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The Role of Sex Hormones and Sex Hormone-binding Globulin in Functional Gatrointestinal Disorders: A Bidirectional Two-sample Mendelian Randomization Study

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ABSTRACT

Background & Aims: Sex hormones and sex hormone-binding globulin (SHBG) have been confirmed to involve in the pathophysiology of functional gastrointestinal disorders (FGIDs), including irritable bowel syndrome (IBS) and functional dyspepsia (FD). However, causal associations have not yet been investigated. Utilizing data from Genome-wide association studies, we conducted bidirectional two-sample mendelian randomization (MR) analyses to assess the causal relationships between sex hormones, SHBG and FGIDs.
Methods: Data for sex hormones including testosterone and estradiol, and SHBG were collected from the UK Biobank and FinnGen study. Relevant single nucleotide polymorphisms (SNPs) were selected as instrumental variables (IVs). Inverse variance weighted (IVW) analysis were performed to assess the causal relationships., supplemented with MR-Egger, weighted median, and weighted mode approaches. Additionally, we used Cochran's Q test to evaluate the heterogeneity of genetic variants and implemented leave-one-out analysis to assess the impact of individual SNPs on the causal estimates. Several sensitivity analyses were conducted to assess the robustness of the results.
Results: Significant negative causal relationship was found between genetically predicted testosterone and the risk of IBS (OR=0.90, 95%CI: 0.83-0.97; p=0.007). SHBG demonstrated an inverse correlation with the risk of IBS (OR=0.82, 95%CI: 0.68-0.98; p=0.035) and FD (OR=0.83, 95%CI: 0.69-0.99; p=0.048). However, no statistically significant association was found between testosterone and FD, while estradiol also showed no causal association with FGIDs.
Conclusions: Our study revealed a negative causal relationship between testosterone and IBS risk, and SHBG appears to be inversely associated with FD. This provided new ideas for the prevention and control of IBS, and future research is warranted to elucidate the underlying mechanisms driving these associations and their potential clinical implications.

Key words: functional gastrointestinal disorders – gonadal hormones – sex hormones-binding globulin – Mendelian randomization analysis.

Abbreviations: CNS: central nervous system; FD: functional dyspepsia; FGID: functional gastrointestinal disorder; GWAS: genome-wide association studies; HPA: hypothalamic-pituitary-adrenal; IBS: irritable bowel syndrome; IV: instrumental variable; IVW: inverse variance weighted; MR: mendelian randomization; SHBG: sex hormone-binding globulin; SNP: single nucleotide polymorphism; UKB: UK Biobank.

INTRODUCTION

Functional gastrointestinal disorders (FGIDs) encompass a diverse array of chronic conditions characterized by recurrent gastrointestinal symptoms in the absence of structural or biochemical abnormalities [1]. According to the Rome Foundation Global Study, the prevalence rate of FGIDs was more than 40% of people worldwide [2]. These disorders, particularly irritable bowel syndrome (IBS) and functional dyspepsia (FD), collectively pose a substantial global health burden, with IBS alone accounting for over $20 billion in direct and indirect expenditures in the United States [1, 3]. Despite this burden, effective treatments remain elusive due to the complex and heterogeneous nature of FGIDs.

Importantly, FGIDs exhibit a notable gender predilection, with women being disproportionately affected compared

to men. For instance, IBS demonstrates a female-to-male prevalence ratio of 5:3, while FD is more prevalent in women (8.7%) than in men (5.8%) [2]. Emerging evidence suggests a pivotal role for sex hormones, particularly testosterone and estradiol, in the pathogenesis of FGIDs. Estradiol, the primary female sex hormone, has been implicated in augmenting visceral sensitivity and modulating gastrointestinal motility, while lower androgen levels may predispose men to IBS [4]. However, the precise mechanistic underpinnings of these asso-ciations remain obscure. Sex hormone-binding globulin (SHBG), a plasma glyco-protein responsible for binding and transporting sex hormones such as estradiol and testosterone [5]. Besides, SHBG is involved in the transport of sex hormones in the blood and the release of these hormones into the target tissues, in order to regulate the bioavailability of sex hormones, adds another layer of complexity to this paradigm [6]. Conflicting reports regarding the relationship between SHBG levels and FGIDs further underscore the need for elucidating its potential causal role [7, 8].

Mendelian randomization (MR), an epidemiological method leveraging genetic variants as instrumental variables (IVs), offers a promising avenue to disentangle causal relationships between modifiable exposures and outcomes in observational data. By capitalizing on the random allocation of alleles during meiosis, MR minimizes biases inherent in traditional observational studies, including confounding and reverse causation. Despite its potential, there remains a dearth of MR studies exploring causal associations between sex hormones, SHBG, and FGIDs [9].

In this study, we conduct bidirectional two-sample MR analyses to investigate potential causal relationships between sex hormones, SHBG, and FGIDs. Our research aimed at uncovering novel etiological mechanisms and identifying therapeutic targets to address the unmet clinical needs in FGIDs.

METHODS

Overview of MR and Study Design

The study employed a two-sample MR approach to assess the causal relation-ships between sex hormones, SHBG, and FGIDs (Fig. 1). MR leverages publicly available data from genome-wide association studies (GWAS) for both exposure and outcome variables [10]. Two-sample MR with summary-level data by using two already conducted, which requires summary statistics for the exposure and outcome to originate from a similar general population, i.e. ethnically and demographically comparable. In addition, two-sample MR uniquely leverages evaluation of underlying assumptions through an array of sensitivity analyses tailored to detect and adjust for heterogeneity, outliers and pleiotropy [11].

Initially, the study investigated the causal links between sex hormones and FGIDs. Exposure variables included levels of testosterone and estradiol, while FGIDs, encompassing IBS and FD, served as outcome variables. Subsequently, the causal association between SHBG and FGIDs was examined. Furthermore, a bidirectional analysis was conducted, whereby FGIDs were considered as exposures and sex hormones as outcomes, exploring the potential impact of FGIDs on sex hormone levels. Finally, the study the effects of FGIDs on SHBG levels.

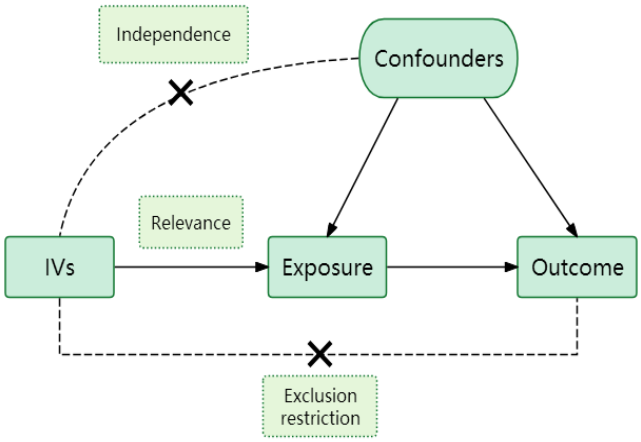

Fig. 1. Graphical representation of the MR assumptions. (i) relevance: IVs must have a robust association with exposure. (ii) independence: IVs must be independent of po-tential confounders. (iii) exclusion restriction: IVs must have no direct effect on the outcome, acting solely through the exposure pathway.

Data Source and Open-GWAS Statistics

The study utilized data from two prominent sources: the UK Biobank (UKB) and the FinnGen study. The UKB, comprising nearly 500,000 participants aged 40 to 69 recruited between 2006 and 2010, provided extensive phenotypic and genotypic in-formation [12]. GWAS data for sex hormones and SHBG were obtained from the UKB, with sample sizes of 194,453 for testosterone, 147,690 for estradiol, and 368,929 for SHBG. The FinnGen study, a comprehensive endeavor integrating pro-spective epidemiological cohorts, disease-based cohorts, and hospital biobank samples in Finland, contributed GWAS data for FGIDs [13]. Specifically, data for IBS and FD were sourced from the FinnGen Consortium. The sample size for IBS was 311,254, comprising 9,323 cases and 301,931 controls, while for FD, it was 329,262, with 8,875 cases and 320,387 controls.

Diagnoses of FGIDs were established using structured diagnostic tools administered by trained interviewers, aligning with international consensus criteria defined by the International Classification of Diseases, 10th Edition (ICD-10). This rigorous diagnostic process ensures the reliability and validity of the FGIDs data used in the study. Detailed information on the GWAS data sources could be found in Table I, including demographics, quality control measures, and phenotype definitions.

Selection for the Instrumental Variables

The selection of IVs adhered to three fundamental assumptions crucial for MR analysis (Fig. 1): (i) relevance assumption: IVs must have a robust association with exposure ($p<5e\times10-8$). (ii) independence assumption: IVs must be independent of potential confounders. (iii) exclusion restriction assumption: IVs must have no direct effect on the outcome, acting solely through the exposure pathway [14].

Instrumental variables were chosen following a rigorous selection process encompassing several key steps. Initially, we

Table I. The list of GWAs included in the MR study

Trait	GWAS ID	Consortium	Sample size
Exposure			
Testosterone	ebi-a-GCST90012113	UK Biobank	194,453
Estradiol	ebi-a-GCST90020091	UK Biobank	147,690
SHBG	ebi-a-GCST90012110	UK Biobank	368,929
Outcome			
IBS	finngen-b-K11_IBS	FinnGen Consortium	311,254
FD	finngen-b-K11_FUNCDYSP	FinnGen Consortium	329,262

GWAS: genome-wide association studies; SHBG: sex hormone-binding globulin; IBS, irritable bowel syndrome; FD: functional dyspepsia.

include single nucleotide polymorphisms (SNPs) that showed a significant association with exposure, using a strict threshold of genome-wide significance (p<5e×10-8). However, given the importance of statistical power in MR studies, we used a more relaxed p-value threshold (p<1e×10-5) to ensure an adequate number of independent SNPs when IVs were less than 10. SNPs with low linkage disequilibrium (R2<0.001, window size=10,000 kb) were then retained to minimize potential bias from correlated genetic variants [15, 16]. Additionally, SNPs directly associated with the outcome (p>0.05) were excluded to ensure specificity to the exposure of interest. Furthermore, to assess the strength of the IVs, the F-statistic was calculated for each genetic variant using the formula: F=r2(N-K-1)/K (1- r2), where r2 represents the SNP-exposure association, N denotes the sample size, and K signifies the number of SNPs. SNPs with an F-statistic less than 10 were considered weak and consequently excluded from further analysis to mitigate potential bias [17].

Statistical Analysis

The primary method employed in this study was inverse-variance weighted (IVW) analysis, which combines Wald ratio estimates for each SNP and is considered the most efficient when genetic variants are not pleiotropic [18]. Additionally, MR-Egger analysis was utilized to relax the assumption of "no horizontal pleiotropy" by allowing a non-zero intercept. This method provides a pleiotropy-robust estimate of causal effects even if individual SNPs violate the assumption [19]. The weighted median method was applied, which derives the median effect estimate from all available SNPs. This method provides consistent results even when up to 50% of the instrumental variables are invalid [20]. Additionally, the weighted mode method was used to estimate the causal effect by clustering SNPs into subsets based on the simi-larity of their causal effects [21]. Several sensitivity analyses were conducted to assess the validity and robustness of the results. These included Cochran's Q test to evaluate heterogeneity among genetic variants [22], MR-Egger intercept test for horizontal pleiotropy, leave-one-out analysis to assess the impact of individual SNPs on causal estimates, and MR-PRESSO analysis to detect and correct for outlier SNPs reflecting likely pleiotropy [23].

Statistical significance was determined using a two-sided P-value threshold of less than 0.05 in all analyses. The TwoSampleMR R package in R software (version 4.3.2) was employed for the implementation of two-sample MR analysis. Results were expressed as odds ratios (ORs) with 95% confidence intervals (CIs), representing the risk of the outcome associated with a unit change in exposure. It is important to note that ORs were interpreted within the context of MR analysis, acknowledging potential limitations or assumptions associated with their interpretation.

RESULTS

Causal Relationship between Sex Hormones and FGIDs

To explore the causal relationship between sex hormones and FGIDs, we conducted MR analyses with sex hormones as exposures and FGIDs as outcomes. After rigorous filtering to ensure the quality of IVs, we selected 152 SNPs for examining the association between testosterone and FGIDs. Additionally, 11 SNPs were chosen to assess the relationship between estradiol and FGIDs (Fig. 2). The primary analysis using the IVW method suggested a significant causal association between genetically predicted testosterone and the risk of IBS (OR=0.90; 95%CI: 0.83-0.97; p=0.007). However, consistent with best practices, we employed multiple MR methods to validate our findings. Surprisingly, while the IVW analysis indicated a significant association with IBS, the results from the alternative methods did not reach statistical significance. Moreover, none of the methods demonstrated a significant association between testosterone and FD. Additionally, MR analysis did not reveal any statistically significant associations between estradiol and FGIDs. We also conducted sensitivity analyses to assess the robustness of our findings. Results from the Cochran's Q test indicated no statistically significant heterogeneity in the effects of sex hormones on FGIDs. Additionally, both MR-Egger and MR-PRESSO tests provided evidence against horizontal pleiotropy (Table II). Furthermore, leave-one-out analysis demonstrated that none of the individual SNPs significantly influenced the robustness of the results (Supplementary file, Table S1-S4).

Causal Relationship between SHBG and FGIDs

MR analysis was employed to investigate the potential causal relationship be-tween SHBG levels and FGIDs, with SHBG as the exposure and FGIDs as the out-come. Following stringent criteria for IV selection, 145 SNPs were chosen for MR analysis between SHBG and FGIDs. As shown in Fig. 2, the primary analysis using the IVW method suggested a

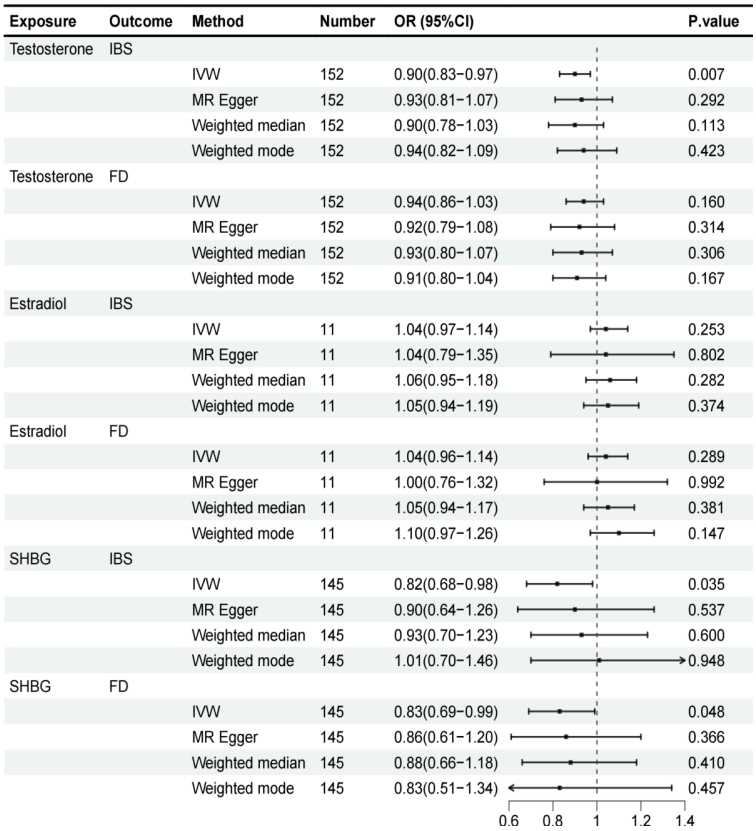

Fig. 2. Forest plot of the causal relationship of sex hormones and SHBG with functional gastrointestinal disorders. IVW: inverse-variance weighted. For the rest of abbreviations see Table I.

significant inverse association between genetically predicted SHBG levels and the risk of IBS (OR=0.82; 95%CI: 0.68–0.98; p=0.035). However, results from alternative MR methods did not consistently support this finding, indicating the need for cautious interpretation. Similarly, MR analysis sug-gested an inverse relationship between SHBG levels and functional dyspepsia (FD), with the IVW estimate indicating an OR of 0.83 (95%CI: 0.69-0.99; p=0.048). However, consistent with IBS analysis, results from other methods varied in their statistical significance. Heterogeneity and horizontal pleiotropy were assessed to evaluate the robustness of the results. For SHBG and IBS analysis, Cochran's Q test indicated the presence of heterogeneity (Q=173.614, p=0.047, Table II), and MR-PRESSO detected horizontal pleiotropy (global test p<0.05, Table II), suggesting potential biases in the results. Conversely, no significant heterogeneity or horizontal pleiotropy was

Table II. Sensitivity analysis results of sex hormones, SHBG and FGIDs

		Cochran's Q test		MR-Egger	MR-PRESSO
Exposure	Outcome	Q	p-value	p-value	Global test p-value
Testosterone	IBS	146.49	0.588	0.552	0.592
Testosterone	FD	177.38	0.070	0.803	0.075
Estradiol	IBS	11.13	0.347	0.921	0.380
Estradiol	FD	11.26	0.338	0.741	0.274
SHBG	IBS	173.61	0.047	0.510	0.043
SHBG	FD	163.31	0.129	0.824	0.125
IBS	Testosterone	24.14	0.395	0.218	0.411
FD	Testosterone	163.31	0.129	0.438	0.081
IBS	Estradiol	23.87	0.299	0.425	0.323
FD	Estradiol	16.92	0.391	0.209	0.430
IBS	SHBG	27.47	0.194	0.166	0.236
FD	SHBG	31.51	0.035	0.691	0.028

MR-PRESSO: MR pleiotropy residual sum and outlier. For the rest of abbreviations see Table I.

observed in the analysis of SHBG and FD. The leave-one-out analysis did not identify any SNPs significantly influencing the robustness of the results (Supplementary file, Table S5-S6).

Causal Relationship between FGIDs and Sex Hormones

To explore the potential causal relationship between FGIDs and sex hormones, FGIDs were utilized as the exposure and sex hormones as the outcome in the MR analysis. Rigorous criteria were applied to select appropriate SNPs as IVs, ensuring high statistical power by utilizing a relaxed p-value threshold ($p<1\times10^{-5}$). All selected IVs exhibited F-values greater than 10, indicating robustness in the analysis. Despite comprehensive analysis using four different MR methods, no discernible effect of FGIDs on sex hormones was observed (Fig. 3). This suggests that FGIDs may not influence sex hormone levels, at least not through the genetic instruments investigated in this study. Sensitivity analyses were conducted to evaluate the robustness of the results. The absence of heterogeneity, as indicated by the Cochran's Q test, along with the absence of horizontal pleiotropy, as demonstrated by MR-Egger and MR-PRESSO results, further support the reliability of the findings (Table II). Additionally, the leave-one-out test confirmed the stability of the results, as no single SNP significantly influenced the overall findings (Supplementary file, Table S6-S10).

Causal Relationship between FGIDs and SHBG

To explore the potential causal relationship between FGIDs and SHBG, FGIDs were designated as the exposure and SHBG as the outcome in the MR analysis. Leveraging data from GWAS, 23 SNPs related to IBS and 20 SNPs related to FD were selected as genetic instruments. The IVs used in the analysis were carefully screened to ensure robustness, with all instrumental variables exhibiting F-values exceeding 10. However, while the MR analysis suggested no significant effect of FGIDs on SHBG levels across the board (Fig. 3), closer examination revealed discrepancies between IBS and FD associations with SHBG. Specifically, the sensitivity analysis indicated the robustness of the results for the relationship between IBS and SHBG (Table II). Conversely, for the association between FD and SHBG, the results were less conclusive. Heterogeneity was detected through the Cochran's Q test (p=0.035), and MR-PRESSO analysis identified the presence of horizontal pleiotropy (p=0.028), suggesting potential biases in the results.

DISCUSSION

In this two-sample MR study, we identified genetically predicted testosterone levels as inversely associated with the risk of IBS. Conversely, we did not find evidence supporting a causal relationship between estradiol and FGIDs. Furthermore, our analysis revealed an association between SHBG levels and the risk of developing IBS, although sensitivity analyses indicated potential limitations in result robustness. Notably, we also identified a significant causal relationship between SHBG levels and the risk of FD.

FGIDs represent a significant health burden due to their high prevalence and lack of effective treatment options, leading to substantial economic and social implications. Gender

Exposure	Outcome	Method	Number	OR (95%CI)	P.value
IBS	Testosterone				
		IVW	24	0.99(0.97−1.01)	0.296
		MR Egger	24	0.97(0.94−1.01)	0.120
		Weighted median	24	0.99(0.96−1.01)	0.234
		Weighted mode	24	0.98(0.95−1.01)	0.290
IBS	Estradiol				
		IVW	22	0.98(0.90−1.06)	0.596
		MR Egger	22	0.92(0.78−1.09)	0.345
		Weighted median	22	0.95(0.85−1.07)	0.401
		Weighted mode	22	0.95(0.82−1.10)	0.534
FD	Testosterone				NA
		IVW	19	0.99(0.97−1.01)	0.487
		MR Egger	19	1.01(0.97−1.05)	0.780
		Weighted median	19	0.99(0.97−1.02)	0.612
		Weighted mode	19	0.97(0.94−1.01)	0.164
FD	Estradiol				
		IVW	17	1.01(0.94−1.10)	0.645
		MR Egger	17	1.10(0.96−1.26)	0.194
		Weighted median	17	1.06(0.96−1.17)	0.273
		Weighted mode	17	1.03(0.92−1.15)	0.629
IBS	SHBG				
		IVW	23	0.99(0.99−1.00)	0.138
		MR Egger	23	0.99(0.98−1.00)	0.060
		Weighted median	23	0.99(0.98−1.00)	0.221
		Weighted mode	23	0.99(0.98−1.00)	0.291
FD	SHBG				
		IVW	20	1.00(0.99−1.01)	0.320
		MR Egger	20	1.00(0.99−1.01)	0.862
		Weighted median	20	1.00(0.99−1.01)	0.884
		Weighted mode	20	1.00(0.99−1.01)	0.955

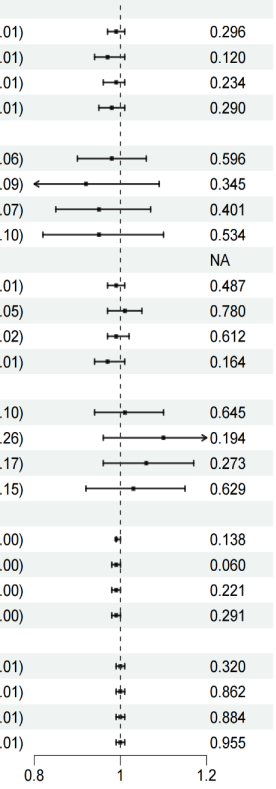

Fig. 3. Forest plot of FGIDs association with and sex hormones and SHBG. For abrreviations see Table I.

disparities in the incidence of FGIDs further underscore the importance of investigating the role of sex hormones in these conditions. For instance, meta-analytical data indicate a higher prevalence of IBS in women compared to men (OR=1.46; 95%CI: 1.33–1.59) [24], and the OR for dyspepsia in women compared with men was 1.24 (95%CI: 1.13-1.36) [25], emphasizing the potential influence of sex hormones. Research suggests that testosterone and estradiol may modulate FGIDs through various pathways, including effects on visceral sensitivity, stress response modulation via the hypothalamic-pituitary-adrenal (HPA) axis, regulation of intestinal motility, and modulation of intestinal immune function [26, 27].

Genetically predicted testosterone levels were found to be protective against IBS in our study, corroborating previous research indicating an inverse relationship between testosterone levels and IBS symptoms [7]. Furthermore, studies have consistently shown that IBS patients exhibit lower blood testosterone levels compared to controls, suggesting a potential link between testosterone deficiency and the severity of IBS symptoms [28, 29]. Additionally, animal studies have demonstrated the necessity of testosterone for normal gastrointestinal transit and the reduction of visceral hypersensitivity, further supporting the causal role of androgens in IBS patho-physiology [29, 30]. However, our analysis did not reveal a significant association between testosterone and FD, possibly due to the differential effects of testosterone on the lower gastrointestinal tract compared to other regions. While testosterone deficiency selectively disrupts colonic motility, it may not affect gastric emptying and small bowel transit [29].

Estradiol is known to play a critical role in visceral sensitivity, a key aspect of FGIDs pathophysiology. This hormone influences the brain-gut axis by binding to receptors in the central nervous system (CNS), spinal cord, and enteric nervous system [31, 32], and it also modulates the HPA axis in response to stress [33]. Additionally, estradiol can impact neurotransmitters, particularly serotonin (5-HT) [34]. However, our study did not find evidence of an association between estradiol and FGIDs. This discrepancy may be attributed to cyclical changes in estradiol levels, which could significantly contribute to FGIDs risk. Notably, women commonly experience IBS symptoms during their late teens to mid-forties [35], corresponding to periods of active cyclical changes in estradiol. Moreover, FGIDs are often associated with the menstrual cycle, with female patients experiencing more severe gastroin-testinal symptoms during menstruation [36, 37]. The lack of verification of the effect of cyclical changes in estradiol on FGIDs risk in our Mendelian randomization analysis underscores the need for further research to elucidate the relationship between estradiol and FGIDs.

To the best of our knowledge, this study represents the first attempt to explore the potential causal relationship between SHBG and FGIDs using MR. Our findings suggest an inverse association between SHBG levels and IBS risk. A previous case-control study reported no differences in SHBG levels between IBS patients and controls [7], while another study indicated higher SHBG levels in IBS patients compared to controls (p=0.03) [8]. Therefore, further investigations are warranted to elucidate the impact of SHBG on IBS. Moreover, our results indicate that higher genetically predicted SHBG levels are causally associated with a reduced risk of FD. As SHBG serves as the primary transport protein for sex hormones, it may influence FD pathogenesis by regulating sex hormone bioavailability.

Our study possesses several strengths, including the utilization of genetic IVs to minimize confounding biases inherent in traditional observational studies. Furthermore, the inclusion of large GWAS sample sizes ensured sufficient statistical power. Additionally, by utilizing GWAS data from patients of European descent, we mitigated the bias of population stratification. Moreover, multiple sensitivity analyses, such as Cochran's Q test, MR-Egger, leave-one-out, and MR-PRESSO, affirmed the robustness of our results. However, certain limitations must be acknowledged. Firstly, the GWAS data were derived from individuals of European ancestry, limiting the generalizability of our findings to broader populations. Secondly, while MR identifies causal statistical associations, it does not provide insights into the underlying biological mechanisms. Lastly, our study did not explore the effects of sex hormones and SHBG on the subtypes of IBS, which warrants further investigation.

CONCLUSIONS

This study provided evidence supporting the causal roles of testosterone and SHBG in FGIDs etiology, thereby shedding light on the mechanisms contributing to the observed gender disparities in these prevalent disorders. These findings may guide future research aimed at developing novel preventive and therapeutic interventions for FGIDs.

Conflicts of interest: None to declare.

Author' contributions: Z.F and C.S conceived and designed the study. Z.K. collected the data and performed the statistical analyses. F.X. and Y.W. analyzed the data and drafted the manuscript. S.Z., B.W. and B.Z. revised it critically for important intel-lectual content. All the authors reviewed the manuscript and approved publishing the final version.

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Supplementary material: To access the supplementary material visit the online version of the *J Gastrointestin Liver Dis* at http://dx.doi.org/10.15403/jgld-5642

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