

# Neural Transcriptomic Analysis of Sex Differences in Autism Spectrum Disorder: Current Insights and Future Directions

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## ABSTRACT

Autism spectrum disorder (ASD) is consistently diagnosed 3 to 5 times more frequently in males than females, a dramatically sex-biased prevalence that suggests the involvement of sex-differential biological factors in modulating risk. The genomic scale of transcriptomic analyses of human brain tissue can provide an unbiased approach for identifying genes and associated functional processes at the intersection of sex-differential and ASD-impacted neurobiology. Several studies characterizing gene expression changes in the ASD brain have been published in recent years with increasing sample size and cellular resolution. These studies report several convergent patterns across data sets and genetically heterogeneous samples in the ASD brain, including elevated expression of gene sets associated with glial and immune function, and reduced expression of gene sets associated with neuronal and synaptic functions. Assessment of neurotypical cortex tissue has reported parallel patterns by sex, with male-elevated expression of overlapping sets of glial/immune-related genes and female-biased expression of neuron-associated genes, suggesting potential roles for these cell types in sex-differential ASD risk mechanisms. However, validating and further exploring these mechanisms is challenged by the available data, as existing studies of ASD brain include a limited number of female ASD donors and focus predominantly on cortex regions not known to show pronounced sex-differential morphology or function. With this review, we summarize convergent findings from several landmark studies of the transcriptome in ASD brain and their relationship to sex-differential gene expression, and we discuss limitations and remaining questions regarding transcriptomic analysis of sex differences in ASD.

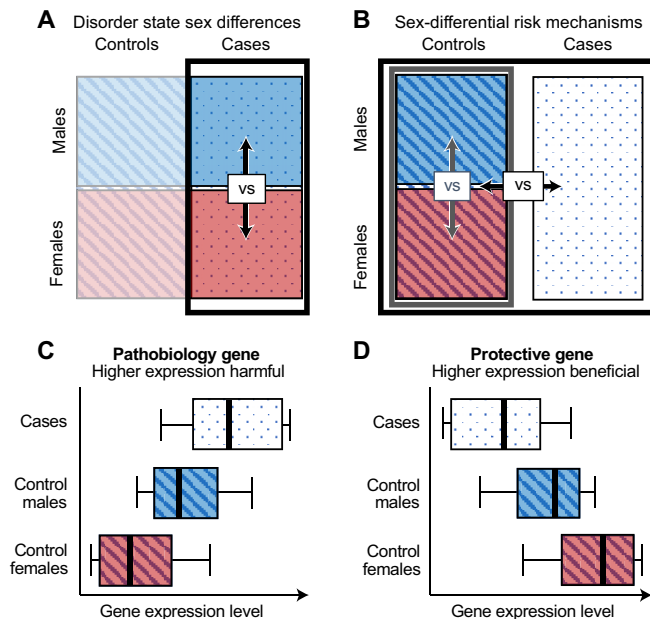
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Autism spectrum disorder (ASD) is a pervasive neurodevelopmental disorder that currently affects about 1 in 54 children in the United States (1). With a consistent 3- to 5-fold greater diagnosis in males relative to females (1,2), ASD is among the most sex-skewed neuropsychiatric conditions. Despite this difference in prevalence, large-scale studies comparing the clinical phenotype of ASD in affected males and females have described reduced restricted and repetitive behaviors in females (3–6) and variable sex effects on social communication traits (4,5), but similar overall severity between the sexes (3,4). Both common inherited variants (7,8) and rare de novo highly deleterious variants (9,10) contribute to ASD risk. Though several X chromosome loci are implicated, including *FMR1* (fragile X syndrome) (11), *MECP2* (Rett syndrome) (12), *NLGN3*, and *NLGN4X* (13), the majority of known risk variants are autosomal (8–10). Several autosomal ASD risk genes are preferentially associated with developmental disorder in one sex, including male-biased incidence in ASD cases of rare deleterious variants in *CHD8*, *MBD5*, and *SYNGAP1* (14), but the mechanisms behind these differences are not fully understood.

By generating an approximate readout of molecular function in tissues and cells at a genomic scale, transcriptomic analyses in the brain are useful for exploring the neurobiology of

brain disorders and can provide information that is complementary to gene discovery work. With the application of appropriate statistical methods, the genomic scale of transcriptome analyses allows unbiased discovery of the biological processes involved in a condition and facilitates bioinformatic comparison across genome-scale data sets to identify points of convergence. Transcriptome studies can also be applied to characterize sex differences in a specific condition or trait, including differences between affected males and females and sex-differential risk mechanisms. Identification of male-female differences in the disorder state, which may be suggestive of sex-differential etiology or response to a disorder, requires sufficiently powered and balanced samples of affected males and females (Figure 1A). Investigation of sex-differential risk requires the integration of case-control comparisons with the characterization of baseline neurotypical sex differences to identify genes and associated biological processes that are influenced by both sex and disorder biology (Figure 1B).

Such genes can be further classified by their direction of effect on risk. Genes with high(er) expression in the disorder state and in the more frequently affected sex (males, for ASD) are more likely to be involved in, or tagging, sex-differential vulnerability mechanisms of disorder pathobiology (Figure 1C). Genes with high(er) expression in the healthy state



**Figure 1.** Study designs and outcomes for transcriptomic analysis of sex differences. **(A)** Characterization of sex differences in the disorder state requires comparisons of male and female cases. **(B)** Identification of genes and processes involved in sex-differential risk requires comparison of neurotypical male and female controls and comparison of control and case cohorts. **(C)** Putative pathobiology genes are predicted to show elevated expression in the disorder state and in the more frequently affected sex. **(D)** Putative protective genes are predicted to show reduced expression in the disorder state and elevated expression in the less frequently affected sex.

and in the less frequently affected sex (females, for ASD) are likely involved in, or tagging, sex-differential protective mechanisms (Figure 1D). A major goal of transcriptomic studies exploring sex-differential risk mechanisms for ASD is to find these genes whose functions are involved in the amplification of male vulnerability and/or the reduction of female risk.

With this goal in mind, this review aims to delineate key results from landmark studies of transcriptomic patterns in ASD brain tissue and to describe how ASD-associated changes intersect with neurotypical sex differences. We also discuss implications of these patterns for sex-differential risk mechanisms, limitations of currently available data sets, and remaining questions for future investigation.

### AVAILABILITY OF TRANSCRIPTOME DATA FROM THE ASD BRAIN

Several landmark studies characterizing transcriptomic differences in ASD versus control brain tissue and cells have been published to date (15–19). However, available brain tissue from ASD donors is sparse, and so these studies have relied on limited sample sizes (Table 1), ranging from just 6 ASD donors in an early gene expression study (15) to 48 ASD donors in the most recent report on bulk brain tissue transcriptomics (18). Critically, study samples were not fully independent. In total, tissues assayed across these 5 studies were derived from 160 unique donors: 69 ASD and 91 control subjects. These are relatively small numbers with which to tackle the known

genetic and phenotypic heterogeneity of ASD, and they also lag behind transcriptome data generation for other neuropsychiatric disorders, such as schizophrenia, for which a recent publication from the CommonMind Consortium included 353 cases (20). Tissue availability limits brain transcriptome studies of both conditions relative to genetic analyses, where recent genome-wide association studies included 18,381 ASD (8) and 36,989 schizophrenia cases (21), and rare variant analyses included 11,986 ASD (10) and 3444 schizophrenia cases (22).

Consistent with ASD's male-skewed prevalence, all data sets are strongly male dominated: of the 69 total ASD donors, only 14 are female. Appropriately, all case and control groups are sex matched, reducing the likelihood of confounding sex with case-control status, but directly limiting the number of female brain transcriptomes available for analysis. This paucity of data from case and control females presents significant challenges for characterizing differential expression patterns 1) between male and female ASD cases, such as has been observed for major depressive disorder (23,24) and post-traumatic stress disorder (25), 2) between female cases and controls, to determine how the female ASD brain diverges from sex-specific expectation, and 3) to identify sex-by-diagnosis interaction effects, in which ASD transcriptomic changes differ in magnitude or direction of effect between the sexes.

### TRANSCRIPTOMIC CHANGES IN THE ASD BRAIN

Despite relatively small sample sizes, studies of bulk brain tissue have successfully described alterations in the ASD neural transcriptome. One of the more consistently observed patterns is the elevated expression of genes associated with astrocyte, microglial, immune, and inflammatory function, which was first reported by Garbett *et al.* (15). Differential expression analysis of microarray data from 6 ASD-control pairs from the Autism Tissue Program (now Autism BrainNet, [www.autismbrainnet.org](http://www.autismbrainnet.org)) revealed increased expression of genes enriched for antigen-specific and cell-specific immune response, inflammation, autoimmunity, and immune-mediated cell death Gene Ontology categories in the ASD superior temporal gyrus. Subsequent larger studies of bulk tissue using microarray (16) or RNA sequencing (17,18) have recapitulated this pattern via the discovery and functional annotation of gene coexpression networks, or modules. Specifically, Voineagu *et al.* (16) identified a module, labeled M16, that was enriched for genes associated with the functions of astrocytes and microglia and upregulated in the ASD brain relative to control. A 2014 study from Gupta *et al.* (17) used a larger sample and signed coexpression networks to resolve M16 into separate astrocyte-associated (Mod7) and activated microglia-associated (Mod5) modules, although only the ASD-elevated expression of Mod5 was significant after multiple testing correction. The latest and largest study, by Parikshak *et al.* (18), further expanded the resolution of this signal by identifying 3 modules with significantly elevated expression in ASD, including one with clear enrichment for astrocyte markers (CTX.M9) and another enriched for microglia markers (CTX.M19).

It is not known whether these putative changes in glial involvement are secondary consequences of an upstream risk exposure or whether astrocytes and microglia are involved in

**Table 1. Tissue Donors and Brain Regions Assayed in Transcriptome Studies of ASD Brain**

Study	Data Type	Total Donors	ASD		Control		Region			
			Female	Male	Female	Male	Frontal Cortex	Temporal Cortex	Occipital Cortex	Cerebellum
Garbett <i>et al.</i> , 2008 (15)	Microarray, bulk tissue	12	2	4	2	4	No	STG	No	No
Voineagu <i>et al.</i> , 2011 (16)	Microarray, bulk tissue	36	5	14	1	16	BA 9	BA 22, BA 41–42	No	Yes
Gupta <i>et al.</i> , 2014 (17)	RNA-seq, bulk tissue	73	8	24	9	32	BA 10, BA 44	No	BA 19	No
Parikshak <i>et al.</i> , 2016 (18)	RNA-seq, bulk tissue	96	9	39	8	40	BA 9	BA 22, BA 41–42	No	Vermis
Velmeshev <i>et al.</i> , 2019 (19)	RNA-seq, single-cell	31	3	12	4	12	PFC, ACC	No	No	No
Total Unique Donors		169	16	59	20	74				

Total number of donor individuals and the brain regions assayed in each study are listed.

ACC, anterior cingulate cortex; ASD, autism spectrum disorder; BA, Brodmann area; PFC, prefrontal cortex; RNA-seq, RNA sequencing; STG, superior temporal gyrus.

the primary, symptom-associated pathology of ASD. None of the studies described here observed enrichment of ASD genetic risk factors, including rare or common variants, within any of the ASD-elevated glia/immune-associated modules, suggesting that the functional changes driving these elevated expression patterns are likely downstream from the immediate effects of genetic risk variants. Regardless of whether these changes are downstream or upstream from causal factors, it remains possible that they are upstream from (and contribute to) ASD symptoms, which would have implications for the utility of glial/immune pathways as treatment targets. Future experimental work is needed to address this possibility.

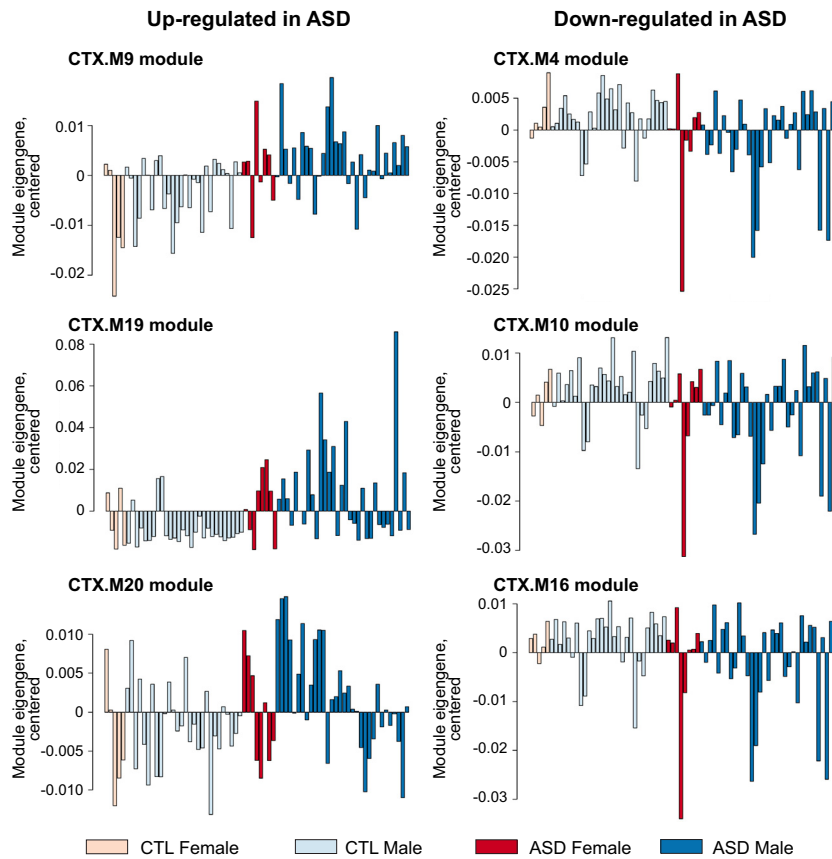
Bulk tissue transcriptomic analyses have also identified modules associated with neuronal and synaptic functions that generally show reduced expression in ASD cortex. Voineagu *et al.* (16) reported an ASD-downregulated neuronal module (M12) associated with synaptic function, vesicular transport, and neuronal projection, and Gupta *et al.* (17) resolved M12 into 3 signed modules, all enriched for synaptic transmission function. Of these 3, Mod6 was significantly upregulated in ASD, while Mod1 genes, found to be associated with inhibitory ion channel activity related to synaptic transmission, were significantly downregulated. Parikshak *et al.* (18) later identified 3 ASD-downregulated modules (CTX.M4, CTX.M10, and CTX.M16) that also overlap with the M12 module and are enriched for neuronal markers and synapse genes, and these authors also implicated neuronal firing rate as a key feature in the CTX.M10 and CTX.M16 modules.

In contrast to the upregulated glial/immune genes, downregulated neuronal/synaptic genes show some evidence of overlap with ASD genetic risk, potentially suggesting that changes in these genes' expression may be closer to ASD's causal roots. Using a permutation test to assess gene set enrichment for common variant association signal from an early ASD genome-wide association study (26), Voineagu *et al.* (16) observed that M12 genes showed significant enrichment for ASD common variant risk. However, findings from Gupta *et al.* (17) and Parikshak *et al.* (18) did not support that initial genetic risk enrichment, instead finding rare variant risk gene enrichment for ASD only in modules that did not show

significant expression changes in ASD, and finding weak enrichment for common variant ASD genome-wide association study signal in the ASD-upregulated CTX.M20 module. These contrasting findings suggest that upstream genetic risk and downstream transcription changes affecting neurons may involve separable gene sets and functions.

Other expression changes reported in bulk tissue analyses of ASD brain include differences between brain regions, similarities between cases of different genetic etiology, and variation across individual donors. Studies that evaluated both cortex and cerebellar tissue (16,18) observed larger-magnitude expression changes in the cortex, leading to a substantially greater number of significant ASD-differentially expressed genes in cortex versus cerebellum [444 vs. 2 genes (16) and 1142 vs. 0 genes (18)], demonstrating far greater sensitivity of the postnatal cortex to transcriptional changes in ASD. Differential expression analysis comparing frontal and temporal cortex tissue found a reduced number of regionally differentially expressed genes in ASD [510 (16) and 551 (18) genes in control samples, vs. 8 (16) and 51 (18) genes in ASD samples], suggesting that cortical patterning is less distinct, and may be disrupted, in ASD. Analysis of data from 9 patients with 15q11.2-13.1 duplication syndrome (dup15q) alongside samples from idiopathic ASD cases reported striking similarities in transcriptomic changes between these two groups of cases (18), suggesting convergent neurobiological changes downstream from heterogeneous genetic risk exposures. Expression changes in individual samples, however, are more variable than group averages may suggest. While tissue from ASD donors is more likely than control samples to show relatively reduced expression of neuronal/synaptic genes and elevated expression of glial/immune genes, this is not universal across all ASD samples (Figure 2). Critically, these directional expression changes do not appear to be male specific, as female samples are similarly likely as male samples to show these same patterns.

Although differentially expressed genes from bulk tissue sequencing can be annotated to the functions of particular cell types, bulk tissue analyses are unable to definitively tease apart the cellular source(s) of these expression changes, nor



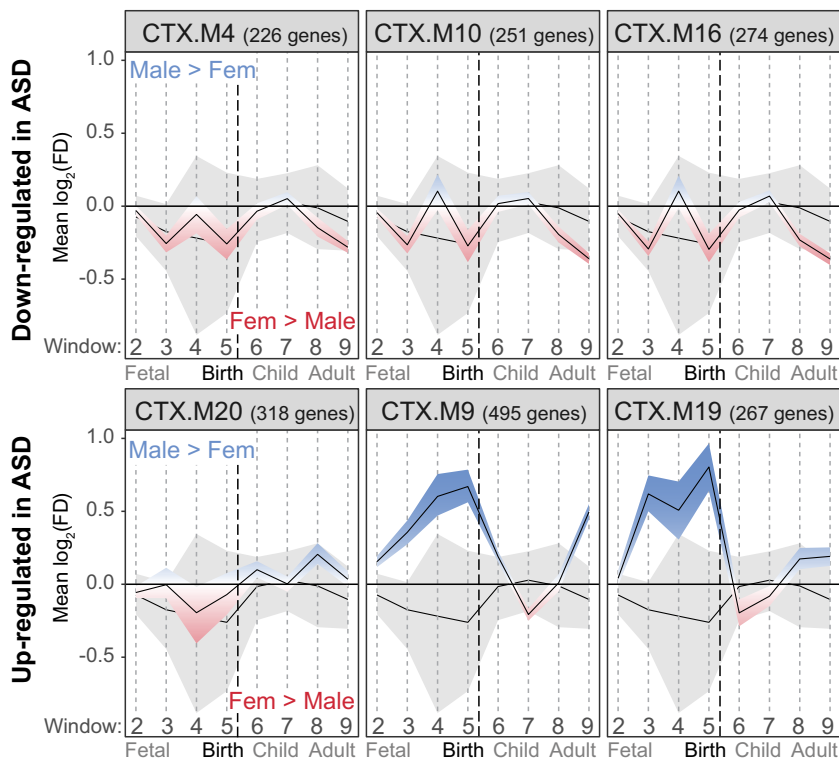
**Figure 2.** ASD-associated gene coexpression modules in the temporal cortex. Visualization of module eigengene values (y-axis) across temporal cortex samples (x-axis). Within each sex-by-diagnosis group, samples are displayed in ascending age order. Modules upregulated in ASD (CTX.M9, CTX.M19, and CTX.M20) are shown to the left and modules downregulated (CTX.M4, CTX.M10, and CTX.M16) in ASD are shown to the right (18). ASD, autism spectrum disorder; CTL, control.

can they definitively distinguish effects of cell number versus cell type–intrinsic expression changes. Single-cell RNA sequencing can begin to address these unknowns. Velmeshev *et al.* (19) published the first such data set for ASD, generating single-nucleus RNA sequencing data from the prefrontal and anterior cingulate cortex in ASD case and control subjects aged 4–22 years. Out of 17 cell type clusters identified, protoplasmic astrocytes were the only cell type found to be relatively more prevalent in ASD than control subjects, suggesting that altered cell type composition may contribute to the elevated glial/immune gene expression seen in ASD brain tissue. Cell type–specific differential expression analyses run within each cluster further showed that the genes most significantly elevated in ASD were predominantly observed in protoplasmic astrocytes or microglia, while genes most significantly reduced in ASD were predominantly observed in cortical layer 2/3 excitatory neurons and vasoactive intestinal polypeptide–positive interneurons. This study also reported enrichment for rare variant–associated ASD risk genes among differentially expressed genes that was significantly greater than expected in layer 4 and layer 2/3 excitatory neurons and vasoactive intestinal polypeptide– and somatostatin–positive interneurons. Similarly, an independent analysis in amygdala (27) reported downregulation of ASD risk genes in amygdala interneurons but did not observe upregulation of ASD-associated genes in microglia and astrocytes of the

amygdala, suggesting that this pattern may be region specific. These early findings are promising, and future data generation from a larger number of individual donors and a wider range of brain regions will provide valuable insight into the molecular changes characterizing ASD at the cellular level and how they relate to genetic susceptibility and overall ASD pathology.

### NEUROTYPICAL SEX-DIFFERENTIAL EXPRESSION AS RELATED TO ASD-CONTROL DIFFERENCES

Transcriptomic alterations in ASD brain tissue as compared with control samples define changes associated with the disorder state, and to elucidate sex-differential risk mechanisms for ASD it is necessary to understand how these disorder-associated patterns intersect or interact with neurotypical sex differences (Figure 1B). A 2016 study (28) addressed this question directly by comparing the results of a series of sex-differential expression analyses from neurotypical cortex tissue with ASD-associated gene expression changes and risk genes. Specifically, Werling *et al.* applied a linear mixed effects model to characterize sex-differential expression in 3 data sets: an adult discovery data set, consisting of 58 cortex samples from 5 male and 5 female donors aged 13–40 years from BrainSpan; a prenatal data set of 86 cortex samples from 4 male and 4 female donors aged 16–22 postconception



**Figure 3.** Sex-differential expression of ASD-dysregulated module genes across developmental time. The average  $\log_2$  fold differences (solid black curves) observed in frontal and temporal neocortex samples from the BrainSpan resource (33) for protein-coding genes belonging to the 6 ASD-downregulated (top) or upregulated (bottom) modules identified by Parikshak *et al.* (18) are displayed at each of 8 developmental windows (gray dashed vertical lines). Red-blue shading displays the 99% confidence interval around the mean  $\log_2$  fold difference for each gene set. The median and interquartile range of the  $\log_2$  fold difference for all 17,222 protein-coding genes expressed in the frontal and temporal cortex samples are displayed in gray behind each modules' sex-differential expression. ASD, autism spectrum disorder; FD, fold difference; Fem, female.

weeks; and an adult replication data set of 13 cortex samples from 5 male and 5 female donors aged 16–56 years.

Genes belonging to either of the ASD-elevated glial/immune modules defined at the time (M16 and Mod5) (16,17) were significantly more likely to show male-biased than female-biased expression in both the adult discovery and prenatal data sets, and M16 genes were also significantly male skewed in the adult replication data. Male-biased genes in the adult and prenatal cortex also showed significant enrichment for astrocyte and microglial marker genes defined by multiple sources (29,30). Conversely, genes belonging to the ASD-downregulated neuronal/synaptic modules (M12, Mod1) were significantly more likely to show female-biased expression in the adult discovery data, although this pattern was not observed in the prenatal or adult replication sets. ASD risk genes, including candidate genes from the SFARI (Simons Foundation Autism Research Initiative) Gene database (<https://gene.sfari.org/>) (31) and genes with rare de novo protein-truncating or missense variants in ASD cases (32), were not enriched for sex-differential expression in any data set, consistent with a working model that the mechanisms driving ASD's sex-biased prevalence operate largely downstream from genetic risk variation (28).

A subsequent analysis of neurotypical sex-differential expression spanning the spatiotemporal range of the BrainSpan data set incorporated 594 samples from 40 donors (23 male, 17 female) aged 8 postconception weeks to 40 years (33). Assessment of sex-differential expression patterns in cortex tissue across developmental time for the 6 ASD-

dysregulated modules reported by Parikshak *et al.* (18) corroborates prior observations: all neuron-associated modules show largely sex-neutral expression across development, while astrocyte- and microglia-associated modules show male-biased expression in mid- to late fetal development, with CTX.M9 additionally male biased in adulthood (Figure 3). Notable male-biased astrocyte-associated genes in CTX.M9 include *APOE* and genes like *RERG* and *SLCO1C1* that are associated with neural responses to hormones. Consistent with CTX.M19 enrichment for microglial functions, top male-biased genes in CTX.M19 include *LYN*, *B2M*, and *RHBDF2*, which are implicated in regulation and responses to immune cell signals and processes. Further addressing prenatal sex differences, a study characterizing transcriptomic patterns in whole brains from 120 second-trimester human donors also found 2756 genes with sex-differential expression (34). In contrast to the findings from BrainSpan described above, these differentially expressed genes showed significant enrichment for high-confidence ASD risk genes, although the direction of sex effects was split, with 7 male-biased and 5 female-biased risk genes.

These ASD- and sex-differential expression patterns seen in cortex tissue suggest the existence of parallel shifts in neurobiological features in neurotypical males (compared with females) and in ASD (compared with control). Male-skewed expression of astrocyte/microglial/immune genes, both separate from and linked to ASD-upregulated expression, is consistently observed, while ASD-downregulated neuronal/synaptic genes are variably skewed female across data sets. In

either functional category, neurotypical tissue from the more frequently affected sex (males) is transcriptionally closer to ASD than females, suggesting that glial/immune and/or neuronal biology may be involved in sex-differential risk mechanisms. Based on the direction of effect in ASD versus control, we hypothesize that increased glial function and/or cell number is associated with ASD pathobiology, while the maintenance or relative elevation of neuronal function or cell number may be protective (Figure 1C, D). Further research, including more extensive single-cell transcriptomics, is needed to determine how cell type composition and molecular function contribute to the sex differences seen in bulk tissue, and neurobiological experiments will be required to investigate the putative pathobiological and protective mechanisms involving these cell types. Importantly, the sex-balanced expression of genes linked to ASD genetic risk in cortex tissue suggests that, at least in the cortex, sex-differential risk-modulatory mechanisms operate downstream from genetic risk factors instead of as upstream regulators of risk gene expression (28). For a condition as genetically heterogeneous as ASD, this is a hopeful observation, as it suggests that interventions targeting downstream pathology may be able to modulate symptoms in patients of diverse genetic etiologies.

### CONCLUSIONS, LIMITATIONS, AND FUTURE DIRECTIONS

Although the studies reviewed here offer insights into ASD neurobiology and sex-differential risk mechanisms, these findings are limited by sample availability across multiple dimensions, including sex, age, brain region, and cell type. With currently available data from only 14 female ASD donors across major transcriptome studies, we are significantly hindered in our ability to characterize sex differences between affected males and females, to assay female-specific shifts from sex-matched norms, and to identify interaction effects between sex and ASD.

This characterization effort is further complicated by age. ASD being a neurodevelopmental but lifelong disorder, gene expression changes in the ASD brain are not static across development. Sex-differential biology is similarly dynamic, unfolding and shifting across defined stages including early sexual differentiation, puberty, and menopause. Thorough understanding of sex-differential risk and neuropathology in ASD will require making sense of the interactions between these two developmentally moving targets. Regarding early development, transcriptomic data from presumably neurotypical human tissue is relatively most available in the midfetal stage, a time when ASD risk genes are also strongly expressed and coexpressed (35,36). However, human tissue samples from late fetal and early childhood stages are sparse, and access to prenatal presumably ASD tissue would require fetal genetic diagnoses for already rare conditions. The ASD study samples described here (Table 1) and developmentally focused data sets like BrainSpan (33) and BrainVar (37) include samples that span puberty, but they all lack phenotypic information regarding donors' pubertal status and lack sufficient numbers of males and females for a well-powered comparison at this stage. Adulthood is well covered by large-scale data sets such as the Genotype-Tissue Expression (GTEx) project

(38), although the latter skews elderly such that a majority of the included females are likely peri- or postmenopausal. Further challenging our ability to thoroughly characterize baseline sex differences in brain transcriptomes, female donors are nearly universally underrepresented across these data sets: for nervous system tissues, the male/female ratio is approximately 2.5:1 in GTEx, 1.3:1 in BrainSpan, and 1.4:1 in BrainVar overall and 2.4:1 for postnatal samples.

Existing transcriptome data for ASD brain is also regionally limited, focused predominantly on the cortex and cerebellum. ASD risk genes are strongly expressed in cortex tissue (35,36) and cortical neurons (10), and transcriptomic changes are evident in ASD (15–19) and neurotypical male (28) cortex, but ASD-associated changes have not yet been characterized in many subcortical brain regions. Therefore, it is not known whether ASD etiology and sex-differential biology interact directly in the cortex, or if other brain regions play a larger role in sex-differential risk mechanisms. A rich body of neuroendocrinology and neurobehavioral work has detailed cellular and morphological sex differences in subcortical brain regions such as the hypothalamus and the bed nucleus of the stria terminalis (39). These subcortical regions could also be directly and robustly involved in ASD-associated pathology, or ASD risk could be modulated through neural circuits that physically connect sex-differential regions to ASD-affected regions. However, bulk tissue transcriptomics in these regions have been largely deprioritized in the past owing to their anatomical complexity (e.g., multiple small and functionally distinct hypothalamic nuclei). Single-cell and spatial transcriptome technologies are now facilitating the characterization of these challenging regions (40) and can be applied to characterize, or rule out, their involvement in sex-differential risk for ASD. Whether in subcortical regions or cortex, single-cell analyses will be essential to better delineate the cell types and/or cellular states that are directly involved in ASD pathology and sex-differential biology, and the likely mechanisms by which these cell types contribute to phenotype. Already, single-cell transcriptome data in the cortex has suggested possible roles for both cell type composition and cell type-specific changes in ASD-associated gene expression patterns (19), but additional data generation is needed to validate and refine these findings.

Moving forward, in vivo and in vitro model systems will also be critical tools for exploring the transcriptomic, functional, and behavioral consequences of ASD-associated genetic variants in both sexes, at precisely selected developmental stages, and in multiple brain regions. Similarly to human cortex, transcriptomic characterization of several mouse models of ASD risk loci, including *Chd8*, *Arid1b*, *Shank3b*, and 16p11.2 deletion, has found downregulation of gene modules associated with neuronal functions of axon guidance and glutamatergic neurotransmission (41–43). Heterozygous mutant lines for *Chd8*, a top ASD risk gene, also consistently show reduced expression of other known ASD risk genes (43–45), and one study observed enrichment of immune response-associated, ASD-upregulated genes in genes upregulated in *Chd8*<sup>+/-</sup> mutants (44), while another observed enrichment of ASD-downregulated, neuron-associated genes among *Chd8*<sup>+/-</sup> downregulated genes (45). These patterns validate, in part, changes reported in human brain, and demonstrate the potential of nonhuman models for linking specific genetic risk

variants to broader downstream transcriptomic consequences. However, these studies have so far focused on disorder-associated changes independent from sex effects, which must be directly addressed in future analyses. Animal work also has yet to demonstrate if elevated expression of glial genes is a cause or a consequence of ASD pathology. Further experimental work that disrupts “hub genes” central to the altered coexpression networks, or that targets the function of the specific cell types linked to these modules, could help to determine if increased glial function (or decreased neuronal function) contributes to, or is simply correlated with, phenotypic changes in brain function and behavior.

Human induced pluripotent stem cells (hiPSCs) derived from ASD patient samples are another useful model, with distinct advantages of being human cells, more readily available than brain tissue, and able to model very early developmental stages. Findings from transcriptomic analyses of hiPSCs differentiated into excitatory and inhibitory neurons follow those of postmortem brain tissue, including dysregulation of gene networks involved in neuronal differentiation, development of neuronal projections and patterning, and synaptic signaling (46,47). However, the neural cell types that can be derived from hiPSCs are currently limited, and it is not yet known which dimensions of sex-differential biology can be recapitulated in a cell culture system, either with or without the application of sex steroid hormones. Exploration of the experimental conditions required for astrocyte- or microglia-neuronal cocultures, and for appropriately modeling sex differences, can advance the utility of hiPSCs for experimental work in this area.

Across the board, from human studies to model systems, limited availability of female ASD samples and limited attention to sex as a biological variable have hindered our ability to describe and understand sex differences in ASD pathology and risk. Further characterization of sex differences in the ASD disorder state and in the general population will be important for ASD and for other neuropsychiatric and neurological disorders with sex-differential prevalence or presentation, and will better set the stage for experimental work delineating mechanisms. Toward this goal, we encourage individual laboratories, funding agencies, and journals to promote or enforce the inclusion of female samples in all human and model work, and to push toward the analysis of sex-balanced sample sets where possible, even for conditions that are sex skewed. Focused attention on this question for transcriptomic studies specifically, and across preclinical and clinical research domains more generally, is important, because understanding sex differences in disorder state and risk has great potential for uncovering fundamental aspects of disorder pathology and for the design of therapeutics to benefit both male and female patients.

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## ARTICLE INFORMATION

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