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Causal effect of sex hormone-binding globulin and testosterone on coronary heart disease: A multivariable and network Mendelian randomization analysis

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ABSTRACT

Background: Although observational studies have shown an association between sex hormone-binding globulin (SHBG), testosterone (T) and cardiovascular diseases (CVD), controversy remains. In this study, we aim to explore the causal effects of SHBG and T on Coronary heart disease (CHD).

Methods: We used univariable, network and multivariable mendelian randomization (MR) analysis to investigate the causal effect of SHBG and T on CHD. We performed inverse variance weighted (IVW) MR as the primary analysis, with the robustness of this approach further tested by other methods in sensitivity analysis. The SHBG and T were collected from the UK Biobank data, about 180,000 men aged 40 to 69 years. CHD was collected from CARDIoGRAMplusC4D 1000 Genomes-based GWAS, which was a meta-analysis including 48 studies and involving 60,801 CHD cases and 123,504 controls.

Results: Using univariable MR-IVW, the results suggested that a one standard deviation (SD) increase in SHBG, the risk of CHD decreased by approximately 14% (OR (95% CI): 0.86(0.76,0.97)), and that a SD increase in total testosterone (TT), the risk also decreased, approximately 8% (OR (95% CI): 0.92(0.85,0.99)). Multivariable MR showed that both SHBG and TT had no direct causal effect with CHD (a SD increase in SHBG: OR (95% CI):0.75 (0.57,1.00), $P = 0.053$; a SD increase in TT: OR (95% CI): 1.05(0.90,1.22), $P = 0.53$). In the network MR analysis, the results suggested that TT might act as mediator in the causal pathway from SHBG to CHD and account for 93% of the total effect of SHBG on CHD, and that SHBG might be a mediator in the causal pathway from TT to CHD and account for 67% of the total effect of TT on CHD.

Conclusions: Genetically predicted SHBG and TT were negatively correlated with CHD in both univariable and network MR, which may provide a causal explanation behind the observed conclusion. In addition, TT and SHBG had a bidirectional causal effect. Further work is required to disentangle the downstream effects of SHBG/TT on CHD and the molecular pathways involved, as the simultaneous regulation of SHBG and TT may make it a viable strategy for the prevention or treatment of CHD.

1. Introduction

With the increase in the average human life span, the morbidity of chronic diseases has also increased. Globally, there were an estimated 422.7 million prevalent cases of CVD and 17.92 million deaths in 2015 [1]. Coronary heart disease (CHD) continues to be the most important cause of premature mortality and a major cause of disability in CVD [1].

In addition, testosterone (T) levels in men decrease with age on account of declining adrenal and testicular function. Consequently, studying the impact of this reduction in testosterone on chronic diseases has become important. And in recent years, there has been controversy about the effect of testosterone on CVD, especially testosterone supplementation. The fundamental reason is that the causal effect between

Abbreviations: CVD, cardiovascular disease; CHD, Coronary heart disease; T, testosterone; TT, total testosterone; BT, bioavailable testosterone; SHBG, Sex Hormone-Binding Globulin; ER^{+ve}, estrogen receptor-positive; MR, Mendelian Randomization; ICD, International Classification of Diseases; SNPs, Single nucleotide polymorphisms; GWAS, Genome wide association study; HWE, Hardy-Weinberg equilibrium; BMI, Body mass index; IVW, Inverse variance weighted; OR, odds ratio; CI, Confidence interval; MAF, Minor allele frequency; cAMP, Cyclic adenosine monophosphate.

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testosterone and CVD is unclear. Some epidemiological and observational studies have shown that low T is associated with increased CVD risk [2–5], but some studies found high testosterone levels are related to the occurrence of CVD [6,7]. The reason for this discrepancy is most likely due to unmeasured confounding and/or reverse causality, a major limitation of observational studies. So it is unclear if this is a causal association or due to low T being a biomarker of poor health. Recently, some studies have explored the causal relationship between total testosterone (TT) and CVD, but SHBG, which is a glycoprotein and the major transporter and putative regulator of androgens that binds sex steroid hormones [8] and has strong genetic correlation with TT (genetic correlation: $r_g = 0.73$) [9] in men, has not been considered. SHBG, however, may also directly influence numerous traits and diseases independently of the hormones it regulates [10]. Some observational evidence has so far linked higher SHBG levels with lower risk of type 2 diabetes [11], asthma [12], estrogen receptor-positive (ER^{+ve})breast cancer [13] etc. Genetic evidence supports the observed associations with type 2 diabetes [14], asthma [15] and ER^{+ve} breast cancer [16].

In this study, we will use genetically predicted TT, bioavailable testosterone (BT) that is not bound to SHBG and largely represents the sum of T unbound to any plasma protein plus human serum albumin bound T and SHBG as exposures, CHD as outcome, and using univariable, multivariable and network Mendelian randomization (MR) analysis to explore the causal association between them. The framework of the MR analysis is described in Fig. 1. MR analysis provides a useful tool for exploring causal effects of endogenous exposures on disease risk without adding any intervention [17]. 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 [17].

2. Methods and materials

2.1. Exposures

The genetic predictors for T (including TT and BT) and SHBG were obtained from the UK Biobank. The UK Biobank is a large, ongoing, prospective cohort study, with median follow up time of 11.1 years [18]. The UK Biobank comprises over 500,000 participants who were aged 40–69 years during the recruitment [19].

In reference 10, the exposures were genetically predicted, normal transformed (log transformed or Inverse normal transformation of rank) serum TT, BT and SHBG (nmol/L) [9]. SHBG and TT were measured by two step sandwich immunoassay analysis on a Beckman Coulter Unicel Dxl 800 [20], BT was calculated from TT, SHBG and albumin [21,22].

In this study, we obtained the SNPs, beta, effect allele etc. from the articles by Ruth, Katherine S et al [9], which was the latest GWAS article on SHBG and testosterone with the largest sample size, recently. Among them, there were 194,453 subjects for TT, 178,782 for BT and 180,726 for SHBG [10]. The sample inclusion/exclusion criteria were as follows: (1) Included white Europeans only. (2) Non-missing values only so excluded participants with testosterone below lower limit of detection. (3) Men who self-reported taking hormone based medication (UK Biobank variables 30,850 and 20,003) [9].

2.2. Outcome

We used CARDIoGRAMplusC4D 1000 Genomes-based GWAS, which does not contain UK Biobank samples, as the outcome data set. CARDIoGRAMplusC4D 1000 Genomes-based GWAS is a meta-analysis of 48 GWAS studies of mainly European descent (about 77%), imputed using the 1000 Genomes phase 1 v3 training set with 38 million variants. The study interrogated 9.4 million variants and involved 60,801 CHD cases and 123,504 control [23]. The call rates of 48 GWAS studies were greater than or equal to 95% [23].

We used CHD as the outcome and extracted SNPs associated with exposures from the D4D CARDIoGRAMplusC4D 1000 Genomes-based GWAS data set.

2.3. Genetic instrumental variables for SHBG and testosterone

MR is based on three basic assumptions, i.e., the genetic variants, specifically here single nucleotide polymorphisms (SNPs), are strongly related to the exposure (relevance), are not associated with confounders of the exposure-outcome relation (independence), and only influence the outcome via the exposure (exclusion-restriction) [24].

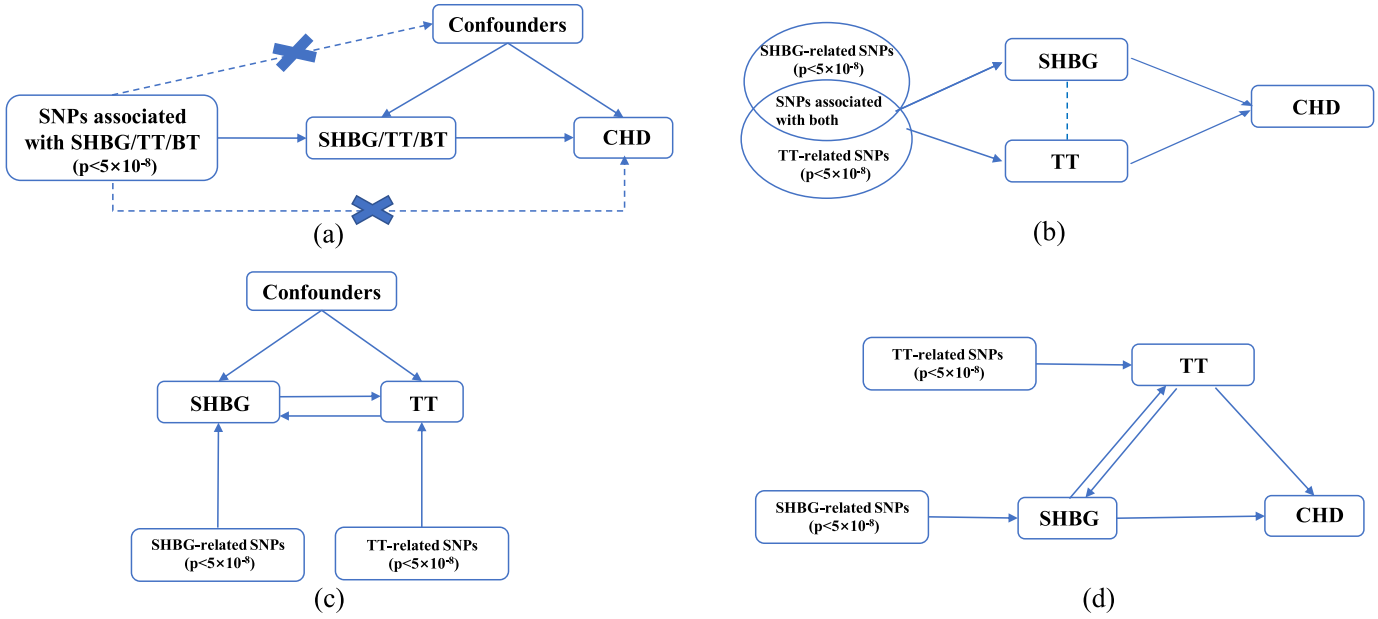

Fig. 1. (a) Univariable Mendelian randomization analysis framework; (b) Multivariable Mendelian randomization analysis framework; (c) Bidirectional Mendelian randomization analysis framework; (d) Network Mendelian randomization analysis framework.

To satisfy these assumptions, we used genome wide significant SNPs ($P < 5 \times 10^{-8}$). The genetic instruments were obtained from the largest, sex-specific genome wide association study (GWAS) conducted in the UK Biobank (178,782 white British men and 230,454 white British women) and replicated in three independent studies (CHARGE Consortium, Twins UK, and EPICNorfolk) [9], and the call rate was greater than or equal to 90% [25]. The GWAS provided 357 ($r^2 < 0.05$) independent SNPs for SHBG in men, with minor allele frequency > 1%, imputation quality score > 0.5 [9].And the GWAS also provided 231 independent SNPs for TT and provided 125 independent SNPs for BT in men [9]. We checked for the Hardy-Weinberg equilibrium (HWE) and dropped any SNP with HWE p value $<1 \times 10^{-4}$. SHBG had strong genetic correlation with TT (genetic correlation: $r_g = 0.73$) [9]. As such, multivariable MR of SHBG and TT may have the greatest validity for these instruments, but for comprehensiveness, we also presented results of univariable and network MR for comparison. To satisfy the assumption of independence, we checked SNPs in PhenoScanner, a R package with comprehensive information on the associations of genotype and phenotype, to test whether these SNPs were associated with the potential confounders, including smoking, alcohol drinking, body mass index (BMI), physical activity, weight, waist circumference, waist hip ratio, and dropped SNP(s) associated with any of these potential confounders at genome wide significance ($P < 5 \times 10^{-8}$).

2.4. Genetic instrumental variables for multivariable MR

Multivariable MR used only for SHBG and TT, because of the strong genetic correlation with TT but weak genetic correlation with BT (genetic correlation: $r_g = 0.73$and -0.05(se = 0.024), $P = 0.04$) [9], respectively). After combining the genetic predictors for SHBG and TT, we dropped overlapping SNPs, correlated SNPs ($r^2 > 0.05$), and SNPs whose correlations with other SNPs not available in MR-Base using "clump" function. The remaining SNPs were used for multivariable MR.

2.5. Mendelian randomization

2.5.1. Statistical analysis for MR estimates using univariable MR

We estimated the total effect of exposures (SHBG, TT or BT) on CHD using univariable Mendelian randomization. Our primary analysis used inverse variance weighting (IVW) estimators with multiplicative fixed effects (if they had heterogeneity using random effects IVW and using I^2 to show their heterogeneity) which assume no directional pleiotropy [26]. And the statistical power was calculated in http://cnsgenomics.com/shiny/mRnd/, using the IVW analysis's result. Specifically, the variance explained by each SNP was calculated using beta [2] × 2 × minor allele frequency (MAF) × (1-MAF), where beta is the standardized beta coefficient for the effect allele and MAF is the minor allele frequency [27].

In sensitivity analysis, we used Weighted median [28] and MR-Egger regression test [29]. The weighted median estimate was robust to invalid instruments and able to provide consistent estimation even when up to 50% of the weight is from invalid SNPs [28]. The MR-Egger regression test was used to evaluate the directional pleiotropy and investigate the null causal hypothesis under the InSIDE (Instrument Strength Independent of Direct Effect) assumption [29]. The intercept of the MR-Egger regression can assess the presence of pleiotropy and the slope of the MR-Egger regression can provide pleiotropy corrected causal estimates [29]. If the intercept of the MR-Egger regression p value <0.05, we used MR-PRESSO to identify outliers with potential horizontal pleiotropy among multiple genetic variants and provide a corrected estimate after removing these outliers [30]. If MR-PRESSO identified outlier(s), we used the corrected estimates from MR-PRESSO instead of IVW in the primary analysis.

To test the sensitivity of SNPs, we designed a leave-one-out analysis. Each SNP was removed to carry out the results of IVW and then evaluated the influence of each SNP on the results. The fluctuation of the results before and after removing each SNP reflects the sensitivity of this SNP. We also drew funnel plots to see the causal effect of each SNP and to test whether the results were affected by potential biases.

2.5.2. Statistical analysis for MR estimates using multivariable MR

Multivariable MR is an extension of basic univariable MR. It uses SNPs to predict two or more exposures and can be used to estimate the direct effect of one exposure after adjusting for the other exposure (s) [31,32], here the effect is SHBG and TT on CHD after adjusting each other, respectively (Fig.1b). In multivariable MR, we used MR IVW to estimate the effects of SHBG and TT on CHD.

In sensitivity analysis, we used MR Egger to detect whether the genetic predictors were acting other than via TT or SHBG (directional pleiotropy) indicated by a non-zero intercept.

2.5.3. Bidirectional MR analysis and Network MR analysis

First, we used bidirectional MR analysis [33] to explore the causal relationship between SHBG and TT. It consists of two univariable MR tests. The framework of the bidirectional MR analysis is described in Fig. 1c. Then if there is a causal relationship between SHBG and TT using bidirectional MR and the univariate MR results for SHBG and TT are significant, the causal mediating effect will be further explored using network MR analysis [34,35]. The framework of the network MR analysis is described in Fig. 1d. It consists of three univariable MR tests that are all described below (I-III) [35].

I. The causal effect of genetically determined SHBG(TT) on CHD is estimated.
II. The causal effects of genetically determined CHD on TT(SHBG) is estimated.
III. The causal effects of the possible mediators on CHD are estimated.

If causal associations are evaluated in all three steps, the conclusion can be drawn that the factor is a mediator.

All statistical analyses were conducted using R version 3.6.1, the "clump" function of MR-Base, and the R package "Mendelian Randomization".

3. Results

3.1. The basic information of instrumental variable and outcomes

Since SHBG and TT had strong genetic correlation (genetic correlation: $r_g = 0.73$) [9] in men, the genetic correlation (genetic correlation: $r_g = -0.06$) was weak in women, and T level was low in women, so this study only analyzed SHBG and T in males.

We used the previously published 357 genome wide significant SNPs for SHBG in men. There were 26 SNPs associated with risk factors, such as smoking, drinking and BMI etc. and 10 SNPs violated HWE, so remaining 313 SNPs were used for matching outcome data. Similarly, we used the previously published 231 genome wide significant SNPs for TT in men. 17 SNPs were associated with risk factors and 12 SNPs violated HWE. So, for TT, 202 SNPs were used. For BT, there were 125 genome wide significant SNPs in the previously GWAS. There were 5 SNPs violated HWE and 9 SNPs were associated with risk factors, So, for BT, 111 SNPs in men were used for matching outcome data. When matching outcome data, 92 SNPs related with SHBG, 69 SNPs related with TT and 44 SNPs related with BT were not found for the outcomes. So, there were remaining 221 SNPs related with SHBG, 133 SNPs related with TT and 67 SNPs related with BT for MR analysis.

The 221 SNPs can explain 2.55% of the variance of SHBG, the 133 SNPs explained 6.88% of the variance of TT, and the 67 SNPs explained 3.08% of the variance of BT in the univariable MR analysis.

In multivariable MR for SHBG and TT, there were 133 SNPs for TT and 221 SNPs for SHBG after quality control (in total 324 SNPs). After excluding 30 duplicate SNPs, 50 correlated SNPs or unclear

correlation information SNPs, 274 SNPs remained and were used in multivariable MR.

3.2. Univariable Mendelian randomization

The I^2 showed that SHBG, TT, and BT had some degree of heterogeneity (I^2 was 47.53%, 47.67% and 23.13%, respectively). Therefore, we used random effect IVW analysis in the univariable MR.

The IVW analysis results suggested that a one standard deviation (SD) increase in SHBG, the risk of CHD decreased by approximately 14% (OR (95%CI): 0.86(0.76,0.97), $P = 0.02$) and its statistical power was close to 1 (Table 1 and Supplementary Fig. 1). The results suggested that a SD increase in TT was also inversely associated with CHD, the risk decreased by approximately 8% (OR (95%CI): 0.92(0.85,0.99), $P = 0.03$) and its statistical power was also close to 1(Table 1 and Supplementary Fig. 2). However, the results suggested that a one SD increase in BT was not associated with CHD (Table 1 and Supplementary Fig. 3).

3.3. Multivariable Mendelian randomization

Exploring the direct causal effect of SHBG or TT on CHD using multivariable MR-IVW, the results suggested that a SD increase in both SHBG and TT had null associations with CHD (SHBG: OR (95%CI): 0.75 (0.57,1.00), $P = 0.053$; TT: OR (95%CI): 1.05(0.90,1.22), $P = 0.53$), but the results of SHBG may have some suggestive significance. Details were presented in Table 2.

3.4. Bidirectional MR analysis and Network MR analysis

In bidirectional MR analysis using IVW, the results suggested that there was bidirectional causality between SHBG and TT (the effect of SHBG on TT: OR (95%CI): 4.71(4.43,5.00), $P < 0.0001$; the effect of TT on SHBG: OR (95%CI): 1.54(1.48,1.60), $P < 0.0001$) (Details were listed in Table 3). In network MR analysis, the results suggested that TT might act as mediator in the causal pathway from SHBG to CHD and account for 93.00% of the total effect of SHBG on CHD(the effect of SHBG on TT is 1.55, the effect of TT on CHD is −0.09, so the mediating effect of TT is equal to $1.55 \times (-0.09) = -0.14$,the mediated proportion was the mediating effect of TT/the total effect of SHBG on CHD $= -0.14/(-0.15) = 93.\%$), and that SHBG might act as mediator in the causal pathway from TT to CHD and account for 71.67% of the total effect of TT on CHD(the effect of TT on SHBG is 0.43, the effect of SHBG on CHD is −0.15, so the mediating effect of SHBG is equal to $0.43 \times (-0.15) \approx -0.06$,the mediated proportion was the mediating effect of SHBG/the total effect of TT on CHD $= -0.06/(-0.09) \approx 67\%$).

4. Sensitivity analysis

4.1. Univariable Mendelian randomization

We conducted a sensitivity analysis using Weighted median and MR-Egger regression. These estimates were consistent in direction with the results of the IVW, the primary analysis and details were presented in Supplementary Table 1. The MR-Egger analysis yielded large p values for the intercept term (P value >0.05), indicating low probability of horizontal pleiotropy and details were presented in Supplementary Table 2.

Table 2
Associations of genetically predicted SHBG and total testosterone in men with ischemic heart disease using multivariable MR(IVW).

Outcomes	Method	exposure	beta	se	OR(95% CI)	P
Coronary heart disease	MV-IVW	SHBG	−0.28	0.15	0.75 (0.57,1.00)	0.053
Coronary heart disease	MV-IVW	total testosterone	0.05	0.08	1.05 (0.90,1.22)	0.525

The results of leave-one-out analysis were similar to the results using all SNPs. No SNPs had strong influence on the results (Supplementary Table 3). And funnel-plot shows that the causal effect of a single SNP is basically symmetrical, indicating that the results are less likely to be affected by potential bias (Supplementary Fig. 4–6). These sensitivity analysis results indicated that our results were stable and reliable.

4.2. Multivariable Mendelian randomization

The multivariable MR-Egger analysis also yielded a large p value for the intercept term (P value 0.64), indicating low probability of horizontal pleiotropy. And the point estimate of the slope was consistent with our main multivariable MR IVW analysis. Details were listed in Supplementary Table 3.

4.3. Bidirectional MR analysis

In the bidirectional MR analysis, we conducted a sensitivity analysis using Weighted median and MR-Egger regression exploring the causal effect of SHBG (TT) on TT (SHBG). These estimates were consistent in direction with the results of the IVW (Table 3). The MR-Egger analysis of SHBG yielded a large p value for the intercept term ($P = 0.52$), indicating low probability of horizontal pleiotropy. Although, the MR-Egger intercept p value of TT was less than 0.05, the corrected causal estimate was consistent with the results of the IVW and details were presented in Table 3.

5. Discussion

Under the univariable MR causal framework, we demonstrate that genetically determined SHBG and TT concentrations are negatively correlated with CHD risk. Under the multivariable MR causal framework, there are no significant difference in the direct causal effect between TT and SHBG on CHD. Under the bidirectional and network MR causal framework, we demonstrate that TT and SHBG have a bidirectional causal effect, which is consistent with the previous study's conclusion that SHBG and TT have a strong genetic correlation (genetic correlation: $r_g = 0.73$) [9], and that genetically determined TT is an important mediator in the causal pathway from SHBG to CHD(the mediated proportion = 93%), and that SHBG is also an important mediator in the causal pathway from TT to CHD(the mediated proportion = 67%). Our results show that SHBG is an important factor for CHD and SHBG has a causal effect on CHD mainly together with TT[10][11][11] [11]. Many observational evidences have shown that SHBG is an important factor affecting a variety of diseases, and its high level was associated with lower risk of type 2 diabetes [11,12], asthma [12], estrogen receptor-positive(ER^{+ve})breast cancer [13] and prostate cancer [36], and some genetic evidences supported the observed associations with type 2 diabetes [14], asthma

Table 1
Associations of genetically predicted SHBG and total testosterone in men with ischemic heart disease using univariable MR-IVW in the UK Biobank.

Eeposure	Outcomes	method	nsnp	beta	se	OR(95%CI)	p	power
SHBG	Coronary heart disease	IVW	221	**−0.15**	**0.06**	**0.86(0.76,0.97)**	**0.017**	**100.0**
Total testosterone	Coronary heart disease	IVW	133	**−0.09**	**0.04**	**0.92(0.85,0.99)**	**0.029**	**99.3**
Bioavailable testosterone	Coronary heart disease	IVW	67	0.04	0.05	1.04(0.95,1.15)	0.399	28.5

Table 3
The Bidirectional MR analysis results between SHBG and TT.

Exposure	Outcome	method	nsnp	beta	se	OR(95%CI)	P	MR-Egger intercept p value
SHBG	TT	IVW	313	1.55	0.03	4.71(4.43,5.00)	<0.001	
SHBG	TT	Weighted median	313	1.63	0.02	5.08(4.85,5.32)	<0.001	
SHBG	TT	MR-Egger	313	1.53	0.04	4.62(4.25,5.02)	<0.001	0.517
TT	SHBG	IVW	202	0.43	0.02	1.54(1.48,1.60)	<0.001	
TT	SHBG	Weighted median	202	0.13	0.01	1.14(1.11,1.17)	<0.001	
TT	SHBG	MR-Egger	202	0.52	0.03	1.68(1.58,1.78)	<0.001	<0.001

[15] and ER^{+ve} breast cancer [16]. The above results are sufficient to indicate that SHBG is an important factor in the development and progression of the disease, but whether SHBG and TT act together in the above-mentioned diseases remains to be determined by further studies, just as in the case of CHD. Studies have shown that cell membranes of many tissues express a receptor for SHBG and that SHBG is found intracellularly [37]. Binding of SHBG to its receptor has been shown to activate cyclic adenosine monophosphate (cAMP) [38], an intracellular signal transduction pathway important for many biological processes [39,40].

In univariable MR, TT is inversely associated with CHD, which is consistent with many observational studies. For example: Ohlsson et al. observed that both serum T levels and SHBG levels were inversely related to the incidence of adverse major cardiovascular events [41]. Lee et al. observed a significant negative correlation between the level of total T and the Framingham Risk Score [2]. But after using multivariable MR to adjust SHBG, it is unlikely to have a large effect on risk of CHD. And our result find that the protective effect of TT is mainly mediated by SHBG. In univariable MR, Studies have shown that SHBG level is negatively correlated with BT, which can bind to the corresponding receptor and cause disease if BT increases a lot [9,42]. The effect of BT on CHD may be coordinated by regulating SHBG level. It follows that SHBG is a very important glycoprotein that coordinates the biological effects of TT and BT. However, our result BT is not associated with CHD, which may be caused by the fact that the BT level is different in male and female populations and the outcome data use both men and women and cannot be verified by gender. Our results show that TT can also regulate SHBG level, and there is a bidirectional causal relationship between them. Based on the results of this study, it may be more meaningful to explore the causal effects of SHBG and TT, especially their mediating effects, on CHD than to study either of them separately.

In previous studies, Luo et al. [43] suggest no independent role of SHBG in CHD risk. This was consistent with our multivariable and network MR results, namely, SHBG had no obvious direct causal effect on CHD and its effect is mainly mediated by TT. However, the instrumental variable they selected only considered the SNPs on the SHBG gene, and the result was the causal relationship between SHBG and CHD determined by these 7 SNPs, which we think may be less comprehensive, because there are hundreds of SHBG related SNPs with genome-wide significance. Similarly, R. Haring et al. found that no causal correlation was found in their MR study [44]. We thought this may also be caused by the use of too little IV, because there were far more than one SNP affecting serum testosterone concentrations, and too little IV would have weak instrumental variable bias, which would affect the results. The Bidirectional MR study of Joel Eriksson et al. [45] showed that there was a causal relationship between BMI and serum testosterone, and lowering BMI could increase serum testosterone level, which indirectly concluded that increasing serum testosterone level could reduce the risk of CHD, because obesity was a recognized risk factor for CHD. It's result was consistent with our results. In addition, Schooling et al. [46] investigated the pleiotropic effect of statins on CHD and found that the effect of statins on CHD was mediated by BT, and they found that BT was positively correlated with CHD, which was consistent with our point estimate of BT.

To our knowledge, this MR study is the first to examine the role of SHBG and TT in CHD by multivariable and network MR, overcoming the potential limitations of univariable MR studies and the first to examine the role of BT in CHD, overcoming the potential limitations of observational studies. Our study has several strengths: first, our genetic instruments were obtained from the largest, most up-to-date, sex-specific GWAS. Second, in the previous MR studies on sex hormones and disease, few studies used multivariable MR to consider the direct effects of two or more exposure, only consider the total effect of only one exposure, only consider the total effect of only one exposure, and few studies used network MR to consider the mediation effect of SHBG/TT on CHD. Third, we use different MR analysis methods and get basically consistent conclusions, which confirm that our results are robust and reliable. But, the associations in Europeans may not apply to other populations, such as Asians. Therefore, in the future, it is necessary to explore their causal relationship in other populations. An additional limitation is that we used CARDIOGRAM-C4D cohort as outcome data which contains men and women and cannot be verified by gender. Therefore, it is necessary to explore the causal relationship between TT and CHD in male and female populations with two samples of MR respectively in the future. Although MR analysis can be used to find the causal effect, the biological mechanism of SHBG and T needs to be determined by further experimental studies.

6. Conclusions

Genetically predicted SHBG and TT were negatively correlated with CHD in both univariable and network MR, which may provide a causal explanation behind the observed conclusion. And TT and SHBG had a bidirectional causal effect. Further work is required to disentangle the downstream effects of SHBG/TT on CHD and the molecular pathways involved, as the simultaneous regulation of SHBG and TT may make it a viable strategy for the prevention or treatment of CHD.

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Competing interests

None.

Ethics approval and consent to participate

The UK Biobank has ethical approval from the Northwest Multi-Center Research Ethics Committee, and all participants provided written informed consent.

Consent for publication

Not applicable.

Availability of data and materials

Researchers could acquire this data by submitting an application to the UK Biobank (https://www.ukbiobank.ac.uk/).

Authors' contributions

Yanxun Liu and Fuzhong Xue contributed to the conception or design of the work. Yunxia Li contributed to the acquisition, analysis, or interpretation of data for the work and drafted the manuscript. All authors participated in the interpretation of the results, edited and reviewed the manuscript and gave final approval and agree to be accountable for all aspects of work ensuring integrity and accuracy.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijcard.2021.06.037.

References

[1] G.A. Roth, C. Johnson, A. Abajobir, et al., Global, regional, and National Burden of cardiovascular diseases for 10 causes, 1990 to 2015[J], J. Am. Coll. Cardiol. 70 (1) (2017) 1–25.
[2] W.C. Lee, M.T. Kim, K.T. Ko, et al., Relationship between serum testosterone and cardiovascular disease risk determined using the Framingham risk score in male patients with sexual dysfunction[J], World J. Mens Health 32 (3) (2014) 139–144.
[3] J.B. Ruige, A.M. Mahmoud, D. De Bacquer, et al., Endogenous testosterone and cardiovascular disease in healthy men: a meta-analysis[J], Heart 97 (11) (2011) 870–875.
[4] A.B. Araujo, J.M. Dixon, E.A. Suarez, et al., Clinical review: endogenous testosterone and mortality in men: a systematic review and meta-analysis[J], J. Clin. Endocrinol. Metab. 96 (10) (2011) 3007–3019.
[5] G. Corona, G. Rastrelli, M. Monami, et al., Hypogonadism as a risk factor for cardiovascular mortality in men: a meta-analytic study[J], Eur. J. Endocrinol. 165 (5) (2011) 687–701.
[6] K.T. Khaw, M. Dowsett, E. Folkerd, et al., Endogenous testosterone and mortality due to all causes, cardiovascular disease, and cancer in men: European prospective investigation into cancer in Norfolk (EPIC-Norfolk) Prospective Population Study[J], Circulation 116 (23) (2007) 2694–2701.
[7] D.J. Lerner, W.B. Kannel, Patterns of coronary heart disease morbidity and mortality in the sexes: a 26-year follow-up of the Framingham population[J], Am. Heart J. 111 (2) (1986) 383–390.
[8] D.C. Anderson, 2-sex-hormone-binding globulin[J], Clin. Endocrinol. 3 (1) (1974) 69–96.
[9] K.S. Ruth, F.R. Day, J. Tyrrell, et al., 14-using human genetics to understand the disease impacts of testosterone in men and women[J], Nat. Med. 26 (2) (2020) 252–258.
[10] D.J. Hryb, M.S. Khan, N.A. Romas, et al., The control of the interaction of sex hormone-binding globulin with its receptor by steroid hormones[J], J. Biol. Chem. 265 (11) (1990) 6048–6054.
[11] E.L. Ding, Y. Song, V.S. Malik, et al., Sex differences of endogenous sex hormones and risk of type 2 diabetes: a systematic review and meta-analysis[J], JAMA 295 (11) (2006) 1288–1299.
[12] Y. Han, E. Forno, J.C. Celedón, Sex steroid hormones and asthma in a nationwide study of U.S. adults[J], Am. J. Respir. Crit. Care Med. 201 (2) (2020) 158–166.
[13] X.Y. He, Y.D. Liao, S. Yu, et al., Sex hormone binding globulin and risk of breast cancer in postmenopausal women: a meta-analysis of prospective studies[J], Horm. Metab. Res. 47 (7) (2015) 485–490.
[14] Q. Wang, A.J. Kangas, P. Soininen, et al., Sex hormone-binding globulin associations with circulating lipids and metabolites and the risk for type 2 diabetes: observational and causal effect estimates[J], Int. J. Epidemiol. 44 (2) (2015) 623–637.
[15] R. Arathimos, R. Granell, P. Haycock, et al., Genetic and observational evidence supports a causal role of sex hormones on the development of asthma[J], Thorax 74 (7) (2019) 633–642.
[16] N.L. Dimou, N. Papadimitriou, D. Gill, et al., Sex hormone binding globulin and risk of breast cancer: a Mendelian randomization study[J], Int. J. Epidemiol. 48 (3) (2019) 807–816.
[17] D.A. Lawlor, R.M. Harbord, J.A. Sterne, et al., Mendelian randomization: using genes as instruments for making causal inferences in epidemiology[J], Stat. Med. 27 (8) (2008) 1133–1163.
[18] C. Sudlow, J. Gallacher, N. Allen, et al., 7-UK biobank: an open access resource for identifying the causes of a wide range of complex diseases of middle and old age [J], PLoS Med. 12 (3) (2015), e1001779.
[19] C. Bycroft, C. Freeman, D. Petkova, et al., The UK Biobank resource with deep phenotyping and genomic data[J], Nature 562 (7726) (2018) 203–209.
[20] UK Biobank, UK Biobank Biomarker Project Companion Document to Accompany Serum Biomarker Data: Version 1.0, 2019.
[21] A. Vermeulen, L. Verdonck, J.M. Kaufman, A critical evaluation of simple methods for the estimation of free testosterone in serum[J], J. Clin. Endocrinol. Metab. 84 (10) (1999) 3666–3672.
[22] M. Chung, S. Gombar, R. Shi, Implementation of automated calculation of free and bioavailable testosterone in epic beaker laboratory information system[J], J. Pathol. Inf. 8 (1) (2017) 28.
[23] M. Nikpay, A. Goel, H.H. Won, et al., A comprehensive 1,000 genomes-based genome-wide association meta-analysis of coronary artery disease[J], Nat. Genet. 47 (10) (2015) 1121–1130.
[24] N.M. Davies, M.V. Holmes, S.G. Davey, 13-Reading Mendelian randomisation studies: a guide, glossary, and checklist for clinicians[J], BMJ 362 (2018) k601.
[25] Genome-wide genetic data on ~500,000 UK Biobank participants[J].
[26] S. Burgess, A. Butterworth, S.G. Thompson, Mendelian randomization analysis with multiple genetic variants using summarized data[J], Genet. Epidemiol. 37 (7) (2013) 658–665.
[27] S. Burgess, F. Dudbridge, S.G. Thompson, Combining information on multiple instrumental variables in Mendelian randomization: comparison of allele score and summarized data methods[J], Stat. Med. 35 (11) (2016) 1880–1906.
[28] J. Bowden, S.G. Davey, P.C. Haycock, et al., Consistent estimation in Mendelian randomization with some invalid instruments using a weighted median estimator[J], Genet. Epidemiol. 40 (4) (2016) 304–314.
[29] J. Bowden, S.G. Davey, S. Burgess, 16-Mendelian randomization with invalid instruments: effect estimation and bias detection through egger regression[J], Int. J. Epidemiol. 44 (2) (2015) 512–525.
[30] M. Verbanck, C.Y. Chen, B. Neale, et al., Detection of widespread horizontal pleiotropy in causal relationships inferred from Mendelian randomization between complex traits and diseases[J], Nat. Genet. 50 (5) (2018) 693–698.
[31] J. Rees, A.M. Wood, S. Burgess, 20-extending the MR-egger method for multivariable Mendelian randomization to correct for both measured and unmeasured pleiotropy [J], Stat. Med. 36 (29) (2017) 4705–4718.
[32] E. Sanderson, G. Davey Smith, F. Windmeijer, et al., An examination of multivariable Mendelian randomization in the single-sample and two-sample summary data settings[J], Int. J. Epidemiol. 48 (3) (2019) 713–727.
[33] M.K. Chung, D.O. Martin, D. Sprecher, et al., C-reactive protein elevation in patients with atrial arrhythmias: inflammatory mechanisms and persistence of atrial fibrillation[J], Circulation 104 (24) (2001) 2886–2891.
[34] Y. Zhan, I.K. Karlsson, R. Karlsson, et al., Exploring the causal pathway from telomere length to coronary heart disease: a network Mendelian randomization study[J], Circ. Res. 121 (3) (2017) 214–219.
[35] S. Burgess, R.M. Daniel, A.S. Butterworth, et al., Network Mendelian randomization: using genetic variants as instrumental variables to investigate mediation in causal pathways[J], Int. J. Epidemiol. 44 (2) (2015) 484–495.
[36] A. Wolk, S.O. Andersson, R. Bergstrom, Prospective study of sex hormone levels and risk of prostate cancer[J], J. Natl. Cancer Inst. 89 (11) (1997) 820.
[37] G.V. Avvakumov, A. Cherkasov, Y.A. Muller, et al., Structural analyses of sex hormone-binding globulin reveal novel ligands and function[J], Mol. Cell. Endocrinol. 316 (1) (2010) 13–23.
[38] W. Rosner, D.J. Hryb, S.M. Kahn, et al., Interactions of sex hormone-binding globulin with target cells[J], Mol. Cell. Endocrinol. 316 (1) (2010) 79–85.
[39] R. Abramovitch, E. Tavor, J. Jacob-Hirsch, et al., A pivotal role of cyclic AMP-responsive element binding protein in tumor progression[J], Cancer Res. 64 (4) (2004) 1338–1346.
[40] B.J. Simpson, A.D. Ramage, M.J. Hulme, et al., Cyclic adenosine 3′,5′-monophosphate-binding proteins in human ovarian cancer: correlations with clinicopathological features[J], Clin. Cancer Res. 2 (1) (1996) 201–206.
[41] C. Ohlsson, E. Barrett-Connor, S. Bhasin, et al., High serum testosterone is associated with reduced risk of cardiovascular events in elderly men. The MrOS (osteoporotic fractures in men) study in Sweden[J], J. Am. Coll. Cardiol. 58 (16) (2011) 1674–1681.
[42] N. Murphy, M. Jenab, M.J. Gunter, Adiposity and gastrointestinal cancers: epidemiology, mechanisms and future directions[J], Nat. Rev. Gastroenterol. Hepatol. 15 (11) (2018) 659–670.
[43] S. Luo, S.L. Au Yeung, J.V. Zhao, et al., Association of genetically predicted testosterone with thromboembolism, heart failure, and myocardial infarction: mendelian randomisation study in UK Biobank[J], BMJ (2019) l476.
[44] R. Haring, A. Teumer, U. Volker, et al., Mendelian randomization suggests non-causal associations of testosterone with cardiometabolic risk factors and mortality[J], Andrology 1 (1) (2013) 17–23.
[45] J. Eriksson, R. Haring, N. Grarup, et al., Causal relationship between obesity and serum testosterone status in men: a bi-directional mendelian randomization analysis[J], PLoS One 12 (4) (2017), e176277.
[46] C.M. Schooling, J.V. Zhao, S.L. Au Yeung, et al., Investigating pleiotropic effects of statins on ischemic heart disease in the UK Biobank using Mendelian randomisation[J], eLife 9 (2020).