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SEX AND GENDER ISSUES IN BEHAVIORAL HEALTH (CN EPPERSON, SECTION EDITOR)

Sex Differences in Autism Spectrum Disorder: a Review

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Abstract

Purpose of Review Neurodevelopmental disorders disproportionately affect males. The mechanisms underlying male vulnerability or female protection are not known and remain understudied. Determining the processes involved is crucial to understanding the etiology and advancing treatment of neurodevelopmental disorders. Here, we review current findings and theories that contribute to male preponderance of neurodevelopmental disorders, with a focus on autism.

Recent Findings Recent work on the biological basis of the male preponderance of autism and other neurodevelopmental disorders includes discussion of a higher genetic burden in females and sex-specific gene mutations or epigenetic changes that differentially confer risk to males or protection to females. Other mechanisms discussed are sex chromosome and sex hormone involvement. Specifically, fetal testosterone is involved in many aspects of development and may interact with neurotransmitter, neuropeptide, or immune pathways to contribute to male vulnerability. Finally, the possibilities of female underdiagnosis and a multi-hit hypothesis are discussed.

Summary This review highlights current theories of male bias in developmental disorders. Topics include environmental, genetic, and epigenetic mechanisms; theories of sex chromosomes, hormones, neuroendocrine, and immune function; underdiagnosis of females; and a multi-hit hypothesis.

Keywords Neurodevelopmental disorders · Autism · Sex differences · Female protective effect · Extreme male brain theory · Fetal testosterone

Introduction

Autism spectrum disorder (ASD) comprises a set of neurodevelopmental disorders that affect 1 in 68 children (https://www.cdc.gov/ncbddd/autism/data.html). ASD is defined by the early developmental onset of persistent, usually lifelong symptoms, primarily social communication deficits, and a pattern of restricted and repetitive behaviors. Heritability estimates of ASD have ranged from 0.5–0.9%. Eight hundred eighty-one genes have been implicated in autism, at least one from every chromosome (see [1] (https://gene.sfari.org/)), however the majority of cases of autism are due to unknown genetic etiology. A few prenatal or perinatal environmental contributors have been identified, but mechanisms of environmental factors are also largely unknown [2].

Despite striking heterogeneity in manifestation and severity of ASD, one of the most highly replicated findings is the male preponderance of the disorder. The male/female ratio of ASD has been reported to be 4.5:1 [3]. However, the ratio of males to females with ASD without intellectual disability is 6–16:1, and the male/female ratio for those with moderate to severe intellectual disability is approximately 1–2:1 [4]. In fact, most neurodevelopmental disorders (NDs) have a male bias, including attention deficit hyperactivity disorder (ADHD), oppositional defiant disorder (ODD), and intellectual disability [4].

Various studies have attributed the male preponderance of ASD to sex-specific single nucleotide polymorphisms

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(SNPs), single-nucleotide variants (SNVs), microdeletions, copy number variants (CNVs), and proteins [4–9]. However, as has been common in studies of the highly heterogeneous ASD, these findings have not been consistently replicated [10•]. A fairly new idea is that risk genes—rather than themselves being sex-specific—may interact with sex-specific pathways, possibly those associated with hormones or immune function [11, 12•]. Another hypothesis is that females may be more sensitive to genetic disruptions and therefore less likely to survive to term, but this remains to be demonstrated [12•]. A recent report described a sex-specific transcriptome based on RNA sequencing data from lymphoblastoid cell lines from a small group of siblings discordant for ASD [13]. This is a fairly new, but promising avenue of study that could shed light on sex bias in ND. In this review, we will summarize recent research on possible mechanisms of the male bias in ASD and promising directions for future study.

Are Females Protected or Are Males Vulnerable or Both?

Female protective effect (FPE) theory attempts to explain the differences in preponderance and severity of ASD between males and females. The FPE includes the greater variability model, which states that males exhibit greater genetic variability, allowing for an increased incidence but decreased severity of ASD [14, 15•]. The FPE also incorporates the liability-threshold model, which states that females who meet diagnostic threshold for ASD will carry a higher mutational load than males, and that relatives of females with ASD are more likely to be affected than relatives of males with ASD [16]. These predictions are supported by several samples of individuals with ASD in which there are more genes within de novo CNVs and higher rates of de novo deletions in females than in males [17, 18]. Larger and more numerous CNVs and SNVs in female versus male individuals with ND and ASD have been reported by several groups [19, 20•]. The median number of genes per CNV identified in autistic females is 10–15, compared to 2–3 in males with ASD [21, 22].

A network-based analysis of genetic associations determined that CNVs affecting females with ASD were significantly more likely to involve genes that are central to the functionality of the gene network, suggesting that a more profound disruption of integral biological pathways is necessary to lead to an ASD phenotype in females [21]. Relatedly, a recent publication reported sex-specific sets of small noncoding RNAs (sncRNAs) in the temporal cortex of individuals with ASD. Specifically, there was more sncRNA dysregulation in brains of females with ASD than in males with ASD, compared to neurotypical controls of the same sex, indicating a necessity for a higher genetic load in ASD females [23]. Therefore, in agreement with the FPE, females seem to require a higher genetic burden to reach diagnostic threshold for ASD, and are more severely symptomatic, on average, than males with ASD [24].

Several studies fulfill the FPE's prediction of increased risk in families of females with mutations as well. Recent reports including very large samples from multiple databases concluded that siblings of females with ASD have higher autistic trait scores and higher recurrence risk than siblings of males with ASD [4, 25–27]. Evidence from large datasets of multiplex families with more than one child affected by autism also supports the FPE. The risk for subsequent children developing autism was higher when at least one of the affected siblings was female versus when the child had only brothers with ASD [28]. Another group similarly hypothesized that female-enriched multiplex families with two or more severely affected females represent a group with an extremely high recurrence risk [29]. These data indicate that affected females had larger or higher penetrance genetic disruptions that are more likely to result in siblings having an autism diagnosis. Similarly, CNV enrichment was identified in mothers of individuals with autism by several groups [19, 20•]. The Autism Science Foundation is currently dedicating substantial research effort to investigating the FPE through the Autism Sisters Project (http://autismsciencefoundation.org/about-asf/media-center/press-releases/autism-sisters-project/).

Other research evaluating groups of individuals with ASD and siblings of individuals with ASD did not find increased genetic burden in females with the disorder or increased incidence in relatives of females [10•, 30, 31]. These differences may be attributable to the heterogeneity of samples and differences in methodology used. Replication with larger groups will be important in the future.

Another theory put forth to explain sex differences in the prevalence of ASD is idea that males are more susceptible to ASD. This concept is not necessarily exclusive of a FPE and may be explained by a proposed increase in genetic variability among males, as mentioned earlier, or other biological factors that confer vulnerability to males in particular [14, 25]. Studies of gene networks found a sex-specific pattern of expression in a typically developing population, and the genes overrepresented in male versus female brains were those often associated with ASD, including genes involved in cytoskeletal and extracellular matrix proteins, immune response, and chromatin [32, 33]. It is possible, therefore, that perturbations in genes that are typically expressed at higher levels in males would have a bigger impact on male brain development.

Animal studies of epigenetic changes point to male vulnerability as well. Prenatal valproic acid (VPA) treatment in rats results in an ASD-like phenotype, as well as a male-specific decrease in the methyl-CpG-binding protein 2 (MeCP2), which binds methylated DNA and can repress transcription via histone acetylation, in the prefrontal cortex. In addition, knockdown of MeCP2 with small interfering RNA (siRNA)

resulted in an increase in PSD95, an important postsynaptic scaffolding protein, in neural progenitor cells derived from males but not females [34]. In conclusion, it is not clear what factors contribute to female protection from and/or male vulnerability to ASD, but they may be environmental, genetic, or epigenetic in nature.

Chromosome and Hormone Mechanisms of Sex Differences and their Effects on Development, Behavior, Brain Function

The developmental origin of the many behavioral and anatomical differences between males and females is in the sex chromosomes. In addition to a maternally inherited X chromosome, there is a paternally inherited sex chromosome complement: a Y in the case of males, and an X in the case of females. In order to correct for gene dosage, whereas females have twice as many X chromosomes as males, one of the X chromosomes in each cell is silenced, known as X-inactivation. However, some genes (~ 10–15%) escape X-inactivation (escape genes) [35]. This earliest source of differences between males and females is discussed below as a possible mechanism for male bias in ND.

Secondary to sex chromosomes is the other main factor that differentiates males and females: sex hormones, primarily testosterone, and estradiol. These steroids are produced by the gonads and have important developmental effects, particularly during two critical periods. First, an organizational period during development in utero results in the body and brain being permanently formed into male or female anatomy. In the case of males, the Y chromosome contains the SRY gene, which encodes the testis-determining factor. The testis-determining factor is a DNA-binding protein that upregulates transcription factors to initiate the formation of testes, which then produce testosterone. This fetal testosterone is responsible for masculinization, whereas the process of feminization mainly requires the absence of hormones. The second critical period is the activational, and it comprises the sex hormone surge during puberty: the cyclical levels of estradiol and progesterone in females until menopause (menstrual or estrous cycle) and the steady surge of testosterone in males until senescence. Sex hormones at that time have more transient effects [36, 37] (Fig. 1). As ND, by definition, manifest early, and are male-biased, fetal testosterone is a strong candidate for male bias in ASD (discussed below).

Testosterone in utero is critical for the development of many observed sex differences, from physical appearance to brain region size, neurotransmitter and receptor levels, neurogenesis, cell death, migration, differentiation, immune function, neuropeptide signaling, and many other factors, some of which will be addressed here. Many of the genes associated with autism encode proteins involved in synapse formation or maintenance, cell adhesion, and scaffolding. These molecules may be targets of hormones during the organizational period of development, resulting in the male preponderance observed in ND [39]. In addition, dendritic spines are mediated by sex hormones in many areas of the brain and are abnormal in several mouse models of ASD and individuals with ASD [40–48]. Finally, animal studies have indicated that DNA methyltransferase (DNMT) and histone deacetylase (HDAC) have sex-specific effects on behavior and can be influenced by sex hormones [49, 50]. These epigenetic mechanisms, along with chromatin modifications, and microRNA expression, are new areas of interest in the ASD field and may be influenced by sex hormones to contribute to susceptibility [51•].

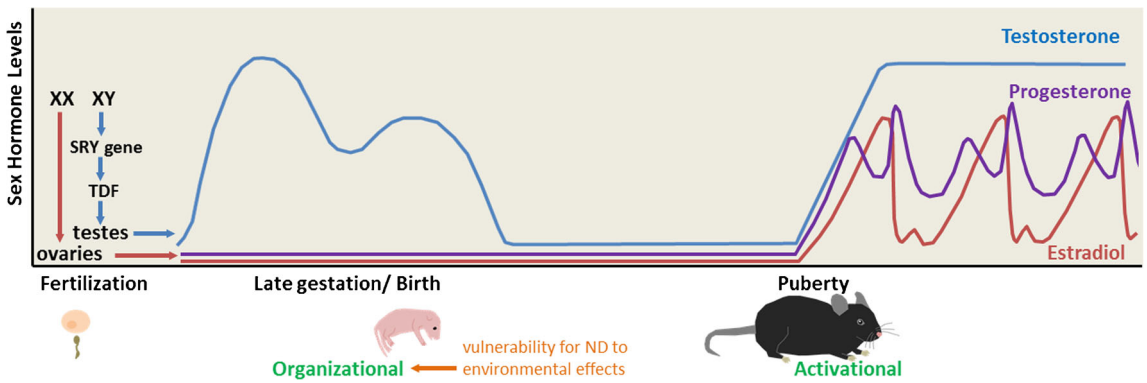

Fig. 1 Sex chromosomes (XX = female; XY = male) determine which gonads will form and which sex hormones (mainly testosterone, estradiol, and progesterone) they will produce. Fetal testosterone is important for permanent masculinization of the male brain and body during the organizational period (late gestation/birth, in rodents). This is the proposed period for vulnerability to ND. Sex hormones then surge during the activational period (puberty) for more transient effects; males have relatively stable testosterone levels, and females have variable hormone levels over the 4–5-day estrous cycle. Modified from [38]

Sex Chromosome Theory: Is XX Protective or Is XY a Risk Factor?

It has been proposed that possessing a Y chromosome, in the case of males, is a risk factor for neurodevelopmental disorders, and/or having a second X chromosome is protective in the case of females. There is some evidence for this in animal models with altered numbers of sex chromosomes, as well as from aneuploid individuals with increased risk for ASD (XYY, XXY, XXYY, XXY) [4, 52–54]. However, aneuploid individuals are relatively rare and often also exhibit sex hormone dysregulation, so these studies can be difficult to interpret, i.e., do not necessarily distinguish the effects of sex chromosomes vs. hormones. Although most genetic mutations associated with ASD susceptibility are autosomal, some ASD-linked genes reside on the X chromosome, including FMRP, MECP2, NLGN3, and NLGN4X [4, 55]. Often, X-linked phenotypes in males are much more severe than in females. For example, loss of function of the MECP2 gene, located on the X chromosome, is associated with Rett syndrome, which causes an ASD-like phenotype. The syndrome is seen almost exclusively in girls, because affected males—with only one copy of *MeCP2* on one X chromosome—are more severely affected, and usually die before or shortly after birth [56]. This led to the idea that escape genes (which survive X-inactivation, discussed above) may be protective to females. Several have been identified [55, 57, 58], but other studies found no differences in X inactivation in females without ASD compared to those with the disorder [59, 60]. Importantly, many of these findings were based on relatively small sample sizes, and have not been replicated. Overall, it seems that the X chromosome theory cannot account for most cases of ASD and may not significantly contribute to the male bias, although a recent report suggested that in addition to rare X-linked loci and Mendelian diseases, sex-specific SNPs on the X chromosome may play a role in ASD risk [10•]. Similarly, the Y chromosome contains few genes and the possibility of a role for these genes in ASD remains understudied. One group found no differences in Y haplotype distribution between individuals with ASD and controls [61]. Another group found some Y haplotypes that were more or less common in ASD individuals compared to a nonaffected population [62]. Thus, a significant contribution of sex chromosomes in the etiology of the majority of cases of ASD seems unlikely but warrants further research.

Sex Hormone Theory: Are Fetal Testosterone Levels Linked to Autism?

Fetal testosterone (fT) affects a number of downstream targets, as discussed earlier, and has been identified as a prime candidate for male-biased risk [39]. Levels of fetal testosterone have been directly measured through amniocentesis. fT is also indirectly approximated by digit ratio (higher levels of fT are associated with lower second to fourth digit length ratio (2D:4D), although this is slightly controversial). Evidence for the effects of fT also come from studies of girls with congenital adrenal hyperplasia (CAH), children of women with polycystic ovarian syndrome (PCOS), and females with male co-twins, all of whom are exposed to higher than normal levels of fT. Correlation of increased fT in these populations with autistic trait scores and ASD diagnosis was demonstrated in many studies but not all [39, 51, 55, 63–71]. A recent study found that at 12 and 36 months, there was no relationship between levels of fT or other androgens in umbilical cord blood of children with older siblings diagnosed with ASD, and autistic traits, except in a small subset of children whose older sibling was a female with ASD [72]. This is an interesting finding that may relate to the liability-threshold model and FPE models (discussed earlier), but the sample sizes were small and the implications should be considered carefully. Finally, maternal blood levels of an endocrine disruptor, which has been shown to decrease testosterone levels, were negatively correlated with social responsiveness in boys and girls at 9 and 10 years of age [73].

A report from the Baron-Cohen group has directly compared fT levels of boys who later received a diagnosis of ASD or Asperger syndrome to typically developing controls. Individuals with ASD had higher amniotic levels of progesterone, 17α-hydroxy-progesterone, androstenedione, testosterone, and cortisol [51•]. There were moderate sample sizes in this study, but it will be important to replicate these findings, especially including female subjects, and investigate the source of the increased levels, which could be environmental or genetic in nature, and could be either maternally or fetally derived. Additionally, although fT levels do not appear to be related to postnatal testosterone levels in young children or adults, some hormone dysfunction appears to be present in both males and females with ASD, including higher rates of precocious puberty and an increased incidence of PCOS and dysmenorrhea [6, 65, 74–80].

A number of studies in the past several years have implicated sex hormones, receptors, and related enzymes in ASD. Human studies are somewhat lacking, as manipulation of fetal hormone levels in humans is of course not possible, but one study looking at postmortem brains of a small group of adolescents demonstrated an interesting pattern of decreased estrogen receptor beta (ERβ), aromatase (an enzyme that converts androgens to into estrogens), and several ER coactivators in the frontal gyrus of individuals with ASD compared to controls ($n = 13$ per group) [81]. Another group has demonstrated decreased aromatase levels in the frontal cortex of individuals diagnosed with ASD and a positive correlation between aromatase levels and the protein product of the ASD-associated gene RORA (retinoic acid-related orphan receptor-alpha) [82–85]. Then, using a neuronal cell line, they showed that

aromatase is a transcriptional target of RORA and that RORA is sex hormone responsive—upregulated by estradiol and downregulated by dihydrotestosterone (DHT) [82]. Finally, using mice, they indicate that males seem to be more susceptible to disruptions in RORA due to reductions in aromatase, resulting in an increase in testosterone and decrease in estradiol [86]. Therefore, genes associated with ASD may be modulated by sex hormones and vice versa, and the enzyme aromatase may be a critical player in the male overrepresentation in autism.

Other animal studies have also pointed to a role of hormones in the development of ASD-like brain or behavioral traits. In a zebrafish model of ASD, animals with a mutation in contactin associated protein-like 2 (CNTNAP2) exhibited hyperactivity and sensitivity to seizures, which were rescued by treatment with a phytoestrogen [87]. A report of heterozygous reeler mice, which exhibit ASD-like social and cognitive deficits and amygdalar abnormalities, found that neonatal administration of estradiol rescued those phenotypes [88]. However, although estradiol is discussed as a "feminizing hormone," a potential shortcoming of this study is that, in fact, estradiol has important masculinizing effects on the developing brain, particularly in rodents, so this may actually result in a "hypermasculinized" state. Treatment of pregnant rats with an aromatase inhibitor, which blocks the conversion of testosterone to estradiol, resulted in pups that emitted decreased numbers of maternal separation-induced ultrasonic vocalizations compared to controls, and a female-specific decrease in social interactions, both of which are considered ASD-relevant traits [89]. Although the authors claim that administration of an aromatase inhibitor will increase masculinization by preventing testosterone conversion to estradiol, this may, in fact, result in "dysmasculinization" instead, as many brain masculinizing effects are due to estradiol. Another recent study, which does not address autism directly, nevertheless has interesting implications for the disorder. The authors reported that prenatal testosterone administration to mice caused a significant increase in spine density [90], which has been associated with ASD [43–48].

In summary, fetal testosterone levels may be predictors of future ASD-related behaviors, but the exact effects of sex hormones during development are quite complex and warrant more study. Perturbations of fT in either direction may increase likelihood for later diagnosis of the disorder.

Extreme Male Brain (EMB) Theory: Is the Autistic Brain a Hypermasculinized Brain?

The extreme male brain theory of autism (EMB) states that there are morphological and functional differences between male and female brains, but the "autistic brain" is a more extreme, or hypermasculinized, version of the male brain, possibly due to elevated fT [39, 55]. Various reports indicate that males are more adept at construction and analysis of rule-based systems, attention to detail, and collecting (systemizing quotient (SQ)), whereas females score higher on tests of empathy, language abilities, and social cognition (empathy quotient (EQ)) [39, 55, 91]. Based on these innate differences, the EMB theory predicts that individuals with ASD will score higher on the SQ than typical males, who will in turn score higher than typical females. Likewise, autistic individuals should score lower on the EQ than typical males, who do not perform as well as typical females [55]. This has been supported by a number of studies [92, 93]. EMB theory also predicts that sex differences found in the general population would be absent or reduced in individuals with ASD, which has also been demonstrated recently with SQ and EQ traits in adults and toddlers with ASD [94, 95].

Some aspects of brain morphology follow the EMB theory as well and demonstrate sex differences in size or laterality, with individuals on the autism spectrum having an exaggerated male pattern [55, 96–98]. Measurements of cortical thickness with MRI indicated that females with a male-typical organization were significantly more likely to have been diagnosed with ASD [99]. Another recent report indicated an exaggeration of a "male brain" phenotype in individuals with ASD, specifically, lower connectivity in the default mode network (DMN), a group of interacting brain areas with correlated activity associated with social processing and dysfunction in ASD [100].

Other findings are counter to what is predicted by the EMB theory or report complex relationships between ASD, sex, and brain morphometry [101–103]. For example, another study of the DMN found sex differences in a control population, but reduced connectivity in the anterior medial prefrontal cortex correlated with autistic traits in males only [104]. In a more recent analysis of DMN and whole-brain connectivity in resting state fMRI, ASD males had a feminized profile of hypoconnectivity compared to control males, and females with ASD had a masculinized expression of increased connectivity. The authors suggest that ASD may not involve hypermasculinization but a general dysfunction of sex differentiation [105]. Finally, an investigation of neuroanatomical features of gray and white matter, some areas of the brains of females with ASD appeared to be masculinized, but those regions did not appear hypermasculinized in males with ASD [106]. Future work is needed to identify underlying factors that drive sex-specific neural expressions of ASD and determine whether they are a cause or effect of ASD.

Neurotransmitters and Signaling Molecules: Do Sex Differences in Synaptic Transmission Contribute to ND?

Sex differences in neurotransmitter signaling may be involved in ASD risk, particularly GABA and glutamate signaling,

which regulate the central excitatory/inhibitory balance, an important factor in ASD. Sex hormones have been shown to influence GABA receptor expression and GABA synthesis, and both suppress and facilitate GABA inhibitory action via age-, sex-, and brain region-specific mechanisms. Likewise, estradiol can cause an increase in glutamate release, affect metabotropic glutamate receptor signaling depending on subtype, and increase NMDA receptor expression. Progesterone can suppress glutamate response and glutamatergic neurons are sexually dimorphic in some brain regions [37, 51•, 107]. Plasma glutamate levels were recently shown to be lower in individuals with ASD [108]. Animal studies have found related results. Juvenile male rats have higher extracellular glutamate levels in the lateral septum at baseline and during social play, and blockade of ionotropic glutamate receptors resulted in a female-specific decrease in social play [109]. Glutamatergic deficits were reported in a VPA-induced model of ASD. Male, but not female, rats exhibited disruptions in NMDAR, AMPAR, and mGluR5 pathways in the prefrontal cortex [34]. In fact, NMDAR deficits have been linked to a number of mouse models of ASD [43, 110–114]. Finally, brain-derived neurotrophic factor (BDNF) and serotonin levels are both influenced by sex hormones and are higher in individuals with autism [39, 115–118].

Can Immune Activation Lead to Sex-Biased Diagnoses?

The role of immune function is becoming more prominent in the ASD field, and immune function is modulated by sex hormones. ASD has been linked to a number of abnormalities in the immune system, including maternal infection, cytokine and chemokine activity [12•, 51•, 119], and many studies have demonstrated the effects of sex hormones on immune responses, including cytokine and microglia responses [120, 121]. In humans, a sex-specific biomarker signature of inflammatory molecules and cytokines identified in blood was significantly different between individuals with Asperger syndrome and controls [122]. PET scans revealed an increase in microglia activation in males with ASD relative to control males [123]. Subsequent studies should include larger group sizes as well as female subjects. Finally, evaluation of the ASD transcriptome revealed a number of genes associated with neuroimmune function [124].

In animal research, lipopolysaccharides (LPS) or polyinosinic:polycytidylic acid (Poly I:C) are administered to pregnant dams to mimic the immune activation of bacterial and viral infections, respectively. Following prenatal LPS or Poly I:C administration, some groups have shown sex-specific ASD-related behavioral deficits in offspring. Only male offspring of mouse dams treated with LPS or Poly I:C exhibit repetitive behavior [125]. Male Swiss mouse offspring of LPS-challenged mothers exhibited repetitive behavior, anxiety, cognitive deficits, and decreased parvalbumin. Some cytokines and immune factors were increased in both sexes, and some in males only [126]. Similarly, IL-6 cytokine was increased in microglia cultures from male more than female rats perinatally exposed to LPS, although other immune responses were not sex-specific [127]. On the other hand, some groups have found immune-related phenotypes in females only. For example, the BTBR mouse model of ASD exhibited female-specific increases in self-grooming behavior, and levels of IL-6 and CD11c [128]. At 6 weeks, wild-type mice prenatally treated with Poly I:C had decreased DNA methylation globally, particularly in females, and decreased methylation at the promoter region of MeCP2, a gene associated with ND [129]. Finally, whole transcriptome profiling in purified microglia from wild-type mice uncovered a delay in gene expression pattern over the course of development in males compared to females. Administration of LPS in adult male mice caused an increase in developmental rate of microglia, indicating that the sex difference may involve immune function. Acceleration of microglial development was also observed in brain transcriptomes of individuals with ASD when compared to controls. Therefore, individuals with ASD appear to exhibit increased immune activation, but the results are contrary to predictions of the EMB theory. [130]. Overall, these data indicate that immune activation may be involved in male ND bias, but the exact mechanisms remain unclear, and continued research will be important.

Neuroendocrine Hypothesis: Neuropeptides Are Responsive to Sex Hormones

Another possible explanation for the male bias in autism falls under the neuroendocrine hypothesis, which posits a role of neuropeptides in sex differences, especially oxytocin (OT), vasopressin, and corticotropin-releasing hormone (CRH). These hormones are synthesized in the hypothalamus, secreted centrally and peripherally, and have expression and response patterns that are sex-specific [131].

OT is known for increasing trust, facilitating bonding between partners as well as mothers and children, and augmenting social interaction, social memory, and social cognition [132–139]. Blood levels of OT are higher in females than males, and its activity, release and receptor expression are regulated by estradiol and progesterone [140–146]. Estrogen receptors are often expressed on oxytocin-containing cells and neurons expressing OT receptors [147, 148].

Blood levels of OT are reduced in individuals with ASD (although this finding has not always been replicated) and OT has been used as a treatment in preclinical trials [146, 149–154]. Animal studies generally support a relationship between sex hormones, OT, and ASD-related phenotypes.

Neonatal testosterone administration to rats decreased early social behaviors as well as OT-expressing cells in the PVN, to a greater extent in males than females [155]. In addition, OT administration may affect males and females differently. OT improved working memory and social gaze only in male, not female, infant macaques [156]. Similarly, intranasal OT increased caudate response in males and decreased it in females participating in a cooperation task during an fMRI, indicating sex-specific alterations in social reward [157]. In contrast, levels of the related neuropeptide, arginine vasopressin (AVP), are higher in males than females. AVP is androgen-dependent; and the gene encoding its receptor has been linked to ASD and social behavior [146, 158–160].

CRH is involved in stress response, cells that produce it are more abundant in males than females, and its receptors exhibit sex-specific signaling [161, 162]. CRH stimulates the release of cortisol, which is increased in children with ASD under baseline conditions and in response to nonsocial environmental stressors, unpleasant stimuli, and neutral social situations, but they have decreased cortisol responses to social stressors compared to controls [51•, 163, 164]. Increases in CRH receptor gene are associated with deficits in rodent social exploration as well [165]. Therefore, some evidence suggests that interactions between neurotransmitters or neuropeptides and sex hormones may contribute to sex differences in ASD.

Are Sex Differences in Autism Overstated? Possible Underdiagnosis of Females

An additional theory growing in popularity purports that the male preponderance of ASD is overstated because of underreporting or underdiagnosis of females with ASD. As mentioned previously, ASD females are more likely to present with comorbid intellectual disability, but they are also more likely to be comorbid for sensory issues, seizures, sleep disturbances, anxiety, and depression [166–170]. Diagnosis with a co-occurring disorder can lead to underdiagnosis of ASD in females (see [15•] for review). Not only are fewer girls diagnosed with autism, but they are diagnosed later than males on average [171, 172]. The reason for this is contentious.

Differences in brain structure, connectivity, or function in ASD indicate that the sexes may manifest the disorder distinctively. There is evidence of this in MRI and fractional anisotropy studies in individuals with ASD [173–179]. fMRI showed that males, but not females, with ASD had decreased activity in the posterior superior temporal sulcus in a social information processing task during fMRI [180]. Likewise, some potential treatments work better in one versus the other sex (see Neuroendocrine Hypothesis section above), including a ketogenic diet in a mouse model of ASD, which rescues social and repetitive abnormalities only in females [181].

Studies have been trying to parse out sex differences in individuals with ASD in order to determine whether the disorder "looks different" in females, which can make diagnosis more difficult. There is disagreement on sex-specific presentations of nearly every behavioral measure, including social aspects, communication, stereotyped and repetitive behaviors, cognition, motor scores, hyperactivity, externalizing and aggressive behaviors, executive function, processing speed, visuospatial skills, and theory of mind (see [15•, 182] for review), with some finding more severe deficits for males [183–187] and others for females [188, 189]. Some report sex differences that include similar overall scores with subtle but specific variations in presentation [190–192]. Most studies conclude there are no, or very minor, sex differences in ASD manifestation, or only an increase in restrictive and repetitive behaviors in males [103, 169, 193–198]. A recent meta-analysis found sex differences in social and cognitive domains in opposite directions depending on the database of participants used [199]. These inconsistencies highlight the high level of heterogeneity in the autism spectrum as a whole, and warrant further research with increased attention to sampling, testing, and analysis to better define patterns of sex differences.

One study found that adult women with ASD self-reported more autistic traits than men with ASD, but clinician observation found no sex differences in social or communication skills [166]. This discrepancy between self-report and clinician observation has important implications for both diagnostic and self-report measures, and also indicates that societal expectations may lead to more self-criticism in females than in males. Besides discrepancies with self-reports, clinicians often find less sex differences in ASD presentation than represented by parent reports, on which much of the literature is based [200–203]. Hiller et al. [198] interestingly point out that parent and teacher reports of behavior often conflict with each other as well, with the parent reports often describing more impairments than teacher reports, particularly for females. This could be because parents are able to pay more attention and can report more accurately. On the other hand, parents may be more worried about their children, resulting in hypersensitivity to any perceived impairments. Results may even differ depending on which parent completes the questionnaire [204]. Other study design and methodology issues may have confounded estimates of sex differences in ASD as well. Some reports are under-sampled and do not control for age and IQ. Many are underpowered to separate males and females with ASD and not enough are longitudinal. In addition, different measures have varying numbers of questions per category, resulting in unintended emphasis on certain symptoms, or alternatively, increased sensitivity to differences. Some assays determine the presence or absence of symptoms, while others assess severity of traits. However, some researchers have argued that current diagnostic tools, when used correctly, are effective and do not exhibit sex bias [205].

Recently, some research has begun to focus on the role of sociocultural norms on the representation of females with ASD [15•, 198]. Males are more likely to act out in classrooms, which makes them more likely to receive attention, diagnoses, and treatment [15•, 169, 206]. In addition, females with ASD are more likely to camouflage their symptoms and perceive them differently [185, 191, 207–213]. Most accepted knowledge of ASD phenotypes and diagnostic criteria is based on research on primarily male subjects, which can make diagnosis in females more difficult. Indeed, a recent report indicates that there were more discrepancies in females than males in meeting diagnostic criteria in the DSM V after previous diagnosis using the DSM IV [198]. In conclusion, there is still not a consensus on whether males and females with ASD present differently and whether the 4.5:1 male to female ratio is accurate. Assessing the influence of social and gender norms will be difficult, but will be an important factor for future treatment strategies.

Multi-Hit Hypothesis: (Epi)Gene × Environment × Sex Interactions

A recently proposed theory, the three-hit hypothesis, combines some of the previously discussed ideas and states that interactions between sex, genes, and environment lead to the male bias in ASD. In the first and only test of this three-hit hypothesis to date, mice showed social deficits and histone modifications most robustly when they were male, deficient for the contactin-associated protein-like 2 gene (*Cntnap2*), and exposed to LPS in utero, which activates maternal immune response. mRNA gene expression assays in the hippocampus revealed that the promoter area of the *Crhr1* gene (a CRH receptor type) may bind SRY (the testis determining factor) and NF-κB, a nuclear factor involved in inflammation, which would confer risk to animals that were male and stressed [214•]. As discussed earlier, maternal immune activation may affect males to a greater extent than females, and especially with regard to social behaviors [215]. However, it is worth noting that mice in this study with two hits performed better in some instances than controls with no hits. Also, other models of autism exhibit deficits with just one or two hits, discussed below.

Many studies indicate that two hits may be sufficient to confer ASD vulnerability, including some combination of a number of possible environmental factors; chromosomal or gonadal/hormonal sex; and/or genetic and epigenetic alterations. Therefore, the combinations of factors potentially resulting in an autism spectrum phenotype under this model are abundant.

Some recent mouse studies indicate that sex is one hit and the second is environmental. Male offspring of obese dams were more severely affected than females, with smaller embryo size and a greater number of dysregulated genes [216]. Additionally, female-specific behavioral deficits and male-specific changes in neuron and glia number in layers 2/3 and layers 5/6 of the mPFC occur after prenatal and early postnatal exposure to propionic acid and bisphenol A, respectively [217, 218]. Other recent reports using mouse models of ASD indicate that two hits—male sex and particular genetic mutations—may be sufficient to result in autism-relevant phenotypes. Findings include sleep disturbances in 16p11.2 del/+ male mice, social and morphological deficits in male Pcdh10$^{+/-}$ mice, and increased social motivation in male Pten mutant mice [43, 219, 220]. Purkinje cell abnormalities were male-specific in the Reeler mouse model of ASD, and the Adnp mouse model of ASD shows sex-specific hippocampal gene expression, behavior alterations, and response to treatment

Multi-Hit Hypothesis

	Female Protective Effect	Both/Either	Male Vulnerability
Genes	Females require higher genetic and symptomatic burden	Sex-specific SNPs, CNVs, SNPs	Genes over-represented genes in male brains implicated in ASD
	Relatives of affected females more likely to be affected	Risk genes interact with sex-specific pathways	Y chromosome haplotypes may increase ASD risk
	X-inactivation genes protective		
Epigenetics	Females need higher epigenetic dysregulation for diagnosis	Sex-specific transcriptome	Male rats show decreased MeCP2 after VPA
Environment	Females have lower fT	Sex differences in neurotransmitters, neuropeptides increase risk	fT affects ASD-related pathways, proteins, processes
		Sex differences in immune function affect males and females differently	Increased immune activation affects males more
		Diagnosis based on male research; females under-represented	

Fig. 2 Summary of theories and phenomena that contribute to the male bias of ASD and ND. *fT* fetal testosterone, *SNP* single nucleotide polymorphism, *SNV* single nucleotide variant, *CNV* copy number variant, *MeCP2* methyl-CpG-binding protein 2, *VPA* valproic acid

[221, 222]. The VPA mouse model shows complex patterns of sex specificity in androgen receptor expression and deep cerebellar nuclei morphology [223, 224].

Therefore, many factors or combinations of factors may lead to sex differences in ASD or ND, and so there is a need for increased research focus on the complex interactions among such factors. As previously mentioned, most studies with human subjects have not included females and have not considered sex as a variable, although several mentioned previously have found sex differences in populations of people with ASD.

Conclusions

The striking sex bias in ASD and other neurodevelopmental disorders is an important phenomenon to examine in order to better understand the underlying biology of ASD and to work toward new and better treatment strategies. There have been many contradictory findings that complicate the question, perhaps due to a combination of the heterogeneity of ASD and the prevalence of underpowered studies. It seems likely that parts of many of the hypotheses and models discussed here interact to confer greater risk to males. In addition, different biological subtypes of ASD almost certainly exist, with different combinations of underlying biological factors involved. See (Fig. 2) for a summary.

Moving forward, more inclusive research is necessary, with increased numbers of human subjects, particularly females, better matched for age and IQ, and with an augmented effort to study different time points in development or utilize longitudinal designs. Using genomic data to parse out more specific sex differences and risk estimates will also be an important avenue for future studies. Replication of the genetic and epigenetic studies discussed here, again, with larger subject pools, will be vital to determine whether there are sex-specific genes and transcriptomes that confer male bias, or whether ND-associated genes interact with sex hormone pathways. Animal studies should include female as well as male subjects, and should be utilized as a tool to investigate manipulation of sex hormones and related enzymes during development.

Finally, care should be taken to try to separate cause and effect–or at least not make unwarranted assumptions about cause and effect—when it comes to ND phenotypes. For example, do ASD-associated gene mutations make children more susceptible to environmental risk factors or are the mutations caused by the environmental factors? Those dynamics can be difficult to disentangle. We should strive for better diagnostic tools and animal research that includes multidisciplinary components. Specifically, genetic, hormonal, environmental, and societal factors should be considered, with an emphasis on multiple "hits," which is a promising future direction.

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Compliance with Ethical Standards

Conflict of Interest Sarah L. Ferri, Ted Abel, and Edward S. Brodkin declare no conflict of interest.

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