Medicine Insights

Article

Gender-Specific Hormonal Profiles: Unveiling the Complex Link to Stroke Risk

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Abstract: Stroke affects women more frequently, suggesting a possible link to sex hormones, yet the evidence for this remains inconclusive. Here, we present a pioneering exploration of the nuanced interplay between genetically predicted sex hormone levels, with a particular focus on testosterone and SHBG, to elucidate their causal relationship with stroke risk. Utilizing Mendelian randomization, we aimed to determine whether SHBG levels causally affect stroke occurrence, with a focus on sex-specific effects and the interplay with established stroke risk factors.

We leveraged genetic variants from comprehensive genome-wide association studies (GWAS), which are known to robustly predict levels of total and bioavailable testosterone, SHBG, and cholesterol. Employing a robust statistical approach, we meticulously analyzed published GWAS summary statistics, applying inverse variance weighting and conducting comprehensive sensitivity analyses with state-of-the-art methods such as MR-Egger, weighted median, and MR-PRESSO. Multivariate analyses further addressed potential confounders, including pleiotropic effects and selection bias.

Strikingly, our results demonstrate that elevated SHBG levels are robustly associated with a markedly reduced stroke risk in women. In contrast, lower SHBG levels were associated with an increased risk of small-vessel ischemic stroke, potentially due to elevated cholesterol. These novel insights not only highlight the intricate interplay among SHBG, cholesterol, and immune system components in the pathogenesis of stroke but also illuminate avenues for future research aimed at unraveling the underlying biological mechanisms

Keywords: sex hormone-binding globulin (SHBG); ischemic stroke; mendelian randomization; genetic susceptibility; hormonal influence; gender disparities; lipid metabolism; immune system

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1. Introduction

Stroke ranks among the top causes of disability and death globally, posing a significant health burden in numerous countries [1,2]. Stroke typically affects male and female patients differently in terms of risk factors, disease severity, mortality, and prognosis [3]. Gender disparities significantly influence the onset and recovery from post-stroke neurological deficits, underscoring the urgent need for sex-specific research to inform clinical practice. Women disproportionately experience the debilitating effects of stroke and its complications, yielding consequences that are typically more severe than those faced by men. Notably, stroke lesions of the same size tend to manifest more pronounced symptoms in women. The disparities are often ascribed to factors such as pre-existing frailty, a higher incidence of comorbidities including depression, socio-occupational stress, and variations in the age of stroke onset [4-7]. Nonetheless, accounting for these factors still falls short of fully explaining the observed gender disparities in disease risk.

Despite the apparent differences in the risk of developing stroke between women and men, sex is often considered a minor variable in clinical case studies, and therefore, there is little effort to adjust its effects. This oversight is problematic, as it frequently overlooks the exploration of the underlying biological mechanisms that differ between males

and females. Additionally, ignorance often leads to the oversight of gender-specific brain injuries. To address this gap, our study utilized a Mendelian gene-wide association group database analysis to meticulously quantify the nuanced differences between female and male stroke patients and to assess the effects of various ischemic stroke types. Expanding upon this, our research delved into sex-specific proteins to shed light on the gender-associated factors influencing stroke risk.

Prior observational research hints at an inverse correlation between sex hormone-binding globulins (SHBG) and stroke risk [8]. SHBG, a regulator of sex hormones, is posited to exert a protective influence on the inflammatory processes initiated in the early stages of stroke [9, 10]. Additionally, diminished SHBG levels have been correlated with alterations in sex steroid hormone levels [11]. On the other hand, testosterone has been implicated in increasing the risk of stroke. Additionally, testosterone and estradiol have been found to be associated with acute inflammation.

Emerging evidence from focused studies confirms that the hypothalamus and pituitary gland are the primary sources of SHBG. Concurrent research indicates that proinflammatory cytokines can alter SHBG levels, underscoring their pivotal role in the inflammatory cascades critical to stroke pathogenesis. Nevertheless, the role of sex hormones in stroke remains unclear and evidence from observational studies may be influenced by confounding and selection bias. Consequently, the present study adopts a more nuanced analytical approach. Precise quantification of uncertainty in variables is imperative for refining medical decision-making, and discerning SHBG level disparities between genders in stroke cases is vital for advancing personalized rehabilitation and therapeutic strategies.

Mendelian randomization (MR) analysis, takes advantage of randomly assigned genetic variation during conception, providing estimates that are free of confounding factors [12, 13]. Previous MR studies investigating total and bioavailable testosterone have shown no significant correlation with stroke [14]. To further investigate the involvement of sex hormone-binding proteins in stroke, a two-sample sex-specific univariate MR study was conducted. The largest and most recent genome-wide association study (GWAS) dataset was utilized to assess the association between total testosterone, bioavailable testosterone, SHBG, and stroke. The possibility of confounding bias was also explored by analyzing potential competing risks in the selection of genes that constitute survivorship and survival. After MR analysis of exposures related to SHBG, the intermediate causality of SHBG was examined by considering hypertension as a bridging tool in the context of small-vessel vasculopathy ischemic stroke.

2. Method

2.1. Research Design

This study was carried out as an MR study, utilizing genetic instrumental variables for analysis. The key hypotheses of MR are: (1) genetic instrumental variables accurately predict exposure variables, (2) genetic instrumental variables are not linked to confounders between exposure and outcome, and (3) genetic instruments are independent of the outcome[12]. In this particular study, univariate MR was applied to examine the associations of genetically predicted total testosterone, bioavailable testosterone, SHBG, and SHBG adjusted for body mass index (BMI) with stroke, taking into account the sex-specific effects.

Due to the varied mechanisms of stroke, this approach may introduce bias. To mitigate this, we performed additional univariate MR analyses using SHBG for different stroke types. We also examined the causal effect of BMI-adjusted SHBG on small-vessel vasculopathy ischemic stroke, considering hypertension as an intermediate factor. Our analysis suggested potential selection bias among stroke survivors with different genetic backgrounds and stroke risks. To address this, multivariate MR was used to adjust for potential bias. Additionally, proxy cases and other participant reports were utilized to minimize bias from selective survival.

This study investigated genetic markers associated with exposure to various factors, such as total testosterone, bioavailable testosterone, SHBG, and BMI-adjusted SHBG. An existing GWAS dataset was utilized comprising 425,097 individuals of European descent from the UK Biobank. In this dataset, sex-specific genetic predictors we successfully identified for total testosterone, bioavailable testosterone, and BMI-adjusted SHBG levels [15].

Next, to forecast the levels of total testosterone, a comprehensive analysis was conducted encompassing 220 distinct single nucleotide polymorphisms (SNPs) unique in females and 159 SNPs in males. Expanding the investigation further, 147 independent SNPs were examined for the accurate prediction of bioavailable testosterone in females, and 98 SNPs exclusive in males. To additionally examine BMI-adjusted SHBG levels, 310 and 338 independent SNPs specifically in females and males, respectively, were meticulously extracted.

Sex-specific genetic predictors of the SHBG were obtained from the UK Biobank GWAS database, consisting of 214,989 individuals of European ancestry. The selection process for predictors involved ensuring their independence ($r^2 < 0.001$) and strength (F-statistics > 10) with genome-wide significance. Additionally, predictors with minor allele frequencies > 0.01 and Hardy-Weinberg equilibrium p-values > 0.001 were included.

For female subjects, a total of 218 independent SNPs were identified and utilized for predicting SHBG levels. For male subjects, 177 independent SNPs were used for the same purpose. If the original SNPs were unavailable, specifically for stroke analysis, a proxy SNP2LDLink (https://ldlink.nci.nih.gov/) with an R-value higher than 0.8 was employed. To evaluate the strength of the association, the SNP-specific F statistic was estimated as the square of β divided by the variance of the SNP exposure in univariate analyses.

Genetic predictors were used in a multivariate MR analysis to explore the causal link between SHBG and stroke, while addressing potential assumption violations. Competing survival-related genes were identified in the stroke population, so survival and competition variables were included to mitigate this issue. Given the complex causes of stroke, additional risk factors like BMI, albumin, and LDL were adjusted using multivariate MR analysis. These factors are independent stroke risks and influence other diseases. Sex-specific genetic predictors for these variables were obtained from previous studies and UK Biobank GWAS summary statistics (http://www.nealelab.is/uk-biobank).

The UK Biobank GWAS dataset offers valuable information on various types of strokes. However, the subjects included in this dataset were predominantly older individuals who had survived stroke events, and this may potentially lead to selection bias. On the other hand, the GWAS analysis of parental proxy cases in the UK Biobank provided sex-specific associations and had larger sample sizes. Nevertheless, the proxy cases in this analysis were based on stroke cases that were not precisely defined.

The current analysis relied on a previously published GWAS dataset that identified genome-wide significant genetic predictors of stroke ($p < 5 \times 10^{-8}$). This dataset consisted of 446,696 individuals of European ancestry, of which 40,585 were stroke cases and 406,111 were control cases (without stroke)[15]. Additionally, genetic association data from the UK Biobank were utilized, which included paternally inherited stroke cases (62,810) and control cases (339,806)（https://gwas.mrcieu.ac.uk/datasets/ukb-b-12777）,as well as maternally inherited stroke cases (60,880) and control cases (364,097)（https://gwas.mrcieu.ac.uk/datasets/ukb-b-4024）.

2.2. Statistical Analysis

Univariate MR was carried out to obtain initial findings. To further analyze associations between SNPs and stroke, a meta-analysis was conducted using the SNP-specific Wald estimates, which are divided by SNP-exposure associations acquired from previously described GWAS. In this analysis, inverse variance weighting and multiplicative random-effects assumptions were employed to account for any pleiotropic effects.

We employed several sensitivity analysis methods, including MR-Egger, weighted median, and MR-PRESSO. MR-Egger is especially useful as it remains valid even with invalid SNPs, given the MR and INSIDE assumptions are met. It also acts as a directed polytomous presence test via its intercept. However, due to MR-Egger's low efficacy, we prioritized the direction of association over statistical significance. The weighted median is effective for value estimation if most data is reliable. In our study, MR-PRESSO identified and removed horizontal pleiotropy, and we checked the funnel plot for asymmetry as an indicator of pleiotropy. A meta-analysis of parental proxy cases estimated the overall effect of sex, showing low heterogeneity for sex-based analyses but random effects in other cases.

3. Multivariate Mendelian Randomization

To address potential confounding factors and gene-specific survival, multivariate MR was employed with inverse variance weighting. This approach accounted for variables such as SHBG, total testosterone, and bioavailable testosterone. Additionally, common factors known to influence stroke risk and survival were controlled, including albumin levels, BMI, and low-density lipoproteins.

To conduct this analysis, the TwoSampleMR R package, version 4.1.3 of the R programming language was utilized. By implementing this rigorous statistical method and software, any biases and confounding effects associated with genotype-selective survival and the competing risk of stroke were mitigated.

Data And Resource Availability

All data used in this study were obtained from publicly available GWAS summary statistics provided by the Genetic Alliance.

4. Results

4.1. Total Testosterone Levels, Bioavailable Testosterone Levels, SHBG, and SHBG Levels Adjusted for BMI and Stroke

We chose SHBG, BMI-adjusted SHBG, total testosterone, and bioavailable testosterone as exposure factors, with male stroke, female stroke, and stroke as outcomes. The forest plot (Fig. 1) showed a significant causal effect of SHBG on stroke and female stroke ($P<0.05$), but not for total or bioavailable testosterone. BMI-adjusted SHBG had a lower p-value for female stroke but was less effective than SHBG for stroke overall. In women, both BMI-adjusted SHBG and SHBG had an OR below 1 and a 95% CI, indicating a protective effect against stroke. The maximum BMI-adjusted 95% CI was smaller than the OR for SHBG. Therefore, BMI-adjusted SHBG and SHBG are protectively linked to stroke in women, while no significant causal relationship was found for other exposure factors.

Figure1: Univariate Mendelian randomization analyses of SHBG, SHBG adjusted for BMI, total testosterone, and bioavailable testosterone for illnesses of father (stroke), illnesses of mother (stroke) and stroke. Data indicates the change in the ratio of these exposures to the ratio of the increase in the ratio per standard deviation of the outcome. p value < 0.05.

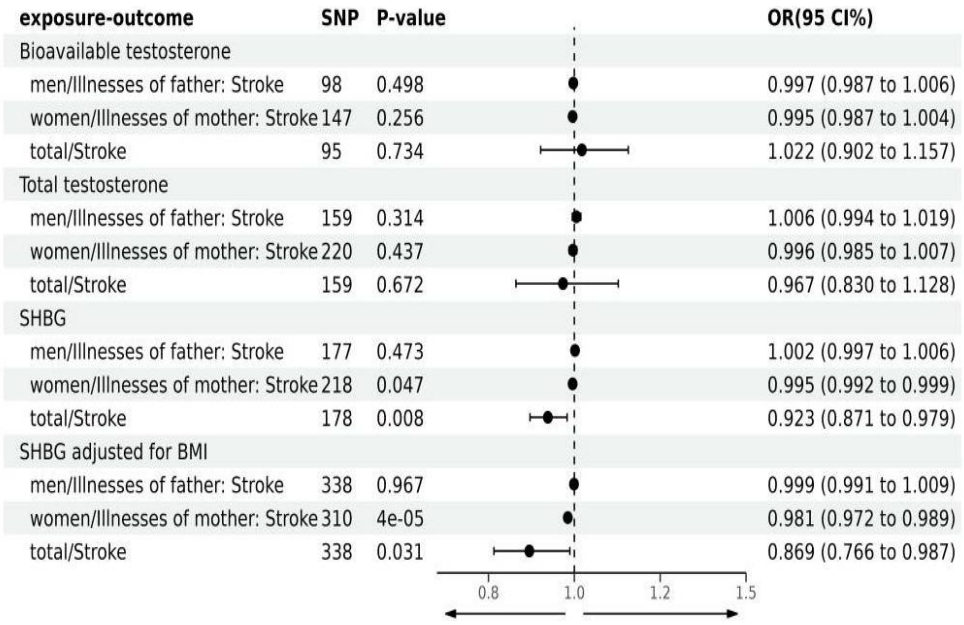

Figure 1. Univariate Mendelian randomization analyses of SHBG.

4.2. SHBG and Stroke

Next, SHBG and BMI-adjusted SHBG were explored as exposure factors, while the outcome factors were the various types of strokes. Results from the forest plot (Fig. 2) revealed a statistically significant causal relationship between SHBG, BMI-adjusted SHBG, and small-vessel ischemic stroke, whereas no statistically significant causal relationship was seen for the other types of strokes. The ORs for SHBG and BMI-adjusted SHBG for small-vessel ischemic stroke cases were less than 1. Moreover, 95% CIs did not include protective factors, and the OR of BMI-adjusted SHBG was lower than SHBG.

Figure2: Univariate Mendelian randomization analyses of SHBG and SHBG adjusted for BMI for ischemic stroke (small vessel), ischemic stroke (atherosclerosis of large arteries), ischemic stroke (cardiac origin), ischemic stroke including SAH, and ischemic stroke excluding SAH. Data indicates the change in the ratio of these exposures to the ratio of the increase in the ratio per standard deviation of the outcome. p value < 0.05.

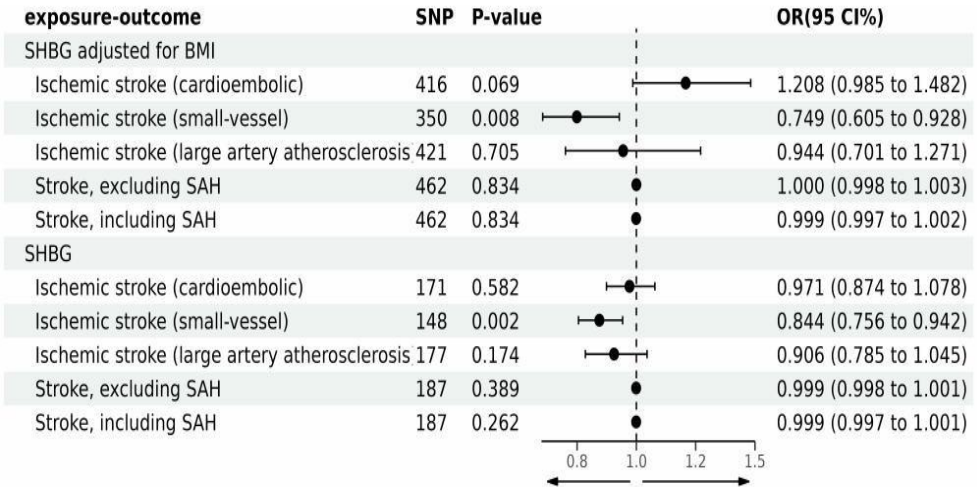

Figure 2. Univariate Mendelian randomization analyses of SHBG and SHBG adjusted for BMI for ischemic stroke.

4.3. Sex Hormone-Binding Globulin Levels Adjusted for BMI, High Cholesterol, High Blood Pressure and Ischemic Stroke (Small-Vessel)

The study evaluated stroke risk factors by analyzing SHBG and BMI-adjusted SHBG, high cholesterol, and hypertension (in men and women) as both exposure and outcome factors. The BMI-adjusted odds ratios of SHBG for hypertension (in men and women) and high cholesterol were all greater than 1, indicating a significant risk factor. As shown in Fig. 3b, a statistically significant causal effect of hypertension on stroke in men, stroke in women, stroke, stroke, and small-vessel ischemic stroke was demonstrated. High cholesterol had a statistically significant causal effect on stroke except for stroke in men. The ORs for all outcomes were greater than 1 and 95% CIs excluded 1, except for the OR for high cholesterol on stroke in men.

Figure3a: Univariate Mendelian randomization analyses of SHBG and SHBG adjusted for BMI for illnesses of father (high blood pressure), illnesses of mother (high blood pressure), high blood pressure, and high cholesterol. Indicates the change in the ratio of these exposures to the ratio of the increase in the ratio per standard deviation of the outcome. p value < 0.05.

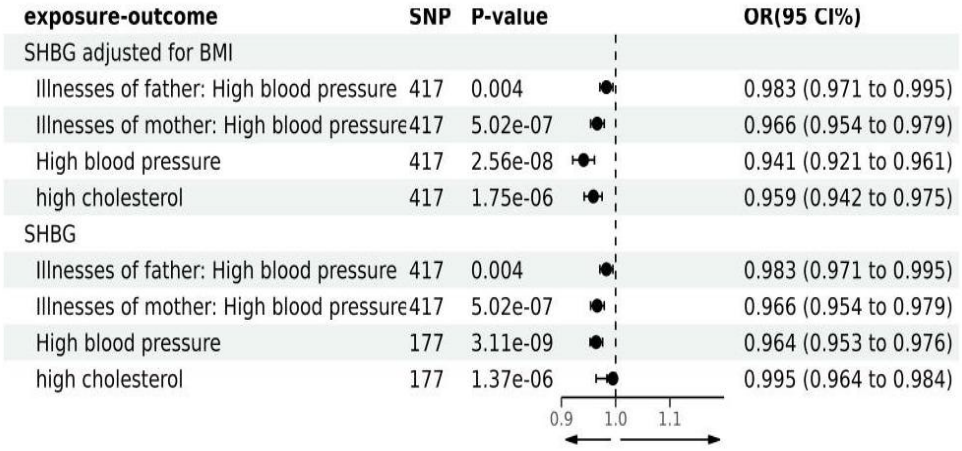

Figure 3a. Univariate Mendelian randomization analyses of SHBG and SHBG adjusted for BMI.

Figure3b: Univariate Mendelian randomization analyses of high blood pressure and high cholesterol for illnesses of father (stroke), illnesses of mother (stroke), stroke and ischemic stroke (small vessel). Indicates the change in the ratio of these exposures to the ratio of the increase in the ratio per standard deviation of the outcome. p value < 0.05.

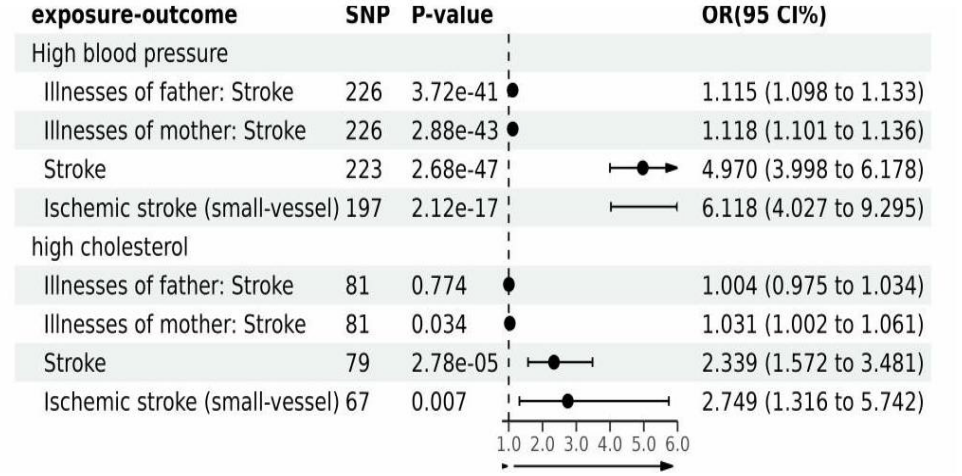

Figure 3b. Univariate Mendelian randomization analyses of high blood pressure and high cholesterol.

4.4. Multivariate Adjusted MR Results

A multivariate analysis of factors (albumin, BMI, LDL) that may compete with SHBG, total testosterone, and bioavailable testosterone for survival risk was performed. Results of the analysis are shown in Fig. 4, and after adjustment, the causal effect of SHBG, total testosterone, and bioavailable testosterone on stroke was found to be not statistically significant and the OR values contained 1.

Multivariate MR analysis of testosterone, SHBG in stroke after adjusting for albumin, LDL, and BMI, ORs indicated a change in the ratio with increased standard deviation, and testosterone, p-value < 0.05.

Figure4: Univariate Mendelian randomization analyses of SHBG adjusted for BMI, total testosterone, bioavailable testosterone, albumin, BMI and LDL for illnesses of father (stroke) and illnesses of mother (stroke). Data indicates the change in the ratio of these exposures to the ratio of the increase in the ratio per standard deviation of the outcome. p value < 0.05.

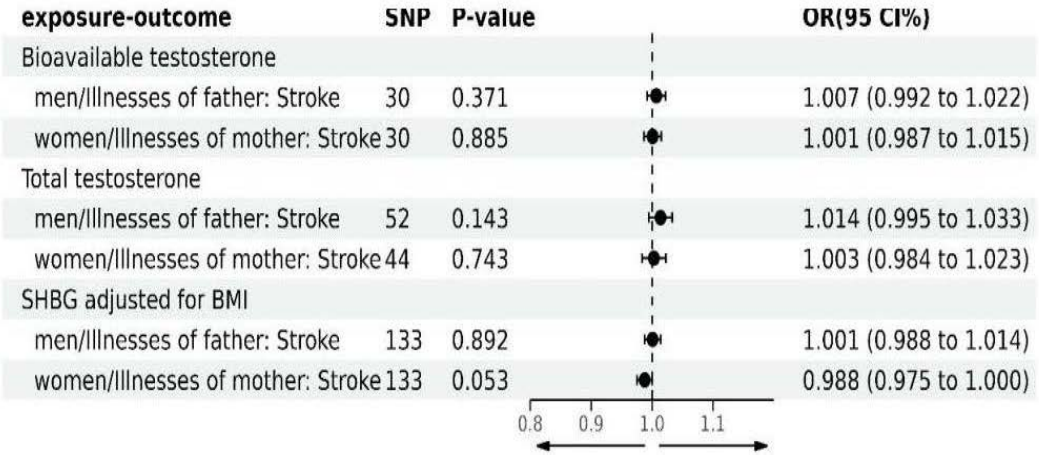

Figure 4. Univariate Mendelian randomization analyses of SHBG.

5. Discussion

The current study demonstrated that higher SHBG was genetically predicted to reduce the risk of stroke and stroke in women. Higher SHBG also reduces the risk of hypertensive ischemic stroke in the population, while SHBG also mediates sex-differentiated behavior in ischemic stroke by modulating cholesterol levels. On the other hand, low SHBG levels can lead to high cholesterol which consequently increases the risk of small-vessel ischemic stroke in women.

In the analysis of risks, a statistically significant causal effect of serum total testosterone and serum bioavailable testosterone levels on stroke was not detected. Nevertheless, it cannot be completely excluded that higher testosterone levels acting on SHBG can elevate the risk of stroke in women. However, in the multivariate analyses, an adjusted causal relationship between SHBG and stroke was also revealed to be statistically insignificant. It was also discovered that the presence of LDL as an exposure factor creates an adjusted factor and a causal relationship between SHBG and high cholesterol. These multivariate results establish an inversed relationship between high cholesterol and SHBG.

The current results are consistent with a previous analysis of a large prospective cohort study, which found a negative association between serum circulating SHBG levels in postmenopausal women and the risk of ischemic stroke [16]. The findings are however, inconsistent with a previous prospective observational study, which found no association between testosterone, estradiol, SHBG levels, and the risk of cerebrovascular disease in men [17]. This study reflects the relationship between stroke and SHBG in men. To address these limitations, a larger GWAS was utilized for sex-specific estimation.

RCTs have investigated SHBG's impact on stroke, yielding inconclusive results. Large studies controlling for BMI show significant variability in SHBG and stroke risk after BMI adjustment. However, our findings differ, suggesting BMI measures body fat. This is suggested as while SHBG is mainly synthesized in the liver, the fat content of the liver may be a potential determinant of circulating SHBG, rather than BMI [18]. Our literature review indicates that liver fat, rather than BMI, significantly influences circulating SHBG levels. Nonetheless, BMI's impact on SHBG cannot be ignored, and more research is needed. Interestingly, the gender difference in SHBG levels becomes insignificant when considering hypertension from ischemic stroke. We hypothesize that SHBG's central effects outweigh its peripheral effects in stroke, but more research is needed to understand SHBG's biological mechanisms.

Methodologically, analyzing epidemiological case studies on exposure and disease involves examining data from unmatched or group-matched designs, individually matched designs, hierarchical exposures, stratified analysis, and multifactorial analysis. This study used a case-control dataset of the entire stroke population, including paternal and maternal stroke surrogates, to gather information for an individually matched design. The surrogate analysis test failed to validate our inferences, and accurately classifying exposure levels in the Mendelian genetic dataset posed challenges. Despite this, we assessed the impact of initial exposure classification on the results.

We conducted stratified analyses to evaluate the association between exposure and disease, focusing on SHBGs with or without BMI adjustment and stroke severity. Multivariate analysis was then used to control for potential confounders and examine these relationships further, incorporating factors related to stroke survival risk and exposure.

Despite larger sample sizes and a control group reducing bias in the current MR analysis, limitations remain. Firstly, instrumental variables must strongly correlate with the exposure, confirmed through genome-wide analysis. Secondly, these variables should be independent and not linked to confounders related to exposures and outcomes. Lastly, it was ensured that instrumental variables were not correlated with outcome variables. These assumptions can be tested using the MR-Egger method, where the intercepts would reflect the presence of horizontal pleiotropy.

Most funnel plots in this study are symmetrical, except for the analysis of bioavailable testosterone for stroke in men. Independent analyses with balanced pleiotropy can still be affected by other pleiotropic factors, causing errors. Adjusting the instrumental variable to account for confounding may introduce another selection bias. To mitigate this drawback, we adjusted the instrumental variable for numerous exposure factors linked to stroke survival. However, not all relevant factors in the sex analysis may have been included, risking overfitting. In our analyses, using proxy cases of parental stroke helped reduce selection bias when identifying potential survivors.

The study's hypotheses were not fully tested in the total population, especially in linear correlation. Future research should conduct multiple sample analyses. The GWAS datasets used lacked clear definitions for stroke and its subtypes, complicating the interpretation of instrumental variables. Additionally, stroke cases identified through instrumental variables were less specific than clinical cases. Thus, caution should be exercised when interpreting the causal relationship between instrumental and outcome variables.

The ethnicity of the population affects all findings, but this was not an issue in the GWAS dataset. The study focused on a European population, so results may not apply to other races. However, the bio-causal effect of SHBG on stroke in women was evident, possibly due to differences in sex hormone-binding protein levels. Additionally, MR analysis requires a large sample size and adequate proxy instrumental variables; otherwise, the results may be impacted. Changes in sex hormone-binding proteins at various life stages, like women's reproductive age, might influence stroke development. However, no current GWAS dataset includes this information.

In addition, genetically predicted sex hormone-binding proteins are negatively associated with cerebrovascular disease, and elucidation of the underlying mechanisms may

reveal better preventive measures. However, there are several probable reasons why sex hormone-binding proteins may have a specific effect on stroke. Firstly, stroke may be a vascular inflammatory disease [19, 20], and sex hormones such as testosterone and estradiol are associated with both acute and chronic inflammation [21,22]. Additionally, low levels of sex hormone-binding proteins may affect the levels of sex steroid hormones.

Taking into account other physiological processes, one of the possible pathways linking sex hormone-binding proteins and stroke is the endocrine action of the hypothalamic-pituitary-gonadal axis [23,24]. Pro-inflammatory cytokines may induce unresponsiveness of the axis during the development of stroke. Recent studies have demonstrated that SHBGs are not just peripherally synthesized proteins, but originate from the hypothalamus and pituitary in the brain, and are spatially and temporally closely associated with prolactin-producing neurons [25]. Meanwhile, previous studies have shown that sex hormones such as testosterone can have anti-inflammatory effects by downregulating microglia function. New studies have, however, found that pro-inflammatory cytokines can lower testosterone levels in acute or chronic inflammation by regulating SHBG. Accordingly, it was found in this study, that the alteration of SHBG precedes the alteration of serum testosterone [26,27].

Studies in the laboratory have also revealed the contribution of cholesterol in stroke through blood-brain barrier disruption, atherosclerosis, and inflammatory response [28, 29]. It has been shown that the expression level of SHBG is negatively correlated with cholesterol levels, i.e., elevated SHBG may reduce cholesterol levels [30]. These findings suggest that sex hormone-binding proteins could serve as a new hormonal biomarker for ischemic stroke risk, offering insights into the relationship between SHBG, cholesterol, and stroke. This could aid early diagnosis and personalized treatment. Further research is needed to explore the interactions between sex hormone-binding proteins, prolactin neurons, SHBG, and cholesterol in stroke.

The current findings suggest that limited evidence links genetically predicted levels of total and bioavailable testosterone, to stroke. Available information on the effects of testosterone is both limited and inconsistent. Testosterone has been shown to influence inflammatory responses and cognitive function, implying that any negative correlation observed with stroke may be influenced by other factors, creating an indirect, rather than a direct effect [31]. A significant association was not found between testosterone levels and stroke.

An investigation was conducted to examine the correlation between SHBG and conventional risk factors associated with stroke. The results revealed a causal association between SHBG levels and established stroke risk factors, namely hypertension and elevated cholesterol levels. These findings imply that SHBG may play a role in regulating the occurrence of stroke through various mechanisms. Additionally, the study suggests that SHBG could potentially mediate sex-specific behaviors in ischemic stroke by influencing cholesterol levels. Notably, low SHBG levels may contribute to increased cholesterol levels, thereby heightening the risk of small-vessel ischemic stroke in females.

However, despite the compelling evidence presented in this study, certain limitations persist. Consequently, future research endeavors must encompass additional prospective cohort studies and laboratory experiments to corroborate and further support the current findings. Furthermore, while certain potential confounding variables were accounted for, it is crucial to acknowledge the possibility of unconsidered factors that may influence the results. Further investigation into the biological role of sex hormone-binding proteins and prolactin neurons in stroke in women could also provide a better understanding of underlying mechanisms and potential causes of stroke. Genetic prediction of higher sex hormone binding protein levels may help reduce the risk of stroke occurring in women.

6. Conclusion

This research aimed to investigate the effect of SHBG on stroke using MR. The analysis showed a significant inverse relationship: higher SHBG levels were linked to lower stroke risk, while lower SHBG levels were associated with higher stroke risk. Gender significantly influenced the relationship between SHBG and stroke risk, with notable differences between men and women. Higher SHBG levels were more effective in reducing stroke risk in women than in men, suggesting that SHBG's impact on stroke risk varies by gender. In summary, our research enhances the understanding of hormonal impacts on stroke risk in women, paving the way for gender-specific prevention strategies. Low SHBG levels in women are linked to a higher risk of small-vessel ischemic stroke, likely due to increased cholesterol and sex differences. This discovery supports further research into SHBG's role in stroke, suggests new targets for personalized treatment, and aids in developing preventive and therapeutic strategies for stroke-related diseases.

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