Commentary

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Unraveling Autism: Using Brain Organoids to Investigate Sex Differences in Brain Development

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Neurodevelopmental changes during the neonatal and childhood periods involve significant, brainwide remodeling, thus rendering the brain susceptible to disruptions that may heighten the risk of neurodevelopmental psychiatric illnesses such as autism spectrum disorder (ASD). ASD is a complex condition defined by challenges in social communication and the presence of restricted repetitive behaviors. Increasing evidence highlights substantial sex differences in the presentation, diagnosis, and underlying biology of ASD. Sex is a fundamental biological variable in humans that significantly influences neurodevelopment. Differences driven by sex chromosomes and hormones distinctly impact brain function at the cellular, circuit, and anatomical levels. These differences underpin the varying susceptibility to psychiatric risks that has been observed between sexes (1).

Previous animal studies have underscored the importance of sex differences in brain development and their implications for neuropsychiatric conditions (2). Nonetheless, the human brain and behavior are more complex, and animal models cannot fully replicate the intricate cellular and molecular mechanisms involved. In the current issue of *Biological Psychiatry: Global Open Science*, Pavlinek *et al.* (2) summarize the impact of sex differences on brain development, the advantages of using organoids in this research, and the ongoing challenges within the field. Additionally, they examine the role of sex hormones such as androgens and estrogens in shaping distinct neurodevelopmental trajectories. At the cellular level, androgens, elevated in typically developing males, increase radial glia proliferation, thereby expanding the progenitor pool and enhancing excitatory neurogenic potential. Estrogens, higher in typically developing females, influence neurogenesis and synapse formation in a sex-dependent manner. These hormonal differences can lead to structural brain variations. Males often have larger brain volumes with denser amygdala, hippocampus, and basal ganglia regions. Females typically show higher frontocortical density and complexity (3). However, some studies dispute these sex-based distinctions, attributing the differences to population-specific changes and the failure to correct for brain size. Further research at the cellular level is necessary to determine whether these changes translate into significant morphological and functional differences (4).

Interestingly, sex chromosomes also play a crucial role in shaping functional and behavioral differences. X-linked inactivation, caused by the presence of 2 X chromosomes in females, underscores the genetic variability in expression patterns. In contrast, the presence of 1 X and 1 Y chromosome in males creates a distinctly different genetic and epigenetic landscape. Neurodevelopmental disorders exhibit marked differences between sexes in prevalence, age of onset, physiology, and symptoms. For example, females are more susceptible to mood disorders, while males are more prone to dopaminergic system deficits, as seen in conditions like ASD, intellectual disability, attention-deficit/hyperactivity disorder, and schizophrenia (5).

The hemizygous nature of X-linked genes likely contributes to the higher prevalence of ASD in males, which has prompted investigations into sex-specific vulnerabilities. Pavlinek *et al.* (2) elucidate how hormonal and genetic factors unique to each sex may influence this disparity. Androgens driving the increased proliferation of excitatory neurons in males could result in excitatory-inhibitory imbalances—a key feature of ASD pathology. Furthermore, their research on X-chromosome inactivation offers a potential explanation for the female protective effect in ASD. The mosaic expression of X-linked genes in females may provide resilience against the disorder, as supported by the lower prevalence of ASD among females. Additionally, RNA sequencing analysis revealed several genes with differential expression between males and females with ASD. Notably, genes involved in synaptic function and neural connectivity, such as *NLGN4X* and *SHANK1*, exhibited sex-specific expression patterns. This finding is consistent with previous studies suggesting that ASD affects neural circuitry differently in males and females, potentially accounting for variations in symptom severity and presentation (6).

Sex differences in neuropsychiatric disorders go beyond X-linked pathologies. Clinical symptoms of neurodevelopmental disorders often reflect complex, multifactorial origins, with sex hormones directly influencing brain development. For example, immune response genes and pathways in ASD appear to be sex specific, with these differences potentially being beneficial or detrimental depending on the context (6). While such findings pave the way for sex-specific therapeutic interventions, Pavlinek *et al.* (2) highlight the lack of suitable models for studying these differences. Additionally, current diagnostic criteria for ASD, which are largely based on male presentations, may lead to underdiagnosis or misdiagnosis in females. Females also often develop compensatory social skills that can mask symptoms. Grasping the relationship between sex hormones and ASD is vital for pinpointing the sex-specific factors that cause the observed differences between males and females in more diverse forms of ASD (3).

Brain organoids mark a significant advancement in modeling human-specific neurodevelopmental processes. Derived from human induced pluripotent stem cells, these organoids mimic the intricate complexity of human brain

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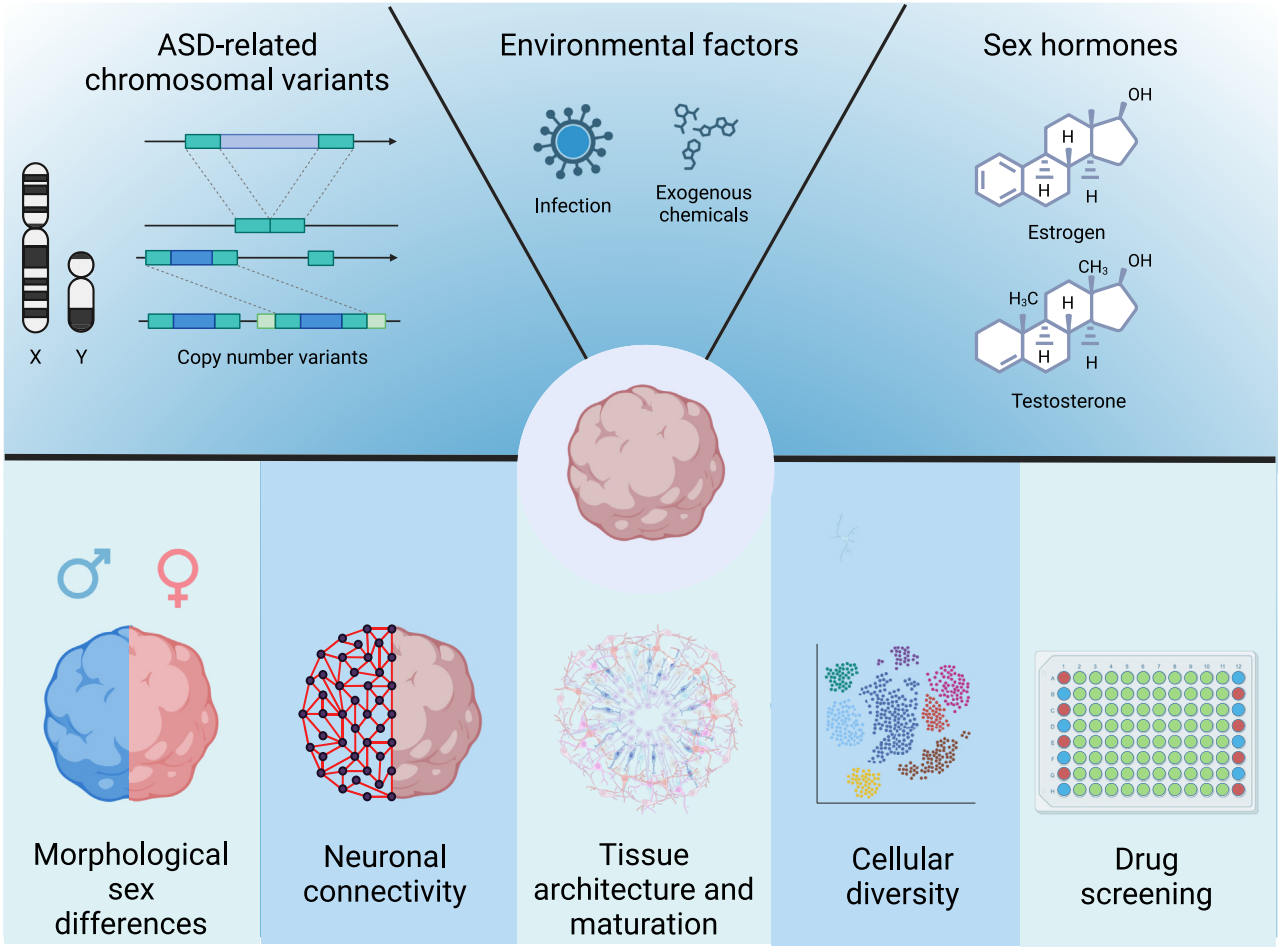

Figure 1. Illustrative representation of modeling sex differences in brain organoids associated with ASD. Brain region–specific organoids can be exposed to various environmental and hormonal factors or studied for the effects of ASD-associated genetic mutations, providing insight into the underlying sex-specific mechanisms in brain development (top lane). These differences in organoids can be characterized by evaluating morphological variations, neuronal connectivity, tissue architecture and maturation, cellular diversity, and in screening of drug candidates (bottom lane). ASD, autism spectrum disorder.

development more accurately. This is particularly vital for studying ASD because human-specific genetic and environmental factors play a crucial role. Pavlinek *et al.* (2) emphasize the significance of utilizing brain organoids to investigate sex-mediated impacts on brain development. This approach helps identify critical changes in neurodevelopmental processes, such as cell proliferation, differentiation, and synapse formation (7). These emerging human cellular models offer a controlled environment to investigate the influence of sex steroids and sex chromosomes on brain development, thereby providing deeper insights into the etiology of ASD (Figure 1). Replicating ASD symptoms in organoids with X-linked perturbations requires the use of genetically penetrant models. Currently, the most effective models are human induced pluripotent stem cell–derived cortical organoids for X-linked conditions such as fragile X syndrome and Rett syndrome (7). These models have elucidated the molecular mechanisms underlying ASD, including impaired neural progenitor proliferation, abnormal differentiation and maturation, increased synapse formation, excitatory/inhibitory imbalances, and disrupted migration. Because these disorders affect different sexes disproportionately, the findings underscore the significant potential of organoids in developing personalized therapies and facilitating targeted drug screening.

Despite their promise, brain organoids come with limitations. Pavlinek *et al.* (2) acknowledge the difficulty in replicating the full complexity of in vivo brain development. Organoids lack the vascularization and cellular diversity of actual brains, which can impact the generalizability of findings. Moreover, the long-term culture and maturation of organoids present technical challenges that must be addressed to fully harness their potential. Furthermore, they also point out the difficulty in accurately modeling X-chromosome inactivation. While organoids offer valuable insights, the stochastic nature of the inactivation process introduces variability that can complicate interpretations.

To overcome these limitations, future research should aim to enhance the complexity and maturity of organoids. Integrating them with microfluidic systems to simulate blood-brain barrier interactions and incorporating various brain cell types could improve their physiological relevance. Additionally, advancements

in single-cell sequencing and emerging spatial biology technologies will enable more detailed analyses of sex-specific differences at the cellular and molecular levels (Figure 1) (7).

While Pavlinek *et al.* (2) discuss the main limitations of using organoids to model sex differences in ASD through X-chromosome inactivation, additional strategies could be further explored and defined. For example, non–X-linked copy number variants serve as penetrant and reliable models for elucidating the mechanisms that underlie ASD phenotypes, such as 22q11.2, 3q29, 1q21.1, and 16p11.2 deletion and or duplication (8). Exposing these human induced pluripotent stem cell–derived copy number variant organoid models to external sex hormones could uniquely illuminate the non–X-linked genetic factors that interact with hormonal sex determinants. Future research should explore the interaction between genetic and environmental factors in the development of ASD. Numerous established maternal exposures significantly increase the risk of ASD. Maternal exposure to harmful substances like heavy metals and pesticides, maternal infections, and the severity and duration of maternal fevers significantly increase the risk of ASD in developing embryos (9). By using organoid models combined with patient-derived cells and factoring in environmental variables, researchers can gain a deeper understanding of the multifaceted nature of ASD.

It is also crucial to examine the complex relationship between early puberty onset in individuals with ASD and the subsequent worsening of ASD symptoms. Accelerated pubertal timing in both males and females suggests that sex-linked factors influence later brain development processes such as myelination and synaptic pruning beyond the neonatal and early developmental stages (10). Additionally, advancements in methodologies such as multiple electrode arrays, which enhance our understanding of organoid functionality, could significantly aid in comprehending the network behavior of organoids. Ultimately, this understanding holds immense potential for developing tailored, sex-specific therapeutic approaches at more precise and manageable stages.

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Article Information

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