SEX REDEFINED

THE IDEA OF TWO SEXES IS SIMPLISTIC. BIOLOGISTS NOW THINK THERE IS A WIDER SPECTRUM THAN THAT.

BY CLAIRE AINSWORTH

As a clinical geneticist, Paul James is accustomed to discussing some of the most delicate issues with his patients. But in early 2010, he found himself having a particularly awkward conversation about sex.

A 46-year-old pregnant woman had visited his clinic at the Royal Melbourne Hospital in Australia to hear the results of an amniocentesis test to screen her baby's chromosomes for abnormalities. The baby was fine — but follow-up tests had revealed something astonishing about the mother. Her body was built of cells from two individuals, probably from twin embryos that had merged in her own mother's womb. And there was more. One set of cells carried two X chromosomes, the complement that typically makes a person female; the other had an X and a Y. Halfway through her fifth decade and pregnant with her third child, the woman learned for the first time that a large part of her body was chromosomally male[1]. "That's kind of science-fiction material for someone who just came in for an amniocentesis," says James.

Sex can be much more complicated than it at first seems. According to the simple scenario, the presence or absence of a Y chromosome is what counts: with it, you are male, and without it, you are female. But doctors have long known that some people straddle the boundary — their sex chromosomes say one thing, but their gonads (ovaries or testes) or sexual anatomy say another. Parents of children with these kinds of conditions — known as intersex conditions, or differences or disorders of sex development (DSDs) — often face difficult decisions about whether to bring up their child as a boy or a girl. Some researchers now say that as many as 1 person in 100 has some form of DSD[2].

When genetics is taken into consideration, the boundary between the sexes becomes even blurrier. Scientists have identified many of the genes involved in the main forms of DSD, and have uncovered variations in these genes that have subtle effects on a person's anatomical or physiological sex. What's more, new technologies in DNA sequencing and cell biology are revealing that almost everyone is, to varying degrees, a patchwork of genetically distinct cells, some with a sex that might not match that of the rest of their body. Some studies even suggest that the sex of each cell drives its behaviour, through a complicated network of molecular interactions. "I think there's much greater diversity within male or female, and there is certainly an area of overlap where some people can't easily define themselves within the binary structure," says John Achermann, who studies sex development and endocrinology at University College London's Institute of Child Health.

These discoveries do not sit well in a world in which sex is still defined in binary terms. Few legal systems allow for any ambiguity in biological sex, and a person's legal rights and social status can be heavily influenced by whether their birth certificate says male or female.

"The main problem with a strong dichotomy is that there are intermediate cases that push the limits and ask us to figure out exactly where the dividing line is between males and females," says Arthur Arnold at the University of California, Los Angeles, who studies biological sex differences. "And that's often a very difficult problem, because sex can be defined a number of ways."

THE START OF SEX

That the two sexes are physically different is obvious, but at the start of life, it is not. Five weeks into development, a human embryo has the potential to form both male and female anatomy. Next to the developing kidneys, two bulges known as the gonadal ridges emerge alongside two pairs of

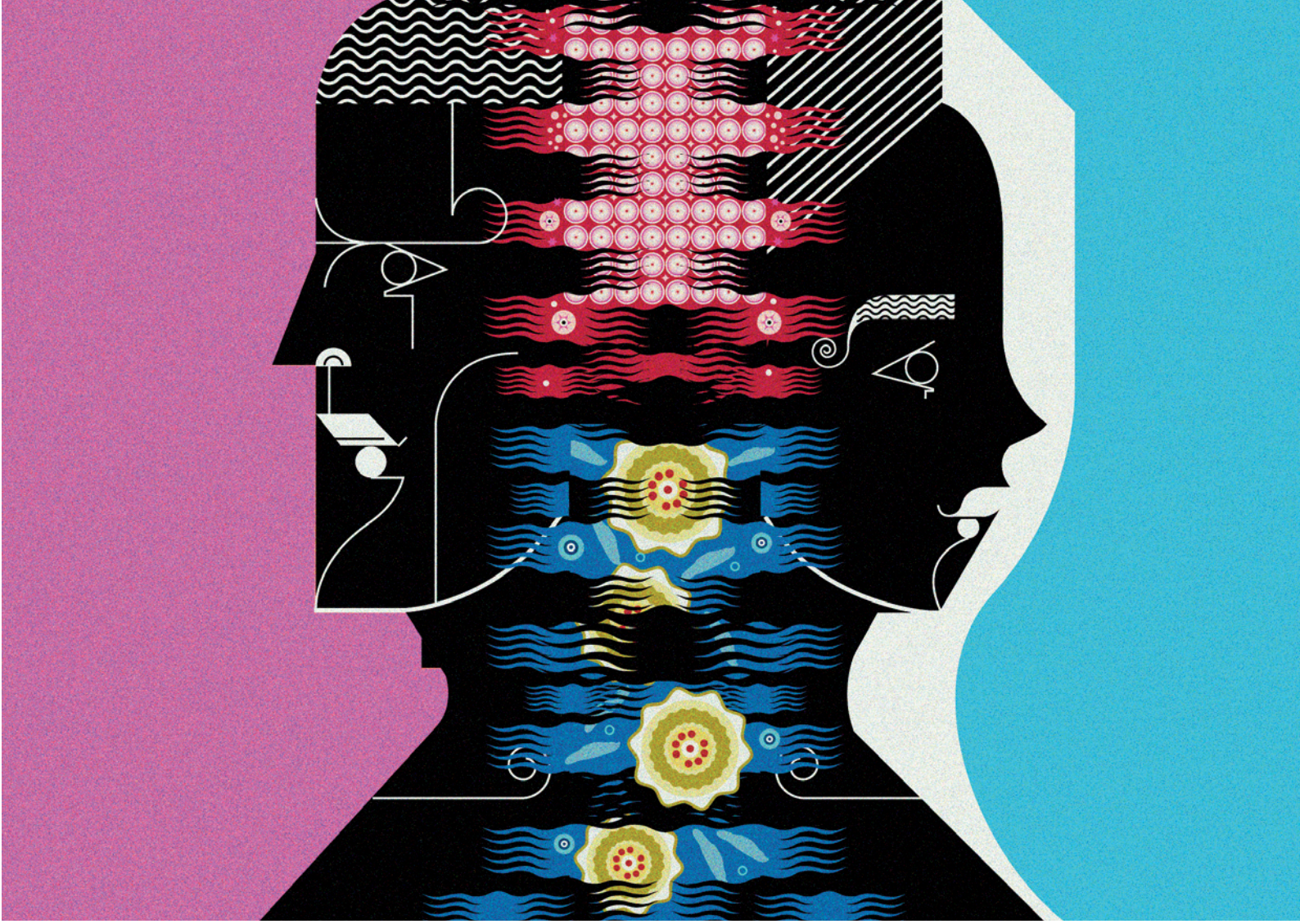

ILLUSTRATION BY JONNY WAN

ducts, one of which can form the uterus and Fallopian tubes, and the other the male internal genital plumbing: the epididymes, vas deferentia and seminal vesicles. At six weeks, the gonad switches on the developmental pathway to become an ovary or a testis. If a testis develops, it secretes testosterone, which supports the development of the male ducts. It also makes other hormones that force the presumptive uterus and Fallopian tubes to shrink away. If the gonad becomes an ovary, it makes oestrogen, and the lack of testosterone causes the male plumbing to wither. The sex hormones also dictate the development of the external genitalia, and they come into play once more at puberty, triggering the development of secondary sexual characteristics such as breasts or facial hair.

Changes to any of these processes can have dramatic effects on an individual's sex. Gene mutations affecting gonad development can result in a person with XY chromosomes developing typically female characteristics, whereas alterations in hormone signalling can cause XX individuals to develop along male lines.

For many years, scientists believed that female development was the default programme, and that male development was actively switched on by the presence of a particular gene on the Y chromosome. In 1990, researchers made headlines when they uncovered the identity of this gene[3,4], which they called *SRY*. Just by itself, this gene can switch the gonad from ovarian to testicular development. For example, XX individuals who carry a fragment of the Y chromosome that contains *SRY* develop as males.

By the turn of the millennium, however, the idea of femaleness being a passive default option had been toppled by the discovery of genes that actively promote ovarian development and suppress the testicular programme — such as one called *WNT4*.

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XY individuals with extra copies of this gene can develop atypical genitals and gonads, and a rudimentary uterus and Fallopian tubes[5]. In 2011, researchers showed[6] that if another key ovarian gene, *RSPO1*, is not working normally, it causes XX people to develop an ovotestis — a gonad with areas of both ovarian and testicular development.

These discoveries have pointed to a complex process of sex determination, in which the identity of the gonad emerges from a contest between two opposing networks of gene activity. Changes in the activity or amounts of molecules (such as WNT4) in the networks can tip the balance towards or away from the sex seemingly spelled out by the chromosomes. "It has been, in a sense, a philosophical change in our way of looking at sex; that it's a balance," says Eric Vilain, a clinician and the director of the Center for Gender-Based Biology at the University of California, Los Angeles. "It's more of a systems-biology view of the world of sex."

BATTLE OF THE SEXES

According to some scientists, that balance can shift long after development is over. Studies in mice suggest that the gonad teeters between being male and female throughout life, its identity requiring constant maintenance. In 2009, researchers reported[7] deactivating an ovarian gene called *Foxl2* in adult female mice; they found that the granulosa cells that support the development of eggs transformed into Sertoli cells, which support sperm development. Two years later, a separate team showed[8] the opposite: that inactivating a gene called *Dmrt1* could turn adult testicular cells into ovarian ones. "That was the big shock, the fact that it was going on post-natally," says Vincent Harley, a geneticist who studies gonad development at the MIMR-PHI Institute for Medical Research in Melbourne.

The gonad is not the only source of diversity in sex. A number of DSDs are caused by changes in the machinery that responds to hormonal

THE SEX SPECTRUM

A typical male has XY chromosomes, and a typical female has XX. But owing to genetic variation or chance events in development, some people do not fit neatly into either category. Some are classed as having differences or disorders of sex development (DSDs), in which their sex chromosomes do not match their sexual anatomy.

- Chromosomes
- Gonads
- Genitals
- Other characteristics/ examples

Typical male	Subtle variations	Moderate variations	46,XY DSD
XY	XY	XY	XY
Testes	Testes	Testes	Testes
Male internal and external genitals	Male internal and external genitals	Male external genitals with anatomical variations such as urethral opening on underside of penis.	Often ambiguous
Male secondary sexual characteristics	Subtle differences such as low sperm production. Some caused by variation in sex-development genes.	Affects 1 in 250–400 births.	The hormonal disorder persistent Müllerian duct syndrome results in male external genitals and testes, but also a womb and Fallopian tubes.

signals from the gonads and other glands. Complete androgen insensitivity syndrome, or CAIS, for example, arises when a person's cells are deaf to male sex hormones, usually because the receptors that respond to the hormones are not working. People with CAIS have Y chromosomes and internal testes, but their external genitalia are female, and they develop as females at puberty.

Conditions such as these meet the medical definition of DSDs, in which an individual's anatomical sex seems to be at odds with their chromosomal or gonadal sex. But they are rare — affecting about 1 in 4,500 people[9]. Some researchers now say that the definition should be widened to include subtle variations of anatomy such as mild hypospadias, in which a man's urethral opening is on the underside of his penis rather than at the tip. The most inclusive definitions point to the figure of 1 in 100 people having some form of DSD, says Vilain (see 'The sex spectrum').

But beyond this, there could be even more variation. Since the 1990s, researchers have identified more than 25 genes involved in DSDs, and next-generation DNA sequencing in the past few years has uncovered a wide range of variations in these genes that have mild effects on individuals, rather than causing DSDs. "Biologically, it's a spectrum," says Vilain.

A DSD called congenital adrenal hyperplasia (CAH), for example, causes the body to produce excessive amounts of male sex hormones; XX individuals with this condition are born with ambiguous genitalia (an enlarged clitoris and fused labia that resemble a scrotum). It is usually caused by a severe deficiency in an enzyme called 21-hydroxylase. But women carrying mutations that result in a milder deficiency develop a 'non-classical' form of CAH, which affects about 1 in 1,000 individuals; they may have male-like facial and body hair, irregular periods or fertility problems — or they might have no obvious symptoms at all. Another gene, *NR5A1*, is currently fascinating researchers because variations in it cause a wide range of effects[10], from underdeveloped gonads to mild hypospadias in men, and premature menopause in women.

Many people never discover their condition unless they seek help for infertility, or discover it through some other brush with medicine. Last year, for example, surgeons reported that they had been operating on a hernia in a man, when they discovered that he had a womb[11]. The man was 70, and had fathered four children.

SURGEONS DISCOVERED THAT THE MAN HAD A WOMB. HE WAS 70.

CELLULAR SEX

Studies of DSDs have shown that sex is no simple dichotomy. But things become even more complex when scientists zoom in to look at individual cells. The common assumption that every cell contains the same set of genes is untrue. Some people have mosaicism: they develop from a single fertilized egg but become a patchwork of cells with different genetic make-ups. This can happen when sex chromosomes are doled out unevenly between dividing cells during early embryonic development. For example, an embryo that starts off as XY can lose a Y chromosome from a subset of its cells. If most cells end up as XY, the result is a physically typical male, but if most cells are X, the result is a female with a condition called Turner's syndrome, which tends to result in restricted height and underdeveloped ovaries. This kind of mosaicism is rare, affecting about 1 in 15,000 people.

The effects of sex-chromosome mosaicism range from the prosaic to the extraordinary. A few cases have been documented in which a mosaic XXY embryo became a mix of two cell types — some with two X chromosomes and some with two Xs and a Y — and then split early in development[12]. This results in 'identical' twins of different sexes.

There is a second way in which a person can end up with cells of different chromosomal sexes. James's patient was a chimaera: a person who develops from a mixture of two fertilized eggs, usually owing to a merger between embryonic twins in the womb. This kind of chimaerism resulting in a DSD is extremely rare, representing about 1% of all DSD cases.

Another form of chimaerism, however, is now known to be widespread. Termed microchimaerism, it happens when stem cells from a fetus cross the placenta into the mother's body, and vice versa. It was first identified in the early 1970s — but the big surprise came more than two decades later, when researchers discovered how long these crossover cells survive, even though they are foreign tissue that the body should, in theory, reject. A study in 1996 recorded women with fetal cells in their blood as many as 27 years after giving birth[13]; another found that maternal cells remain in children up to adulthood[14]. This type of work has further blurred the sex divide, because it means that men often carry cells from their mothers, and women who have been pregnant with a male fetus can carry a smattering of its discarded cells.

Microchimaeric cells have been found in many tissues. In 2012, for example, immunologist Lee Nelson and her team at the University of Washington in Seattle found XY cells in post-mortem samples of women's brains[15]. The oldest woman carrying male DNA was 94 years old. Other studies have shown that these immigrant cells are not idle; they integrate into their new environment and acquire specialized functions, including (in mice at least) forming neurons in the brain[16]. But what is not known is how a peppering of male cells in a female, or vice versa, affects the health or characteristics of a tissue — for example, whether it makes the tissue more susceptible to diseases more common in the opposite sex. "I think that's a great question," says Nelson, "and it is essentially entirely unaddressed." In terms of human behaviour, the consensus is that a few male microchimaeric cells in the brain seem unlikely to have a major effect on a woman.

Scientists are now finding that XX and XY cells behave in different ways, and that this can be independent of the action of sex hormones. "To tell you the truth, it's actually kind of surprising how big an effect of sex chromosomes we've been able to see," says Arnold. He and his colleagues have shown[17] that the dose of X chromosomes in a mouse's body can affect its metabolism, and studies in a lab dish suggest[18] that XX and XY cells behave differently on a molecular level, for example with different metabolic responses to stress. The next challenge, says

	Ovotesticular DSD	46,XX testicular DSD	Moderate variations	Subtle variations	Typical female
Chromosomes	XX, XY or mix of both	XX	XX	XX	XX
Gonads	Both ovarian and testicular tissue	Small testes	Ovaries	Ovaries	Ovaries
Genitals	Ambiguous	Male external genitals	Female internal and external genitals	Female internal and external genitals	Female internal and external genitals
Other characteristics/ examples	Rare reports of predominantly XY people conceiving and bearing a healthy child.	Usually caused by presence of male sex-determining gene *SRY*.	Variations in sex development such as premature shutdown of ovaries. Some caused by variation in sex-development genes.	Subtle differences such as excess male sex hormones or polycystic ovaries.	Female secondary sexual characteristics

Arnold, is to uncover the mechanisms. His team is studying the handful of X-chromosome genes now known to be more active in females than in males. "I actually think that there are more sex differences than we know of," says Arnold.

BEYOND THE BINARY

Biologists may have been building a more nuanced view of sex, but society has yet to catch up. True, more than half a century of activism from members of the lesbian, gay, bisexual and transgender community has softened social attitudes to sexual orientation and gender. Many societies are now comfortable with men and women crossing conventional societal boundaries in their choice of appearance, career and sexual partner. But when it comes to sex, there is still intense social pressure to conform to the binary model.

This pressure has meant that people born with clear DSDs often undergo surgery to 'normalize' their genitals. Such surgery is controversial because it is usually performed on babies, who are too young to consent, and risks assigning a sex at odds with the child's ultimate gender identity — their sense of their own gender. Intersex advocacy groups have therefore argued that doctors and parents should at least wait until a child is old enough to communicate their gender identity, which typically manifests around the age of three, or old enough to decide whether they want surgery at all.

This issue was brought into focus by a lawsuit filed in South Carolina in May 2013 by the adoptive parents of a child known as MC, who was born with ovotesticular DSD, a condition that produces ambiguous genitalia and gonads with both ovarian and testicular tissue. When MC was 16 months old, doctors performed surgery to assign the child as female — but MC, who is now eight years old, went on to develop a male gender identity. Because he was in state care at the time of his treatment, the lawsuit alleged not only that the surgery constituted medical malpractice, but also that the state denied him his constitutional right to bodily integrity and his right to reproduce. Last month, a court decision prevented the federal case from going to trial, but a state case is ongoing.

"This is potentially a critically important decision for children born with intersex traits," says Julie Greenberg, a specialist in legal issues relating to gender and sex at Thomas Jefferson School of Law in San Diego, California. The suit will hopefully encourage doctors in the United States to refrain from performing operations on infants with DSDs when there are questions about their medical necessity, she says. It could raise awareness about "the emotional and physical struggles intersex people are forced to endure because doctors wanted to 'help' us fit in," says Georgiann Davis, a sociologist who studies issues surrounding intersex traits and gender at the University of Nevada, Las Vegas, who was born with CAIS.

Doctors and scientists are sympathetic to these concerns, but the MC case also makes some uneasy — because they know how much is still to be learned about the biology of sex[19]. They think that changing medical practice by legal ruling is not ideal, and would like to see more data collected on outcomes such as quality of life and sexual function to help decide the best course of action for people with DSDs — something that researchers are starting to do.

Diagnoses of DSDs once relied on hormone tests, anatomical inspections and imaging, followed by painstaking tests of one gene at a time. Now, advances in genetic techniques mean that teams can analyse multiple genes at once, aiming straight for a genetic diagnosis and making the process less stressful for families. Vilain, for example, is using whole-exome sequencing — which sequences the protein-coding regions of a person's entire genome — on XY people with DSDs. Last year, his team showed[20] that exome sequencing could offer a probable diagnosis in 35% of the study participants whose genetic cause had been unknown.

Vilain, Harley and Achermann say that doctors are taking an increasingly circumspect attitude to genital surgery. Children with DSDs are treated by multidisciplinary teams that aim to tailor management and support to each individual and their family, but this usually involves raising a child as male or female even if no surgery is done. Scientists and advocacy groups mostly agree on this, says Vilain: "It might be difficult for children to be raised in a gender that just does not exist out there." In most countries, it is legally impossible to be anything but male or female.

Yet if biologists continue to show that sex is a spectrum, then society and state will have to grapple with the consequences, and work out where and how to draw the line. Many transgender and intersex activists dream of a world where a person's sex or gender is irrelevant. Although some governments are moving in this direction, Greenberg is pessimistic about the prospects of realizing this dream — in the United States, at least. "I think to get rid of gender markers altogether or to allow a third, indeterminate marker, is going to be difficult."

So if the law requires that a person is male or female, should that sex be assigned by anatomy, hormones, cells or chromosomes, and what should be done if they clash? "My feeling is that since there is not one biological parameter that takes over every other parameter, at the end of the day, gender identity seems to be the most reasonable parameter," says Vilain. In other words, if you want to know whether someone is male or female, it may be best just to ask. ■

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