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A neurodevelopmental perspective on sex-differentiated genetic effects on behavior

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More than 35,000 peer-reviewed scientific articles have been published investigating human “sex differences” AND “behavior” OR “behaviour” in the fields of Psychology, Neuroscience, Biochemistry, Genetics and Molecular Biology (Scopus search dated April 20th, 2021). And yet, the biological mechanisms underlying sex differences in complex traits are still not understood. Martin and colleagues (1) took on this interesting challenge and reported, in this issue of *Biological Psychiatry*, results of a thorough investigation to test whether sex differences exist in the known common genetic architecture of behavior. They examined 20 neuropsychiatric and behavioural traits, including autism, a heritable neurodevelopmental condition that has three times a higher prevalence in males than females (2). They tested for sex differences in Single Nucleotide Polymorphism heritability (SNP-h^2) and at the individual SNP/gene level, and then explored whether pairs of traits showed similar sex differences in genetic architecture in mostly adult cohorts.

Their findings offer the opportunity to reflect on various possibilities for how autosomal variants could contribute to sex differences in complex traits such as autism. This commentary is centred around three main observations; first, genetic variants that have sex-differentiated effects on behavioural traits might have sex-specific expression profiles in the brain earlier in development. Second, some autosomal variants have sex-specific effects in brain development and neural function via epigenetic mechanisms. Third, gender, rather than sex, might be more crucially associated with differences in the developmental trajectory of complex traits. I suggest that research in developmental samples and using longitudinal designs would clarify whether autosomal common genetic variants have sex-

differentiated effects early in life that contribute to shape neurodevelopmental trajectories.

Analytic approaches have been proposed to evaluate genetic models that could explain sex differences in behaviour (3). Differences in SNP-h^2 and a small genetic correlation between the two sexes could indicate that the two sexes have different genetic liability. Further, if sex-stratified Genome-Wide Association Studies (GWAS) lead to different results, this could reflect a different genetic architecture for the two sexes, with individual variants or genes associated with a phenotype in one sex and not the other (3). Martin and colleagues (1) found no convincing evidence for a significantly different SNP-h^2 or genetic architecture between sexes for the examined traits. They also found no genome-wide significant difference between sexes at the individual SNP-level and genetic correlation between sexes was moderate to high in all traits.

For autism, different results were obtained with two methods based on different assumptions on the genetic architecture, with the first method reporting similar SNP-h^2 between males and females and the second reporting higher SNP-h^2 for autistic males. However, the authors note that the discrepancy of results was seen for the phenotypes with smaller samples, cautioning the reliability of the statistical results. Thus, although sex differences exist for many of the neuropsychiatric and behavioural traits studied by Martin et al. (1), their results do not provide consistent evidence of sex differences in common genetic influences on these traits. This leaves open the question of what other factors play a role in behavioural sex differences. I discuss the developmental effects of gene expression, DNA methylation and gender as three possible answers.

To uncover mechanisms underlying behavioural differences between males and females, Martin et al. (1) conducted a gene-level analysis to test whether genes that show sex-differentiated effects for neuropsychiatric and behavioural traits were differentially expressed in the brain. They found genes with sex-differentiated effects for some of the examined traits. However, these sex-differentiated genes were not significantly enriched in the brain. This null result does not necessarily rule out the possibility that expression pattern of autosomal genes in the brain contribute to sex differences in behavior. For example, Shi et al. (4) report sex differences in gene expression developmental profiles in the brain. They observed male-biased expression in most brain regions prenatally and during puberty, while the majority of the brain regions showed a female-biased gene expression in the first four years of life. No significant sex differences were observed in adulthood. Thus, genes that are associated with a trait might have a sex-specific gene expression profile during critical developmental periods. Another possibility is that an association between behavioural differences and gene expression levels, rather than genotype, is present in genes that show a sex-differentiated expression pattern in the brain. This is suggested by Werling and colleagues (5) with regard to autism. They showed that candidate autism-genes were not differentially expressed in the two sexes. Rather, sex-differentiated functional networks of co-expressed genes involved in neuronal connectivity showed an altered expression pattern in autism post-mortem brain tissue. The authors interpreted their findings as suggestive of shared biological mechanisms between autism and typical sex differences in brain development (5).

The hypothesis that autosomal common variants contribute to sex differences in brain development is supported by Martin et al. (1)'s pathway analysis of 100 genes harbouring the top sex-differentiated SNPs. They found that some of the gene sets

enriched for sex-differentiated effects played a role in nervous system development and neuron differentiation. Understanding the mechanisms through which these genes contribute to sex differences in the developing brain will require studying the interaction between genetic, epigenetic, hormonal and environmental factors. Evidence from animal models indicate that sex differences in the brain depend on epigenetic changes to key genes (e.g., estrogen and progesterone receptor genes) induced by sex hormones at specific developmental times. Further, DNA methylation plays a specific role in brain feminization, inhibiting masculinization (6). In humans, sex differences in DNA methylation profiles in the prenatal human cortex are observed in a number of loci in the autosomal and X chromosomes, including the SHANK1 autism-gene (7). Lying “on top of the genome”, prenatal epigenetic changes, in interaction with hormonal signals, might mediate the effect of genotype on brain development in a sex-specific way from early in life.

There is evidence that the interaction between genetic factors and environmental exposure (which includes the effect of hormones influencing the cellular environment in which genes are transcribed and translated into protein) during critical developmental periods might have an effect on gender-typed behaviour. For example, Hines and colleagues (8) compared 4- to 11-year-old girls with a genetic condition that causes abnormally elevated androgen exposure prenatally (congenital adrenal hyperplasia or CAH) with typically developing girls and boys and with CAH boys (who are not exposed to elevated androgens, but experience other abnormalities). Differently from the other groups, CAH girls did not declare a preference for toys that were labelled for their sex (i.e., ‘for girls’) or that were chosen by adults of the same sex (i.e., women). They also did not spontaneously play with these toys. This provides evidence that diversity in gender-type behaviour can be

influenced by exposure to hormonal levels in critical periods (8). Although research has only started to investigate biological contributions to gender identity and gendered behaviour, twin studies so far indicate that genetic factors account for between 17 and 57% of the variance in gender-typed behaviour in 3- and 4-year-olds, and above 70% of the variance in cross-gender behaviour and identity (i.e., 'behaves like opposite sex' and 'wishes to be of opposite sex') at 7 and 10 years of age (9). Warrier et al. (10) recently found that transgender and gender-diverse individuals not only showed 3 to 6 times higher prevalence of autism, autistic traits and neurodevelopmental conditions than cisgender males and females, but were also more likely to feel they had undiagnosed autism. It is possible that current autism diagnostic criteria may fail to recognise autistic symptoms in certain individuals, which would render current sex bias estimates in autism unreliable. Specifically, investigating gender differences in autism characteristics may offer new insights on the mechanisms underlying behavioural differences.

So far, research suggests that the interplay between genetic, epigenetic, hormonal and environmental factors (possibly different between the two sexes) produces unique developmental pathways for each individual, such that the entire population exist along a spectrum for a neuropsychiatric or behavioural trait, with overlapping but shifted distributions for males and females (3). Martin et al. (1)'s findings are in line with the idea that the effect of autosomal common genetic variation on sex differences in human behaviour is likely to be mediated by the biological and social contexts associated with the individual's sex and gender in which genetic factor operates (see (3) and Fig. 1).

Importantly, sex differences in complex traits account only for a small portion of individual variability in behaviour and in some cases considering sex as the main explanatory variable might be misleading. "*No one could put me into a box – outwardly I was quirky but everything a girl should be. People could not understand why my hobbies were so unladylike",* writes D.F.H. sharing her experience of growing up as an autistic woman (www.aspiringtobeu.com). The systems-biology and developmental neuroscience lenses might be needed to see the big picture illustrating the biological mechanisms underlying sex and gender differences in complex traits.

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Figure legend

Fig. 1. The developmental landscape in which autosomal common genetic variants interact with other factors contributing to individual differences in complex traits. The figure illustrates the effects of gene expression (4), DNA methylation (7) and hormones exposure (8) on brain and gender-type behavior development mentioned in this commentary (that only partially describes the multi-factorial mechanisms underlying sex differences in brain and behaviour, for more complete accounts see (6) and (3), respectively). Created with BioRender.com.