# SFARI Sex Differences Collaboration Gathers for Third Annual Meeting

By Michele Solis

April 23, 2025

wacomka

On January 27–28, Simon Foundation Executive Vice President of Autism and Neuroscience Kelsey Martin and SFARI Senior Scientist Alan Packer opened the annual meeting of the SFARI Sex Differences Collaboration (SSDC). Formed in 2021, the collaboration seeks to understand the male bias in autism spectrum disorders (ASD), which could provide important clues to ASD's etiology in general. Over 70 participants attended in person, and a similar number watched virtually.

The SSDC is composed of five working groups, each of which presented new data in three talks, followed by a forward-looking talk from the group director.

## Chakravarti Collaboration

Led by Aravinda Chakravarti of New York University, the first group focused on how dysregulations of the X chromosome contribute to ASD sex bias. Yang Sui of Evan Eichler's lab at the University of Washington has been looking for the genes behind unexplained neurodevelopmental disorders (NDDs) in females. Short-read sequencing of the X chromosome has revealed *de novo* variants in eight genes that occur more often in females than in males. Using long-read sequencing methods, Sui has assembled 192 complete genomes to help detect pathogenic variants in females with unexplained ASD; this revealed a trend for an excess of structural variants in ASD. While these kinds of experiments help explain cases of ASD in females, the same approach can be applied to males to account for ASD's male bias.

Kelsey Hennick of Tomasz Nowakowski's lab at the University of California, San Francisco gave an update on identifying patterns of gene expression, chromatin accessibility and other epigenetic marks that differ between males and females during human fetal cortex development. Many of these differences vary by cell type, which highlights potential circuit components involved in ASD. Some differentially-expressed genes are among those showing sex-biased enrichment for mutation in NDDs. This enrichment, along with other properties related to gene regulation (such as sex-biased chromatin accessibility, enhancers and estrogen-sensitive elements), could contribute to sex-dependent penetrance of ASD.

In discussion, Daniel Geschwind of the University of California, Los Angeles noted that ASD is more pronounced in males with high IQ than in males with NDDs that are accompanied by intellectual disability. Evan Eichler of the University of Washington countered that genes associated with NDDs could still explain sex bias in those less severely affected with ASD if a milder disruption (hypomorph) to an NDD gene was involved.

Rebecca Meyer-Schuman of Huda Zoghbi's lab at Baylor College of Medicine provided a framework for understanding this kind of mechanism through work done on X-linked *MeCP2*, for which mild mutations cause male-biased ASD, but loss-of-function mutations are necessary to cause Rett syndrome in females. To understand the regulatory regions that govern *MeCP2* expression, she has used a massively parallel reporter assay (MPRA) to probe the functional effects of disrupting these regions. This has refined the boundaries and functions of known and candidate elements and provided evidence for enhancers, repressors and promoters. ASD variants found in males near some of these regions also altered reporter activity, which suggests that male-biased ASD variants may work through noncoding variation that governs *MeCP2* expression.

Having established an enrichment in females for *de novo* mutations, as well as sex-biased patterns of gene expression and open chromatin in the developing cortex, the group wants to continue a detailed survey of sex chromosomes. In his director's talk, Chakravarti proposed a comprehensive search for variants on the X and Y chromosomes with long-read sequencing. This would identify hypomorphic, pathogenic variants in genes and regulatory elements related to ASD in males, create a catalog of sex chromosome regulatory elements, and identify the relevant transcription factors and regulatory elements operating on target genes.

## Sanders Collaboration

Stephan Sanders of the University of Oxford leads a collaboration focused on understanding the cells, brain regions, and circuits underlying sex differential neurobiology and ASD neurobiology, given that an interaction between the two may help explain ASD's sex bias. They primarily focus on the roles of sex hormones and their receptors.

To understand how sex hormone receptors give rise to sex-differential brain regions, Xin Jin of Scripps Research has been mapping sex hormone receptor expression pre- and postnatally at times when sex hormone surges occur in mice and humans. Developing a method for whole brain imaging in the mouse brain, she reports broad expression of the estrogen receptor 1 (ESR1) and the androgen receptor, but sparse expression of estrogen receptor 2 (ESR2). Through single cell RNA sequencing, she finds ESR1 enriched in layer 6 neurons of the cortex at the same time and place that ASD-implicated genes are highly expressed. Mice engineered to lack ESR1 show deficits in social behavior in males but not females, which suggests males rely on different circuitry for some social behavior.

Donna Werling of the University of Wisconsin presented on behalf of the group studying how ASD risk gene expression intersects with sex-differential gene expression at the level of cell type, asking where the sex-differential cells in the brain reside and whether they express ASD risk genes. Their studies focus on the cortex and three subcortical areas known to express sex hormone receptors: the bed nucleus of the stria terminalis (BNST), the ventrolateral division of the ventromedial nucleus of the hypothalamus (VMHvl), and the medial

amygdala (MeA). She presented single-nucleus RNA sequencing data from mouse brain at postnatal day 14, just after a neonatal testosterone surge. She reported good cell type identification and sex hormone receptor gene expression, but limited evidence for differential gene expression between sexes within cells in these proof-of-concept datasets. Data from human postmortem cortex shows sex hormone receptor expression trajectories that also differ between cortical cell types, but not between sexes. Current work with Nenad Sestan and Tomasz Nowakowski will examine subcortical regions in the human brain.

Vikaas Sohal and Devanand Manoli of the University of California, San Francisco presented their efforts to identify neural circuitry that mediates social behavior in sex-differential ways. In mice, Sohal described activity in the prefrontal cortex during social interactions that seems to encode more information in females than in males and was mediated by the subcortical BNST. Manoli presented results from prairie voles, which form pair bonds with mates. Losing one copy of *SCN2A*, an ASD risk gene, has sex-specific results for male prairie voles and changed neural physiology in the BNST. Among ESR1-containing neurons in the BNST, *SCN2A* expression was higher in males than in females, suggesting this region may play a role in sex-differentiated behavior.

Sanders reviewed evidence for different hypotheses for ASD's sex bias. A cortex-centered view, in which the bias arises solely from cortical mechanisms, is supported by results from Jin and Werling that found sex hormone receptors co-localizing with ASD-related gene expression in the cortex. A second hypothesis, in which sex differences in subcortical areas drive differential activity in the cortex, requires closer examination of subcortical areas; however, the sex-differential expression of *SCN2A* in BNST neurons expressing ESR1 align with this idea. The third hypothesis, which posits that sex bias arises from an interaction between sexually dimorphic biology and ASD biology, was supported by Sohal's work, which showed that BNST inputs to the cortex shape female encoding during social interactions. Sanders noted that these three hypotheses were not mutually exclusive and that systematic data would allow relative contributions to be assessed.

Future work would continue characterizing the cells and circuits in humans and rodents across development to look for gene expression differences between the sexes (including spatial transcriptomics to survey tiny subcortical regions) as well as similar circuits in humans or non-human primates.

## Dougherty Collaboration

Coordinated by Joseph Dougherty of Washington University, this collaboration focuses on systematic exploration of interactions between genes and environment to account for ASD's sex bias.

Lauren Weiss of the University of California, San Francisco presented data regarding potential mechanisms for male bias in ASD. Having ruled out a liability threshold model in which males and females have different thresholds to cross for ASD (i.e., in which females start further away from the ASD threshold),[1] Weiss proposed an alternative model in which males have larger variance in liability. This idea has been supported by greater variation in males with ASD risk heritability,[2] social responsiveness measures[3] and polygenic risk scores.

Because scanning males and female genomes for differences can be laborious, Tychele Turner of Washington University in St. Louis has developed computational tools to quickly and comprehensively evaluate different kinds of genetic variations. She presented a new tool called Copy Number Private Investigator (CNPI) which can detect an individual's copy number variants (CNVs), genotype, digital karyotype and sex chromosomes from whole genome data in a mere 1.75 seconds. Preliminary analysis did not find a difference in CNV numbers between males and females with ASD, but did find differences between them and their parents, and a gene-level analysis highlighted a role for non-coding RNAs in ASD.

Simona Sarafinovska of Washington University in St. Louis presented her work on the neurobiological

underpinnings of differences in social motivation, which could contribute to ASD's social deficits. Using a method developed to assay social motivation in mice,[4] Sarafinovska and colleagues have found that individual mice differ in their drive for social reward. Combining these measures with single cell genomics from the hypothalamus of male and female autism model mice, she reported a novel link between social reward-seeking behavior and specific neuronal populations in these mice. She then confirmed altering activity of these cells changed social behavior.

In his director's talk, Dougherty emphasized the need to systematically explore interactions between genes and sex. As part of this, his group has found a role for sex hormones in driving social motivation in mice[5] and optimized massively parallel reporter assays (MPRAs) to enable examination of interactions between sex and gene expression in multiple mouse models. He also highlighted opportunities from new large-scale NIH funded projects powered for sex by gene studies. In humans, the group is finding ways to connect the dots between sex, hormones, metabolites and ASD. In collaboration with the Marrus and Nowakowski labs, they are now analyzing brain samples as well as cerebrospinal fluid (CSF) collected from neonates to measure up to 6,000 metabolites and to see how these vary by sex. Behavioral follow-up will provide data on whether the infants later develop ASD. In collaboration with Nowakowski, the group is also looking at fetal human brain samples to identify the genes responding to hormones during brain development.

## Baron-Cohen Collaboration

The second day of the meeting began with presentations from the Autism and Prenatal Sex Differences (APEX) collaboration, led by Simon Baron-Cohen of the University of Cambridge. Given the differences in sex hormone exposure prenatally (males experience a surge in testosterone in the second trimester, whereas females do not), APEX has focused on prenatal origins for the sex bias in autism.

Previous work has supported a role for the placenta in the sex bias given its involvement in steroid production and in mediating sex differences in pregnancy: prenatal estrogen levels are higher in boys who develop ASD[6] and placental growth factor (PlGF) is associated with autistic traits.[7] In new work, Alex Tsompanidis of the University of Cambridge reported 172 differentially expressed genes between males and females in the placenta, and some of these include X-linked and Y-linked autism-associated genes. This was combined with an analysis of millions of birth records in Sweden that found widespread sex differences in pregnancy complications based on the sex of the fetus, as well as an interaction between these sex differences and autism likelihood (e.g., for moderately premature birth). These findings are consistent with a role for the placenta in mediating the sex bias in autism likelihood.

Jonathan Mill of the University of Exeter presented several approaches his group has taken to understand potential gene regulation contributions to the sex bias in autism. Long read-sequencing of human cortex samples has turned up diverse and novel transcripts in known ASD-associated genes, and some are sex-specific.[8] When tracking genome-wide methylation patterns by cell type, he reported sex differences that include ASD-associated genes and hormone-sensitive elements.[9] Similarly, data from Autism BrainNet (ABN) human brain tissue find differential DNA methylation patterns in autism that vary by sex and cell type. For example, microglia showed the most differentially methylated positions in autism, and this was particularly pronounced between females and males. Preliminary data also showed increased variation of methylation at the X-linked AR gene.

Two speakers highlighted how APEX has used brain organoids to model sex differences in autism and the effects of prenatal hormone surges.[10] Jose Gonzales-Martinez of Madeline Lancaster's lab at the Medical Research Council presented new data from experiments that model interactions between genes and hormones in ASD in cortical brain organoids. Starting with organoids engineered to carry ASD-related mutations in genes

like *KDM5B* or *CHD7*, he finds that a dose of androgen shifts the proportions of progenitor cells, resulting in more excitatory neurons and fewer inhibitory ones. Adam Pavlinek of Deepak Srivastava's lab at King's College London presented work on brain organoids from cells derived from a Klinefelter syndrome (XXY) donor. This allows the generation of organoids that contain either XX or XY chromosomes, with identical genetic background. Having generated over 550 of these so far, Pavlinek noted similarities between XX and XY brain organoids in tissue patterning, size, and synaptic activity, but differences in gene expression. A dose of estrogen provoked differential gene expression in XY organoids, including in genes known to be estrogen-responsive; it also altered glutamate receptor location in synapses.

In his director's talk, Baron-Cohen laid out the rationale of the APEX research and reviewed previous studies that showed how prenatal testosterone alters brain development and is associated with autistic-like traits in children.[11] APEX has been working to identify a source for higher-than-usual testosterone levels, which may be the fetus, the placenta or the mother. Baron-Cohen traced his lab's work back to the first studies demonstrating autistic people have elevated prenatal androgens and estrogens, measured in amniotic fluid stored in the Danish Biobank and linked to the Danish Psychiatric Register.[12, 13]. Tsompanidis then gave an overview of ongoing clinical studies, which include looking at maternal serum and amniotic fluid sampled prenatally to ask which sex steroids are associated with autism, as well as looking at how hormones interact with genetics and how some autism genetic variants may be related to steroid sex sensitivity. Deep Adhya of the University of Cambridge finished with APEX's future plans, which include examining the effects of estrogen on brain organoids, looking at pre- versus postnatal differences in gene expression in the brain and placenta, enhancer mapping in the genome and imaging to detect hormone effects on synapses, connectivity and network activity.

## Page Collaboration

Director David Page of the Whitehead Institute and the Massachusetts Institute of Technology (MIT) introduced his group's work, which explores a role for the inactive X chromosome in producing a female protective effect in ASD. Previous work has found that the inactive X substantially modulates gene expression on the X chromosome[14] and on autosomes.[15] These effects are cell-type-specific, particularly pronounced in microglia [16] and appear to be mediated in part by transcription factors ZFX and ZFY. Future work will characterize these effects on the epigenome, metabolome and proteome, as well as in multiple animal models and cell types. The group plans to investigate the targets of ZFX and ZFY, integrate transcriptomic and epigenomic datasets and examine the effects of Y chromosome dosage in the brain with iPSC models.

Chris Glass of the University of California, San Diego described research investigating the regulatory actions of *MEF2C* in microglia. *MEF2C* is a transcription factor that, when haploinsufficient, is linked to ASD. Microglia are of interest because they are sexually-dimorphic cells and because immune activation has been linked to ASD. Looking in microglia from humans, macaques and mice reveal MEF2C binding motifs across the genome, which are enriched in regulatory regions. Loss of *MEF2C* in human microglia derived from iPSCs results in an activated phenotype. This approach could also be used to understand cell type- and sex-specific regulation of other ASD genes.

Olivia Corradin of the Whitehead Institute and MIT presented her work to computationally extract information about the context in which ASD-related variants operate. Looking at genome-wide associated loci for ASD and the linkage disequilibrium block surrounding them, she showed how enhancers clustered in these regions can give information about the cell type in which a variant might act. In preliminary data, this approach pointed to cortical neurons, medium spiny neurons, amygdala neurons, and microglia. She also presented analyses of data generated by the Coufal, Glass and Page labs in which enhancer clustering was documented across species and cell types. Human-specific regulatory elements in microglia and neurons explained more ASD heritability than genetically conserved regions.

Nicole Coufal of the University of California, San Diego gave an update on her studies of Y chromosome modulation of human microglia. Previous work using cells with Y chromosome aneuploidies show a dose-dependent effect of Y chromosomes on gene expression in a cell-specific manner[17] as well as on microglia function. Transplanting these human microglia into mice shows an environmental effect,[18] with new results showing cell type-specific effects on autosomal gene expression, including genes involved in inflammatory pathways.

## Discussion

At the end of the meeting, the group leaders joined to discuss the status and future of the SSDC as a whole. Highlights included firming up a role for prenatal sex hormones in the sex bias in ASD (which comes with additional questions about hormone sources and sensitivity), sex-differential effects of ASD-associated genes, advances in getting a comprehensive view of the X chromosome and indications that subcortical brain regions may contribute to ASD. Participants mentioned the need to catalog other types of genetic variation (beyond common variants or rare *de novo* variation), such as hypomorphs of moderate effect. Another question was whether data should track the IQ of a person with ASD, and whether different theories about ASD's sex bias are needed to account for ASD with or without intellectual disability.

Martin and Packer then concluded the meeting by thanking everyone for their participation and acknowledging the richness of the data and ideas shared, as well as the need to support robust and high-quality datasets.

## References


1. Dougherty J.D. *et al. Neuron* **110**, 3243–3262 (2022) PubMed
2. Sandin S. *et al. JAMA Psychiatry* **81**, 673–680 (2024) PubMed
3. Moreno-De-Luca A. *et al. JAMA Psychiatry* **72**, 119–126 (2015) PubMed
4. Maloney S.E. *et al. Cell Rep. Methods* **3**, 100504 (2023) PubMed
5. Chaturvedi S.M. *et al. Biol. Sex Differ.* **16**, 13 (2025) PubMed
6. Baron-Cohen S. *et al. Mol. Psychiatry* **25**, 2970–2978 (2020) PubMed
7. Tsompanidis A. *et al. Transl. Psychiatry* **13**, 256 (2023) PubMed
8. Bamford R.A. *et al. bioRxiv* (2024) Preprint
9. Franklin A. *et al. bioRxiv* (2025) Preprint
10. Pavlinek A. *et al. Biol. Psychiatry Glob. Open Sci.* **4**, 100343 (2024) PubMed
11. Lombado M.V. *et al. Biol. Psychiatry* **72**, 839–847 (2012) PubMed
12. Baron-Cohen S. *et al. Mol. Psychiatry* **20**, 369–376 (2015) PubMed
13. Baron-Cohen S. *et al. Mol. Psychiatry* **25**, 2970–2978 (2020) PubMed
14. San Roman A.K. *et al. Cell Genom.* **3**, 100259 (2023) PubMed
15. San Roman A.K. *et al. Cell Genom.* **4**, 100462 (2024) PubMed
16. Blanton L.V *et al. Cell Genom.* **4**, 100628 (2024) PubMed
17. San Roman A.K. *et al. Cell Genom.* **4**, 100462 (2024) PubMed
18. Han C.Z. *et al. Immunity* **56**, 2152–2171 (2023) PubMed