Sex-specific Behavioral Features of Rodent Models of Autism Spectrum Disorder

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Sex is an important factor in understanding the clinical presentation, management, and developmental trajectory of children with neuropsychiatric disorders. While much is known about the clinical and neurobehavioral profiles of males with neuropsychiatric disorders, surprisingly little is known about females in this respect. Animal models may provide detailed mechanistic information about sex differences in autism spectrum disorder (ASD) in terms of manifestation, disease progression, and development of therapeutic options. This review aims to widen our understanding of the role of sex in autism spectrum disorder, by summarizing and comparing behavioral characteristics of animal models. Our current understanding of how differences emerge in boys and girls with neuropsychiatric disorders is limited. Information derived from animal studies will stimulate future research on the role of biological maturation rates, sex hormones, sex-selective protective (or aggravating) factors and psychosocial factors, which are essential to devise sex precision medicine and to improve diagnostic accuracy. Moreover, there is a strong need of novel strategies to elucidate the major mechanisms leading to sex-specific autism features, as well as novel models or methods to examine these sex differences.

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the resulting differential expression of genes that are transcribed to express molecules such as sex hormones could shape the sexual dimorphisms described above, but no clear mechanism has been presented. Besides, there is ample evidence that factors other than sex hormones are also involved in sex differences in brain function [6, 7], which makes delineating the sex difference issue in normal and pathological conditions a formidable challenge. Lovell-Badge and Robertson first described the four core genotypes (FCG) mice, characterized by the deletion of the testis-determining gene Sry from the Y chromosome (XY females) and the insertion of an Sry gene into the autosomes (XX males) [8, 9]. Thus, the FCG mice produce XX and XY gonadal males and XX and XY gonadal females. The juvenile social play in FCG mice has been studied to determine sex differences and chromosomal effects in social interaction behaviors. In normal C57BL/6 mice, non-sibling, same-sex pairing revealed that males have a tendency to be more social than females, as manifested by close physical contact with their partners, while, on the other hand, female mice spent more time engaged in anogenital sniffing and play solicitation than males. In FCG mice, both wild-type male and female mice (XX females and XY males) have greater social behaviors than their heterochromosomal counterparts (XY females and XX males), and the XY male group showed the highest social behavior compared to any other group. Indeed, the interaction of sex chromosomes and gonadal sex affects the social behavior in FCG mice, with the possible additional influence of sex hormones in social behaviors [10]. Other neurobehavioral features such as aggression [11], parenting [12], nociception [13], and drug abuse [14] observed in FCG mice indicate that sex differences in such behaviors is partly due to a sex chromosome effect as well.

Remarkably, some disease conditions have sexual dimorphism at least in the prevalence rate, although the underlying causes are still unexplained. In some cases of autoimmune diseases like systemic lupus erythematosus, multiple sclerosis or Sjögren’s syndrome, there is a marked skewness in the prevalence toward females [15, 16], while in neurodevelopmental disorders like language impairments and attention deficit hyperactivity disorder (ADHD), the prevalence is higher in males [17, 18]. ASD is one of the neurodevelopmental disabilities with male preponderance. However, the higher male prevalence in ASD also remains an unsolved puzzle. Recent efforts are geared towards understanding the mechanistic underpinnings of sex differences in ASD.

According to the Center for Disease Control and Prevention (CDC), the male-to-female prevalence ratio of ASD is estimated to be about 4.5 [19]. In a recent meta-analysis of prevalence studies from 54 publications, the general male-to-female odds ratio (MFOR) was estimated to be 4.20, while stricter conditions and selection of high-quality studies only gave an MFOR of about 3.1 [20]. The discrepancy between these two values could be attributed to the apparent diagnostic bias between sexes, and specifically to a tendency not to clinically diagnose females who meet the ASD diagnosis criteria. Interestingly, females have fewer externalizing problems and restricted and repetitive behaviors than males, suggesting that the discrepancy in phenotypes between males and females with ASD may contribute to the biased prevalence [21, 22]. The impression is that the female population, characterized by fewer diagnoses of ASD, may display weaker manifestations of the core and accompanying symptoms of autism, and that a higher genetic liability is required to induce full-blown autism in females [22, 23]. Efforts to explain the differences have identified a potential protective mechanism in females against de novo mutations, and suggested that a higher genetic load is needed to induce a diagnostic level of autistic phenotypes [24]. Thus, the “female protective model” emerged as one of the possible underlying mechanisms that could explain how female genetic or epigenetic architecture can shield from loads of genetic interruptions enough to cause ASD in males [25].

Another prevailing hypothesis to explain the sex differences in autism is the “Empathizing-Systemizing (ES) Theory of Psychological Sex Differences” [25]. This theory provides evidence that males have higher systemizing scores, while females have higher empathizing scores, and that these tendencies make the male more susceptible to social impairments when exposed to environmental insults or genetic mutations during the early developmental period. Empathy involves an emotional response to another individual’s behavior, whereas systemizing represents a more intellectual and logical approach to understanding the objects and the environment [26]. Thus, the greater empathizing ability of females may alleviate the social alterations, so that a higher percentage of diagnosed or undiagnosed females with ASD, especially those with average IQ scores, have better socialization and communication abilities than their male counterparts [27, 28]. One underlying factor possibly explaining the male-specific features is fetal testosterone during development, which has a negative correlation with social behaviors but a positive correlation with restricted interests [29].

In line with clinical findings, the study of sex difference in ASD animal models will be an important tool to elucidate various mechanistic aspects of the differences. However, interest in this area increased only recently, since most of the preclinical studies typically used only males. Factors such as hormonal differences, especially the estrus cycle of females, are just one of the reasons encouraging the more frequent use of males in research. However, the presentation of sex differences in behavior underscores the
need to include female animals in basic research studies. Similarly, sex differences in the presentation, treatment, and outcomes of mental illnesses are often overlooked. Keeping in mind the theories putatively explaining the sex difference in human ASD development, this review is concerned in how researchers have tried to address the sex difference of ASD in animal models, and whether the results support the theories or not. We look at the current progress of sex difference studies in animal models of ASD, and identify the areas where most improvement is needed in future work.

**SEX DIFFERENCES IN ENVIRONMENTAL MODELS OF AUTISM**

**Valproic acid-exposed animal models**

The valproic acid (VPA)-exposed animal model is one of the most widely used models of ASD because of its known clinical relevance and validity [30-32]. Most studies using this model have predominantly exploited the male sex, and few studies have delved into the sex differences in behaviors and brain molecular changes. Clinically, the occurrence of ASD in children exposed to VPA *in utero* is found to be two-fold higher in males than females [31], while another study found almost equal prevalence in the two sexes, although, in the latter case, the authors analyzed the clinical results of subjects exposed to antiepileptic drugs in general and not just to VPA [32]. With these seemingly inconsistent results in clinical studies, the study of sex difference in VPA-exposed animal models will help elucidate possible phenotypic differences and underlying mechanisms of ASD.

The animal studies, in rat models, which took into account possible sex differences found that social impairment, increased electric-shock induced seizure susceptibility, reduced pain sensitivity and increased anxiety-like behaviors were significantly observed in the male offspring prenatally exposed to VPA but not in their female littermates [33-35]. On the other hand, increased repetitive/stereotyped behaviors was observed in both male and female VPA-exposed rats [35]. In the visuospatial attention and sensorimotor gating behaviors, both male and female VPA rats showed similar levels of abnormalities with minor differences in specific parameters [36]. Mice exposed to VPA also exhibited a male-prevalent social deficit, manifested by reduced sniffing of a stranger mouse in the three-chamber assay, although both sexes have similar abnormalities in the open field and elevated plus maze tasks [37]. Combined together, the experimental results so far suggest that VPA male animals have greater impairment in social interaction, while other behaviors may or may not be different, suggesting that social interaction is the most sensitive behavioral domain affected in a sex-specific manner. Therefore, a female protective or compensatory mechanism towards the development of normal social capability could be modulating the sex differences in VPA rodent models.

Interestingly, the prefrontal cortex region of VPA-exposed rat offspring revealed a male-specific reduction of methyl CpG binding protein 2 (MeCP2) expression from as early as embryonic day 14 (two days after VPA injection), which was sustained up to the second week of postnatal period [33, 38]. This phenomenon could also have driven the male-biased increased levels in the expression of postsynaptic density protein 95 (PSD95), N-Methyl-D-aspartate (NMDA) receptor subunits, and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor subunits during the neonatal and juvenile period of development, which has important implications on the excitatory-inhibitory imbalance in the affected brain [33, 38]. In mice, neuronal cell loss in the prefrontal cortex and transient increase in apoptotic-like cell death in the neocortex were observed following exposure to VPA during the embryonic period for both sex [37]. These results suggest that differences in the sophisticated mechanisms governing neural differentiation or synaptic development, but not the general massive cellular toxicity against ASD-inducing stimuli (in this case, prenatal VPA exposure) underlie the observed sex differences in behavioral characteristics.

Similarly, RNA-sequencing and proteomics analysis were performed in prenatally VPA-exposed male juvenile rats, and showed reduced expression of genes such as *CDK1* and *MAPK8*, which are related to pathways such as neural development and cellular growth [39]. Changes in the expression of genes such as *MAP3K11, HDAC9, and GRIN2A* were observed in the immune, calcium signaling, Rho GTPase and protein kinase A signaling pathways [39]. These results demonstrate that prenatal exposure to VPA can be considered a proper model for studying ASD-related symptoms and the related molecular pathways.

Another context that could be involved in the sex differences displayed by the VPA rat model is the neuroendocrine and immune system. According to Schneider and colleagues [35], male VPA rats have reduced thymus weight, decreased proliferation response of splenocytes to mitogenic stimulation, and increased NO production by peritoneal macrophages in the basal and lipopolysaccharide (LPS) stimulated states, whereas a decreased IFN-γ/IL-10 ratio was noted in both sexes. In addition, male, but not female, VPA rats have elevated corticosterone levels versus controls under basal conditions. Indeed, some of the findings of immune dysregulation in the VPA rat model have also been observed in autistic children [40], and it has been suggested that the neuroimmune system together with masculinization factors, such as sex hormones, may make males more vulnerable to developing ASD [41].
several lines of evidence suggest that VPA can affect both the reproductive and the endocrine systems, including sex hormone levels, of men and women [42-45]. Thus, the sexual dimorphism in the endocrine and immune response of rats to VPA exposure may be an interesting area to help explain the unresolved sex bias of autism prevalence in humans. More interestingly, a recent review by McCarthy et al. [46] suggested that sexual dimorphism in brain development could be mediated by neuroinflammatory dynamics, which might be driven by divergent neuroepigenetic profiles between sexes. Future studies in these areas will provide insight into the role of immunology in various neurodevelopmental disorders like ASD and in the unresolved issue of sex differences.

Brain metabolic activities in VPA rat models have been studied recently by Cho and colleagues [34] using independent component analysis (ICA) of cross-sectional Positron Emission Tomography (PET) data to look at changes in metabolic networks. This method revealed changes caused by VPA treatment in the metabolic activity of the various brain regions examined, showing decreased activities of the olfactory bulb and thalamus, and increased activity of the left caudoputamen in both male and female rats when compared to their respective controls [34]. In addition, some areas of connectivity between brain regions were affected by VPA treatment in both sexes, including those connecting the olfactory bulb to the nucleus accumbens, the somatosensory cortex to the insular cortex, and the olfactory bulb to the orbitofrontal cortex and the cerebellum. Interestingly, sex differences in the effect of VPA treatment on brain connectivity, but not metabolic activity, were found. These include the connections between visual cortex and cerebellum, and between retrosplenial cortex, nucleus accumbens and olfactory bulb, which were less increased in VPA-exposed males than in VPA-exposed females; and connections between anterodorsal hippocampus and caudoputamen, and between medulla and insular/motor cortex, that were more increased in VPA-exposed males than females. Thus, the metabolic activity and connectivity changes caused by VPA may clarify the connection between the behavioral and cellular alterations in the pathophysiology of ASD, and reveal subtle differences in the sex bias aspect of ASD. How similar changes in metabolic activity can result in differential brain connectivity needs to be further investigated in future studies.

Taken together, the VPA animal model of ASD continues to be a useful tool to help understand yet unknown pathways in the development of ASD and the differences between male and female mechanisms, and especially in explaining the differences in behavioral outcomes such as social, emotional and cognitive function.

**Prenatal zinc deficiency (PZD) models**

Zinc is an essential micronutrient with an important role in brain development processes such as neurogenesis, neuronal differentiation, and migration, as well as synaptic plasticity [47, 48]. Zinc deficient mice models have been generated due to the possible association between zinc deficiency and autism [49, 50], wherein zinc-dependent Shank scaffold proteins and the zinc-metalloprotease-2 (BDNF) axis could be strongly involved in the pathophysiology [51, 52]. Animals who were zinc-deficient during the prenatal period exhibited behavioral abnormalities such as anxiety-like behaviors, impaired social-related behaviors, aggression, and altered cognition [53].

One research group addressed possible sex differences in the prenatal zinc deficiency (PZD) mice model [54]. During the neonatal period, the PZD mice showed reduced zinc levels in the brain, which however started to normalize after being cared for by foster mothers. Thus, at the time of experiment, during adulthood, the PZD mice already had a normal range of zinc levels in the brain. Behaviorally, the PZD mice have increased anxiety, reduced nest-building capability, delayed latency to call but a normal number of calls during a reciprocal social interaction test, and decreased latency to fall during the rotarod test, which were not sex-specific. Notably, both male and female PZD mice have normal sociability behaviors, normal spontaneous ability during the Y maze test and normal self-grooming behaviors. Concerning sex differences, the females show increased freezing behavior during transfer to a new environment or cage, more significant reduction of nest-building capacity, impaired preference to social novelty, reduced oral-tooral interaction during reciprocal social interaction, and reduced marble burying behavior. On the other hand, male PZD mice seem to have longer sniffing duration in response to a social stimulus than control males. In general, according to this study, the few sex-specific effects of PZD seem to have more negative effects in female than male mice. This is surprising, considering that most of the sex difference effects in many autism models are more prevalent in males. Moreover, there are weak abnormalities or even enhancing effects of PZD on social and self-grooming behaviors, especially in males, suggesting that more studies will be needed to clearly validate this model of ASD. Although the PZD model sometimes shows contradictory social and self-grooming behaviors, unexpected in an ASD models, the causative relationship of zinc deficiency to other etiologic factors of ASD, such as Shank proteins, make it an interesting model to delve into the mechanistic examination of sex differences, and to examine whether zinc deficiency is more debilitating in the female autistic population.
Maternal immune activation (MIA) model

Maternal infection causing immune activation during pregnancy is one of the many environmental risk factors for ASD [55-58]. Both viral and bacterial infections were associated with increased risk of ASD development in the offspring [59, 60]. To strengthen the involvement of maternal immune activation (MIA) in ASD development, the measurement of an inflammatory biomarker, C-reactive protein (CRP), in the mothers of autistic and normal children found direct proportionality between CRP levels and autism risk [61]. Thus, the development of MIA model in animals eventually emerged, using immunogens such as LPS and poly-cytidylacyclic acid (poly (I:C)) to mimic bacterial and viral infections, respectively [62]. The use of MIA as animal models of ASD has been widely discussed in the literature [63]. Here, we gathered the few studies that have specifically tackled the sex-difference effects of MIA models in ASD core and accompanying symptoms. The offspring of C57BL/6 mice prenatally treated with both LPS and/or poly (I:C) during the gestation period displayed behavioral impairments with treatment and sex-specific effects [64-66]. Foley and colleagues [67] reported the sex-differential effects of prenatal exposure to lipopolysaccharide (LPS), or pre- and postnatal exposure to propionic acid (PPA), in the acoustic startle response and prepulse inhibition in Long-Evans rats. Prenatal LPS exposure resulted in hypersensitivity to acoustic startle only in male offspring, while both pre- and postnatal exposure to PPA induced initial changes in startle response in both male and female rats. Meanwhile, a female-biased deficit in prepulse inhibition was observed in PPA-exposed offspring. Overall, these studies concluded that prenatal, postnatal and the combination of pre- and postnatal exposures to LPS or PPA can induce subtle and sex-differential effects in the sensory processing of adolescent rats.

Xuan and Hampson [64] found that the locomotor activity of MIA-exposed mice was reduced in both sex for poly (I:C) but not LPS exposure. In the three-chamber social test paradigm, female mice exposed to both immunogens showed reduced preference towards a social stimulus, while males were socially affected in poly (I:C) but not in LPS-exposed conditions [64]. Furthermore, marble burying was increased only in males exposed to both immunogens, but females were not affected, and grooming behaviors were not changed in either sex [64]. In contrast, Ruskin et al. [66] showed that poly (I:C) exposure could reduce social interaction and increase grooming behavior in male but not female mice. Yet another study by Hui et al. [65] revealed that prenatal poly (I:C) exposure could impair social interaction and increase marble burying in both male and female offspring, whereas increased anxiety and decreased prepulse inhibition were observed only in males. Furthermore, one study found no, or at most few, sex-specific effects of MIA exposure in autism-relevant behaviors [68]. These differences in behavioral phenotypes among studies could be explained by the variability in dosage and timing of poly (I:C) injection during pregnancy. Therefore, agreement between results and methods among researchers would be needed to better understand sex differences in the effects of MIA in autism, and to maximize the translational perspective [63]. Of particular interest is the idea that the interaction of genetic liability with environmental factors (such as MIA) could be another promising area of investigation for the study of sex difference in ASD [69]. Only a few studies have attempted to elucidate the possible sex differences in the effects of MIA at the molecular and structural level. In the study by Hui et al. [65], prenatal exposure to poly (I:C) affected structural and molecular features in the brain in a sex-dependent manner. In the hippocampus, altered morphology and distribution of the microglia were noted in male but not female mice. Moreover, alterations in the expression of pro-inflammatory genes both in the cerebral cortex, cerebellum, and hippocampus were mostly male-biased [65]. Indeed, more in-depth mechanistic studies are needed to understand the pathophysiologic implications of the differences in relevant behaviors between male and female MIA-exposed animals. It should also be noted that MIA has been introduced as a ‘neurodevelopmental disease primer’, indicating that it can have broad effects and induce other neurologic conditions as well [56].

SEX DIFFERENCE IN GENETIC MODELS OF AUTISM

MeCP2 and sex difference

The Methyl CpG binding protein 2 (MECP2) gene is widely known for its role in determining brain morphology in both humans and mice [70-73]. It is located on Xq28, and encodes the methyl CPG binding protein 2. Expressed in two isoforms with different lengths [74], MECP2 is thought to undergo complex developmental regulation in neuronal cells and possibly to have a different role [75, 76] in the neurons than in other cells. MeCP2 expression peaks in mature postnatal neurons, with some variation between different brain regions [77].

MeCP2, generally regarded as a transcriptional repressor, binds methylated CpG dinucleotides, resulting in the tightening of the chromatin coil leading to a decrease in transcription. It also recruits histone deacetylases (HDACs) [78, 79]. However, depending on the temporal and tissue context, it was also recently shown to behave as either a repressor or a translational activator, enhancing the expression of 85% of its target genes [80-82]. Most of MeCP2’s binding sites are associated with active transcription, with only a small percentage of these sites being methylated cytosines [82].

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Mutations of MeCP2 were shown to be the genetic cause of Rett syndrome [83]. However, these mutations are also associated with other phenotypically similar neurodevelopmental disorders [84-86], indicating the need for a correct MeCP2 dosage to ensure neuronal functional integrity [87-90]. The protein has been suggested to play a vital role in modulating changes in gene expression in response to neuronal activity [91]. For example, MeCP2 phosphorylation regulates activity-dependent BDNF expression [92, 93]. Its phosphorylation at S421 is dependent on neuronal activity, and is necessary for MeCP2-modulated dendritic growth and spine maturation [92].

Expression of MeCP2 was found to be sex-, and time-dependent. During the critical period for brain sexual differentiation in rodents, approximately between embryonic day 18 (E18) and postnatal day 10 (PN10) [94, 95], females expressed more MeCP2 in the amygdala and hypothalamus (at PN1). This difference in expression levels finally equilizes between the sexes by day 10 (PN10) [96]. In another study, this difference in protein expression in rat prefrontal cortex (PFC) was observed from E14 to PN7, but did not differ between sex at PN14 [97]. Interestingly, mutations in the MeCP2 gene were found in autism [91, 98-100], albeit they are not common [101, 102].

These discoveries prompted researchers to ask whether disruption of this sex-specific MeCP2 expression is responsible for the male bias observed in autism. In a study conducted by Kurian et al. [103], a transient knockdown of MeCP2 protein by siRNA in the amygdala of female rats revealed no changes in play behavior; however, knockdown in male rats resulted into a significant decline of juvenile social play behavior, which resembles female-typical play behavior. While both male and female rats were observed to have reduced MeCP2 expression, altered juvenile social behavior was only evident in males. This male-specific sensitivity to MeCP2 disruption during neonatal period provides an intriguing link to the observed male bias associated with reduced MeCP2 expression, which may again point to the so-called female protective mechanism against disruptive stimuli for neural development and synaptic function.

Moreover, there are sex-dependent features of the transcriptome in MeCP2 knockout (KO) mice. Our transcriptome analysis comparing male and female mice model of MeCP2 KO, available from Gene Expression Omnibus (GEO) (Male datasets: GSE105045; Female datasets: GSE90736) revealed that there are 149 differentially expressed gene (DEGs) for male mice and 430 DEGs for female mice, with a base 2 logarithmic fold changes greater than 1.5 (p-value<0.04, Fig. 1A). For both datasets we used 22-24 weeks old MeCP2 KO mice. RNA sequencing was performed with Illumina HiSeq. In the GSE105045 and GSE90736 datasets we analyzed the transcriptome of cortex tissue and microglia, respectively. We explored the mouse phenotypes and Gene Ontology categories shared by common DEGs. Phenotypes enriched in both male and female KO models were mostly related to morphological categories. The Gene Ontology analysis performed with the Database for Annotation, Visualization and Integrated Discovery (DAVID), showed that DEGs shared by both male and female mice were, among biological processes, mainly related to transport processes (Fig. 1B). Phenotypes from male DEGs were enriched in those related to the increase of body weight (Fig. 1C). One study reported that male MeCP2 hypomorphic mice showed significantly increased body weight from 9 weeks of age [104]. On the other hand, most DEGs from female MeCP2 KO mice had a phenotypical link to decreased body weight (Fig. 1D), and it is known clinically that female MeCP2 patients have lower weight than normal people [105]. Gene Ontology (GO) analysis also revealed that DEGs from male MeCP2 KO mice were significantly associated with sensory perception of pain (Fig. 1C). Interestingly, it was shown that sensory pain perception can be regulated by MeCP2 [106]. In addition, Gene Ontology results indicated angiogenesis as one of the most strongly enriched biological processes in female MeCP2 KO mice (Fig. 1D). Experimental results on senescent endothelial progenitor cells (EPCs) revealed that MeCP2 might moderate angiogenesis [107]. These analytical results suggest that the transcriptomic differences are closely related to the phenotypic sex differences in an animal model of MeCP2.

Based on evidence that ASD is one of the many neurodevelopmental disorders involving excitatory/inhibitory imbalance (E/I imbalance), Meng et al. [108] investigated the effects of MECP2 on specific neuronal types. Male mice without MECP2 in their glutamatergic neurons, induced through conditional knockout, were observed to display tremors, anxiety-related behaviors, abnormal seizure-like brain activity and severe obesity. On the other hand, female Mecp2-heterozygous mice were less affected, and were shown to have normal lifespans [109]. Accordingly, only female mice demonstrated relief from ataxia when MeCP2 was restored in glutamatergic neurons [108].

A decrease in excitatory synapses was also observed in MECP2 shRNA-transfected neurons [110]. In a separate study, transgenic male mice displayed an increased number of glutamatergic synapses compared to MECP2-null mice [88]. Likewise, Kim et al. [97] traced the male preponderance of the VPA animal model of autism to the influence of MeCP2 in postsynaptic development. Interestingly, they hypothesized that MeCP2 downregulation was the effect of the proteasomal degradation properties of VPA, which again may be dependent on its HDACi activity, based on previous studies reporting VPA to have induced degradation of
Sex Differences in Autism Mouse Models

Fig. 1. Sexually differential gene expression analysis from MeCP2 KO mice. (A) Venn diagram of differentially expressed genes in the MeCP2 KO mice. A total of 149 and 430 DEGs were sorted in MeCP2 KO male and female mice, respectively. Each graph represents enrichment in mouse phenotypes and gene ontologies in the mouse genome informatics (MGI) (B) at common DEGs (C) DEGs only from male MeCP2 KO mice and (D) only from female MeCP2 KO mice.
some proteins via the proteasomal pathway [111, 112]. On the other hand, an increase in NR2A mRNA level was observed in both cerebellum and hypothalamus in MECP2-deficient male mice [80, 113]. Similarly, increased expressions of excitatory synaptic protein marker PSD-95 and neural stem cell marker Tuj-1 were also observed in cultured rat neural progenitor cells transfected with siRNA against MeCP2 [97]. However, the absence of MECP2 also altered the basal inhibitory rhythms of the hippocampal CA3 circuit, resulting in increased susceptibility to hyperexcitability [114]. These contradicting results imply MECP2’s capacity to broadly regulate excitatory neuronal development and function, which recommends specific investigations of the role of this important epigenetic regulator in a neuronal context-dependent manner to gain a finer understanding of its sex-specific functions related to ASD.

MeCP2 is also linked to the regulation of vasopressin (AVP) expression in the hypothalamus [115]. AVP, in itself, is associated with a number of social behaviors [116-118] and AVP in the amygdala is vital in governing these social behaviors [119]. In the mammalian brain, higher levels of AVP are found in the male than in the female amygdala [120], implying it as a potential target of MeCP2 in modulating social behavior in males [121]. This was shown in male rats with transient reduction of MeCP2 levels in their amygdala during their first three days of postnatal life. A decrease of AVP expression was observed in the first two weeks of life, which persisted to adulthood. This was not demonstrated in their female counterparts [122]. Male brains appear to be more sensitive to changes in molecules associated with epigenetic regulation during early brain development. These findings call for further investigations of the epigenetic mechanisms regulating sexual differentiation of the brain, which would clarify why there is a male-bias in the susceptibility to autism, while females appear to be more resilient to the disorder.

**Euchromatin histone methyltransferase 1 (Ehmt1)**

Euchromatin histone methyltransferase 1 (EHMT1 or G9a-like protein, GLP), together with its paralog, EHMT2 (G9a), forms a chromatin remodeling complex that catalyzes the dimethylation of histone H3 at lysine 9 (H3K9me2), a post-translational modification associated with repression of gene transcription [123]. H3K9me2 is an epigenetic mark that is dynamically regulated in the hippocampus and nucleus accumbens during contextual fear memory formation, addiction, and stress [124]. Additionally, Ehmt1 is highly expressed in the embryonic brain, and in restricted areas of the adult brain, providing a plausible mechanism through which EHMT1 can affect neurodevelopmental processes and, as a result, higher cognitive functions.

Kleefstra syndrome (KS) is a neurodevelopmental disorder caused by the haploinsufficiency of the EHMT1 gene. KS is characterized by intellectual disability, general developmental delay, childhood hypotonia, craniofacial abnormalities and autistic-like behavioral problems. In addition, mutations in EHMT1 have also been associated with ASD [125] and schizophrenia [126]. Many of the human clinical features of KS are recapitulated in mice carrying constitutive haploinsufficiency of Ehmt1 (Ehmt1+/-), making it a highly valid mammalian model for the human disease [127, 128].

Both male and female Ehmt1+/- mice showed increased anxiety, decreased sniffing and approaching time to novel objects or mice, and normal locomotor activity that was not sex-specific [129]. The sex differences observed in these mice include decreased juvenile playing time in adolescent males, and prolonged and delayed preference for social novelty in adult males, but no preference for social novelty in adult females. Male mice showed no preference for stranger mice, but such preference was recovered for a period of time. On the contrary, the females showed no preference for the stranger during all the test sessions, implying that female mice might be more severely affected in their social preference compared to their male counterparts. On the other hand, both male and female mice seem to display more sniffing time in presence of social stimulus than in the empty compartment, indicating higher sociability compared to the wild-type mice [129]. Another core symptom of autism, stereotyped/repetitive behavior, was not clearly confirmed in Ehmt1+/- mice since they showed no differences in spontaneous T maze alternation compared to the wild-type group [129].

Since EHMT1 is required for H3K9me2-mediated gene repression, impaired H3K9me2 by EHMT1 deficiency is suspected to be the main cause of the autism-like behaviors as well as KS [130]. Expression of BDNF and Rab3b is known to be affected by H3K-9me-2 mediated gene repression, which mediates homeostatic excitatory and inhibitory synaptic plasticity both in vitro and in vivo, respectively [131-133]. Thus, altered dosage of those targets might be one of the etiological factors of autism and KS. Notably, the special importance of H3K9me2 and EHMT1 expression in male germ cell development and function was reported [134, 135]. In male mice, EHMT1 is expressed from intermediate spermatogonia until the pre-leptotene stage, and H3K9me2 persists during the synopsis stage. On the other hand, in females, EHMT1 is expressed until the leptotene, and the H3K9me2 methyl marks are maintained during the diplotene. Post pachytene male germ cells complete the meiosis and differentiate into sperm rapidly, while female meiosis stops at the diplotene stage and lasts until ovulation. Thus, male-specific rapid turnover of H3K9me2 at the pachytene
stage might be a driving force for the sex-specific features of development. Another intriguing possibility is that EHMT1 is recruited by the transcriptional repressor element-1 (RE1) silencing transcription factor (REST), and assembled with other corepressors such as HDAC1, HDAC2, and the Rett-syndrome-related protein MECP2 onto the promoters of target genes modulating epigenetic remodeling and gene repression [136, 137]. MECP2 is also known to be implicated in sex differences in neurodevelopment (more details are discussed in the MeCP2 section). The possibility that EHMT1 and MeCP2 play a cooperative role in the regulation of neural development implies a crucial role of epigenetic regulation in ASD and in sex differences.

**Methylenetetrahydrofolate reductase (Mthfr)**

Methylenetetrahydrofolate reductase (Mthfr) plays a role in processing amino acids, the building blocks of proteins, and in the production of the vitamin folic acid (also called vitamin B9) [138]. Specifically, this enzyme converts a molecule called 5,10-methylenetetrahydrofolate to a molecule called 5-methyltetrahydrofolate [138]. This reaction is required for the multistep process that converts the amino acid homocysteine to another amino acid, methionine. Defects in Mthfr gene regulation and abnormal homocysteine–folate metabolism have been reported to increase the risk of birth defects such as neural tube defects, oral clefts, and Down syndrome [139]. Furthermore, an increased risk has been reported for neuropsychiatric and neurodegenerative diseases such as depression, obsessive-compulsive disorder, Alzheimer’s disease, Parkinson’s disease, schizophrenia, and autism [140, 141].

In mice, because of lethality in the homozygotes, behavioral features were confirmed using Mthfr+/- mice. They showed hyperactivity, anxiety, cognitive deficits and low sociability in both sexes [142, 143]. However, the anxiety level in the open field task and social preference deficit was much higher in females compared to males, which might be related to the dysregulated excitatory and inhibitory synaptic protein levels. Interestingly, Mthfr deficiency-mediated enhancement of cortical reelin and glutamatergic signaling in female mice might be a major factor mediating social and anxiety-related problems [143]. These effects would be affected by sex hormones that influence synaptogenesis and synaptic plasticity, such as estradiol [144]. Estradiol recruits PSD95, neuroligin 1, and the NMDA receptor subunit GluN1 to the dendrite spine, resulting in increased excitatory synapses and altering the excitatory/inhibitory neurotransmitter balance in primary neurons [144]. Notably, this sexual hormone-induced synaptogenesis works in female but not male mice [145], but the underlying mechanism is unclear. This may also explain the sex-dependent enhancement of excitatory synapses in Mthfr+/- females. Another plausible mechanism is GABA signaling, which is directly regulated by estradiol [146]. Bluskin et al. [143] showed the sex-dependent GAD activation and potentiation of the GABAergic system in Mthfr+/- females, suggesting a deficit in basal inhibitory function. Overall, sex-specific Mthfr deficiency is critical for the excitatory and inhibitory synaptic plasticity balance that affects cognition and social behavior. Even though the sex differences in neural development may be context-specifically regulated, depending on the etiological factor involved, at least it is obvious that recurrent pathological findings in many neurodevelopmental psychiatric disorders such as E/I imbalance are preserved in Mthfr+/- mice. However, the results from the Mthfr+/- male and females mice clearly indicate that sex selective neural development and autistic behaviors are not invariably biased in one direction, in which females are always protected, but may be governed by the complex interplay of etiological factors, each of which may have differential roles in males and females.

**BTBR T+tf/J (BTBR)**

The BTBR T+tf/J mouse model is an inbred strain, commonly used to study ASD, which, according to previous studies, exhibits behavioral phenotypes with face validity to all three diagnostic symptom categories of autism: lower reciprocal social interactions as juveniles and adults, lower social approach behaviors, reduced social transmission of food preference, unusual patterns of ultrasonic vocalizations, and high repetitive self-grooming as compared to the C57BL/6J strain (B6) [147, 148]. Only a few studies have examined possible sex differences in this strain. One such study was undertaken to examine the effect of Poly IC, a substance which induces maternal immune activation, in C57/BL6 and BTBR mice. Male BTBR mice showed ASD-like symptoms such as social approach, ultrasonic vocalization, marble burying and self-grooming behaviors significantly more than female mice [149].

In another sociability study, male B6 and female BTBR mice displayed a preference for sex- and strain-matched conspecific stimulus while their sex counterpart did not [150]. Although there was no significant interaction between sex and strain in the social proximity test, a significant main effect of sex indicated that female mice showed higher levels of frontal contact (nose tip-to-nose tip) and lower levels of posterior investigation (nose-to-anogenital) in comparison to male mice, altogether suggesting different motivations for sociability in males and females. Systemic administration of the anxiolytic diazepam decreased the frequency of two behaviors associated with anxiety and defensiveness, upright and jump escape, as well as crawl-under behavior [150]. These findings may be interpreted as similar to the gaze aversion response that is commonly reported in autistic individuals, contributing to the view that BTBR mice constitute an excellent model for the analy-

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sis of autistic-like behaviors. These results suggest that the BTBR model can be used to study social aspects of autism, including sex-specific behaviors.

**Glutathione S-transferase Mu 1 (Gstm1)**

Glutathione (GSH) is the most abundant low molecular weight thiol [151]. It is a tripeptide formed by the amino acids L-glutamate, L-cysteine and L-glycine, capable of inhibiting damage to essential cell components caused by reactive oxygen species, such as heavy metals, peroxides, liquid peroxides, and free radicals [152, 153]. Atypical expression of genes of the oxidative stress pathways and an increase in oxidative stress have been reported in ASD [154, 155]. Phenotypic differences of genes associated with the metabolism of glutathione, e.g. GSTP and GSTM1 (glutathione-S-transferase M1), have been correlated with autistic spectrum disorder [156].

GSTM1 is found in humans and in mice [157]. The deletion or removal of GSTM1 may cause an organism to become more susceptible to environmental insults. Therefore, when combined with an environmental insult, alternative GSTM1 genotypes may result in alterations of neurological development and behaviors that mimic those seen in individuals with autism [158].

Yochum et al. [159] used this model to determine if genetically altered mice are more sensitive to toxicant exposure early in life. They used mice with GSTM1 deletion and wild-type controls exposed to valproic acid on PN14. As expected, both GTSM1 knockout male and female mice demonstrated behavioral phenotypes reminiscent of that of autism. Considering that female GTSM1 knockout mice also showed autistic phenotypes, deletion of GTSM1 may also leave females vulnerable to VPA-induced neurotoxicity. Interestingly, female GTSM1 wild-type mice treated with VPA had a decreased number of apoptotic cells from TUNEL staining at P14, suggesting that a neuroprotective effect is exerted in females against VPA damage. Of note, differences in the expression of GSTM1 between sex, with increased expression in females, are observed in both liver and colon in human and mouse [160, 161]. A significantly higher incidence of the GSTM1-null genotype was found in female multiple sclerosis patients compared with male patients, and it has been suggested that GSTM1’s role may possibly be sex-dependent [162]. These studies point out that GSTM1 may exert a role in neural cell survival in female mice during early development, making them less susceptible to environmental insults compared to male mice. Overall, the neurobehavior of GSTM1 knockout mice shows sex- and stage-specific difference and further mechanistic studies are needed.

**Mouse model of 16p11.2 hemideletion**

A clinical study on individuals diagnosed with ASD revealed deletions and duplications in chromosomal region 16p11.2 [163]. These alterations, which occur with frequency similar to that of the duplication of the Prader-Willi/Angelman region, are a common cause of autism and predispose the individual to neurodevelopmental disorders including ADHD [164, 165]. To further uncover the possible mechanisms and pathophysiology of the disorder and explain the sex-difference in striatal dysfunction, a mouse model of 16p11.2 hemideletion was produced [166]. According to Ted Abel’s group, del/+ males, but not females, displayed deficits in reward-directed learning and maintaining motivation to work for rewards, accompanied by increased striatal ERK1 phosphorylation [166]. Since ERK1 is involved in functional repression of striatum, while ERK2 has the opposite effect [167], ERK1 up-regulation probably induced the operant learning impairment which is, on the other hand, not manifested by female del/+ animals. Since ERK1 is located within the 16p11.2 region, the mRNA of ERK1 was reduced by half in del/+ animals [166]. However, the striatal ERK1 in del/+ males, but not in females, is hyperphosphorylated under basal conditions, and this effect is significantly exacerbated by reward, implying that ERK1 phosphorylation is linked to the goal-directed learning deficit in the striatum, in a sex dependent manner. The underlying mechanism which causes sex differences in the ERK pathway in the striatum remains unclear, but there are several candidates including protein phosphatase-kinase intracellular signaling, and the Y-chromosome specific USP9Y protein ubiquitination/degradation factor [168].

Another significant finding in their study is the overexpression of the mRNA of dopamine receptor 2 and adenosine receptor 2a in the striatum in male animals with the deletion. These proteins are regarded as markers of medium spiny neurons signaling via the indirect pathway, which is linked to behavioral inhibition. Hence, in this case, the obvious influence of sex in the genetic lesion was connected with neurodevelopmental disorders, involving intracellular signaling mechanisms in the brain to explain the male-specific susceptibility and female-specific protective ability [166].

Additional information about sex-biased behavioral deficits was sought by examining whether 16p11.2 del/+ animals would exhibit sex-specific sleep and activity alterations, two co-morbid symptoms present in some neurodevelopmental disorders, including ASD and ADHD [169]. The data revealed home-cage hyperactivity in both sexes, but sex-biased alterations in sleep tests. 16p11.2 del/+ male, but not female, mice were observed to spend significantly more time awake and significantly less time in non-rapid eye movement (NREM) sleep during the 24 h period than...
their wild-type littermates [169].

Beside the male-specific behavioral deficits exhibited by the mouse model of 16p11.2 hemideletion (del/+), another study investigated sex-specific neuroanatomical endophenotypes, due to the commonly accepted notion that brain structural changes are a relevant factor in the pathogenesis of neurodevelopmental disorders [170]. Twenty-seven genes are deleted in del/+ animals, and their expression patterns were analyzed using the Allen Mouse Brain Atlas, showing that they spatially overlapped the brain regions affected by structural changes [171]. These results confirmed an apparent elevation of fractional anisotropy in the medial fiber tracts proximal to the striatum in male del/+ mice alone, supporting the previous study of sex-specific differences [166]. Interestingly, the genes overexpressed in regions of the male del/+ endophenotypic changes are associated with the regulation of neurite outgrowth and the activity of MAPK pathway, two credible mechanisms for the pathogenesis of neurodevelopmental disorders, suggesting the interconnection between neural activity and neuroanatomical endophenotypic changes in the sex-biased regulation of ASD-like behaviors [171].

**Chromodomain-helicase-DNA-binding protein 8 (CHD8)**

Chromodomain-helicase-DNA binding protein 8 (CHD8) is one of the chromatin remodeling proteins that can regulate various autism risk genes [172-174]. It has been reported that heterozygous mutations of CHD8 in males show the behavioral features of ASD [175, 176]; however, the involvement of sex dimorphism has not been investigated in detail except in one recent study [177]. Jung et al. [177] showed male-biased behavioral abnormalities in social communication in pups, mother attachment behaviors in juvenile, and increased self-grooming in adults, but these phenotypes did not appear in females. The authors demonstrate that neural activity is suppressed in the female mice under both basal and stress conditions, but the male CHD8 mutants exhibited normal baseline activity and excessive neural activity under the stress condition.

Moreover, transcriptomic analysis showed many GO terms underlying the sex-dependent features in CHD8 mutants such as extracellular matrix (ECM), that regulates the development, function, and plasticity of synapses [177, 178]. The ECM-related genes in the females showed upregulation, but downregulation in the males, which results in the differential neural activity as shown by the c-Fos activity. Collectively, male-biased ASD behavioral properties in CHD8 mutant mice are linked to the sexual DEGs in the transcriptome, and to differential neuronal activities, making these mice a useful tool, especially for the sex-specific behavioral changes regulated by gene expression.

**RAR-related orphan receptor alpha (RORA)**

Hu et al. [179, 180] identified a number of genes with dysregulated expression through gene expression and methylation profiling of lymphoblastoid cell lines (LCL) from monozygotic twins and pairs of siblings discordant for ASD diagnosis. RAR-related orphan receptor alpha (Rora), a nuclear hormone receptor which serves as a transcriptional regulator, was among the many to be found hypermethylated and consequently downregulated [179, 180]. Decreased Rora expression was also observed in the prefrontal cortex and cerebellum of these patients [181]. Rora has been associated with circadian rhythm regulation [182], which is disrupted in patients with ASD [183]. In addition, Rora directly regulates *NLGN1* and *NTRK2*, genes associated with increased susceptibility to ASD [184, 185]. *RORA* is implicated primarily as a candidate gene that could contribute to restricted interest and repetitive behaviors [186]. In mouse models, decreased Rora expression resulted in perseveration in limited maze patrolling [187], reduced exploration [188], and spatial disorientation [189]. Purkinje cell degeneration, also implicated in ASD, is also associated with Rora deficiency [190].

Rora plays a role in the translational regulation of CYP19A1 [191], which codes for aromatase, an enzyme responsible for converting testosterone to estradiol [192]. Aromatase is considered crucial in modulating male and female sex hormone levels in various tissues, including the brain [193]. Reciprocally, estradiol was found to upregulate RORA expression, while the androgen dihydrotestosterone (DHT) downregulates it [193].

Correlation studies have revealed that Rora and a number of its transcriptional targets may display sex-dependent expression in certain brain regions of both humans and mice [194]. Investigation of the correlation coefficients between Rora expression and its transcriptional target genes (CYP19A1, A2BP1, ITPR1, and *NLGN1*) showed higher correlation in the cortex of male mice compared to females. Such higher correlation implies that defects in Rora expression may pose a greater burden on the neurological development and function in males than in females. A strong positive correlation between levels of Rora and aromatase proteins was observed in the cortex of control human males and females as well as ASD males, but not in ASD females [194]. In the presence of Rora deficiency, the ensuing deficiency in aromatase will more likely result in increased levels of testosterone, which was also reported in particular cases of ASD [195, 196].

Interestingly, Sarachana and colleagues [193] reported decrements of Rora in the brain tissue of both male and female subjects with ASD and suggested that while *RORA* is a relevant candidate gene for autism, it is not necessarily sex-specific. However, it is possible that estrogen in females may have compensated for Rora
deficiency, thereby nullifying the effects of RORA downregulation. Rora and estrogen receptor protein (ER) have the same consensus binding site on the DNA, and thus share their target genes. Having common target genes, females, with higher levels of estrogen, may have a higher tolerance for Rora deficiency, making them less susceptible to autism [194].

While the above-mentioned studies seem to implicate the disruption of Rora expression and its transcriptional targets in the sex differences in autism, there were no studies so far directly demonstrating that dysregulated Rora expression could result in the development of autism-related behavioral phenotypes. Prenatal hormonal disruption and the resulting Rora dysregulation should also be considered in a context of gene-environment interplay [197], where biological sex acts as a regulator. Epidemiologically-based endocrine studies listed increased prenatal steroidogenic activity as an early ‘environmental’ risk for the later diagnosis of autism in males [198]. Etiological investigation for autism should also focus on such context, considering the male-specific environmental risk factors that were identified, and the proposed correlation between genes and environment.

**Gene × environment × sex hypothesis**

As we described in the previous sections, some of the genes related to autism are found in the sex chromosomes and some may also indirectly result in sex-specific consequences. We also identified environmental factors whose effects are seemingly skewed towards the male sex. While the contributing genes vary in different patients and many gene variants at different loci are necessary for the disorder to develop, the concentration of most of the suggested environmental risk factors is not enough to induce the disease [199]. These facts suggest a possible interaction or perhaps synergism between genetic and environmental factors in increasing the risk for autism and its reported male preponderance.

Schaafsma et al. [69] put the “triple hit” (gene×environment×male sex) hypothesis to the test by inducing maternal immune activation in a contactin-associated protein-like 2 (Cntnap2) mouse model, and investigated the autism-like effects in male and female animals, thus reproducing the three “hits” of ASD-related etiological factors. These hits demonstrated cumulative effects on ultrasonic vocalization and resulted in deficits in social behavior. The synergistic effects of these hits also significantly altered the expression of the corticotropin-releasing hormone receptor-1 (Crh1) in the left hippocampus, while simultaneously altering histone H3 N-terminal lysine 4 trimethylation (H3K4me3). These findings suggest that both gene and environmental factors may cooperate with male-biased biological mechanism to determine the final outcome of ASD phenotypes. This hypothesis, while increasingly gaining recognition, still needs further exploration, in particular of the quantitative contribution of each factor to the manifestation of ASD-like traits.

**CONCLUSIONS**

Since the origin of research on sex differences, it has been hypothesized that sex differences in behavior are at least in part caused by hormonal influences on the brain [200]. Of course, not all sex differences can be attributed to sex hormones, but genetic, epigenetic, and chromosomal effects also play an important role [201]. At the moment, it is very important to note that sex per se (and probably the sex hormone levels as well) and the interaction with the genetic predisposition and environmental stimuli govern the final outcomes of neural development and synaptic function, which eventually affect sex differences in behavior.

In addition, studying sex-dependent innate behaviors such as sociability, cognition or empathy is useful to understand the underlying mechanisms of sex- or circumstance-dependent neurobiological behaviors in mice. Using a model of pre-gestational stress, a study explored the effects of perinatal exposure to fluoxetine, a selective serotonin reuptake inhibitor, on social play behavior and the hypothalamic pituitary adrenal (HPA) axis. Perinatal administration of fluoxetine resulted in diminished negative effect of maternal stress on sibling play behavior, but increased social aggressive play with a novel conspecific in both sexes and decreased grooming of a novel conspecific in males. Furthermore, it increased serum corticosteroid binding globulin levels, hippocampal serotonin levels, and pre-synaptic density probed using synaptophysin in the dentate gyrus. Pre-gestational maternal stress, in itself, produced a decline in hippocampal neurogenesis rates and synaptophysin density in the dentate gyrus of pre-adolescent males, but not females [202]. This study demonstrated how pre-gestational maternal stress, perinatal selective serotonin reuptake inhibitors (SSRIs), and sex exert their effects on the developing social behavior and the related hippocampal plasticity of the pre-adolescent offspring.

Known as major modulators of the excitatory/inhibitory balance in the brain, glutamate and GABA signaling systems play a vital role in ASD pathophysiology, and these neurotransmission systems are not free from sex biases. Both glutamate and GABA regulation are influenced by sex hormones. In fact, studies reported the neuroprotective effects of female sex hormones, prolactin (PRL) and estrogen, against hippocampal neurodegeneration caused by glutamate excitotoxicity [203-205]. Furthermore, progesterone acts on non-NMDA receptors (AMPA and kainite) and suppresses glutamatergic excitatory activity (reviewed in [206]). Al-Suwailem
et al. [205] demonstrated a significantly lower level of glutamate in the brains of female Wistar albino rats than their male counterparts and suggested that the male bias observed in autism may be explained by the reduced susceptibility of females to glutamate excitotoxicity. Upon the onset of testicular activity in males, estradiol, converted from testosterone by aromatase, initializes a cascade of differentiating effects on the neuronal substrate, giving rise to the masculine brain [207]. Estradiol has the capacity to both boost and prolong the duration of the initial developmental excitatory effects of GABA [207]. On the other hand, progesterone enhances GABAergic inhibitory transmission through its interaction with GABA_A receptors in the mature brain (reviewed in [206]).

Indeed, animal studies also reflect the sexually dimorphic regulation of glutamatergic and GABAergic signaling. In our study [208], we observed aberrations in the kinetic profile of NMDAR, AMPAR, and mGluR5 pathways in the prefrontal cortex of VPA-exposed male rats. These observations were coupled with a reduction of MeCP2 expressions in both the prefrontal cortex of the male offspring and neuronal progenitor cells isolated by sex. We observed the same male bias in the TERT-tg mice, which shows autistic-like phenotypes, more pronounced increase in the expression of VGluT1, a presynaptic marker, and increases in postsynap-

| Table 1. Sex-specific behavioral features in rodent autism spectrum disorder (ASD) models |
|-----------------|------------------|-----------------|---------------|
| ASD Model       | Male phenotype   | Female phenotype| References     |
| VPA             | Reduced sociability and social recognition | Repetitive behavior | Kim et al., 2013 |
|                 | Increased seizure susceptibility |                 | Cho et al., 2017 |
|                 | Reduced pain sensitivity |                 | Schneider et al., 2008 |
| PZD             | Repetitive behavior | Reduced nest-building | Grabrucker et al., 2016 |
|                 | Increased sociability | Impaired social novelty | |
|                 | Increased anxiety | Decreased social interaction | |
|                 | Hyperactivity | Repetitive behavior | |
|                 | Anxiety-like behavior | Increased anxiety | |
| MIA             | Abnormal startle response | Abnormal startle response | Xuan et al., 2014 |
|                 | Reduced social preference | Social preference deficit | Hui et al., 2018 |
|                 | Increased repetitive behavior |                  | Ruskin et al., 2017 |
|                 | Hyperactivity | Breathing problem | Foley et al., 2015 |
| MeCP2           | Declined juvenile social play | Abnormal motor function | Kurian et al., 2008 |
|                 | Anxiety-like behavior | Hyperactivity | Meng et al., 2016 |
|                 | Abnormal seizure-like tremor | Cognitive deficit | Patterson et al., 2016 |
|                 | Motor abnormality | Decreased social preference | |
| Ehmt1           | Anxiety-like behavior | Anxiety-like behavior | Balemans et al., 2010 |
|                 | Reduced sociability and preference | Decreased sociability and novelty | |
|                 | Decreased juvenile social play | No social novelty preference |
| Mthfr           | Anxiety | Anxiety-like behavior | Levav-Rabkin et al., 2011 |
|                 | Hyperactivity | Hyperactivity | Blumkin et al., 2011 |
|                 | Cognition problem | Cognitive deficit | |
|                 | Reduced social preference | Decreased social preference | |
| BTBR            | USV problem | Mild USV problem | Schwartz et al., 2013 |
|                 | Repetitive behavior | Mild repetitive behavior | |
|                 | Reduced sociability | Mild reduced sociability | |
| Gstm1           | Repetitive behavior | Mild repetitive behavior | Yochum et al., 2010 |
|                 | Reduced sociability | Mild reduced sociability | Singhal et al., 1992 |
| 16p11.2 hemideletion | Reward-directed learning deficit | Hyperactivity | Grissom et al., 2018 |
|                 | Hyperactivity | Sleep deprivation | Angelakos et al., 2017 |
| CHD8            | Social communication problem | No specific behavior features | Jung et al., 2018 |
|                 | Hypoactivity | Hyperactivity | Platt et al., 2017 |
| RORA            | Repetitive behavior | Not determined in mouse model | Goodall et al., 1987 |
|                 | Reduced exploration |                           | Lalonde et al., 2003 |

VPA, valproic acid; PZD, prenatal zinc deficiency; MIA, maternal immune activation; MeCP2, Methyl CpG binding protein 2; Ehmt1, Euchromatin histone methyltransferase 1; Mthfr, Methylene tetrahydrofolate reductase; BTBR, BTBR T+tf/J; Gstm1, glutathione-S-transferase M1; CHD8, Chromodomain-helicase-DNA-binding protein 8; RORA, RAR-related orphan receptor alpha.
tic markers of the NMDA and AMPA receptor subtypes [209].

As we described in the previous sections (Table 1), the strong male preponderance in ASD together with a greater genetic load in female ASD led to a female protective effect (FPE) hypothesis [210]. Similarly, sex chromosomes and hormones have been suggested as the underlying mechanisms. However, the basic mechanisms of sex-specific phenotypes from many animal models leave plenty of room for further investigation. For example, experiments using sex chromosome aneuploidy models, or genetic and pharmacological gonadal steroid perturbation models, in combination with ASD models may provide better insight into the role of sex chromosomes and hormones in sex-biased ASD manifestation. Obviously, the unsolved questions in the field of sex skewness of ASD may enormously benefit from experimental results, which might need large number of biological replicates, but not much from merely speculative assumptions about the differences in the structural, functional, and behavioral domains of neural correlates of sex. Overall, combining the knowledge from cellular, molecular, and animal studies on how sex, together with other parameters, influences neural development and neurological functions such as altered neurotransmitter systems, we can expand our understanding and examine the underlying hypotheses, including FPE, about sex-specific neurodevelopmental and psychiatric behaviors and improve diagnosis and intervention through increased precision.

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