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Sex-Specific Genetic Architecture of Human Disease

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Abstract

Sexual dimorphism in anatomical, physiological, and behavioural traits characterize 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.

Differences between males and females in anatomical, physiological, and behavioral traits characterize many vertebrate species, including humans. Although some may be apparent at birth, striking differences between the sexes most often emerge at or around the time of sexual maturation. It is thought that these are, in large part, due to sex hormone levels that differ in males and females beginning *in utero* and continuing throughout life¹ (Figure 1). The genetic contribution to sexual dimorphism was, until recently, less studied. Indeed, whereas genes on sex chromosomes contribute to many sexually dimorphic traits, the autosomal genome is generally assumed to be similar among the males and females of a species. Mechanisms for dosage compensation in HETEROGAMETIC species further assure that genetic contributions from the shared sex chromosome (X chromosome in mammals) is equivalent among males and females, at least for most genes².

Recent studies have challenged this paradigm, however, suggesting that natural variation within the autosomal genomes of many species also affects anatomical, physiological, and behavioral traits differently in males and females^{3–5}. In this context, sex can be considered an ‘environmental’ variable that includes the cellular, metabolic, physiological, anatomical, and even behavioral differences between boys and girls (in childhood) or between men and women (in adulthood). Sex, then, may interact with genotype in a manner similar to other environmental factors (Figure 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 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 the predominance of men with cardiovascular disease throughout adult life but a higher rate of occurrence in post-menopausal women compared to men⁶, the higher prevalence of asthma among boys in childhood and higher occurrence of new cases among girls around and following puberty⁷, and the increased prevalence of autoimmune diseases in women throughout life but particularly for diseases that onset during or immediately following the reproductive years⁸ (Figure 3). In addition to those diseases highlighted in Figure 3, significant sex differences have been described for many common birth defects, 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 boys⁹. In adulthood, major depression and Alzheimer disease are more common in women^{10,11} whereas schizophrenia, Parkinson disease, and colorectal cancer are more common in men¹²⁻¹⁴.

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 females¹⁵, as many of these differences could be due to hormonal profiles, particularly with regard to sex steroids (Figure 1), or to behaviors that differ between the sexes (e.g., exposure to cigarette smoke)¹⁶. 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-menopausal (cardiovascular disease) have implicated sex hormones as important mediators of disease pathogenesis and contributors to sex differences in prevalence rates and progression.

Importantly, differences in the immune systems of males and females have been observed as early as in the first few years of life, suggesting a developmental component to sex-specific differences in disease risk¹⁷. Such differences could result in sex-specific thresholds of susceptibility to immune-mediated diseases throughout life. Interestingly, immune responses may be modulated by sex hormones^{18,19}. In fact, the transient rise in sex steroid levels ('minipuberty') that occurs in early infancy¹ (Figure 1), could pattern immune cells differently in boys and girls. Thus, both the immune and endocrine systems likely contribute to sexual dimorphism in the epidemiology of many common diseases. However, recent evidence suggests that some of the differences between males and females may also be due to differences in genetic architecture. The review henceforth will focus on such sex-specific genetic effects.

Sex Effects on Disease Risk through Gene Regulation

Contribution of sex chromosomes

The contributions of the sex chromosomes to sex-specific GENETIC ARCHITECTURE of human disease has long been appreciated. For example, an excess of boys express X-chromosome-linked recessive diseases, and skewed patterns of X chromosome inactivation resulting in varied expression of disease phenotypes are seen in female carriers of X-linked mutations²⁰. 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 harbors relatively few genes, most of which are expressed exclusively in the testes and others that are typically thought of as 'housekeeping' genes (of the latter, most have X chromosome homologues that escape X inactivation)²¹. Thus, it is perhaps unlikely that Y-linked genes *per se* directly affect disease risk, other than constituting major contributors to genetic causes of male infertility²². However, Y-linked genes may 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^{23–26}. 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 worms²⁷, flies^{28,29}, fish³⁰, rodents^{25,31}, and primates²³. 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 level²⁶. 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 Parsch²⁶ 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 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* species³². Similarly, Reinus et al. found a signature of evolutionary conserved sexually dimorphic gene expression in the brain of three primate species, including humans²³. 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 underlie important phenotypic differences (developmental, physiological and/or behavioural) between the sexes. These conserved sexually dimorphic gene expression patterns suggest the existence of constant regulatory differences between males and females, which may be beneficial to each sex but can also contribute to different gene–environment interactions in the two sexes. In turn, such differences may 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 females³³.

A second interesting observation is that sexually dimorphic gene expression patterns are often tissue-specific²⁵, whereby a gene may be differentially expressed between the sexes in some but not in other tissues. This important observation suggests that a different architecture of regulatory interactions may underlie gene expression patterns in males and females in different tissues. Hence, it is likely that entire regulatory networks may 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 may account for genotype–sex interactions that affect other measurable phenotypes as well as 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 long-term moderate/high fat diet on mice. They found phenotypic differences between males and females, including differences in gene regulation, following the application of identical diet in the two sexes³⁴.

Bhasin et al. provided additional support for this hypothesis by mapping sex-specific EXPRESSION QTL (eQTL) in mice³⁵. They identified SNPs in putative *cis* regulatory elements that 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 among the sexes, differences in the use of *cis* regulatory elements between the sexes indicate sex-specific differences in *trans* elements (e.g., transcription factors and co-factors). Sex steroid receptors may be one example of sex-specific *trans* regulatory elements³⁶. Similar analyses of human eQTL data have not been performed to date, yet the findings of Bhasin et al.³⁵ are consistent with a growing number of observations^{23,25,28,30,32} suggesting that ignoring sex in studies of gene expression will underestimate, perhaps quite dramatically, the affect of genetic variations on gene regulation and mRNA abundance.

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

Evidence of 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 (i.e., genetic differences between individuals) is referred to as the *HERITABILITY* of the trait. The genetic variance can be further divided into the variance due to additive genetic effects, to non-additive genetic effects (e.g., dominance, recessiveness, epistasis), as well as be assigned to autosomes or sex chromosomes. The proportion of the variance 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 derivation of the individual variance components has recently been reviewed³⁷. 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 in males and females, many of which are associated with common diseases, was investigated in a large multigenerational pedigree comprised of >500 members of the Hutterites, a founder population that practices a communal lifestyle^{41,42}. 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 and rare, meals are eaten and prepared in a communal kitchen, and large families desired⁴³. Moreover, because all relative pairs in the extended pedigree are considered in the analysis, it was possible to estimate both additive and dominance variance components⁴⁴.

In this population, sex was a significant predictor of the trait value in a linear regression model for 16 of the 19 phenotypes, including cardiovascular disease-associated traits (HDL cholesterol, lipoprotein[a], triglycerides, diastolic and systolic blood pressure), asthma-associated traits (FORCED EXPIRATORY FLOW AT 1 SECOND, (FEV₁), the ratio of FEV₁ to forced vital capacity [FVC], eosinophil count, total serum IgE level, percent lymphocytes), anthropometrics (body mass index, percent fat, fat free mass, adult height), and signaling molecules (morning serum cortisol, whole blood serotonin). Sex was not a significant predictor of three phenotypes (LDL cholesterol, lymphocyte count, fasting insulin).

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, triglycerides) or in both sexes (lipoprotein[a], whole blood serotonin). Interestingly, four traits had significant non-X sex interactions in which either the estimates of heritability were significantly different between males and females (LDL-cholesterol, FEV₁:FVC) or the best-fitting heritability model was different between males and females (HDL-cholesterol, fat free mass). Thus, the genetic architecture of nine (of 19) 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 Figure 4.

Taken together, these data suggest that the genetic architecture (additive, dominant, X-linked) and/or the overall genetic contribution (heritability) significantly differs between males and females for a large number of quantitative phenotypes, many of which are risk factors for common diseases, consistent with other studies of sex-specific heritabilities of common disease-associated quantitative phenotypes^{45,46}. Although this data set is limited to only 19 quantitative traits, it further suggests that X chromosome genes may 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 families⁴⁵, and for bone mineral density in a number of recent studies (reviewed in Karasik and Ferrari⁴⁶).

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.

Accumulating 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 risk⁴⁷. 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. On the other hand, the roughly halving of the sample size to conduct sex-specific analysis reduces the power to detect an effect. For example, a study with 80% power for a main effect will have only 29% power to detect an interaction of the same magnitude⁴⁸, 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 studies¹⁵. 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 (unadjusted for multiple testing). Sixty of those claims were judged to have good internal validity, including the one association that was replicated. This was the association between the deletion/insertion (D/I) polymorphism in the angiotensinogen converting enzyme (*ACE*) gene with hypertension in men only^{49–52} (discussed below).

Despite these limitations, a number of 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 while many linkage studies have reported sex effects, only few have shown that increased 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 Effects in Model Systems

The most compelling and consistent evidence for genotype–sex interaction effects comes from studies of physiological, anatomical, and behavioral traits in model organisms, including fruitflies^{54,55}, mice^{56–59}, and rats⁶⁰. 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 Mackay and Anholt⁵⁵).

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^{56,57}. Other examples include knocking out the cytochrome P2J5 gene in C57BL/6 mice, and the resulting sex-specific effects on blood pressure and renal phenotypes⁵⁸, and studies of CONSONIC STRAINS of mice that revealed sex-specific effects of individual chromosomes on fear conditioning⁵⁹. Similarly, in consomic rat strains, sex–specific effects on phenotypes related to hypertension and kidney disease predominate⁶⁰.

It should be noted that many of the mapping studies claiming sex effects in model organisms suffer from the same limitations as those described above for human studies. However, the experimental toolbox available for studies of model organisms allows for a more thorough dissection of sex-specific genetic architecture, which has, in many cases, 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

Example 1: Hypertension and Blood Pressure

Hypertension is a major risk factor for cardiovascular disease, stroke, and end-stage renal disease⁶¹. In 2005 the prevalence of hypertension of the adult population worldwide was 26%⁶². Blood pressure is higher in men compared to 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^{63,64}, similar to overall trends for cardiovascular disease (Figure 1). Genes involved in the renin-angiotensinogen system are functional candidates for blood pressure regulation and hypertension, and have been associated with these phenotypes with varying success (reviewed in Kato et al.⁶⁵). A 250 bp deletion/insertion (D/I) polymorphism in intron 16 of the angiotensin converting enzyme (*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 levels⁶⁶. *ACE* was considered 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 (ref.⁵⁰ and references therein).

Studies in a rat model suggested genotype–sex interactions⁶⁷. Both male and female rats heterozygous for an inactivating mutation in *Ace* had lower *Ace* protein levels compared to wild type animals (23% reduction in males and 35% reduction in females). However, only heterozygous males had a reduced blood pressure compared to the wild type males; heterozygous females had blood pressures similar to wild type females. Therefore, low *Ace* levels 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^{49–51} (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 even in the absence of a genotype–sex interaction in quantitative trait variation (in this example, *ACE* protein levels⁶⁶), a genotype–sex interaction can still occur with respect to an associated physiological trait (e.g., blood pressure)^{50,52} and a disease phenotype (e.g., hypertension)^{49–51}. Lastly, these studies further 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 (models B and E, respectively, in Figure 2), suggesting a quantitative (blood pressure) threshold effect for expression, or *PENETRANCE*, of a common disease (hypertension) that is sex-specific.

Example 2: Schizophrenia

Schizophrenia is a common psychiatric disorder with significant sex differences in prevalence, age of onset and morbidity⁶⁸. For example, most cases occur between the ages of 16 and 25 years in men and between the ages of 25 and 30 years among women. Overall, the male to female sex ratio is 1.4^{12,69}. Estimates of heritability for this complex disease is approximately 0.80⁷⁰, 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 replicated¹⁵.

Shifman and colleagues conducted a genome-wide association study for schizophrenia using a novel DNA pooling strategy⁷¹. One hundred ninety four SNPs were selected for further studies based on their ranking and statistical significance in the studies in pooled DNA, and their biological plausibility⁷¹. 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 (G→A), for which the frequency of the GG genotype was 0.76 in female patients compared to 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 or related phenotypes (Ref72 and references therein). In the Ashkenazi Jewish sample, rs7341475 showed high LINKAGE DISEQUILIBRIUM (LD) with other SNPs in the third and fourth intron of the *RELN* gene, but the LD did not extend to neighboring genes, suggesting that the association with schizophrenia is with 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 U.K., U.S., Ireland, and China. The predicted direction of effect was present in all the samples, but differences were only significant in the U.K. 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 (Figure 2, **panel D**).

Although the association with rs7341475 did not meet criteria for genome-wide significance (i.e., corrected for multiple testing), the supportive data from four replication samples and the biological plausibility of the involvement of *RELN* in brain abnormalities⁷³ 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, however, higher expression of the *RELN* gene (in layer I neurons) in women compared to men and a reduction of *RELN* expression (in the superficial interstitial white matter neurons) in men with schizophrenia but not in females with schizophrenia⁷⁴, suggests sex-specific gene regulation. Whether the schizophrenia-associated variation is also associated *RELN* expression differences in women remains to be determined.

Example 3: 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^{75,76}, it is likely that this process is highly regulated⁷⁷. Recently, the rate of recombination⁷⁸ and location of recombination⁷⁹ were shown to be heritable phenotypes in human pedigrees.

Recombination rate itself 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^{80,81}. A recent genome-wide association study of recombination rates in 1887 Icelandic men and 1702 Icelandic women identified a locus that showed significant sex-specific effects⁷⁸. 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 associated with low recombination rates in men (allele C at rs3796619 and allele T at rs1670533) were 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 3135 men and 3365 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 rs3796619 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 suggested that *RNF212* is an excellent candidate for a human recombination gene because it is homologous to a gene involved in recombination in yeast, although 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 (Figure 2, Panel C). 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, where 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^{54,82-84}.

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 represents an important area for future research. Because it 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 mechanism. Standing 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 age⁸⁵, can result in shifting differences in disease susceptibility between the sexes, for example, as has been observed for asthma (Figure 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 above¹⁵. 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 have demonstrated that differences in lod scores between males and females are significant or tested directly for interactions. On the other hand, 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 favor 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 no pharmacogenetic study to date has looked for genotype–sex interactions on drug response, although sex-specific responses to drugs are well known^{86–88}, or that genotype–sex interactions on development has not been explored.

An alternative approach for discovering genotype–sex interactions, in particular 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 (e.g., Figure 2). With the availability of many data sets with both dense SNP typing and measurements of global gene expression in the same individuals^{89–92}, it should be possible to directly assess sex-specific genotype effects on heritable variation in mRNA abundance using eQTL mapping approaches^{90,93}. Moreover, because the ultimate goal of eQTL mapping is to identify regulatory variation that results in physiological or disease phenotypes⁹⁴, this approach can be extended to study the sex-specific architecture of these phenotypes (Figure 5). Traits or diseases with sex-specific genetic architecture, such as those shown in Figures 3 and 4, would be excellent candidates for these studies.

Understanding genotype–sex interactions at the level of gene expression would not only shed light on mechanism, but may also identify “signatures” for variations that participate in sex-specific gene regulation. Such knowledge may 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.

Box 1. Sexual Dimorphism

Following Darwin's (1859) observation that males and females may have the same “general habits of life” but “differ in structure, color, or ornament”⁹⁵, research on sexual dimorphism progressed gradually from qualitative descriptions of conspicuous anatomical and behavioral traits in animals⁹⁶ to elegant experiments probing the sex-specific neural circuitry of reproductive behavior in flies^{97,98} and mice⁹⁹. The results of this century-and-a-half of research demonstrated that sexual dimorphism was taxonomically widespread and

remarkably variable in the magnitude and form of its expression^{100,101}. It is now quite obvious that sex-specific differences occur not only in conspicuous morphological traits (i.e. size, shape, and coloration) but also in a diverse suite of behavioral^{97-99,102}, psychological^{102,103}, biochemical^{69,103}, and gene expression^{23,24,26} phenotypes.

Variation in the magnitude of sexual dimorphism among closely related species, and sometimes within a species, motivated biologists to test Darwin's (1859) hypothesis that sex-specific differences were largely due to sexual selection, particularly male–male competition, in dozens of different taxa¹⁰¹. The results of these studies consistently reaffirmed the importance of sexual selection (via male–male competition and/or female choice) as a major driver of sexual dimorphism, but also suggested a significant role for natural selection and non-selective forces, i.e. genetic, ecological, and developmental pressures and constraints, in the evolution of sex-specific phenotypic divergence^{100,104}. Indeed, future research into the nature and consequences of intersexual genetic correlations¹⁰⁵ and intersexual ONTOGENETIC CONFLICT¹⁰⁶ will lead to a more sophisticated understanding of the evolution and expression of sexual dimorphism.

Box 2. Microchimerism and Disease

As a result of bi-directional cell trafficking between the mother and fetus during pregnancy, mothers may harbor cells from their children and children may harbor cells from their mother will into adulthood. This mixture of a small amount of cells from a genetically disparate individual is referred to as microchimerism¹⁰⁷. The persistence of maternal cells in her children, called maternal microchimerism, has been detected in the peripheral blood mononuclear cells in approximately 22% of healthy individuals¹⁰⁸⁻¹¹¹. The persistence of fetal cells in the mother is called fetal microchimerism, which has been detected in peripheral blood mononuclear cells in 30% to 55% of healthy women, depending on the outcome of the pregnancy¹¹². 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, monocyte/macrophages, and natural killer cells¹¹³. 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 types¹¹⁴⁻¹¹⁶. While 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 autoimmune disease^{110,117-124}, including most of the diseases shown in Figure 3c. The idea that the destructive immune response causing disease may be directed at the chimeric cells raised the suggestion that some autoimmune diseases may in fact be ALLOIMMUNE¹²⁵. Lastly, the onset of many autoimmune diseases in women during and immediately following the reproductive years has been attributed to microchimerism¹²⁶, suggesting that exposure to fetal cells during pregnancy is a sex-specific risk factor for autoimmune disease.

Box 3. Genetic Imprinting and Parent-of-Origin Effects

One mechanism for sex-specific transmission of disease or 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 (i.e., allele-specific silencing). The best studied silencing mechanism is methylation, and differential methylation between alleles is considered the hallmark feature of an imprinted locus¹²⁷. 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 previously reviewed¹²⁷⁻¹³⁰.

In roughly half of imprinted genes the maternally-inherited allele is silenced (i.e., 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 bi-allelic and monoallelic expression is observed between individuals^{131,132}. 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, behavior and learning, and carcinogenesis^{127,128}.

A census of imprinted genes in 2005 suggested that approximately 41 genes in 16 chromosomal regions are imprinted in humans, compared to 71 genes in 22 chromosomal regions in mice (29 of the same genes are imprinted in both humans and mice)¹³⁰. The authors speculated 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. They cite in support of the latter 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 for disease in the child differs depending on whether the 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 may 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 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 which arose or persisted into adulthood¹³³. Therefore, some of the sex-specific parent-of-origin effects observed in complex human diseases, such as those mentioned above, may be attributable to genomic imprinting.

Glossary

Heterogametic (species), Refers to species that produce gametes that differ with respect to sex chromosomes. In mammals, males are the heterogametic sex (XY) and females are homogametic (XX), whereas in birds, females are heterogametic (ZW).

Pyloric stenosis (infantile hypertrophic 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.

Genetic architecture, Refers to the underlying genetic basis for a trait.

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. Competition for fertilization can occur through competition among the same sex (mate competition) or through attraction to the opposite sex (mate choice).

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 (FEV₁), The volume exhaled in the first second of a forced expiratory maneuver. This index is used to assess airway obstruction, bronchoconstriction, or bronchodilation.

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

Multiple testing, When multiple independent hypotheses are tested, the combined probability of type I error increases in an unadjusted analysis.

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, 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.

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.

eQTL, Loci at which genetic allelic variation is associated with variation in gene expression.

Ontogenetic conflict, occurs when an allele is advantageous at one stage of development and disadvantageous at another stage, or when it is advantageous in one sex and disadvantageous in the other sex, so that the allelic effects are antagonistic with respect to fitness.

Alloimmune, An immune reaction against cells from another individual of the same species.

Alloimmunity can occur during transfusion or transplantation, or during pregnancy.

Odds ratio (OR), Compares the likelihood of an outcome (e.g., a disease) between two groups (e.g., 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: $p(1-q)/q(1-p)$, where p is the probability of the event occurring for the first group and q the probability for the second group.

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Figure 1. Approximate mean sex steroid levels in plasma in males and females

Variation in steroid levels is shown as percent of the maximum mean testosterone (T) in males and the maximum mean estradiol (E) in females across the life stages. 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 5-fold during the pre-ovulatory and luteal phases of the menstrual cycle. Note the drop in levels of all sex steroids at birth and the transient 'minipuberty' in early infancy. Free testosterone falls more with aging (to approximately 50% of the maximum in 80 year old men) than the total testosterone¹³⁴, which is shown here. Modified from Alonso and Rosenfield 2002¹, Khosla et al. 1998¹³⁵, Winters et al. 2000¹³⁶.

Figure 2. Models of genotype-sex interactions reflecting genotype effects that differ between males and females. [I'll arrange for 'B' to be changed to 'a' in this figure]

For any measured phenotype or disease risk (y axes), the genotypic effects may be apparent only in females (red; panels a, d), only in males (blue; panels b, e), or be present in both sexes but with opposite directions of effects (panels c, f). The genotype effects can be additive (panels a-c) or recessive (panels d-f). Other models (e.g., dominant) or interactions (e.g., same direction of effect but differences in magnitude of effect) are not shown. Examples discussed in this review illustrate panel e (relationship between the DD genotype of the angiotensinogen converting enzyme (*ACE*) and hypertension), panel b (relationship between the DD genotype of *ACE* and blood pressure), panel d (relationship between the reelin (*RELN*) rs7341475-GG genotype and schizophrenia), and panel c (relationship between chromosome 4p16.3 SNPs rs3796619 and rs1670533 SNPs and recombination rate). Red lines track phenotypic values by genotype in females; blue lines track phenotypic values by genotype in males.

Figure 3. Sex-specific prevalence rates, age of onset, and sex ratios for common sex-skewed diseases
 The key for background colors is shown in Figure 1 [I'll ask for the key to be copied to this figure]. **a** Cardiovascular disease in the U.S. (from the National Health and Nutrition Examination Survey (NHANES) III 1988–1994)⁶. Note the increase in female prevalence rates in the post-menopausal period. **b** Asthma in the U.S. from 1998–2006 (Center 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 U.S. and Europe^{137,138}. Note the female skewing at all ages, with the largest skew and number of diseases with onset during and immediately following the reproductive years. T1D, type 1 diabetes; JIA, juvenile idiopathic arthritis; JDM,

juvenile dermatomyositis; MS, multiple sclerosis; MG, myasthenia gravis; GD, Grave's disease; SLE, systemic lupus erythematosus; SSc, systemic sclerosis (scleroderma); AD, Addison disease; DM, dermatomyositis/polymyositis; RA, rheumatoid arthritis; TH, thyroiditis; SS, Sjögren's disease.

Figure 4. Sex-specific heritabilities in males and females (data from Pan et al. 2007⁴¹)

Six quantitative traits with significant sex-specific genetic architecture show differences between males and females in the overall estimates of H^2 (e.g., LDL cholesterol, lipoprotein [a], systolic blood pressure) and/or with respect to the best-fitting model (triglycerides, HDL cholesterol, systolic blood pressure, height) are shown.

a GWAS results for a disease-associated quantitative trait **b eQTL studies of associated SNPs** **c Effect of eQTL on disease**

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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 genome-wide association study for a disease-associated QTL (such as those shown in Figure 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 data⁸⁹⁻⁹², eQTL that reside within the 50 kb region with sex-specific effects on expression levels can be identified. Each copy of the T allele at this eQTL is associated with increased expression in females but has no effect on expression in males (Figure 2a). **c)** ODDS RATIOS for disease risk by genotype. Validation of a role for the 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 Figure 2a.

Table 1
***ACE* D/I genotype–sex interaction on hypertension**
 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 multivariate model adjusted for other covariates.

	Sample Size (Cases/Controls)		OR (95% CI) Relative to Genotype II		
Sample			DD	DI	Reference
Men					
U.S. Caucasian	689/755		1.59 (1.13, 2.23)	1.18 (0.87,1.62)	O'Donnell ⁵⁰
Japanese	604/1736		1.75 (1.21, 2.53)	1.14 (0.87, 1.51)	Higaki ⁴⁹
Serbian	98/112		+2.05 (1.07, 3.91)	NA	Stanković ⁵¹
Women					
U.S. Caucasian	705/945		1.00 (0.70, 1.44)	0.78 (0.56, 1.09)	O'Donnell ⁵⁰
Japanese	596/2079		1.17 (0.79, 1.72)	0.87 (0.65, 1.17)	Higaki ⁴⁹
Serbian	77/98		+0.72 (0.33, 1.60)	NA	Stanković ⁵¹

NA, information not available.

⁺ Relative to genotype II.

Table 2
Genotype-sex interaction effects of the *Reelin* SNP rs75341475 (G→A) on schizophrenia

$P_{\text{interaction}}$ for all samples combined = 1.6×10^{-5} (from ref.⁷¹).

Sample	Sample Size (Cases/Controls)	Freq. GG (Cases/Controls)	OR* (95% CI) GG Relative to GA+AA
Men			
Ashkenazi	470/1988	0.606/0.619	0.95 (0.77, 1.17)
U.K.	320/1439	0.709/0.725	0.93 (0.71, 1.21)
U.S.	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	--	0.96 (0.85, 1.10)
Women			
Ashkenazi	265/656	0.755/0.610	1.97 (1.43, 2.71)
U.K.	155/1488	0.813/0.702	1.85 (1.22, 2.81)
U.S.	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	--	1.58 (1.31–1.89)