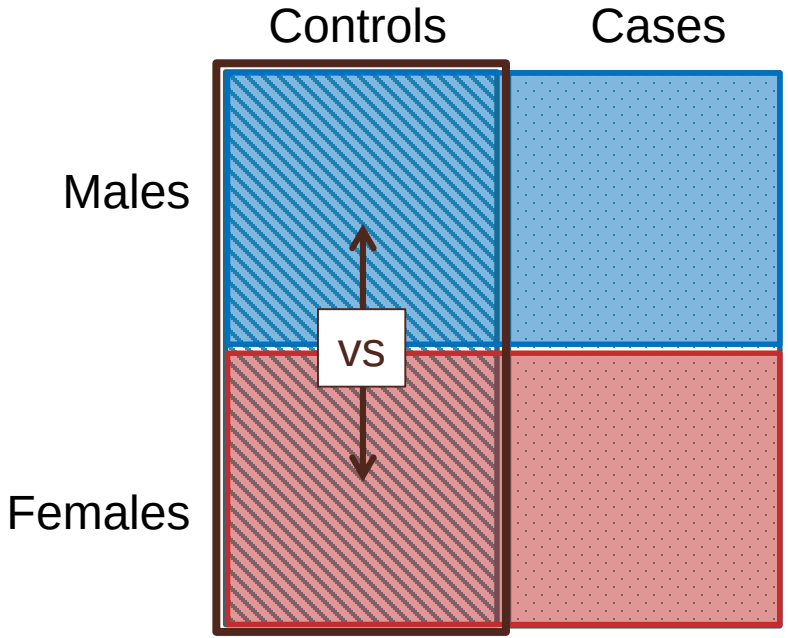


2) In risk mechanisms:



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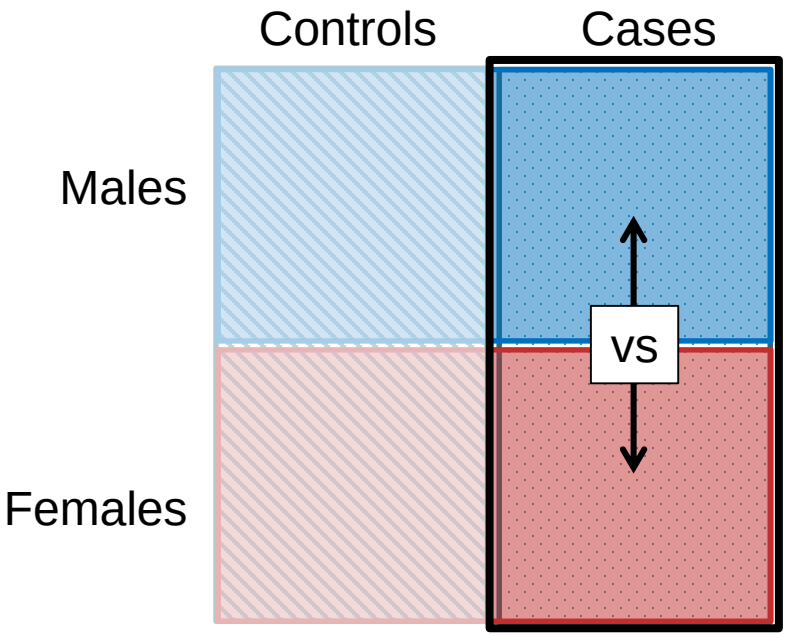

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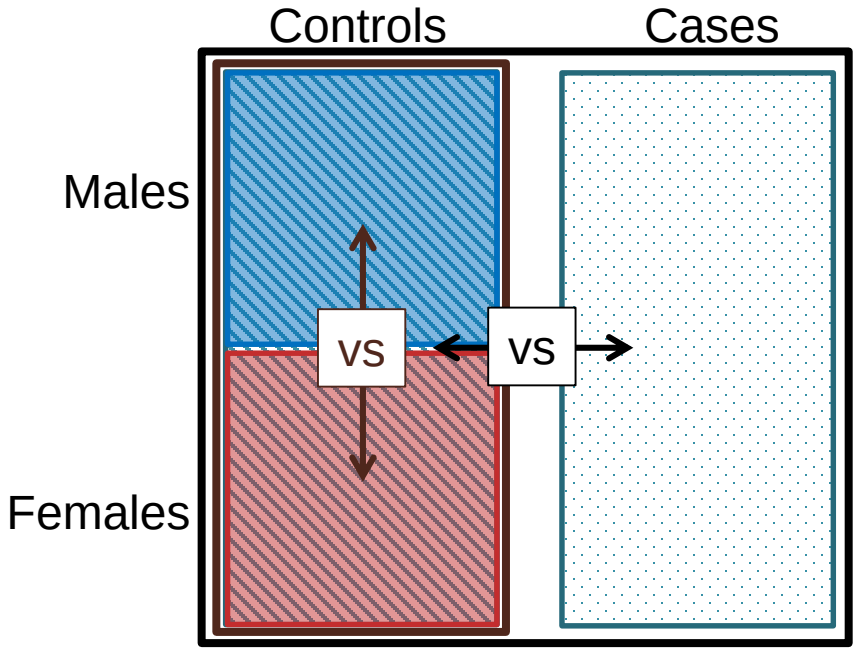
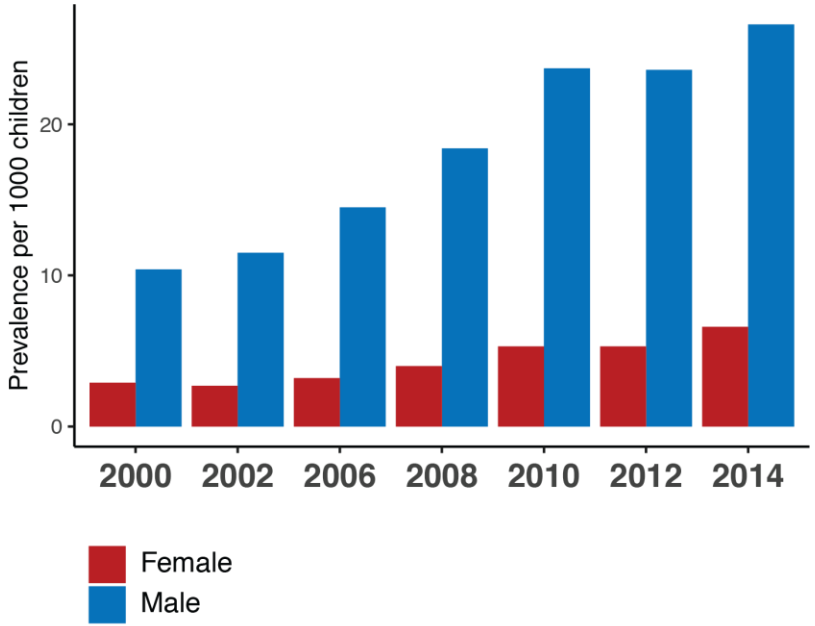
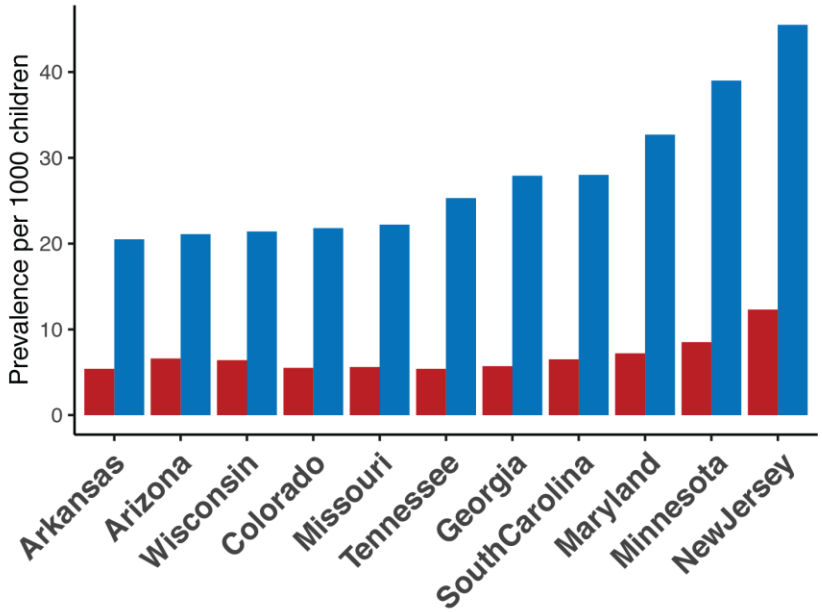


ASD prevalence is consistently ~4-fold higher in males than females

Over the years:

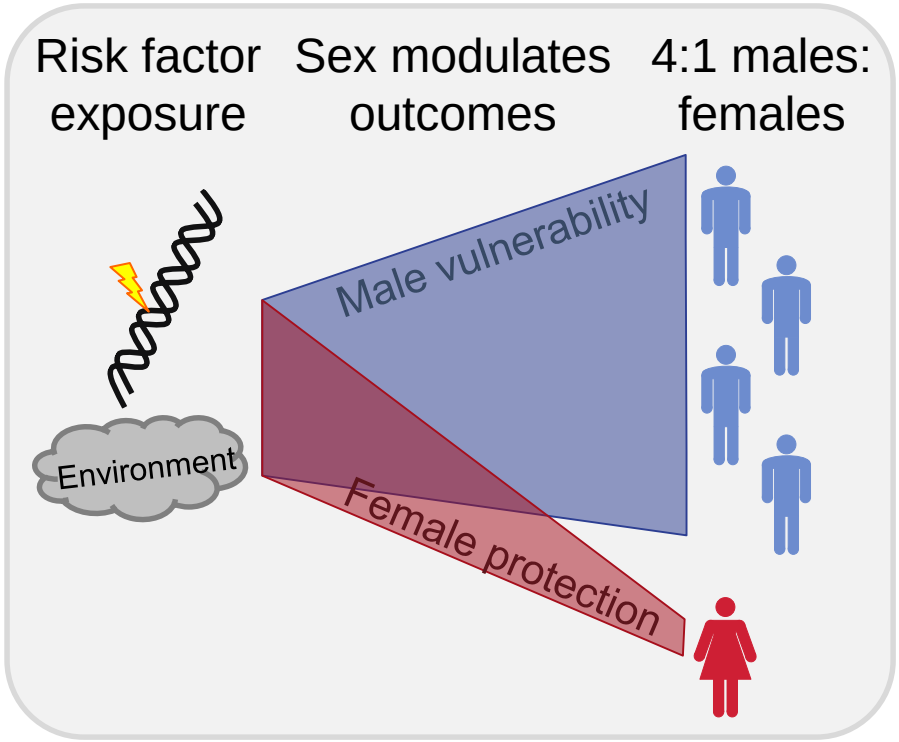


In different locations:

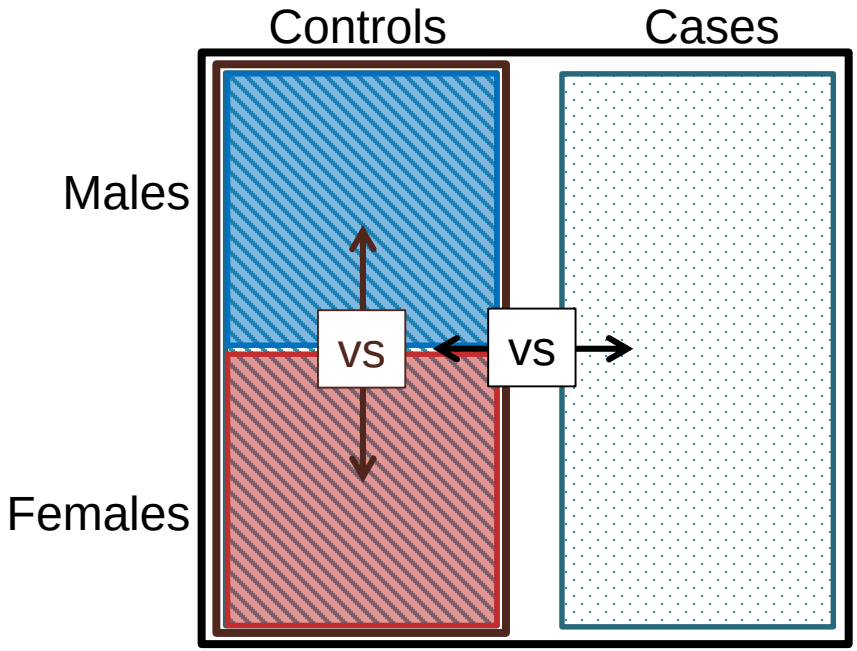


CDC ADDM, MMWRs: Baio *et al*, 2018; Christensen *et al,* 2016; Baio *et al,* 2014; Baio *et al,* 2012; Rice *et al,* 2009; Rice *et al,* 2007

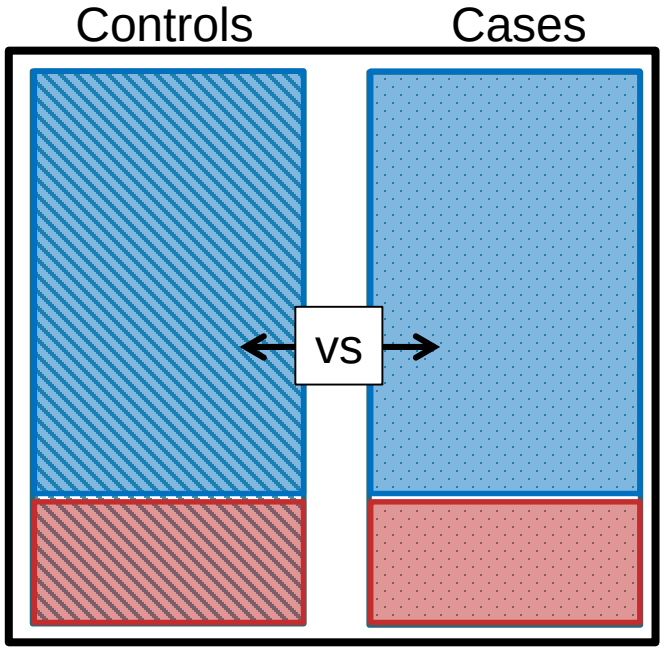
Genomics + sex differences: Transcriptome analyses can be used to characterize sex differences



In risk mechanisms:



ASD vs. Control differences identified by transcriptomics



Landmark studies of gene expression in ASD brain have used increasing, but still limited sample sizes

Year	Author	Total	ASD	M	F	Controls	M	F
2011	Voineagu et al	36	19	14	5	17	16	1
2014	Gupta et al	73	32	24	8	41	32	9
2016	Parikshak et al	96	48	39	9	48	40	8
2019	Velmeshev et al	31	15	12	3	16	12	4

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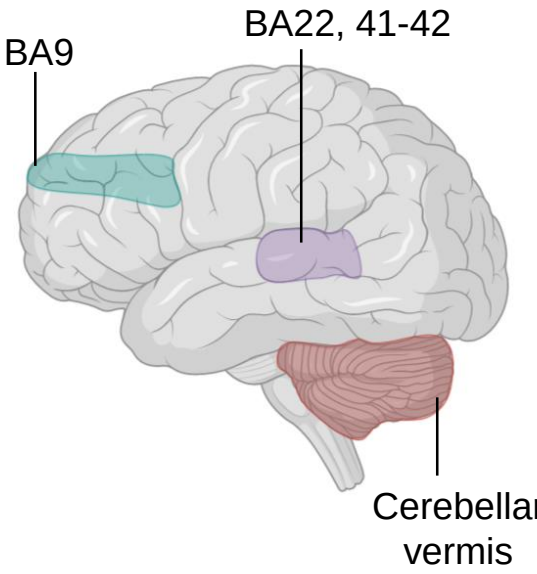
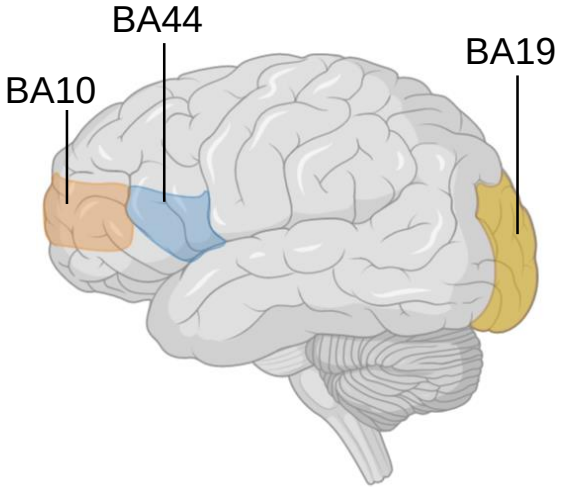
2019	Velmeshev et al	31	15	12	3	16	12	4
	~~Total:~~	~~236~~	~~114~~	~~89~~	~~25~~	~~122~~	~~100~~	~~22~~
	Total:	**160**	**69**	**55**	**14**	**91**	**71**	**20**

Landmark studies of gene expression in ASD brain have focused on a limited set of brain regions

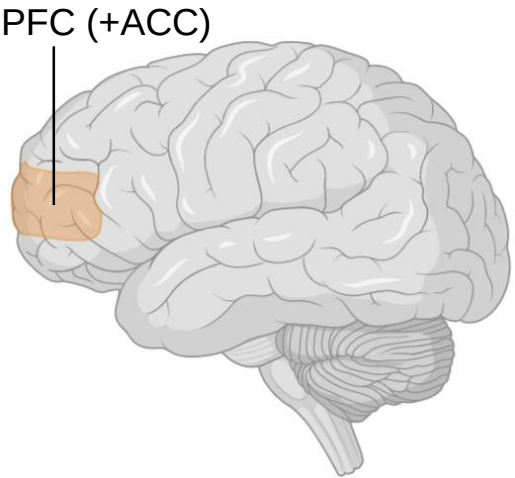
Voineagu et al, 2011
Parikshak et al, 2016



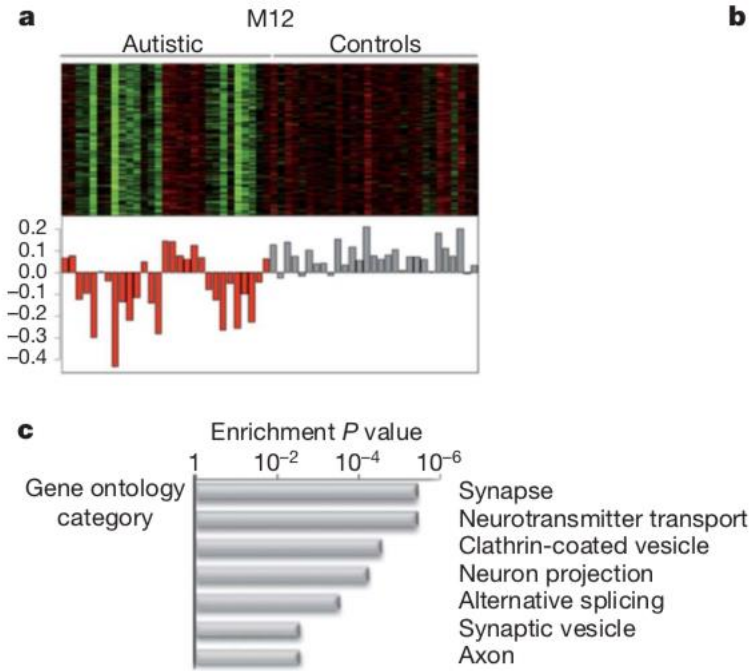
Gupta et al, 2014

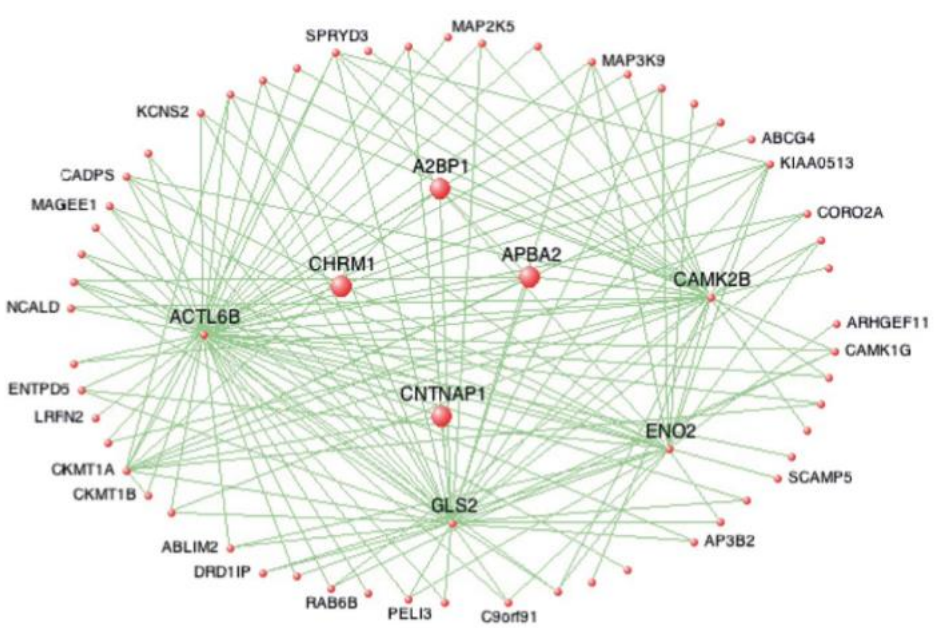


Velmeshev et al, 2019



Cases vs. controls, Voineagu et al (2011), N ASD = 19: Neuronal/synapse-associated modules show reduced expression in ASD cortex

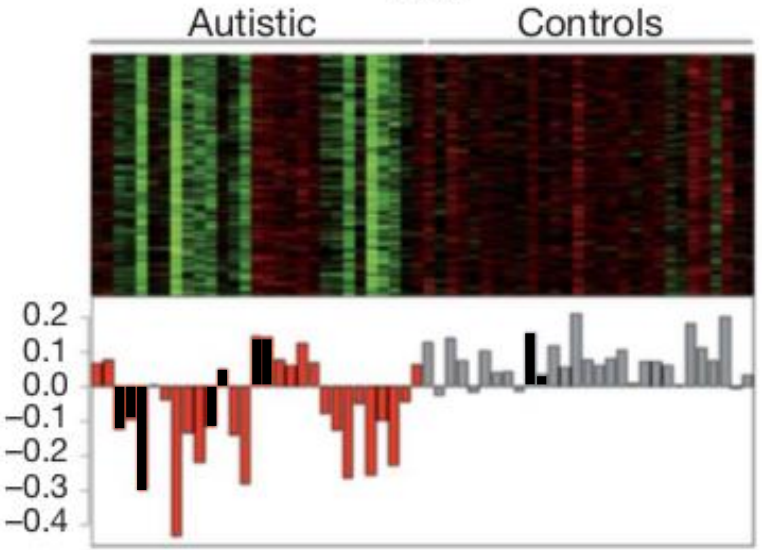




Voineagu et al, *Nature,* 2011

Cases vs. controls, Voineagu et al (2011), N ASD = 19: Neuronal/synapse module ↓**ASD is not a male-specific pattern**

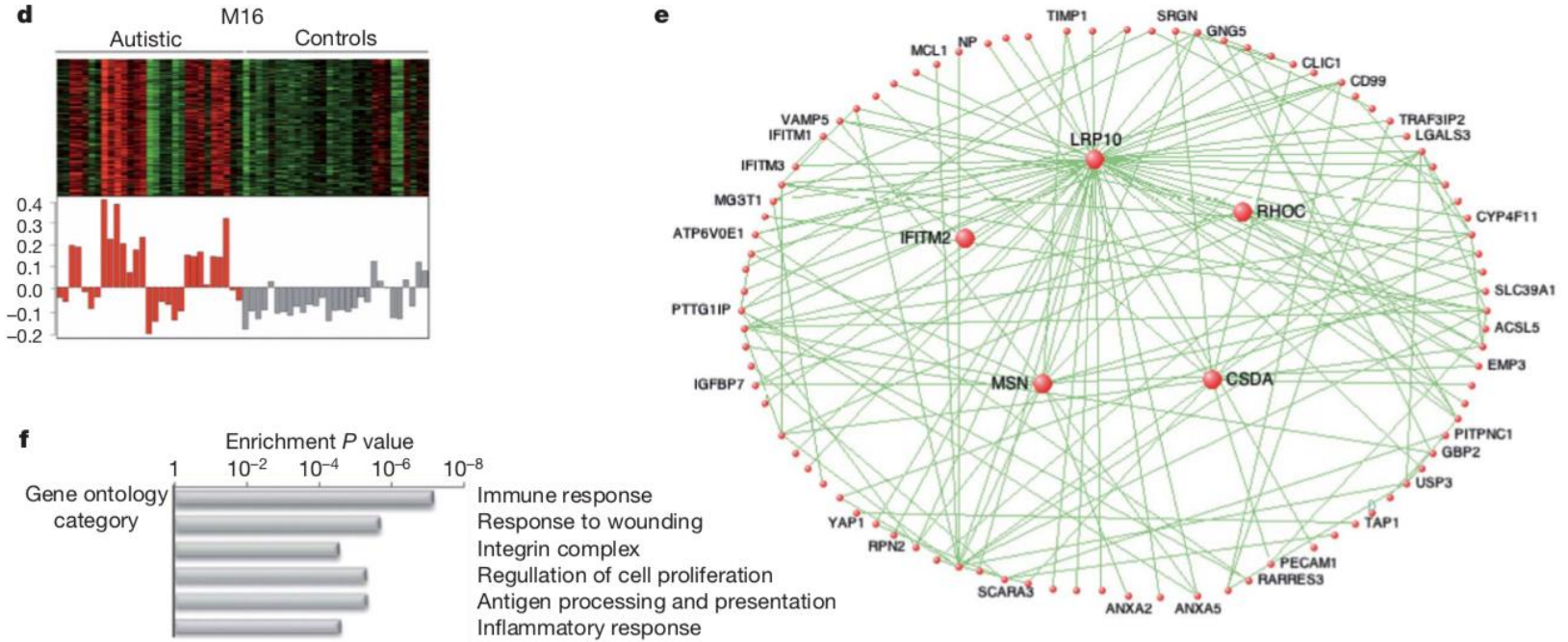
M12: Synaptic, neuronal genes



Females show ↓ expression comparable to males

Voineagu et al, *Nature,* 2011

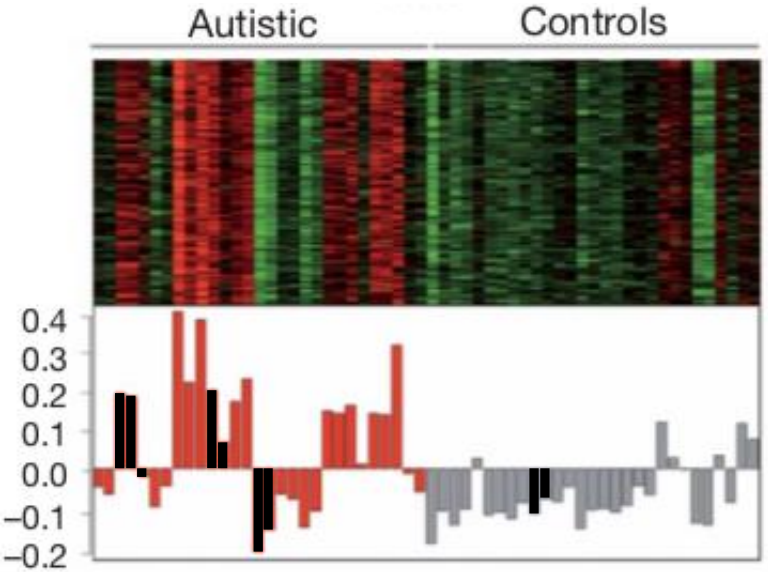
Cases vs. controls, Voineagu et al (2011), N ASD = 19 : Immune/inflammatory/astrocyte/microglia-associated modules show elevated expression in ASD cortex



Voineagu et al, *Nature,* 2011

Cases vs. controls, Voineagu et al (2011), N ASD = 19: Immune/glial module ↑**ASD is not a male-specific pattern**

M16: Immune/glial genes



Females show ↑ expression comparable to males

Voineagu et al, *Nature,* 2011

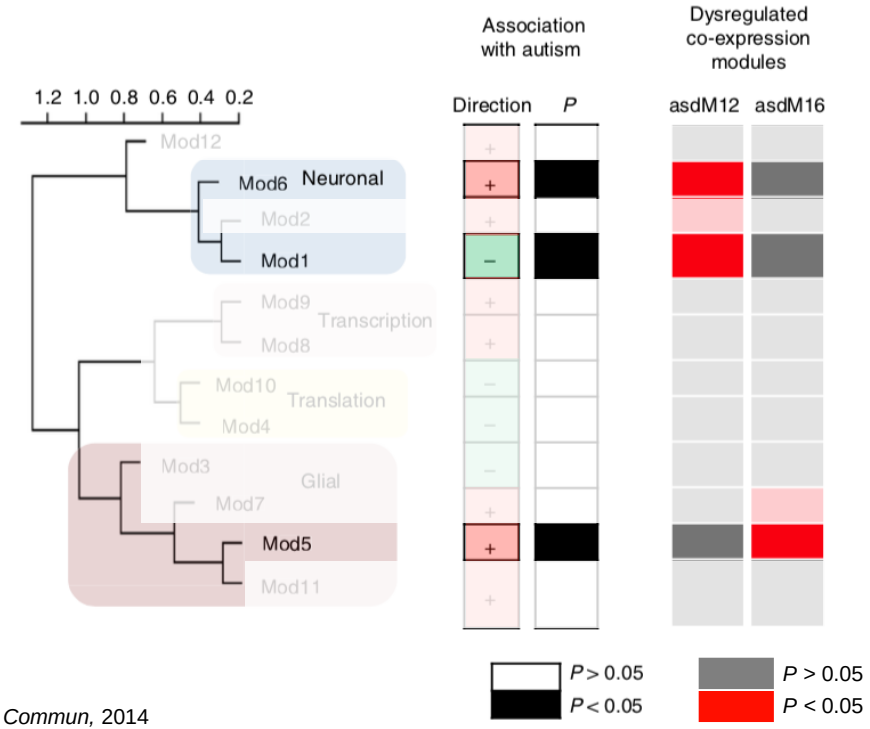
Cases vs. controls:

In ASD frontal and temporal cortex:

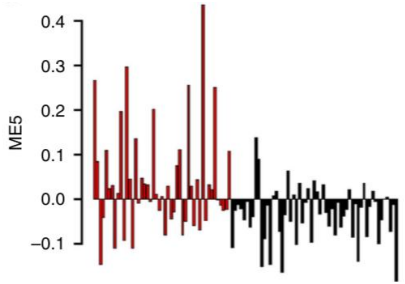
- ↓ Neuronal/synaptic genes
- ↑ Immune/astrocyte/microglia genes

These same patterns are validated and refined in later studies with larger sample sizes.

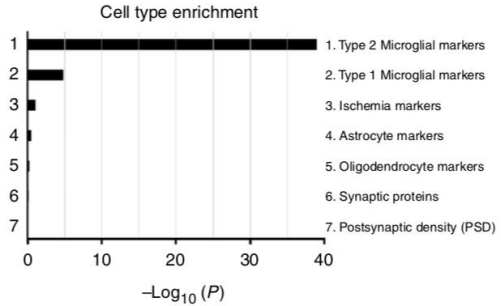
Cases vs. controls, Gupta et al (2014), N ASD = 32:
3 ASD-associated modules
ASD↓ neuronal/synaptic & ASD↑ immune/glial genes



Mod5 up-regulated in ASD



Mod5 enriched for Type 2 microglial markers

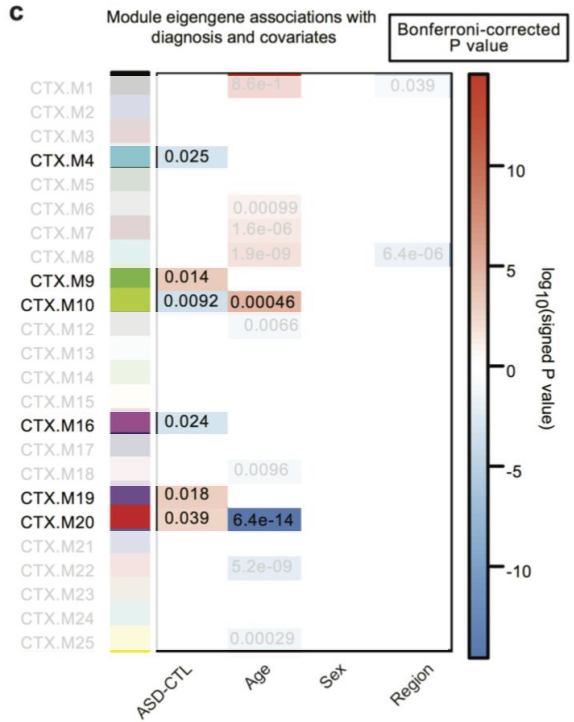


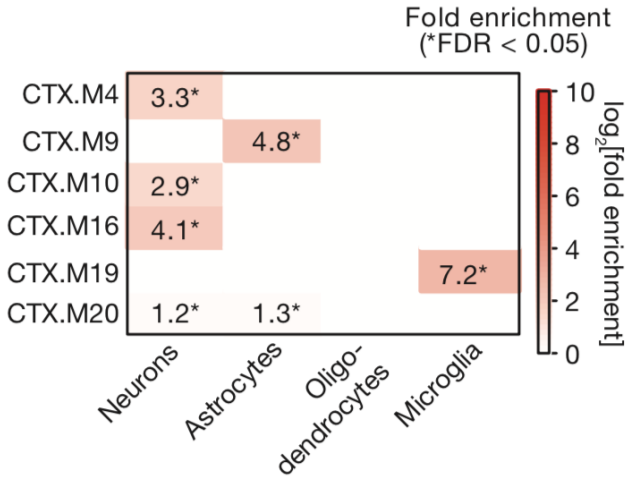
Gupta et al, *Nat Commun,* 2014

Cases vs. controls, Parikshak et al (2016), N ASD = 48:

6 ASD-associated modules

ASD↓ neuronal/synaptic & ASD↑ immune/glial genes





Parikshak, Swarup, Belgard et al, *Nature,* 2016

Summary, cases vs. controls:

In ASD frontal and temporal cortex:

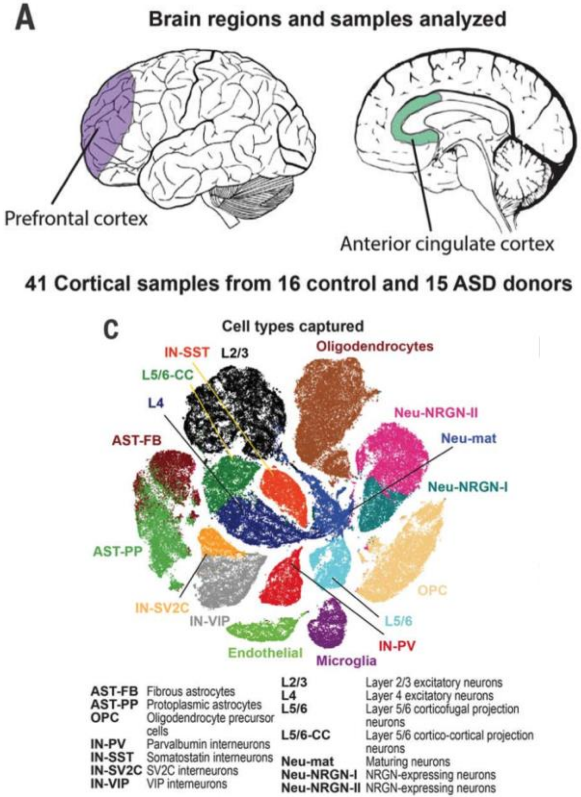
↓ Neuronal/synaptic genes

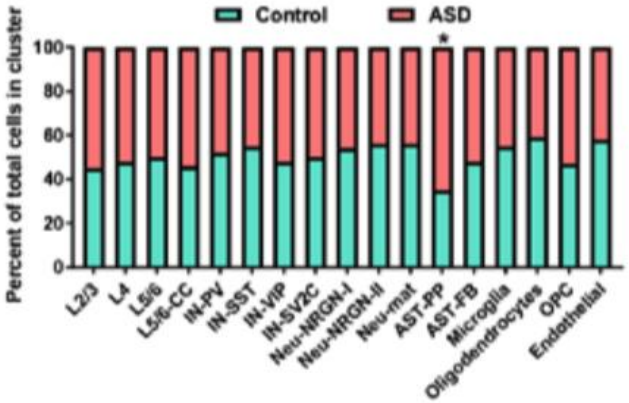
↑ Immune/astrocyte/microglia genes

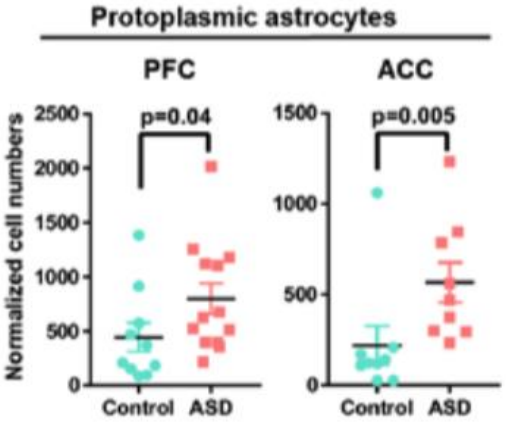
Do these patterns indicate alterations in cell type composition or function?

See: Single cell expression

Cases vs. controls, single cell RNA-seq (2019), N ASD = 15: Larger proportion of protoplasmic astrocytes in ASD





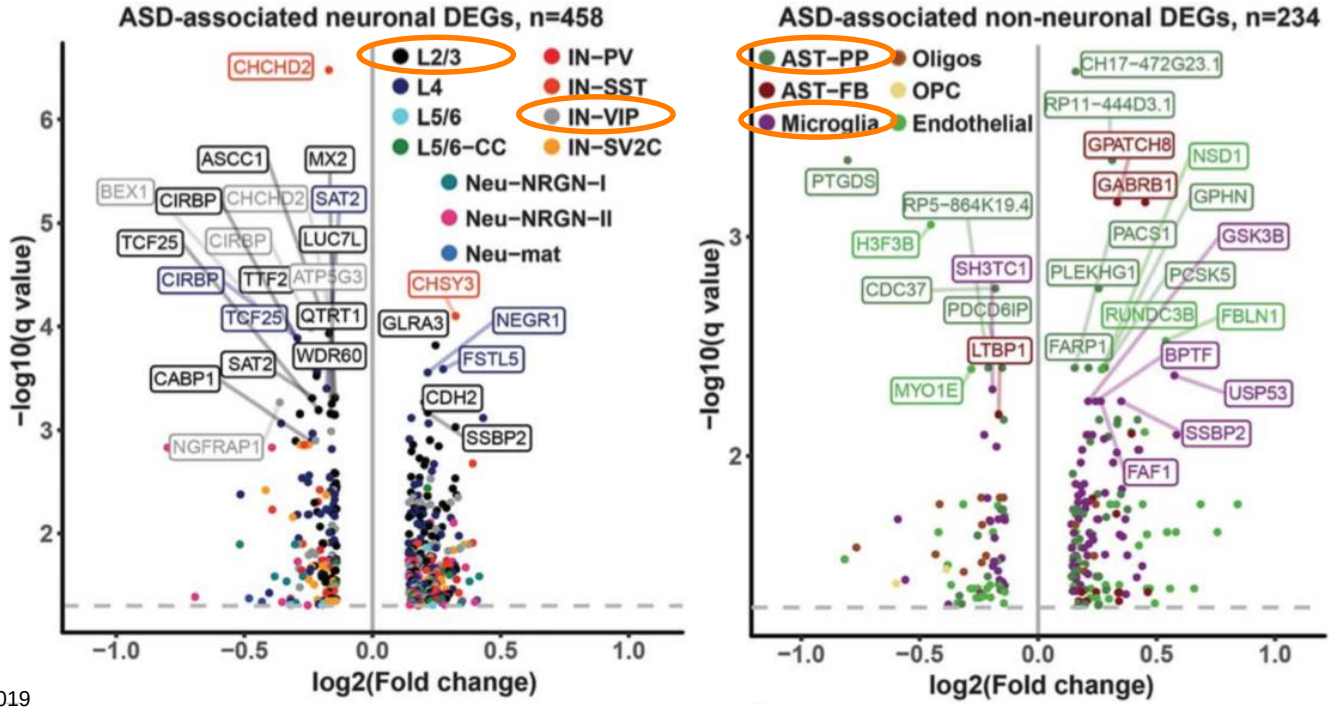


Velmeshev et al, *Science,* 2019

Cases vs. controls, single cell RNA-seq (2019), N ASD = 15:

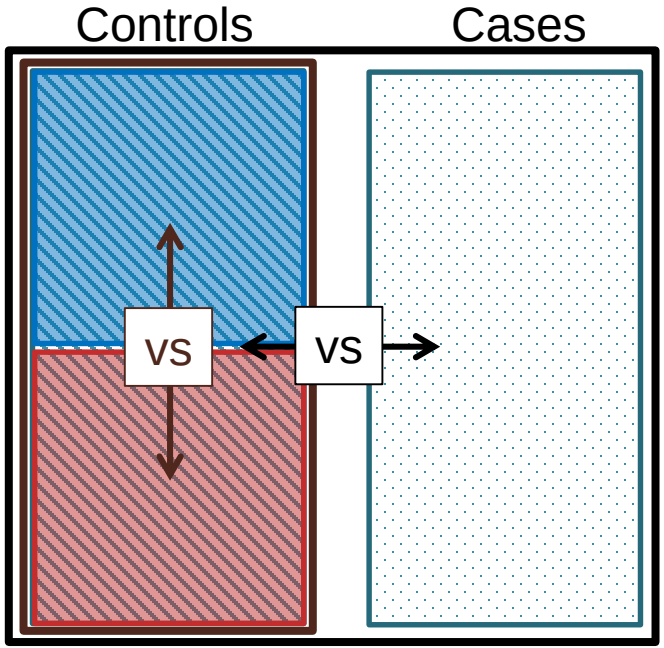
ASD↓↓ genes in L2/3 excitatory neurons & VIP interneurons

ASD↑↑ genes in protoplasmic astrocytes & microglia



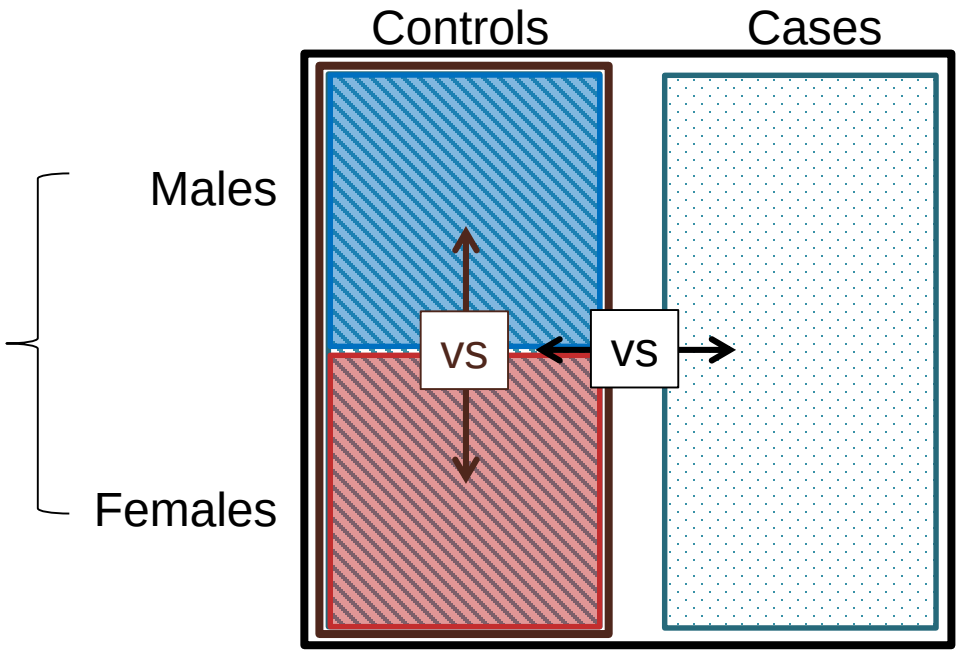
Velmeshev et al, *Science*, 2019

Neurotypical sex differences and their relationship to ASD-control differences



Males vs. females + cases vs. controls:

BRAINSPAN
ATLAS OF THE DEVELOPING HUMAN BRAIN

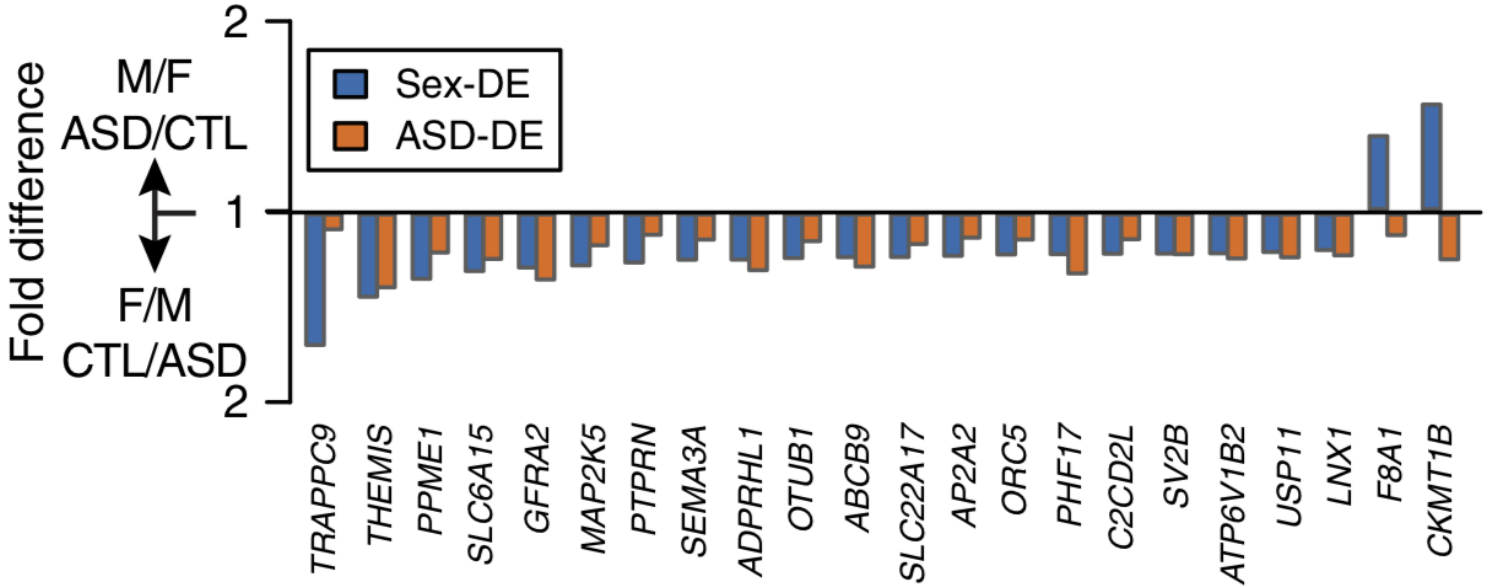


Werling et al, *Nat Commun,* 2016

Males vs. females + cases vs. controls:

ASD↓ synaptic/neuronal module genes have female-biased expression in cortex

M12: Synaptic, neuronal genes

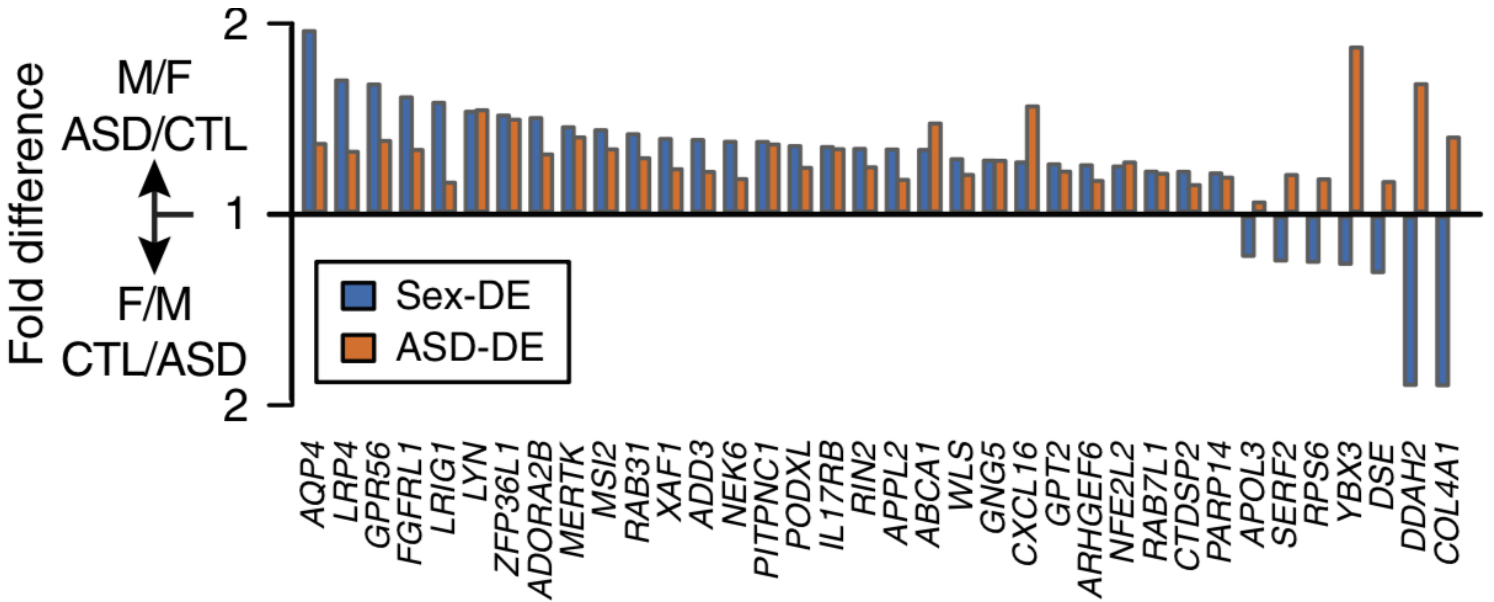


Werling et al, *Nat Commun,* 2016

Males vs. females:

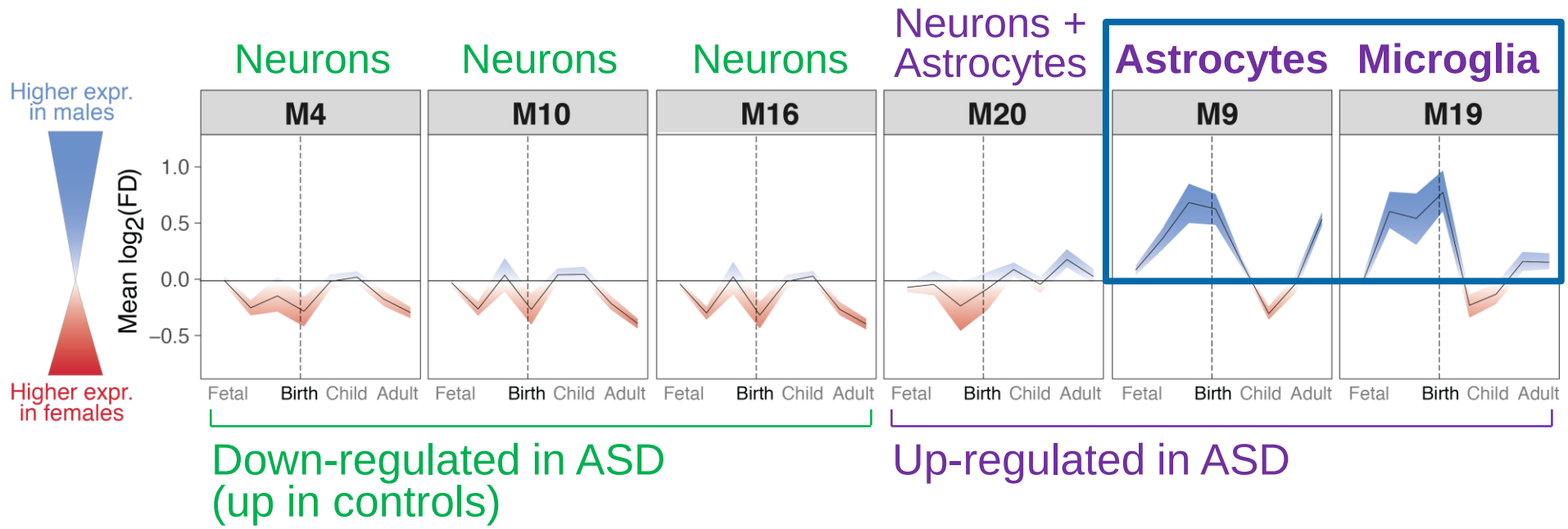
ASD↑ immune/glia module genes have male-biased expression in cortex

M16: Immune/glial genes



Werling et al, *Nat Commun,* 2016

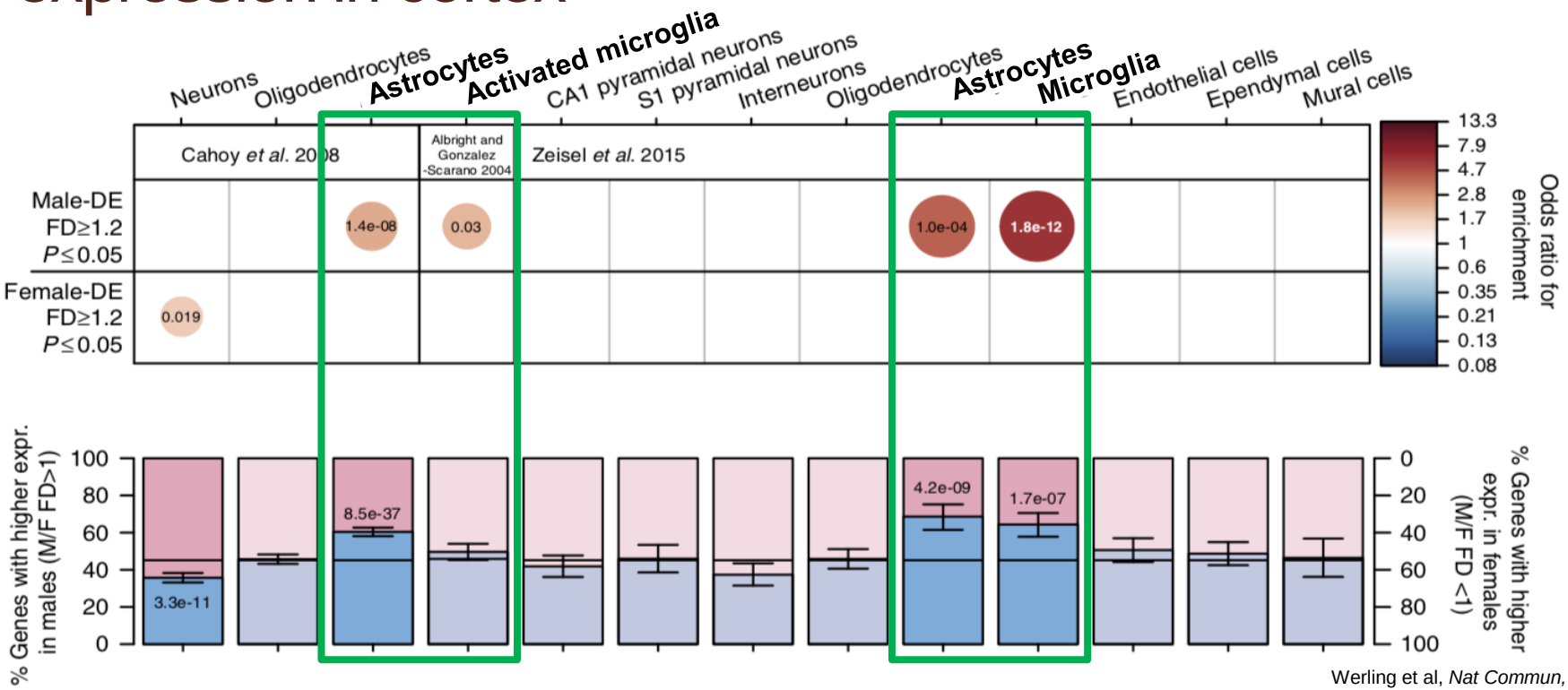
Genes up-regulated in the ASD brain show prenatal, male-biased expression



ASD-Control analysis: Parikshak, Swarup, Belgard *et al, Nature,* 2016

Males vs. females:

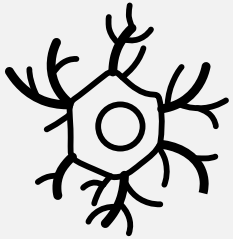
Astrocyte and microglia marker genes have male-biased expression in cortex



Werling et al, *Nat Commun,* 2016

Sex-differential function of glia and/or neurons may contribute to ASD pathobiology and/or protective mechanisms

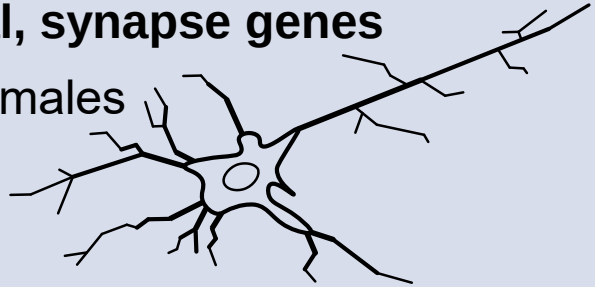
Microglia, astrocyte genes



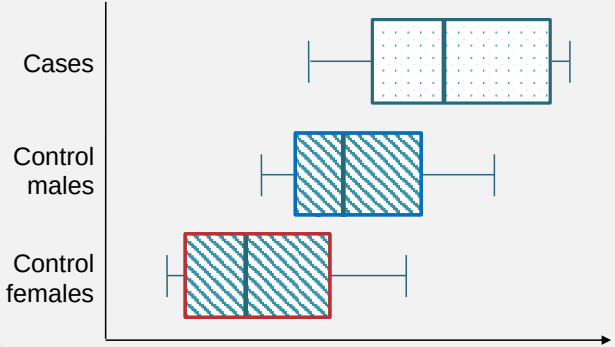
↑ ASD, ↑ Males

Neuronal, synapse genes

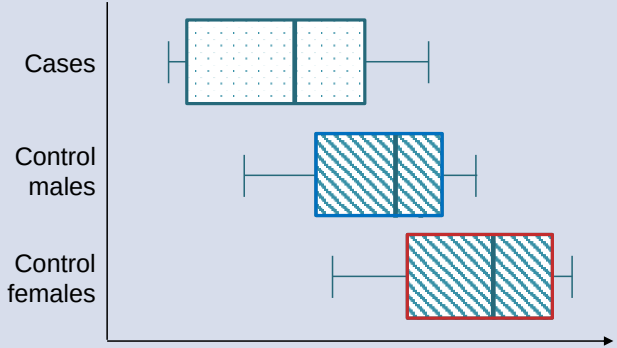
↓ ASD, ↑ Females



Pathobiology mechanisms



Protective mechanisms



Current limitations and future directions

- **Sample size:** Transcriptome-level characterization of **female ASD** is severely **limited by sample size**
- **Brain region:** Transcriptome analyses have focused primarily on **cortex and cerebellum**, while subcortical regions show more robust sex differences
- **Cell type:** Single cell data can help resolve patterns from bulk tissue that suggest roles for specific cell types in pathobiology or protection
- **Sample age:** Current data from postnatal, child-adult donors; data from fetal development, infancy, and defined stages of sexual development is sparse

We need more information about baseline sex differences in healthy individuals, across **cell types**, **brain regions**, and **developmental stages**

Acknowledgements

Werling lab:
- Lee Kissel
- Nicole Montag
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- Sanders lab

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- Jinmyung Choi
- Forrest Gulden
- Xuming Xu
- Mingfeng Li
- Rob Kitchen

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

USC:
- Jim Knowles

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NIH National Institute of Mental Health

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