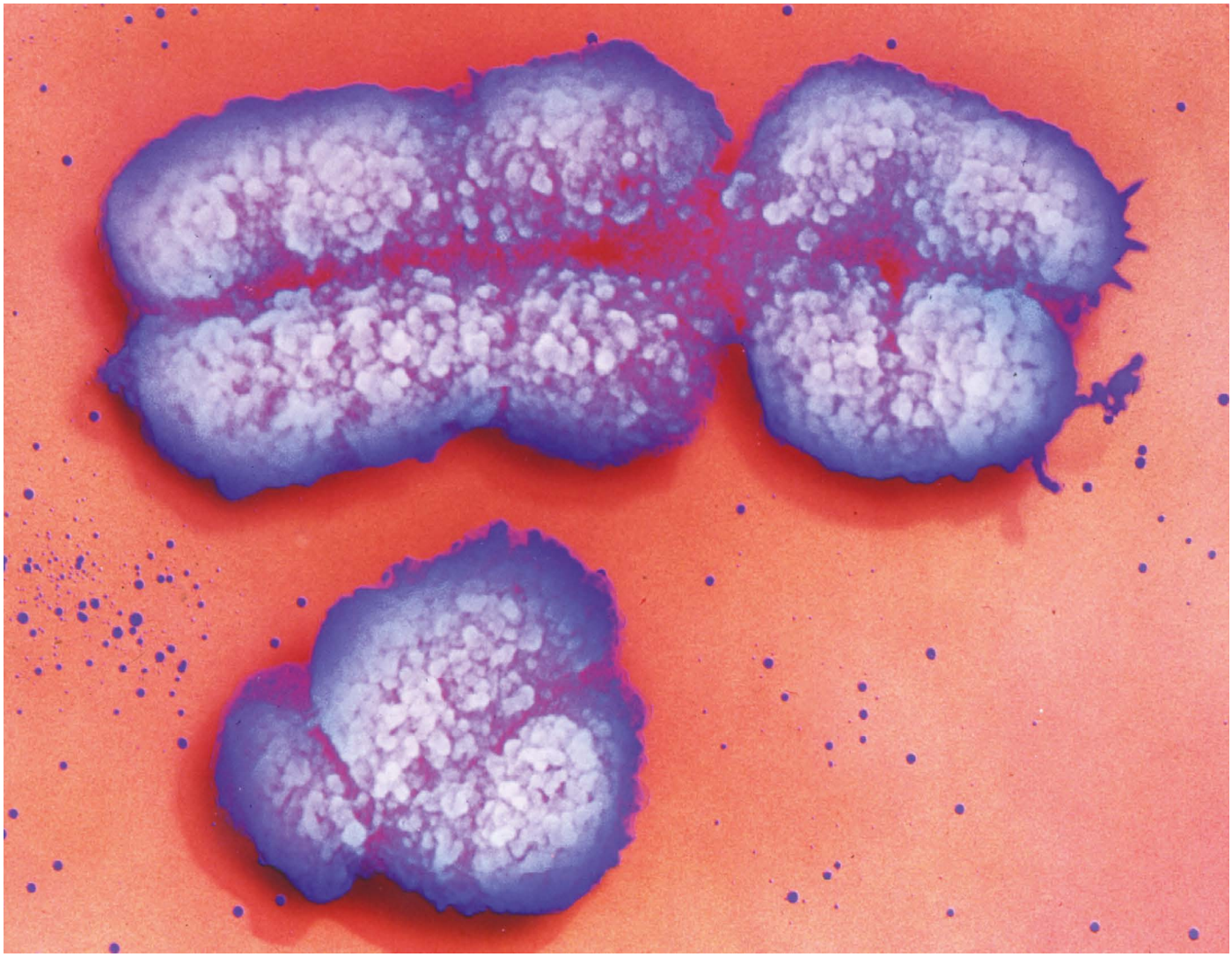

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The X chromosome and its diminutive partner, Y, play a bigger part in human health than was previously thought.

THE RADICAL RETHINK ON SEX CHROMOSOMES AND DISEASE

Long underestimated, the X and Y chromosomes are transforming our understanding of sex differences in health. **By Claire Ainsworth**

Cholesterol-lowering statins are among the most widely prescribed drugs in the world. They can have side effects, however, including muscle pain that affects twice as many women as men. When Karen Reue, a geneticist at the David Geffen School of Medicine at the University of California, Los Angeles (UCLA), set out to discover why, she expected that the answer would lie in the sex hormones, such as oestrogen and testosterone, which are produced by the ovaries and testes.

But her data pointed to a different culprit: the X chromosome. Whether they had female or male gonads, mice with two Xs were more susceptible to statin-induced side effects[1]. "I was amazed when we got our results," says Reue. "It was just clear-cut as could be." A single gene on the X chromosome was the main contributor to the difference in how female mice respond to the drug. Her work has even pointed to a potential way to mitigate the side effects in women. Fish oil contains a fatty

acid called DHA, which is depleted in women taking the drugs, and the supplement reverses some of the metabolic side effects of statins in female mice.

Reue's findings are just one of a wave of discoveries showing that genes on the sex chromosomes, and how they are regulated, can have a substantial impact on health and disease. Women usually have two X chromosomes, men an X and a Y. In women, one of the X chromosomes is typically 'silenced' to ensure that both sexes have a roughly equivalent number of X-linked genes expressing proteins in each cell. But some genes manage to escape the silencing process.

Although these escapees have been known about for a while, the fundamental nature of their influence is only now emerging, says Edith Heard, who studies X-chromosome inactivation at the Francis Crick Institute in London. Escape genes on the X chromosome – and Y genes as well – are revealing themselves to be key contributors to observed sex differences in conditions such as autoimmunity, cancer, cardiovascular disease, metabolic disease, dementia and autism and other neurodevelopmental disorders. These genes are also spotlighting the yawning gap in our understanding of women's health. "Female biology has been neglected for so long," says Heard. "Finally, we actually have the tools to find out what is different in an XX and an XY context other than just the hormones."

The origins of X and Y

Human sex differentiation might look simple, but it is deeply complicated, emerging from the collective action of genes, sex chromosomes and hormones. Variations in any of these can mean that individuals might develop a mix of female-typical and male-typical traits, resulting in a spectrum of outcomes in the population. In individuals with typical development, some sex traits, such as gonad type, are a one-or-the-other affair, whereas others, such as gene-expression patterns or disease risk, appear in ranges that are biased towards one sex. And although some sex differences are permanent, others can change over time. Further complicating things is gender – the societal roles and expectations associated with an individual's sex – and gender identity, which might not align with a person's sex.

What is clear is that a main cause of sex differences stems from the unequal sets of genes on the X and Y chromosomes. According to the conventional view on sex differentiation, the presence or absence of a gene called *SRY*, which is found only on the Y chromosome, determines whether individuals develop testes or ovaries. Then the gonads take over, directing changes in the rest of the body through the secretion of hormones – before birth, during puberty and beyond.

But this focus on gonads has blinkered scientists' understanding of sex differentiation, says Art Arnold, who studies sex-chromosome biology at UCLA. Over the past 20 years, it has become clear that sex differences are also being driven by the unequal effects of the other genes found on the X and Y. This imbalance exists, Arnold says, "because of evolutionary pressures that don't really have to do with reproduction".

Before about 200 million years ago, the precursors of X and Y were an ordinary pair of chromosomes, or autosomes. But then, one of them acquired a hard-wired gonadal sex determination gene, *SRY*, and the once close-knit couple began to drift apart. The Y eventually lost large chunks of DNA, leaving it with a mere 27 distinct protein-coding genes, 17 of which have similar, but not identical, counterparts on the X. Its towering partner, the X, has about 1,000 genes.

Evolution came up with a solution to compensate for this difference in gene number (see 'The story of X'). In males, gene activity, or transcription, is ramped up on the sole X to make up for the missing genes. In females, to balance out that ramped-up gene expression, one of the X chromosomes, Xa, stays active whereas the other (or others, if there is more than one other X), becomes inactive. This is called Xi.

Inactive lifestyle

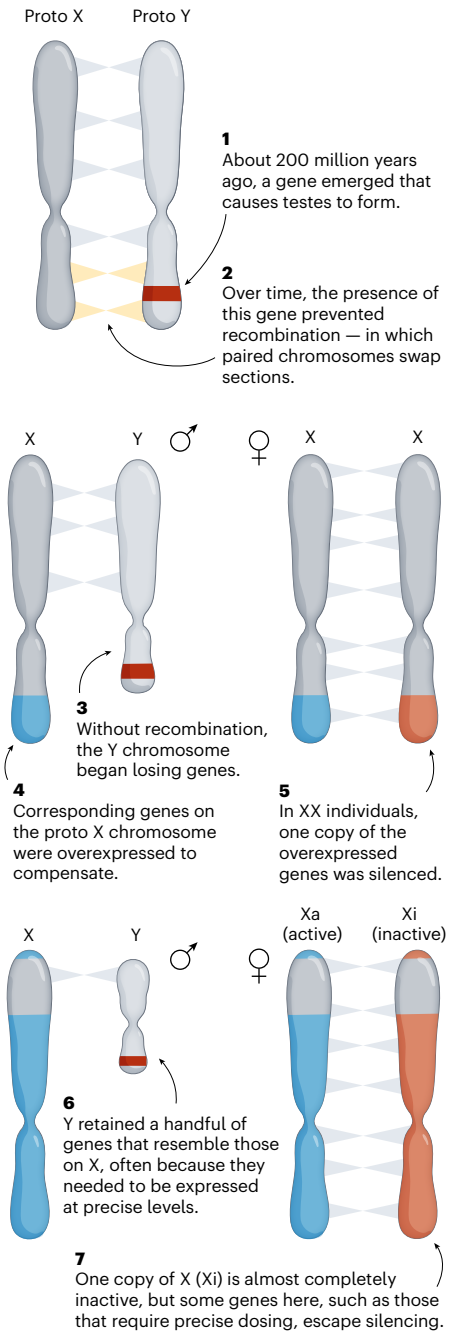
X inactivation takes place early in embryonic development, and happens when copies of a large RNA molecule, called Xist, coat one X chromosome at random. This coating summons a set of proteins to shut down gene activity. But it would be misleading to call Xi inactive. In humans, the Xi is clearly crucial for survival: around 99% of embryos with a solitary X chromosome die. And individuals born XXY (Klinefelter syndrome) have an Xi and an elevated risk of developing certain conditions, such as autoimmune disorders that are associated with women.

Researchers are now getting to the bottom of how the number of X chromosomes someone has affects their health, says James Turner, who studies sex chromosomes at the Crick. "And I'd say there's been huge amounts of progress." At least 20% of Xi genes can escape silencing, and their expression can have profound effects on the rest of the genome. In Reue's study on statin side effects, for example, the culprit was an Xi escape gene called *Kdm5c*. This extra bit of expression in XX mice made the biosynthesis of fatty acids, such as DHA, more prone to disruption by statin drugs[1]. Removing the sex difference by halving the number of *Kdm5c* genes in females reversed the side effects.

Xi escapees include some of the 17 genes that the X and Y still share. David Page, a geneticist at the Whitehead Institute in Cambridge, Massachusetts, and his team have shown that

THE STORY OF X

X inactivation, seen in most mammalian species, shuts off duplicate genes on one X chromosome for XX individuals. But some genes escape this silencing. This is how the process evolved.



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these genes can have a profound impact on the expression of other genes across the genome. They studied cells taken from people with unusual numbers of sex chromosomes — individuals with one, two or three X chromosomes, and zero to four Ys[2]. The researchers found that the presence of Xi and Y affects the expression of 21% of all genes expressed in the cell types tested. How the shared X–Y gene pairs, which closely resemble each other, create sex differences as well as similarities is not yet clear, but subtle differences in how they are regulated could be key.

Not all escapees are equal, either. Some escape in all tissues and all individuals, and are known as constitutive escapees. The other kind, the variable escapees, differ between individuals and between tissues. Even working out what counts as 'escape' is challenging, says Carolyn Brown, a geneticist at the University of British Columbia in Vancouver, Canada. There is a continuum from low to high expression, and conventional definitions set the cut-off at anything above 10% of expression from the corresponding Xa gene. Moreover, Page argues, the view of inactivation as the default state is clouding researchers' understanding of escape genes. "It conjures up all kinds of incorrect ideas, as if there's something illegal or roguish about these genes," he says. Inactivation evolved gene-by-gene, Page says. "The genes that we describe as escaping X inactivation were never subject to X inactivation."

Sex differences

Differential expression of genes on the Xi might nevertheless be related to sex biases for several common diseases, including obesity, heart disease and diabetes. In mice, for example, more expression of *Kdm5c* promotes greater fat mass and diet-induced weight gain. Reue's team has lowered the gene's expression in fat cells, or adipocytes. "We see dramatic effects on metabolism just by changing in this one tissue," she says. In humans, high expression of *KDM5C* in adipose tissue is associated with an increased body mass index[3], and knocking out the gene in developing mouse adipocytes reduces the formation of fat tissue and shifts it to an energy-burning form known as brown fat[4]. "It's kind of exciting to us because this is exactly what you would like," says Reue. "You would like to be able to squelch that *KDM5C* level a little bit and cause this shift."

Another disease for which sex differences are important is cancer. Men are more prone to cancer than are women, and the sexes respond differently to anticancer therapies. In 2022, Heard and her colleagues showed that the loss of the Xist RNA that inactivates the X chromosome is common in aggressive breast tumours in women[5]. Losing Xist caused only a slight increase in the levels of certain proteins, but this was enough to stymie normal breast cell development. "So that shows that escape really matters," says Heard, "and even a little bit more expression of some of these key factors actually makes a difference."

One of the biggest sex differences in medicine concerns autoimmunity. Around 80% of people with autoimmune disease are women, and it's becoming increasingly clear that Xi is a central player. One of its escapees is a gene encoding an immune receptor called TLR7. In most XX cells, one copy of *TLR7* is silenced, but in a subset of immune cells, it escapes. A higher dose of protein production from this gene might confer an advantage; XX immune cells mount a stronger antiviral response than do XY ones. But if TLR7 levels climb too high, then the B immune cells start producing antibodies to the body's own RNA, sparking the development of the autoimmune disease known as systemic lupus erythematosus (SLE). "What's striking is that the X has a lot of very important immunity-related genes on it," says Montserrat Anguera, an epigeneticist at the University of Pennsylvania in Philadelphia. Her team has shown that deleting *Xist* in B cells increases the chances of female mice developing SLE[6].

> "THE X HAS A LOT OF VERY IMPORTANT IMMUNITY-RELATED GENES ON IT."

But there's a surprising twist: over the course of an individual's life, X inactivation can change for some cells, such as certain immune cells. Anguera's team has shown, for example, that in resting B cells, Xi has a different structure from that in other cell types and is not coated with Xist[7]. But when the cell is immunologically stimulated, Xist coats the Xi, which reorganizes its structure. The Xi in T cells is also affected by immunological activation[8]. This plasticity suggests that the immune system can draw on a reservoir of X-linked immune genes for a rapid response. In chronic inflammation, says Anguera, the integrity of Xi silencing might be progressively eroded, driving autoimmune pathology.

Right to Xist

Meanwhile, Heard and her colleagues have shown in mice that Xist continues to regulate Xi gene activity throughout life — a finding that might also apply to humans[9]. There are natural variations in Xist levels between individuals and tissues that might influence X gene dosage, and there are hints that Xist's actions extend beyond the X chromosome, such as by regulating some autosomal genes[10,11].

This is prompting some scientists to think that Xist does more than just balance the dosage of X-linked genes — and that it contributes substantially to sex differentiation in tissues beyond the gonads. "Xist is making females different from males in an analogous fashion to *SRY* making males different from females," says Arnold.

A complete understanding of sex differentiation means uniting the effects of the sex chromosomes and the gonads. Arnold suggests that sex hormones can counterbalance — or amplify — the effects of the sex chromosomes. And when oestrogen plummets at menopause in women, or testosterone slowly declines with age in both sexes, the effects of the sex chromosomes become more apparent. "A lot of our lifetime, we don't have much in terms of gonadal hormones. So the sex-chromosome effects in that situation for sure are going to be important determinants," says Reue.

But it's not all about disease susceptibility. The influence of the Xi and Y on the genome could confer resistance to some conditions. Page and his colleagues, for example, suggest that the Xi escapees could help to explain why autism is more common in males than in females: the extra dose of Xi genes might buffer the effects of autism-associated gene variants[12].

Turner is excited about what he sees as a new era in sex-chromosome biology: understanding how the stability of these chromosomes varies over a person's lifespan and how this influences disease. "Y chromosomes can get lost with age, inactive X chromosomes can get lost," says Turner. Loss of the Y has been linked to cancer, neurodegeneration and heart disease, for example, whereas X loss has been associated with leukaemia.

There is the possibility of intervening using therapies such as CRISPR-based treatments, epigenetic drugs and drugs that target escape-gene proteins. And many scientists are eager to watch as chromosomes once deemed either silent, like Xi, or a one-trick genetic wasteland like Y, are becoming not only central players in common diseases, but also targets to alleviate them. "We're not there yet," says Heard, "but I think we'll be there quite soon."

Claire Ainsworth is a science journalist based in Hampshire, UK.

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