

Causal Associations of Sjögren's Syndrome with Sex Hormones: Case-control and Mendelian Randomization Study

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Additional Keywords:	case-control study, mendelian randomization, genome-wide association study
Abstract:	<p>Objective: To identify the association between sex hormones and Sjögren's Syndrome (SS).</p> <p>Methods: A case-control study was conducted from January 2018 to January 2024 at Nanjing Drum Tower Hospital to investigate the relationship between sex hormones and SS. Two-sample Mendelian randomization (MR) was then performed to identify the causal association by using the public genome-wide association study (GWAS) data from the UK biobank and the FinnGen consortium. Results: In the case-control study, a total of 93 cases diagnosed with SS were compared to 90 SS-like non-SS controls in the population of women after natural postmenopausal age. A strong direct relationship was observed between hypo-estradiol (hypoE2) and SS (aOR, 2.195; 95%CI:1.156-4.165; $p = 0.016$). Regarding the adjusted estimates, each 1 unit increase in E2 level was related to a 1.6% decrease (1-aOR) in the odds of SS (aOR, 0.984; 95% CI, 0.971-0.997; $p = 0.018$). However, MR analysis revealed no significant associations were observed for the effects of E2 on SS susceptibility. In turn, a heightened risk of SS was associated with decreased E2 levels in females, as indicated by inverse-variance weighted (IVW) (OR, 0.954; 95% CI, 0.917-0.992; $p = 0.019$).</p> <p>Conclusion: A strong direct relationship was observed between hypoE2 and SS in the population of women after natural postmenopausal age. However, this relationship may be due to the direct effect of SS on the genetic variation at low E2 levels. Keywords: Sex hormones; Sjögren's</p>

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	Syndrome; case-control study;mendelian randomization; genome-wide association study



Causal Associations of Sjögren's Syndrome with Sex Hormones: Case-control and Mendelian Randomization Study

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Abstract

Objective: To identify the association between sex hormones and Sjögren's Syndrome (SS).

Methods: A case-control study was conducted from January 2018 to January 2024 at Nanjing Drum Tower Hospital to investigate the relationship between sex hormones and SS. Two-sample Mendelian randomization (MR) was then performed to identify the causal association by using the public genome-wide association study (GWAS) data from the UK biobank and the FinnGen consortium.

Results: In the case-control study, a total of 93 cases diagnosed with SS were compared to 90 SS-like non-SS controls in the population of women after natural postmenopausal age. A strong direct relationship was observed between hypo-estradiol (hypoE2) and SS (aOR, 2.195; 95%CI: 1.156-4.165; $p = 0.016$). Regarding the adjusted estimates, each 1 unit increase in E2 level was related to a 1.6% decrease (1-aOR) in the odds of SS (aOR, 0.984; 95% CI, 0.971-0.997; $p = 0.018$). However, MR analysis revealed no significant associations were observed for the effects of E2 on SS susceptibility. In turn, a heightened risk of SS was associated with decreased E2 levels in females, as indicated by inverse-variance weighted (IVW) (OR, 0.954; 95% CI, 0.917-0.992; $p = 0.019$).

Conclusion: A strong direct relationship was observed between hypoE2

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and SS in the population of women after natural postmenopausal age.

However, this relationship may be due to the direct effect of SS on the

genetic variation at low E2 levels.

Keywords: Sex hormones; Sjögren's Syndrome; case-control study;

mendelian randomization; genome-wide association study

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65 **Introduction**

66 Sjögren's Syndrome (SS) is a systemic autoimmune disorder
67 characterized by the lymphocyte infiltration in exocrine glands [1, 2].

68 Although the exact pathogenesis of SS remains unknown,
69 epidemiological features of SS such as a higher prevalence in women,
70 onset usually around menopause, and often triggered by significant

71 stressful events, suggest an important role of hormones in the
72 development of SS [3-6].

73 Previous studies have shown that reduced production of ovarian
74 estrogens (E) and adrenal pro-hormone dehydroepiandrosterone (DHEA)
75 are closely related to the onset of SS, especially in postmenopausal
76 women [6-10]. However, the findings of different studies are controversial,
77 and the specific association between E2 and SS remains unclear. Based

78 on the previous findings, we conducted a matched case-control study,
79 and hypothesized that there is an association between low E2 levels and

80 an increased risk of SS. To validate the results, the Mendelian
81 randomization (MR) method was further used to analyze the exact causal
82 relationship between sex hormones (testosterone, E2, and sex
83 hormone-binding globulin (SHBG)) and SS.

84
85 **Materials and methods**

A matched case-control study was performed. The study protocol was approved by the Ethics Committee of Nanjing Drum Tower Hospital (No. 2022-529-04). Our study was conducted in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines [11]. The checklist is presented in [Supplementary Table S1](#).

Selection of Cases and Controls

A total of 189 cases were randomly selected from Nanjing Drum Tower Hospital between January 2018 and January 2024. Of these, 6 cases with missing data on key variables were excluded, leaving 183 valid samples for subsequent analysis. All cases consisted of postmenopausal women. The case group (n = 93) consisted of patients newly diagnosed with SS who visited our Rheumatology Outpatient Clinic, while the control group (n = 90) included sex-, age-, and race-matched patients who visited our department with SS-like symptoms but were not diagnosed with SS. In both groups, blood samples were collected during the patients' first visit to our department, before any treatment was administered. The timing of menopause in all cases fell within the natural age range for menopause based on a large population-based epidemiological survey [12]. The classification of SS was based on the 2002 American-European Consensus Group (AECG) criteria or 2016 American College of Rheumatology/European League Against

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Rheumatism classification criteria for primary Sjögren's syndrome, with all cases categorized under ICD-10 M35.0 [13, 14]. Individuals with other autoimmune disorders, premature ovarian failure, estrogen replacement therapy, polycystic ovarian syndrome, and hysterectomy without bilateral oophorectomy were all excluded from the controls/cases.

Measurement of Exposure and Outcomes

For the exposure, laboratory values including neutrophilic granulocyte (NE), hemoglobin (HB), platelet (PLT), complement3 (C3) and complement4 (C4), immunoglobulin G (IgG), fasting blood glucose (FBG), thyroxine levels, estradiol (E2), testosterone, SHBG, follicle stimulating hormone (FSH), luteinizing hormone (LH) and prolactin (PRL) were all recorded. The cut-off value of E2 levels < 89.76 poml/L was classified as hypo-estradiol (hypoE2) by ROC analysis. Age, menopausal age, body mass index (BMI), smoking, tea/coffee addiction, medical history of hypertension and diabetes and thyroxine levels were considered possible confounding factors. All samples for sex hormone were obtained from the patients' early morning fasting blood. For the outcome, the main clinical manifestations of the patients in the case group were collected on the basis of EULAR Sjögren's syndrome disease activity index (ESSDAI) score by means of questionnaires. All of the laboratory results were tested by the Clinical Laboratory Center of

129 Nanjing Drum Tower Hospital Affiliated to Medical College of Nanjing

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University according to the instructions of the corresponding kit.

Mendelian randomization analyses

Supplementary Figure S1 presents a brief summary of the bidirectional MR design that investigated the relationship between sex hormones and SS. Two MR analyses were conducted using summary statistics from a genome-wide association study (GWAS) to explore this relationship. In the forward MR analyses, sex hormones were considered as the exposure and SS as the outcome, whereas in the reverse MR analyses, SS was considered as the exposure and sex hormones as the outcome. MR analyses use publicly available summary statistics and do not require ethical approval. This part followed the Strengthening the Reporting of Observational Studies in Epidemiology–Mendelian Randomization reporting guidelines (Supplementary Table S2), available on the JAMA Network at <https://jamanetwork.com/journals/jama/fullarticle/2785494> [15].

Instrumental Variable Selection for MR Analyses

For each exposure factor, SNPs were filtered based on the three primary assumptions of MR. Initially, SNPs were included if they reached genome-wide significance ($p < 5 \times 10^{-8}$). Due to the insufficient number of SNPs for estradiol analysis, more lenient thresholds were opted for ($p < 5 \times 10^{-7}$). All the F -values are greater than 10 (Supplementary Table S3). Subsequently, only variants with the lowest p-values were retained as

independent instruments, considering linkage disequilibrium (LD) with an r^2 threshold ($r^2 > 0.1$ in the European 1000 Genome Reference Panel). Finally, we calculated F-statistics to assess the strength of the instrumental variables. We recommend a threshold of F-statistics >10 for MR analysis.

Sources of Data and Selection of Instrumental Variables for Sex Hormones

Sex hormones, including testosterone, E2, and SHBG, were obtained from the summary statistics. The summary statistics for female (N = 230,454) and male (N = 194,553) testosterone were obtained from a previous extensive GWAS that used genotype and phenotype data from the UK Biobank [12]. Estradiol summary statistics were sourced from another GWAS that used data from the UK Biobank [16], encompassing 163,985 samples in women and 147,690 in men. Ruth et al. provided data on SHBG, which involved 214,989 samples in women and 185,221 in men [17]. [Supplementary Table S4](#) contains comprehensive details of the summary data for all the GWAS.

Data Sources and Selection of Instrumental Variables for SS

Summary-level statistics for SS were obtained from the FinnGen consortium in 2023, which included 392,423 samples (2495 cases and 389,928 controls). The complete GWAS dataset for SS is publicly

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173 available through the FinnGen research project

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(<https://www.finngen.fi/en>), which aims to identify novel therapeutic targets by assessing genotype-phenotype correlations. The diagnosis of Sjögren's syndrome, unspecified, was established using International Classification of Diseases codes, specifically ICD-10 M35.0.

Statistical analysis

In the case-control study, normal data were presented as the means \pm standard deviations, skewed data were presented as the medians (interquartile ranges), and categorical data were presented as the absolute values. The independent-samples T test and the Mann-Whitney U test were used to compare the continuous parameters between groups, while the chi-square test was used to compare the categorical data. The logistic regression analysis was conducted to predict the relationship between sex hormones levels and the risk of SS. $P < 0.05$ was considered significant in the two-tailed tests. Data analysis was conducted by using SPSS software (Version 26.0). In the MR study, the inverse-variance weighted (IVW) method was used to explore potential bidirectional causal links between sex hormones and SS, with additional checks using MR-Egger, Weighted Median, and MR-Pleiotropy Residual Sum, and Outlier (MR-PRESSO) to address potential biases like invalid instruments and pleiotropy. A p -value exceeding 0.05 indicates the absence of horizontal pleiotropy [18]. Heterogeneity was assessed using Cochran's Q test. We applied the IVW random effects method [19] to

estimate the main effect. MR-PRESSO identified outliers influencing heterogeneity [20], which were subsequently excluded. A meta-analysis synthesized MR results across sexes for sex-stratified hormone levels and SS using *R* software (Version 4.30), with the Two-Sample MR and Meta packages utilized for the respective analyses.

Results

The relationship between Sex Hormones and SS

The characteristics of the 93 patients included in the case group are shown in Table 1.

The distributions of all the study variables in the cases and controls are presented in Table 2. SS cases showed significantly lower mean total E2 levels than controls (84.41 pmol/L vs 91.57 pmol/L). Similarly, the rate of hypoE2 was significantly higher in the SS group (62.37% vs 46.67%). The crude odds ratio (cOR) and adjusted odds ratio (aOR) between SS and both E2 levels and hypoE2 are shown in Table 3. According to the results of univariate logistic regression analyses, each 1 unit increase in E2 levels corresponded to a 1.2% decrease (1-cOR) in the risk of SS (cOR, 0.988; 95% CI, 0.975-1.000; $p = 0.047$) and hypoE2 seemed to act as a risk factor against SS (cOR, 1.894; 95% CI, 1.050-3.415; $p = 0.034$). Regarding the adjusted estimates, each 1 unit increase in E2 level was related to a 1.6% decrease (1-aOR) in the odds of SS (aOR, 0.984; 95%

217 CI, 0.971-0.997; $p = 0.018$) . Consistent with the findings, a strong direct

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relationship between hypoE2 and SS was observed in the adjusted analysis, with an aOR value of 2.195 (95%CI, 1.156-4.165; $p = 0.016$). However, no significant relationship was found for other hormone levels at the onset of SS ($p > 0.05$), which suggests a weak association between SS and other hormone levels.

The Casual Effect of Sex Hormones on SS

The impact of each hormone on susceptibility to SS was examined individually. However, no significant association was observed, as shown in [Figure 1](#). To further validate these findings, scatter plots, the leave-one-out test, funnel plot, and forest plot were employed, offering additional confirmation of the results (see [Supplementary Figures S2-S7](#)).

The Causal Influence of SS on Sex Hormones

The [Figure 2](#) showed that a heightened risk of SS was associated with decreased E2 levels in females, as indicated by the IVW method (OR, 0.954; 95% CI, 0.917-0.992; $p = 0.019$). However, this effect was not observed in males. In relation to testosterone levels, SS was identified as a risk factor for testosterone in both sexes (OR, 0.993; 95% CI, 0.987-1.000; $p = 0.047$). However, no significant difference was observed in the sex-stratified testosterone levels. Additional confirmation of the results was obtained through the use of scatter plots, the

239 leave-one-out test, funnel plot, and forest plot ([Supplementary Figures](#)

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S8-S13).

Assessment of Heterogeneity, Pleiotropy, and Sensitivity Analysis

In addition, we conducted sensitivity analyses to support a causal relationship between sex hormones and SS. The Cochran's Q test did not show any detectable heterogeneity of effects across the instrumental variables (Table 4). Furthermore, the F statistics for all instrumental variables exceeded 10, indicating the absence of weaknesses in the selected instruments. No signs of horizontal pleiotropy were detected as the intercept of MR-Egger did not significantly deviate from zero. Additionally, the MR-PRESSO analysis did not identify any potential instrumental outliers. The leave-one-out results suggest that the causal effect was not solely influenced by a single instrumental variable.

Discussion

SS is an autoimmune disease that primarily affects the exocrine glands, particularly the salivary and lacrimal glands. Follicular cells of the external glands are damaged and destroyed in SS, resulting in reduced secretion of saliva and tear fluid [21]. A number of clinical studies and animal studies have suggested that decreased level of sex hormones may increase the risk of SS [22-25]. In this part of case-control study, we found a strong direct relationship between low E2 exposure and SS in

261 menopausal women which is consistent with the previous studies.

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Furthermore, each unit increase in E2 was associated with a 1.4% reduction in the odds of SS as shown in our results.

Estrogens exert their influence on specific intracellular estrogen receptor subtypes (ERs) found within every cell of the immune system [26-28], especially in modulating the development and function of lymphocytes [29, 30]. Estrogens may facilitate protective effects that counteract harmful changes following inflammatory responses. For example, human lymphocyte cultures have shown a reduction in CD4+/CD8+ T-cell-subset ratios after estrogen treatment [31]. Estrogen can directly affect the subsets of B lymphocytes subsets. Studies in animals have shown that estrogens can increase the population of bone marrow progenitor B cells by protecting them from apoptosis [29, 32] and by improving the survival of B cells [29]. It has been suggested that estrogens have a protective effect on secretory glandular acinar cells by shielding them from apoptosis. Additionally, testosterone can be converted to dihydrotestosterone (DHT) in exocrine glands. DHT has anti-apoptotic properties, which protect acinar cells against apoptosis [10]. In menopausal women, a lack of local intracellular DHT may increase susceptibility to SS when estrogen levels are low [33]. The above findings had prompted a strong interest in hormone replacement therapy (HRT) as a prospective treatment for SS [34-36].

283 Due to the natural limitations of case-control study, we further

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284 analysed by MR, and revealed no significant associations were observed
285 for the effects of E2 on SS susceptibility. In turn, a heightened risk of SS
286 was associated with decreased E2 levels in females, which is also
287 consistent with the findings of a number of studies. In a nested
288 case-control study, the modified composite estrogen scores (mCES)
289 demonstrated no significant association with SS in adjusted models,
290 considering 546 SS cases and 1637 age-matched controls [24]. In both
291 human and mouse studies, it has been observed that the number of X
292 chromosomes, rather than sex-steroid hormones, is associated with an
293 increased risk or susceptibility to develop autoimmunity, particularly
294 rheumatic diseases like SS. This observation suggests that the number
295 of X chromosomes plays a crucial role in the development of
296 autoimmunity [37]. Research findings suggest that the differences in the
297 immune system between sexes can be attributed to inherent composition.
298 Women generally exhibit a higher count of T lymphocytes CD4/CD8, B
299 lymphocytes, and plasma cells (PC), whereas men demonstrate a higher
300 proportion of Natural Killer cells (NK), CD14, and CD16 monocytes [38].
301 Genetic mechanisms linked to sex chromosomes have been identified as
302 possible factors contributing to sex differences in immune responses
303 across different age groups [39, 40]. Individuals with Klinefelter syndrome
304 (47,XXY) exhibit a susceptibility to lupus similar to that of females (46, XX)
305 [41]. Conversely, females with Turner syndrome (45, X0) appear to have

a protective effect [42]. Therefore, the dosage of the X chromosome seems to be involved in the development of lupus pathology, scleroderma, and SS. Certain immune-related genes can escape X chromosome inactivation (XCI) to varying degrees, resulting in bi-allelic expression in immune cells. This ultimately increases the likelihood of developing immune-related disorders [37].

The efficacy of hormone therapy in SS has also varied significantly in the results of a number of clinical trial studies. For instance, numerous studies conducted on post-menopausal women have yielded conflicting results regarding the efficacy of estrogen use in managing SS symptoms. While some studies found that estrogen replacement therapy have no significant effect on osmolarity, tear volume, breakup time, or ocular symptoms [43, 44], others have shown that therapy can alleviate ocular symptoms in this population [45-47]. Notably, one study revealed that 3 months of estrogen replacement therapy in post-menopausal women without SS symptoms led to the development of dry eyes, with symptomatic patients showing no improvement after the same duration of therapy [48]. Therefore, there are further observations concerning the interplay between estrogen treatment and SS signs and symptoms. Regarding androgens, individuals with SS exhibit lower serum levels of DHEA, dihydrotestosterone (DHT), and dehydroepiandrosterone sulfate (DHEA-S) compared to healthy controls [6]. For example, in a case study,

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the use of testosterone cream applied to the eyelids was suggested to effectively reduce SS symptoms and improve the lipid layer breakup time and its thickness to normal levels [49]. Similarly, another case study indicated that systemic androgen and testosterone therapy in post-menopausal women resulted in a reduction in dry eyes [50]. However, a study found that DHEA showed no evidence of efficacy in SS. Due to the lack of evidence for its efficacy, patients with SS should avoid using unregulated DHEA supplements, as the long-term adverse consequences of exposure to this hormone are unknown [51]. Taken together, our results suggest that early sex hormone replacement therapy for normal menopausal women may not be helpful in the prevention of SS; moreover, previous observational studies may have overlooked the direct effect of SS on genetic variation in estrogen itself.

Our study has several limitations. First, our collection of sex hormone levels only once before SS diagnosis and lack of follow-up, causality still need to be clarified by designing longitudinal prospective studies or retrospective nested cohorts. Second, the GWAS datasets used in this study were not stratified by age. And the accuracy of our findings may be affected by the age-specific nature of SS. The impact of the genetic variation may be underestimated. Then, the limited sample size in the case-control cohort, consisting of only 93 SS cases and 90 controls, may reduce the statistical power of our analysis and limit the

generalizability of the findings. Last, the observational study used a Chinese postmenopausal female cohort, while the MR analysis relied on European GWAS summary data. Due to genetic differences across populations, this cross-ancestry approach may impact the validity of the causal inferences.

Conclusion

A strong direct relationship was observed between hypoE2 and SS in the population of women after natural postmenopausal age. However, this relationship may be due to the direct effect of SS on genetic variation at low E2.

Ethics statement

The case-control study conformed to approved guidelines, and all experimental protocols were approved by the Ethics Committee of Nanjing Drum Tower Hospital (No. 2022-529-04). Our study complied with the Declaration of Helsinki, and all subjects provided written informed consent. In the MR study, ethical review and approval were not deemed necessary for this study involving human subjects, in

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369 accordance with local laws and institutional regulations. Additionally,
370 written informed consent for participation was not required for this study,
371 aligning with national laws and institutional guidelines.

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Author contributions

JZ and SL conceptualized and designed the study; JZ and QC conducted the statistical analysis; A-NW, M-GP, ZL and W-WW collected the clinical specimens; JZ and QS organized the data, drafted the manuscript, and revised it; QC and SL edited the manuscript and supervised the study. All authors contributed to the article and approved its final version.

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This study had no funding.

Conflict of Interest

The authors affirm that there are no competing interests that could

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391 be construed as influencing the impartiality of this study.

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393 **Data availability**

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For the data of Mendelian randomization: The data that support the findings of this study are openly available in the UK Biobank and the FinnGen research project (<https://www.finnngen.fi/en>). For the data of case-control: The data that support the findings of this study are available on request from the corresponding author, SL, upon reasonable request.

Supplementary material

The Supplementary Material for this article can be found online.

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Table 1. Characteristics of the patients in the case group

Characteristics	Case Group (n = 93)
Dichotomous variables, No. (%)	
Fever	2 (2.15)
Weight loss	1 (1.08)
Large lymph node	2 (2.15)
Swelling parotid/lacrimal/submaxillary glands	2 (2.15)
Joint pain	6 (6.45)
Synovitis	1 (1.08)
Erythema	3 (3.23)
Interstitial lung changes	2 (2.15)
Proteinuria > 0.5g/24h	2 (2.15)
Active myositis	0 (0.00)
Abnormal peripheral sensation	0 (0.00)
Central neuropathy	0 (0.00)
NE < 1 (10×9) /L	1 (1.08)
HB < 120 g/L	3 (3.23)
PLT <100 (10×9) /L	4 (4.30)
Continuous variables	
C3, g/L	1.02 ± 0.24
C4, g/L	0.22 ± 0.07
IgG, g/L	13.20 (10.75, 15.85)
ESSDAI	0 (0, 2)

Dichotomous variables data are presented as n (%), normal variables are presented as mean ± standard deviation, and non-normal variables are presented as median (interquartile).

NE: neutrophilic granulocyte; HB: hemoglobin; PLT: platelet; C3: Complement 3; C4: Complement 4; IgG: immunoglobulin G.

Normal Range: C3 (0.80-1.60) g/L; C4 (0.20-0.40) g/L; IgG (8.00-16.00) g/L.

Table 2. Descriptive Statistics of the Study Variables in Controls and Cases

Variables	Cases (n=93)	Controls (n =90)	p value
Dichotomous variables, No. (%)			
Smoker	1 (1.08)	2 (2.22)	0.541
Tea/Coffee addiction	3 (3.23)	4 (4.44)	0.667
Alcohol addiction	2 (2.15)	3 (3.33)	0.624
Hypertension	15 (16.13)	14 (15.56)	0.865
Diabetes	10 (10.75)	9 (10.00)	0.828
E2 < 89.76 (pmol/L)	58 (62.37)	42 (46.67)	0.033
Continuous variables			
Age (years)	56.00 (53.00, 63.00)	57.00 (55.00, 63.25)	0.230
Age at menopause (years)	47.00 (43.50, 52.00)	48.50 (44.75, 52.25)	0.188
BMI	22.22 ± 2.64	21.68 ± 2.24	0.133
FBG (mmol/L)	4.65 (4.27, 5.27)	5.08 (4.12, 6.12)	0.260
FT4 (pmol/L)	17.17 ± 3.36	16.58 ± 3.43	0.247
FT3 (pmol/L)	5.05 ± 1.19	5.04 ± 1.17	0.924
TSH (mIU/L)	2.30 ± 1.24	2.13 ± 1.17	0.349
E2 (pmol/L)	84.41 ± 25.25	91.57 ± 22.62	0.045
Testosterone (nmol/L)	0.88 (0.70, 1.17)	0.91 (0.73, 1.20)	0.741
SHBG (nmol/L)	96.42 ± 4.61	93.55 ± 4.74	0.678
FSH (mIU/mL)	39.67 ± 34.99	39.98 ± 24.95	0.946
LH (mIU/mL)	22.18 ± 17.58	19.16 ± 13.18	0.191
PRL (ug/L)	14.61 (8.91, 50.99)	13.10 (6.78, 133.00)	0.205

Dichotomous variables data are presented as n (%), normal variables are presented as mean ± standard deviation, and non-normal variables are presented as median (interquartile).

BMI: body mass index; FBG: fasting blood-glucose; FT3: free triiodothyronine; FT4: free (unbound) thyroxine; TSH: thyroid stimulating hormone; E2: estradiol; SHBG: sex hormone-binding globulin; FSH: follicle stimulating hormone; LH, luteinizing hormone; PRL: prolactin.

Table 3. Crude and Adjusted OR Values for the Association Between SS and Each Hormone

Variables	Crude Estimates		Adjusted Estimates	
	cOR (95%CI)	p-value	aOR ^a (95%CI)	p-value
OR values for E2 levels and SS				
E2	0.988 (0.975-1.000)	0.047	0.984 (0.971, 0.997)	0.018
E2 < 89.76 (pmol/L)	1.894 (1.050,3.415)	0.034	2.195 (1.156, 4.165)	0.016
OR values for the remaining hormone levels				
SHBG (nmol/L)	1.001 (0.995, 1.008)	0.676	1.000 (0.994, 1.007)	0.965
FSH (mIU/mL)	1.000 (0.990, 1.009)	0.946	1.001 (0.991, 1.011)	0.887
LH (mIU/mL)	1.013 (0.994, 1.032)	0.192	1.013 (0.993, 1.034)	0.200
Testosterone (nmol/L)	0.952 (0.437, 2.075)	0.901	0.867 (0.379, 1.983)	0.736
PRL (ug/L)	1.000 (0.998, 1.002)	0.807	1.000 (0.998, 1.002)	0.847
TSH (mIU/L)	1.123 (0.882, 1.431)	0.347	1.105 (0.859, 1.421)	0.436

OR: odds ratio; SS: Sjögren's Syndrome; aOR: adjusted odds ratio; CI: confidence interval; cOR: crude odds ratio; SHBG: sex hormone-binding globulin; FSH: follicle stimulating hormone; LH: luteinizing hormone; E2: estradiol; PRL: prolactin; TSH: thyroid stimulating hormone.

^a The following variables were also included in each model: age, menopausal age, body mass index (BMI), smoking, tea/coffee addiction, medical history of hypertension and diabetes and thyroxine levels.

Table 4. Sensitivity analysis of the causal relationship between sex hormones and Sjögren's Syndrome

Sex hormones	Heterogeneity		Horizontal pleiotropy		Outlier examination by MR-PRESSO (p-value)			
	IVW Q	IVW Q df	IVW p-value	Intercept	SE	p-value	Before correction	After correction
Sjögren's Syndrome as outcome								
	257.46				0.00			
Testosterone in females	0	132.000	0.000	0.004	7	0.586	0.206	0.392
	161.41				0.00			
Testosterone in males	0	129.000	0.028	0.007	6	0.240	0.346	NA
					0.03			
E2 in females	5.785	6.000	0.448	0.013	5	0.726	0.103	NA
					0.03			
E2 in males	13.867	10.000	0.179	0.046	5	0.222	0.932	NA
	202.19				0.00			
SHBG in females	9	149.000	0.002	-0.003	6	0.674	0.651	0.521
	187.10				0.00			
SHBG in males	4	162.000	0.086	-0.009	5	0.098	0.674	NA
Sjögren's Syndrome as exposure								
Testosterone in females	16.201	5.000	0.006	0.004	3	0.165	0.128	NA
					0.00			
Testosterone in males	16.849	5.000	0.005	0.011	6	0.112	0.314	NA
E2 in females	1.235	3.000	0.745	-0.007	0.02	0.747	0.035	NA

					0			
					0.03			
E2 in males	7.992	3.000	0.046	0.028	7	0.525	0.923	NA
					0.00			
SHBG in females	0.595	4.000	2.783	0.000	5	0.953	0.149	NA
					0.00			
SHBG in males	2.121	4.000	0.714	-0.002	5	0.714	0.513	NA

IVW: inverse-variance weighted; E2: estradiol; SHBG: sex hormone-binding globulin; NA: not applicable

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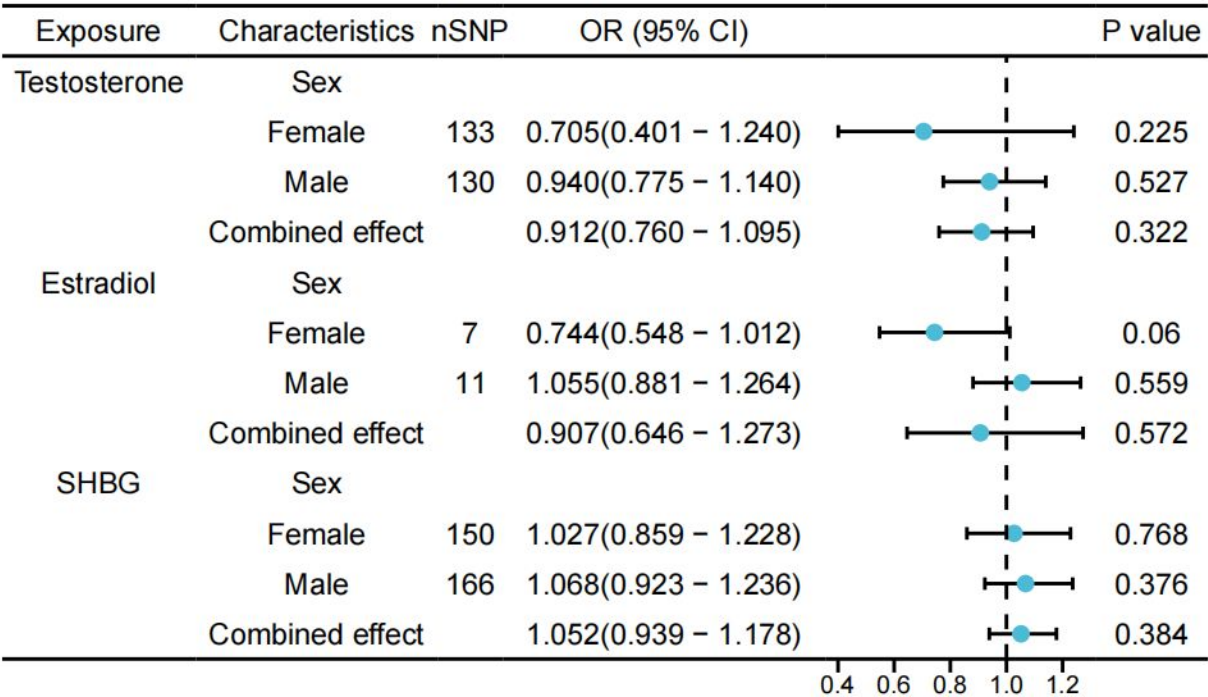


Figure 1. MR estimates for the relationship between genetically instrumented sex hormones and SS

nSNP: number of single nucleotide polymorphisms; OR: odds ratio; CI: confidence interval; SHBG: sex hormone-binding globulin.

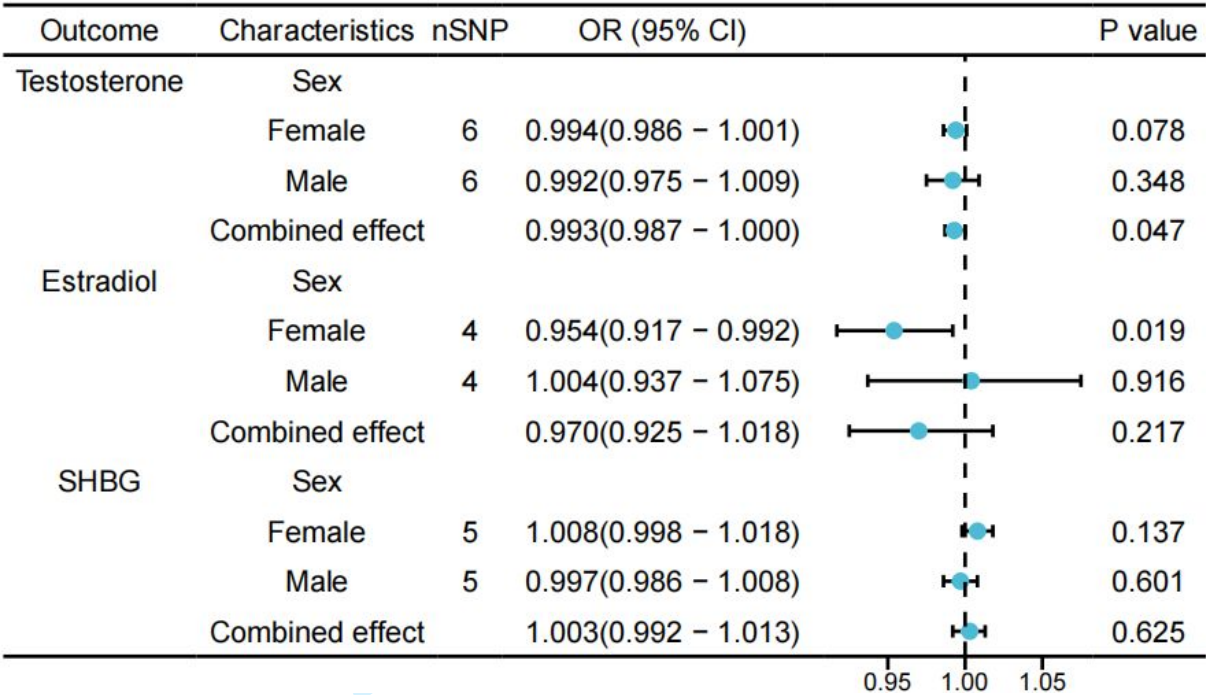


Figure 2. MR estimates for the relationship between genetically instrumented SS and sex hormones

nSNP: number of single nucleotide polymorphisms; OR: odds ratio; CI: confidence interval; SHBG: sex hormone-binding globulin.

Table S1. STROBE Statement—Checklist of items that should be included in reports of case-control studies

	Item No	Recommendation	Page No
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract	1
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	2-3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	4-5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5
Participants	6	(a) Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls	5
		(b) For matched studies, give matching criteria and the number of controls per case	5
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5-6
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	5-6
Bias	9	Describe any efforts to address potential sources of bias	5
Study size	10	Explain how the study size was arrived at	5
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	5
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	9
		(b) Describe any methods used to examine subgroups and interactions	not applicable
		(c) Explain how missing data were addressed	not applicable
		(d) If applicable, explain how matching of cases and controls was addressed	9
		(e) Describe any sensitivity analyses	not applicable
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	5
		(b) Give reasons for non-participation at each stage	not applicable
		(c) Consider use of a flow diagram	not applicable
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	10
		(b) Indicate number of participants with missing data for each variable of interest	not applicable
Outcome data	15*	Report numbers in each exposure category, or summary measures of exposure	5

Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	10
		(b) Report category boundaries when continuous variables were categorized	10
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	not applicable
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	not applicable
Discussion			
Key results	18	Summarise key results with reference to study objectives	12
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	16-17
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	16
Generalisability	21	Discuss the generalisability (external validity) of the study results	not applicable
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	not applicable

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at <http://www.strobe-statement.org>.

Table S2. STROBE-MR checklist of recommended items to address in reports of Mendelian randomization studies

Item No.	Section	Checklist item	Page No.
1	TITLE and ABSTRACT	Indicate Mendelian randomization (MR) as the study's design in the title and/or the abstract if that is a main purpose of the study	1-3
INTRODUCTION			
2	Background	Explain the scientific background and rationale for the reported study. What is the exposure? Is a potential causal relationship between exposure and outcome plausible? Justify why MR is a helpful method to address the study question	5
3	Objectives	State specific objectives clearly, including pre-specified causal hypotheses (if any). State that MR is a method that, under specific assumptions, intends to estimate causal effects	5
METHODS			
4	Study design and data sources	Present key elements of the study design early in the article. Consider including a table listing sources of data for all phases of the study. For each data source contributing to the analysis, describe the following:	
	a)	Setting: Describe the study design and the underlying population, if possible. Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection, when available.	6-7
	b)	Participants: Give the eligibility criteria, and the sources and methods of selection of participants. Report the sample size, and whether any power or sample size calculations were carried out prior to the main analysis	not applicable
	c)	Describe measurement, quality control and selection of genetic variants	7-8
	d)	For each exposure, outcome, and other relevant variables, describe methods of assessment and diagnostic criteria for diseases	7-8
	e)	Provide details of ethics committee approval and participant informed consent, if relevant	not applicable
5	Assumptions	Explicitly state the three core IV assumptions for the main analysis (relevance, independence and exclusion restriction) as well assumptions for any additional or sensitivity analysis	6-7
6	Statistical methods: main analysis	Describe statistical methods and statistics used	
	a)	Describe how quantitative variables were handled in the analyses (i.e., scale, units, model)	not applicable
	b)	Describe how genetic variants were handled in the analyses and, if applicable, how their weights were selected	6-9
	c)	Describe the MR estimator (e.g. two-stage least squares, Wald ratio) and related statistics. Detail the included covariates and, in case of two-sample MR, whether the same covariate set was used for adjustment in the two samples	6-9
	d)	Explain how missing data were addressed	not applicable
	e)	If applicable, indicate how multiple testing was addressed	not applicable
7	Assessment of	Describe any methods or prior knowledge used to assess the assumptions or justify	6-8

	assumptions	their validity	
8	Sensitivity analyses and additional analyses	Describe any sensitivity analyses or additional analyses performed (e.g. comparison of effect estimates from different approaches, independent replication, bias analytic techniques, validation of instruments, simulations)	6-8
9	Software and pre-registration		
	a)	Name statistical software and package(s), including version and settings used	9
	b)	State whether the study protocol and details were pre-registered (as well as when and where)	not applicable
RESULTS			
10	Descriptive data		
	a)	Report the numbers of individuals at each stage of included studies and reasons for exclusion. Consider use of a flow diagram	not applicable
	b)	Report summary statistics for phenotypic exposure(s), outcome(s), and other relevant variables (e.g. means, SDs, proportions)	Table 4, figures 1-2 and supplementary Figures 1-13
	c)	If the data sources include meta-analyses of previous studies, provide the assessments of heterogeneity across these studies	not applicable
	d)	For two-sample MR: i. Provide justification of the similarity of the genetic variant-exposure associations between the exposure and outcome samples ii. Provide information on the number of individuals who overlap between the exposure and outcome studies	Table 4 and figures 1-2
11	Main results		
	a)	Report the associations between genetic variant and exposure, and between genetic variant and outcome, preferably on an interpretable scale	11-12
	b)	Report MR estimates of the relationship between exposure and outcome, and the measures of uncertainty from the MR analysis, on an interpretable scale, such as odds ratio or relative risk per SD difference	11-12
	c)	If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	not applicable
	d)	Consider plots to visualize results (e.g. forest plot, scatterplot of associations between genetic variants and outcome versus between genetic variants and exposure)	Table 4, figures 1-2 and supplementary Figures 1-13
12	Assessment of assumptions		
	a)	Report the assessment of the validity of the assumptions	12, Table 4 and figures 1-2
	b)	Report any additional statistics (e.g., assessments of heterogeneity across genetic variants, such as I^2 , Q statistic or E-value)	Table 4 and figures 1-2
13	Sensitivity analyses and additional analyses		

	a)	Report any sensitivity analyses to assess the robustness of the main results to violations of the assumptions	Table 4 and figures 1-2
	b)	Report results from other sensitivity analyses or additional analyses	Table 4 and figures 1-2
	c)	Report any assessment of direction of causal relationship (e.g., bidirectional MR)	11-12
	d)	When relevant, report and compare with estimates from non-MR analyses	not applicable
	e)	Consider additional plots to visualize results (e.g., leave-one-out analyses)	Table 4, figures 1-2 and supplementary Figures 1-13
DISCUSSION			
14	Key results	Summarize key results with reference to study objectives	12-13
15	Limitations	Discuss limitations of the study, taking into account the validity of the IV assumptions, other sources of potential bias, and imprecision. Discuss both direction and magnitude of any potential bias and any efforts to address them	16-17
16	Interpretation		
	a)	Meaning: Give a cautious overall interpretation of results in the context of their limitations and in comparison with other studies	12
	b)	Mechanism: Discuss underlying biological mechanisms that could drive a potential causal relationship between the investigated exposure and the outcome, and whether the gene-environment equivalence assumption is reasonable. Use causal language carefully, clarifying that IV estimates may provide causal effects only under certain assumptions	12-16
	c)	Clinical relevance: Discuss whether the results have clinical or public policy relevance, and to what extent they inform effect sizes of possible interventions	15-16
17	Generalizability	Discuss the generalizability of the study results (a) to other populations, (b) across other exposure periods/timings, and (c) across other levels of exposure	not applicable
OTHER INFORMATION			
18	Funding	Describe sources of funding and the role of funders in the present study and, if applicable, sources of funding for the databases and original study or studies on which the present study is based	not applicable
19	Data and data sharing	Provide the data used to perform all analyses or report where and how the data can be accessed, and reference these sources in the article. Provide the statistical code needed to reproduce the results in the article, or report whether the code is publicly accessible and if so, where	18
20	Conflicts of Interest	All authors should declare all potential conflicts of interest	18

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Table S3. Univariate Mendelian randomization analysis for the effects of E2 on Sjögren's Syndrome risk.

SNP	EAE	OAE	EAO	OAO	exposure			outcome			F
					se	beta	pval	se	beta	pval	
ALL											
rs1073548	G	T	G	T	0.0189315	0.106682	1.75E-08	0.0453519	0.00113479	0.980037508	31.76
rs11160915	A	G	A	G	0.0132441	-0.164053	3.08E-35	0.0296124	-0.005112	0.862941967	153.43
rs112881196	G	C	G	C	0.0300433	0.302154	8.52E-24	0.0946876	-0.216239	0.022388592	101.15
rs113047993	T	C	T	C	0.0271096	-0.153581	1.47E-08	0.0604196	-0.0918301	0.128542975	32.09
rs3751591	G	A	G	A	0.0168483	0.107544	1.73E-10	0.0428018	0.0124049	0.771952475	40.74
rs45446698	G	T	G	T	0.034576	-0.209456	1.38E-09	0.0642233	0.104477	0.103784426	36.70
rs56196860	A	C	A	C	0.0337074	0.233712	4.10E-12	0.130167	-0.233534	0.072795511	48.07
rs600038	C	T	C	T	0.0163807	-0.126217	1.31E-14	0.0342139	-0.051297	0.133795067	59.37
rs62059839	T	C	T	C	0.0144126	0.0935541	8.53E-11	0.0318731	0.0621088	0.051339922	42.13
rs7173595	T	C	T	C	0.0139251	0.22874	1.24E-60	0.0299847	0.00207077	0.944941154	269.83
rs7662029	G	A	G	A	0.0129643	-0.112665	3.61E-18	0.0288158	0.00065421	0.981887047	75.52
rs7855247	C	T	C	T	0.0128633	-0.0671445	1.79E-07	0.0287208	-0.000572157	0.984106117	27.25
rs145292296	T	C	T	C	0.0472937	-0.238539	4.56E-07	0.0723391	0.0837929	0.246727418	25.44
rs145893930	C	T	C	T	0.0683425	-0.348858	3.32E-07	0.392552	-0.110371	0.778585574	26.06
rs16991615	A	G	A	G	0.0237994	0.130028	4.67E-08	0.0998847	0.0449093	0.652990665	29.85
rs2345568	G	A	G	A	0.0127206	0.064701	3.66E-07	0.0295611	-0.0604542	0.04084902	25.87
rs4764934	T	C	T	C	0.0156467	-0.0847521	6.07E-08	0.0317555	0.0142795	0.652948308	29.34
rs734518	A	G	A	G	0.0120323	-0.0650688	6.38E-08	0.028731	-0.023684	0.409748622	29.24
Male											
rs1073548	G	T	G	T	0.0189315	0.106682	1.75E-08	0.0453519	0.00113479	0.980037508	31.76
rs11160915	A	G	A	G	0.0132441	-0.164053	3.08E-35	0.0296124	-0.005112	0.862941967	153.43
rs112881196	G	C	G	C	0.0300433	0.302154	8.52E-24	0.0946876	-0.216239	0.022388592	101.15
rs113047993	T	C	T	C	0.0271096	-0.153581	1.47E-08	0.0604196	-0.0918301	0.128542975	32.09
rs3751591	G	A	G	A	0.0168483	0.107544	1.73E-10	0.0428018	0.0124049	0.771952475	40.74
rs45446698	G	T	G	T	0.034576	-0.209456	1.38E-09	0.0642233	0.104477	0.103784426	36.70
rs56196860	A	C	A	C	0.0337074	0.233712	4.10E-12	0.130167	-0.233534	0.072795511	48.07
rs600038	C	T	C	T	0.0163807	-0.126217	1.31E-14	0.0342139	-0.051297	0.133795067	59.37
rs62059839	T	C	T	C	0.0144126	0.0935541	8.53E-11	0.0318731	0.0621088	0.051339922	42.13

rs7173595	T	C	T	C	0.0139251	0.22874	1.24E-60	0.0299847	0.00207077	0.944941154	269.83
rs7662029	G	A	G	A	0.0129643	-0.112665	3.61E-18	0.0288158	0.00065421	0.981887047	75.52
rs7855247	C	T	C	T	0.0128633	-0.0671445	1.79E-07	0.0287208	-0.000572157	0.984106117	27.25
Female											
rs145292296	T	C	T	C	0.0472937	-0.238539	4.56E-07	0.0723391	0.0837929	0.246727418	25.44
rs145893930	C	T	C	T	0.0683425	-0.348858	3.32E-07	0.392552	-0.110371	0.778585574	26.06
rs16991615	A	G	A	G	0.0237994	0.130028	4.67E-08	0.0998847	0.0449093	0.652990665	29.85
rs2345568	G	A	G	A	0.0127206	0.064701	3.66E-07	0.0295611	-0.0604542	0.04084902	25.87
rs45446698	G	T	G	T	0.0301572	-0.206441	7.62E-12	0.0642233	0.104477	0.103784426	46.86
rs4764934	T	C	T	C	0.0156467	-0.0847521	6.07E-08	0.0317555	0.0142795	0.652948308	29.34
rs734518	A	G	A	G	0.0120323	-0.0650688	6.38E-08	0.028731	-0.023684	0.409748622	29.24

SNP: single nucleotide polymorphism; EAE: effect allele exposure; OAE: other allele exposure; EAO: effect allele outcome; OAO: other allele outcome; SE: standard error; F:F value;

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Table S4. Summary data from all GWAS used in current study.

Phenotype	Sample sizes	Number of SNPs	Study	Ethnicity
Testosterone in females	230,454	16577312.00	Schmitz et al.	European
Testosterone in males	194,553	16,580,850	Schmitz et al.	European
E2 in females	163,985	7,871,694	Schmitz et al.	European
E2 in males	147,690	7,870,546	Schmitz et al.	European
SHBG in females	214,989	12,321,875	Ruth et al.	European
SHBG in males	185,221	12,321,875	Ruth et al.	European
Sjögren's Syndrome	392423	201,700,11	Finngen biobank	European

GWAS: genome-wide association studies; SNPs: single-nucleotide polymorphisms; E2: estradiol;
SHBG: sex hormone-binding globulin

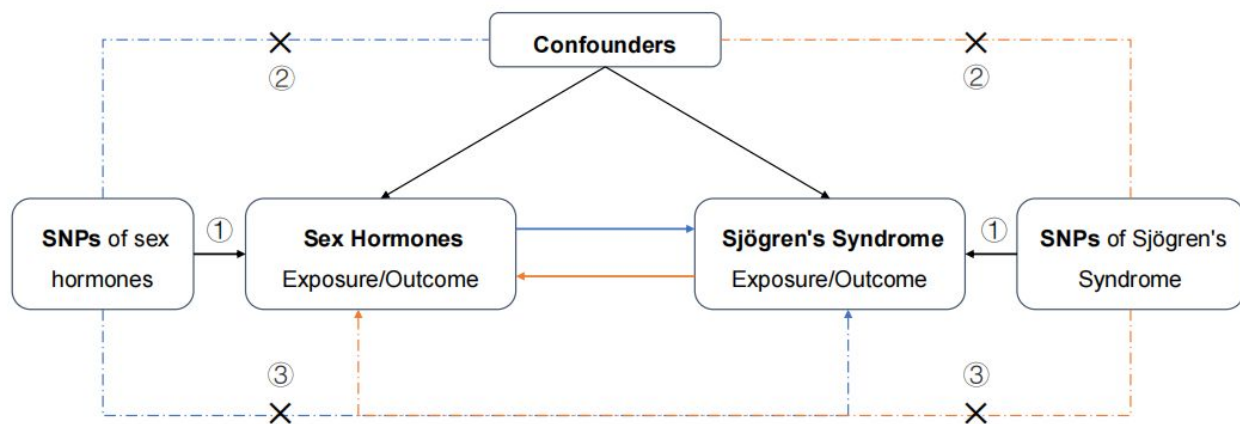


Figure S1. An overview of the study design for MR

Directed acyclic graph of the MR framework examining the cause-and-effect relationship between sex hormones and the risk of SS.

Assumption 1: Instrumental variables must be strongly associated with exposure. Assumption 2: Instrumental variables should not be associated with any potential confounders. Assumption 3: Instrumental variables should not be associated with the outcome except through the exposure.

SNPs: single nucleotide polymorphisms; SS: Sjögren's Syndrome; MR: Mendelian Randomization.

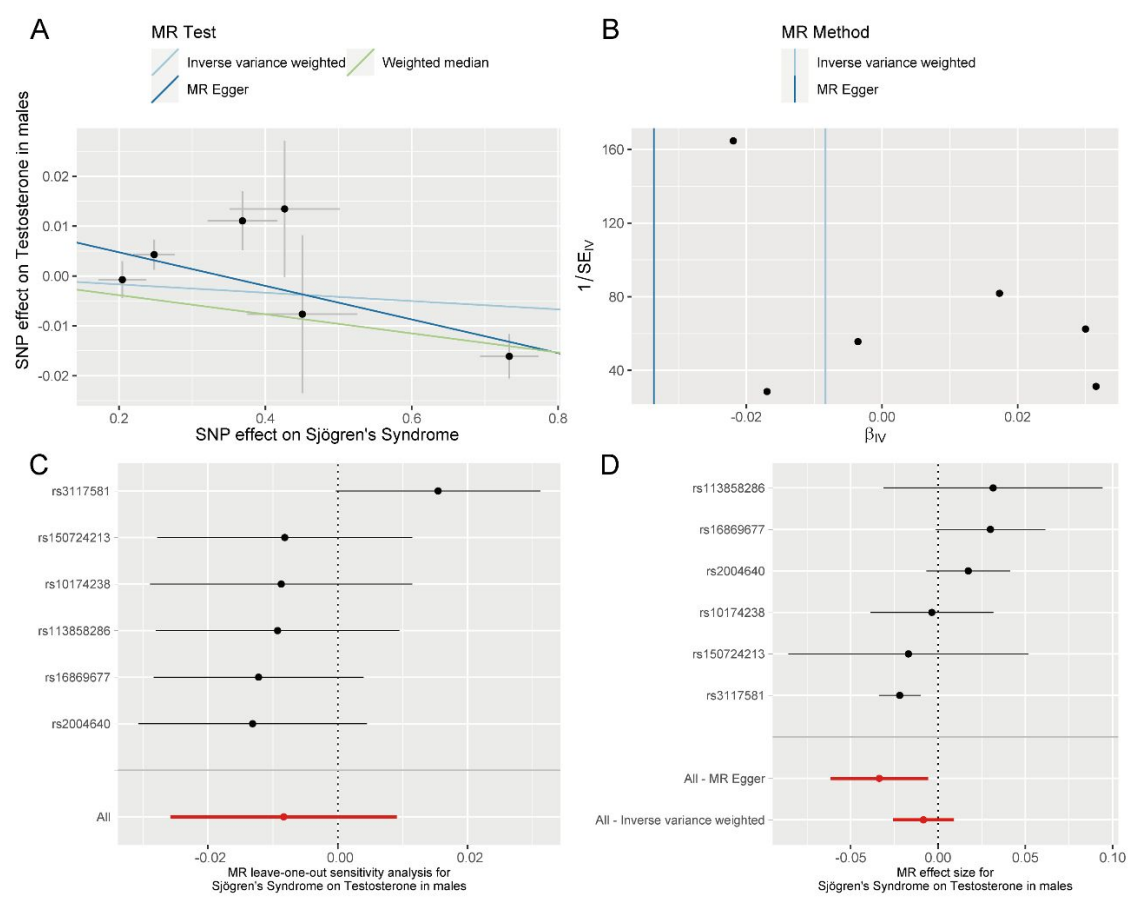


Figure S2 (A) Scatter plot of genetic correlations of testosterone in females and SS; (B) Funnel plot for the causal effects of testosterone in females on SS; (C) Forest plot of the causal effects of testosterone in females on SS; and (D) Forest plot for leave-one-out analysis of the causal effects of testosterone in females on SS.

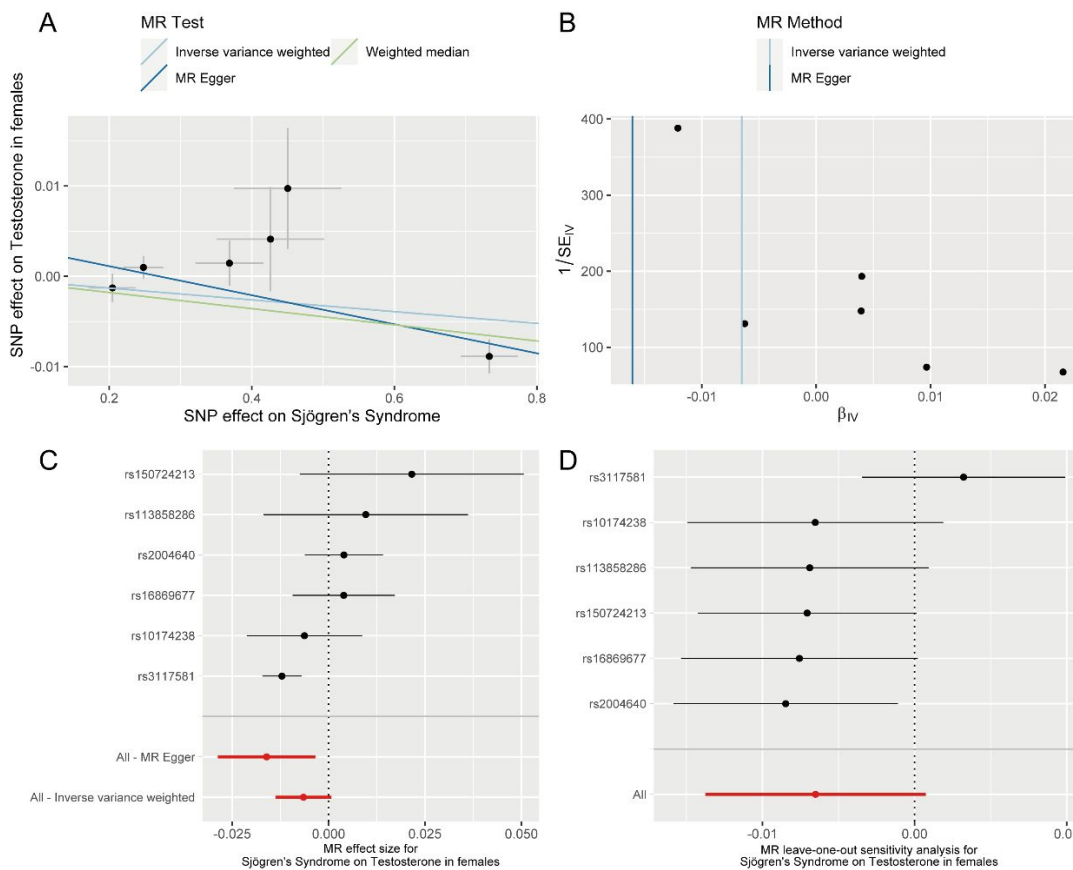


Figure S3 (A) Scatter plot of genetic correlations of testosterone in males and SS; (B) Funnel plot for the causal effects of testosterone in males on SS; (C) Forest plot of the causal effects of testosterone in males on SS; and (D) Forest plot for leave-one-out analysis of the causal effects of testosterone in males on SS.

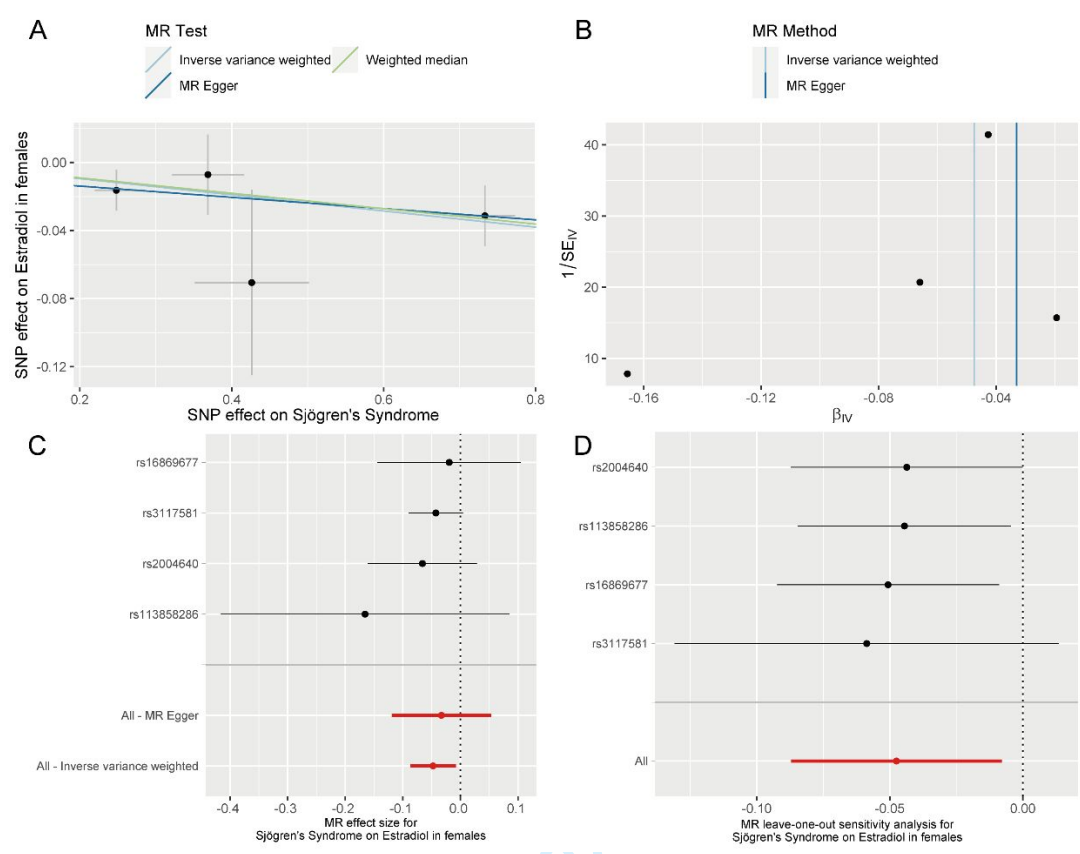


Figure S4 (A) Scatter plot of genetic correlations of estradiol in females and SS; (B) Funnel plot for the causal effects of estradiol in females on SS; (C) Forest plot of the causal effects of estradiol in females on SS; and (D) Forest plot for leave-one-out analysis of the causal effects of estradiol in females on SS.

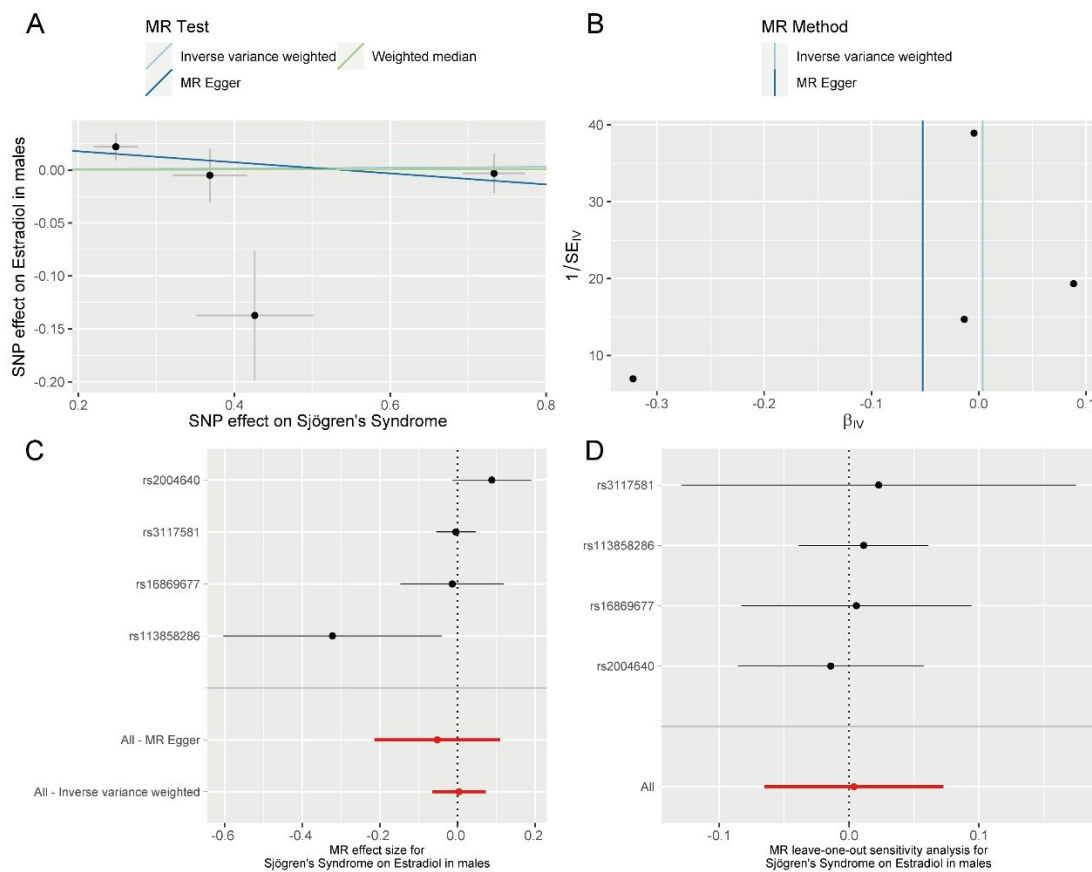


Figure S5 (A) Scatter plot of genetic correlations of estradiol in males and SS; (B) Funnel plot for the causal effects of estradiol in males on SS; (C) Forest plot of the causal effects of estradiol in males on SS; and (D) Forest plot for leave-one-out analysis of the causal effects of estradiol in males on SS.

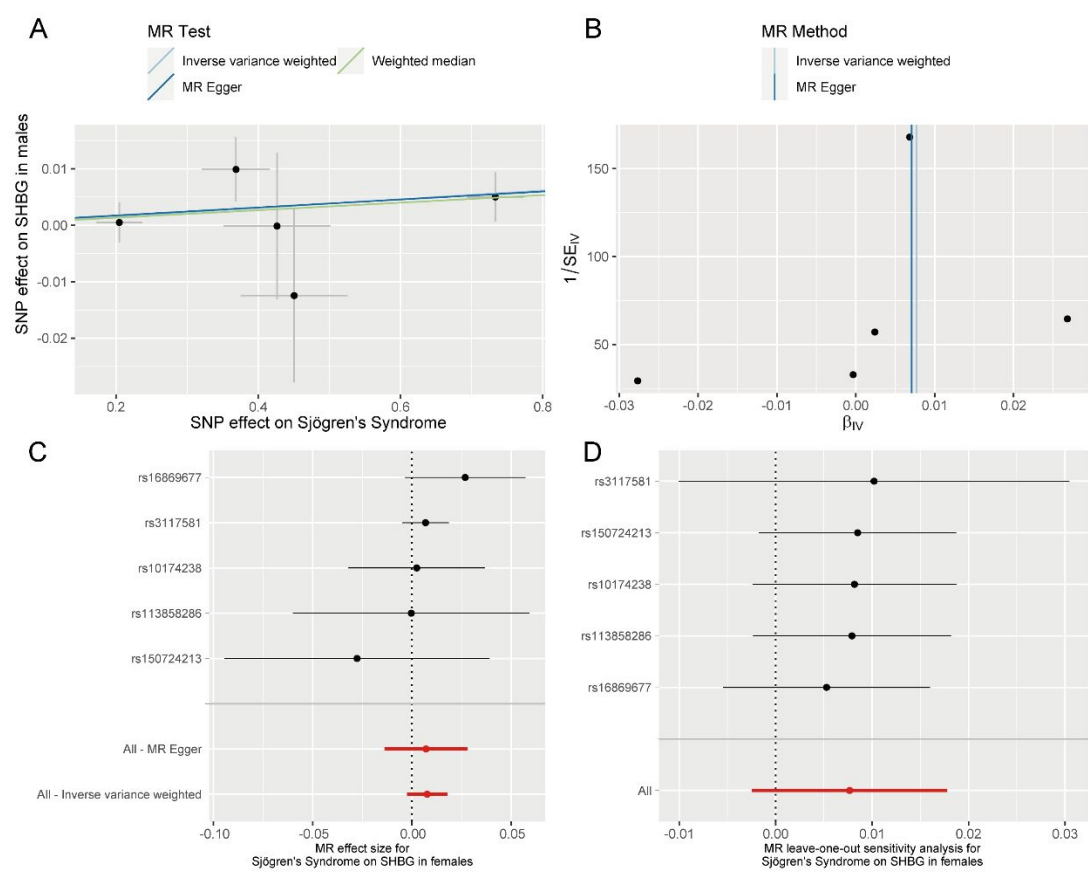


Figure S6 (A) Scatter plot of genetic correlations of SHBG in females and SS; (B) Funnel plot for the causal effects of SHBG in females on SS; (C) Forest plot of the causal effects of SHBG in females on SS; and (D) Forest plot for leave-one-out analysis of the causal effects of SHBG in females on SS.

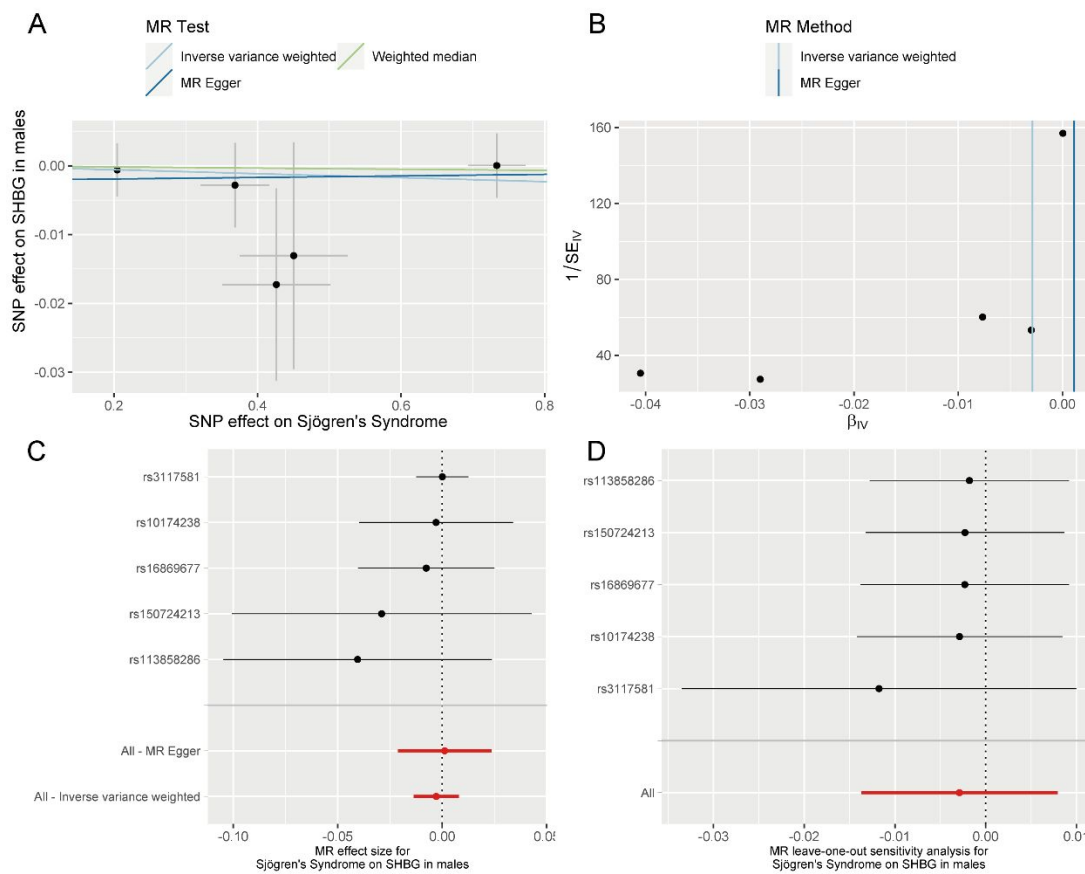


Figure S7 (A) Scatter plot of genetic correlations of SHBG in males and SS; (B) Funnel plot for the causal effects of SHBG in males on SS; (C) Forest plot of the causal effects of SHBG in males on SS; and (D) Forest plot for leave-one-out analysis of the causal effects of SHBG in males on SS.

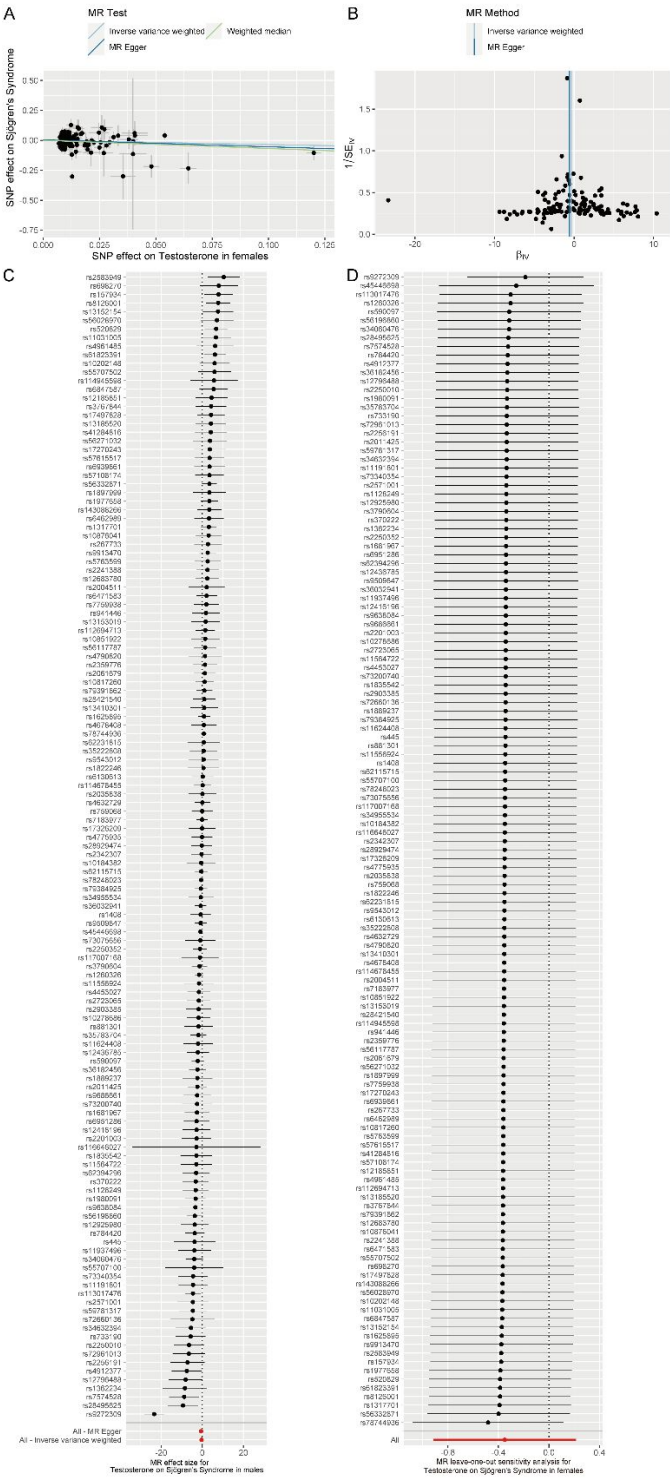


Figure S8 (A) Scatter plot of genetic correlations of SS and testosterone in females; (B) Funnel plot for the causal effects of SS on testosterone in females; (C) Forest plot of the causal effects of SS on testosterone in females; and (D) Forest plot for leave-one-out analysis of the causal effects of SS and testosterone on females.

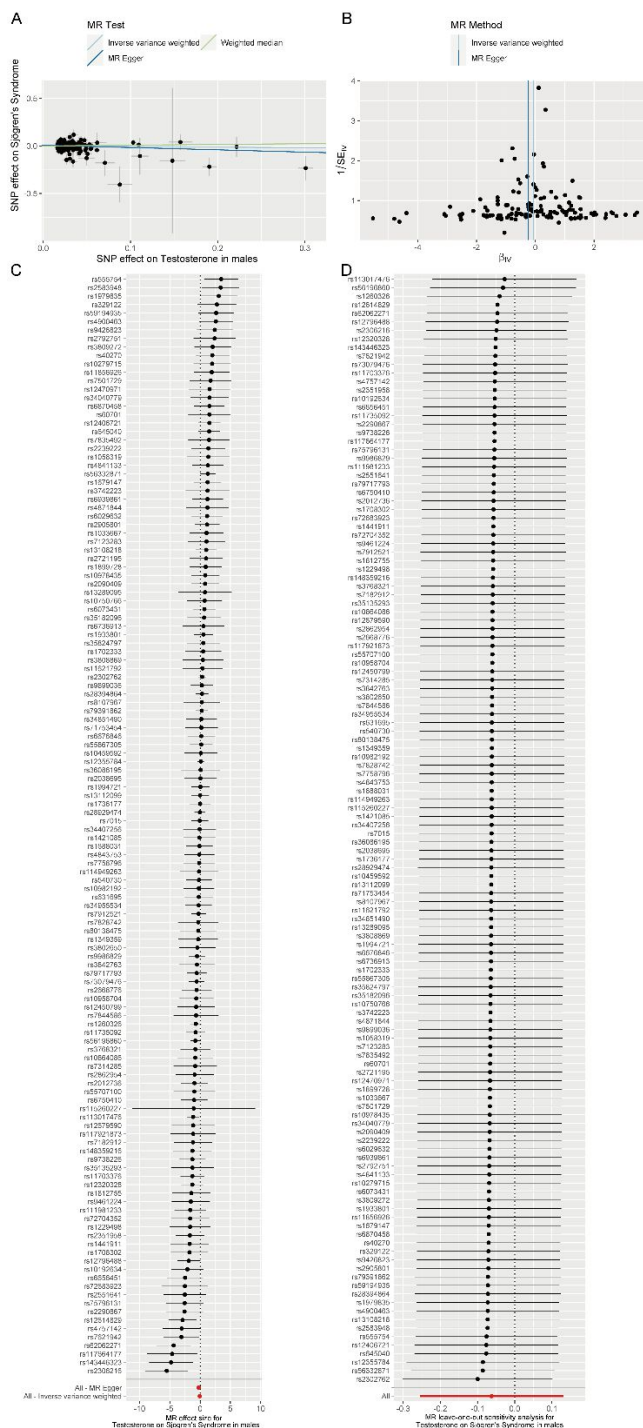


Figure S9 (A) Scatter plot of genetic correlations of SS and testosterone in males; (B) Funnel plot for the causal effects of SS on testosterone in males; (C) Forest plot of the causal effects of SS on testosterone in males; and (D) Forest plot for leave-one-out analysis of the causal effects of SS and testosterone on males.

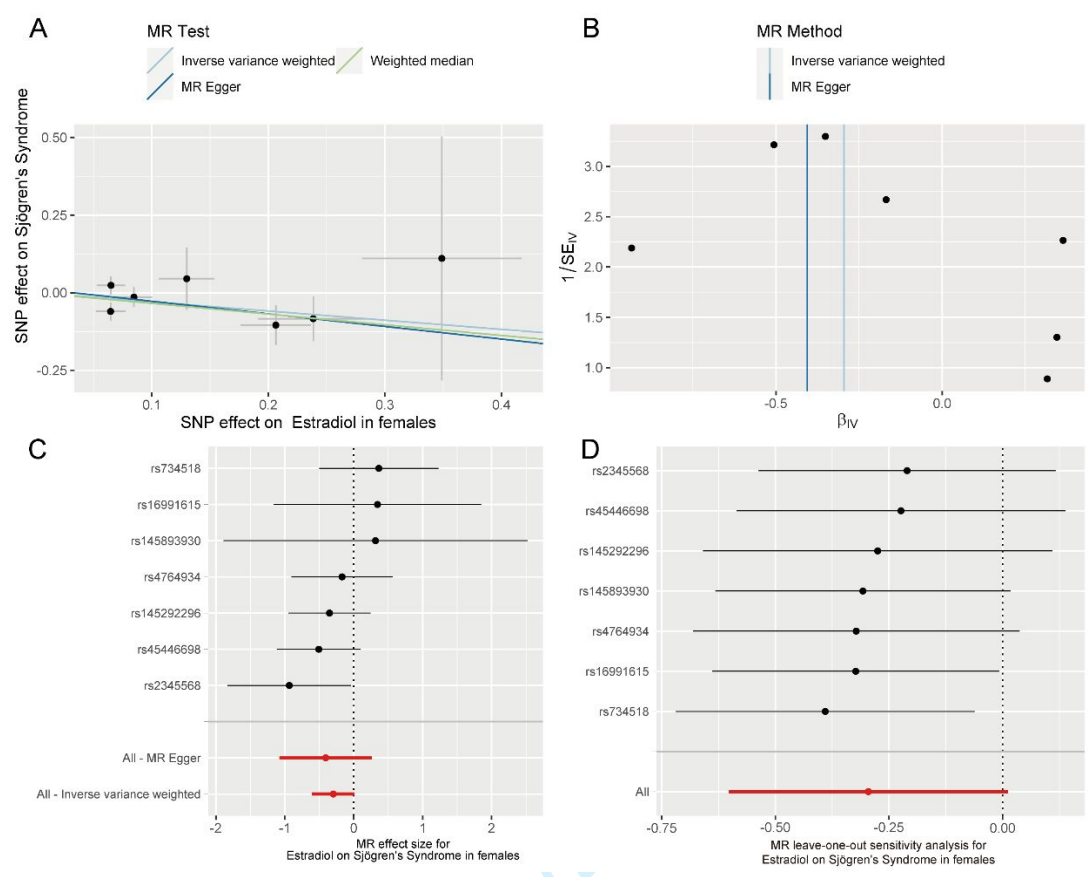


Figure S10 (A) Scatter plot of genetic correlations of SS and estradiol in females; (B) Funnel plot for the causal effects of SS on estradiol in females; (C) Forest plot of the causal effects of SS on estradiol in females; and (D) Forest plot for leave-one-out analysis of the causal effects of SS and estradiol on females.

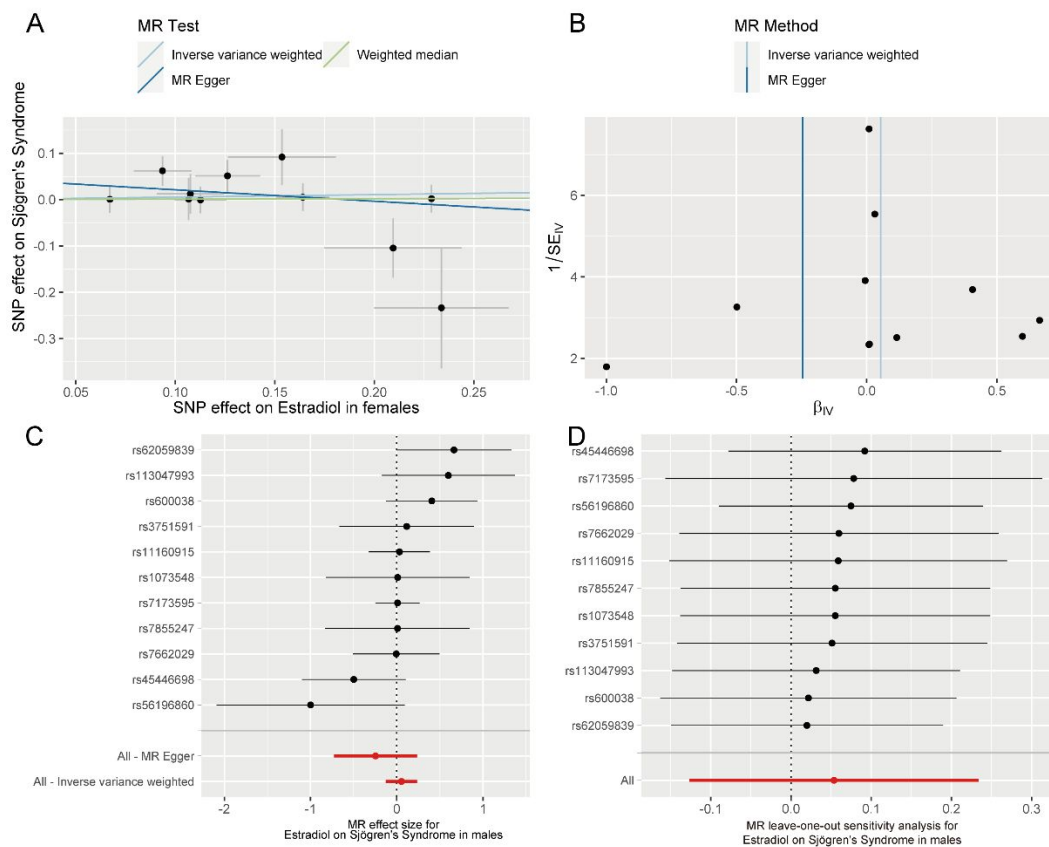


Figure S11 (A) Scatter plot of genetic correlations of SS and estradiol in male; (B) Funnel plot for the causal effects of SS on estradiol in males; (C) Forest plot of the causal effects of SS on estradiol in males; and (D) Forest plot for leave-one-out analysis of the causal effects of SS and estradiol on males.

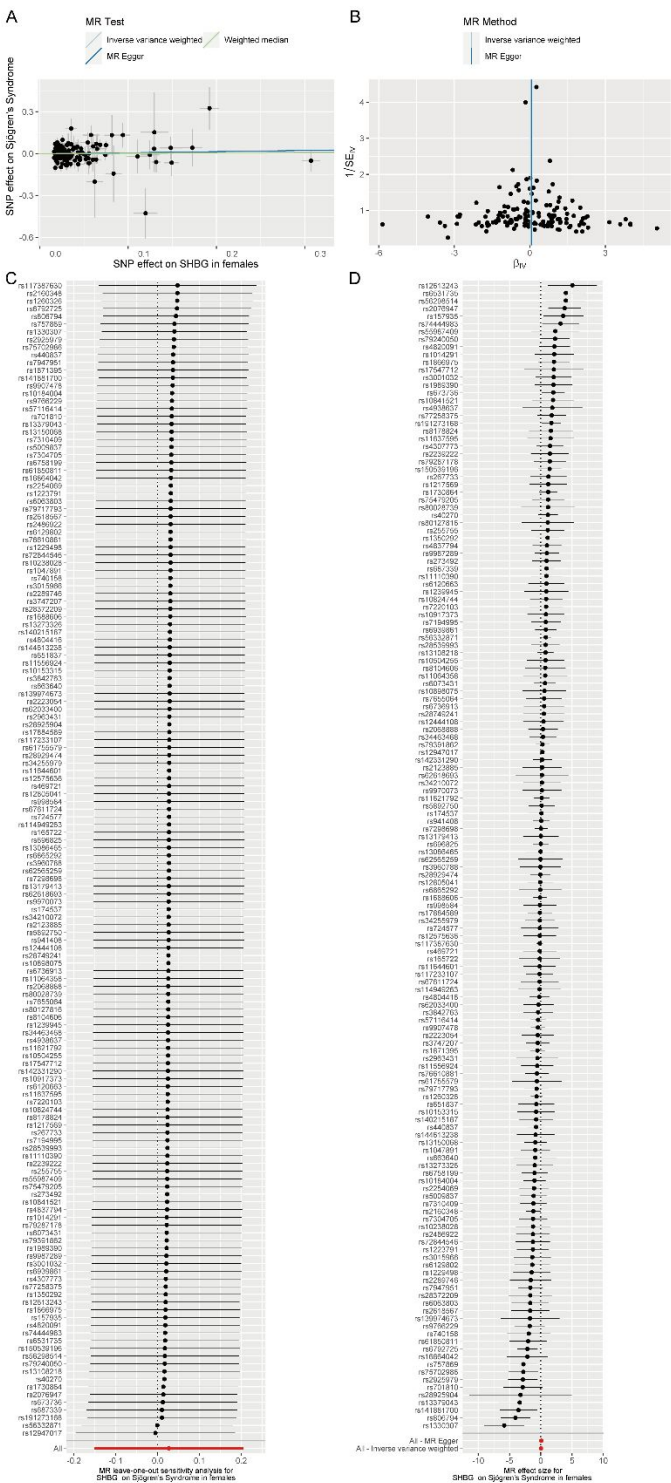


Figure S12 (A) Scatter plot of genetic correlations of SS and SHBG in females; (B) Funnel plot for the causal effects of SS on SHBG in females; (C) Forest plot of the causal effects of SS on SHBG in females; and (D) Forest plot for leave-one-out analysis of the causal effects of SS and SHBG on females.

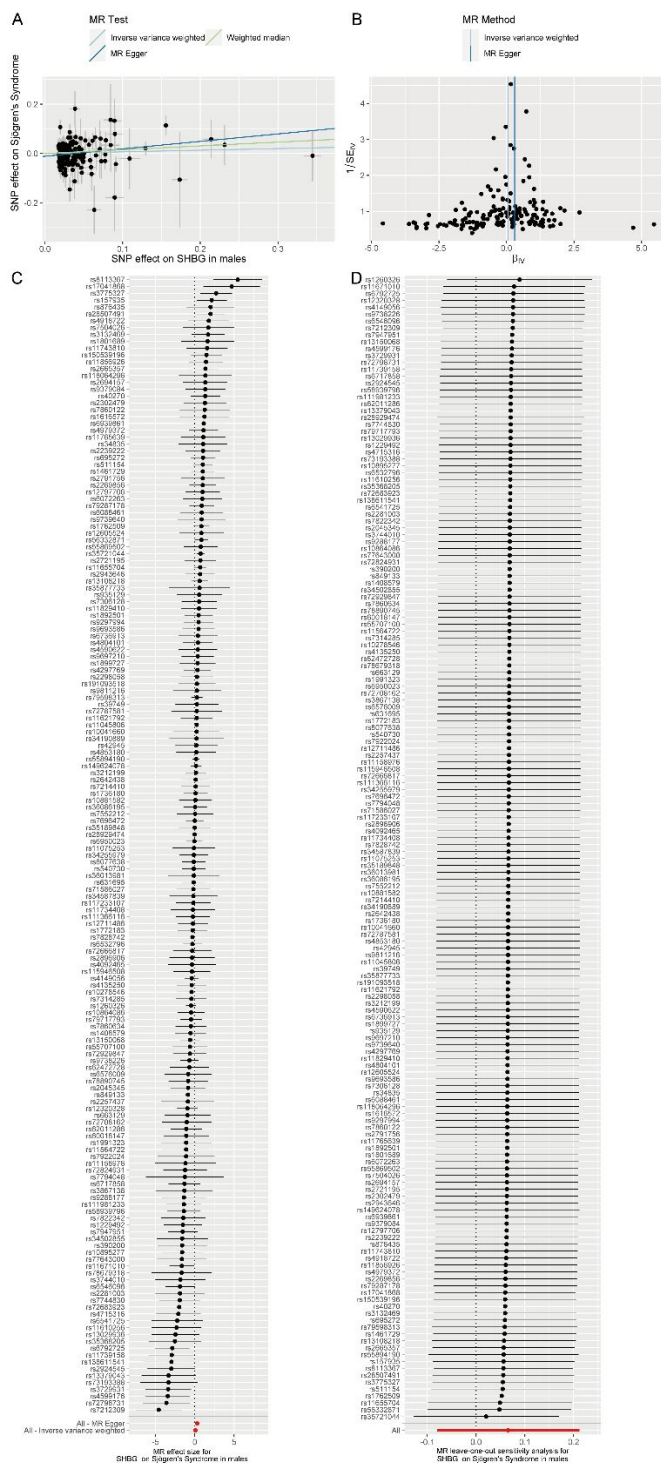


Figure S13 (A) Scatter plot of genetic correlations of SS and SHBG in males; (B) Funnel plot for the causal effects of SS on SHBG in males; (C) Forest plot of the causal effects of SS on SHBG in males; and (D) Forest plot for leave-one-out analysis of the causal effects of SS and SHBG on males.