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Using human genetics to understand the disease impacts of testosterone in men and women

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Testosterone supplementation is commonly used for its effects on sexual function, bone health and body composition, yet its effects on disease outcomes are unknown. To better understand this, we identified genetic determinants of testosterone levels and related sex hormone traits in 425,097 UK Biobank study participants. Using 2,571 genome-wide significant associations, we demonstrate that the genetic determinants of testosterone levels are substantially different between sexes and that genetically higher testosterone is harmful for metabolic diseases in women but beneficial in men. For example, a genetically determined 1 s.d. higher testosterone increases the risks of type 2 diabetes (odds ratio (OR) = 1.37 (95% confidence interval (95% CI): 1.22–1.53)) and polycystic ovary syndrome (OR = 1.51 (95% CI: 1.33–1.72)) in women, but reduces type 2 diabetes risk in men (OR = 0.86 (95% CI: 0.76–0.98)). We also show adverse effects of higher testosterone on breast and endometrial cancers in women and prostate cancer in men. Our findings provide insights into the disease impacts of testosterone and highlight the importance of sex-specific genetic analyses.

The role of testosterone in disease is largely unknown, despite its strong epidemiological correlations with many health conditions and the widespread use of testosterone supplements. Previous studies have shown protective associations with testosterone on type 2 diabetes (T2D) and related metabolic traits in men, but harmful associations in women[1,2]. However, such phenotypic observations are prone to confounding due to the substantial effects of ageing and adiposity on circulating testosterone concentrations[3].

More than 3% of US men aged 30 yr or older received a prescription for testosterone in 2013, just before a US Food and Drug Administration safety communication on its possible cardiovascular risks[4], and rates of prescribing are even higher in Canada[5]. Testosterone therapy has established positive effects in randomized controlled trials (RCTs) on sexual function, lean mass, muscle strength and bone mineral density, and reductions in whole-body and intra-abdominal fat[6]. These body composition changes should predict benefits of testosterone on T2D and cardiometabolic disease. Conversely, testosterone is known to promote growth and metastasis of prostate cancers and observational studies have shown that testosterone replacement therapy might increase susceptibility to future prostate cancer[7–9]. However, even the largest trials of testosterone have too few cases of incident T2D, cardiovascular disease or prostate cancer to provide informative data on these risks[10]. Furthermore, experimental studies of testosterone therapy in men, with or without T2D, surprisingly report no or modest improvements in insulin sensitivity and no change in glycemic control[11,12]. Similarly, in women, experimental evidence of testosterone administration is insufficient to confirm the apparently metabolically harmful associations in observational studies between testosterone and higher adiposity, risk of polycystic ovary syndrome (PCOS) and other cardiovascular disease risk markers[13,14].

Mendelian randomization (MR) is a genetic approach to understand the causal effects of putative risk factors on disease. Given alleles are both randomly assigned and fixed at conception, genetic risk can be used as an epidemiological exposure to reduce the effects of confounding and reverse causality. Previous studies have used this approach to test the role of sex hormones in disease, but were largely limited to *cis* variants in the sex hormone-binding globulin (SHBG) protein-coding gene. Such studies reported that SHBG-raising alleles were associated with lower risk of T2D, but did not test effects separately in men and women in large numbers[15,16]. Furthermore, because higher SHBG reduces levels of bioavailable testosterone, separation of the apparent effects of testosterone from those of SHBG on disease is a major challenge.

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Table 1 | Heritability of and genetic correlations between sex hormone traits included in the genome-wide association analyses

	Heritability (s.e.m.), %	SHBG (Women)	Testosterone (Women)	Bioavailable T (Women)	Estradiol (Women)	SHBG (Men)	Testosterone (Men)	Bioavailable T (Men)	Estradiol (Men)
SHBG (men)	21 (1.2)	**0.83**	**−0.02**	**−0.59**	**0.29**	-			
Testosterone (men)	17 (1)	**0.69**	**0.001**	**−0.49**	**0.40**	**0.73**	-		
Bioavailable T (men)	12 (0.7)	**0.06**	**0.01**	**−0.03**	**0.21**	**−0.05**	**0.60**	-	
Estradiol (men)	2 (0.4)	**0.04**	**0.24**	**0.10**	**0.07**	**0.19**	**0.32**	**0.19**	-
SHBG (women)	20 (0.01)	-							
Testosterone (women)	13 (0.8)	**−0.06**	-						
Bioavailable T (women)	14 (0.8)	**−0.74**	**0.65**	-					
Estradiol (women)	1.6 (1)	**0.45**	**−0.25**	**−0.51**	-				

To identify additional genetic variants that can be used to test the effects of testosterone, large genome-wide association studies (GWASs) are needed. Previous GWASs for sex hormone levels in men and women were small[17–20], identifying only a handful of associated loci. This study substantially advances our understanding of the genetic regulation of sex hormone levels, increasing the number of known genetic determinants by two orders of magnitude. We use these genetic variants to demonstrate likely causal associations with metabolic disease and cancer outcomes, with many divergent effects of testosterone between men and women.

Results

After extensive quality control (see Methods), serum levels of SHBG, total testosterone and estradiol were available in up to 425,097 individuals with genetic data in UK Biobank (UKBB) (Supplementary Table 1). We additionally estimated bioavailable (free/unbound) testosterone in 382,988 individuals (see Methods). Genetic association testing was performed in European ancestry individuals and within each sex for the four traits, using a linear mixed model to control for relatedness and population structure. We identified a heritable component for all traits except estradiol levels in women (genetic heritability, $h^2_g = 1.6\%$; s.e.m. = 1%) (Table 1). As the majority (78%) of women had estradiol levels below the limit of detection (as expected, given most women in UKBB are postmenopausal), analysis of this trait was limited by low sample numbers and a bias towards detecting age at menopause-associated loci. Therefore, assessment of estradiol levels in women was not considered further.

To identify independent genetic determinants for sex hormone measures, we next performed distance-based clumping and approximate conditional analysis (see Methods). In total, we identified 2,571 genome-wide significant trait–signal pairs (Supplementary Tables 2–11). These trait–signal pairs ranged from 22 signals for estradiol in men, to 658 for SHBG in a sex-combined analysis. To validate these findings, we performed replication using three available datasets (see Methods)—a previously published GWAS meta-analysis of SHBG levels in 21,791 individuals[19], 9,138 individuals with testosterone measurements from the European Prospective Investigation of Cancer (EPIC)-Norfolk study and published data on 2,913 individuals from the Twins UK study with nine sex hormones measured[20]. Whilst these studies were substantially smaller than UKBB, we found strong directional consistency with our results. Assessment of our SHBG-associated loci in the published meta-analysis (Extended Data Fig. 1) demonstrated that 236 of 278 (85%, binomial $P = 6.1 \times 10^{-34}$) of the captured male SHBG signals had consistent direction of effect (77 with $P < 0.05$), and 241 of 283 in women (85%, binomial $P = 4.2 \times 10^{-35}$, 60 at $P < 0.05$). In Twins UK, all identified genome-wide significant variants in aggregate were significantly associated and directionally concordant for the respective sex hormone traits (Supplementary Table 12). Finally, in the EPIC-Norfolk study we estimated the magnitude of effect that a genetic risk score for SHBG and testosterone had on the respective trait levels (Extended Data Fig. 2). Men with the 5% highest genetic risk have 0.69 s.d. (95% confidence interval (95% CI): 0.53–0.85) and 1.27 s.d. (1.12–1.41) (equivalent to 2.55 nmol l^{-1} and 21.34 nmol l^{-1}) higher total testosterone and SHBG, respectively, than men with the 5% lowest scores; the equivalent difference in women is 0.45 s.d. (0.26–0.64) (0.28 nmol l^{-1}) and 1.29 s.d. (1.12–1.45) (35.91 nmol l^{-1}), respectively.

To putatively map each identified variant to its effector gene, we first identified any nonsynonymous variant highly correlated (linkage disequilibrium $r^2 > 0.7$) with a lead index variant. This implicated one or more genes at 482 of 2,571 (19%) single nucleotide polymorphism (SNP)–trait pairs, highlighting 291 unique genes (Supplementary Tables 2–11). To identify the likely tissue(s) and cell type(s) of action for sex hormone-associated loci, we integrated our data with gene expression data across 53 tissues available from the Genotype–Tissue Expression (GTEx) consortium using linkage disequilibrium score regression (see Methods). In both sexes, liver was the most enriched tissue (Extended Data Fig. 3), consistent with its established role as the site of SHBG production. Skeletal muscle in men and adrenal gland in women were the next most strongly enriched tissues. In contrast to findings for other reproductive traits, we found no evidence for enrichment of gene expression in any brain cell type (Extended Data Fig. 3). Within the three prioritized tissue types (liver, skeletal muscle and adrenal gland), we identified 161 unique expression quantitative trait loci (eQTL)-linked genes mapping within 300 kb to 200 of 2,571 SNP–trait pairs (see Methods and Supplementary Tables 2–11). We note that further evidence from experimental studies is needed to confirm our putative genes, but the current findings should help to guide such work.

Distinct genetic architectures of testosterone regulation between sexes. Despite similar heritability estimates (Table 1), the genetic component to variation in circulating testosterone levels was very different between sexes, as indicated by null genome-wide correlations between sexes (Table 1) and limited overlap of genome-wide significant signals between sexes (Supplementary Tables 13 and 14). This discordance was partly due to opposing effects between sexes at several individual loci, rather than solely null associations in one sex. For example, of the 254 signals for total testosterone in women, 72 were also at least nominally associated ($P < 0.05$) with total testosterone in men; however, of these, 33 (46%) showed directionally opposing effects between sexes (Supplementary Table 7). Notably, several variants had genome-wide significant but directionally opposing effects on testosterone in men and women (Supplementary Table 5), including the missense variants: rs56196860 in *FKBP4*, which encodes a regulator of androgen receptor transactivation activity[21]; and rs28929474 in *SERPINA1*, which encodes one of a family of proteins that are reported to regulate steroidogenesis in testicular Leydig cells[22].

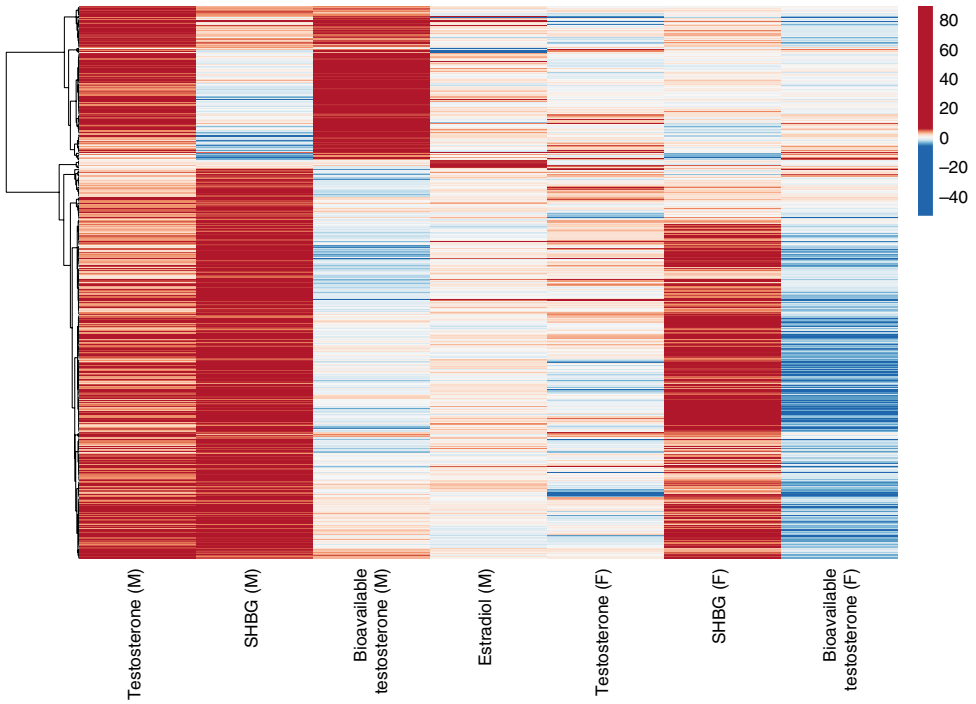

Fig. 1 | Cluster analysis of male identified sex hormone signals. All *Z* score effects are aligned to the male total testosterone-increasing allele. F, female; M, male.

Several other signals showed sex-specific effects (Supplementary Table 5). Notably, seven of nine X-chromosome signals for total testosterone in men and women combined altered levels only in men, including five variants located in/near genes associated with androgen insensitivity (*AR*), hypogonadotropic hypogonadism (*ANOS1*), failure of sex steroid 11 beta-hydroxylation (*HPRT1*), disrupted steroidogenesis (*STARD8*) and hypospadias (*DGKK*) (Supplementary Table 5). Notable autosomal male-specific testosterone signals were located at key regulators of puberty timing (for example, *LIN28B*-rs7759938; *TACR3*-rs528845403 and *KISS1*-rs201416723) and androgen secretion (*NR0B2*-rs182050989) or biosynthesis (*SRD5A2*-rs113017476) (Supplementary Table 5).

Among many signals with apparent female-specific effects on testosterone were five signals in/near to genes encoding enzymes in the cytochrome P450 family with reported roles in testosterone hydroxylation (*CYP3A7*-rs45446698, *CYP2D6*-rs5751229, *CYP2C8*/*CYP2C9*-rs11572082, *CYP11B2*-rs6471583 and *POR*-rs17853284) (Supplementary Table 5). Other signals with female-specific effects on testosterone included: reported PCOS susceptibility loci at *FSHB* (rs12294104) and *THADA* (rs58839393); *CYP17A1* (rs11441374) encoding 17,20-lyase, the decisive step in androgen synthesis, and its critical cofactor cytochrome b5 (*CYB5A*-rs17089026/rs79384925); and also near genes encoding luteinizing hormone subunit beta (*LHB*-rs78248023) and hormone receptors for glucocorticoids (*NR3C1*-rs34632394) and prolactin (*PRLR*-rs112694713) (Supplementary Table 5).

In contrast to testosterone traits, the genetic architecture of SHBG levels was highly concordant between men and women (genetic correlation, $r_g = 0.84$ (0.81–0.87), $P < 1 \times 10^{-100}$) (Table 1); 315 (88%) of the 359 genome-wide significant variants in women were also at least nominally associated ($P < 0.05$) with SHBG in men (Supplementary Table 4).

Genetic overlap between sex hormone traits within sexes. Among men, we found partially overlapping genetic determinants between the different sex hormone traits. This was reflected by positive genetic correlations between all four sex hormone measures (Table 1; $r_g = 0.19$–0.73), with the exception of a weak negative correlation between SHBG and bioavailable testosterone ($r_g = -0.048$ (s.e.m. = 0.024), $P = 0.04$). These genetic correlations were very similar to the observed phenotypic correlations (Supplementary Table 15). In contrast to men, among women, there was a weak negative genetic correlation between total testosterone and SHBG ($r_g = -0.06$ in women; 0.73 in men), a strong negative correlation between bioavailable testosterone and SHBG (−0.74 in women; −0.05 in men) and a similar positive correlation between total and bioavailable testosterone (0.65 in women; 0.60 in men), again closely reflecting the observed phenotypic correlations (Table 1 and Supplementary Table 15).

Cluster analysis identifies loci with primary SHBG or testosterone effects. Testosterone levels are dependent on SHBG levels but genetic variants may allow us to separate distinct components of variation in sex hormone levels. To identify signals with primary effects on individual sex hormone traits, we performed a cluster analysis of all 525 signals that reached genome-wide significance for one or more sex hormone measure in men, identifying three clusters (Fig. 1 and Supplementary Table 16). The largest cluster (362 signals) was characterized by loci with relatively strong positive associations with SHBG; in combination, SNPs in this cluster also increased total testosterone, reduced bioavailable testosterone and increased estradiol in men (Supplementary Table 17). Hence, this cluster (termed 'male SHBG cluster'; see Methods) represents a genetic instrument with primary SHBG-increasing effects, and secondary divergent effects on total (higher) and bioavailable testosterone (lower) that are consistent with the known hormone-regulatory role of SHBG.

Among men, the second identified cluster (122 loci) was consistently associated with higher total and bioavailable testosterone levels in a dose-response manner. In combination, SNPs in this cluster

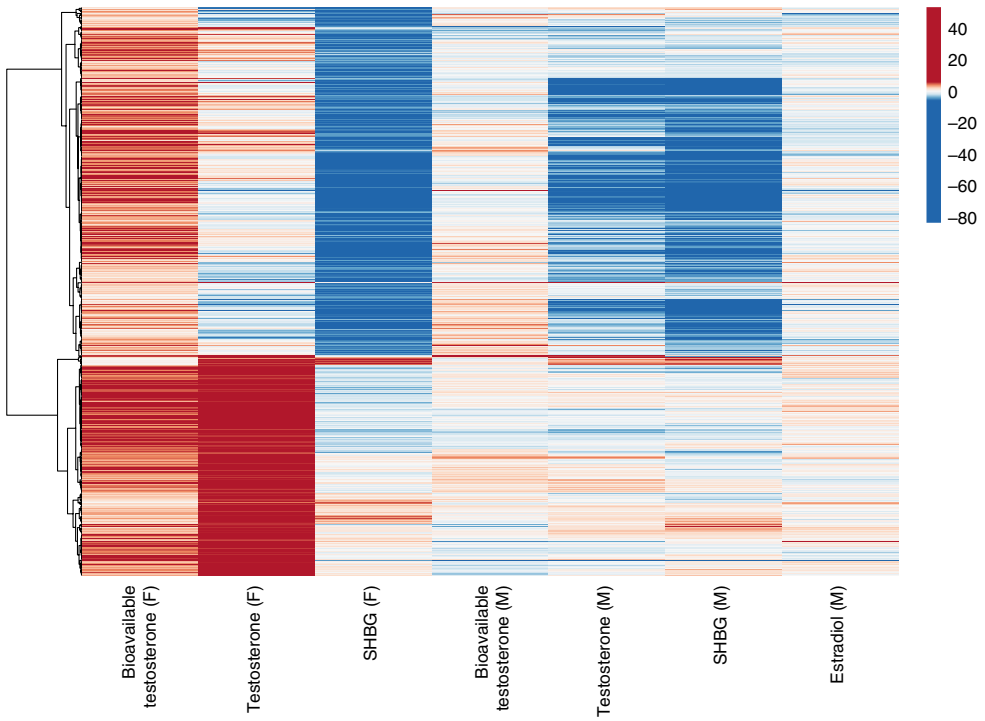

Fig. 2 | Cluster analysis of female identified sex hormone signals. All *Z* score effects are aligned to the female bioavailable testosterone-increasing allele.

also increased estradiol levels, but had no effect on SHBG ($P=0.66$) (Supplementary Table 17). Hence, this cluster (termed 'male specific testosterone cluster') represents a genetic instrument with primary (total and bioavailable) testosterone-increasing effects and with secondary estradiol-increasing effects (consistent with the physiological conversion of androgens to estrogens), but independent of SHBG.

Among men, a third small cluster (14 signals) strongly increased estradiol, but not other sex hormone measures (Supplementary Table 17). The most prominent signal in this cluster (rs781858752) was uniquely associated with estradiol in men ($P=7.6\times10^{-15}$) but not with any other sex hormone measure in men or women (all $P>0.05$), and influenced expression of *IGHV3-9* and *IGHV1-8* in the liver (Supplementary Table 11).

In addition to separating testosterone from SHBG effects, defining such clusters is an important step for downstream analyses to minimize the pleiotropic effects of SNPs that may have much stronger effects on other sex hormones. For example, the apparent strong male estradiol association ($P=1.5\times10^{-35}$) at the X-chromosome rs111386834 locus, ~200 kb from *KAL1*, is clearly secondary to a stronger effect of this signal on bioavailable ($P=3\times10^{-670}$) and total testosterone ($P=1.5\times10^{-372}$), consistent with the known role of this gene on the hypothalamic–pituitary reproductive axis.

As in men, in women, cluster analysis of all 614 signals for any of the three sex hormone measures in women (Fig. 2) identified two main clusters, representing genetic instruments with (1) primary SHBG effects and secondary directionally opposing effects on total and bioavailable testosterone ('female SHBG cluster', 373 signals) and (2) consistent testosterone effects but no aggregate effect on SHBG ('female specific testosterone cluster', 241 signals) (Supplementary Table 18). Hence, in both men and women, cluster analyses resulted in genetic instruments that allowed us to test specific testosterone-increasing effects, independent of SHBG.

Understanding the impact of sex hormone measures on disease outcomes. Having identified over 2,500 associations between genetic variants and sex hormone measures, we designed a set of MR analyses (see Methods) to inform the causal effects of sex hormones on two broad categories of disease outcomes—(1) T2D, insulin resistance, body composition and related metabolic disease risk factors; and (2) hormone-sensitive cancers. Given the lack of overlap between men and women in sex hormone-associated variants (Table 1 and Supplementary Tables 13 and 14), and the possible different metabolic effects of these hormones between sexes, we focused analyses on sex-specific disease outcomes. As exposures, we tested total and bioavailable testosterone and SHBG in both men and women, and also estradiol in men. For each outcome trait, we identified the largest published sex-specific GWAS meta-analysis with publicly available data (Supplementary Table 19). We then performed a series of MR analyses using two-sample inverse variance-weighted (IVW), Egger and weighted median models (see Methods). We additionally modeled different genetic risk scores by (1) Steiger filtering to exclude variants with larger effects on metabolic traits than the tested sex hormone, and (2) cluster filtering using variants in the clusters as defined in "Cluster analysis identifies loci with primary SHBG or testosterone effects" representing primary effects on SHBG or testosterone independent of SHBG. To further inform the role of SHBG, we additionally tested the two *cis* variants in *SHBG* as an instrument for SHBG.

Using these genetic instruments, in men and women separately, we could infer causal positive effects of testosterone levels on lean body mass and number of lifetime sexual partners (Supplementary Tables 20–22 and Extended Data Fig. 4). These findings are consistent with the established positive effects of testosterone on these traits in RCTs[6] and therefore support the validity of our genetic instrument analyses.

MR analyses in men. In men, we found evidence of beneficial effects of higher testosterone on metabolic traits (Fig. 3, Extended Data Figs. 4 and 5 and Supplementary Tables 20 and 23). For T2D and related traits, the evidence of a protective effect of testosterone was most consistent when using the cluster-specific genetic instrument representing a primary (total and bioavailable) testosterone-increasing effect independent of SHBG. Using data from 34,990 men with

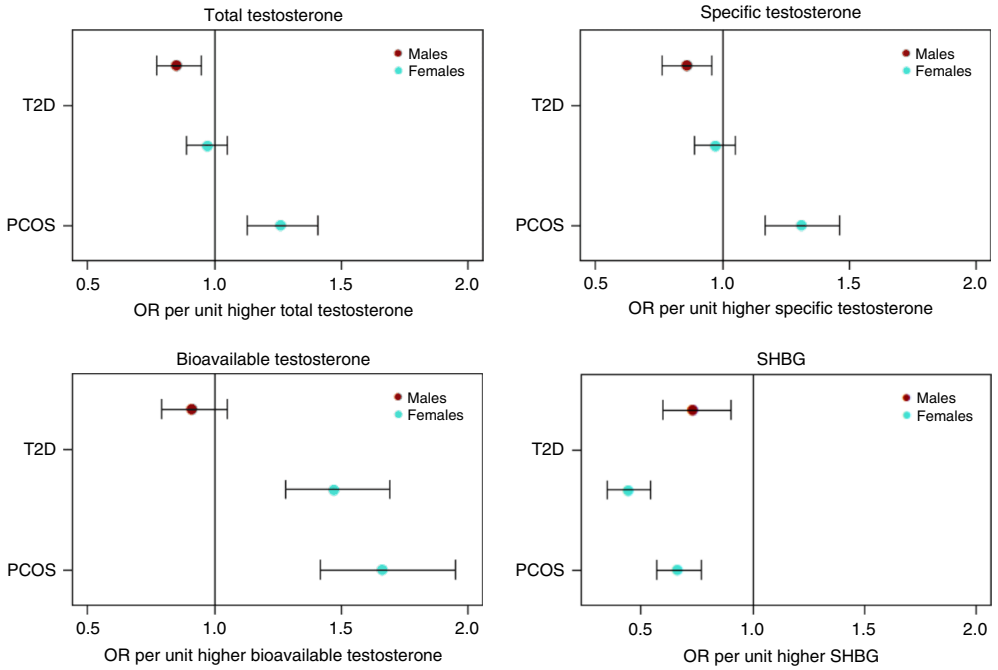

Fig. 3 | Plots showing the odds of T2D and PCOS per unit higher testosterone and SHBG using genetic instruments in MR analyses. Unit measurements for the individually transformed exposure traits can be found in Supplementary Table 1. Specific testosterone refers to a total testosterone score that has no aggregate effect on SHBG. Bars indicate 95% CIs around the point estimates from IVW analyses. Analyses are based on association statistics generated in a maximum of: total and specific testosterone, $n=194{,}453$ men and $n=230{,}454$ women; bioavailable testosterone, $n=178{,}782$ men and $n=188{,}507$ women; SHBG, $n=180{,}726$ men and $n=189{,}473$ women; T2D, $n=34{,}990$ cases and $n=150{,}760$ controls in men and $n=17{,}790$ cases and $n=243{,}645$ controls in women; PCOS, $n=10{,}074$ cases and $n=103{,}164$ controls. Numbers of genetic variants included in the analyses are given in Supplementary Tables 20 and 21.

T2D and 150,760 male controls[23], and 67,506 nondiabetic men with fasting glucose levels available (Lagou, V. et al., manuscript in preparation), exposure to higher testosterone, independent of SHBG, conferred lower T2D risk and lower fasting glucose: each 1 s.d. higher testosterone level (approximately 3.7 nmol l^{-1}) was associated with a 15% lower T2D risk in men (total testosterone OR: 0.85; 95% CI: 0.77–0.95; cluster-specific testosterone OR: 0.86; 95% CI: 0.76–0.98). These metabolically beneficial associations were directionally consistent, but did not reach nominal significance ($P<0.05$), in all sensitivity analyses (Supplementary Tables 20 and 24 and Extended Data Fig. 5).

In contrast to these apparent beneficial metabolic effects, MR analyses indicated that testosterone increases prostate cancer risk in men: each 1 s.d. higher bioavailable testosterone level increased prostate cancer risk by 23% (OR: 1.23; 95% CI: 1.13–1.33), with consistent findings across all testosterone genetic instruments (unfiltered, Steiger-filtered and cluster-filtered) (Fig. 4, Supplementary Table 25 and Extended Data Fig. 6).

We found no compelling evidence for an effect of estradiol in men on any metabolic or body composition trait; however, confidence intervals were wide (Supplementary Tables 20, 23 and 25).

MR analyses in women. Despite evidence for a positive effect of total testosterone on lean body mass in women as well as men, testosterone was associated with several adverse metabolic outcomes in women (Supplementary Table 21).

We found consistent evidence supporting a causal effect of testosterone on higher PCOS risk in women. These effects were most evident with bioavailable testosterone, with positive findings across all MR models and all instruments (unfiltered, Steiger-filtered and cluster-filtered) (Extended Data Figs. 7 and 8 and Supplementary Table 21). These effects equated to an OR of 1.51 (95% CI: 1.33–1.72) per 1 s.d. higher bioavailable testosterone.

MR analyses also showed a causal effect of bioavailable testosterone on higher T2D risk and higher fasting insulin in women (using unfiltered and Steiger-filtered instruments) (Supplementary Table 21 and Extended Data Figs. 4 and 9). Risk of T2D was increased by 37% (OR: 1.37; 95% CI: 1.22–1.53) per 1-s.d. higher bioavailable testosterone. We also found evidence for protective effects of SHBG on T2D across all MR models using Steiger-filtered and cluster-filtered instruments, and apparent protective effects of SHBG on fasting insulin levels, and central fat measures, android and visceral, but not total body fat (consistently across unfiltered, Steiger-filtered and cluster-filtered instruments) (Supplementary Tables 21 and 26 and Extended Data Figs. 4 and 9). These effects equated to an OR for T2D of 0.65 (95% CI: 0.58–0.72) per 1 s.d. (approximately 30.3 nmol l^{-1}) higher SHBG. The lack of association with the testosterone-specific cluster (representing higher testosterone independent of SHBG) for T2D or fasting insulin (Supplementary Table 21) indicates that the above associations with bioavailable testosterone and SHBG in women might be driven by direct effects of SHBG; however, we did not have a genetic instrument that was specific to SHBG.

We found evidence that testosterone increased the risk of estrogen receptor (ER)-positive but not ER-negative breast cancer, with consistent findings across all MR models and instruments (Extended Data Fig. 6 and Supplementary Table 27). Furthermore, testosterone increased the risk of endometrial cancer but reduced the risk of ovarian cancer, again with consistent findings across sensitivity models (Extended Data Fig. 6 and Supplementary Table 27). There was also evidence for a protective effect of SHBG on risk of endometrial cancer in women, which was consistent across all models, but a risk-increasing effect of SHBG on ER-negative breast cancer.

Cis variants in the *SHBG* gene provide a confirmatory test of higher circulating SHBG levels, independent of potential confounding

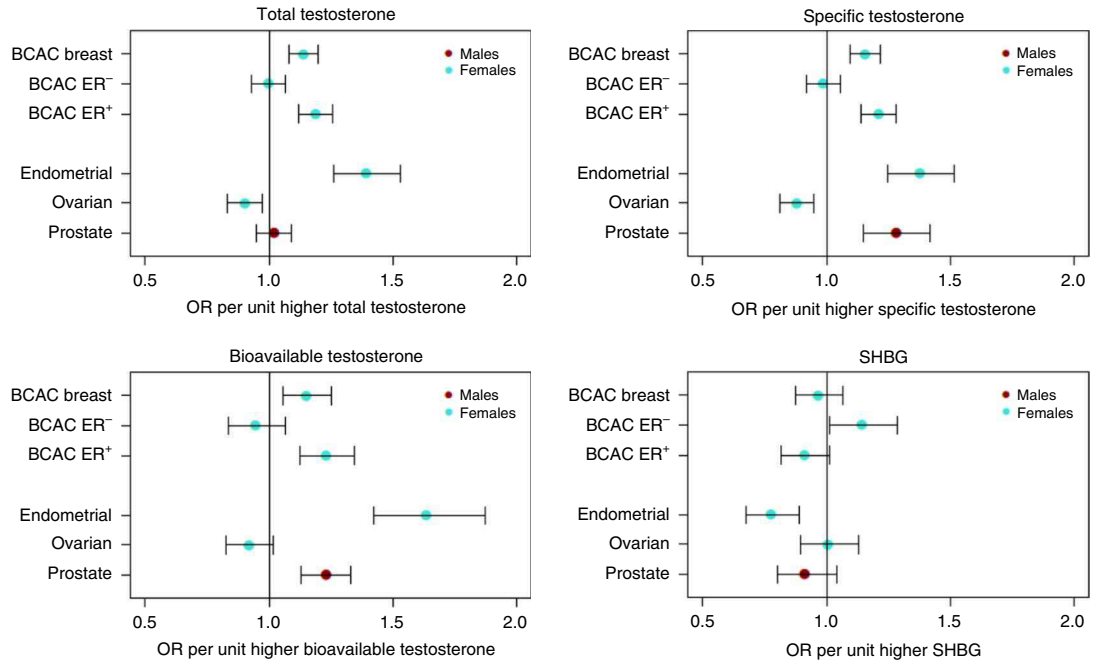

Fig. 4 | Plots showing the odds of cancer per unit higher testosterone and SHBG using genetic instruments in MR analyses. Unit measurements for the individually transformed exposure traits can be found in Supplementary Table 1. Specific testosterone refers to a total testosterone score that has no aggregate effect on SHBG. Bars indicate 95% CIs around the point estimates from IVW analyses. Analyses are based on association statistics generated in a maximum of: total and specific testosterone, $n=194{,}453$ men and $n=230{,}454$ women; bioavailable testosterone, $n=178{,}782$ men and $n=188{,}507$ women; SHBG, $n=180{,}726$ men and $n=189{,}473$ women; breast cancer, $n=105{,}974$ cases and $n=122{,}977$ controls; ER-negative subtype breast cancer, $n=21{,}468$ cases and $n=100{,}594$ controls; ER-positive subtype breast cancer, $n=69{,}501$ cases and $n=95{,}039$ controls; endometrial cancer, $n=12{,}270$ cases and $n=46{,}126$ controls; ovarian cancer, $n=25{,}509$ cases and $n=40{,}941$ controls; prostate cancer, $n=67{,}158$ cases and $n=48{,}350$ controls. Numbers of genetic variants included in the analyses are given in Supplementary Tables 25 and 27.

by adiposity and insulin resistance, but including effects of reciprocally lower bioavailable testosterone. Results using two *cis* variants were generally consistent with our main analyses (Supplementary Table 24), with consistent associations with the low-frequency missense *SHBG* variant on PCOS and T2D in women, and directionally consistent but smaller effects of the common noncoding variant.

Discussion

We identify >2,500 genetic variant sex hormone associations and provide insights into the genetic architecture of sex hormone regulation and its relevance to disease. We see limited overlap between the genetic variants identified in men and women for all sex hormone traits except SHBG, and even overlapping signals often showed divergent effects. Cluster analyses across all identified variants distinguished, in each sex, groups of variants with testosterone-increasing effects either dependent or independent of SHBG. These clusters helped inform genetic causal inference analyses by showing primary metabolic effects of testosterone that were beneficial in men (lower fasting glucose and lower T2D risk) but harmful in women (higher PCOS risk). In contrast, associations that are seen only with bioavailable testosterone and SHBG (for example, T2D in women) could be driven by effects of SHBG, directly or in combination with testosterone.

Testosterone Trials in men, the largest RCTs of testosterone administration to date, found clear benefits of testosterone on sexual function and body composition in men, but insufficient data on disease outcomes due to sparse numbers of such outcomes even in the largest trials. While RCT evidence remains the gold standard, genetic instrumental variable analyses provide a more robust evidence base than phenotypic observational study designs, as they are less prone to confounding and reverse causality. For example, while adverse effects of testosterone on prostate cancer risk might be expected, given the established role of testosterone-reducing agents in the treatment of prostate cancer, the evidence from observational studies is remarkably diverse: out of 45 papers, 18 reported positive associations between testosterone and prostate cancer, 17 reported negative associations and 10 reported no association[8]. Furthermore, in a recent analysis of 20 prospective studies, low bioavailable testosterone predicted lower risk of low-grade prostate cancers but higher risk of high-grade cancers[9]. Therefore, our findings advance our understanding of the risks and benefits of this widely used therapy in men.

Our findings that higher testosterone increases the risk of PCOS in women is important in demonstrating the etiological role of testosterone in this common disorder, rather than simply being a consequence of upstream defects in ovarian dysfunction and insulin signaling. Androgen-blocking agents are widely used to treat symptoms of hyperandrogenism in women with PCOS, but evidence is lacking for the role of androgens in the etiology and prevention of this condition[24]. Similarly, experimental evidence of the effects of testosterone administration in women arises from several RCTs, albeit using substantially lower doses than in men and often topical routes of administration, which substantiate the positive effects of testosterone on the primary outcome, sexual function. However, even in combination, these RCTs include insufficient disease events to inform about its potential effects on cardiometabolic traits and cancer risks[25].

Our findings positively link testosterone to number of sexual partners and lean body mass in men and women, which provides reassurance about the validity of our approach. However, some limitations need to be acknowledged. While we could distinguish a cluster-specific genetic instrument for testosterone that was independent of SHBG, the effects of this, and our other testosterone instruments, might be mediated at least in part by downstream conversion of testosterone to estradiol. This has been hypothesized to explain the observed phenotypic associations between testosterone

and higher risk of ER-positive breast cancer. However, regardless of downstream mechanisms, our findings provide evidence to inform the consequences of real-world differences in testosterone on health outcomes. Similarly, while our SHBG-related clusters in men and women were not independent of testosterone, and therefore cannot inform the debate about SHBG-specific metabolic effects, they reflect the actual downstream biological effects of SHBG on (higher) total testosterone and (lower) bioavailable testosterone. A second limitation, common to all MR analyses, is that genetic instruments represent lifelong exposures to the risk factor, and so may have different effects to short-to-medium term pharmacological interventions even if they achieve the same difference in circulating concentrations. A third limitation is that the discovery of genetic variants was performed in a single large study that is known to be enriched for healthier and older individuals, potentially influencing (likely underestimating) the effect size of associated variants. Finally, the MR approach depends on some key assumptions which we attempted to assess using a range of sensitivity analyses. Associations across these sensitivity analyses were generally directionally consistent, but did not always reach $P < 0.05$. We note that our findings do not preclude an additional bi-directional effect of disease status on testosterone or suggest that other factors are not important causal determinants of the tested outcomes.

Our study highlights three important methodological considerations. First, in light of the substantial overlap between genetic determinants of testosterone and SHBG within each sex, our cluster-based analyses allowed us to identify subsets of variants that alter testosterone independent of SHBG. This effectively removes potential direct biological effects of SHBG and its confounding by adiposity and insulin resistance[26]. Second, we used Steiger filtering of our genetic instruments to exclude variants with stronger effects on metabolic traits compared with their effects on sex hormones. This approach helped reduce the possibility of reverse causality, an issue that is increasingly important in large-scale GWASs[27].

Finally, our findings show the importance of sex-specific analyses, both in the discovery of genetic variants for sex hormone traits and in the analyses of downstream traits. The apparently sex-divergent effects of testosterone on T2D were obfuscated by sex-combined data. Available large-scale sex-specific data on T2D were invaluable for our study—unfortunately, similar sex-specific data for cardiovascular disease are not yet available, which will be critically important to understand the wider cardiometabolic impact of testosterone. Hence, while the findings relating to adverse metabolic effects of testosterone in women may inform clinical practice, it is premature to infer wider beneficial metabolic effects in men.

In conclusion, our findings provide unique insights into the disease impacts of testosterone and highlight the importance of sex-specific analyses of disease risk.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41591-020-0751-5.

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Methods

Phenotype preparation in UKBB. Discovery analyses were performed in the full UKBB study which has been described extensively elsewhere[28]. All UKBB participants provided written informed consent, the study was approved by the National Research Ethics Service Committee North West–Haydock and all study procedures were performed in accordance with the World Medical Association Declaration of Helsinki ethical principles for medical research. At baseline, a panel of 34 biomarkers were measured across the full ~500,000 study participants. We selected three sex hormone traits—SHBG, testosterone and estradiol—and additionally calculated a measure of bioavailable testosterone using the Vermeulen equation[29,30]. Individual trait transformations and exclusion criteria are detailed in Supplementary Table 1.

Genetic discovery analysis. We used genetic data from the 'v3' release of UKBB[28], containing the full set of Haplotype Reference Consortium (HRC) and 1000 Genomes imputed variants. In addition to the quality control metrics performed centrally by UKBB, we defined a subset of 'white European' ancestry samples using a *K*-means clustering approach applied to the first four principal components calculated from genome-wide SNP genotypes. Individuals clustered into this group who self-identified by questionnaire as being of an ancestry other than white European were excluded. After application of quality control criteria, a maximum of 425,097 UKBB participants were available for analysis with genotype and phenotype data. Association testing was performed using linear mixed models implemented in BOLT-LMM[31] to account for cryptic population structure and relatedness. Only autosomal genetic variants that were common (minor allele frequency (MAF) > 1%), passed quality control in all 106 batches and were present on both genotyping arrays were included in the genetic relationship matrix.

Across each of the four sex hormone traits, we performed GWAS discovery analyses both within and across sexes, with the exception of estradiol where analyses were performed only in men. To help improve reproducibility of results, analyses were conducted independently at two sites and compared for consistency, with any discrepancies investigated. A decision on which dataset to use for each discovery GWAS was made based on strength of association of the previously reported *SHBG* gene locus variants[19].

Genotyping chip, age at baseline and ten genetically derived principal components were included as covariates in all models, in addition to specific covariates used for individual traits detailed in Supplementary Table 1. For SHBG we included body mass index (BMI) as a covariate, which was previously demonstrated to increase statistical power by reducing trait variance. To avoid any effects that may be attributed to collider bias[32], we compared BMI-adjusted estimates with BMI-unadjusted estimates across all identified genome-wide significant SHBG signals. We discarded from further consideration any loci that changed effect direction between models and/or had large changes in effect estimate and statistical significance. For downstream analyses, genetic loci from the BMI-adjusted analyses were used with corresponding effect estimates from the BMI-unadjusted analyses.

Replication was performed using three independent datasets. Firstly, a previously published Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) consortium meta-analysis of SHBG (age- and BMI-adjusted) in 21,791 individuals (9,390 women, 12,401 men)[19]. Given these data used HapMap 2 imputation, we found proxy HapMap 2 variants with a minimum $r^2 > 0.5$ to align (Supplementary Table 28). Secondly, a previously published GWAS of 2,913 individuals from the Twins UK resource[20] with measured dehydroepiandrosterone, total testosterone, follicle stimulating hormone, luteinizing hormone, estradiol, progesterone, prolactin and SHBG, and calculated free androgen index. Finally, replication of the genetic scores was attempted with measurements of total testosterone (5,334 men and 3,804 women) and SHBG (5,694 men and 5,476 women) from the EPIC-Norfolk study[33]. Here, regression models were conducted on ventiles of the score, and were controlled for ten genetic principal components and additionally menopausal status in women (Extended Data Fig. 2). Given the relatively small sizes of these replication studies, we used these data to validate genetic instruments in aggregate rather than as individual loci (Supplementary Table 28).

Signal selection and genetic instrument generation. We defined statistically independent signals (described as lead or index variants) using 1 megabase distance-based clumping across all imputed variants with $P < 5 \times 10^{-8}$, an imputation quality score > 0.5 and MAF > 0.1%. Although several studies[42,43] have suggested other *P* value thresholds for genome-wide significance more stringent (for example, $P < 6 \times 10^{-9}$) than the currently accepted community standard ($P < 5 \times 10^{-8}$), as our primary focus of this paper was the production of genetic risk scores (rather than focus on individual genetic variants), we felt the more liberal threshold was acceptable to help maximize variance explained. We note that multiple trait correction would likely be overconservative given the correlation structure between traits.

Genome-wide significant lead variants that shared any correlation with each other due to long-range linkage disequilibrium ($r^2 > 0.05$) were excluded from further consideration. These loci were additionally augmented with additional independent signals (described as secondary signals) using approximate conditional analyses implemented in Genome-wide Complex Trait Analysis (GCTA)[34]. Here, secondary signals were only considered if they were (1) uncorrelated ($r^2 < 0.05$) with a previously identified index variant, (2) genome-wide significant pre- and postconditional analysis, and (3) had an effect estimate that did not change by more than 10% pre- and postconditional analysis.

For downstream analyses we produced genetic instruments using two approaches. Firstly, used the genome-wide significant signal SNPs that we identified from our signal selection process for a given trait and sex to derive seven genetic instruments:

1. 'SHBG-Men' ($N = 357$)—individually genome-wide significant SNPs for SHBG in men, discovered using BMI-adjusted analysis but using weights from a BMI-unadjusted analysis.
2. 'SHBG-Women' ($N = 359$)—as above, but in women.
3. 'Total T-Men' ($N = 231$)—individually genome-wide significant SNPs for total testosterone in men, weighted by individual SNP beta estimate for total testosterone.
4. 'Total T-Women' ($N = 254$)—as above, but in women.
5. 'Bioavailable T-Men' ($N = 125$)—individually genome-wide significant SNPs for bioavailable testosterone in men, weighted by individual SNP beta estimate for bioavailable testosterone.
6. 'Bioavailable T-Women' ($N = 180$)—as above, but in women.
7. 'Estradiol-Men' ($N = 22$)—individually genome-wide significant SNPs for estradiol in men, weighted by individual SNP beta estimates for estradiol.

Secondly, given the genetic overlap between traits, we observed that some signals were shared between sex hormone traits but appeared to have much stronger effects in one versus others. To help derive additional genetic risk scores that reflected this, we took all genome-wide significant signals within each sex but across traits, and performed ward-based hierarchical clustering[35] on individual variant *Z* scores. We used the observed clusters from these analyses to produce additional genetic instruments (Supplementary Table 16):

8. A 'male SHBG cluster' ($N = 362$) formed from SNPs with dominant effects on SHBG in men. Each SNP in this genetic instrument is weighted by its effect from the BMI-unadjusted SHBG analysis.
9. A 'male testosterone cluster' ($N = 122$) formed from SNPs with dominant effects on both total and bioavailable testosterone in men. Each SNP in this genetic instrument is weighted by its effect on total testosterone.
10. A 'male estradiol cluster' ($N = 14$) formed from SNPs with dominant effects on estradiol in men.
11. A 'female SHBG cluster' ($N = 373$) formed from SNPs with dominant effects on SHBG in women. Each SNP in this genetic instrument is weighted by its effect from the BMI-unadjusted SHBG analysis.
12. A 'female testosterone cluster' ($N = 241$) formed from SNPs with dominant effects on both total and bioavailable testosterone in women. Each SNP in this genetic instrument is weighted by its effect on total testosterone.

Gene prioritization. We used the SMR software package[36] to systematically map associated genetic variants to genes via expression effects (eQTLs). For all analyses we included expression data from liver, in addition to skeletal muscle in men and adrenal gland in women. All expression data were generated by the GTEx consortium (v7), made available from the SMR website resource section (https://cnsgenomics.com/software/smr/#DataResource). Only genes passing multiple test correction and exhibiting no statistically significant evidence of coincidental eQTL overlap (assessed by the SMR HEIDI metric) were considered. The same data were additionally used to perform global tissue enrichment using linkage disequilibrium score regression applied to specifically expressed genes (LDSC-SEG)[37].

MR analyses. For outcome traits, we limited analyses (1) to traits that were previously reported as associated with circulating sex hormone levels, (2) to traits that have sex-specific associations with sex hormones and (3) to traits where sex-specific GWAS data were available in large non-UKBB studies (see Supplementary Table 19). Given the potential for bias in MR studies when a large proportion of genetic variants are discovered in the same sample as the outcome is measured, we used non-UKBB GWAS data as the primary outcome data. This resulted in us considering as an outcome six diseases: T2D, PCOS, prostate cancer, breast cancer, ovarian cancer and endometrial cancer; two glycemic traits: fasting insulin as a measure of insulin resistance and fasting glucose; and four main measures of body composition: BMI, waist-to-hip ratio adjusted for BMI, and, using dual energy X-ray absorptiometry (DEXA) measures, total body fat and total lean mass. Where we observed positive associations for total fat or lean mass, we tested six more specific measures of body composition; android fat, gynoid fat, android lean mass, gynoid lean mass, and subcutaneous and visceral fat from DEXA data. Outcome data for number of sexual partners were based on previously analyzed data from UKBB[38].

Each of the 12 genetic instruments listed in "Signal selection and genetic instrument generation" was used as an exposure instrumental variable in our subsequent MR analyses. Where a signal was not present in the outcome GWAS, we identified a 1000 Genomes or HapMap proxy with $r^2 > 0.5$ within 250 kb either

side of the signal and its relevant weight was included in our genetic instrument (Supplementary Table 28).

In each MR test we assessed a number of widely used methods—IVW, weighted median and MR–Egger[39,40]. MR relies on some key assumptions. These assumptions include (1) that alleles are randomly assigned among people and (2) that alleles that influence exposure do not influence the outcome via any pathway other than through the exposure. The use of the most robust models available (linear mixed models), as implemented in BOLT-LMM, to ensure that alleles are not stratified within the UKBB provides reassurance that the first assumption holds. To address the second assumption, we performed several additional analyses. We used two additional MR methods (MR–Egger and median MR), both of which are more robust to pleiotropy—directionally consistent results strengthened our causal inference. We used the MR–Egger intercept, with a P value of $P < 0.05$, to provide evidence that pleiotropy could be affecting the MR results. Furthermore, we implemented an approach known as Steiger filtering. In this test, we excluded variants with larger effects on outcome traits or traits known to be closely associated with outcome traits compared with their effects on the sex hormone exposure trait[41]. Given the strong association between SHBG and adiposity and insulin resistance, and the large discovery sample size, it was possible that many variants could be associated with sex hormone levels via an outcome trait, rather than having direct effects on sex hormones, so invalidating the MR assumptions. We excluded between 2% and 40% of variants (depending on the sex hormone) if they had larger effects (based on standardized beta) on any one of 11 metabolic traits available in the UKBB (fasting glucose, T2D, coronary artery disease, HDL-C, LDL-C, triglycerides, total-cholesterol, and diastolic and systolic blood pressure, BMI and waist-to-hip ratio adjusted for BMI). A full list of which variants were excluded and why is given in Supplementary Table 29.

Furthermore, we considered only *cis* variants at the *SHBG* gene locus (Supplementary Table 24). Here, we used two variants in low linkage disequilibrium as more specific but less powerful genetic instruments. Variants in *cis* with a gene likely represent the most specific test of the causal role of a circulating protein encoded by that gene. One of these variants (rs1799941) is common and has been used in several previous MR studies of SHBG[16,19], whilst the other (rs6258) is rare (~1% MAF) and alters SHBG's binding affinity for testosterone.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

All data used in discovery analyses are available from UK Biobank upon request (https://www.ukbiobank.ac.uk).

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Author contributions

K.S.R., F.R.D., J.T., D.J.T., J.R.B.P. and T.M.F. analyzed the data. K.S.R., F.R.D., J.T., J.R.B.P., T.M.F., K.K.O. and A. Murray drafted the manuscript. D.J.T., A.R.W., A. Mahajan, R.N.B., L.W., S.M., A.S.B., A.M.E., B.H., T.A.O'M., M.I.M., C.L., D.F.E., N.J.W., S.B. and the ECA consortium contributed data and advised on analysis. J.R.B.P., T.M.F., K.K.O., A. Murray and S.B. designed and led the study. All co-authors commented on and revised the manuscript.

Competing interests

The views expressed in this article are those of the authors and not necessarily those of the NHS, the NIHR or the Department of Health. M.I.M. has served on advisory panels for Pfizer, NovoNordisk and Zoe Global, and has received honoraria from Merck, Pfizer, Novo Nordisk and Eli Lilly, and research funding from Abbvie, Astra Zeneca, Boehringer Ingelheim, Eli Lilly, Janssen, Merck, NovoNordisk, Pfizer, Roche, Sanofi Aventis, Servier and Takeda. As of June of 2019, M.I.M. is an employee of Genentech, and a holder of Roche stock. T.M.F. holds an MRC CASE studentship with GSK and has consulted for Sanofi, Servier and Boerhinger Ingelheim.

Additional information

Supplementary information is available for this paper at https://doi.org/10.1038/s41591-020-0751-5.

Extended data is available for this paper at https://doi.org/10.1038/s41591-020-0751-5.

Correspondence and requests for materials should be addressed to T.M.F. or J.R.B.P.

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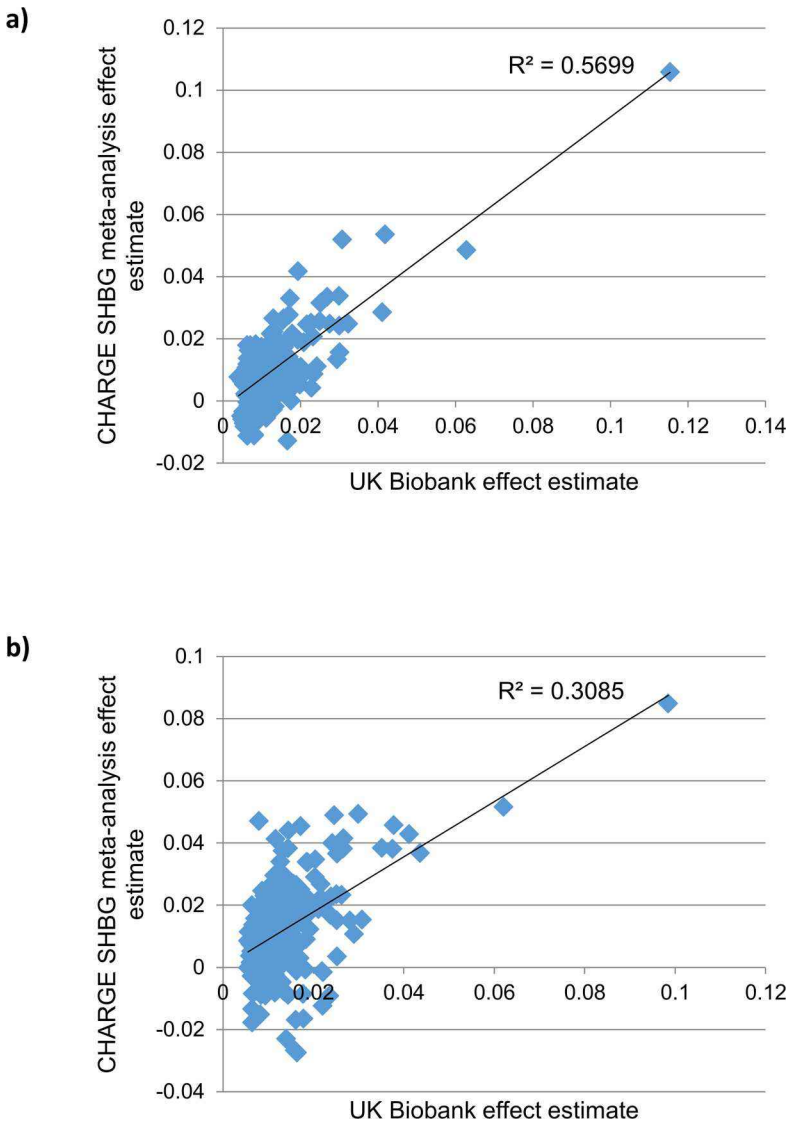
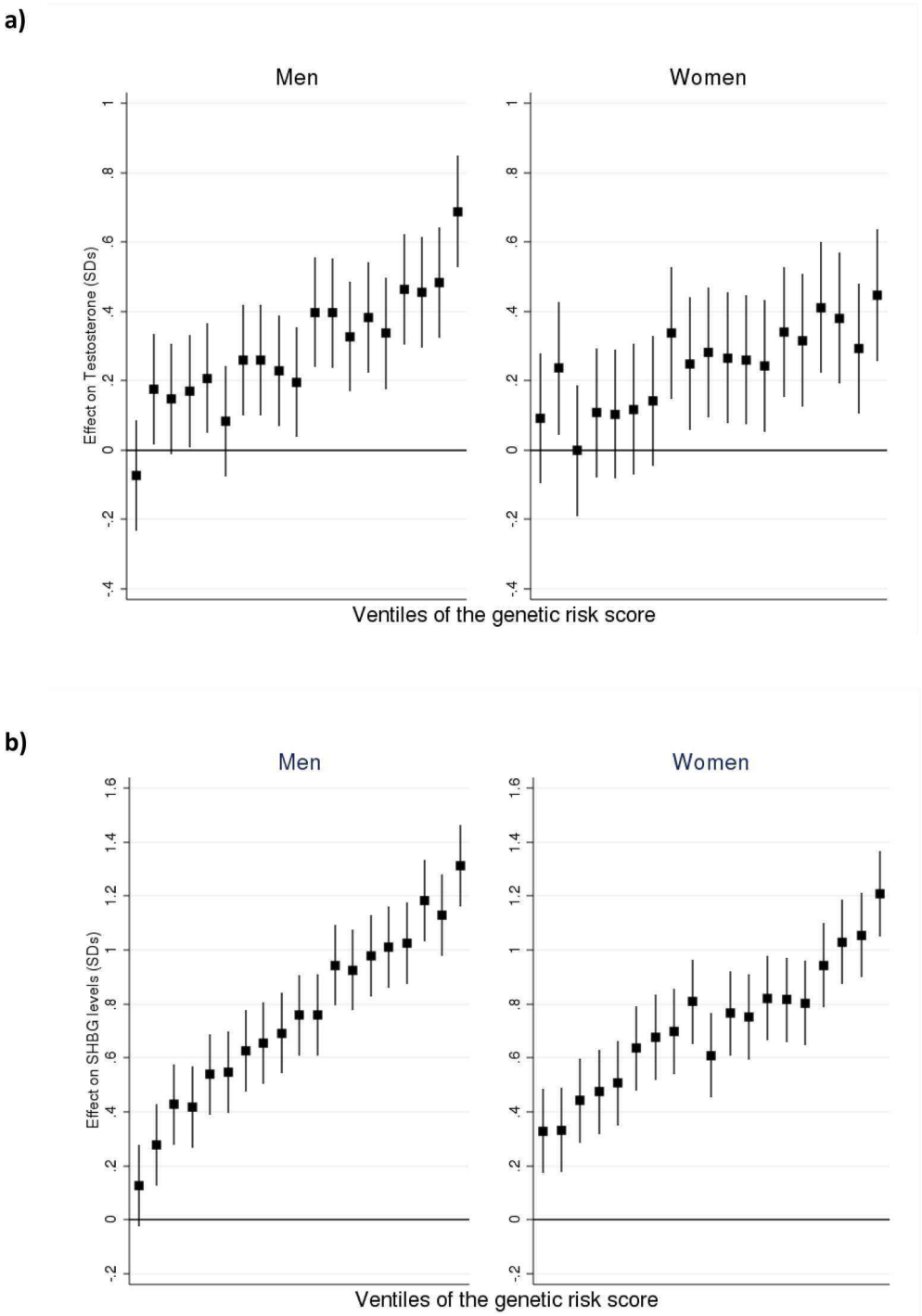
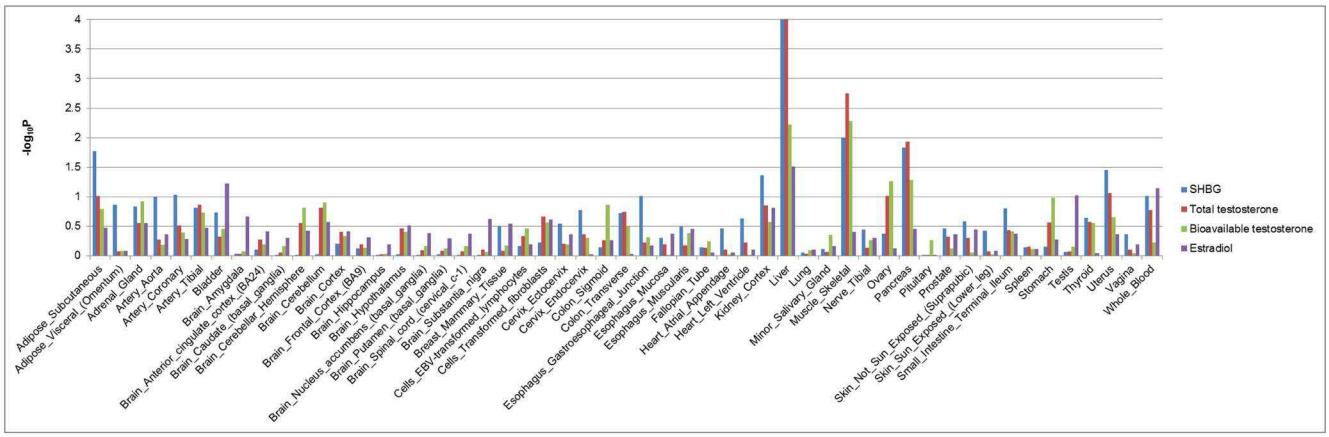


Extended Data Fig. 1 | Replication of identified SHBG signals in CHARGE meta-analysis. a) Effect size comparison performed against published estimates from the CHARGE male SHBG meta-analysis (N = 12,401). **b**) Effect size comparison performed against published estimates from the CHARGE female SHBG meta-analysis (N = 9,390). The SHBG *cis* locus (which had a concordant effect direction) has been excluded to maintain an appropriate scale.



Extended Data Fig. 2 | Relationship between measured sex hormone levels in the EPIC-Norfolk study and polygenic score for increased sex hormone level. a) Total testosterone levels in the EPIC-Norfolk study by polygenic score for increased total testosterone (n = 5,334 men; n = 3,804 women). **b**) SHBG levels in the EPIC-Norfolk study by polygenic score for increased SHBG (n = 5,694 men; n = 5,476 women). Bars denote the standard error around the point estimate of the mean. Effect on hormone is given in standard deviations (SDs). SHBG = sex hormone binding globulin.

a)



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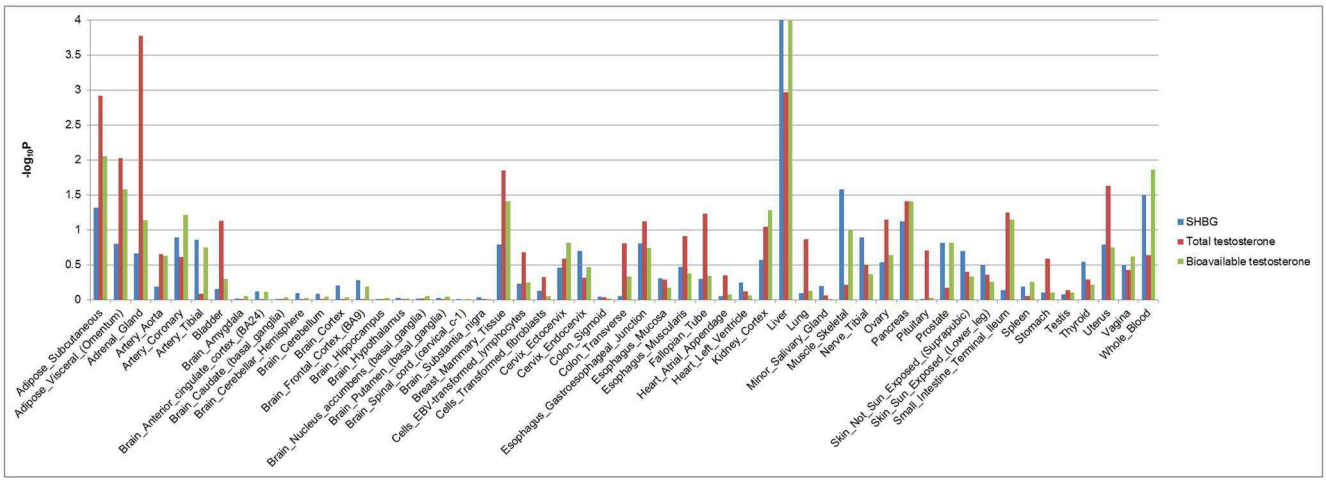
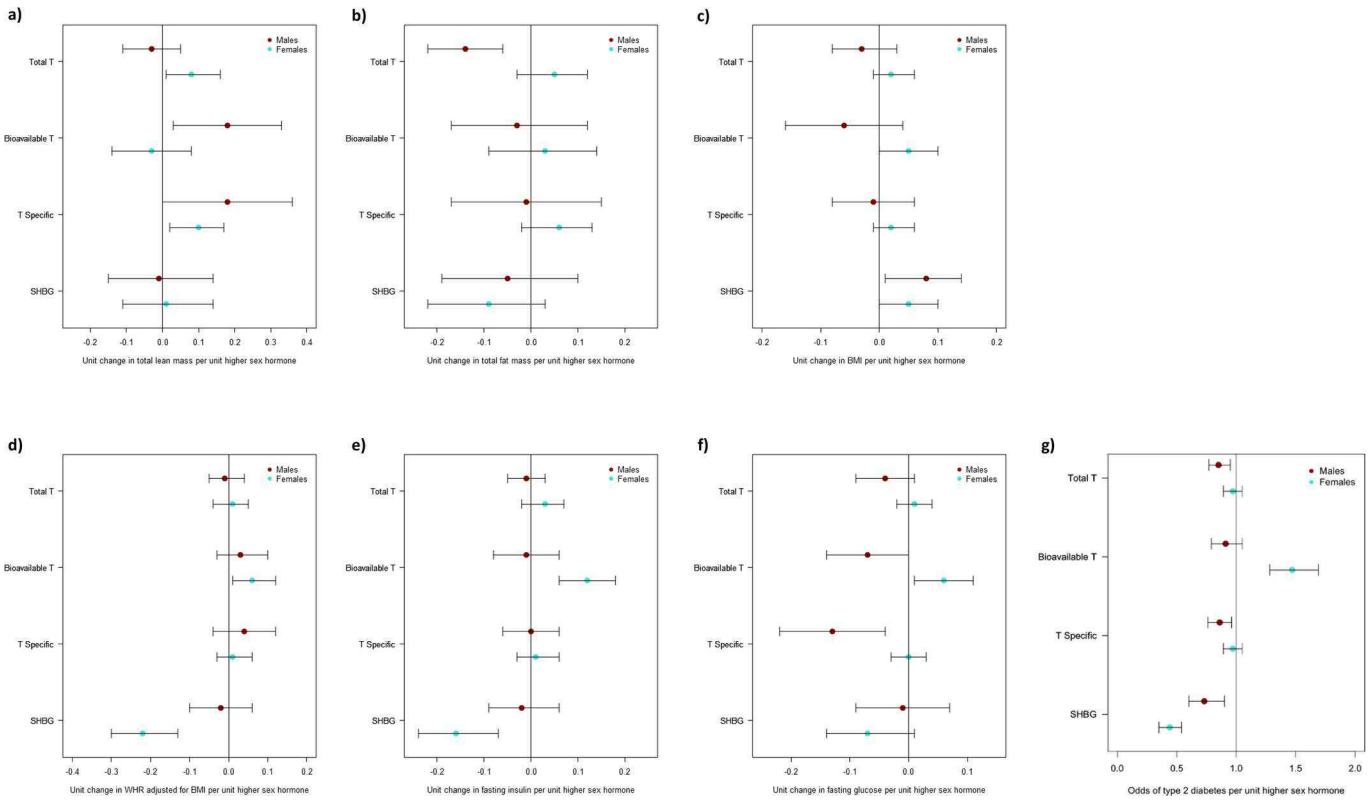
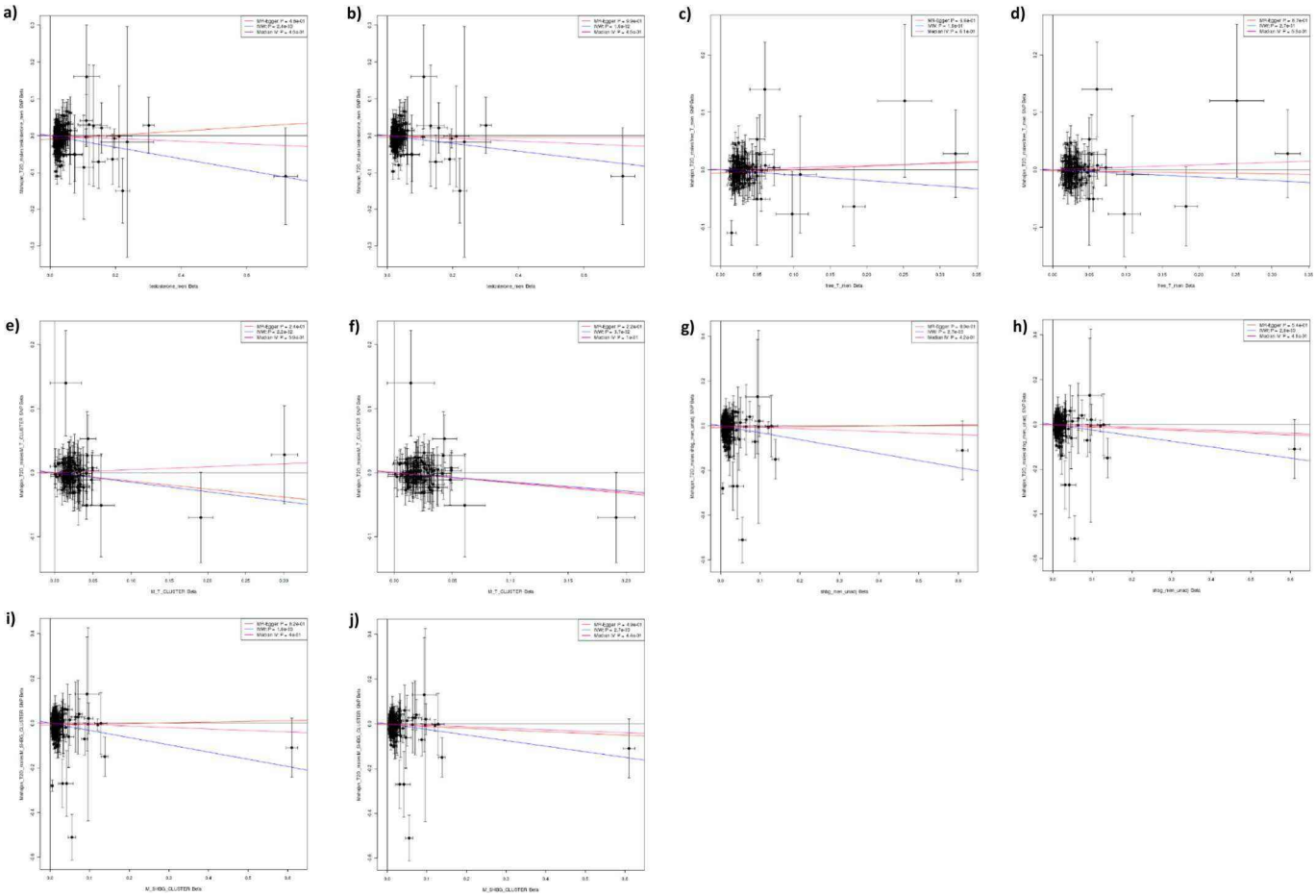


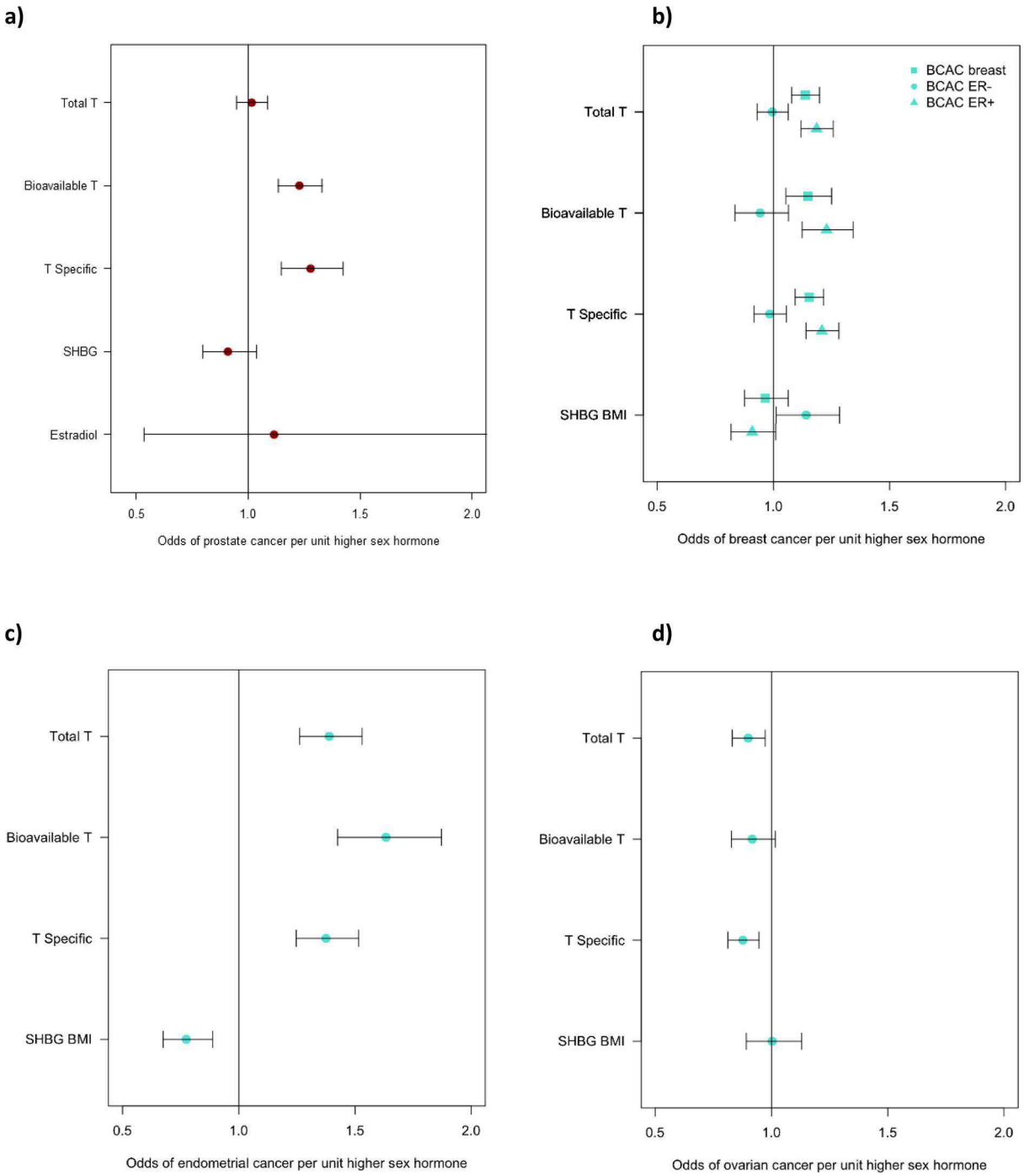
Extended Data Fig. 3 | LD score regression analysis of enrichment of sex hormone signals in 53 GTEx tissues and cell types. a) Analysis in men. **b**) Analysis in women.



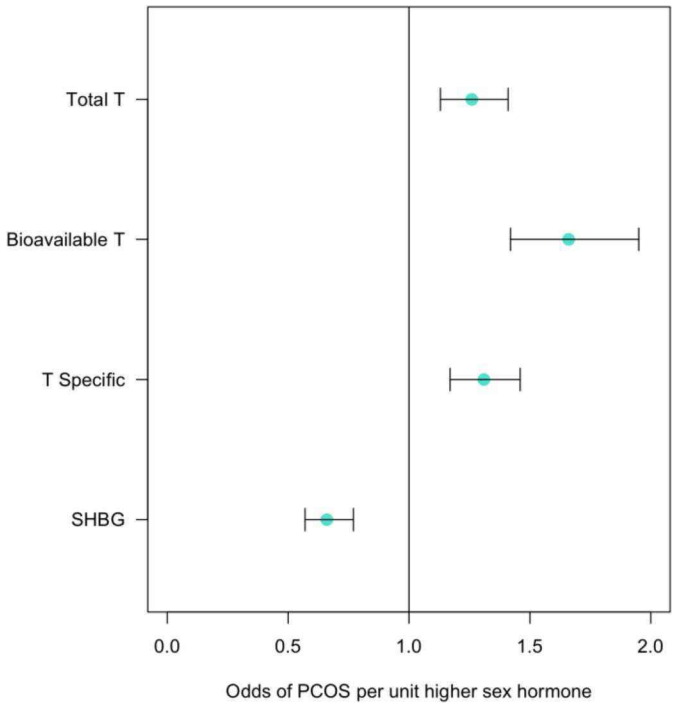
Extended Data Fig. 4 | Results of inverse-variance weighted Mendelian randomization analysis of sex hormone genetic instruments on metabolic traits and body composition outcomes. Dot plots representing the change in the following metabolic outcomes and body composition traits in males and females per unit higher sex hormone: **a**) Total lean mass. **b**) Total fat mass. **c**) BMI. **d**) Waist-hip ratio adjusted for BMI. **e**) Fasting insulin. **f**) Fasting glucose. **g**) Type 2 diabetes. Bars indicate 95% confidence interval around the point estimate from inverse-variance weighted analysis. Analyses are based on association statistics generated in a maximum of: total and specific testosterone, n = 194,453 men and n = 230,454 women; bioavailable testosterone, n = 178,782 men and n = 188,507 women; SHBG, n = 180,726 men and n = 189,473 women; total lean mass and total fat mass, n = 9,102 men and n = 10,406 women; BMI, n = 152,893 men and n = 171,977 women; WHR adjusted for BMI, n = 93,480 men and n = 116,742 women; fasting insulin, n = 47,806 men and n = 50,404 women; fasting glucose, n = 67,506 men and n = 73,089 women; T2D, n = 34,990 cases and n = 150,760 controls in men and n = 17,790 cases and n = 243,645 controls in women. Numbers of genetic variants included in the analyses are given in Supplementary Table 20, 21, 23 and 26. BMI = body mass index; SHBG = sex hormone binding globulin; T = testosterone; T Specific = testosterone cluster; WHR = waist-hip ratio.



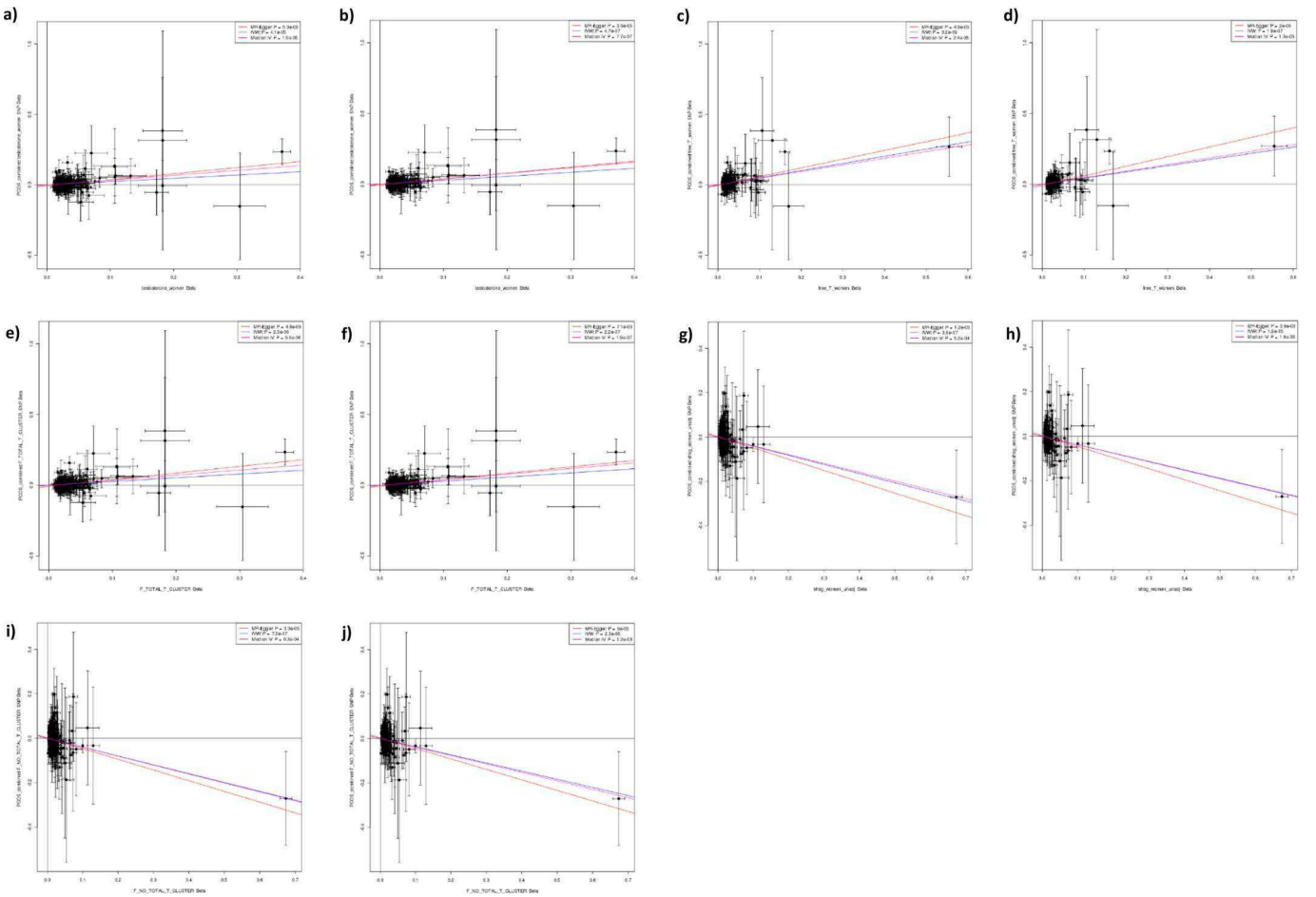
Extended Data Fig. 5 | Results of Mendelian randomization analysis in men of genetic instruments for testosterone and SHBG on the outcome of Type 2 diabetes. Plots show effect on ln(odds) of Type 2 diabetes (y axes) in men of the following sex hormone genetic instruments (x axes; effect size in units). **a**) Total testosterone. **b**) Steiger filtered total testosterone. **c**) Bioavailable testosterone. **d**) Steiger filtered bioavailable testosterone. **e**) Testosterone specific cluster. **f**) Steiger filtered testosterone specific cluster. **g**) SHBG. **h**) Steiger filtered SHBG. **i**) SHBG specific cluster. **j**) Steiger filtered SHBG specific cluster. P-values and effect size estimates (indicated by lines) are from Egger (pink), IVW (blue), and median IV (red) Mendelian randomization analyses. Bars indicate 95% confidence interval around the point estimate for each genetic variant. Analyses are based on association statistics generated in a maximum of: total testosterone (including specific and Steiger filtered), n = 194,453; bioavailable testosterone (including Steiger filtered), n = 178,782; SHBG (including specific and Steiger filtered), n = 180,726; T2D, n = 34,990 cases and n = 150,760 controls. Numbers of genetic variants included in the analyses are given in Supplementary Table 20. SHBG = sex hormone binding globulin.



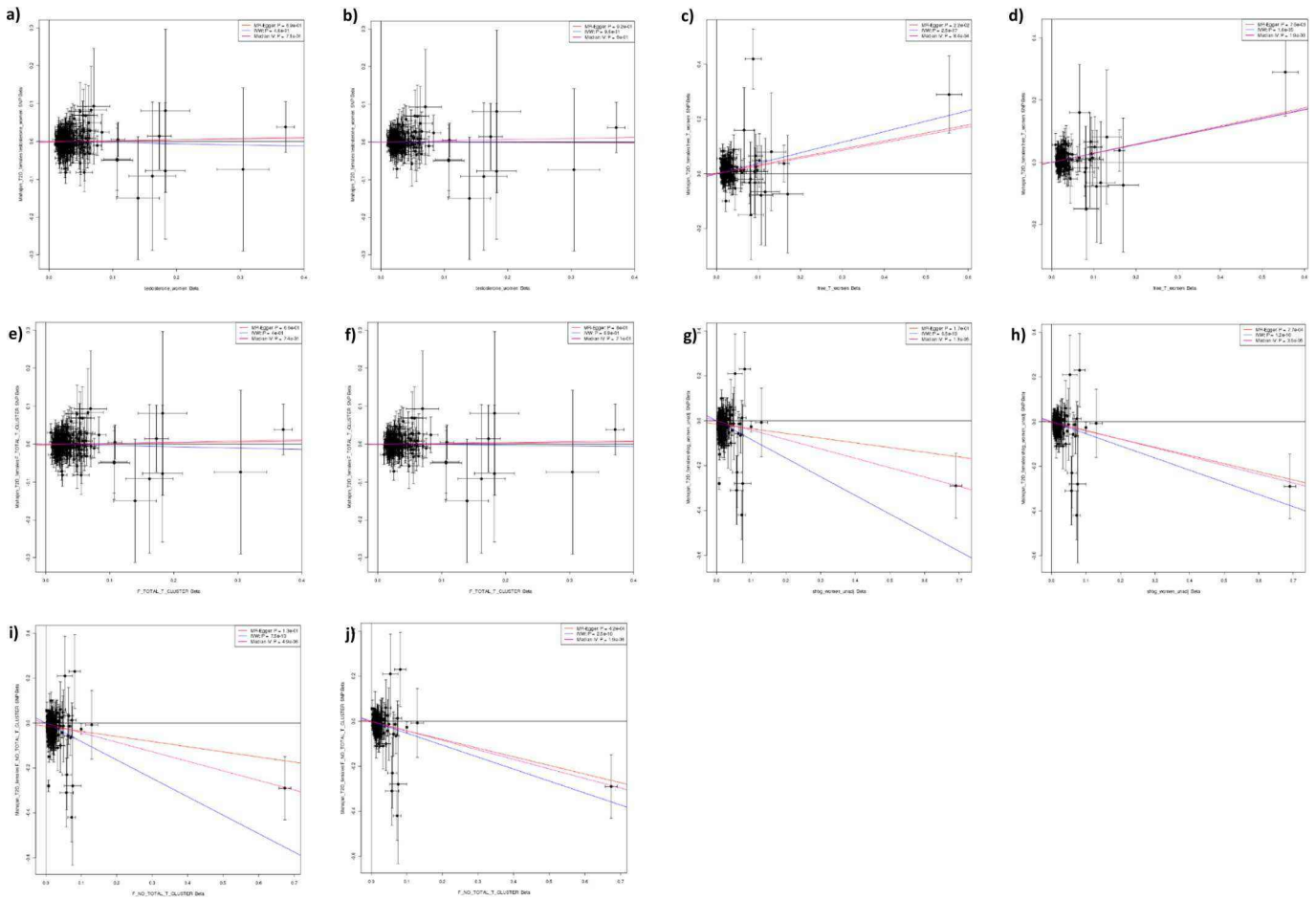
Extended Data Fig. 6 | Results of inverse-variance weighted Mendelian randomization analysis of sex hormone genetic instruments on cancer outcomes. Dot plots representing the change in the odds of the following cancers per unit higher sex hormone in males or females, as appropriate. **a**) Prostate cancer in males. **b**) Breast cancer (all types) and estrogen receptor positive (ER+) and negative (ER-) subtypes in females. **c**) Endometrial cancer in females. **d**) Ovarian cancer in females. Bars indicate 95% confidence interval around the point estimate from inverse-variance weighted analyses. Analyses are based on association statistics generated in a maximum of: total and specific testosterone, n = 194,453 men and n = 230,454 women; bioavailable testosterone, n = 178,782 men and n = 188,507 women; SHBG, n = 180,726 men and n = 189,473 women; estradiol, n = 206,927 men; prostate cancer, 67,158 cases and 48,350 controls; breast cancer, n = 105,974 cases and n = 122,977 controls; ER negative subtype breast cancer, n = 21,468 cases and n = 100,594 controls; ER positive subtype breast cancer, n = 69,501 cases and n = 95,039 controls; endometrial cancer, n = 12,270 cases and n = 46,126 controls; ovarian cancer, n = 25,509 cases and n = 40,941 controls. Numbers of genetic variants included in the analyses are given in Supplementary Table 25 and 27. SHBG = sex hormone binding globulin; T = testosterone; T Specific = testosterone cluster.



Extended Data Fig. 7 | Results of inverse-variance weighted Mendelian randomization analysis in females of sex hormone genetic instruments on PCOS. Dot plot represents the odds of PCOS per unit higher sex hormone. Bars indicate 95% confidence interval around the point estimate from inverse-variance weighted analyses. Analyses are based on association statistics generated in a maximum of: total and specific testosterone, n = 230,454; bioavailable testosterone, n = 188,507; SHBG, n = 189,473; PCOS, n = 10,074 cases and n = 103,164 controls. Numbers of genetic variants included in the analyses are given in Supplementary Table 21. PCOS = polycystic ovary syndrome; SHBG = sex hormone binding globulin; T = testosterone; T Specific = testosterone cluster.



Extended Data Fig. 8 | Results of Mendelian randomization analysis in women of genetic instruments for testosterone and SHBG on the outcome of PCOS. Plots show effect on ln(odds) of PCOS (y axes) of the following sex hormone genetic instruments in women (x axes; effect size in units). **a**) Total testosterone. **b**) Steiger filtered total testosterone. **c**) Bioavailable testosterone. **d**) Steiger filtered bioavailable testosterone. **e**) Testosterone specific cluster. **f**) Steiger filtered testosterone specific cluster. **g**) SHBG. **h**) Steiger filtered SHBG. **i**) SHBG specific cluster. **j**) Steiger filtered SHBG specific cluster. P-values and effect size estimates (indicated by lines) are from Egger (pink), IVW (blue), and median IV (red) Mendelian randomization analyses. Bars indicate 95% confidence interval around the point estimate for each genetic variant. Analyses are based on association statistics generated in a maximum of: total testosterone (including specific and Steiger filtered), n = 230,454; bioavailable testosterone (including Steiger filtered), n = 188,507; SHBG (including specific and Steiger filtered), n = 189,473; PCOS, n = 10,074 cases and n = 103,164 controls. Numbers of genetic variants included in the analyses are given in Supplementary Table 21. PCOS = polycystic ovary syndrome; SHBG = sex hormone binding globulin.



Extended Data Fig. 9 | Results of Mendelian randomization analysis in women of genetic instruments for testosterone and SHBG on the outcome of Type 2 diabetes. Plots show effect on ln(odds) of Type 2 diabetes in women (y axes) of the following sex hormone genetic instruments in women (x axes; effect size in units). **a**) Total testosterone. **b**) Steiger filtered total testosterone. **c**) Bioavailable testosterone. **d**) Steiger filtered bioavailable testosterone. **e**) Testosterone specific cluster. **f**) Steiger filtered testosterone specific cluster. **g**) SHBG. **h**) Steiger filtered SHBG. **i**) SHBG specific cluster. **j**) Steiger filtered SHBG specific cluster. P-values and effect size estimates (indicated by lines) are from Egger (pink), IVW (blue), and median IV (red) Mendelian randomization analyses. Bars indicate 95% confidence interval around the point estimate for each genetic variant. Analyses are based on association statistics generated in a maximum of: total testosterone (including specific and Steiger filtered), n = 230,454; bioavailable testosterone (including Steiger filtered), n = 188,507; SHBG (including specific and Steiger filtered), n = 189,473; T2D, n = 17,790 cases and n = 243,645 controls. Numbers of genetic variants included in the analyses are given in Supplementary Table 21. SHBG = sex hormone binding globulin.

nature research

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Reporting Summary

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For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed	
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☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☐	☒	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	N/A - no custom code was used for data collection
Data analysis	BOLT-LMM (v2.3.2), LDSC (v1,0), SMR (v0.712), GCTA (v1.91.6beta), FUSION-TWAS (v1.0)

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

All data used in discovery analyses is available from UK Biobank upon request (https://www.ukbiobank.ac.uk).

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	We used the full available sample in UK Biobank (with the exception of exclusions below) for discovery analyses. Previously published analyses reported positive findings in an order of magnitude fewer samples, so we were confident the current study was appropriately powered.
Data exclusions	Individuals failing standard genotyping quality control parameters defined initially by the UK Biobank study or individuals of non-european ancestry were excluded from analysis. Additionally, we excluded any individuals taking hormone replacement therapy medication. These decisions were made prior to performing any downstream analysis.
Replication	Replication was performed in three independent studies described fully in the methods text. All attempted replication has been reported in the manuscript without exception
Randomization	N/A - randomization occurred naturally as genetic variants were the exposure.
Blinding	N/A - genetic association testing does not require blinding.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology
☒	☐ Animals and other organisms
☐	☐ Human research participants
☒	☐ Clinical data

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Human research participants

Policy information about studies involving human research participants

Population characteristics	UK Biobank is a national resource that has been described extensively elsewhere (https://www.ukbiobank.ac.uk). Individuals were not directly selected for inclusion in the study on the basis of any disease or health parameter.
Recruitment	All people aged 40–69 years (men and women) who were registered with the National Health Service and living up to 25 miles from one of the 22 study assessment centers were invited to participate in 2006–2010. Overall, about 9.2 million invitations were mailed to recruit 503,325 participants (a response rate of 5.47%).
Ethics oversight	All UK Biobank participants provided written informed consent, the study was approved by the National Research Ethics Service Committee North West–Haydock and all study procedures were performed in accordance with the World Medical Association Declaration of Helsinki ethical principles for medical research

Note that full information on the approval of the study protocol must also be provided in the manuscript.