The role of sex in the genomics of human complex traits

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Abstract | Nearly all human complex traits and disease phenotypes exhibit some degree of sex differences, including differences in prevalence, age of onset, severity or disease progression. Until recently, the underlying genetic mechanisms of such sex differences have been largely unexplored. Advances in genomic technologies and analytical approaches are now enabling a deeper investigation into the effect of sex on human health traits. In this Review, we discuss recent insights into the genetic models and mechanisms that lead to sex differences in complex traits. This knowledge is critical for developing deeper insight into the fundamental biology of sex differences and disease processes, thus facilitating precision medicine.

Sex differences
Significant differences in the means of a phenotype between males and females — also includes sexual dimorphism.

Genetic liability
The total contribution of the risk or trait-influencing alleles for a given trait.

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Throughout the human lifespan, sex is among the most important characteristics, but despite decades of research, the role of sex in health and disease remains poorly understood. Without understanding the effects of sex on disease risk, prognosis and treatment efficacy, efforts towards precision medicine are likely to suffer in multiple domains. As a case in point, biological factors that differentially impact women's health have been understudied, contributing to growing disparities in health care for women across the globe (for example, conditions of pregnancy, mental health, immune-mediated conditions and drug response)[1]. Indeed, the study of sex differences (BOX 1) is a crucial component of ensuring equitable medicine for all, for example, by determining the best therapeutic strategies in each sex for health conditions exhibiting sex differences[2].

Despite the critical importance of sex as a biological variable (SABV) in development and disease, relatively few studies address the complexity of sex differences in the genetics of human disease. Methodological challenges associated with the analysis of sex chromosomes[3,4] and low statistical power for inferring sex differences have impeded progress towards understanding SABV (BOX 2). Several efforts are underway to increase awareness of the importance of considering sex and gender in studies of human disease[2,5,6] and to provide tools, statistical frameworks and guidance for implementation[3,7–9]. For working definitions of sex and gender, see BOX 3.

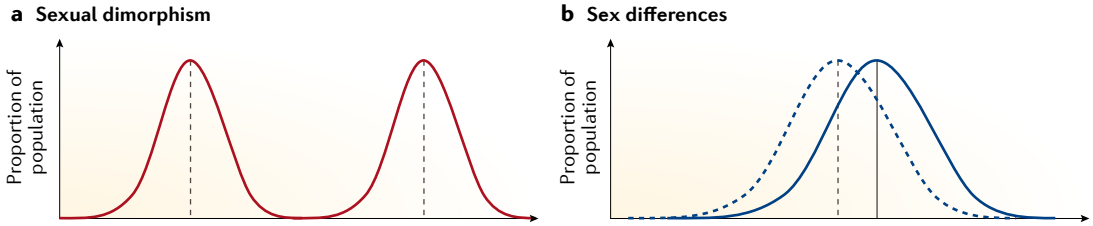
In this Review, we summarize the current understanding of the role of sex in human complex traits (FIG. 1). We first describe genetic models that have been proposed to underlie the biological differences between the sexes. In doing so, we also evaluate recent findings from large-scale epidemiological studies and provide examples of recent discoveries reported as a result of testing these models. We go on to describe functional genomic evidence for sex differences at the mechanistic molecular level. Finally, we provide perspectives on future challenges, opportunities for discovery and implications for clinical care.

Models proposed to explain sex differences

Here, we discuss evidence for three genetic models that are hypothesized to explain sex differences observed in epidemiological studies. Although we describe three distinct genetic models that may explain sex differences observed in complex traits, we stress that these models are not mutually exclusive, and indeed, it is likely that all three affect disease outcomes in both males and females. To date, much attention has focused on the sex chromosomes as the primary source of variance between the sexes (model 2 below). Comprehensive reviews on the role of the sex chromosomes and gonadal hormones have already been written[10–12], and thus our treatment of the sex chromosomes is comparatively brief in order to expand on genome-wide models of sex differences that are less frequently reviewed.

Model 1: the Carter effect and sex-dependent liability thresholds. In the 1960s, Cedric Carter observed that females, while less commonly affected with pyloric stenosis (a thickening of the pylorus muscle between the stomach and the small intestine, with onset in infancy), were more likely than males with pyloric stenosis to have children affected with the disorder[13,14]. Carter posited that perhaps females were protected in some way from developing pyloric stenosis and therefore required a greater genetic liability or increased number of risk alleles to develop the disease. Thus, females who became affected with pyloric stenosis, despite the presence of protective factors, should also be more likely than affected males to transmit these risk alleles

Box 1 | **Sexual dimorphism, sex differences and examples**



The term sexual dimorphism has been widely misused to describe not only two distinct forms of a phenotype but also sex differences. Traditionally, sexual dimorphism (see the figure, part **a**), meaning having two forms, has been used to describe two distinct and non-overlapping traits of males and females from the same species. In animals, sexually dimorphic traits may include ornamentation, coloration, size and behaviour. In humans, sexually dimorphic anatomical features include gonads, internal and external genitals, and breasts. However, beyond these specific examples where two forms can be fully differentiated, a multitude of other sex differences exist on a spectrum (see the figure, part **b**). Thus, we encourage researchers to adopt the term 'sex differences' in reference to overlapping but shifted phenotypic distributions present in both males and females (see the figure, part **b**) and to reserve 'sexual dimorphism' for the description of two distinct and fully differentiated forms of a trait (see the figure, part **a**). Sex differences in humans exist both in physiological traits (for example, height, hormone levels and immune cell composition) and in disease.

Sex differences in complex traits and diseases can vary in presentation and change over time. Specifically, there may be sex differences in incidence or prevalence; age of onset; clinical presentation and diagnostic criteria; disease severity, progression, prognosis and outcome; susceptibility; response to treatment; and pharmacological adverse events. Some prominent examples are as follows:

- Women have a lower incidence of cardiovascular disease, such as stroke, before menopause, after which the incidence surpasses that of men[54].
- The age of onset for asthma peaks in boys between ages 2–8 years. However, in adults, the incidence is higher in women[52,53].
- Clinical presentation of obsessive–compulsive disorder varies between males and females[194].
- Differences in the severity and symptoms have been reported for males and females with schizophrenia[26], obsessive–compulsive disorder[195,196], Tourette syndrome[197] and autism spectrum disorders[198].
- Males have increased susceptibility to disorders arising from mutations on the sex chromosomes, as exemplified by X-linked cardiomyopathies and muscular dystrophies[199].
- Pharmacokinetics differ between males and females for most drugs[188], and failure to investigate these differences has led to potentially dangerous dosing guidelines for women, as in the case of zolpidem (Ambien).
- Women have a higher rate and severity of adverse drug reactions[185–187].

to their offspring. Furthermore, if a greater genetic liability was required for females to manifest the disease, then, among females, the genetic variance should also account for more of the phenotypic variance. In other words, the heritability of the trait should also be higher in the sex with the lower prevalence[13,14]. Although Carter's original observation of the protective effect was in females, this effect need not be specific to females but in fact may occur in either sex with the lower disease prevalence. This phenomenon is often referred to as the female (or male) protective effect, the Carter effect or the sex-dependent liability threshold (BOX 4).

Under the model of a female protective effect, siblings of female probands are also more likely than siblings of male probands to be affected themselves. In other words, the sibling recurrence risk is higher for siblings of female probands than for siblings of male probands. For example, siblings of female probands with neurodevelopmental disorders (such as autism spectrum disorder[15] or attention deficit and hyperactivity disorder (ADHD)[16,17]) are more likely than siblings of male probands with neurodevelopmental disorders to be diagnosed themselves. This suggests that females may indeed experience some protection against these early-onset neurodevelopmental disorders. Studies also report that siblings of female probands show significantly higher rates of ADHD and autism spectrum disorder symptomatology (not only diagnosis) than siblings of male probands[15,17]. Similarly, males with idiopathic scoliosis or multiple sclerosis, two disorders more commonly diagnosed in females, demonstrate the same pattern predicted by a male protective effect[18,19].

Despite evidence of protective genetic effects observed in both sexes, several well-powered investigations of sex-dependent liability in humans suggest that heritability estimates for males and females are largely similar for most traits. A recent large-scale epidemiological study by Ge et al.[20] examined sex differences in both trait prevalence and heritability estimates across hundreds of phenotypes in the UK Biobank ($N \approx 150{,}000$). In phenome-wide analyses of sex differences across human health conditions, of 551 traits for which sex-specific heritability could be estimated with reasonable power, only 14 (2.5%) exhibited significantly different male and female heritabilities[20]. Within the United States, large-scale analyses of electronic health and insurance records are also underway. For example, Wang and colleagues[21] used insurance claim data to reconstruct 128,898 families and estimate the proportion of trait variance explained by genetic and environmental variables.

Sexual dimorphism
Two distinct forms of a trait that differentiate members of the same species by their sex.

Heritability
The proportion of the total phenotypic variance in a population that can be attributed to genetic variance in the population.

Box 2 | **Challenges and recent methodological advances for studying sex as a biological variable**

The major challenges for studying sex differences include adequate sample sizes for well-powered analyses of each sex and a lack of appropriate experimental and analytical approaches. For example, in genome-wide association studies (GWAS), only recently have study cohorts reached sufficient sample sizes to be well-powered for sex-stratified and interaction analysis. However, for a majority of GWAS performed to date, there may be challenges to retrospectively analysing available cohorts, as those studies were not specifically designed for this purpose. For traits and disorders with a large difference in prevalence between the sexes, splitting the currently available cohorts by sex or including an interaction term would result in an underpowered analysis as well as potentially different ascertainment between the sexes. In the future, recruitment of cohorts for GWAS should be performed in a sex-aware manner to ensure sufficient statistical power to assess sex differences.

The sex chromosomes contribute to the genetic basis of some sexually differentiated phenotypes but have historically been excluded from GWAS, mainly owing to the lack of statistical approaches to analyse the haploid Y chromosome (ChrY) and to account for dosage compensation and inactivation of the X chromosome (ChrX). Recently, statistical methods and analytical packages have been developed for ChrX phasing, imputation, quality control and analysis; for example, the XWAS tool[3] and XYalign[9], along with ChrX genotype data, have been added to reference panels such as the 1,000 Genomes Project[200] and the Haplotype Reference Consortium[201]. Tools are still lacking for analysis of ChrY in GWAS. In the meantime, association of ChrY haplogroups with disease traits may improve our understanding of the contribution of this sex chromosome to the risk of disease.

Technical challenges and considerations for analysis of the sex chromosomes include the following:

Genotyping. The sex chromosomes exhibit lower genotyping accuracy than autosomes, owing to both the shared origin[202] and complex history of ChrX and ChrY, which present unique challenges for genome assembly and analysis[9], as well as to the overall lower intensity of ChrX signal in males for array-based genotyping. Studies have demonstrated that genotype clustering algorithms that account for sex result in more accurate genotyping than those that do not[203]. The male-to-female ratio in cases and controls also affects single-nucleotide polymorphism (SNP) quality control, as the accuracy is affected by sample size. Additional challenges for ChrX genotyping include the higher frequency of ChrX anomalies than in autosomes, as well as historical underrepresentation of ChrX SNPs on genotyping arrays[4].

X chromosome inactivation. It is not yet possible using standard sequencing technologies to discern which genetic variants are on the silenced version of ChrX[6], thus complicating interpretation.

Power. Power for association analysis depends on both sample size and male-to-female ratio in cases and controls[204] and is affected by sex differences in genotyping accuracy.

Population substructure. Sex-biased demographic events can result in different population structure on ChrX and the autosomes and thus result in differential population stratification. Population structure of ChrX needs to be considered to accurately correct for population stratification in genetic association study of ChrX, particularly in the case of admixed populations[41].

Best practices and tools. Despite the challenges described, approaches have been developed to facilitate genetic analysis of the sex chromosomes, including tools to facilitate sex chromosome alignment and quality control[9], sex-specific SNP quality control and genotype imputation[41], as well as a variety of association algorithms that are specific for ChrX[7,205,206].

Transcriptome analysis. RNA sequencing (RNA-seq) and microarrays have different sensitivity to lowly expressed transcripts[207], which has an impact on the characterization of ChrX transcripts. Additionally, filters based on expression of all genes (autosomes and sex chromosomes) disproportionately exclude ChrX transcripts and can influence downstream results and conclusions[208]. Importantly, RNA-seq data can be affected by ChrX and ChrY homology because of their shared history, which can affect mapping and variant calling. The XYalign framework[9] can be applied to RNA-seq data to appropriately account for the specific characteristics of sex chromosome transcriptomes. It is worth noting that this is a newly released framework; thus, previously published and released RNA-seq data from the sex chromosomes have not been analysed with this toolset.

Resources such as these provide an opportunity to estimate sex-stratified heritability, as has been done by Ge et al.[20], and compare whether those estimates are stable across populations, health-care systems, and ascertainment methods. Similarly to Ge et al., in an independent meta-analysis of 2,335,920 twin pairs, only 1% of 2,608 surveyed traits demonstrated significant sex differences in heritability[22,23]. Traglia et al.[25] also found no evidence of sex differences in heritability due to common genotyped single-nucleotide polymorphisms (SNPs) (that is, SNP-based heritability) for nine phenotypes collected by the Wellcome Trust Case–Control Consortium. In addition, the genome-wide genetic correlations between males and females, for many traits, are not significantly different from one[24,25]. Consistent with this observation, Vink and colleagues[26] found evidence for differing genetic architecture (that is, different frequency or effect size of trait-associated genetic variants in males and females) in only 4% of 122 complex traits examined with a twin design that compared same-sex dizygotic twins to opposite-sex twin pairs. However, notable exceptions to this trend include post-traumatic stress disorder (PTSD), rheumatoid arthritis, hypertension, hay fever (allergic rhinitis) and self-reported miserableness[20,27,28]. Interestingly, for phenotypes exhibiting sex differences in heritability estimates, females consistently tend to show higher heritability than males, with the exception of self-reported miserableness[20] (FIG. 2a,b). A recent network analysis of 22.1 million electronic health records also showed that females have a greater

Genetic architecture
The number, allele frequency and effect size of genetic variants that influence a trait.

Box 3 | **Definitions of sex and gender**

Sex or sex assigned at birth

For the majority of births, a physician, nurse, midwife, doula or other person assisting in the birth examines the genitals of the neonate and assigns male sex or female sex based on this observation. Typically, sex is treated as a binary trait, but exceptions occur in the case of intersex individuals, who may be born with varying presentations of male and female genitalia. Occasionally, sex may be determined by examining the sex chromosomes. Females typically present with two X chromosomes, while males present with one X and one Y chromosome. However, again, variation in the number of X and Y chromosomes is not uncommon.

Gender

The umbrella term 'gender' is often used to refer to distinct concepts including gender identity, gender expression, gender roles and gender stereotypes. It is most commonly meant to refer to gender identity, which the American Psychological Association refers to as "a person's deeply felt, inherent sense of being a boy, a man, or a male; a girl, a woman, or a female; or an alternative gender (for example, genderqueer, gender nonconforming, gender neutral) that may or may not correspond to a person's sex assigned at birth or to a person's primary or secondary sex characteristics". Evidence suggests that gender identity is a complex multifactorial trait, independent from sex assigned at birth, influenced by both environment and polygenic factors (for a thorough review, see REF. [209]). Gender expression refers to the ways in which gender identity is expressed in society and is thought to be primarily driven by the social expectations of gender within a society. Gender roles and stereotypes are again social constructs and refer to the set of behaviours that are culturally expected of males and females.

number of comorbidities and stronger correlations (t value = 12.67, $P < 0.0001$) between disease diagnoses than do males in a hospital setting[29], which might be consistent with greater genetic liability in females for certain conditions.

Several hypotheses offer to explain the apparent paradox between evidence of a female (or male) protective effect in epidemiological studies and the lack of sex differences observed in SNP-based heritability studies. One possibility is that population or sampling differences may impact studies of sex differences. We undertook a simple comparative analysis of supplementary data from the UK Biobank study of Ge et al.[20] and data from the insurance claim study of Wang et al.[21] to determine the similarity of prevalence estimates across different populations, health-care systems and ascertainment strategies. FIGURE 2c shows that female-to-male prevalence ratios of 239 traits present in both data sets were extremely similar ($r = 0.95$) between the two studies after removing a single outlier, indicating that the sex differences in the prevalence of disease traits are largely stable. In addition, comparison of the sex difference in trait prevalence to the sex difference in trait heritability shows no significant correlation (FIG. 2d,e), again corroborating earlier observations[25]. Under this model, very large differences in prevalence may exist with indistinguishable differences in heritability. Others have suggested that genetic risk may manifest in different symptoms or be diagnosed differently in males than in females and that this could drive ascertainment differences that may also influence heritability estimates[30]. Still others hypothesize that sex-differentiated environmental factors may contribute differently to the phenotypic variance observed in males and females[20,31]. This may be more complex than the simple differential effects of a sex-specific environmental risk factor and may also include sex-biased genotype–environment correlations (BOX 4). Clearly, this is an area of research with many opportunities for hypothesis testing and discovery. We believe the field will benefit from improved power (that is, larger sample sizes) and comparative analyses across multiple ascertainment strategies (for example, registries, biobanks and cohort collections) that include symptom-level data as well as diagnostic information.

Model 2: sex chromosome effects. In humans, biological sex is genetically defined by the sex chromosomes, typically XX for females and XY for males; however, aneuploidies of the sex chromosomes are also common. The sex chromosomes primarily determine the sexual differentiation of gonads (ovaries in females and testes in males) and the expression of sex steroid hormones. Sex hormone expression is one of the drivers of sexual differentiation at the molecular level (see the 'Mechanisms' section below) and the phenotypic level. In addition to determining the hormonal milieu within each cell, regions outside the pseudoautosomal regions (PARs) of sex chromosomes exhibit unique properties impacting phenotypic sex differences arising from sex chromosome effects. These properties include different dosage of non-PAR X chromosome (ChrX) genes (one copy in males versus two copies in females); parental imprinting of non-PAR ChrX genes; the presence, absence or skewing of ChrX inactivation; and the presence or absence of non-PAR Y chromosome (ChrY) genes. The contributions of these sex chromosome effects are briefly outlined below, while REFS[10–12] provide a more comprehensive review on this topic.

ChrX genes outside of the pseudoautosomal region have one copy silenced in females to ensure dosage compensation of the hemizygous X-linked genes in males. This process of X chromosome inactivation (XCI) results in approximately half of the female cells expressing only the genes from the maternally inherited ChrX and half expressing only the genes from the paternally inherited ChrX. Thus, whereas males who have one copy of ChrX and ChrY are highly susceptible to the impact of mutations and deletions on these chromosomes (for example, haemophilia A (*FVIII* gene), Duchenne muscular dystrophy (*DMD* gene), Rett syndrome (*MECP2* gene), fragile X syndrome (*FMR1* gene), red–green colour blindness and male-pattern baldness), females who carry a deleterious allele would not be affected by ChrX mutations if the expression is sufficiently balanced by a functional allele on the second X chromosome that is expressed in half of the cells. However, when recessive mutations and deletions in regions subject to XCI or ChrX imprinting cannot be sufficiently buffered, they can lead to disorders and altered phenotypes in females[32]. In humans, escape from XCI is known to result in sex-biased gene expression, and incomplete XCI is estimated to affect approximately 23% of ChrX genes[33,34]. This partial biallelic expression of ChrX genes in females may lead to sex differences in gene expression that are subtler than the above effects of heterozygous versus hemizygous X-linked single-gene mutants and may contribute to polygenic complex disease. However, the contribution of sex chromosomes to non-syndromic complex traits has been less well characterized.

Pseudoautosomal regions
(PARs). Homologous regions on the X and Y chromosomes that recombine and are not inherited in a sex-dependent manner.

Imprinting
An epigenetic mechanism of transcriptional silencing of a gene in a gamete inherited from the mother or the father, leading to a parent-of-origin specific imbalance in gene expression of the two inherited copies.

Dosage compensation
A process by which gene expression is balanced between two members of the same species (typically between two biological sexes). In humans, this is accomplished by silencing of one of the copies of the X chromosome in females.

Hemizygous
A haploid zygosity state in which only one copy of a gene is present, such as Y chromosome genes, which do not recombine with the X chromosome

X chromosome inactivation
(XCI). A process by which one of the copies of an X chromosome is silenced in each female cell through epigenetic modification, such as DNA methylation.

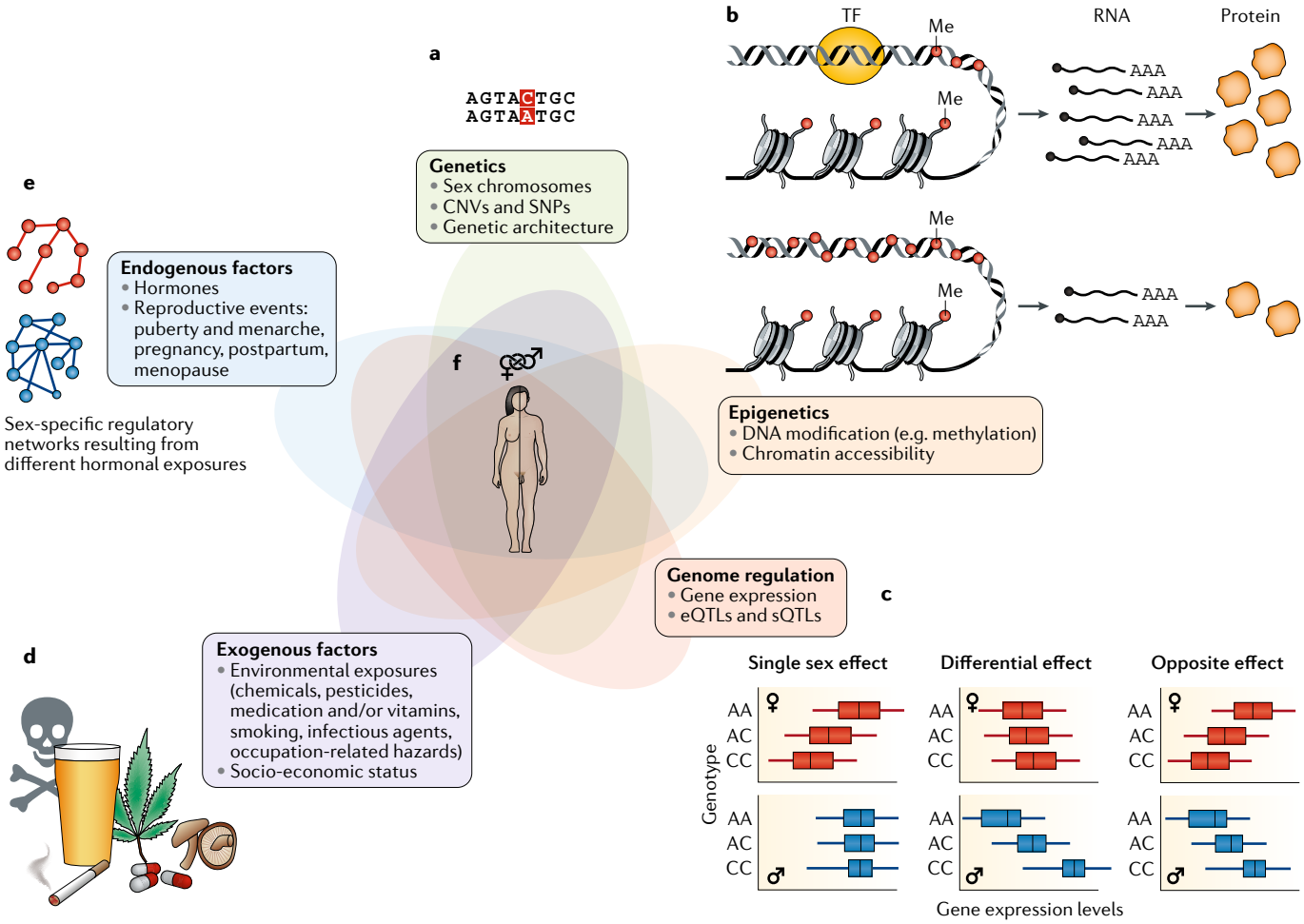


Fig. 1 | **Factors contributing to phenotypic sex differences.** Disease and non-disease human traits are shaped by the combined effects of an individual's genome, the environment and the interaction between the two. **a** | The genetic components that contribute to heritable phenotypes include sex chromosomes, genetic variation (single-nucleotide polymorphisms (SNPs) and copy number variants (CNVs)) and de novo mutations. Sex differences at the DNA sequence level primarily consist of the sex chromosomes. Indeed, with the exception of a female bias for large, rare CNVs, there are no large sex differences in SNP minor allele frequencies. **b,c** | Sex differences can exist in DNA accessibility and methylation status, as well as in levels and patterns of gene expression. Epigenetic variation and transcriptome regulation, which can be altered by both endogenous and exogenous environmental factors, form endophenotypes between an individual's genetic sequence and phenotype. In the illustrative example shown in part **b**, promoter DNA hypomethylation in one sex allows transcription factor (TF) binding to promote transcription, whereas the DNA in the other sex is hypermethylated, thus repressing transcription. Part **c** shows how the effect of genotype on gene expression can vary between males and females. **d,e** | Exogenous factors such as environmental exposures and occupation-related hazards (part **d**) and endogenous factors such as hormones and reproductive events (part **e**) influence molecular phenotypes that may ultimately contribute to sex differences in higher-order phenotypes. As an example in part **e**, sex-specific hormone exposure can lead to sex-biased gene expression and formation of sex-specific regulatory networks. **f** | As depicted in the Venn diagram, any one factor or a combination of factors may contribute to sex differences. eQTLs, expression quantitative trait loci; sQTLs, splicing quantitative trait loci.

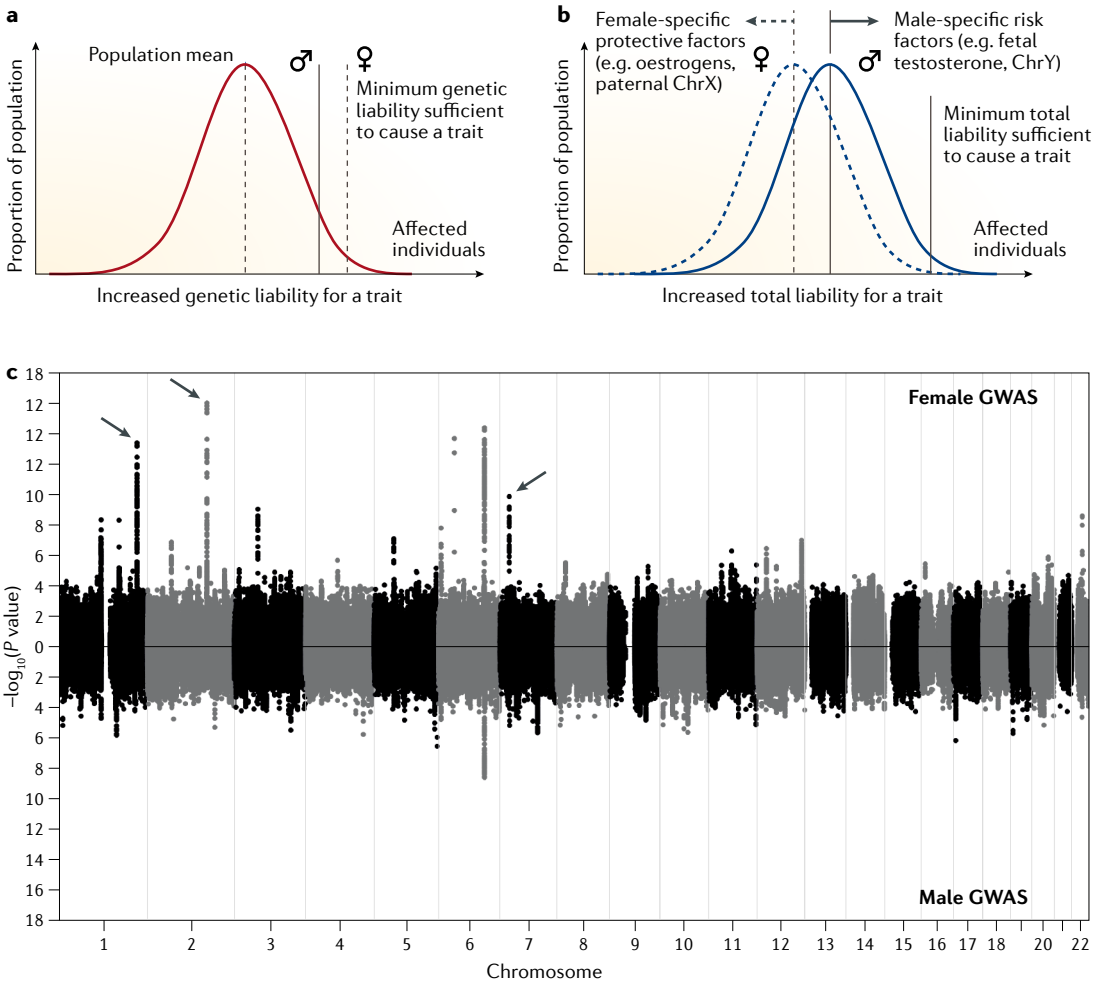
Furthermore, in humans, sex chromosome aneuploidy is known to influence gene expression[35] and disease phenotypes, including Turner Syndrome[36] and Klinefelter syndrome[37]. Historically, it has been challenging to assess the isolated effects of sex chromosomes owing to confounding factors of the gonadal hormones. However, model systems are available, including gonadless mice (for example, *Sf1* knockout) and 'four core genotypes' mice (XX mice with ovaries or testes and XY mice with ovaries or testes). Although not without caveats, these model systems facilitate disentangling the effects of the sex chromosomes from those of hormones and prioritizing the genes contributing to those effects, as reviewed in REF.[38].

The vast majority of published genome-wide association studies (GWAS) of complex traits exclude the sex chromosomes. This exclusion is in part due to the paucity of analytical approaches specifically designed to account for the unique characteristics of the sex chromosomes, including but not limited to the haploid nature of ChrY, unequal numbers of ChrX in males and females, the shared history of ChrX and ChrY, and XCI (BOX 2). Before the development of analytical tools and approaches specific to genetic analysis of the sex

Sex-biased gene expression
A term that encompasses various gene regulatory phenomena that may differ between sexes, including differential expression and differential splicing.

Aneuploidy
Abnormal number of chromosomes in a cell.

Box 4 | **Liability threshold model and sex-specific genetic architecture**



A sex-differentiated trait may be explained by multiple models that consider both genetics and environment. These may include a sex-dependent liability threshold (see the figure, part **a**), a multifactorial model accounting for sex or gender differences in environmental exposures (see the figure, part **b**) or sex differences in genetic architecture (see the figure, part **c**). The sex-dependent liability threshold model and multifactorial model are characterized by sex differences in the proportion of genotypic and environmental contributions to phenotypic variation. Part **a** of the figure illustrates the multi-threshold model, in which genetic liability for a trait is normally distributed in the population, and the minimum genetic liability that is sufficient to cross the threshold for diagnosis differs between sexes[210,211]. In this scenario, heritability is expected to be higher in females, as they require more trait-associated or disease-risk alleles to develop a trait or a disease. In the multifactorial model illustrated in part **b**, female-specific genetic and environmental factors shift females' total liability distribution away from — and male-specific factors shift males' distribution towards — the diagnostic threshold (part **b**). In this scenario, the heritability can be the same across sexes, as the environmental component varies.

Furthermore, even where males and females exhibit equivalent heritability for a trait, the underlying genetic architecture may vary between sexes (part **c**). From genome-wide association studies (GWAS) data for hip circumference adjusted for body mass index, a Miami plot for sex-stratified results (male results shown at the top and female results at the bottom) demonstrates that a genetic association with a trait may be sex-specific (arrows)[80]. Finally, even when the heritability and genetic architecture are the same across the sexes, investigation of the genetic contribution to trait variance can be used to highlight important environmental contributions to sex differences.

ChrX, X chromosome; ChrY, Y chromosome. Panels in parts **a** and **b** are adapted with permission of Wolters Kluwer, from: Sex differences in autism spectrum disorders, Werling, D. M. & Geschwind, D. H., *Curr. Opin. Neurol.* **26**, 146–153 (2013), REF.[210]; permission conveyed through Copyright Clearance Center, Inc. Data in part **c** are from REF.[80].

chromosomes (for example, ChrX-wide association studies (XWAS) and others)[3,7,9], the few studies analysing ChrX in GWAS utilized approaches designed for the autosomes[4]. This exclusion and use of sex-agnostic analytical tools and association models have persisted in whole-genome and whole-exome studies of complex disease[6]. Reanalysis with tailored approaches is warranted for such studies, as well as those that did not originally analyse ChrX. These endeavours have potential to be impactful; several recent studies demonstrated the importance of including ChrX in GWAS and gene-based approaches by discovering novel

Total liability
The combination of genetic and environmental factors that contribute to the development of a complex trait.

Missing heritability
The observation that for most complex traits, the sum of the identified trait-associated genetic variation contributes only a proportion of the estimated trait heritability.

Sexually differentiated
(Also known as sex-specific or sex-biased). A term used to describe a phenotype exhibiting a quantitative or qualitative sex difference.

ChrY haplogroups
Groups of haplotypes that map to the same common ancestor on the patriline.

Interaction
A phenomenon in which the effect of one variable depends on the value of another variable (for example, gene-by-environment interaction).

Type II error
A false negative finding, that is, a failure to reject a false null hypothesis.

Copy number variants
(CNVs). Regions of the genome that may be duplicated or deleted and for which the number of copies vary between individuals.

Sexually antagonistic selection
A situation in which selection on an allele acts in opposite directions in males and females because opposite phenotypes associated with the allele are optimal in each sex.

Genetic correlation
An estimate of the proportion of genetic variance shared by two traits, measured from 0 to 1, with 1 indicating complete genetic correlation.

Anthropometric traits
Physical properties of the human body including but not limited to secondary sex characteristics such as height, waist and hip measurements.

trait-associated and disease-associated loci[39–42] (FIG. 3) and by demonstrating that variants on ChrX contribute to the 'missing heritability' of some complex traits[43]. Careful analysis of ChrX will be essential for understanding sexually differentiated traits. Beyond statistical methods for association analysis, there is also a need for method development in genotyping, variant calling and transcriptome analysis of sex chromosomes (BOX 2). To date, no genome-wide associations have been reported on ChrY; however, several studies have reported that ChrY haplogroups contribute to disease phenotypes in both humans and mice[44–48].

Model 3: gene-by-environment interactions. Sex is both an important biological factor and an important environmental factor, as it has a direct impact on the endogenous environment (for example, the cellular environment in which genes are transcribed and translated into protein) and influences exposure to exogenous factors (for example, contraceptive use). Additionally, gender moderates multiple environmental exposures, including occupation-related hazards, stress and smoking[49–51]. The direct action of hormones throughout the lifespan is one mechanism by which a sex-dependent cellular environment may impact health. In females, oestrogen levels vary with the phase of the menstrual cycle, are high during pregnancy and are low after menopause. It has been noted that life stages marked by changes in hormone levels (for example, puberty, pregnancy, postpartum and menopause) are inflexion points for risk of some diseases. For example, the incidence of asthma peaks early in boys; however, after puberty, the incidence in women is double that of men[52,53]. Women have lower incidence of cardiovascular disease, such as stroke, before menopause, after which the incidence of stroke surpasses that of men[54].

Furthermore, reproductive events accompanied by hormonal changes are known to exacerbate or even lead to onset of some phenotypes including asthma[55,56], obsessive–compulsive disorder[57,58] and depression[59,60]. Moreover, sex steroid hormones (for example, oestradiol, progesterone and androgens) regulate components of innate and adaptive immunity[61] and thus are critical contributors to some immune-mediated diseases. On the basis of evidence from patients with asthma and in vivo mouse experiments, a recent study suggests that increases in testosterone levels in males during puberty protect against asthma by reducing the number of group 2 innate lymphoid cells and the cytokines these cells produce[62]. One possible explanation for this observation is that hormones influence gene regulatory mechanisms, as described in the 'Mechanisms' section below.

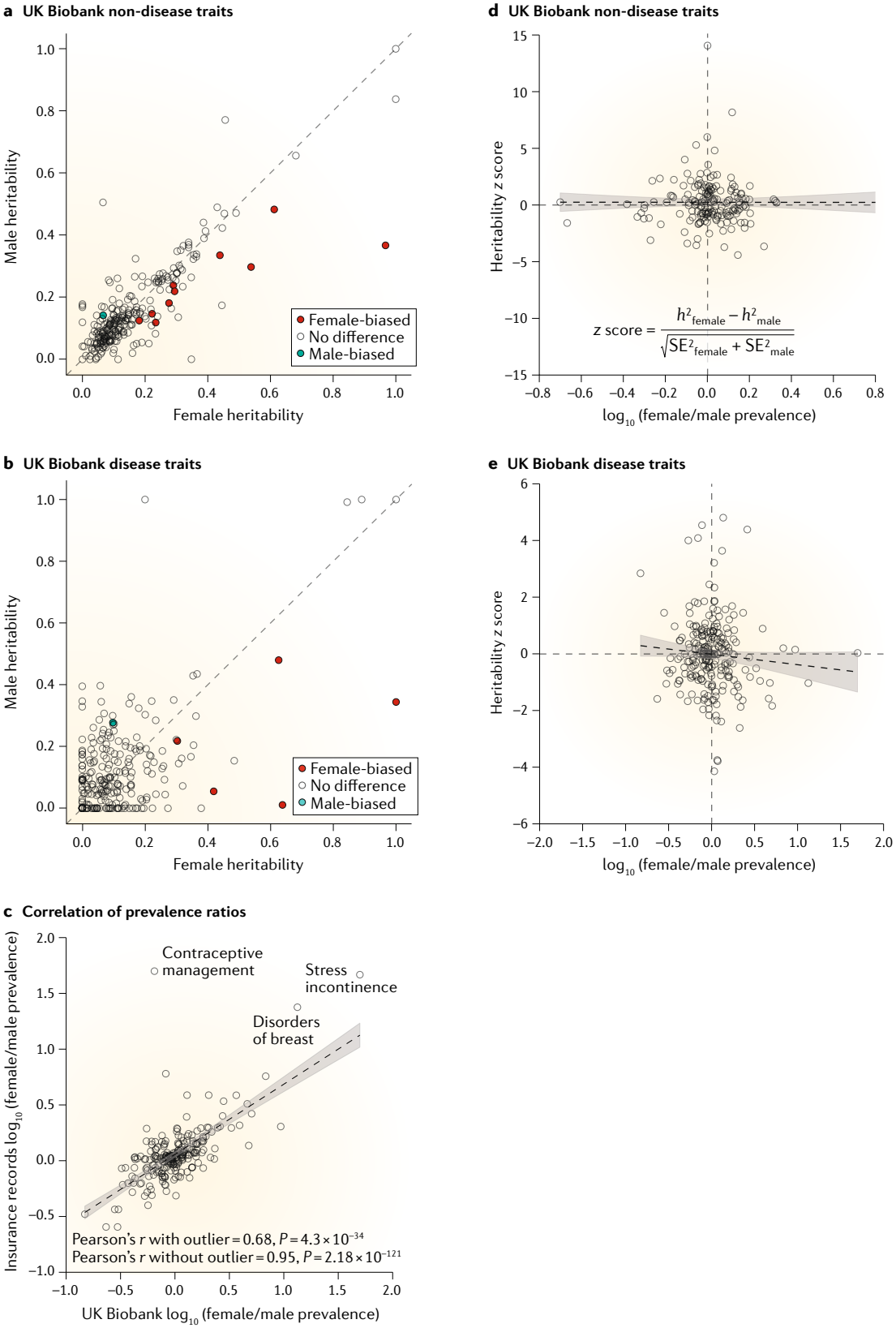
Evidence from genome-wide association studies

GWAS can be used to investigate sex-differentiated genetic effects at individual loci. For example, statistical tests of interaction can be employed to determine whether sex (or variables correlated with sex, such as hormones) alters the effect of genotype on phenotype. This approach assesses whether there is a difference between the sexes in the effect of genotype on phenotype and is distinct from a sex-stratified analysis, which can identify genetic associations within a single sex. Gene-by-sex interaction tests are prone to type II error because very large sample sizes are required to detect differences between two non-zero effect estimates[63]. Nevertheless, some well-powered studies demonstrate a lack of widespread evidence for gene-by-sex interactions in mice[64], whereas others focused on specific phenotypes report significant gene-by-sex interactions[65–69]. Several recent reports in humans are described below.

In a unique study, Boraska and colleagues[70] set out to identify alleles that differ in frequency between males and females and may contribute to the slight imbalance in male-to-female (1.06) birth ratios. Their GWAS, in which males and females were compared instead of the more typical case-control design, was well powered ($N = 114{,}863$) to identify variants that are moderately differentiated between the sexes (odds ratios >1.13). They identified no significant associations, suggesting that common (that is, older) genetic variation observed in both sexes with at least a 5% minor allele frequency is not strongly sexually differentiated. These findings have important implications for the design of common-variant discovery studies. For instance, studies searching for common alleles contributing to sexually differentiated traits, such as breast cancer or anorexia (that is, with few affected males), may not need to restrict to female-only controls. By contrast, studies of copy number variants (CNVs) demonstrate that females carry significantly more large, rare CNVs and that within those CNVs, there are a greater number of affected genes[71,72]. This suggests that the frequency of younger genetic variation, perhaps with larger effect sizes on fitness traits, may be subject to sexually antagonistic selection, as has been demonstrated in model organisms[73–76] and is further described in REF.[77], but with limited evidence to date in humans.

In the absence of allele frequency differences, loci with sex-biased effects on phenotypes may also give rise to sex differences in the genetic architecture of complex traits. For example, it is possible for heritability estimates to be the same in both sexes even when the genetic correlation between them is less than one, which would suggest that different loci contribute to the same total heritability in each sex[22]. Sex-stratified GWAS can be used to discover the effects of individual variants within each sex and to identify novel loci that may have been previously undetected in sex-combined GWAS owing to the heterogeneity of SNP effects between sexes[78–81]. These strategies have been particularly successful in studies of anthropometric traits and blood-based biomarkers (lipid levels and white blood cell counts), in which sex-stratified GWAS, particularly in females, have yielded novel significant associations. These results raise the question of whether anthropometric traits and blood biomarkers are unique among complex traits in their sex-specific architecture or whether sex differences in genetic architecture will be discovered for additional traits when future studies approach similar sample size.

By way of illustration, Randall and colleagues[80] identified seven loci with genome-wide significant associations to anthropometric traits (that is, waist-to-hip ratio adjusted for body mass index (BMI) and



waist circumference adjusted for BMI) in females that exhibited no association in males. Although this study reported no SNPs with opposite effects in males and females and no male-specific associations, a meta-analysis including >320,000 individuals discovered 44 loci with significant sex-specific effects, including 11 variants with opposite effects on waist-to-hip ratio and 5 variants with larger effect sizes in males than in females[82]. A more recent meta-analysis of >690,000 individuals reported that both heritability was higher and

◀ Fig. 2 | **Epidemiological insights into sex-biased disease prevalence and heritability from biobank and insurance claims data. a,b** | Sex-stratified heritability (h^2) estimates for 269 non-disease traits (part **a**) and 282 disease traits from the UK Biobank[20] (part **b**). Phenotypes exhibiting a significant difference in heritability estimates between sexes after multiple testing correction are shown as coloured data points; red are female-biased, and cyan are male-biased. **c** | The graph shows correlations of disease prevalence for 239 complex traits from the UK Biobank[20], ascertained by questionnaire (*x* axis), versus prevalence determined from insurance claims records in the United States from 128,898 families (*y* axis)[21]. With the exception of a single outlier, contraceptive management, the correlation between estimates from each of the two data sets is very high, Pearson's $r = 0.95$, $P = 2.18 \times 10^{-121}$. **d,e** | For 173 non-disease traits and 245 disease traits shown in part **a** and part **b**, prevalence in each sex was also determined. Plots (non-disease traits in part **d** and disease traits from the UK Biobank in part **e**) of phenotypic variance between sexes as measured by $\log_{10}$(female/male prevalence) versus heritability *z* score (calculated as shown in part **d**) demonstrate lack of correlation between the difference in prevalence between sexes and heritability estimates. This indicates that for the majority of traits with sex differences in prevalence, a sex-dependent liability threshold model is not the driving mechanism. For further information on the data analysis to generate this figure, see Supplementary information. SE, standard error of heritability estimates.

variant effects were generally stronger in women than in men and that approximately one-third of all genetic association signals were sexually differentiated[83]. Two follow-up studies reported that the genome-wide genetic correlation for anthropometric traits between males and females is significantly less than one for height, BMI, waist circumference, hip circumference and waist-to-hip ratio, providing further evidence for sex differences in the genetic architecture of these traits[24,25]. These studies, together with the aforementioned UK Biobank heritability studies, indicate that gene-by-sex interactions exist for some complex traits and that sexually antagonistic selection may be acting on anthropometric phenotypes.

In addition to the discovery of fundamental biology that may differ between males and females, these findings demonstrate the importance of sufficient statistical power for detecting robust modifying effects of sex on the relationship between genotype and phenotype. Similar results have been observed in sex-stratified GWAS of coronary artery disease[84,85], Crohn's disease[84,86], type 1 and type 2 diabetes[86], rheumatoid arthritis[87], childhood acute lymphoblastic leukaemia[88] and autism spectrum disorders[78]. It is interesting to note that, consistent with heritability studies, most sex-specific genome-wide associations discovered to date are female-specific.

Pleiotropy can influence phenotypes in a sex-dependent manner. Recent analyses of genetic correlation between human complex traits[89–91] indicate that, in aggregate, some pairs of traits exhibit a high degree of sharing of genome-wide genetic effects. For example, the genetic correlation between schizophrenia and bipolar disorder is 0.79 (standard error (SE) = 0.04, $P = 7.45 \times 10^{-94}$), and that between adult BMI and childhood obesity is 0.73 (SE = 0.05, $P = 2.95 \times 10^{-57}$)[89]. Pleiotropy, one possible explanation for these observations, has been identified for several metabolic traits[92,93] and psychiatric conditions[94]. Recent studies suggest that pleiotropic effects can also act in a sex-dependent manner. Among the top genetic associations from a female-only autism GWAS, Mitra et al.[78] identified an enrichment of variants previously shown to exhibit large differences in effect size between male-specific and female-specific GWAS for anthropometric traits[80]. Similarly, top variants associated with endometriosis were found to be enriched for significant associations from female-only GWAS for waist-to-hip ratio adjusted for BMI[95]. These findings suggest that the same genetic factors contributing to differences in anthropometric traits may also contribute to sex differences in several other complex traits.

Support for genetic models of sex differences. Evidence exists in support of each of the described models; however, because comprehensive testing of all models has been applied to only a few phenotypes[25,30,78,96], it is difficult to assess the extent to which each model contributes to the genetics of sex-differentiated phenotypes. Despite this, it is clear from heritability studies that the genetic basis of complex traits differs between males and females in only a small proportion (less than 5%) of phenotypes[20,22,23]. Most studies are still underpowered to detect small differences in the magnitude (or even direction) of individual gene or genetic variant effects that may also contribute to disease risk differences. Investigation of the sex chromosomes has yielded some associations with disease but alone is unlikely to explain a large proportion of the differences between males and females for highly polygenic traits. Gene-by-environment interactions, which encompass sex differences in the cellular environment as well as the external environment, may be more likely to contribute to observed sex differences. These types of sex-differentiated gene-by-environment interactions can be tested at the individual variant or gene level to provide insight into sex-differentiated disease mechanisms. Furthermore, studies at the level of polygenic risk scores hold promise for the detection of population-level risk factors that are moderated by sex.

Mechanisms

As described above, the combined effects of genetics, hormones and response to the cellular and external environment can contribute to sexually differentiated traits. These factors mediate their effects through molecular pathways. Indeed, sex differences at the molecular level are common, and characterization of these differences in endophenotypes such as the transcriptome and epigenome has provided valuable insights into causal mechanisms and altered biological functions and pathways.

Sex differences in genome function and regulation. Sex-biased gene expression has both quantitative and qualitative components. It is a fundamental characteristic that is common across species[97–99]. In humans, genes demonstrate sex-biased expression within and between tissues[33,100–107], in primary cells[108], in cell lines[109–111], across developmental stages[112], in the context of disease[113–115] and under different environmental conditions[116,117]. Several common themes emerge from this work. First, sex-biased gene expression is a common characteristic of genes encoded both on the sex chromosomes and on the autosomes, with ChrX enriched for sex differentially expressed (sex-DE) genes. Second, fold-change between male and female expression levels is typically small for significant sex-DE genes. Third, genes

Pleiotropy
A phenomenon in which a single gene or genetic variant influences more than one phenotype.

Endophenotypes
Intermediate measurable phenotypes between an individual's genotype and a phenotype, for example, characteristics of the transcriptome.

Sex differentially expressed
(sex-DE). A situation in which mean mRNA levels of a gene differ between tissues or cells derived from males or females.

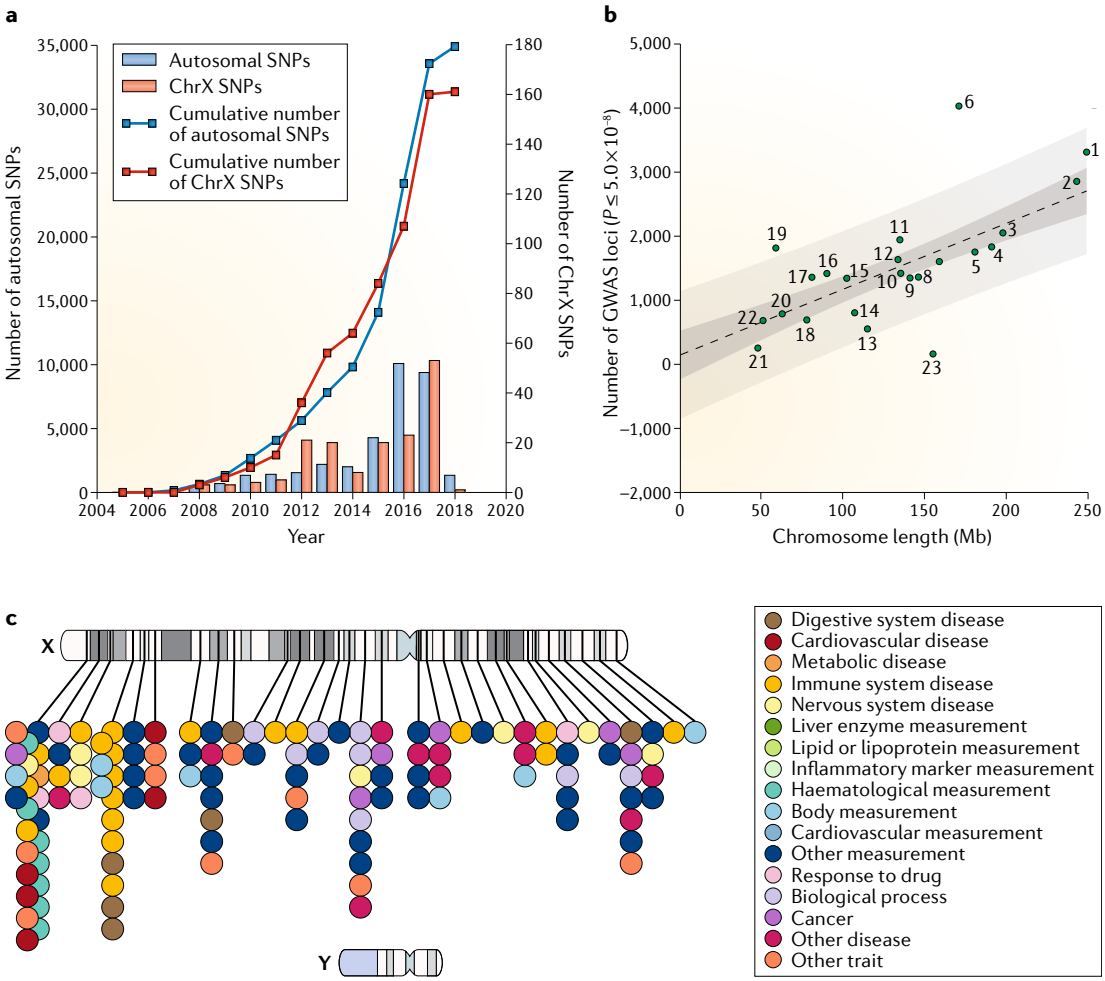


Fig. 3 | **GWAS loci identified on autosomes and the X chromosome. a** | Genome-wide association study (GWAS) single-nucleotide polymorphism (SNP)–trait associations with $P \leq 5.0 \times 10^{-8}$ on the autosomes (left *y*-axis scale) and the X chromosome (ChrX) (right *y*-axis scale) over time (published in the US National Human Genome Research Institute (NHGRI)–European Bioinformatics Institute (EBI) GWAS Catalogue, accessed 4 May 2018)[39]. The first significant ChrX association was reported in June 2008. The number of reported ChrX loci has increased over time but at a slower rate than for autosomes owing to the number of autosomes (see the *y*-axis scale difference). **b** | Scatter plot of chromosome length (*x* axis) versus number of significant GWAS loci (*y* axis). Each dot is labelled with the chromosome number. The dashed line represents the linear regression line. The darker grey area represents the 95% confidence interval (CI) for the regression, whereas the lighter grey area represents the prediction interval, indicating that there is a 95% probability that the real value for the number of loci for a given chromosome length lies within the prediction interval. Chromosome 6 is an outlier owing to overrepresentation of associations in the major histocompatibility complex (MHC) region. ChrX (number 23) is an outlier owing to the small number of loci identified to date. Regression and CI are estimated excluding outliers. **c** | NHGRI–EBI GWAS Catalogue schematic of ChrX trait-associated loci, colour coded by phenotype. No genome-wide associations have been reported on the Y chromosome (ChrY). For further information on the data analysis to generate this figure, see Supplementary information.

exhibiting sex-biased expression include those implicated in human phenotypes, many of which have some degree of sex bias in prevalence or clinical presentation. Finally, sex-biased gene expression varies across tissues, developmental stages and environmental conditions (endogenous or exogenous) at both the individual gene level and on the genome-wide scale.

Analysis of sex differences in gene expression provides information about specific genes, pathways and biological functions that differ between sexes and may form the basis of differentiated traits. The high prevalence of autoimmune diseases in females[118] provides motivation for functional studies of blood to understand the molecular basis of the sex difference in a tissue enriched for immune cells. A study of sex-biased gene expression in whole blood from >5,000 individuals found that 3% of all expressed genes are differentially expressed between males and females, including 51, 16 and 572 genes encoded on ChrX, ChrY and autosomes, respectively[107]. Female-biased genes are enriched for several immune system gene ontology (GO) categories, genes linked to rheumatoid arthritis and genes regulated by oestrogen, whereas male-biased genes are enriched for genes linked to renal cancer[107]. Together, these observations suggest that sex-biased gene expression may contribute to sex differences in common diseases.

In addition to blood and the human immune system, sex differences in human brain structure, neurochemistry,

Hormone response elements
Short segments of DNA in gene promoters to which hormone receptor complexes bind and regulate gene expression.

Sex-biased splicing
A situation in which different transcript splicing isoforms (or different ratios of them) are present in tissues or cells derived from males or females.

behaviour and neurological and neurodegenerative disease have inspired investigations to discover the molecular mechanisms contributing to sex differences in the human brain. In a study of the post-mortem transcriptome of 12 brain regions of 137 adult donors, Trabzuni et al.[106] report sex-biased gene expression for 448 genes (2.6% of expressed genes). This study also highlighted differences among brain regions with respect to the degree of sexual differentiation at the transcriptome level, with the primary visual cortex exhibiting the most differences. This study suggests that sex-biased gene expression is likely to have functional consequences that are relevant to human disease, as there is a significant enrichment of disease-related genes among the significant sex-DE genes.

To assess patterns of sex-biased gene expression within and between human tissues, Mayne et al.[100] performed sex-DE analysis of publicly available data sets comprising 2,500 samples from 15 different human tissues and 9 different organs and reported strong differential expression between males and females in several tissues, including anterior cingulate cortex (1,818 genes), heart (375 genes), kidney (224 genes), colon (163 genes) and thyroid (163 genes). Most sex-DE genes exhibited small differences in expression levels between males and females; 64% of sex-DE genes had a magnitude $\log_2$-fold change <1. Even though differential expression can be influenced by hormones, only 32% of the sex-DE autosomal genes contained androgen or oestrogen hormone response elements, revealing that two-thirds of sex-DE autosomal genes are not under the direct influence of sex hormones[100]. One important consideration in interpreting sex differences in gene expression from human tissues is that sex-DE genes might reflect sex differences in cell-type proportions rather than sex-biased gene regulation per se.

Animal studies have demonstrated that sex-biased gene expression is highly tissue-dependent[119,120]. In humans, several recent studies have assessed differential gene expression in multiple tissues and cell lines derived from the RNA sequencing (RNA-seq) data of the Genotype-Tissue Expression (GTEx) project; as many as 60% of autosomal genes demonstrated sex-biased gene expression, with the patterns varying greatly across tissues[101–103]. In analysis of GTEx autosomal gene expression, breast mammary gland tissue displayed the most sex-biased expression, with approximately 6,500 sex-DE genes in the breast, which is more than 40 times the number of sex-DE genes of the next most sex-differentiated tissues (skin, thyroid, brain and adipose tissues). Within tissues, 10–60% of autosomal genes were differentially expressed, with approximately 1,500 sex-DE genes in multiple tissues. Interestingly, sex hormone receptors do not exhibit sex-biased expression in most tissues, but network analyses demonstrated that they and other transcription factors are involved in sex differences in regulatory targeting, thereby resulting in considerable differences in the structure of male and female gene regulatory networks[102]. Given that differential expression of the hormone receptors themselves did not drive these observed sex-differentiated networks, it is possible that sex differences in post-transcriptional mechanisms underlie these observations. Furthermore, it is important to note that differences in network regulatory structure can be independent of differential expression of transcription factors or target genes and allow the possibility that sex-differentiated phenotypes might arise from perturbations that affect the regulatory processes in one sex, altering the expression of the downstream genes.

As noted above, sex differences in gene expression are thought to play a role in complex disease and might contribute to those with sexually differentiated characteristics. Indeed, disease-associated and phenotype-associated variants are found disproportionately near or in genes that are sex-DE in a tissue-specific manner. For example, gene sets previously associated with neurodegenerative disorders and immune-related diseases are enriched among brain sex-DE genes, whereas genes associated with heart block, syncope, ventricular arrhythmia, atrial fibrillation and palpitations are enriched among heart sex-DE genes[102]. Across tissues, both male-biased and female-biased genes are enriched among GWAS catalogue genes[39] that have previously been identified to have an association with disease or non-disease complex traits. Consistent with the enrichment of sex-DE genes involved in immune response and inflammatory disease, many immunoglobulin genes exhibit higher expression in females[121]. Collectively, these observations suggest that genes exhibiting sex-biased gene expression may be involved in biological processes linked to human diseases and phenotypes.

It is worth noting that the studies and results described above are derived from analyses of tissues and cells of adults. In an analysis of sex-biased gene expression in human brains along developmental stages (prenatal, early childhood, puberty and adult), >2,000 sex-DE genes were identified for each stage, with the largest number (4,164) of sex-DE genes identified in pubescent brains[112]. Little overlap of sex-DE genes was found across developmental stages, suggesting a highly dynamic gene regulation program throughout the course of brain development. Collectively across stages, male-biased brain-expressed genes, that is, those with higher expression in males, were enriched for genes involved in neurological and psychiatric disorders, including schizophrenia, autism and Alzheimer disease, which are traits with known sex differences in prevalence and clinical characteristics. These findings suggest that sex differences in susceptibility to brain disorders may be caused in part by sex-biased gene expression regulation during brain development. Collectively, these analyses indicate an important role of sex-biased genes in brain development and neurodevelopmental disorders.

In addition to sex differences in gene expression levels, sex-biased splicing might contribute to sex-differentiated phenotypes. Nearly all genes in the human genome undergo alternative splicing, thereby greatly expanding the transcriptional complexity derived from a single DNA sequence. Interindividual variation in RNA splicing plays an important role in the development of complex traits[122,123,125]. Sex differences in splicing have been reported in skeletal muscle[125], human brain[106] and liver[126], with 95% of the sex-biased spliced genes residing

Expression quantitative trait loci
(eQTLs). Regions of the genome containing genetic variants associated with gene expression levels in a given tissue or cell type.

Sex-biased eQTLs
Expression quantitative trait loci (eQTLs) at which the allelic effect size differs between females and males.

on the autosomes. Approximately 50% of sex-biased spliced genes are associated with a Mendelian disease[127] exhibiting a sex difference in incidence, involved in the reproductive system, or implicated in prostate, breast or ovarian cancers, suggesting a role for sex-biased splicing in disease[106].

Sex differences in genetic regulation (eQTLs and sQTLs). Gene expression variation has a heritable component; hence, sex differences in the genetic contribution to gene expression phenotypes might influence sex-differentiated phenotypes. Indeed, expression quantitative trait loci (eQTLs) are highly enriched among complex trait and disease risk loci[128–130], indicative of genetic variants that exert their effects on complex traits through regulatory mechanisms. Studies integrating GWAS and eQTLs have identified putative causal genetic variants, causal genes and mechanisms underlying complex phenotypes[131–137]. Genetic variants also affect gene expression by influencing RNA splicing; such variants are referred to as splicing quantitative trait loci (sQTLs). Many eQTLs and sQTLs are context-specific, meaning that they act as eQTLs or sQTLs only under certain conditions (as described in REF.[138]). Context-specific genetic effects on gene expression appear to play a role in the genetic basis of complex disease[131,132,139]. In humans, eQTLs vary across populations[140,141], tissues and cell types[125,130,133,134,139,142–146], cellular activation state[132,135–137] and sex[147–150]. Thus, sex differences in eQTL and sQTL effects might underlie sex-specific or sex-differentiated associations with higher-order complex phenotypes.

Sex-biased eQTLs may act as an eQTL in one sex but not in the other sex or could be shared by both sexes but with different effect sizes or even allelic direction in males and females. This could result in sex differences in mean expression levels or differences in expression variance (FIG. 1c). Yao et al.[147] tested the hypothesis that sex-biased eQTLs contribute to the genetic basis of complex traits. They evaluated 11,672 trait-associated SNPs[151] for function as sex-biased eQTLs in whole blood and identified 14 eQTLs with significant genotype-by-sex interactions on gene expression (13 autosomal and 1 ChrX *cis*-eQTLs, that is, those eQTLs that act locally). Of 14 genes targeted by sex-biased eQTLs, only 7 exhibited sex-biased mean expression differences. The sex-biased eQTLs regulating the remaining genes either exhibited a difference in allelic direction between sexes or may be explained by sex differences in variance but with no accompanying difference in mean expression. Interestingly, these sex-biased eQTLs are associated with traits that are also known to exhibit sex differences, including systemic lupus erythematosus, obesity, blood pressure and lipid traits. Extending these analyses, Kukurba et al.[148] performed a genome-wide analysis of sex-biased eQTLs in whole blood and identified six sex-biased eQTLs (four autosomal and two ChrX *cis*-eQTLs). Two of the six eQTLs were replicated in other cohorts, including the sex-biased eQTLs for *NOD2* (REF.[147]) and *BSCL2* (REFS[152,153]) expression. Interestingly, the replicated *NOD2* sex-biased eQTL in whole blood may not be a true case of a sex-biased eQTL. In *cis*-eQTL studies of sorted blood cell types, there is no evidence for a sex-biased eQTL for *NOD2*. However, the allelic direction (that is, which allele is associated with higher expression) is reversed between neutrophils and monocytes and T cells[139,154,155]. Therefore, the *NOD2* sex-biased eQTL reported in whole blood might be derived from the known sex difference in the proportions of neutrophils[156] rather than a true effect of sex on genetic regulation of the gene. To date, sex-biased sQTLs have received little attention.

Studies characterizing sex-biased eQTLs have reported many fewer significant associations than have standard eQTL studies. This result is expected, as the genetic regulation of most genes is unlikely to be influenced by sex. In fact, despite early studies suggesting sex-biased eQTLs might be relatively common[150], the more recent, better-powered and statistically controlled studies have reported mere handfuls of significant sex-biased eQTLs, with the majority failing to replicate across studies. The paucity of sex-biased eQTLs may reflect the true underlying biology, that is, that there are very few interactions of this type, as suggested by Kassam et al.[157], or may be a result of some combination of small effect size, relatively low power for interaction tests and between-study and interindividual heterogeneity. Future studies will interrogate much larger cohorts and will assess the degree of tissue sharing of sex-biased eQTLs and sQTLs. Indeed, the sex Analysis Working Group of the GTEx Consortium is currently identifying sex-biased eQTLs and sQTLs across GTEx tissues and characterizing their role in disease. As suggested by Lindén et al.[149], it may be the case that large-effect sex-biased eQTLs do not have large individual effects on disease but that an accumulation of small-effect sex-biased genetic disease risk could be polygenic across a broad range of variants and genes. Better-powered functional genomics studies specifically designed for these purposes will play an important role in assessing such models in the future.

Hormones contribute to sex-biased gene expression. In addition to eQTLs and sQTLs, another mechanism driving sex-biased gene expression is hormone regulation. Despite the fact that the majority of sex-DE genes do not contain hormone response elements[100], for approximately one-third of sex-DE genes, hormones may directly regulate transcription[158,159] and have consequences on phenotypes. Menopause, characterized by changes in female hormone levels, has been shown to influence the expression levels of several immune and metabolism genes in adipose[160] and bone[161] tissues. In studies of model organisms, removal of male hormones in male mice by castration changes the expression of sex-biased genes in the hypothalamus and, consequently, results in complex sex differences in behaviour[158]. Xu et al.[158] also found that the expression profile of castrated males is plastic, such that provision of testosterone reverts the gene expression to the levels of intact males. Differential expression of genes regulated by hormones has a direct impact on health, as exemplified by the protection of males from central nervous system autoimmune disease through an androgen-induced upregulation of autoimmune regulator protein (encoded by the *AIRE* gene)[162].

Sex differences in the epigenome influence gene regulation. Another important mechanism contributing to sex differences is sex-biased epigenetic regulation of gene expression. Sex differences in DNA methylation patterns have been reported[163–167]. Several thousand sex differentially methylated autosomal CpG sites have been identified in whole blood and replicated in independent cohorts[168,169]. The sex differentially methylated CpG sites are enriched both in CpG island shores (which are thought to regulate gene expression through silencing)[170] and in imprinted genes[168]. Given this observation and the acknowledged role of imprinting in several diseases (for example, Prader–Willi syndrome, Beckwith–Wiedemann syndrome and Silver–Russell syndrome), it is possible that sex differences in DNA methylation may also contribute to sexually differentiated traits. It is worth noting that many epigenome-wide studies have not analysed ChrX, which might prove valuable in the context of gene–environment interactions. Indeed, method development for appropriate analysis of the epigenome of ChrX is needed.

Sex-biased gene expression may also arise from sex differences in chromatin accessibility, which have been documented in mice[171–173] and humans[148]. Not surprisingly, sites with sex-specific chromatin accessibility are enriched for genes with sex-biased expression and for genetic variants with genotype-by-sex interactions on gene expression levels[148]. For example, sex-dependent DNaseI-hypersensitivity sites in mouse liver are associated with sex-biased gene expression[171]. A subsequent study integrating chromatin state maps and epigenetic marks with genome-wide transcription factor binding and gene expression data revealed that sex-biased gene expression results from a complex interplay between sex-biased interaction of transcription factors with sex-biased chromatin modifications[172]. Although the mechanisms for sex-specific chromatin accessibility remain poorly understood in humans, one potential hypothesis is that the difference may arise from hormone receptor transcription factor binding. In a model organism study, hormonal feminization of male mice suppressed the majority of male-specific sites and revealed some female-specific sites[171], indicating that hormones modulate chromatin accessibility. Studies such as these reinforce the need to consider SABV in large studies aiming to define the chromatin landscape and the three-dimensional chromatin interactome across human cell types and tissues.

An integrative approach to studying sex differences. The mechanisms leading to sex differences described in this Review are likely to work in concert to influence the sex differences observed in complex traits and disease. Although sex differences in expression at the individual gene level may be small, in aggregate, these differences may be amplified through the joint effect of many genes acting in networks[174] and pathways to influence system-level biology[175]. Furthermore, sex differences in one tissue might affect the function of other tissues or organs. In complex traits, interactions between broad biological systems have been described, as exemplified by the role of the immune system in neuropsychiatric disorders[176–178] and neurodegenerative diseases[179,180].

Implications for medicine

Perhaps the most urgent translation of knowledge gained in this field is to drug discovery and therapeutic implementation. Molecular mechanisms that differ by sex might suggest novel targets for therapeutic intervention[181–183] and should be leveraged in the early stages of drug development. Knowledge of sex-differentiated disease mechanisms has not played a predominant role in drug discovery to date. Instead of targeting drug development with a sex-aware approach, most drugs have been developed using a one-size-fits-all approach that has resulted in increased adverse events and reduced efficacy in females for some drugs[184–187]. Optimizing therapeutics to perform equally well in males and females can rightfully be thought of as ground zero for personalized or precision medicine.

It is well known that interindividual differences in pharmacokinetics and pharmacodynamics have a genetic basis but also differ by sex[188]. In order to realize personalized medicine using pharmacology, it is imperative to understand whether genetic variation contributes to drug responses in a sex-specific manner, not only for drug-metabolizing enzymes and transporters but also for drug targets. Improved knowledge of the interaction of sex, genetic variation and drugs can provide clinically useful information for evaluating an individual's likelihood of drug toxicity or therapy efficacy. For example, a study of Korean patients identified different genetic variants associated with alcohol dependence in men and women[183]. In addition, an allele in the μ-opioid receptor gene *OPRM1* was present at higher frequency in female patients than in male patients[189]. This same variant influences the effectiveness of the drug naltrexone, a μ-opioid receptor antagonist, which raises the possibility that sex-differentiated genetic effects contribute to sex differences in naltrexone efficacy[189]. Further evidence of this phenomenon is observed in the sex-biased responses to statins, driven by gene-by-sex interactions in a key drug-metabolizing gene (*SLCO1B1*)[190,191]. Drug efficacy may also be modulated by effects of hormones or other sex-differentiated traits, as observed in murine models in which inhibition of PARP1, a mediator of cell response to cellular stress, protects male mice from infarction and ischaemic cell death but exacerbates injury in female mice[192]. These findings indicate that, whereas inhibition of PARP1 as a therapy may be beneficial for males with stroke and other inflammation-mediated disorders, it could exacerbate disease in females.

The evidence accumulated to date for sex differences in the genetics and molecular underpinnings of human disease is enough to suggest the possibility of future sex-based drug development and therapy implementation. Although we cannot be certain that this approach will improve outcomes, there are sufficient data to justify further SABV research studies to assess its value. Importantly, hindsight suggests that, in some cases, when SABV is not considered in clinically relevant research, it can lead to serious consequences. High-profile examples of such consequences include the case of zolpidem (Ambien), a common sleep aid for which the US Food and Drug Administration (FDA) recommended a lower dosage for females[193] after reports of

Table 1 | **Resources for learning about SABV**

Resource	Link	Description
US NIH Office of Research on Women's Health		
Online course series	https://sexandgendercourse.od.nih.gov/	• The basic science and biological basis for sex-related and gender-related differences • Sex and gender differences in health and behaviour • The influence of sex and gender on disease expression and treatment
Policies	https://grants.nih.gov/grants/guide/notice-files/NOT-OD-15-103.html	Enhancing reproducibility through rigour and transparency
	https://grants.nih.gov/grants/guide/notice-files/NOT-OD-15-102.html	Consideration of sex as a biological variable in NIH-funded research[5]
Guidance on implementing the NIH SABV policy	https://orwh.od.nih.gov/resources/pdf/NOT-OD-15-102_Guidance.pdf	A compilation of literature reviews, along with links to research and training materials, methods and techniques, and research summaries
CIHR		
Sex, gender and health research guide: a tool for CIHR applicants	http://www.cihr-irsc.gc.ca/e/32019.html	Sex, gender and health research guide: online training modules, fact sheets, videos and webinars
Training module	http://www.cihr-irsc-igh-isfh.ca/	• Sex and gender in biomedical research • Sex and gender in primary data collection with human participants • Sex and gender in secondary data collected from human participants
Other resources		
Sex and Gender Equity in Research Guidelines	https://researchintegrityjournal.biomedcentral.com/articles/10.1186/s41073-016-0007-6	Guidelines developed by a panel of 13 experts representing 9 countries through a series of teleconferences, conference presentations and a 2-day workshop[5]
Biology of Sex Differences	https://bsd.biomedcentral.com/	Specialized journal for highlighting SABV
GenderMed database (available in English and German)	http://gendermeddb.charite.de/index.php?site=faq&lang=eng	Systematic collection of scientific publications in the medical field analysing sex and gender differences
Organization for the Study of Sex Differences	http://www.ossdweb.org/	Promotes the field of sex and gender differences research through education, mentoring and outreach
International Society of Gender Medicine	http://www.isogem.eu/	Encourages and facilitates interdisciplinary research on sex and gender in basic and clinical frameworks

CIHR, Canadian Institutes of Health Research; NIH, US National Institutes of Health; SABV, sex as a biological variable.

next-day impairment (including sleep-driving) linked to a slower drug metabolism in females. It is our hope that careful attention to sex differences in preclinical and clinical research before the release of new therapeutics will prevent such tragic consequences.

Conclusions and future perspectives

Sex is a fundamental biological characteristic that influences nearly all human traits. Understanding the biological underpinnings leading to sex differences observed in human disease is critical for developing sex-informed diagnostics and therapeutics and for realizing the promise of precision medicine. The field of human genomics is just beginning to develop a comprehensive framework for approaching genetic studies of sexually differentiated traits. We strongly recommend that future genome-wide studies of complex traits include sex-stratified, gene-by-sex interaction and heritability analyses including the sex chromosomes to enable comprehensive characterization of the role of sex in the genetic basis of complex traits. Assessment of phenotypes for which there are no apparent sex differences would also benefit from such sex-aware analysis, as there could potentially be differences between the sexes in the genetic architecture and mechanisms influencing phenotypic variation but not affecting prevalence. Given the widespread evidence of sex differences in phenotypes at the organismal and molecular levels, future studies of complex traits should integrate genetic analyses with transcriptomics, proteomics, epigenomics, metabolomics, microbiome and other omics to enable integrative analyses that can elucidate the cascade of molecular effects contributing to sexually differentiated traits. Large cohorts of genotyped and phenotyped individuals, as well as biobanks, population registries and associated genomic data, constitute necessary resources for these future studies. Collaboration and open data sharing should be even more strongly encouraged by funding agencies and journals to facilitate the combination of existing data sets and results to improve the power for SABV-relevant analyses. Training opportunities (TABLE 1) for how to best design SABV studies and collect, analyse and share data will enable the expansion of SABV research.

In summary, this Review highlights examples of sex differences in the genetic architecture of human complex traits and mechanisms contributing to sexually differentiated traits. Further expansion of SABV research holds great promise both for improving our understanding of biological sex at a fundamental level and for enabling sex-targeted approaches to improving health and battling disease.

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