Sex-specific causal effects of serum sex hormones on COVID-19 susceptibility and severity: evidence from the UK Biobank and COVID-19 Host Genetics Initiative

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Abstract

Several medications and treatments are being investigated for their potential effectiveness against coronavirus disease 2019 (COVID-19), including androgen and other sex hormones. However, the causal relationships between serum sex hormones and COVID-19 susceptibility and severity, particularly with regards to potentially sex-specific effects, remain largely unknown. In this study, we used the latest data from the UK Biobank (up to 424,907 individuals) and COVID-19 Host Genetics Initiative (up to 1,878,143 individuals) to systematically assess the sex-specific causal effects of serum sex hormone levels on COVID-19 outcomes within a two-sample Mendelian randomization (MR) framework. The inverse-variance weighted method was used in the main MR analysis. We additionally performed a series of sensitivity analysis to assess the robustness of MR effect estimates to potentially invalid genetic variants. Our MR analysis revealed novel causal associations between serum estradiol and bioavailable testosterone levels and SARS-CoV-2 infection in women, but not men, except for a suggestive inverse causal association between estradiol levels and COVID-19 severity in men. These novel findings improve our understanding of the sex-specific causal nature of sex hormones in relation to COVID-19 outcomes, and suggest that sex hormones may serve as potential therapeutic targets for preventing SARS-CoV-2 infection and improving patient outcomes.

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Abstract

Several medications and treatments are being investigated for their potential effectiveness against coronavirus disease 2019 (COVID-19), including androgen and other sex hormones. However, the causal relationships between serum sex hormones and COVID-19 susceptibility and severity, particularly with regards to potentially sex-specific effects, remain largely unknown. In this study, we used the latest data from the UK Biobank (up to 424,907 individuals) and COVID-19 Host Genetics Initiative (up to 1,878,143 individuals) to systematically assess the sex-specific causal effects of serum sex hormone levels on COVID-19 outcomes within a two-sample Mendelian randomization (MR) framework. The inverse-variance weighted method was used in the main MR analysis. We additionally performed a series of sensitivity analysis to assess the robustness of MR effect estimates to potentially invalid genetic variants. Our MR analysis revealed novel causal associations between serum estradiol and bioavailable testosterone levels and SARS-CoV-2 infection in women, but not men, except for a suggestive inverse causal association between estradiol levels and COVID-19 severity in men. These novel findings improve our understanding of the sex-specific causal nature of sex hormones in relation to COVID-19 outcomes, and suggest that sex hormones may serve as potential therapeutic targets for preventing SARS-CoV-2 infection and improving patient outcomes.

Keywords

Sex hormone, COVID-19, Causal effects, Mendelian randomization, Therapeutic targets

1 INTRODUCTION

It is necessary to explore medications for the prevention of severe cases of coronavirus disease 2019 (COVID-19) and for improving patient outcomes, owing to the low rates of uptake for vaccine boosters (for example, only 54.8% and 14.3% of the total population in European Union/ European Economic Area countries received the first and second booster doses, respectively) [1], the waning efficacy of vaccines [2], and the emergence of new variants[3]. Various medications and treatments for COVID-19, including corticosteroids, immunomodulatory agents, monoclonal antibodies against the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), have been investigated [4].

Notably, it has been hypothesized that serum sex hormone concentrations, particularly testosterone levels, and androgen receptor activation may contribute to the immunoregulatory response and higher risks of COVID-19 susceptibility and severity [5-8]. Androgen was considered as a potential hormone therapeutic target for COVID-19. However, androgen suppression drugs (e.g., degarelix and enzalutamide) did not significantly improve the COVID-19 outcomes in recent randomized clinical trials (RCTs)[9,10]. Meanwhile, a recent study provided conflicting evidence that there was an association between lower testosterone levels and severe COVID-19 in men [11]. Thus, so far, little evidence has been found to support a protective role of androgen receptor signaling inhibition against COVID-19 [12]. Furthermore, other RCTs (e.g., NCT04865029, NCT04853069 and NCT04539626 as listed on ClinicalTrials.gov) investigating the efficacy of estradiol and progesterone therapy on the COVID-19 outcomes are still onging without clear conclusions [13,14]. Causal relationships between serum sex hormones and COVID-19 susceptibility and severity, particularly potentially sex-specific effects, remain largely unclear.

The present study aimed to systematically investigated the sex-specific causal effects of serum sex hormone levels on COVID-19 outcomes using data from the UK Biobank (UKB) and COVID-19 Host Genetics

Initiative (HGI).

2 PATIENTS AND METHODS

Study design and data sources

We conducted a two-sample Mendelian randomization (MR) analysis to assess the causal associations of sex hormones with COVID-19 susceptibility and severity. MR is a causal inference approach, which uses germline genetic variants as instrumental variables (IVs) to estimate possible causal effects of modifiable risk factors on health outcomes. This approach is less prone to non-genetic confounding and reverse causation bias [15,16].

We used data from the UKB and COVID-19 HGI. Summary statistics on sex hormones levels (including estradiol, total testosterone (TT), bioavailable testosterone (BT), and sex hormone binding globulin (SHBG)) were obtained from the largest genome-wide association studies (GWASs) of sex hormones [17,18], in up to 230,454 women and 194,453 men of European ancestry in the UKB.

In the estradiol GWAS, individuals' estradiol levels were analyzed as a binary phenotype, with values equal to or above the detection limit (175 pmol/L) considered as one group, and values below the limit as another group [18]. Moreover, for quantitative analysis, individuals with estradiol levels below the detection limit were included by using censored regression modeling with a Tobit type I technique [19]. This approach allowed analyzing estradiol levels as a continuous phenotype in a total of 163,985 women and 147,690 men [18].

Testosterone and SHBG levels were measured and analyzed as continuous phenotypes. In the original GWAS of SHBG levels, body mass index (BMI) was unadjusted and adjusted for, in order to assess the potential impact of collider bias [20]. In this study, we took potential collider bias into account by using summary data from GWAS of SHBG levels, where BMI was unadjusted and adjusted for, to estimate the causal effects of genetically predicted SHBG on COVID-19 susceptibility and severity, respectively.

For the outcomes in this study (i.e., COVID-19 susceptibility and severity), summary statistics were obtained from the latest and largest GWAS of COVID-19 outcomes in European ancestry conducted by HGI with data freeze 6 (excluding UKB and 23andMe participants)[21]. Three COVID-19 related phenotypes were selected as the outcomes: (1) severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection (as cases) and the general population (as controls) (74,614 cases and 1,803,529 controls); (2) COVID-19 hospitalization (as cases) and the general population (as controls) (14,925 cases and 1,393,029 controls); and (3) COVID-19 critical illness (as cases) and the general population (as controls) (4,297 cases and 378,521 controls) (**Table 1**). Due to a lack of European ancestry GWAS of COVID-19 critical illness in data freeze 6, summary statistics on this outcome were obtained from GWAS data freeze 5 instead. This study used publicly available data and was not subject to institutional review board approval.

Genetic instruments selection

Single nucleotide polymorphisms (SNPs) that were genome-wide significant ($P < 5 \times 10^{-8}$) were extracted from each GWAS of sex hormones. A stringent linkage disequilibrium (LD) clumping strategy was then applied, using a window of 10 Mb and r^2 threshold of less than 0.001. We used the 1000 Genomes Project European samples as the LD reference in PLINK 1.9. After LD clumping, statistically independent SNPs were selected as genetic instruments for sex hormones.

If genetic instruments were not available in the summary data of COVID-19 outcomes, we used a proxy variant that was identified in 1000 Genomes Project European samples using LDlink (https://ldlink.nci.nih.gov/?tab=ldproxy). We harmonized the SNP-exposure and SNP-outcome summary datasets using a previously described method [22]. During the data harmonization process, SNPs with a minor allele frequency > 0.49 were excluded. The number of genetic variants after LD clumping and IVs for each sex hormone are listed in **eTable 1 and eTable 2** .

Genetic instruments mapped to androgen signaling genes

To further investigate the causal effects of androgen signaling on COVID-19 susceptibility and severity, we used three top hits (rs112881196, rs17703883, and rs12944649) for BT levels in men and six hits (rs112635299, rs17580, rs78081080, rs727428, rs1050541, and rs9895413) in women as genetic instruments, respectively. These hits were located within 1Mb of eight androgen signaling genes (as shown in**Table 2**), which were identified using the high-throughput drug screen in the study by Samuel et al [8].

Statistical analysis

Main MR analysis

For the main MR analysis, we used the inverse-variance weighted (IVW) method [23]. This method assumes that all instruments are valid, although it can still provide unbiased estimates in some scenarios where the IV assumptions are violated (e.g., balanced horizontal pleiotropy). In this study, our focus is on effect size and precision, and we interpret p-values as indicators of the strength of evidence [24,25]. Since we examined four sex hormones (estradiol, TT, BT and SHBG) in the MR analysis, we considered a p-value less than 0.0125 (0.05/4) as strong evidence for MR estimates, and a p-value between 0.0125 and 0.05 as suggestive causal evidence.

MR Sensitivity analysis

To assess the robustness of MR effect estimates to potentially invalid genetic variants, we conducted a sensitivity analysis using the weighted median and MR-Egger methods when at least five genetic instruments were available for sex hormones. The weighted median method produced a consistent causal effect estimate even when up to half of the genetic variants included in the MR analysis were invalid instrumental variables[26]. When a weaker assumption (i.e., Instrument Strength Independent of Direct Effect, INSIDE) was met, the MR-Egger method was able to estimate causal effects while allowing all SNPs to be pleiotropic [27].

Previous studies have suggested that obesity may be a potential cause of COVID-19 susceptibility and severity [28,29]. To minimize the risk of bias resulting from violating the exclusion restriction assumption (i.e., IVs can only affect the outcome through exposure rather than alternative pathways), we identified genome-wide significant SNPs associated with obesity traits such as BMI and waist-hip ratio based on published GWASs in the PhenoScanner V2 database (http://www.phenoscanner.medschl.cam.ac.uk/), and removed these SNPs from the IVW analysis [30].

3 RESULTS

Key characteristics of each GWAS data used in the study are presented in**Table 1** . MR analysis showed that higher genetically predicted serum estradiol levels were suggestively associated with a decreased risk of SARS-CoV-2 infection in women (odds ratio (OR), 0.88 for log odds of estradiol detection; 95% CI, 0.78, 0.98; $P = 0.021$; OR 0.66 per standard deviation (SD) increased estradiol; 95% CI, 0.47 to 0.94; $P = 0.021$) (**Figure 1, panel A**). However, little evidence was found to support a causal effect of genetically predicted serum estradiol levels on COVID-19 hospitalization and critical illness using the binary exposure (i.e., estradiol levels were detected or not). In contrast, suggestive inverse causal associations of continuous estradiol levels with COVID-19 hospitalization (OR 0.50 per SD increased estradiol; 95% CI, 0.25 to 0.98; $P = 0.044$) and COVID-19 critical illness (OR 0.69 per SD increased estradiol; 95% CI, 0.48 to 0.99; $P = 0.043$) in men were observed (**Figure 1, panels B and C**).

A causal effect of genetic predisposition to higher levels of serum BT on SARS-CoV-2 infection was observed in women (OR, 1.08 per SD higher BT; 95% CI, 1.02 to 1.14; $P = 0.005$), where SNPs mapped to androgen signaling genes were used as genetic instruments (**Figure 2, panel A**). Furthermore, there was no evidence of a causal association between serum testosterone levels and COVID-19 severity outcomes, such as hospitalization and critical illness (**Figure 2, panels B and C**).

Notably, potential causal effects of serum estradiol and BT levels were mostly observed in women but not men, except for a suggestive inverse causal association of estradiol levels with COVID-19 severity in men, suggesting a sex-specific causal nature in relation to COVID-19 outcomes. Furthermore, there is no evidence

supporting a causal association of serum SHBG levels with COVID-19 susceptibility and severity outcomes, whether including or excluding obesity-related SNPs (**Figure 3**).

4 DISCUSSION

In this study, to the best of our knowledge, we used data from the largest GWASs of serum sex hormones and COVID-19 outcomes to date, as well as the MR analysis technique, to systematically investigate the potential sex-specific causal associations of serum sex hormones with COVID-19 susceptibility and severity. Androgen signaling inhibition has been debated regarding its potential to reduce the susceptibility and severity of COVID-19 in men; however, accumulating evidence does not support this potential [12], which is consistent with the MR analysis results in our study. Interestingly, our results suggest that serum estradiol and bioavailable testosterone may serve as potential intervention or therapeutic targets for preventing SARS-CoV-2 infection in women.

The role of sex hormones in modulating COVID-19 susceptibility and severity has generated intense research interest given that sex-specific differences in these COVID-19 outcomes have been reported globally[31-33]. However, inconsistent conclusions have been drawn from various studies. For testosterone, it has been reported that lower levels of testosterone are associated with a higher risk of developing severe COVID-19 disease in men [11,34,35]. Moreover, men who had severe COVID-19 or needed intensive care have higher total testosterone levels than the mild COVID-19 or the control groups [36]. In contrast, estradiol levels were elevated in men with severe COVID-19 compared to the controls, but no significant difference was observed in women with critical COVID-19[37].

Interestingly, the findings of a previous MR analysis study did not support the causal effects of estradiol levels in men and women on COVID-19 susceptibility and severity [38], where a total of 10 SNPs were selected to genetically predict the estradiol levels in both men and women. In our study, we conducted a stringent strategy and LD clumping procedure for genetic instruments selection. Finally, two genome-wide significant SNPs (i.e., rs45446698 and rs16991615) for estradiol levels in women and seven SNPs for estradiol levels in men, respectively, were used for MR analysis (see **eTable 2** for details). GWAS summary data on binary and continuous estradiol levels were used in our analysis [18]. It is worth noting that our results suggested a protective effect of estradiol levels in women on COVID-19 infection. Moreover, suggestive causal effects of elevated estradiol levels in men on COVID-19 hospitalization and critical illness were observed. These findings may be explained by previous studies and animal experiments that show estrogens have anti-inflammatory effects and can affect immune systems [39-41]. Experiments revealed the suppressive function of 17β-estradiol (E2) on the production of proinflammatory cytokines by macrophages and monocytes, and its ability to inhibit chemokines to prevent innate immune cells, such as neutrophils and monocytes, from concentrating into inflamed areas [42]. Additionally, E2 could stimulate CD4+ T-helper cells to produce anti-inflammatory cytokines and produce antibodies by affecting B cells [39,42,43].

In addition, the angiotensin-converting enzyme 2 (*ACE2*) and the transmembrane serine protease 2 (*TMPRSS2*) in the lung have been well-studied as the entry receptor and for the spike (S) protein priming of SARS-CoV-2 [44]. Recently, researchers found that elevated estradiol levels downregulate the expression of *ACE2* and *TMPRSS2* messenger ribonucleic acid (mRNA) in lung epithelial cells [14,45,46]. This may support the protective role of increased estradiol levels on COVID-19 infection and critical illness revealed in our MR analysis.

Although several ongoing or completed clinical trials are targeting sex hormone pathways, for example, estrogen therapy (NCT04539626), estradiol (NCT04853069), estradiol and progesterone therapy (NCT04865029) and non-steroidal androgen receptor antagonist (NCT04870606), hormone therapy has not been authorized for COVID-19 treatment. A recent clinical trial did not support the use of androgen suppression treatment for improving severe COVID-19 illness and hospitalization in men[10]. This finding was consistent with our results, which showed that total and bioavailable testosterone levels in men did not causally associate with the risk of COVID-19 infection or severe illness. These results are also supported by previous MR studies[38,47]. The potential implications of our results for COVID-19 prevention/ treatment warrant further validation in

larger clinical trials.

There were several limitations in our study. Firstly, due to the lack of large-scale sex-specific COVID-19 GWAS, we utilized sex-combined GWAS summary statistics of COVID-19 susceptibility and severity as the outcomes in the MR analysis. Secondly, the findings in this study were generated using GWAS data of European ancestry. Therefore, the conclusions of this study may not be generalizable to populations of other ancestries. Thirdly, due to the lack of individual data, we were unable to test the potential non-linear causal associations of serum sex hormones with COVID-19 susceptibility and severity.

In summary, our MR analysis revealed novel causal associations of serum estradiol and bioavailable testosterone with SARS-CoV-2 infection in women but not men, except for a suggestive inverse causal association of estradiol levels with COVID-19 severity in men. This suggests a sex-specific causal nature of sex hormones in relation to COVID-19 outcomes and their potential roles as therapeutic targets for preventing SARS-CoV-2 infection and improving patient outcomes. Further validation in larger clinical trials is warranted.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

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Table 1 Key characteristics of GWAS summary statistics used in Mendelian randomization analysis.

Exposures/outcomes	Sample size
Exposure	
Estradiol (binary)	
Estradiol levels in women	37,461 women above/equal to the detection limit[a]
	126,524 women below the detection limit[a]
Estradiol levels in men	13,367 men above/equal to the detection limit[a]
	134,323 men below the detection limit[a]
Estradiol (continuous)	
Estradiol levels in women	163,985 women (37,461 women above/equal to and 126,524 women below the
Estradiol levels in men	147,690 men (13,367 men above/equal to and 134,323 men below the detectio
Total testosterone (TT)	
TT levels in women	230,454 women
TT levels in men	194,453 men
Bioavailable testosterone (BT)	
BT levels in women	188,507 women
BT levels in men	178,782 men
Sex hormone-binding globulin (SHBG)	
SHBG levels adjusted for BMI in women	188,908 women
SHBG levels adjusted for BMI in men	180,094 men
SHBG levels unadjusted for BMI in women	189,473 women
SHBG levels unadjusted for BMI in men	180,726 men
Outcome	
COVID-19[b]	
SARS-CoV-2 infection	74,614 cases
	1,803,529 controls
COVID-19 hospitalization	14,925 cases
	1,393,029 controls
COVID-19 critical illness	4,297 cases
	378,521 controls

a. The detection limit for estradiol is 175 pmol/L.

b. Summary statistics were obtained from GWASs of COVID-19 outcomes after excluding UK Biobank and 23andMe participants.

Table 2 Eight androgen signaling genes identified in the previous drug screen study[8].

Gene	Chromosome	Position (Start)	Position (End)

Gene	Chromosome	Position (Start)	Position (End)
SRD5A2	2	31749656	31806040
NCEH1	3	12348434	172429008
SRD5A1	5	6633499	6669675
NR3C1	5	142657495	142783254
IL6	7	22766765	22771621
SERPINA6	14	94770584	94789688
CYP19A1	15	51500253	51630795
SHBG	17	7531286	7536701

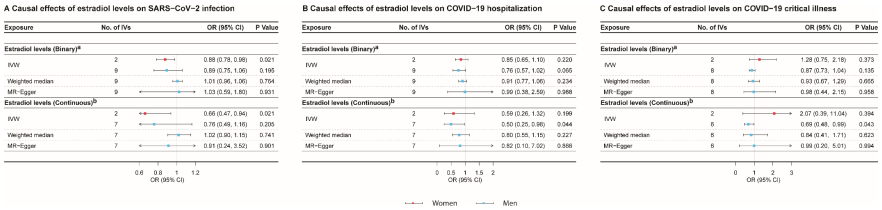


Figure 1 MR analysis results on the causal effects ofserum estradiol levels on COVID-19 susceptibility and severity.

Panel A. Causal effects of serum estradiol levels on SARS-CoV-2 infection in men and women. Panel B. Causal effects of serum estradiol levels on COVID-19 hospitalization in men and women. Panel C. Causal effects of serum estradiol levels on COVID-19 critical illness in men and women.

a. Estradiol levels above or below the detection limit (175 pmol/L) were analyzed as a binary phenotype.

b. Estradiol levels were analyzed as continuous phenotypes (individuals with estradiol levels below the detection limit (175 pmol/L) were included in the censored regression (Tobit type I) model as if continuous estradiol levels were measured in all participants) [18].

Abbreviations: CI, confidence interval; IV, instrumental variable; OR, odds ratio.

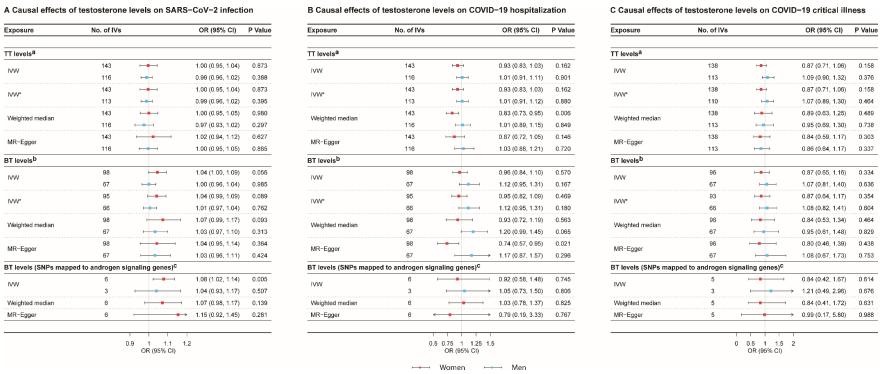


Figure 2 MR analysis results on the causal effects of serumtestosterone levels on COVID-19 susceptibility and severity.

Panel A. Causal effects of serum testosterone levels on SARS-CoV-2 infection in men and women. Panel B. Causal effects of serum testosterone levels on COVID-19 hospitalization in men and women. Panel C. Causal effects of serum testosterone levels on COVID-19 critical illness in men and women.

a. Total testosterone levels was measured in nmol/L and analyzed as a continuous phenotype.

b. Bioavailable testosterone levels was measured in nmol/L and analyzed as a continuous phenotype.

c. SNPs mapped to androgen signaling genes (within 1,000 kb distance) identified in the previous drug screening work done by Samuel et al. were used as instrumental variables [8].

* IVW estimate after excluding obesity-related SNPs.

Abbreviations: BT, bioavailable testosterone; CI, confidence interval; IV, instrumental variable; OR, odds ratio; TT, total testosterone.

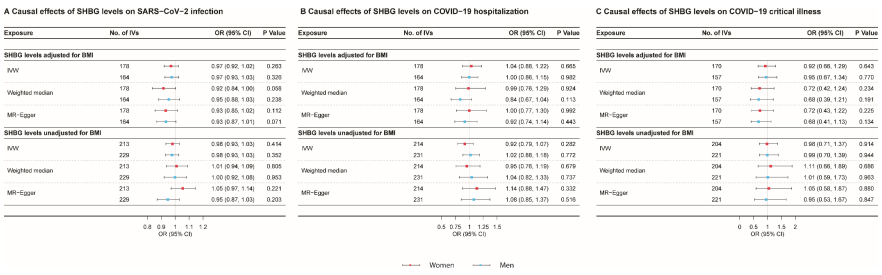
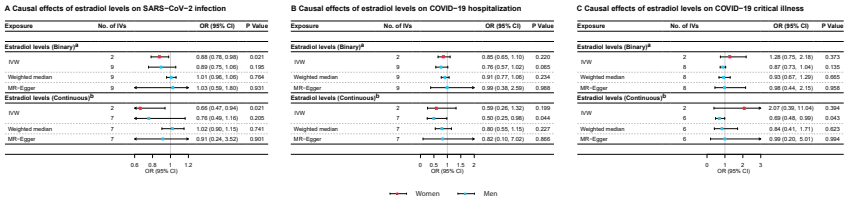


Figure 3 MR analysis results on the causal effects of serumSHBG levels on COVID-19 susceptibility and severity.

Panel A. Causal effects of serum SHBG levels on SARS-CoV-2 infection in men and women. Panel B. Causal effects of serum SHBG levels on COVID-19 hospitalization in men and women. Panel C. Causal effects of serum SHBG levels on COVID-19 critical illness in men and women. SHBG levels were measured in nmol/L and analyzed as a continuous phenotype.

Abbreviations: BMI, body mass index; CI, confidence interval; IV, instrumental variable; OR, odds ratio; SHBG, sex hormone binding globulin.



A Causal effects of testosterone levels on SARS−CoV−2 infection

Exposure	No. of IVs	OR (95% CI)	P Value
TT levels[a]			
IVW	143	1.00 (0.95, 1.04)	0.873
	116	0.99 (0.96, 1.02)	0.388
IVW*	143	1.00 (0.95, 1.04)	0.873
	113	0.99 (0.96, 1.02)	0.395
Weighted median	143	1.00 (0.95, 1.05)	0.980
	116	0.97 (0.93, 1.02)	0.297
MR−Egger	143	1.02 (0.94, 1.12)	0.627
	116	1.00 (0.95, 1.05)	0.885
BT levels[b]			
IVW	98	1.04 (1.00, 1.09)	0.055
	67	1.00 (0.96, 1.04)	0.985
IVW*	95	1.04 (0.99, 1.09)	0.089
	66	1.01 (0.97, 1.04)	0.762
Weighted median	98	1.07 (0.99, 1.17)	0.093
	67	1.03 (0.97, 1.10)	0.313
MR−Egger	98	1.04 (0.95, 1.14)	0.364
	67	1.03 (0.96, 1.11)	0.424
BT levels (SNPs mapped to androgen signaling genes)[c]			
IVW	6	1.08 (1.02, 1.14)	0.005
	3	1.04 (0.93, 1.17)	0.507
Weighted median	6	1.07 (0.98, 1.17)	0.139
MR−Egger	6	1.15 (0.92, 1.45)	0.281

B Causal effects of testosterone levels on COVID−19 hospitalization

Exposure	No. of IVs	OR (95% CI)	P Value
TT levels[a]			
IVW	143	0.93 (0.83, 1.03)	0.162
	116	1.01 (0.91, 1.11)	0.901
IVW*	143	0.93 (0.83, 1.03)	0.162
	113	1.01 (0.91, 1.12)	0.880
Weighted median	143	0.93 (0.73, 0.95)	0.006
	116	1.01 (0.89, 1.15)	0.849
MR−Egger	143	0.87 (0.72, 1.05)	0.146
	116	1.03 (0.88, 1.21)	0.720
BT levels[b]			
IVW	98	0.96 (0.84, 1.10)	0.570
	67	1.12 (0.95, 1.31)	0.167
IVW*	95	0.95 (0.82, 1.09)	0.469
	66	1.12 (0.95, 1.31)	0.180
Weighted median	98	0.93 (0.72, 1.19)	0.563
	67	1.20 (0.99, 1.45)	0.065
MR−Egger	98	0.74 (0.57, 0.95)	0.021
	67	1.17 (0.87, 1.57)	0.296
BT levels (SNPs mapped to androgen signaling genes)[c]			
IVW	6	0.92 (0.58, 1.48)	0.745
	3	1.05 (0.73, 1.50)	0.806
Weighted median	6	1.03 (0.78, 1.37)	0.825
MR−Egger	6	0.79 (0.19, 3.33)	0.767

C Causal effects of testosterone levels on COVID−19 critical illness

Exposure	No. of IVs	OR (95% CI)	P Value
TT levels[a]			
IVW	138	0.87 (0.71, 1.06)	0.158
	113	1.09 (0.90, 1.32)	0.376
IVW*	138	0.87 (0.71, 1.06)	0.158
	110	1.07 (0.89, 1.30)	0.464
Weighted median	138	0.89 (0.63, 1.25)	0.489
	113	0.95 (0.69, 1.30)	0.738
MR−Egger	138	0.84 (0.59, 1.17)	0.303
	113	0.86 (0.64, 1.17)	0.337
BT levels[b]			
IVW	96	0.87 (0.65, 1.16)	0.334
	67	1.07 (0.81, 1.40)	0.636
IVW*	93	0.87 (0.64, 1.17)	0.354
	66	1.08 (0.82, 1.41)	0.604
Weighted median	96	0.84 (0.53, 1.34)	0.464
	67	0.95 (0.61, 1.48)	0.829
MR−Egger	96	0.80 (0.46, 1.39)	0.438
	67	1.08 (0.67, 1.73)	0.753
BT levels (SNPs mapped to androgen signaling genes)[c]			
IVW	5	0.84 (0.42, 1.67)	0.614
	3	1.21 (0.49, 2.96)	0.676
Weighted median	5	0.84 (0.41, 1.72)	0.631
MR−Egger	5	0.99 (0.17, 5.80)	0.988

A Causal effects of SHBG levels on SARS−CoV−2 infection

Exposure	No. of IVs	OR (95% CI)	P Value
SHBG levels adjusted for BMI			
IVW	178	0.97 (0.92, 1.02)	0.263
	164	0.97 (0.93, 1.03)	0.326
Weighted median	178	0.92 (0.84, 1.00)	0.058
	164	0.95 (0.88, 1.03)	0.238
MR−Egger	178	0.93 (0.85, 1.02)	0.112
	164	0.93 (0.87, 1.01)	0.071
SHBG levels unadjusted for BMI			
IVW	213	0.98 (0.93, 1.03)	0.414
	229	0.98 (0.93, 1.03)	0.352
Weighted median	213	1.01 (0.94, 1.09)	0.805
	229	1.00 (0.92, 1.08)	0.953
MR−Egger	213	1.05 (0.97, 1.14)	0.221
	229	0.95 (0.87, 1.03)	0.203

B Causal effects of SHBG levels on COVID−19 hospitalization

Exposure	No. of IVs	OR (95% CI)	P Value
SHBG levels adjusted for BMI			
IVW	178	1.04 (0.88, 1.22)	0.665
	164	1.00 (0.86, 1.15)	0.982
Weighted median	178	0.99 (0.76, 1.29)	0.924
	164	0.84 (0.67, 1.04)	0.113
MR−Egger	178	1.00 (0.77, 1.30)	0.992
	164	0.92 (0.74, 1.14)	0.443
SHBG levels unadjusted for BMI			
IVW	214	0.92 (0.79, 1.07)	0.282
	231	1.02 (0.88, 1.18)	0.772
Weighted median	214	0.95 (0.76, 1.19)	0.679
	231	1.04 (0.82, 1.33)	0.737
MR−Egger	214	1.14 (0.88, 1.47)	0.332
	231	1.08 (0.85, 1.37)	0.516

C Causal effects of SHBG levels on COVID−19 critical illness

Exposure	No. of IVs	OR (95% CI)	P Value
SHBG levels adjusted for BMI			
IVW	170	0.92 (0.66, 1.29)	0.643
	157	0.95 (0.67, 1.34)	0.770
Weighted median	170	0.72 (0.42, 1.24)	0.234
	157	0.68 (0.39, 1.21)	0.191
MR−Egger	170	0.72 (0.43, 1.22)	0.225
	157	0.68 (0.41, 1.13)	0.134
SHBG levels unadjusted for BMI			
IVW	204	0.98 (0.71, 1.37)	0.914
	221	0.99 (0.70, 1.39)	0.944
Weighted median	204	1.11 (0.66, 1.89)	0.686
	221	1.01 (0.59, 1.73)	0.963
MR−Egger	204	1.05 (0.58, 1.87)	0.880
	221	0.95 (0.53, 1.67)	0.847