

# Sex-specific genetic architecture of human disease

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**Abstract** | Sexual dimorphism in anatomical, physiological and behavioural traits are characteristics of many vertebrate species. In humans, sexual dimorphism is also observed in the prevalence, course and severity of many common diseases, including cardiovascular diseases, autoimmune diseases and asthma. Although sex differences in the endocrine and immune systems probably contribute to these observations, recent studies suggest that sex-specific genetic architecture also influences human phenotypes, including reproductive, physiological and disease traits. It is likely that an underlying mechanism is differential gene regulation in males and females, particularly in sex steroid-responsive genes. Genetic studies that ignore sex-specific effects in their design and interpretation could fail to identify a significant proportion of the genes that contribute to risk for complex diseases.

## Heterogametic

Refers to the sex that produces gametes that have two different sex chromosomes. In mammals, males are the heterogametic sex (XY) and females are homogametic (XX), whereas in birds females are heterogametic (ZW).

## Genetic architecture

Refers to the underlying genetic basis for a trait.

Differences between males and females in anatomical, physiological and behavioural traits are characteristics of many vertebrate species, including humans. Although some can be apparent at birth, striking differences between the sexes usually emerge at or around the time of sexual maturation. It is thought that these are mainly due to sex-hormone levels that differ between males and females, beginning *in utero* and continuing throughout the lifetime of the organism<sup>1</sup> (FIG. 1). The genetic contribution to sexual dimorphism was, until recently, studied less than the hormonal contribution. Indeed, whereas genes on sex chromosomes contribute to many sexually dimorphic traits, the autosomal genome is generally assumed to be similar between the males and females of a species. Mechanisms for dosage compensation in heterogametic species further ensure that genetic contributions from the shared sex chromosome (the X chromosome in mammals) is equivalent among males and females, at least for most genes<sup>2</sup>.

However, recent studies have challenged this paradigm, suggesting that natural variation within the autosomal genomes of many species also affects anatomical, physiological and behavioural traits differently in males and females<sup>3–5</sup>. In this context, sex can be considered an ‘environmental’ variable that includes the cellular, metabolic, physiological, anatomical and even behavioural differences between boys and girls (in childhood) or between men and women (in adulthood). Therefore, sex might interact with the genotype

in a manner similar to other environmental factors (FIG. 2). However, unlike most other environmental factors, sex is easily observable and usually unambiguous. Such sex-specific genetic architecture suggests new models of susceptibility for common diseases and sheds light on potential mechanisms of sexual dimorphism (BOX 1) in human phenotypes.

In this Review, we argue that sex-specific genetic architecture is common in humans and that genotype–sex interactions contribute to differences in the prevalence, course and severity of diseases, as well as to other quantitative phenotypes. We provide recent examples of genotype–sex interactions as evidence to support this argument and to illustrate how patterns of tissue-specific gene expression differ markedly between males and females. Lastly, we discuss the importance of considering sex in the design and analysis of genetic studies.

## Evidence of sex effects

Accumulating evidence suggests that nearly all human diseases have sex-specific differences in prevalence, age of onset and/or severity. Classic examples include: cardiovascular disease, which is predominant in men throughout adulthood but has a higher rate of occurrence in post-menopausal women compared with men<sup>6</sup>; asthma, which is more prevalent among boys in childhood but shows a higher occurrence of new cases among girls around and following puberty<sup>7</sup>; and autoimmune diseases, which are more prevalent

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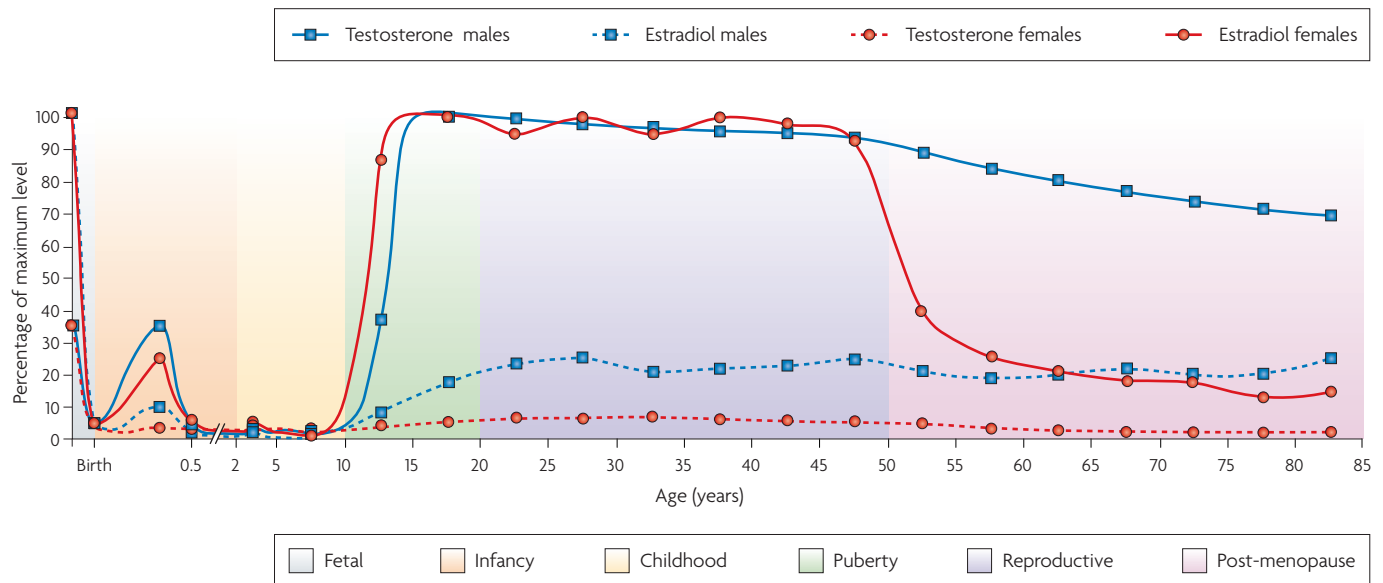


Figure 1 | **Approximate mean sex-steroid levels in plasma in males and females.** Variation in steroid levels is shown as a percentage of the maximum mean testosterone and the maximum mean estradiol across the life stages shown. The figure does not show diurnal, cyclic (female) or possible seasonal fluctuations. Female estradiol levels refer to the mean for the mid-follicular phase of the menstrual cycle; estradiol production transiently increases about fivefold during the pre-ovulatory and luteal phases of the menstrual cycle. Note the drop in the levels of all sex steroids at birth and the transient ‘minipuberty’ in early infancy. Free testosterone in men falls more with ageing (to approximately 50% of the maximum in 80-year-old men) than the total testosterone<sup>134</sup>, which is shown here. This figure is drawn using data from REFS 1, 135, 136.

in women throughout life, but particularly for diseases that begin during or immediately following the reproductive years<sup>8</sup> (FIG. 3). In addition to the diseases highlighted in FIG. 3, significant sex differences have been described for many common birth defects, for neurological and psychiatric disorders, as well as for some common cancers. For example, in infancy or childhood, neural tube defects, congenital dislocation of the hip and scoliosis are more common among girls, whereas autism, stuttering and pyloric stenosis are more common among boys<sup>9</sup>. In adulthood, major depression and Alzheimer disease are more common in women<sup>10,11</sup>, whereas schizophrenia, Parkinson disease and colorectal cancer are more common in men<sup>12–14</sup>.

It should be noted that differences in prevalence rates or age of onset do not necessarily imply that genetic variation leads to different effects in males and females<sup>15</sup>, as many of these differences could be due to hormonal profiles, particularly with regard to sex steroids (FIG. 1), or to behaviours that differ between the sexes, such as exposure to cigarette smoke<sup>16</sup>. For example, the consistent associations between increased risk for disease among females during and following puberty (asthma), during the reproductive years (autoimmune disease) or post-menopause (cardiovascular disease) have implicated sex hormones as important mediators of disease pathogenesis and as contributors to sex differences in prevalence rates and progression.

Importantly, differences between the immune systems of males and females have been observed as early as the first few years of life, suggesting a developmental

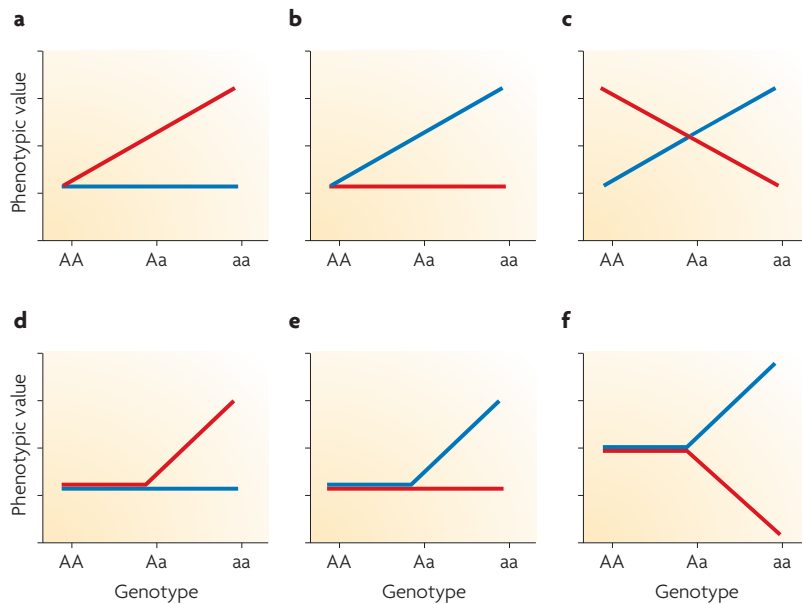
component to sex-specific differences in disease risk<sup>17</sup>. Such differences could result in sex-specific thresholds of susceptibility to immune-mediated diseases throughout life. Interestingly, immune responses might be modulated by sex hormones<sup>18,19</sup>. In fact, the transient rise in sex-steroid levels (‘minipuberty’) that occurs in early infancy<sup>1</sup> (FIG. 1) could pattern immune cells differently in boys and girls. Thus, both the immune and endocrine systems probably contribute to sexual dimorphism in the epidemiology of many common diseases. However, recent evidence suggests that some of the differences between males and females might also be due to differences in genetic architecture. This Review will focus on such sex-specific genetic effects.

### Sex effects through gene regulation

**Contribution of sex chromosomes.** The contribution of the sex chromosomes to sex-specific genetic architecture of human disease has long been appreciated. For example, males often express X chromosome-linked recessive diseases, and female carriers of X-linked mutations show skewed patterns of X-chromosome inactivation that often result in varied expression of disease phenotypes<sup>20</sup>. More generally, dosage differences in X-linked genes between the sexes probably account for some of the sex-specific genetic architecture of common diseases and phenotypes. In turn, the Y chromosome in males harbours few genes, most of which are expressed exclusively in the testes; the other genes are typically thought of as ‘housekeeping’ genes, most of

#### Pyloric stenosis

A common birth defect that results from the narrowing of the pylorus (lower part of the stomach), which prevents food and other stomach contents from passing into the intestine. This condition causes severe vomiting in infancy. Also called infantile hypertrophic pyloric stenosis.



**Figure 2 | Models of genotype–sex interactions reflecting genotype effects that differ between males and females.** For any measured phenotype or disease risk (y axes), the genotypic effects might be apparent only in females (**a,d**), only in males (**b,e**), or be present in both sexes but with opposite directions of effects (**c, f**). The genotype effects can be additive (**a–c**) or recessive (**d–f**). Other models (for example, dominant) or interactions (for example, same direction of effect but differences in magnitude of effect) are not shown. Red lines track phenotypic values by genotype in females, blue lines track phenotypic values by genotype in males. Examples discussed in this Review can be illustrated using these graphs: the relationship between the DD genotype of *ACE* and blood pressure (**b**); the relationship between two SNPs on chromosome 4p16.3 (rs3796619 and rs1670533) and recombination rate (**c**); the relationship between the reelin gene (*RELN*) rs7341475–GG genotype and schizophrenia (**d**); and the relationship between the DD genotype of the angiotensin converting enzyme (*ACE*) gene and hypertension (**e**).

which have X-chromosome homologues that escape X inactivation<sup>21</sup>. Thus, it is perhaps unlikely that Y-linked genes directly affect disease risk, other than being major contributors to genetic causes of male infertility<sup>22</sup>. However, Y-linked genes might interact with autosomal genes to differentially affect disease risk in males and females.

**Contribution of autosomes.** In contrast to the sex chromosomes, the autosomal genome is shared by both sexes. However, although the DNA sequence, gene structure and frequency of polymorphisms on the autosomes do not differ between males and females, the regulatory genome is sexually dimorphic<sup>23–26</sup>. That is, sex-specific differences in gene regulation, rather than gene content, probably underlie most phenotypic sexual dimorphism, including sex-specific effects on human diseases. Indeed, at the mRNA level, sexually

dimorphic gene expression has been observed in a wide range of organisms, including worms<sup>27</sup>, flies<sup>28,29</sup>, fish<sup>30</sup>, rodents<sup>25,31</sup> and primates<sup>23</sup>. Although genes with sex-biased expression are enriched on the sex chromosomes, thousands of sex-biased genes are also found on the autosomes.

**Sexually dimorphic gene expression patterns are conserved.** Interestingly, genes with sex-biased expression patterns tend to evolve rapidly at the coding-region level<sup>26</sup>. This observation is consistent with the notion that many differences in gene expression between the sexes are the result of sexual selection (BOX 1). The evolution of sex-biased genes was recently reviewed by Ellegren and Parsch<sup>26</sup> and will not be discussed in detail here. However, it is relevant to note that although sex-biased genes often evolve rapidly at the protein-coding level, differences in gene regulation between the sexes

#### Regulatory genome

The total set of different DNA molecules of an organelle, cell or organism that are involved in the regulation of gene expression.

#### Sexual selection

Differential reproductive success resulting from the competition for fertilization, which can occur through competition among the same sex (mate competition) or through attraction to the opposite sex (mate choice).

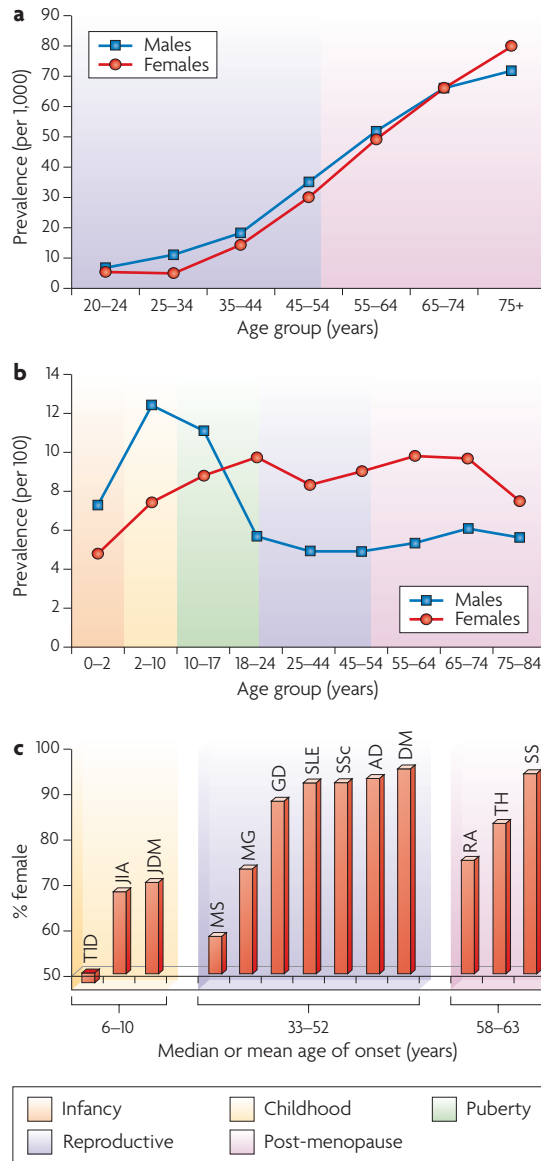
#### Ontogenetic conflict

Occurs when the same allele has different fitness consequences in juveniles and adults or in males and females.

#### Box 1 | Sexual dimorphism

Following Darwin's observation in 1859 that males and females might have the same "general habits of life" but "differ in structure, colour, or ornament"<sup>95</sup>, research on sexual dimorphism progressed gradually from qualitative descriptions of conspicuous anatomical and behavioural traits in animals<sup>96</sup> to elegant experiments probing the sex-specific neural circuitry of reproductive behaviour in flies<sup>97,98</sup> and mice<sup>99</sup>. The results of these ~150 years of research demonstrated that sexual dimorphism was taxonomically widespread and remarkably variable in the magnitude and form of its expression<sup>100,101</sup>. It is now obvious that sex-specific differences occur not only in conspicuous morphological traits such as size, shape and colouration, but also in a diverse suite of behavioural<sup>97–99,102</sup>, psychological<sup>102,103</sup>, biochemical<sup>69,103</sup> and gene expression<sup>23,24,26</sup> phenotypes.

Variation in the magnitude of sexual dimorphism among closely related species, and sometimes within a species, motivated biologists to test Darwin's hypothesis that sex-specific differences were largely due to sexual selection, particularly male–male competition, in dozens of different taxa<sup>101</sup>. The results of these studies consistently reaffirmed the importance of sexual selection (via male–male competition and/or female choice) as a major driving force of sexual dimorphism, but also suggested a significant role for natural selection and non-selective forces, that is, genetic, ecological and developmental pressures and constraints, in the evolution of sex-specific phenotypic divergence<sup>100,104</sup>. Indeed, future research into the nature and consequences of intersexual genetic correlations<sup>105</sup> and intersexual ontogenetic conflict<sup>106</sup> will lead to a more sophisticated understanding of the evolution and expression of sexual dimorphism.



**Figure 3 | Sex-specific prevalence rates, age of onset and sex ratios for common sex-skewed diseases.**  
**a** | Cardiovascular disease rates in the United States (from the *National Health and Nutrition Examination Survey* (NHANES) III 1988–1994)<sup>6</sup>. Note the increase in female prevalence rates in the post-menopausal period.  
**b** | Asthma in the United States from 1998–2006 (from the *Centers for Disease Control National Health Interview Survey* (CDC NHIS)). Note the increase in female prevalence rates during and following puberty.  
**c** | Sex ratios (% female) by mean or median age of onset for autoimmune diseases in the United States and Europe<sup>137,138</sup>. Note the female skewing at all ages, with the largest skew and number of diseases during and immediately following the reproductive years. AD, Addison disease; DM, dermatomyositis/polymyositis; GD, Graves disease; JDM, juvenile dermatomyositis; JIA, juvenile idiopathic arthritis; MG, myasthenia gravis; MS, multiple sclerosis; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; SS, Sjögren disease; SSc, systemic sclerosis (scleroderma); T1D, type 1 diabetes; TH, thyroiditis. Figure is drawn using data from REFS 6, 137, 138. Part **a** is modified, with permission, from REF. 6 © (2007) Elsevier.

**Expression QTL (eQTL).** Loci at which genetic allelic variation is associated with variation in gene expression.

are often conserved in evolution. For example, Zhang and colleagues showed that sexually dimorphic expression patterns of a large number of genes are conserved across seven *Drosophila* species<sup>32</sup>. Similarly, Reinius *et al.* found a signature of evolutionarily conserved sexually dimorphic gene expression in the brains of three primate species, including humans<sup>23</sup>. Specifically, they compared gene expression profiles in the occipital cortex of male and female humans and cynomolgus macaques (*Macaca fascicularis*), and identified hundreds of genes with sex-biased expression patterns in both species.

**Phenotypic consequences of sexually dimorphic gene expression patterns.** The observations of conserved sex-specific regulation suggest that at least a subset of the sexual dimorphism in gene expression underlies important phenotypic differences between the sexes, including developmental, physiological and/or behavioural differences. These conserved sexually dimorphic gene expression patterns suggest the existence of constant regulatory differences between males and females, which can be beneficial to each sex but can also contribute to different gene–environment interactions in the two sexes. In turn, such differences might result in sex-specific susceptibility to disease. For example, potential sexual dimorphism in the regulation of oxidative stress response pathways could differentially affect susceptibility to cardiovascular diseases in males and females<sup>33</sup>.

A second interesting observation is that sexually dimorphic gene expression patterns are often tissue specific<sup>25</sup>, whereby a gene is differentially expressed between the sexes in some tissues but not in others. This important observation suggests that a different architecture of regulatory interactions might underlie gene expression patterns in males and females in different tissues. Therefore, it is likely that entire regulatory networks differ between the sexes, interacting with functional genetic variation, such as expression quantitative traits, in a sex-specific manner. Such differences in gene regulation between the sexes might account for genotype–sex interactions that affect other measurable phenotypes in addition to disease risk. A clear example of a sex-specific response to an environmental variable was recently provided by Zammaretti *et al.*, who investigated the effects of a long-term moderate to high-fat diet in mice. They found phenotypic differences between males and females, including differences in gene regulation, when both sexes were fed an identical diet<sup>34</sup>.

Bhasin *et al.* provided additional support for this hypothesis by mapping sex-specific expression QTLs (eQTLs) in mice<sup>35</sup>. They identified SNPs in putative *cis*-regulatory elements, which were associated with variation in gene expression within individuals from one sex but not the other, indicating that some loci have a regulatory role in males but not in females, or in females but not in males. Because the SNPs are shared between the sexes, sex-specific differences in the use of *cis*-regulatory elements indicate the existence of differences in *trans*-regulatory elements (for example, transcription

## Box 2 | Microchimerism and disease

As a result of bidirectional cell trafficking between the mother and fetus during pregnancy, mothers can harbour cells from their children, and children can harbour cells from their mother well into adulthood. This mixture of a small amount of cells from a genetically disparate individual is referred to as microchimerism<sup>107</sup>. The persistence of maternal cells in an individual, called maternal microchimerism, has been detected in the peripheral blood mononuclear cells in approximately 22% of healthy individuals<sup>108–111</sup>. The persistence of fetal cells in the mother is called fetal microchimerism, and it has been detected in peripheral blood mononuclear cells in 30% to 55% of healthy women, depending on the outcome of the pregnancy<sup>112</sup>. Maternal microchimerism is found less often than fetal microchimerism in unselected peripheral blood mononuclear cells as well as in cellular subsets, such as T and B lymphocytes, monocytes and macrophages, and natural killer cells<sup>113</sup>. Moreover, microchimerism has been found in many human tissues and has the capacity to differentiate into tissue-specific cells, including myocytes, hepatocytes and other cell types<sup>114–116</sup>. Although some studies have reported differences in the prevalence of microchimerism between healthy individuals and patients with autoimmune disease, a more striking difference has often been an increase in the quantity of microchimerism in patients with an autoimmune disease<sup>110,117–124</sup>, including most of the diseases shown in FIG. 3c. The idea that the destructive immune response that causes these diseases is directed at the chimeric cells raised the suggestion that some autoimmune diseases could in fact be alloimmune<sup>125</sup>. Lastly, the onset of many autoimmune diseases in women during and immediately following the reproductive years has been attributed to microchimerism<sup>126</sup>, suggesting that exposure to fetal cells during pregnancy is a sex-specific risk factor for autoimmune disease.

factors and cofactors). Sex-steroid receptors might be one example of sex-specific *trans*-regulatory elements<sup>36</sup>. Similar analyses of human eQTL data have not been performed to date, but the findings of Bhasin *et al.*<sup>35</sup> are consistent with a growing number of observations<sup>23,25,28,30,32</sup>. This suggests that ignoring sex in studies of gene expression will underestimate, perhaps dramatically, the affect of genetic variations on gene regulation and mRNA abundance.

Genetic mechanisms other than gene regulation might also contribute to sex-specific disease risk or sexual dimorphism in quantitative phenotypes (BOXES 2, 3). However, regardless of the mechanism, abundant evidence now exists for a significant role of sex-specific genetic architecture.

### Sex-specific genetic architecture in humans

**Estimating heritability.** One way to estimate the relative contribution of genes to a trait is through variance component analysis in related individuals. In this approach, the total variance in a quantitative, or measured, phenotype is divided into its genetic and environmental components. The proportion of the total phenotypic variance attributed to genetic factors (that is, genetic differences between individuals) is referred to as the heritability of the trait. The genetic variance can be further divided into the variance that is due to additive genetic effects and to nonadditive genetic effects (for example, dominance, recessiveness and epistasis), it can also be assigned to autosomes or sex chromosomes. The proportion of the variance that is due to additive genetic effects is referred to as narrow heritability ( $h^2$ ); the overall proportion of genetic variance is referred to as broad heritability ( $H^2$ ). The theoretical basis for heritability estimates and the derivation of the individual variance components has recently been reviewed<sup>37</sup>. The heritabilities of many human traits have been estimated, although most studies are limited to estimates of narrow heritabilities in combined samples of males and females (for examples, see REFS 38–40).

**Sex-specific genetic architecture of human quantitative traits: a case study.** Recently, the sex-specific genetic architecture of 19 human quantitative traits, many of which are associated with common diseases, was investigated in males and females of a large multigenerational pedigree comprising >500 members of the Hutterites, a founder population that practices a communal lifestyle<sup>41,42</sup>. Because of the remarkably uniform environment and lifestyle between individuals of both sexes in this community, the authors argued that sex-specific genetic architecture might be easier to detect. For example, smoking is prohibited, meals are eaten and prepared in a communal kitchen, and large families are desired<sup>43</sup>. Moreover, because all relative pairs in the extended pedigree are considered in the analysis, it was possible to estimate both additive and dominant variance components<sup>44</sup>.

In this population, sex was a significant predictor of the trait value in a linear regression model for 16 of the 19 phenotypes, which can be placed into five groups: cardiovascular disease-associated traits — high-density lipoprotein (HDL) cholesterol, lipoprotein(a) and triglyceride levels, and diastolic and systolic blood pressure; asthma-associated traits — forced expiratory flow at 1 second ( $FEV_1$ ), the ratio of  $FEV_1$  to forced vital capacity ( $FEV_1:FVC$ ), eosinophil count, total serum immunoglobulin E levels and percent lymphocytes; anthropometrics — body mass index, percent fat, fat-free mass and adult height; and signalling molecules — morning serum cortisol and whole-blood serotonin. Sex was not a significant predictor of three phenotypes — low-density lipoprotein (LDL)-cholesterol, lymphocyte count and fasting insulin levels.

The narrow and broad heritabilities of each of these traits were estimated in a unified model. Five traits had significant X-chromosome variance components either in males only (systolic blood pressure, adult height and triglyceride levels) or in both sexes (lipoprotein(a) and whole-blood serotonin). Interestingly, four traits

#### Alloimmune

An immune reaction against cells from another individual of the same species. Alloimmunity can occur during transfusion or transplantation, or during pregnancy.

#### Heritability

The proportion of the total phenotypic variance for a given trait that can be attributed to genetic variation among individuals.

#### Forced expiratory volume at 1 second

( $FEV_1$ ). The volume exhaled in the first second of a forced expiratory manoeuvre. This index is used to assess airway obstruction, bronchoconstriction or bronchodilation.

**Box 3 | Genetic imprinting and parent-of-origin effects**

One mechanism for sex-specific transmission of disease or of quantitative phenotypes is genomic imprinting, which refers to the transcriptional silencing of a gene in the gamete inherited from either the mother or the father, but not both (that is, allele-specific silencing). The best studied silencing mechanism is methylation, and differential methylation between alleles is considered the hallmark feature of an imprinted locus<sup>127</sup>. The cellular mechanisms for sex-specific gene silencing and the impact of such parent-of-origin effects on human disease and gene evolution have been reviewed elsewhere<sup>127–130</sup>.

In approximately half of all imprinted genes the maternally inherited allele is silenced (that is, imprinted), and in the other half the paternally inherited allele is silenced. In a few interesting cases, the imprinting itself is polymorphic so that both biallelic and monoallelic expression can be observed<sup>131,132</sup>. Mutations in or deletions of the expressed allele at imprinted loci in humans or mice have a wide range of phenotypic consequences, including effects on growth and development, on behaviour and learning, and carcinogenesis<sup>127,128</sup>.

A census of imprinted genes in 2005 suggested that approximately 41 genes in 16 chromosomal regions are imprinted in humans, compared with 71 genes in 22 chromosomal regions in mice (29 genes are imprinted in both humans and mice)<sup>130</sup>. The authors speculate that the total numbers of imprinted genes are probably not much greater than these estimates, although they acknowledged the possibility that additional imprinted genes with more subtle phenotypic effects probably exist. In support of the existence of imprinted alleles with subtle effects the authors cite the large number of complex diseases with parent-of-origin effects, including asthma, autism, type I and type II diabetes, Alzheimer disease and schizophrenia. For these diseases, the risk of disease in an individual depends on whether their mother or father is likewise affected, or whether a particular risk allele is inherited from the mother or from the father. Some of these effects might reflect as yet unidentified imprinted loci. In fact, a recent genome-wide analysis of genomic imprinting in mice revealed evidence for parent-of-origin effects that are due to genomic imprinting on a wide range of quantitative phenotypes related to body size and growth rates, and for imprinting effects that varied over time and that arose or persisted into adulthood<sup>133</sup>. Therefore, some of the sex-specific parent-of-origin effects observed in complex human diseases, such as those mentioned above, might be attributable to genomic imprinting.

had significant sex interactions that did not involve the X chromosome, in which either the estimates of heritability were significantly different between males and females (LDL-cholesterol and FEV<sub>1</sub>:FVC), or the best-fitting heritability model was different between males and females (HDL-cholesterol and fat-free mass). Thus, the genetic architecture of nine common phenotypes had significant sex-specific genetic architecture. The best-fitting heritability model for six representative traits with sex-specific architecture is shown separately for males and females in FIG. 4.

Taken together, these data suggest that the genetic architecture (additive, dominant or X-linked) and/or the overall genetic contribution (that is, heritability) significantly differs between males and females for a large number of quantitative phenotypes, many of which are risk factors for common diseases. This conclusion is consistent with other studies of sex-specific heritabilities of common disease-associated quantitative phenotypes<sup>45,46</sup>. Although this data set is limited to only 19 quantitative traits, it also suggests that X-chromosome genes might contribute disproportionately more to common phenotypes and quantitative trait variation in males than in females, not unlike Mendelian disease genes. Indeed, subsequent studies supported these conclusions, demonstrating significant sex differences in estimates of the autosomal narrow heritability for 13 (of 539) cardiovascular disease-associated quantitative traits in French Canadian families<sup>45</sup>, and for bone-mineral density in a number of recent studies (reviewed in REF. 46).

Thus, standing natural variation in the human genome contributes to quantitative phenotypes in a

sex-specific manner. That many of these phenotypes are also risk factors for common diseases further suggests that significant sex-specific genetic architecture contributes to risk for common diseases.

**Evidence for genotype–sex interactions**

Demonstrating genotype–sex interaction effects on human diseases has been challenging because until recently most study designs did not allow a systematic search for sex-specific genetic contribution to quantitative variation or disease risk<sup>47</sup>. Moreover, in most linkage and association studies that address sex-specific architecture, analyses are performed in each sex separately (usually in addition to studies in the combined sample), adding to the number of statistical tests and increasing the likelihood of a type I error if multiple testing is not properly taken into account when assessing significance. In addition, the approximate halving of the sample size to conduct sex-specific analysis reduces the power to detect an effect. For example, a study in both sexes with 80% power for a main effect will have only 29% power to detect an interaction of the same magnitude in a study of one sex<sup>48</sup>, making replication of genotype–sex interactions particularly challenging.

It is, therefore, not surprising that a recent meta-analysis of 188 genetic association studies claiming sex effects in their title found only one association that was consistently replicated in at least two studies<sup>15</sup>. Among 188 claims of a sex difference, 83 were significant ( $p < 0.05$ ), although 44 of those had modest  $p$ -values between 0.01 and 0.05 (which were unadjusted for multiple testing). Sixty of those claims were judged to have good internal validity, including the one association that was

**Type I error**

The probability of rejecting the null hypothesis when it is true, also referred to as a false positive.

**Multiple testing**

An analysis in which multiple independent hypotheses are tested. Multiple testing must be taken into account during statistical analysis, as the combined probability of type I error increases in an unadjusted analysis.

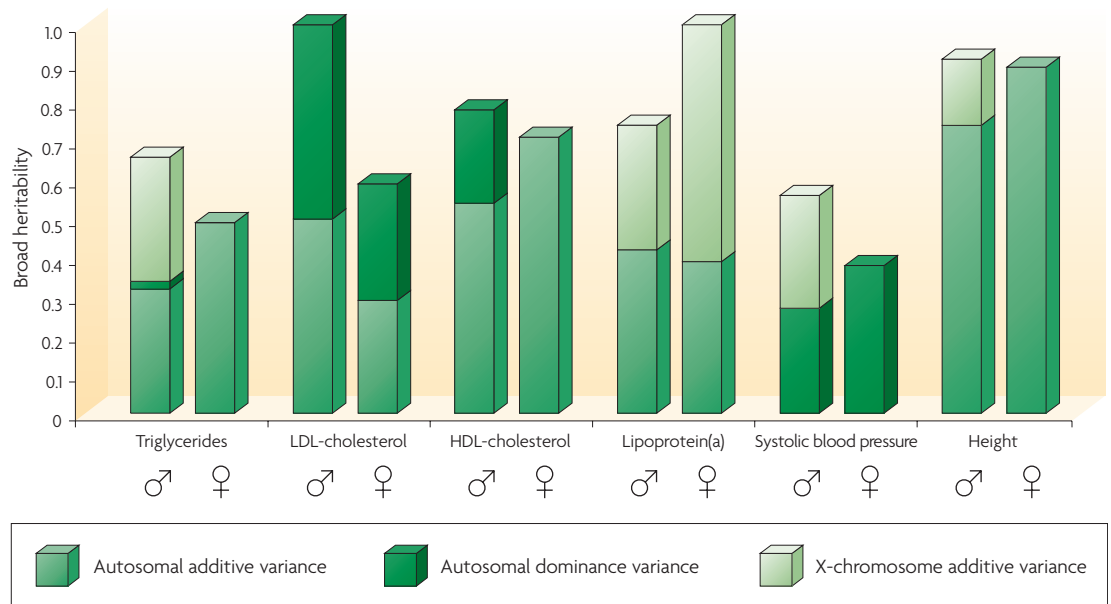


Figure 4 | **Sex-specific heritabilities in males and females.** Six quantitative traits with significant sex-specific genetic architecture show differences between males and females in the overall estimates of broad heritability ( $H^2$ ) (low-density lipoprotein (LDL)-cholesterol, lipoprotein(a) and systolic blood pressure) and/or with respect to the best-fitting model (triglycerides, high-density lipoprotein (HDL)-cholesterol, systolic blood pressure and height). This figure is drawn using data from REF. 41.

replicated. This was the association between the deletion–insertion (D–I) polymorphism in the angiotensin converting enzyme gene (*ACE*) and hypertension in men only<sup>49–52</sup> (discussed below).

Despite these limitations, several recent studies suggest the importance of genotype–sex interactions in the genetic architecture of quantitative phenotypes and common diseases, which should motivate the development of robust methods for both assessing and routine testing of genotype–sex interactions in genetic studies. It should be noted, however, that although many linkage studies have reported sex effects, only a few have shown that increased logarithm of odds (LOD) scores in one sex are not due to chance findings resulting from splitting samples and performing multiple tests, or have explicitly tested for genotype–sex interactions (see REFS 45,53 for exceptions). As a result, linkage studies will not be reviewed here. Instead, we first review evidence for genotype–sex interactions in model organisms, and then highlight three recent examples of genotype–sex interactions in human association studies.

#### Genotype–sex interaction in model systems

The most compelling and consistent evidence for genotype–sex interaction effects comes from studies of physiological, anatomical and behavioural traits in model organisms, including fruitflies<sup>54,55</sup>, mice<sup>56–59</sup> and rats<sup>60</sup>. For example, sex-specific effects in which QTLs have significantly different effects in males and females are a near-ubiquitous characteristic of the genetic architecture of complex traits in the *Drosophila* genus (reviewed in REF. 55).

In mice, studies of sex-specific effects include alcohol preference, which in the C57BL/6 strain has been shown to be nearly entirely controlled by sex-specific effects<sup>56,57</sup>. Other examples include the sex-specific effects on blood pressure and renal phenotypes that result from knocking out the cytochrome P2J5 gene in C57BL/6 mice<sup>58</sup>, and studies of consomic strains of mice that revealed sex-specific effects of individual chromosomes on fear conditioning<sup>59</sup>. Similarly, in consomic rat strains, sex-specific effects on phenotypes related to hypertension and kidney disease are apparent<sup>60</sup>.

It should be noted that many of the mapping studies claiming sex-specific effects in model organisms suffer from the same limitations as described above for human studies. However, the experimental toolbox that is available for studies of model organisms allows for a more thorough dissection of sex-specific genetic architecture, which in many cases has directly implicated specific genes or chromosomes in genotype–sex interactions. Overall, genotype–sex interaction effects on diverse biological processes are common in model organisms and often account for a significant proportion of the phenotypic variability. The extent of sex-specific genetic architecture in the human genome has yet to be determined, although we predict that humans are similar to other organisms in this respect.

#### Genotype–sex interaction effects in humans

**Hypertension and blood pressure.** Hypertension is a major risk factor for cardiovascular disease, stroke and end-stage renal disease<sup>61</sup>. In 2005 the prevalence of hypertension in the adult population worldwide was 26%<sup>62</sup>. Blood pressure is higher in men compared

#### Consonic strain

Inbred strain in which a chromosome has been replaced by a homologous chromosome from another inbred strain.

**Penetrance**

The probability of observing a specific phenotype in individuals carrying a particular genotype.

**Linkage disequilibrium**

(LD). The nonrandom association of alleles at two or more loci. The pattern of linkage disequilibrium in a given genomic region reflects the history of natural selection, mutation, recombination, genetic drift, and other demographic and evolutionary forces.

with women among adults under the age of 45, but this trend switches and at 70–79 years of age women have higher blood pressure than men<sup>63,64</sup>, similar to overall trends for cardiovascular disease (FIG. 1). Genes that are involved in the renin–angiotensin system are functional candidates for blood pressure regulation and hypertension, and have been associated with these phenotypes with varying success (reviewed in REF. 65). A 250 bp D–I polymorphism in intron 16 of the *ACE* gene accounts for approximately 47% of the variance in plasma *ACE* protein levels, with each copy of the D allele associated with an approximately 30% increase in *ACE* levels<sup>66</sup>. *ACE* was considered as a candidate gene for blood pressure and hypertension, but results of case–control association studies with blood pressure were conflicting, and family-based studies failed to demonstrate linkage between the *ACE* locus and hypertension<sup>50</sup>.

Studies in a rat model suggested genotype–sex interactions<sup>67</sup>. Both male and female rats that were heterozygous for an inactivating mutation in *Ace* had lower *ACE* protein levels compared with wild-type animals (23% reduction in males and 35% reduction in females). However, heterozygous males had a reduced blood pressure compared with the wild-type males, whereas heterozygous females had blood pressures similar to wild-type females. Therefore, low *ACE* levels that were due to an inactivating mutation in the *Ace* gene did not affect blood pressure in female rats but protected against hypertension in male rats. The authors suggested that interactions with sex should be evaluated in genetic studies of the human *ACE* gene. Indeed, subsequent studies in humans have replicated this interaction<sup>49–51</sup> (TABLE 1).

Collectively, these studies provide convincing evidence for an *ACE* genotype–sex interaction effect on hypertension and possibly on blood pressure, although the mechanisms for these effects are still unknown. Moreover, these studies demonstrate that in the absence of a genotype–sex interaction in quantitative trait variation (in this example, *ACE* protein levels<sup>66</sup>) a genotype–sex interaction can still occur with respect to an associated physiological trait (for example, blood

pressure)<sup>50,52</sup> and a disease phenotype (for example, hypertension)<sup>49–51</sup>. Lastly, these studies provide an example in which the genetic model underlying the interaction can differ between the physiological trait and the disease: in males, the effect of the D allele of *ACE* is additive on blood pressure, but recessive on hypertension (FIG. 2b,e), suggesting a sex-specific quantitative (blood pressure) threshold effect for expression, or penetrance, of a common disease (hypertension).

**Schizophrenia.** Schizophrenia is a common psychiatric disorder with significant sex differences in prevalence, age of onset and morbidity<sup>68</sup>. For example, most cases occur between the ages of 16 and 25 years in men and between the ages of 25 and 30 years in women. Overall, the male to female sex ratio is 1.4 (REFS 12,69). Estimates of heritability for this complex disease are approximately 0.80 (REF. 70), indicating that a significant proportion of disease risk is attributable to genetic variation. A number of sex-specific genetic associations with schizophrenia risk have been reported, but none has been consistently replicated<sup>15</sup>.

Shifman and colleagues conducted a genome-wide association study for schizophrenia using a novel DNA-pooling strategy<sup>71</sup>. 194 SNPs were selected for further investigation on the basis of their ranking and statistical significance in the pooled-DNA studies, and because of their biological plausibility<sup>71</sup>. These SNPs were then individually typed in 745 patients and 759 controls from the Ashkenazi Jewish population. The smallest p-value corresponded to SNP rs7341475 (a G to A transition), for which the frequency of the GG genotype was 0.76 in female patients compared with 0.59 in female controls ( $p = 9.8 \times 10^{-5}$ ). There was no association in males ( $p = 0.47$ ), yielding a significant genotype–sex interaction ( $p = 0.0053$ ) (TABLE 2). This SNP is located on chromosome 7 in intron 4 of the *reelin* gene (*RELN*), which had previously been studied as a candidate for schizophrenia and related phenotypes<sup>72</sup>. In the Ashkenazi Jewish sample, rs7341475 showed high linkage disequilibrium (LD) with other SNPs in the third and fourth intron of *RELN*, but the LD did not extend to neighbouring genes, suggesting that the association with schizophrenia is because of variation in the *RELN* gene.

To confirm that rs7341475 is a female-specific risk factor for schizophrenia, the investigators assessed whether the GG genotype was increased in women with schizophrenia in four other samples — from the United Kingdom, United States, Ireland and China. The predicted direction of effect was present in all the samples, but differences were only significant in the UK sample (TABLE 2). In the combined samples (with and without the primary Ashkenazi Jewish sample), the recessive (GG) genotype was a significant risk factor for schizophrenia in females only (FIG. 2d).

Although the association with rs7341475 did not meet criteria for genome-wide significance (that is, when corrected for multiple testing), the supportive data from four replication samples and the biological plausibility of the involvement of *RELN* in brain abnormalities<sup>73</sup>

Table 1 | **ACE D–I genotype–sex interaction on hypertension**

Sample	Sample size (cases/controls)	OR (95% CI)		Refs
		DD	DI	
<b>Men</b>				
US Caucasian	689/755	1.59 (1.13, 2.23)	1.18 (0.87, 1.62)	50
Japanese	604/1736	1.75 (1.21, 2.53)	1.14 (0.87, 1.51)	49
Serbian	98/112	2.05 (1.07, 3.91)*	NA	51
<b>Women</b>				
US Caucasian	705/945	1.00 (0.70, 1.44)	0.78 (0.56, 1.09)	50
Japanese	596/2079	1.17 (0.79, 1.72)	0.87 (0.65, 1.17)	49
Serbian	77/98	0.72 (0.33, 1.60)*	NA	51

In three independent studies, the D allele at the angiotensin converting enzyme (*ACE*) locus was associated with risk for hypertension in men but not in women. Odd ratios (ORs) and confidence intervals (CIs) from a multivariate model adjusted for other covariates. \*Relative to genotype II. D–I, deletion–insertion; NA, information not available.

Table 2 | Genotype–sex interaction effects of the reelin SNP rs75341475 on schizophrenia

Sample	Sample size (cases/controls)	Frequency of GG genotype (cases/controls)	OR (95% CI)* GG relative to GA+AA
<b>Men</b>			
Ashkenazi	470/1988	0.606/0.619	0.95 (0.77, 1.17)
UK	320/1439	0.709/0.725	0.93 (0.71, 1.21)
US	295/202	0.692/0.698	0.97 (0.66, 1.43)
Irish	669/337	0.750/0.733	1.10 (0.81, 1.48)
Chinese	222/229	0.806/0.830	0.85 (0.53, 1.38)
Combined	1976/4195	NA	0.96 (0.85, 1.10)
<b>Women</b>			
Ashkenazi	265/656	0.755/0.610	1.97 (1.43, 2.71)
UK	155/1488	0.813/0.702	1.85 (1.22, 2.81)
US	109/232	0.725/0.638	1.50 (0.91, 2.46)
Irish	311/245	0.762/0.731	1.18 (0.80, 1.73)
Chinese	193/229	0.845/0.825	1.15 (0.69, 1.93)
Combined	1033/2850	NA	1.58 (1.31–1.89)

\* $P_{\text{interaction}}$  for all samples combined =  $1.6 \times 10^{-5}$  (from REF. 71). CI, confidence interval; NA, information not available; OR, odds ratio.

make these results particularly intriguing. However, mechanistic studies demonstrating functionality of the associated intronic SNP, or a SNP in LD with rs7341475, are still needed. Interestingly, sex-specific gene regulation is suggested by two other observations: the higher expression of *RELN* in layer I neurons in women compared with men; and a reduction of *RELN* expression in the superficial interstitial white-matter neurons in men with schizophrenia, but not in females with schizophrenia<sup>74</sup>. Whether the schizophrenia-associated variation is also associated with *RELN* expression differences in women remains to be determined.

**Recombination rate.** Meiotic recombination is one of the most fundamental biological mechanisms to ensure normal embryonic development. Because too few recombination events can result in nondisjunction and aneuploidy, and ectopic exchange can result in chromosomal rearrangements<sup>75,76</sup>, it is likely that this process is highly regulated<sup>77</sup>. Recently, the rate of recombination<sup>78</sup> and location of recombination<sup>79</sup> were shown to be heritable phenotypes in human pedigrees.

Recombination rate is a sexually dimorphic trait, with overall higher rates in female germ cells in humans, except at the telomeres of chromosomes where male recombination rates exceed those of females<sup>80,81</sup>. A recent genome-wide association study of recombination rates in 1,887 Icelandic men and 1,702 Icelandic women identified a locus that showed significant sex-specific effects<sup>78</sup>. Three SNPs in a block of LD spanning 200 kb on chromosome 4p16.3 showed genome-wide significant evidence of association in men ( $p < 10^{-10}$ ) and two of those SNPs were also genome-wide significant in women ( $p < 10^{-7}$ ). Surprisingly, the combination of alleles that was found to be associated with low recombination rates in men (allele C at rs3796619 and allele T at rs1670533) was

associated with high recombination rates in women. The opposite effect of these SNPs on male versus female recombination was replicated in a second sample of 3,135 men and 3,365 women from Iceland ( $p < 10^{-8}$  in men and  $p < 10^{-4}$  in women). Relative to the average recombination rate in the population, each copy of the rs3796619 C allele decreased recombination rate by 2.62% in men, whereas each copy of the rs1670533 T allele increased recombination by 1.8% in women. The former allele explained 3.5% of the variance in recombination rate in men and the latter allele explained 1.7% of the recombination rate in women.

The associated SNPs were in an 'LD block' that included two genes, spondin 2 (*SPON2*) and ring finger protein 212 (*RNF212*). The authors suggest that *RNF212* is an excellent candidate for a human recombination gene because it is homologous to a gene that is involved in recombination in yeast. However, further studies are required to determine which SNP and which gene influence recombination rates as well as the exact mechanism for the sex-specific effect. Nonetheless, these results illustrate a genotype–sex interaction of alleles with additive and opposite effects in males and females (FIG. 2c). Loci with this type of genotype–sex interaction effects would never be detected in a genome-wide association study in a combined sample of men and women, in which the opposite nature of the association in the two sexes would cancel out any observable effect in combined samples, similar to other genotype–environment interactions<sup>54,82–84</sup>.

### Summary and future directions

Significant sexual dimorphism in prevalence, age of onset, severity or genetic risk is observed for most common human diseases. Elucidating the underlying mechanisms for these observations remains challenging, but is an important area for future research. Because it

#### Nondisjunction

The failure of chromosomes to separate at anaphase.

#### Aneuploidy

The presence of an abnormal number of chromosomes, either more or less than the diploid number.

#### Ectopic exchange

Homologous recombination between non-allelic chromosomal regions.

#### Odds ratio

(OR). Compares the likelihood of an outcome (for example, a disease) between two groups (for example, cases and controls). It is measured as the ratio of the odds in one group to the odds in the second group and can be calculated by the following formula:  $OR = \frac{p(1-q)/q(1-p)}{p(1-q)/q(1-p)}$ , where  $p$  is the probability of the event occurring for the first group and  $q$  the probability for the second group.

is unlikely that sexually dimorphic traits are due to differences in the structure of genes in males and females (with the possible exception of genes on the Y chromosome), the importance of the regulatory genome in this context becomes central to understanding the mechanism(s) of sex-specific effects. Existing variation in regulatory elements that contribute to sexually dimorphic traits could result in sex-specific gene-environment interactions. In addition, sexually dimorphic developmental processes, such as sex-specific changes in gene regulation with age<sup>85</sup>, can result in shifting

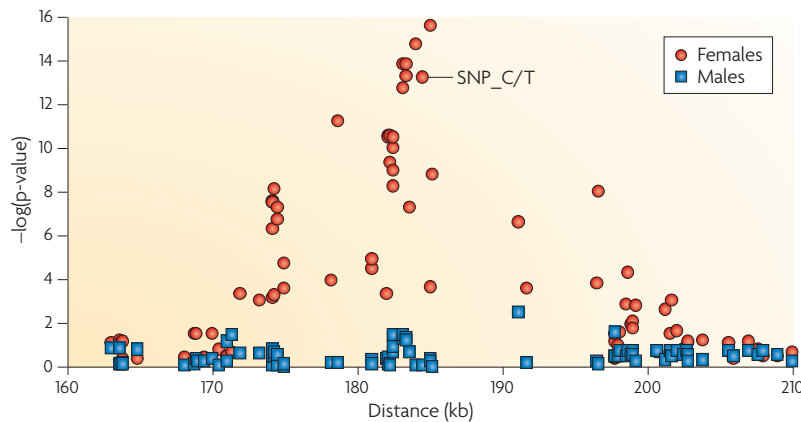
differences in disease susceptibility between the sexes, as has been observed for asthma, for example (FIG. 3).

To date, studies of genotype–sex interactions have interrogated genetic associations that have different effects in males and females on physiological or disease traits. These studies have had varying success, as discussed above<sup>15</sup>. Even among the more prominent examples discussed in this Review, it has not yet been possible to relate the associated polymorphisms to sex-specific differences in the regulation of gene expression. Moreover, a large number of studies of diseases or QTLs in families have reported sex-specific linkages. However, as mentioned above, few studies have demonstrated that differences in LOD scores between males and females are significant or have tested directly for interactions. By contrast, animal-model studies suggest that genotype–sex interactions are widespread and that many important genes will be missed if such interactions are ignored. In that context, we favour testing for genotype–sex interactions in association studies, particularly for sexually dimorphic phenotypes, although appropriate significance testing is required to avoid type I errors. For example, it is striking that genotype–sex interactions on development has not been explored, or that no pharmacogenetic study has looked for genotype–sex interactions on drug response, even though sex-specific responses to drugs are well known<sup>86–88</sup>.

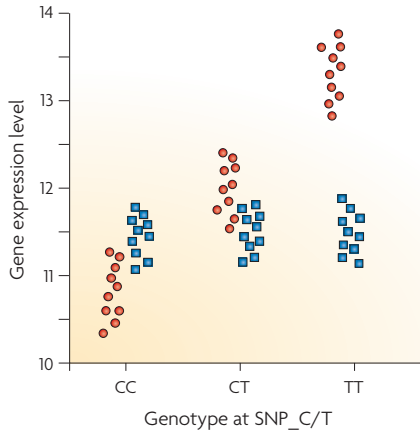
An alternative approach for discovering genotype–sex interactions, particularly in the context of gene regulation, is to directly study gene expression as a quantitative phenotype and identify genetic variation that is associated with expression levels differently in males and females. Because thousands of gene expression phenotypes can be measured simultaneously, it is likely that genotype–sex interaction effects on gene expression will be easier to detect than studies of physiological and disease traits, and that all types of interactions will be present (for example, FIG. 2). With the availability of many data sets with both dense SNP typing and measurements of global gene expression in the same individuals<sup>89–92</sup>, it should be possible to directly assess sex-specific genotype effects on heritable variation in mRNA abundance using eQTL-mapping approaches<sup>90,93</sup>. Moreover, because the ultimate goal of eQTL mapping is to identify regulatory variation that results in physiological or disease phenotypes<sup>94</sup>, this approach can be extended to study the sex-specific architecture of these phenotypes (FIG. 5). Traits or diseases with sex-specific genetic architecture, such as those shown in FIGS 3,4, would be excellent candidates for these studies.

Understanding genotype–sex interaction effects at the level of gene expression would not only shed light on the mechanism of these effects, but might also identify ‘signatures’ for variations that participate in sex-specific gene regulation. Such knowledge might also inform studies of physiological and disease traits by allowing variants to be categorized as more or less likely to participate in the sex-specific regulatory genome, and by identifying genes that are differentially regulated as candidates for sexually dimorphic traits.

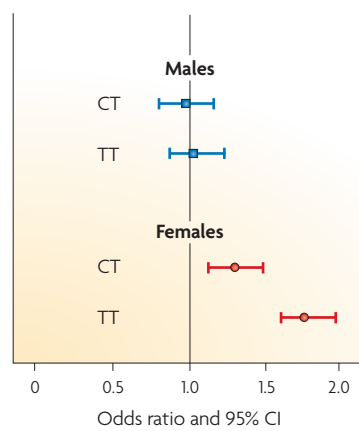
**a** GWAS results for a disease-associated quantitative trait



**b** eQTL studies of associated SNPs



**c** Effect of eQTL on disease



**Figure 5 | Strategy for discovering sex-specific eQTLs contributing to sexual dimorphism in disease risk.** Red symbols are results for females and blue symbols are results for males. **a** | Results of a hypothetical genome-wide association study (GWAS) for disease-associated QTLs (such as those shown in FIG. 4). Analyses in sex-stratified samples identify an association with SNPs spanning a 50-kb region in females but not in males. **b** | mRNA expression level by genotype. Using publicly available expression data<sup>89–92</sup>, eQTLs that reside within the 50-kb region with sex-specific effects on expression levels can be identified; changes in gene expression caused by SNP\_C/T are shown here. Each copy of the T allele at this eQTL is associated with increased expression in females but has no effect on expression in males (FIG. 2a). **c** | Odds ratios and 95% confidence interval (CI) for disease risk by genotype at SNP\_C/T. Validation of a role for the SNP\_C/T eQTL on disease risk is determined by directly demonstrating a genotype-specific risk for disease in one sex only, in a direction that is consistent with the patterns observed with the associated QTL and eQTL. In this example, each copy of the T allele is associated with increased risk for disease in females. The SNP is not associated with disease risk in males. This model of association is also represented in FIG. 2a.

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## DATABASES

Entrez Gene: <http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene>  
ACE | RELN | RNF212 | SPON2

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Carole Ober's homepage: <http://www.genes.uchicago.edu/ober.html>  
Yoav Gilad's homepage: <http://www.genes.uchicago.edu/gilad.html>  
Centers for Disease Control National Health and Nutrition Examination Survey (NHANES) III: <http://www.cdc.gov/nchs/nhanes.htm>  
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