NEWS

Searching for the biology behind autism's sex bias

BY **GRACE HUCKINS**

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Autism comes with a stereotype: shy, awkward, obsessed with tech, decidedly male. It's an image that erases the many girls and women who are autistic, but it also reflects the reality that far more boys than girls have a diagnosis. Several studies put that **sex ratio** at about 3 to 1; among psychiatric conditions, only eating disorders show a more skewed distribution.

Though researchers might debate whether the condition's diagnostic criteria accurately **characterize autistic girls**, they concur that autism **affects boys and girls differently**. And this conspicuous divide remains largely unexplained.

One major clue has emerged in the past decade or so: **Autistic girls and women** carry more **rare, uninherited** — or de novo — variants in autism-linked genes than autistic boys and men do, studies have repeatedly found; a similar pattern **holds for common variants** tied to the condition, according to a study awaiting peer review. Scientists theorize that women are "protected" from the combined effects of autism-linked genes, such that it takes a larger number of random genetic events for them to manifest autism traits.

But as reliable an observation as this 'female protective effect' is, it leaves lingering a major question: Why might women be protected in the first place? Many autism researchers assume the answer must **lie in the basic biological differences** between men and women — their chromosomal makeups and hormone levels. By comparing the genes and gene-expression patterns of men and women, both autistic and non-autistic, some scientists are working to uncover the mechanisms behind such an effect.

“Given how consistent the sex bias and autism prevalence is, if we were to understand the mechanisms that are responsible for that male predominance of autism diagnoses, then we would understand something really fundamental about the biology of autism itself,” says **Donna Werling**, assistant professor of genetics at the University of Wisconsin-Madison.

Even if scientists are successful, fully disentangling the biological and social roots of autism’s sex bias may be impossible, Werling and others caution. Diagnosis — ultimately a social phenomenon — depends on not only the biology of sex and autism but also the rich and messy environmental factors that lead to the **expression of gender and autism** in a social world. And biological studies of the autism sex ratio typically don’t consider transgender or intersex individuals, despite the fact that the autism community is **extremely gender diverse**. (For this reason, the words “men” and “women” are used in this article to refer exclusively to cis men and cis women.)

“It’s a very vexing problem, and it’s a high-dimensional kind of problem, and it’s hard to know where we’re going to get traction,” says **John Constantino**, professor of psychiatry and pediatrics at Washington University in St. Louis, Missouri. “But it’s very important.”

At first blush, the most straightforward explanation for the female protective effect would seem to be chromosomal makeup. Chromosomes ultimately account for all other sex differences, including those in hormones, genitalia, hair growth patterns and even hemoglobin levels. And with two copies of the X chromosome, women can compensate if they carry a broken copy of a gene, whereas men, with only one X, cannot; the tiny Y chromosome doesn’t provide them with any backups.

This difference explains the sex bias in **fragile X syndrome**, a rare, autism-linked condition that is both more common and more severe in men and is caused by mutations in the **FMR1** gene, on the X chromosome. These mutations make the X chromosome look ‘fragile’ under a microscope, hence the condition’s name. **Rett syndrome**, which also often co-occurs with autism, similarly results from mutations in an X-chromosome gene, **MECP2**. Because people need at least one functional copy of MECP2 to survive, Rett syndrome is seen almost exclusively in girls.

Most cases of autism are thought to arise from an accumulation of genetic mishaps in multiple genes. But even in these cases, the X chromosome could take some credit for shielding women from the mutations’ full effects: For example, if a particular X-chromosome gene made autism less likely and women had two copies whereas men had only one, it could take more mutations for autism to manifest in women — which is precisely what scientists observe.

Constantino and his colleagues went looking for such an X-chromosome gene in a 2015 study but **came up empty-handed**. Since then, the sex chromosomes have received too little attention, says **Tychele Turner**, assistant professor of genetics at Washington University in St. Louis, Missouri.

“People will say they don’t contribute,” Turner says. “But they do.” In 2019, Turner and her colleagues **discovered seven genes** with a statistically significant link to autism and other neurodevelopmental conditions only in girls and women; five are located on the X chromosome.

These genes don’t necessarily mediate the female protective effect. For example, mutations in **DDX3X**, a gene that regulates RNA and showed the most extreme sex effect in the study, are, like those in MECP2, thought to be fatal for male embryos.

But, she says, these genes help build the case that the X chromosome plays an important role in the autism sex ratio.

“If you look at those genes and what they do, they often regulate other genes,” Turner says. A single mutation on the X chromosome, then, could have broad enough effects to meaningfully contribute to a complex condition such as autism.

What lies downstream of X-chromosome genes — patterns of gene expression — could also help account for the female protective effect.

“You could imagine a scenario where perhaps there’s a gene that’s more abundant in one sex than the other,” says **Jessica Tollkuhn**, assistant professor at Cold Spring Harbor Laboratory in New York. “And if you have a mutation in that gene, maybe you’re fine, because you have more of that transcript to begin with.” If, for example, women naturally produce higher levels of some autism-linked protein than men do, they may not experience adverse effects if the gene that codes for that protein is mutated to become less effective.

Werling looked for signs of exactly this pattern by comparing the expression of autism-linked genes in cortical tissue — from the brain’s wrinkly surface — from autistic men and women. She **didn’t find any differences** in the autism-linked genes themselves, but some of these regulate other genes. And among those other genes, those that were upregulated in men’s brains compared with women’s were also upregulated in autistic brains compared with non-autistic ones, she found. The men’s brains were like a river swollen with snowmelt: Its high water level doesn’t cause more precipitation, but it does mean that even a modest rainfall is more likely to cause flooding.

The differences are small. “The sex differences in gene expression that we see tend to be really subtle, small magnitude changes that indicate some slight but consistent shifts in the way certain processes are operating in the male and female brain,” Werling says.

However tiny, these differences may contribute to autism’s sex ratio, says **Stephan Sanders**, professor of psychiatry at the University of California, San Francisco, who was not involved in Werling’s study. “It is conceivable that a small shift in RNA — like a 3 percent shift between sexes — could be enough to shift the distribution, leading to a dramatic sex bias,” he says.

If so, these small transcriptional differences could also hint at how that dramatic bias ultimately comes to be. Many of the genes upregulated in both men and autistic people are associated with astrocytes and **microglia**, two types of glial cells that help support neurons. Microglia in particular function as the brain's immune cells, and so Werling's finding converges with a **growing body of evidence** that **links autism** and the **immune system**. But it's still unclear, Werling says, why those genes are upregulated in men; men could simply have more glial cells, she says, or their individual cells could each express more glia-linked proteins.

As new brain transcriptome data have become available — a slow process because those data must **come from donated brains** — Werling and others have been reexamining the differences between male and female cortical gene expression. What they have found so far, Werling says, validates her original conclusions.

But another team has found **12 genes strongly linked to autism** that are differentially expressed in male and female fetal brains. The discrepancy with her own findings, Werling says, comes down to one major difference between the studies: Whereas hers focused only on the cortex, home to complex skills such as problem-solving and social behavior, the other looked across the entire brain. The additional differences in autism-linked gene expression probably came not from the cortex, but from subcortical tissue, she says.

Subcortical brain regions, which are responsible for more basic functions, such as movement and threat detection, show some dramatic sex differences, it turns out. "Where sex seems to be living in the brain [is] these subcortical regions that are rich in hormone receptors," Tollkuhn says. Hormones are thought to give rise to most of the differences between male and female brains, so they could conceivably help shield female brains from the effects of autism-linked mutations.

During gestation, male infants start to secrete testosterone, which is then converted to estrogen and binds with estrogen receptors in the brain. In unpublished work, Tollkuhn and her colleagues were able to track just how estrogen **prompts changes in gene expression** in the mouse brain. In subcortical cells that had the estrogen receptor protein, gene expression varied significantly between males and females.

"We just really do not know still where in the [human] brain these hormone receptors are expressed," Tollkuhn says, but her results establish subcortical regions as a promising horizon for further research.

To date, subcortical regions have received much less attention than the cortex when it comes to autism because they aren't responsible for higher cognitive processes. But subcortical regions are closely connected with the cortex, Sanders says, so there's no reason that those regions couldn't play a major role. For example, the hypothalamus and the pituitary — both subcortical — control stress responses, which strongly influence behavior.

“The idea that a subcortical region can be important to behavior is, without a doubt, true,” Sanders says. “We say autism is cortical largely because we’ve only looked in the cortex.”

Despite the findings thus far, researchers have no guarantee that biology can ever fully explain why autism is so much more common in boys. “That liability is [a product of] genetics and environment,” Turner says. That means that the female protective effect doesn’t just have to come down to genes, she says. “But that’s the way that we’ve all thought about it.”

Some of the underlying reasons for the female protective effect could be purely social in nature, says **Kevin Pelphrey**, professor of neurology at the University of Virginia in Charlottesville. How girls tend to be raised — encouraged to socialize with peers, practice social graces and play with toys that resemble people — is “almost like an early intervention program for being highly social,” he says.

To try to distinguish such environmental influences from biology — if that’s even possible — researchers would need much more information about the life experiences of the people whose tissues they study. Werling can observe when the expression of a given gene is elevated in male versus female brains, for example, but she can’t necessarily tell whether that elevation is due to regulation from genes on the X chromosome, regulation from hormones, or gendered life experiences.

“Unfortunately, with the availability of donated brain tissue that we’re looking at today, the amount of information that we have about each donor’s life is very limited,” Werling says. In particular, the data she uses tell her only the person’s sex assigned at birth, although the autistic community is particularly **gender diverse**.

Making progress on this devilishly complex issue could ultimately come down to something relatively simple: larger sample sizes with more thorough phenotyping. Werling hopes to eventually undertake studies in which she compares the transcriptomic profiles of autistic boys, autistic girls, non-autistic boys and non-autistic girls. But to analyze sex and autism status at the same time, she would need an equal number of donors in each of those four groups — and there are very, very few autistic female brain samples.

“We don’t really have great datasets for putting those two variables together,” she says. “We really need more autistic females in studies.”

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