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Genetic Mechanisms Leading to Sex Differences Across Common Diseases and Anthropometric Traits

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ABSTRACT Common diseases often show sex differences in prevalence, onset, symptomology, treatment, or prognosis. Although studies have been performed to evaluate sex differences at specific SNP associations, this work aims to comprehensively survey a number of complex heritable diseases and anthropometric traits. Potential genetically encoded sex differences we investigated include differential genetic liability thresholds or distributions, gene–sex interaction at autosomal loci, major contribution of the X-chromosome, or gene–environment interactions reflected in genes responsive to androgens or estrogens. Finally, we tested the overlap between sex-differential association with anthropometric traits and disease risk. We utilized complementary approaches of assessing GWAS association enrichment and SNP-based heritability estimation to explore explicit sex differences, as well as enrichment in sex-implicated functional categories. We do not find consistent increased genetic load in the lower-prevalence sex, or a disproportionate role for the X-chromosome in disease risk, despite sex-heterogeneity on the X for several traits. We find that all anthropometric traits show less than complete correlation between the genetic contribution to males and females, and find a convincing example of autosome-wide genome-sex interaction in multiple sclerosis ($P = 1 \times 10^{-9}$). We also find some evidence for hormone-responsive gene enrichment, and striking evidence of the contribution of sex-differential anthropometric associations to common disease risk, implying that general mechanisms of sexual dimorphism determining secondary sex characteristics have shared effects on disease risk.

KEYWORDS sex differences; gene–sex interactions; heritability; sex heterogeneity; hormone-responsive genes

SEX differences are a major predictor in many common diseases, used in diagnosis, prognosis, and treatment recommendations. We know much about the biological basis of sex determination (She and Yang 2014), and research in model organisms allows us to separate the effects of sex chromosomes and hormonal differences (Arnold and Chen 2009; Cox *et al.* 2014). However, we do not fully understand how the biology of sex shapes disease risk and outcomes in humans (Ober *et al.* 2008; Ngo *et al.* 2014; Austad and Bartke 2015). While some studies in model organisms suggest major roles for gene–sex interaction in complex traits (Mackay 2009; Lehtovaara *et al.* 2013; Bearoff *et al.* 2015; Parks *et al.* 2015), a recent study using mouse models found few true sex interaction effects (Krohn *et al.* 2014). Human studies of disease-relevant quantitative traits in founder populations suggested major sex differences in heritability and identifiable genetic loci (Weiss *et al.* 2006), as well as a major role for the X-chromosome (Pan *et al.* 2007). Twin studies have been used to investigate gene–sex interaction in a variety of complex diseases and traits, with a range of findings from little to substantial sex difference (Vink *et al.* 2012; Mitchem *et al.* 2014; Richmond-Rakerd *et al.* 2014). Additionally, several studies have examined loci identified in combined-sex samples to identify gene–sex interactions in these candidate regions (Avery *et al.* 2006; Silander *et al.* 2008; Loisel *et al.* 2011; Gilks *et al.* 2014; Yao *et al.* 2014;

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de Castro-Catala *et al.* 2015; Mersha *et al.* 2015). However, few studies have applied more sophisticated genome-wide methodologies for assessing association and determining additive SNP-based heritability to comprehensively assess sex differences (Zillikens *et al.* 2008; Chiu *et al.* 2010; Luo *et al.* 2010; Myers *et al.* 2014).

In this work, we selected nine common diseases, and nine heritable traits, with rich genetic datasets available and a variety of sex biases, to investigate several genetic hypotheses about the drivers of sexual dimorphism. For discrete traits, we examine consistent adherence to liability threshold (LT) models (Hayeck *et al.* 2015; Weissbrod *et al.* 2015), which are commonly used in contemporary heritability analyses (Cross-Disorder Group of the Psychaitric Genomics Consortium 2013; Lee *et al.* 2011). Under an LT model of disease, individuals have an underlying normally distributed phenotype, ϕ, called the *liability*. When an individual's liability is greater than a threshold, t, they are a case, and are a control otherwise. In order to induce a sex-biased disease prevalence, the liability distributions, and/or thresholds, must differ between males and females. When males and females have identical distributions of liability, but a sex-biased prevalence exists due to a difference in sex-specific thresholds, the lower prevalence sex will have an enrichment of genetic associations due to the increased genetic load required to exceed the higher threshold. This is mathematically equivalent to one sex having an environmental risk factor, (*e.g.*, if androgens affect the mean liability, the disease prevalence will differ between males and females), and again the lower prevalence sex will have a relatively increased genetic load among cases.

To evaluate this LT model (hypothesis 1, Figure 1), we consider autosomal genetic load in both sexes to determine whether differences in polygenic burden can account for sex differences in prevalence. We use enrichment of autosome-wide association signal in male-specific and female-specific datasets (1a) and polygenic additive SNP-based heritability estimates (h^2_g) to determine whether males and females have different genetic loads for common disease and anthropometric traits (1b), and whether the genetic patterns follow those expected under the liability threshold model based on prevalence differences, or imply the existence of nongenetic sex differences in the mean or variance of liability or trait distributions.

Second, we globally test for evidence of gene–sex interaction (hypothesis 2, Figure 1) to determine whether similar or different autosomal loci might contribute to disease risk across the sexes. Much of the gene–sex interaction literature is focused on specific genetic loci that might differ in their effects by sex. We assess more globally whether evidence exists for sex-heterogeneity in association signal (2a), significant sex-interaction terms in h^2_g models, or a genetic correlation <1 for the same trait across the sexes (2b). We also use simulation to examine the effects of liability variance differences between sexes on disease prevalence that can occur in the presence of gene–sex interactions.

Third, we dissect the role of the X-chromosome (hypothesis 3, Figure 1), the major genomic sex difference (Ross *et al.* 2005). The X-chromosome is gene-rich, contrasting with the small gene-poor Y chromosome (Ellis and Affara 2006; Mulugeta *et al.* 2016), and, despite dosage-compensation mechanisms, shows gene expression differences across the sexes (Jansen *et al.* 2014). The X-chromosome has been proposed to contribute to autoimmune disease; metabolic and cardiovascular traits such as fasting insulin, blood pressure, and cholesterol levels; and anthropometric traits such as height (Pan *et al.* 2007; Chen *et al.* 2012; Gao *et al.* 2014; Tukiainen *et al.* 2014). We examine sex differences in association signal on the X-chromosome between males and females (3a).

Finally, we postulate that gene-environment interactions might generate sexual dimorphism (hypothesis 4, Figure 1), and, at the cellular level, steroid hormones could be a significant contributor. Thus, we specifically consider genes whose expression is known to be responsive to androgens or estrogens, and assess whether SNPs in these genes contribute disproportionately to association signal (4a), or heritability (4b), in complex disease or anthropometric traits. Similarly, we ask whether the same underlying biology is responsible for secondary sex characteristics like height, weight, and body proportions, and we assess whether SNPs showing differential association to anthropometric traits by sex contribute markedly to common, complex heritable disease (Roach *et al.* 2015).

Materials and Methods

Samples

WTCCC1 and WTCCC2 data were gathered from the Wellcome Trust Case Control Consortium (WTCCC; http://www.wtccc.org.uk/). WTCCC1 includes BD (prevalence 0.005), CAD (prevalence 0.06), CD (prevalence 0.001), HT (prevalence 0.26), RA (prevalence 0.005), T1D (prevalence 0.005), and T2D (prevalence 0.08); WTCCC2 includes AS (prevalence 0.003) and MS (prevalence 0.001) (Table S1).

Genetic Investigation of Anthropometric Traits (GIANT) genome-wide meta-analyzed data were gathered from the Broad Institute (http://portals.broadinstitute.org/collaboration/giant/index.php/GIANT_consortium_data_files). The 2015 datasets were used for all anthropometric traits, except for when unavailable [such as for Height and Weight; 2012 uploads were used instead (Yang *et al.* 2012)] (Table S1).

Software

PLINK v 1.90b2n and R v 3.0.1, and METASOFT v2.0.0 were used in association enrichment and heterogeneity analyses. GCTA v 1.24.2 was used for REML estimates of heritability. PCGC regression was used for Haseman-Elston regression estimates heritability. HAPI-UR v 1.01 was used for prephasing, and IMPUTE v 2.3.0 was used for imputation.

Creation of the hormone-responsive gene sets

An androgen-responsive gene list was gathered from ARGDB (Jiang *et al.* 2009) (Table S2), with duplicates in name removed for a total of 2616 genes. Of these 2616 genes,

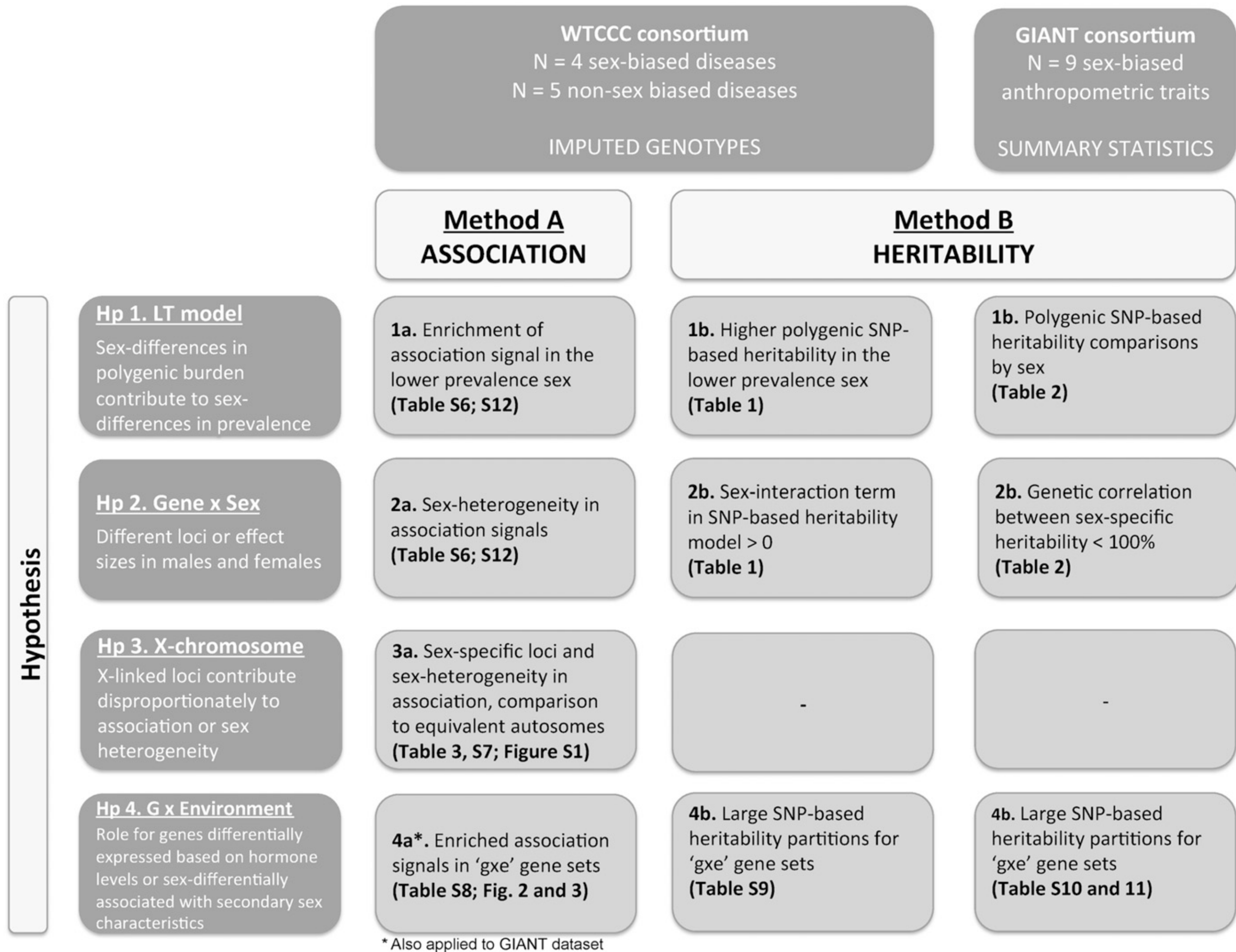

Figure 1 Analyses flow chart. The scheme reports four main hypothesis tested with two approaches (method A: association; method B: heritability). Method A was applied to the imputed genotypes available for WTCCC dataset (when possible also to summary statistics available for GIANT dataset), whereas method B was applied to both WTCCC and GIANT datasets. For each hypothesis and method, the results are reported in the *Results* section, and summarized in figures and tables: results for hypothesis 1 tested in the WTCCC dataset are reported in Table 1 and in the GIANT dataset in Table 2. Table 1 and Table 2 also report results for hypothesis 2 tested in the WTCCC dataset and GIANT dataset, respectively. Results for hypothesis 3 are shown in Table 3. Finally, hypothesis 4 results are shown in Figure 2 and Figure 3.

2508 are autosomal. *CNTNAP2* was removed from androgen-responsive analyses due to difficulty of matching on gene-length in gene-permutations, as detailed below.

An estrogen-responsive gene list was gathered from ERGDB (Tang *et al.* 2004) (Table S3), with duplicates in name removed for a total of 1431 genes. Of these genes, 1150 are autosomal. *GRID2* was removed from estrogen-responsive analyses due to difficulty of matching on gene-length in gene-permutations, as detailed below.

Creation of top heterogeneous anthropometric hits

METASOFT was used on GIANT consortium data (further elaborated on below) to ascertain the most significantly heterogeneous SNPs between males and females for each anthropometric trait separately ($P \leq 0.001$). These markers were combined for a total of 8423 SNPs. This list contained no duplicates, and was LD-pruned ($r^2 = 0.5$) for a final total of 8162 SNPs.

Determination of sex

PLINK –sex-check flags were used to determine the sexes of all subjects within the WTCCC1/2 datasets. Ambiguously identifiable individuals per PLINK's *–sex-check* flag were excluded from sex-specific analyses.

Imputation

WTCCC data were prephased using three runs of HAPI-UR with default parameters, and merged by consensus vote. Phased genomes were then imputed to the 1000 Genomes (phase 1 integrated v3) reference panels in 1-MB windows using default parameters. For chrX imputation, only the non-pseudoautosomal reference was used. The X chromosome

contained $\sim$10,000 and $\sim$14,000 genotyped SNPs in the WTCCC1 and WTCCC2 data, respectively. After imputation, 64 and 69% of all reference SNPs with MAF $>$1% were imputed with high accuracy (INFO score $>$0.9) in the respective cohorts; indicative of sufficient SNP density to impute a majority of the reference variants with high quality. After imputation, variants with INFO score $>$0.5 were retained, and dosages were rounded to hard calls. An additional round of stringent QC was performed, removing any variants with: MAF $<$0.01; missingness $>$5%; Hardy-Weinberg equilibrium $P < 0.01$; or case-control missingness $P < 0.05$.

HLA removal in autoimmune diseases

For all autoimmune diseases—rheumatoid arthritis, Crohn's disease, type-1 diabetes, multiple sclerosis, and ankylosing spondylitis—the HLA region was removed due to the known significant enrichment associated within that region (of extended linkage disequilibrium) that violates the polygenic assumption of many modest effects across the genome. The HLA region was defined as chr6:26,000,000:34,000,000 (de Bakker *et al.* 2006).

Method A—association analyses

We applied two different approaches, *i.e.*, association enrichment (method A) and SNP-based heritability (method B), to test our four main hypotheses. A complete analysis flowchart is reported in Figure 1.

Association analyses and false discovery rate plots: In each complex trait, we analyzed the sexes combined and separately using the appropriate *–filter-males* and *–filter-females* PLINK flags where needed. Through PLINK, we ran logistic regressions, while using as covariates the first 10 principal components of each phenotype to account for differences in genetic ancestry. Sex-specific association signal enrichment was also tested separately for the nonpseudoautosomal X chromosome (and chromosome 7 and 17 for comparison). The X chromosome was coded in standard PLINK format, where male genotypes are $A = 0$ and $B = 1$, and female genotypes are $AA = 0$, $AB = 1$, and $BB = 2$. In order to compare across permutations, we set a false discovery rate (FDR) threshold, and assessed the percent of SNPs with q-value exceeding this threshold. A threshold of $q = 0.7$ was chosen to maximize the power of this comparison in small datasets. This was determined after calculating the percentage of autosomal SNPs above a range of FDR thresholds (0.1, 0.3, 0.5, 0.7, and 0.9) in the male, female, and combined-sex datasets for WTCCC diseases. For each FDR threshold, we calculated the number of datasets with SNP proportion below 0.0001%, and above 1%. An FDR threshold 0.7 minimized datasets for which our comparisons would lack power, or be based on implausibly high proportions of SNPs (Table S4). Note that we do not use this FDR threshold to assess significance, only as a means of comparison across permuted datasets, as recommended previously (Liu *et al.* 2012a). The proportion of SNPs with q-values that meet or fall below the FDR threshold is referred to here as the *original proportion*.

This procedure was implemented for genome-wide, chromosome-specific, hormone-responsive, and sex-heterogeneous SNP set analyses.

Sex permutations: To assess whether association signals are truly female- or male-specific, we ran a series of sex permutations. The null hypothesis we assess is that differences observed in male-specific *vs.* female-specific datasets are random sampling differences; our alternative hypothesis is that sex is driving a difference. For each complex trait, we randomly permuted sex labels within case and control strata. We produced a complete list of permuted individuals, combining sex-permuted controls with the sex-permuted cases. We repeated this 100 times for a total collection of 100 permuted male lists and 100 permuted female lists. *R* was used to generate these lists in the format of phenotype files for consequent association analyses in PLINK, and then multiple-test corrections via FDR in *R*, as described above. As a result of this step, each permutation results in a data point—a proportion of SNPs that meet or fall below the FDR threshold. Empirical *P*-values were calculated by tallying the number of permuted sets with a proportion of significant SNPs that exceeded the original proportion. For analysis with nominally significant and/or borderline significant empirical *P*-values, $P \leq 0.1$, we repeated the random selection 1000 times, and we obtained 1000 permuted male lists, and 1000 permuted female lists, and we estimated empirical *P*-values as described earlier. We replaced initial *P*-values estimated with 100 permutations with empirical *P*-values obtained with 1000 permutations.

Test for heterogeneity: METASOFT was used to assess extent of significant heterogeneity between the sexes by means of Cochran's Q test for heterogeneity. To discern whether a phenotype was particularly (read: significantly) heterogeneous, we used the above FDR test and sex-permutation approach. Empirical *P*-values were used to determine significance. This analysis was done on the Wellcome-Trust cohorts, and in genome-wide and X-linked data. For significantly heterogeneous phenotypes, we applied a binomial sign test to assess the top 0.1% of hits in sex-specific datasets for effect directions matching across the sexes. Empirical *P*-values were calculated with the sex-permutation approach. For analysis with nominally significant empirical *P*-values $P \leq 0.1$, we repeated the sex-permutation approach 1000 times, and we estimated empirical *P*-values as described earlier. We replaced initial *P*-values estimated with 100 permutations with empirical *P*-values obtained with 1000 permutations.

Gene/SNP permutations: Similar to the purpose of the sex permutations, gene permutations are meant to assess whether elevated enrichment when investigating a subset of genes is due to those genes, and not to other factors like gene size. In gene permutations, we sample genes that met the following criteria: (1) the sampled gene locus includes at least one SNP that is represented within our dataset, and (2) the sampled gene is matched on gene length, selected within a window of 100 genes closest in length to the original gene of interest.

SNPs for these genes (all SNPs within 5 kb of longest transcript) were then compiled into lists. Because association analyses were previously run, the SNPs in each permuted list were extracted with their corresponding *P*-value and quantified for the proportion of SNPs that met or fell below the FDR threshold. In other words, the same FDR analysis was conducted on each permuted gene list as it had been for the original gene set of interest. We repeated this 100 times for a total collection of 100 permuted estrogen-responsive gene lists, and 100 permuted androgen-responsive gene lists.

For sex-heterogeneous SNPs, SNP permutations were performed using a similar procedure, but matching individual SNPs for test statistic in the combined-sex dataset of origin (*i.e.*, a SNP with sex-heterogeneity for height will be matched on its test statistic for combined-sex association for height, but tested for association enrichment in each anthropometric trait).

Empirical *P*-values were determined as described earlier. For analysis with nominally significant empirical *P*-values $P \leq 0.1$, we repeated the random selection 1000 times, and we obtained 1000 permuted estrogen-responsive gene lists, 1000 permuted androgen-responsive gene lists, and 1000 permuted sex-heterogeneous SNPs. We estimated empirical *P*-values as described earlier. We replaced initial *P*-values estimated with 100 permutations with empirical *P*-values obtained with 1000 permutations.

Reference gene annotations for sampling were downloaded from UCSC's genome annotation database. For WTCCC1/2 analyses, we used the hg19 refGene.txt.gz annotation file. The file was truncated to contain: (1) only autosomes, (2) the longest version of a gene when duplicates were found, and (3) removal of *CNTNAP2* and *GRID2* due to their inability to be properly matched.

Method B—heritability analyses

Variance-component estimation: SNP-heritability ($h^2{}_g$) was estimated using variance-components and restricted maximum-likelihood (REML) (Yang *et al.* 2011) for studies with individual-level data. Briefly, the variance-component model assumes the phenotype is drawn from a multivariate normal distribution, with variance modeled by a linear combination of components computed from the SNPs and a normal residual. For each annotation (*e.g.*, AR genes) a genetic relatedness matrix (GRM) from SNPs in that annotation was jointly evaluated with a GRM from all remaining SNPs and the identity matrix to estimate the corresponding variance parameters σ_a^2, $\sigma_{a'}^2$, and σ_e^2. The heritability proportion was then computed as $\%h_a^2 = \sigma_a^2/(\sigma_a^2 + \sigma_{a'}^2)$, and the corresponding SE estimated using the delta method. Twenty principal components were always included as fixed-effects in the estimation procedure to account for population structure. We observed a significant association between sex and the top 20 PCs for 2/9 cohorts: AS ($P = 0.003$, $R^2 = 0.008$) and MS ($P = 4 \times 10^{-21}$, $R^2 = 0.011$). However, because the R^2 is low (explaining $\sim$1% of the sex label), and principal components were always included as fixed effects, we do not expect this to impact the results. We separately evaluated the potential confounding effect of case-control ascertainment by estimating genome-wide SNP-heritability using Haseman-Elston regression (Golan *et al.* 2014), where the product of individual phenotypes is regressed on the corresponding off-diagonal GRM entries (Table S5). The same GRMs, individuals, and principal components were used for both estimation procedures.

A similar procedure was used for estimating autosomal heritability of SNPs-by-sex (h_{gxs}^2). The sex GRM (K_{gxs}) was computed by setting entries in the standard GRM (K_g) to 0 for individuals of different sex. The phenotype was then modeled as $y = h_g^2K_g + h_{gxs}^2K_{gxs} + e$, and significance of h_{gxs}^2 assessed by likelihood ratio test against the one-component model.

We did not use a bivariate variance-components model to evaluate genetic correlation between traits due to small sample size. Power calculations showed that, for the average trait evaluated here (1300 cases and 1700 controls), the SE on the genetic correlation is expected to be 0.21, yielding little power to detect deviations.

Heritability permutations: To evaluate significance of the difference in $h^2{}_g$ between males and females, sex label permutation was again used. For each trait, sex labels were randomly shuffled and sex-specific partitioned heritability re-estimated to get the estimates from a single permutation. The permutation procedure was performed 1000 times, and the fraction of instances where the absolute permuted difference was higher than the absolute observed difference used to compute the *P*-value. The same variance-components and fixed effects were used in the permutations as in the real data.

Summary statistic-based estimation: SNP-heritability was estimated using LD-score regression (LDSC) for studies with only summary-level data. For a single trait and functional annotation, LDSC regresses the χ^2 (or Z^2) association statistic from each SNP onto the "LD-score" of that SNP: computed as the sum of LD across all neighboring SNPs from a reference panel. Under assumptions of independent causal effect sizes, the slope of this regression is then proportional to the SNP-heritability, and the intercept is proportional to the effects of population stratification. For multiple functional annotations, the model naturally extends to include annotation-specific LD-scores computed only to the neighboring SNPs that belong to the given annotation. The coefficients from this multiple regression are then proportional to the partitioned SNP-heritability for each annotation. For multiple traits, replacing the χ^2 statistic with the product of association βs from each trait in either the single or multiple LD-score regression yields an estimate of genetic correlation between the traits.

LDSC was run on sex-specific summary statistics to estimate total $h^2{}_g$ and genetic correlation using default parameters and default LD-scores (computed in the 1000 Genomes EUR samples). The GIANT GWAS data were imputed (by the original study) to $\sim$2 M HapMap3 variants. This is the recommended SNP set to use for LD-score regression, and has been shown to yield comparable results to high-quality

1000G imputation (Finucane *et al.* 2015). SE were estimated using the weighted block-jackknife. For the novel annotations of AR genes, ER genes, and GIANT heterogeneous SNPs, additional LD-scores were computed using the 1000 Genomes EUR samples, and used to partition the heritability and genetic correlation. Heritability partitioning was evaluated with and without the "baseline" annotations from Finucane *et al.* (2015) to account for potential background enrichment from overlapping functional categories. When baseline annotations were included, the *P*-value of the coefficient is reported, which corresponds to the significance of the given annotation beyond all other annotations in the model. For the AR/ER annotations this will yield a conservative estimate because multiple "genic" annotations are already in the baseline model. In contrast to the previous studies with raw data, we are not aware of any method to evaluate G $\times$ Sex heritability from summary data, and could not assess this effect for the anthropometric traits.

Data availability

The authors state that all data necessary for confirming the conclusions presented in the article are represented fully within the article and are publicly available.

Results

Autosomal genetic load

We studied nine common, complex diseases from the WTCCC studies (WTCCC 2007; Evans *et al.* 2011; Sawcer *et al.* 2011), and nine anthropometric traits from the GIANT studies (Randall *et al.* 2013; Shungin *et al.* 2015), to capture a variety of sex biases (or lack thereof). Male biased diseases included ankylosing spondylitis (AS, with M:F prevalence of 2:1) (Chen *et al.* 2011; Haroon *et al.* 2014), and type 1 diabetes (T1D, 3:2) (Gale and Gillespie 2001; Liu *et al.* 2012a; Orozco *et al.* 2012). Female biased diseases included multiple sclerosis (MS, 1:3) (Pakpoor and Ramagopalan 2014) and rheumatoid arthritis (RA, 1:2) (Emery *et al.* 2014). Disorders estimated to have similar lifetime prevalence by sex included bipolar disorder (BD) (Almeida-Filho *et al.* 1997; Negash *et al.* 2005; Diflorio and Jones 2010), coronary artery disease (CAD) (Sharma and Gulati 2013), Crohn's disease (CD) (Liu *et al.* 2012b; Law and Li 2014), hypertension (HT) (Nwankwo *et al.* 2013), and type 2 diabetes (T2D) (Orozco *et al.* 2012; Hilawe *et al.* 2013), although sex differences often exist in age of onset, subtype, and comorbidities. Anthropometric traits with different means by sex were body mass index (BMI), hip circumference (hip), hip adjusted for BMI (hip-a), waist circumference (WC), WC adjusted for BMI (WC-a), waist-hip-ratio (WHR), WHR adjusted for BMI (WHR-a), height, and weight.

Under a threshold difference LT model of sex bias, we would anticipate that diseases with increased prevalence in one sex would have more association enrichment due to increased genetic load in the opposite sex. This pattern did not occur for any of the disorders with sex-biased prevalence, when sex-specific datasets were compared with sex-permuted datasets [Figure 1 (Method 1a) and Table S6]. We excluded the HLA region for autosomal analysis of autoimmune diseases, as its large effect could bias interpretation. However, when we tested the HLA region separately, we observed greater association enrichment in the HLA region in AS males ($P < 0.001$). As is male-biased in prevalence, neither finding supports the threshold difference LT model (Table S6).

In order to confirm our observations using an independent approach, we evaluated polygenic variance components for differences in total heritability, gene set specific heritability, and genetic correlation [Figure 1 (Method 1b)]. These analyses consider both "quantitative" differences between sexes due to different genetic variance, and "qualitative" differences due to different regions contributing to heritability. First, we estimated SNP-based additive heritability (${h^2}_g$) on the liability scale, utilizing imputed datasets for each disease trait, and utilizing summary statistics for each anthropometric trait. This estimate is a ratio of the SNP-genetic and environmental terms, and resulting ${h^2}_g$ differences between sexes can be indicative of quantitative differences in either component. The HLA region was excluded from autosomal heritability estimates for MHC-linked autoimmune disorders as in our association enrichment results, as its major effect (with extensive LD) violates the polygenic model, and could thus impact interpretation.

As in our association enrichment results, we did not find evidence for a threshold difference LT model based on heritability estimates. Of the two disorders with higher male prevalence (AS, T1D), neither showed increased heritability in the lower-prevalence sex (Table 1), with similar heritability estimates for each sex in AS, and higher estimate in males for T1D, which was not significantly different from female estimates. Of the disorders with higher female prevalence (MS, RA), we observed similar estimates across the sexes for MS, and a higher estimate of heritability for males with RA, which was not significant.

One disease thought to have similar lifetime prevalence showed evidence for sexually dimorphic heritability, with HT showing higher heritability in females in the WTCCC dataset ($P < 0.001$). We re-evaluated the dichotomous traits using a regression-based method that is not biased by case-control ascertainment, and observed no substantial change in sex differences (see *Materials and Methods*, Table S5). Several anthropometric traits showed higher heritability in males compared with females, including WC-a, hip-a, WHR ($P < 0.05$) (Table 2).

Gene $\times$ sex

We did not detect autosome-wide heterogeneity by comparing the distribution of Cochran's Q [Figure 1 (Method 2a)] to the distribution of sex-permuted datasets. However, when we separately assessed the HLA region, we observed significant sex-heterogeneity for association signal on the HLA region for MS ($P < 0.001$) (Table S6). For traits with, or without,

Table 1 Autosomal SNP-based heritability and G × Sex interaction in WTCCC diseases

Disease	Heritability[a] M h^2_g (SE)	F h^2_g (SE)	G × Sex Interaction[a] h^2_{gxs} (SE)
AS[b]	0.11 (0.04)	0.15 (0.07)	0.01 (0.05)
BD	0.18 (0.08)	0.22 (0.06)	−0.01 (0.06)
CAD	0.25 (0.09)	0.50 (0.22)	0.12 (0.11)
CD[b]	0.14 (0.06)	0.17 (0.04)	0.03 (0.05)
HT	0.18 (0.21)	0.73 (0.17)***	0.19 (0.17)
MS[b]	0.17 (0.02)	0.17 (0.02)	0.06 (0.01)****
RA[b]	0.20 (0.11)	0.08 (0.05)	0.03 (0.06)
T1D[b]	0.14 (0.06)	0.08 (0.06)	0.04 (0.06)
T2D	0.31 (0.12)	0.37 (0.15)	−0.06 (0.12)

h^2_g, heritability; SE, standard error. **** $P < 0.0005$, *** $P < 0.001$.
[a] Empirical P-value for difference between M and F h^2_g: estimation based on 1000 permutations.
[b] HLA locus was tested separately from the rest of the autosomes.

polygenic heritability differences, gene–sex interaction could occur at specific loci [Figure 1 (Method 2b)]. For example, a disease could have equivalent overall heritability but with different loci contributing in males *vs.* females; a difference in environmental contribution could result in different heritability, with the same loci contributing, or different loci could result in disparate heritability estimates. For traits with individual-level data, we were able to include an overall G × Sex interaction term in the heritability estimates. We observed highly significant heritability of G × Sex for MS (0.06 SE 0.01; $P = 1 \times 10^{-9}$); this was striking, given the remaining additive SNP-heritability was 0.13 (SE 0.01) (Table 1). Although we could not specify an interaction term due to lack of genotype data for the anthropometric traits, we assessed traits for which genetic correlation between male-specific and female-specific datasets was significantly <1, which occurred for every anthropometric trait ($P < 0.05$) (Table 2). This estimate of genetic correlation is not affected by quantitative differences in total genetic variance, and only reflects differences in individual effect-sizes.

X-chromosome

Genetic sex differences not contained on the autosomes could be attributable to the sex chromosomes. Since the X-chromosome is relatively gene-rich, contrasting with the small gene-poor Y chromosome (Ellis and Affara 2006; Mulugeta *et al.* 2016), and our disease datasets had available data, we assessed the contribution of the X-chromosome. We detect a relatively modest contribution from the X-chromosome to this group of common, complex diseases [Figure 1 (Method 3a)]. Association enrichment signal appears similar to autosomes matched by physical length (chr7), and SNP content (chr17) (Table S7).

We next assessed sex differences on the X-chromosome. We note that, although females have twice the number of alleles, and thus increased power compared to males, one might expect greater impact of nonpseudoautosomal loci in males, who are hemizygous, and thus express an associated allele in every cell as the sole copy. AS and CAD showed increased male signal ($P = 0.050$ and $P = 0.037$, respectively) (Table 3).

Of all the equivalent sets of permutations performed on both chromosomes 7 and 17, only one showed a significant female increase (T2D, chr7 $P = 0.046$) (Table S7). To support these data suggesting sex differences specific to the X-chromosome, we assessed the chromosome-wide heterogeneity via Cochran's Q, and compared the distribution to sex-permuted datasets. AS, CAD, and MS showed suggestive or significant sex-heterogeneity for association signal on the X-chromosome ($P = 0.059, P = 0.004$, and $P = 0.016$, respectively) (Table 3 and Figure S1). It is difficult to consider equivalent male and female statistical models considering X-inactivation (or escape), because the biology of mosaicism is challenging to predict and compare with the typical biallelic models (or hemizygosity in males). We thus performed the binomial test to compare the direction of association, as direction should not be impacted by model specification, regardless of effect size estimates. While MS did not show differences in direction of association in the top 0.1% of X-chromosome SNPs, male and female top associations show significant differences in AS ($P = 0.01$ females) and in CAD ($P = 0.04$ males; $P = 0.02$ females). For AS and CAD, while male top X-chromosome results showed an excess of top SNPs in the same direction in females, female top X results showed a significantly decreased proportion of top SNPs in the same direction in males compared with permutations.

Gene set "environmental" contribution analyses

At the biological level, a major "environmental" influence on the genome might be differences in levels of steroid hormones, beginning early in development, and influencing the expression of many genes. The first hypothesis we wanted to assess with regard to steroid-responsive genes (Tang *et al.* 2004; Jiang *et al.* 2009) (Tables S2 and S3) was whether these genes contribute disproportionately to association signals compared to matched gene sets [Figure 1 (Method 4a)]. In fact, compared with permuted gene sets matched for gene length (which resulted in matching for SNP number), androgen-responsive genes contributed disproportionately to association signal in CD, RA, hip-a, WC-a, WHR, WHR-a, height, and weight, in at least one sex (Figure 2 and Table S8). Although the set of estrogen-responsive genes is smaller, we identified significant contribution to association signal in CAD, T1D, WC-a, and WHR-a (Figure 2 and Table S8).

The second putative mechanism would be gene–sex interaction with respect to steroid-responsive genes, such that these sets of genes would show evidence of sex difference in their trait association [Figure 1 (Method 4a)]. We observed sex differences significant by sex permutation for androgen-responsive genes in WC-a (males), WHR (females), WHR-a (females), height (both sexes), and weight (males), in association signal (Table S8). We observed sex differences for estrogen-responsive genes in CAD (females) and T1D (females), and WHR-a (males) in association signal (Table S8).

Next, we wondered whether the same SNPs showing sexual dimorphism in anthropometric traits ($P < 10^{-3}$, Randall *et al.* 2013) would show excess contribution to

Table 2 Autosomal SNP-based heritability and genetic correlation in GIANT anthropometric traits

	Heritability			Genetic Correlation		
Phenotype	$M\ h^2_g$ (SE)	$F\ h^2_g$ (SE)	*P*-value[a]	cov_g (SE)	r_g (SE)	*P*-value[a]
BMI	0.18 (0.01)	0.17 (0.01)	NS	0.15 (0.01)	0.89 (0.02)	5.6×10^{-10}
HIP	0.19 (0.02)	0.18 (0.01)	NS	0.17 (0.01)	0.90 (0.03)	6.2×10^{-4}
HIP-a	0.21 (0.01)	0.15 (0.01)	5.5×10^{-3}	0.17 (0.01)	0.92 (0.03)	2.6×10^{-3}
WC	0.18 (0.02)	0.16 (0.01)	NS	0.16 (0.01)	0.91 (0.03)	1.6×10^{-3}
WC-a	0.18 (0.01)	0.11 (0.01)	2.1×10^{-5}	0.11 (0.01)	0.75 (0.05)	2.0×10^{-8}
WHR	0.14 (0.01)	0.11 (0.01)	4.0×10^{-2}	0.09 (0.01)	0.74 (0.05)	9.2×10^{-7}
WHR-a	0.13 (0.01)	0.11 (0.01)	NS	0.08 (0.01)	0.67 (0.06)	2.3×10^{-9}
Height	0.26 (0.02)	0.25 (0.02)	NS	0.25 (0.02)	0.95 (0.02)	2.0×10^{-2}
Weight	0.19 (0.01)	0.19 (0.01)	NS	0.18 (0.01)	0.93 (0.03)	1.0×10^{-2}

h^2_g, heritability; SE, standard error; cov_g, genetic covariance; r_g, genetic correlation; NS, not significant.
[a] Empirical *P*-value for difference between *M* and *F* h^2_g: estimation based on block jackknife.

common disease and trait genetic signal (compared with SNPs matched on test statistic in the combined sample) [Figure 1 (Method 4a)]. With the exception of AS, MS, HT, and T1D, we observed a disproportionate enrichment of SNPs with sex-heterogeneous effects in the remaining diseases and every trait (Figure 2, Figure 3, and Table S8).

We partitioned the heritability to quantify the contribution of all SNPs in AR/ER gene regions (Tables S9 and S10) [Figure 1 (Method 4b)]. In contrast to sex differences in total h^2_g, partitioned heritability is normalized by total heritability, and therefore will be unaffected by differences in the overall environmental component. An additional 2 kb flank was added to include nearby regulatory elements in the promoter, and these heritability estimates should be interpreted as corresponding to SNPs in or near the genes (Table S11). For the anthropometric traits that are well-powered to model overlapping annotations, we included "baseline" annotations (Finucane *et al.* 2015) to account for potential background enrichment from overlapping functional categories, such as our selection of genic regions. In this joint model, AR genes account for a significantly increased proportion of heritability in both sexes for height, and for females in BMI. ER genes account for increased proportion of heritability in males in WHR-a and weight.

We observed significant sex differences in heritability for androgen-responsive genes in CAD (females) and RA (females) (Table S9). Genetic correlation in AR genes was significantly <1 between males and females for BMI, hip, WC-a, WHR, WHR-a, height, and weight, suggesting heterogeneity in this functional category (Table S10). Sex differences in heritability for estrogen-responsive genes were significant for WHR-a (females), weight (males), and CAD (females) (Tables S9 and S10). Genetic correlation in ER genes was significantly <1 between males and females in WHR-a but the analysis could not be performed in hip or weight as the estimate for one sex was nonsignificant (Table S10). Despite the tiny size of the sex-heterogeneous SNP set, heritability was significantly different between the sexes after permutation in MS, RA, and T2D ($P < 0.05$) (Table S9).

We separately analyzed each of the "baseline" annotations—which include major regulatory and evolutionary functional groups—for deviations in genetic correlation. After correcting for the 51 annotations tested, conserved regions (Lindblad-Toh *et al.* 2011) were the only annotation that remained significant, with genetic correlation <1 for BMI, hip, WC, and WHR. Conserved regions have previously been identified as enriched for heritability across many traits (Finucane *et al.* 2015), and this depletion in genetic correlation implicates conserved regions in harboring sex-specific effects. The depletion is striking given that these regions harbor 43% of the anthropometric trait h^2_g on average.

Interpretation of results under the LT model

To better understand the results of the analyses presented above, we consider several LT models that can lead to sex biased prevalence. As we observed little difference in genetic load by sex (Table 1), we evaluated the effects of variance instead of threshold differences. The variance of the liability can have dramatic effects on disease prevalence, which will be larger in the higher variance sex if the mean liabilities and thresholds are equal. To compute the disease prevalence for a given threshold and variance, we computed the area under the normal curve with mean 0, and specified variance that falls beyond the specified threshold. This was achieved using the pnorm function in the R statistical package (Table S12). For rare diseases, even a small increase in the variance of the liability can have a dramatic effect on disease prevalence. Consider a disease similar to MS with prevalence 0.1%. If the liability variance is increased to 1.2 in females *vs.* 1.0 in males, the disease prevalence will be 2.4 times higher in females.

Gene–sex interactions arising from the autosomes as we observed for MS (or dominance effects on the sex chromosomes, not evaluated here) can result in differences in liability between sexes without having large-scale effects on heritability estimates. We simulated a disease under an LT model with population prevalence 2%. (We used a prevalence of 2% instead of 0.1% for computational efficiency, but the same principles will hold at any prevalence.) We sampled 1000 individuals genotyped at 10 SNPs, which were drawn from a binomial distribution with allele frequencies drawn uniformly between 0.05 and 0.95. Males and females were sampled randomly, and an unobserved environmental factor was drawn for each individual from a normal

Table 3 Sex-differences in X-chromosome association signal and heterogeneity in WTCCC diseases

Disease	Prev M:F	Association		Heterogeneity P^b	Sign Test[a]	
		M P^b	F P^b		M%[c]	F%[d]
AS	2:1	0.0500[e]	NS	0.0600[e]	77.3	9.3**
BD	1:1	NS	NS	NS	—	—
CAD	1:1	0.0370[e]	NS	0.0040[e]	71.3*	8.0*
CD	1:1	NS	NS	NS	—	—
HT	1:1	NS	NS	NS	—	—
MS	1:3	NS	NS	0.0016[e]	82.0	75.7
RA	1:2	NS	0.1000[e]	NS	—	—
T1D	3:2	NS	NS	NS	—	—
T2D	3:2	NS	NS	NS	—	—

Prev, ratio in prevalence.
[a] Sign test based on 0.1% of male and female top hits applied only to significant heterogeneity results.
[b] Empirical *P*-value estimation based on 100 permutations.
[c] Empirical *P*-value estimation based on 100 permutations showing increase.
[d] Empirical *P*-value estimation based on 100 permutations showing decrease.
[e] Empirical $P \leq 0.1$ were replaced with empirical *P*-values estimated with 1000 permutations. ** $P < 0.01$; * $P < 0.05$

distribution, with mean 1.0 in males and 2.0 in females, and variance 1.0 in both sexes. The total additive heritability was 20%, and the G $\times$ Sex interaction heritability was 30%. In each of 100 simulations, we estimated the heritability in the entire population as well as males and females separately. We also estimated the prevalence of the disease in each sex. As expected (Hill *et al.* 2008), we observed similar average heritabilities on the liability scale of 35.3, 34.9, and 36.1% in all individuals, females, and males, respectively. Our observed G $\times$ Sex heritability in MS was only 6%, suggesting that the absorption of this effect into the sex specific estimates is even more likely to occur.

Discussion

In this study, we set out to perform a survey of the influence of sex on the genetic risk for common, complex heritable disease, and anthropometric traits. Utilizing SNP GWAS results (method A), we assessed sex-specific association enrichment (hypothesis 1), global gene–sex interaction (hypothesis 2), sex differences on the X-chromosome (hypothesis 3), and the influence of steroid-responsive genes and sex-heterogeneous loci associated with secondary sex characteristics (hypothesis 4). We examined polygenic heritability (method B) to substantiate our observations. We found that sex-specific mechanisms were not limited to diseases or traits with notable prevalence or mean differences. In general, we sought to perform a broad survey of potential mechanisms for sexual dimorphism, and to examine patterns of genetic evidence supporting these mechanisms across traits. Because many tests were performed, individual results with respect to a given disease/trait should be interpreted with caution in the absence of further follow-up. However, general principles of sexual dimorphism are substantiated by observations replicated in several complex traits.

When assessing sex differences in genetic load, we did not find global evidence for the predicted threshold difference LT model (hypothesis 1), *e.g.*, the lower-prevalence sex showing higher genetic load. We found an example of association enrichment in the HLA locus in males for male-biased AS. We also found evidence that hypertension shows increased heritability in females despite similar prevalence to males (Biino *et al.* 2013). Sex specific heritability was previously evaluated in much smaller sample for the anthropometric traits, with inconsistent effects, and the large studies used here resolves this difference (Shungin *et al.* 2015). We found evidence for sex differences in heritability in several anthropometric traits, although, interestingly, for traits with potentially greater selective impact for females (hip-a, WC-a, and WHR), males have significantly higher heritability, in contrast to some previous findings for fitness traits (Pettay *et al.* 2005). While the LT is a popular model, alternatives exist, and these may better explain the observed data. Further research should include delineating the impact of sex differences in genetically or environmentally mediated trait or liability variance on heritability estimates, and assessment of the influence of ascertainment, which may differ by sex for case-control traits (Zaitlen *et al.* 2012a, 2012b; Yang *et al.* 2014). The extent to which data do, or do not, reflect given sex bias models constrains the set of possible biological mechanisms inducing sex-biased prevalence. Further, clinical interpretations of sex-specific risk should consider these results. For example, counseling relatives of a proband of the lower-prevalence sex that they are at increased risk compared to relatives of a proband of the higher-prevalence sex is based on the threshold difference LT model. In the context of GWAS, alternative analysis strategies could both provide biological insights as well as improve power. Random effects meta-analysis (Han and Eskin 2011) can reveal variants with different effect sizes between sexes, and retrospective likelihood models can reduce power-loss when sex is included as a covariate in ascertained studies (Zaitlen *et al.* 2012b).

We estimated a substantial G $\times$ Sex interaction term in heritability for MS. MS is by far the largest and best-powered study evaluated, and, based on estimated SE, we still cannot rule out the presence of interaction for other diseases. For every anthropometric trait, genetic correlation appeared significantly <1. Although the genetic correlation between sexes was still relatively high for many of these traits, the large anthropometric datasets allowed for powerful tests, confirming that less than complete correlation in genetic contribution to males and females is pervasive (hypothesis 2). Similarly, a study (Rawlik *et al.* 2016) published while this work was under review found that the genetic correlation between the traits measured in men and women was significantly below 1 (complete correlation) across several quantitative traits including height, BMI, WC, HIP, and WHR, supporting our evidence for G $\times$ Sex interaction. There is precedent in both model organism (Nuzhdin *et al.* 1997; Dilda and Mackay 2002; Leips and Mackay 2002;

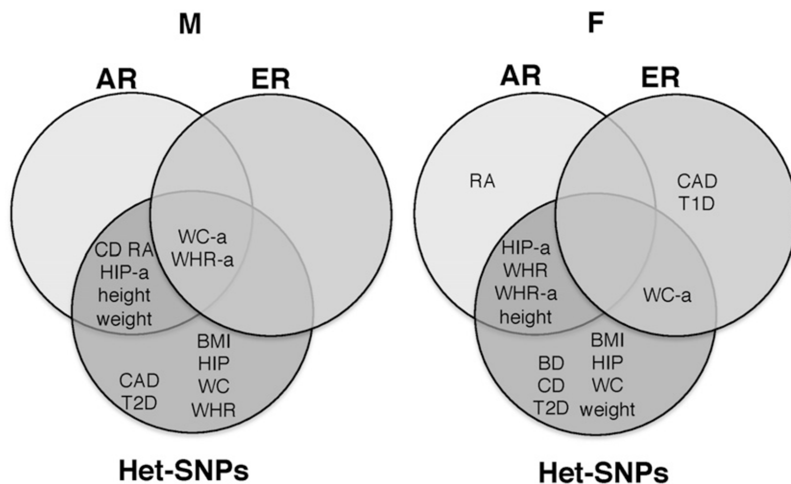

Figure 2 Venn diagram of association enrichment of androgen-responsive (AR) and estrogen-responsive (ER) genes and heterogeneous SNPs (Het-SNPs) in WTCCC diseases and GIANT anthropometric traits. Diseases/traits with significant association enrichment in males (M) are represented in the left diagram, diseases/traits with significant association enrichment in females (F) are represented in the right diagram.

Mackay and Anholt 2006) and gene expression (Trabzuni *et al.* 2013; Yao *et al.* 2014; Kukurba *et al.* 2016) data for autosomal G × Sex interaction, supporting the plausibility of our results. In addition, consistent with Rawlik *et al.* (2016), our well-powered study highlighted no significant differences for male and female heritability for BMI and height. However, we observed significant male enrichment for WHR heritability, in contrast with the female enrichment showed by Rawlik *et al.* (2016). Heritability estimates can differ due to a different amount of genetic variance present in a population, or different amounts of nongenetic variance in a trait. The study designs for GIANT (meta-analysis of 34 studies from different populations) and Rawlik *et al.* (2016) (UK Biobank) likely lead to the observed differences in heritability estimates. Population-specific and sex-specific properties of environment, such as lifestyle, diet, and smoking status, are important for anthropometric measures, and could contribute to differences between studies, as could populations with different genetic ancestry leading to differing genetic variation.

In order to better understand our lack of evidence for threshold differences and strong evidence for G × Sex interaction, we performed simulations. Our simulated models showed a small difference in estimated heritability, but, in the presence of G × Sex interaction, led to an increase in variance in one sex. We thus demonstrated how a 66.6% increase in disease prevalence can exist between sexes with only a 1.1% difference in additive heritability estimates. Given that the phenotypic variance of many traits is different between the sexes in the GIANT data (Randall *et al.* 2013; Shungin *et al.* 2015), difference in variance of disease-related risk factors, as well as differences in mean values of risk factors, may contribute to observed differences in prevalence. In the context of GWAS, a difference in variance between sexes, as opposed to difference in mean, is not captured by a standard fixed effect regression term for sex. Performing sex-stratified analyses, or using a double generalized linear model to account for the difference in variance between sexes, will improve power.

When assessing the most obvious difference in the genome, the X-chromosome, in contrast to previous studies (Pan *et al.* 2007), we did not find strikingly disproportionate contribution from the X chromosome, although we did observe sex differences in X-chromosome signal, including evidence for female-limited X associations and effect size heterogeneity (hypothesis 3). A previous linkage study concluded there is no role for the X-chromosome in AS (Hoyle *et al.* 2000), in contrast to our results suggesting association enrichment on the X specific to males. Although blood pressure and cholesterol levels have been associated with X-linked loci, similar evidence for X-chromosome sex differences have not previously been associated with cardiovascular disease (Chang *et al.* 2014), and a study of stroke specifically suggested a role for hormones, but not sex chromosomes (Manwani *et al.* 2014; Winham *et al.* 2015). Additionally, a recent well-powered X-WAS meta-analysis showed no evidence of genome-wide X-chromosome loci contributing to CAD (Loley *et al.* 2016). Consistently, we did not find any genome-wide associated locus on X chromosome (Figure S1). However, our study was designed to assess global rather than locus-specific genetic contributions to disease, thus we were able to identify significant chromosome-wide heterogeneity across sexes and male association enrichment for CAD on the X chromosome. Likewise, MS has been suggested to have X-linked risk via mouse models and association with X aneuploidy (D'Alessandro *et al.* 1990; Smith-Bouvier *et al.* 2008; Seminog *et al.* 2015), but, to our knowledge, not genome-wide studies before ours (Chang *et al.* 2014). Additionally, a recent study (Chang *et al.* 2014) hypothesized sexually dimorphic effect sizes for X-linked genes in autoimmune diseases. Although the latter authors showed individual X-linked genes with sexually dimorphic association, our study did not detect significant chromosome-wide sex heterogeneity for the same disorders, *i.e.*, CD, RA, and T2D. Our power for single-chromosome analyses was extremely limited, and these data were not available for the anthropometric data—our most powerful datasets. For example, we observe a large estimated difference in RA,

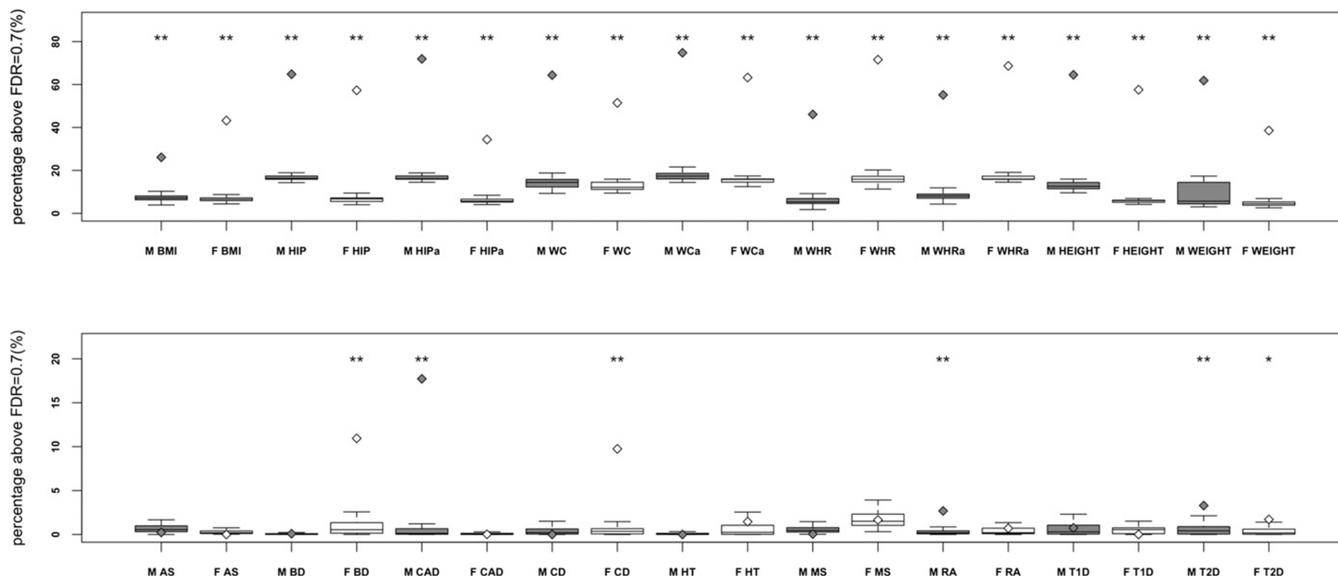

Figure 3 True enrichment and permutation-based enrichment for heterogeneous markers in WTCCC diseases and GIANT anthropometric traits. Enrichment distributions for 100 permuted males and 100 permuted females are represented with boxplots. Diamonds show the true significant enrichment for males and females. Males are represented in light grey, females in white. ** $P < 0.01$, *$P < 0.05$.

but it does not reach significance. Thus larger data sets are needed to robustly estimate the contribution of the X-chromosome to prevalence differences through changes in mean and variance of the liability. The X-chromosome may have a different role in traits subject to strong natural selection, such as those directly related to reproduction, in comparison to common diseases with onset primarily after historic reproductive ages (Kosova *et al.* 2010).

In order to assess potential consequences to genetic risk of sex differences in steroid hormone levels, we assessed the contribution of steroid-responsive genes to each trait. We found a strong contribution of hormone-responsive genes to several diseases and anthropometric traits, with examples of sex-interaction in this contribution (hypothesis 4). Recently, a gene involved in androgen synthesis was associated with RA (Stark *et al.* 2015), and we show that androgen-responsive genes across the genome are enriched for association signal and sex differences. Although estrogen biology has been associated with comorbidities of T1D (Ryba *et al.* 2011; Ryba-Stanisławowska *et al.* 2014; Słomiński *et al.* 2015), our results are the first to suggest a global impact on risk. Interestingly, we observed several examples of an increased proportion of heritability in AR genes in females, and for ER genes in males. These results could imply that increased levels or variance in androgens in males and estrogens in females reduce the relative impact of genetic variation in genes responsive to these hormones.

Finally, and strikingly, we observed that SNPs showing heterogeneity in association with anthropometric traits make an exceptional contribution to common, complex traits, including those without major prevalence differences. Although there has been a previously noted relationship between hypertension and height, our findings suggest that many observed disease differences by sex are generated by the same mechanisms determining secondary sex characteristics. Our observation is one of association enrichment for anthropometric sex-heterogeneous SNPs, and thus does not directly correspond with sex-heterogeneity in the target disease, but does highlight the pleiotropy of the biology involved in sexual dimorphism. This implies that sex differences in disease prevalence, symptoms, and outcomes may be governed by universal biological pathways, rather than disease-specific pathophysiology or environmental/behavioral risk factors. Further research may clarify whether identification of these putative factors could improve sex-specific diagnosis, treatment, or prevention.

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