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RESEARCH ARTICLE

Women's reproductive traits and ischemic stroke: a two-sample Mendelian randomization study

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Abstract

Objective: We conducted a Mendelian randomization (MR) study to disentangle causal associations between women's reproductive behaviors and ischemic stroke (IS) and investigate the roles of two modifiable risk factors (body mass index (BMI) and educational attainment (EA)) in these associations. **Methods**: Using summary-level data from large-scale genome-wide association studies, we performed univariable MR to examine whether there is genetic evidence that women's reproductive traits are causally associated with IS and its subtypes. Multivariable MR and MR mediation analysis were used to investigate whether BMI and EA are common mechanisms or mediators for these associations. A set of sensitivity analyses were conducted to test valid MR assumptions. **Results**: We observed consistent and statistically significant associations across female and sex-combined analyses for earlier age at first birth (AFB) and age at first sexual intercourse (AFS) with a higher risk of IS and large-artery atherosclerotic stroke (LAS) risk in the primary analysis. The odds ratios of IS per 1 SD increase in genetically predicted early AFB and AFS were 0.93 (95% CI, 0.86–0.99; $p = 0.046$) and 0.83 (95% CI, 0.70–0.97, $p = 0.020$), respectively. Further analyses indicated that BMI played a shared role in AFS and IS/LAS while EA played a shared role in AFS/AFB and IS/LAS as well as a mediator in the path from AFS to IS/LAS. **Interpretation**: These findings may inform prevention strategies and interventions directed toward relative women's reproductive behaviors and IS. Future studies are warranted to explore other factors related to EA which are responsible for these causalities.

Introduction

Globally, stroke remained the second-leading cause of death and the third-leading cause of death and disability combined.[1] Nearly 87% of all strokes are ischemic strokes (IS), and women account for over half (56%) of all persons who have experienced a stroke by current estimates.[2] Beyond conventional risk factors for IS, mounting evidence has identified distinct sex-specific risk factors related to female reproductive and pregnancy history for women.[3]

Women's reproductive traits (including age at first birth (AFB), age at first sexual intercourse (AFS), age at menarche (AAM), age at natural menopause (ANM), and pregnancy loss) have important implications for evolutionary fitness and later-life health.[4] To date, the relationship between women's reproductive traits and the risk of IS has been reported in several studies; however, the results are inconsistent and incomplete. Some studies have suggested that early menarche and menopause are associated with an increased risk of cardiovascular disease (CVD) in later life[5–7] while a meta-analysis further noted that early menopause was associated with a higher risk of hemorrhagic stroke (HS) but no statistically significant association with IS.[8] Several studies also identified that women who have experienced infertility, miscarriage, and stillbirth are at a higher risk for both non-fatal and fatal stroke, which could be explained by genetic factors or endocrine disruptions.[9,10] Moreover, earlier AAM, ANM, earlier AFB, and a history of miscarriage have been

reported to be independently associated with a higher risk of incident CVD in later life, suggesting a long-term adverse effect of certain factors relating to reproduction and pregnancy.[11] Nonetheless, it is still unclear whether these changes in female reproductive behaviors causally increase the risk of IS, or whether they are both caused by another, single underlying cause.

The rapid development of genome-wide association studies (GWAS) has led to the increasing application of mendelian randomization (MR) analysis using single nucleotide polymorphisms (SNPs) strongly associated with phenotypes as instrumental variables (IV).[12] Since SNPs are randomly assigned at conception, results from MR studies are less susceptible to measurement error, confounding, and reverse causation.[13] The genetic regulations in AFB, AFS, AAM, ANM, and pregnancy loss have been recently highlighted by different discoveries from large-scale GWAS and provided a valuable opportunity to investigate the long-term effects of female reproductive traits on IS. IS is known as a polyetiological disease with remarkable differences in risk factors among its subtypes (large-artery atherosclerosis stroke (LAS), small vessel stroke (SVS), and cardioembolic stroke (CES)).[14] Recent studies suggested that younger AFS was significantly associated with an increased risk of intracerebral hemorrhage or SVS[15] while older AFB was associated with longevity and decreased incidence of CVD.[16] What's more, previous studies have implicated two risk factors (BMI and educational attainment (EA)) in both women's reproductive health and stroke, separately.[17–20] Strong genetic correlations with AFB and AFS that may limit the ability to consider their distinct influences on disease risks,[16] while few studies took them into consideration either in observational or MR studies. Therefore, there is a paucity of well-designed MR studies assessing the causal relationships between genetically predicted female reproductive traits and IS.

In this study, we performed MR analysis to clarify whether women's reproductive traits (AFB, AFS, AAM, ANM, and pregnancy) have causal effects on IS and its subtypes independent of BMI and EA. To that end, we used genetic variants as instruments and performed relevant MR analysis to obtain causal estimates and characterize the causal structure by assessing mediation or shared effects of BMI and EA.

Materials and Methods

Study design

A brief description of the multiple MR designs was displayed in Figure 1. We performed univariable mendelian randomization (UVMR) and multivariable mendelian randomization (MVMR) analyses to clarify whether women's reproductive traits (AFB, AFS, AAM, ANM, and pregnancy) have causal effects on IS and its subtypes independent of BMI and EA. UVMR rests on three main assumptions: (1) the genetic variant selected as the instrumental variable is robustly associated with the exposure; (2) the genetic variant is not associated with confounders; (3) genetic variants affect the outcome only through the exposure, not other pathways.[21] Compared with the assumptions of UVMR, the first assumption of MVMR was the genetic variants associated with one or more of the exposures and other assumptions were consistent with UVMR.[22]

In this study, we used summary-level data from published Genome-wide association studies (GWASs) of five women's reproductive traits, BMI, EA, IS, and its subtypes. First, we selected genetic variants for each women's reproductive trait to infer the causality from each women's reproductive traits to IS and its subtypes using UVMR. Second, we integrated GWAS summary statistics and additional genetic variants on BMI and EA and conducted MVMR models to estimate the direct effect of reproductive factors on the outcomes controlling for the effect of BMI and EA. Third, we explored the role of BMI and EA (mediated or common mechanism) playing in the relationships of AFB and AFS on IS and LAS by two-step MR and UVMR. To test the relationships between AFB and AFS, we additionally examined whether evidence supports AFB as a mediator for AFS on IS and LAS using a two-step MR analysis.

Data sources

In the current study, the exposures were women's reproductive traits, including AAM, ANM, AFB, AFS, and pregnancy loss. The coprimary study outcome was IS. Secondary outcomes included LAS, CES, and SVS, which were common etiological subtypes of IS.[23] For each trait, summary-level data (effect estimates, standard errors, and *p*-values) were obtained from recent large European GWASs (Table 1). There was no overlap between exposure and outcome samples since exposures and outcomes data were obtained from different consortia.

Women's reproductive traits

Genetic variants of AAM were obtained from a meta-analysis of GWASs including 329,345 individuals of European ancestry from UK Biobank, 23andMe, and ReproGen consortium.[24] Each dataset tested SNPs using an additive linear regression model for association with AAM, adjusted for age at study visit and other study-

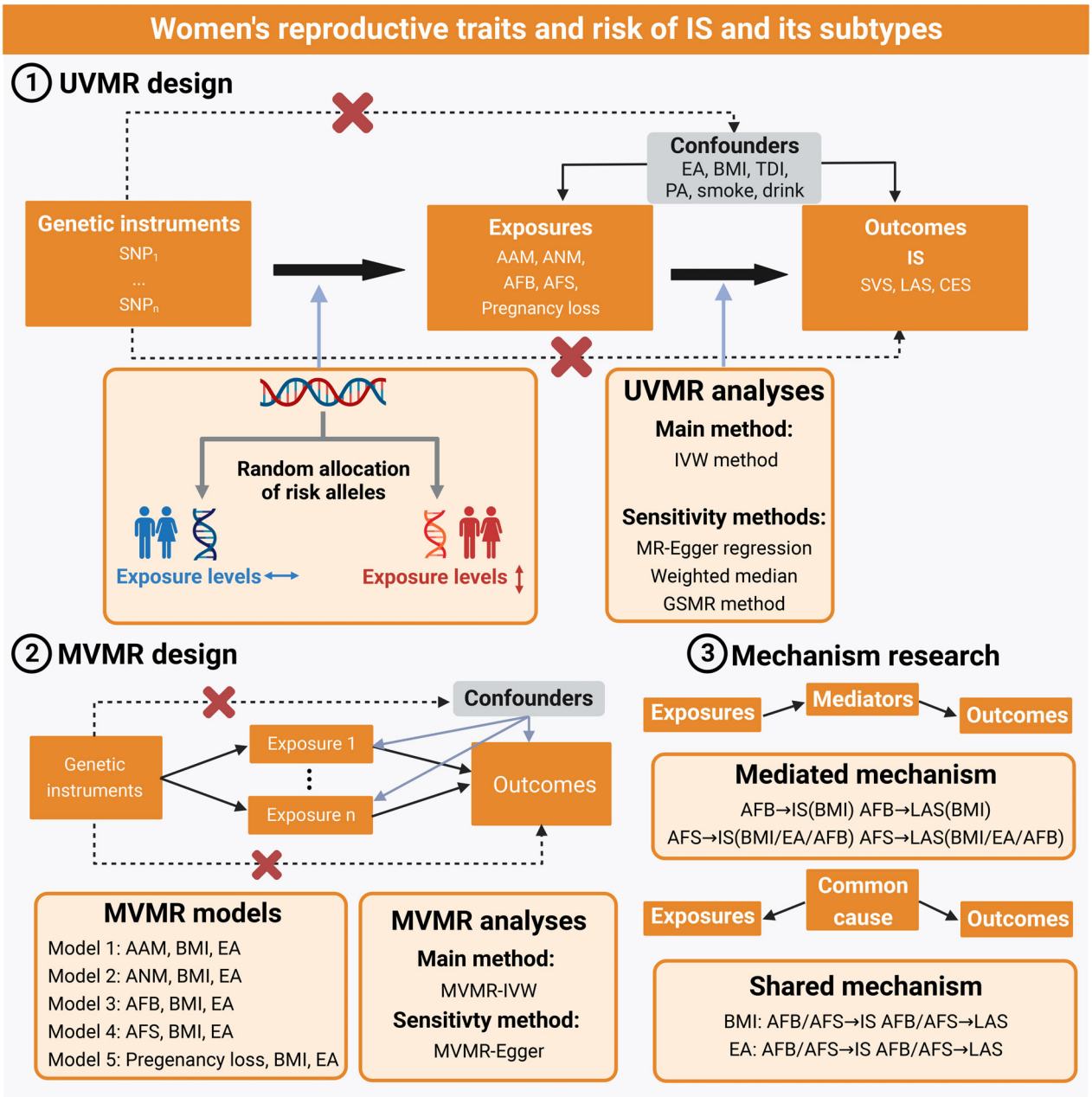

Figure 1. Assumptions and study design of the MR study of the associations between five women's reproductive traits and IS and its subtypes. AAM, age at menarche; AFB, age at first birth; AFS, age at first sexual intercourse; ANM, age at natural menopause; BMI, body mass index; CES, cardioembolic stroke; EA, educational attainment; GSMR, generalized summary-data-based Mendelian randomization; IS, ischemic stroke; IVW, inverse-variance weighted; LAS, large-artery atherosclerotic stroke; PA, physical activity; SVS, small vessel stroke; TDI, Townsend deprivation index; UVMR, univariable Mendelian randomization. In the part of mediated mechanism, we assume the risk factor in the brackets might be the mediator. In the part of shared mechanism, we explored whether BMI and EA could be a shared role in the relationships of AFB and AFS on IS and LAS.

specific covariates. To obtain the genetic associations between SNPs and AAM, ReproGen consortium results were combined with data from the UK Biobank and 23andMe studies using an inverse-variance-weighted meta-analysis method.[24]

Genetic predictors of ANM were obtained from the up-to-date GWAS meta-analysis in ~200,000 women of European ancestry.[25] ANM was defined by the age at last naturally occurring menstrual period followed by at least 12 consecutive months of amenorrhea and derived from self-

Table 1. Data sources used in the MR analyses for the current study.

Phenotype	Participants included in analysis	Ancestry	Unit	Adjustments	Source
Exposures					
AAM	329,345 females	European	Year increase in AAM	Age at study visit and other study-specific covariates	ReproGen, UK Biobank, 23andMe
ANM	201,323 females	European	Year increase in ANM	Genetic principal components matrix	UK Biobank, BCAC Consortium, 1000 Genomes imputed studies
AFB	418,758 females	European	Year increase in AFB	Birth year of the respondent, its square, its cubic, and top principal components	36 studies
AFS	214,547 females	European	SD increase in AFS	Birth year of the respondent, its square, its cubic, and top principal components	UK Biobank
Pregnancy loss	191,252 females (60,565 cases and 130,687 controls)	European	Log odds increase in pregnancy loss	Age, up to 20 genetic principal components	UK Biobank
Outcomes					
IS	34,217 cases and 406,111 controls	European	/	Age, sex, up to 20 genetic principal components	MEGASTROKE Consortium
SVS	5386 cases and 406,111 controls	European	/	Age, sex, up to 20 genetic principal components	MEGASTROKE Consortium
LAS	4373 cases and 406,111 controls	European	/	Age, sex, up to 20 genetic principal components	MEGASTROKE Consortium
CES	7193 cases and 406,111 controls	European	/	Age, sex, up to 20 genetic principal components	MEGASTROKE Consortium

AAM, age at menarche; AFB, age at first birth; AFS, age at first sexual intercourse; ANM, age at natural menopause; CES, cardioembolic stroke; IS, ischemic stroke; LAS, large-artery atherosclerotic stroke; SVS, small vessel stroke.

reported in each study. Genetic associations between SNPs and ANM were obtained from additive linear regression model adjusted for genetic principal components matrix.

From 36 studies of European descent in total, Mills et al. reported the largest meta-analysis of GWASs, including 418,758 females for AFB.[16] AFB was treated as a continuous measure, assessed for those who have ever given birth to a child. Genetic variants of AFS were also obtained from the largest GWAS, including 214,547 females of European ancestry from UK Biobank.[16] AFS was treated as a continuous measure, with individuals considered to be eligible if they had given a valid answer with ages lower than 12 years excluded and was transformed by inverse rank-normal. Both genetic associations between SNPs and AFB or AFS were adjusted for birth year of the respondent, its square, its cubic, and top principal components.[16]

Summary-level data for pregnancy loss was derived from the UK Biobank study.[26] In UK Biobank, pregnancy loss was defined by the history of having stillbirth, spontaneous miscarriage, or termination. We used the second round of Neale Lab's GWAS in UK Biobank, including191,252 women (60,565 cases and 130,687 controls). Genetic associations were adjusted for 20 genetic principal components, and age.

Ischemic stroke and its subtypes

Summary-level data for IS and its subtypes were derived from the meta-analysis of GWAS from MEGASTROKE

consortium, including 521,612 primarily European (~86%) participants from 29 cohorts.[23] To reduce the heterogeneity caused by multi-ancestry, we used the summary-level data involving European participants only (40,585 cases; 406,111 controls). Detailed definitions of phenotype for IS and its subtypes were described elsewhere.[23] The GWAS meta-analyses were performed for IS (N = 34,217), LAS (N = 4373), CES (N = 7193), and SVS (N = 5386) using additive genetic models with sex, age, and up to 20 genetic principal components as covariates.

Ethical approvals

All studies included in cited publicly GWASs had been approved by a relevant ethical review board. All samples that participated in cited GWASs gave informed written consent.

Selection of genetic instrumental variables

To satisfy the MR assumptions (Fig. 1), all SNPs for UVMR and MVMR are strongly and independently (R^2 <0.001 within 10 Mb) predicted exposures from the published GWAS at genome-wide significance ($p < 5 \times 10^{-8}$). Since no genome-wide significant SNPs for pregnancy loss, we adopted a less stringent threshold of 5×10^{-6} to obtain more SNPs for pregnancy loss.

Using the publicly available GWAS summary data, we examined whether any of these SNPs were associated with confounders (BMI, EA, physical activity, Townsend deprivation index, smoking, and drinking) and outcomes at a p-value of 1×10^{-5} for UVMR. The associations of these SNPs with inverse-normally transformed BMI were obtained from a meta-analysis of GWASs in approximately 700 000 participants of European ancestry.[27] The associations of these SNPs with alcoholic drinks per week and cigarettes smoked per day were obtained from a meta-analysis of GWASs in up to 1.2 million individuals of European ancestry.[28] Summary-level data on Townsend deprivation index was obtained from the MR-Base platform, including 462,464 individuals of European ancestry in UK Biobank.[29] Summary-level data for physical activity was derived from a GWAS of 377,234 individuals from UK Biobank.[30] Genetic associations with EA were obtained from the meta-analysis of GWASs on 3 million individuals of European ancestry contributed by a previous meta-analysis of 69 cohorts, 23andMe, and UK Biobank.[31] Similar to UVMR, we also examined whether any of these SNPs were associated with confounders (physical activity, Townsend deprivation index, smoking, and drinking) and outcomes for MVMR.

At last, we quantified the strength of SNPs for UVMR using mean F-statistic[32] and assessed the strength of our MVMR SNPs using conditional F-statistic.[33] Mean F-statistic and conditional F-statistic >10 suggested sufficient strength to ensure the validity of the SNPs for the exposures in UVMR and MVMR, respectively.

Statistical analysis

Univariable MR

SNPs that were unavailable in the outcome datasets were replaced by proxies at $R^2 > 0.80$ in LDlink (https://ldlink.nci.nih.gov/). After extracting and harmonizing the data, we performed UVMR to estimate the causal effect of women's reproductive traits on IS and its subtypes. In the main analysis, we calculated a Wald ratio estimate for each genetic variant and summarized the estimates using the inverse-variance weighted (IVW) method. The IVW with multiplicative random effects method provides a concise estimation and takes into account potential heterogeneity among the Wald ratio estimates from SNPs.[34] Thus, if there is heterogeneity, random-effects IVW models are applied; otherwise, the fixed-effect IVW model is applied. To assess the robustness of the findings, we also performed sensitivity analyses using methods with different assumptions about horizontal pleiotropy, including MR-Egger regression, weighted median, and generalized summary-data-based MR (GSMR). MR-Egger analysis provides an assessment of instrumental variable pleiotropy, with a non-zero intercept indicating that the IVW estimate is biased.[35] The weighted median approach that selects the median MR estimate as the causal estimate was taken into consideration for multiple genetic variants to be invalid or present pleiotropy.[36] GSMR method employed a slightly different formula from IVW by modeling variance of the effect of SNPs on exposures and outcomes and also accounts for horizontal pleiotropy but operates based on excluding outlier or heterogenous genetic instruments that are likely pleiotropic (HEIDI-outlier method).[37] Heterogeneity in the IVW estimates was examined by the Cochran Q test and I^2 index.

Multivariable MR

Next, since obesity and EA might play confounding or mediated roles in the pathway from women's reproductive traits to IS phenotypes, we performed MVMR to examine the direct effect of women's reproductive traits on IS and its subtypes, controlling for BMI and EA.[16,38] For MVMR, we used an extension of the IVW MR method, performing MVMR-IVW and selecting random effects or fixed effects based on heterogeneity as described in UVMR. In sensitivity analyses, we additionally used an MVMR-Egger to correct for both measured and unmeasured pleiotropy.

A strong genetic correlation (r_g = 0.811) was reported between AFB and AFS among women.[39] To account for the genetic correlation and to explore the independent effects of each exposure on IS and LAS, we performed MVMR including both AFB and AFS as exposures.

Exploration on the common cause and mediator

Upon initial analysis, EA and BMI significantly weakened the association of AFB and AFS with IS and LAS, thus they may play mediated or common-cause effect for the associations. We conducted multiple studies to explore possible mechanisms of BMI, and EA play in the association between AFS and IS (Data S1 and S2). Notably, we only test the mediated role of EA in the associations of AFB on IS due to individual's first birth most often occurring after they have completed their education. We also investigated whether AFB played mediated role in the association between AFS and IS due to the strong evidence for the direct effect of AFB on IS in the MVMR analysis including both AFB and AFS as exposures (Data S1). The hypotheses of role of BMI and EA were consistently investigated in the analysis of association between AFB on IS (Data S2). Same analysis methods were applied to the outcome of LAS.

Sensitivity analysis

Because we used sex-specific SNPs for women's reproductive traits, whereas GWASs of IS and its subtypes were assessed in both males and females. We repeatedly performed UVMR and MVMR analyses using sex-combined SNPs for AFB and AFS as sensitivity analysis to assess the impact of the issue for sex heterogeneity of SNPs when conducting sex-specific two-sample MR studies.

Results of the effects of AAM, ANM, AFB, and EA on IS and its subtypes are presented as ORs (95% CIs) per year genetic predicted traits increase and the effect of pregnancy loss on IS and its subtypes is presented as OR (95% CI) per log odds of pregnancy increase. The effects of AFS and BMI on IS and its subtypes are presented as OR (95% CI) per one SD genetic predicted AFS and BMI increase. For UVMR, the Bonferroni method was used to correct for multiple testing, therefore, we considered associations with p-values below 0.01 (0.05/5) as strong evidence of associations and results with p-values between 0.01 and 0.05 were regarded as suggestive associations. For other analyses, results with p-values <0.05 indicated a statistically significant causal association. All analyses were two-sided and conducted using TwoSampleMR (version 0.5.6), Mendelian randomization (version 0.5.1), MVMR (version 0.3), and GSMR (version 1.0.9) packages in R software (version 3.6.3). Reporting of the study follows the STROBE-MR statement.

Results

UVMR analyses of each women's reproductive trait on IS and its subtypes

We selected 319, 270, 25, 58, and 9 SNPs as genetic instruments for AAM, ANM, AFB, AFS, and pregnancy loss, respectively (Table S1–S5). The mean F-statistics for women's reproductive traits ranged from 22.78 to 95.07, suggesting the weak instruments bias is minimal (Table S8). One year genetically higher AFB was suggestively associated with a 7.0% (1.0%–14.0%) lower risk of IS (p = 0.046) and strongly associated with a 26.0% (12.0%–38.0%) lower risk of LAS (p < 0.001; Fig. 2). Genetically higher AFS was suggestively associated with lower risk of IS (OR 0.83, 95% CI 0.70–0.97; p = 0.020) and lower risk of LAS (OR 0.63, 95% CI 0.42–0.94; p = 0.023; Fig. 2). There was no evidence for the causal effect of AFB and AFS on other outcomes (Fig. 2). Primary IVW method also showed no evidence for the causal effect of AAM, ANM, and pregnancy loss on IS and its subtypes (all p > 0.05; Fig. 2). The results of MR-Egger regression and weighted median methods were similar to IVW method, and no horizontal pleiotropy was detected using MR-Egger regression (Table S9). GSMR also showed similar results after removing outliers detected by HEIDI-outliers test (Table S9). Although heterogeneity was detected in the UVMR of AAM and ANM on IS and its subtypes, we used IVW with random effects to alleviate the problem (Table S9).

MVMR analyses of each women's reproductive trait on IS and its subtypes

In MVMR, we integrated additional genome-wide significant genetic variants on EA, BMI with each women's reproductive trait (Table S6 and S7). The conditional F-statistics for other women's reproductive traits, BMI and EA ranged from 10.61 to 33.27, except for pregnancy loss (F-statistics = 6.71; Table S8). After controlling for the effect of BMI and EA, the effect size of AFB and AFS on IS and LAS was reduced and the association became non-significant compared with results of UVMR (all p > 0.05; Fig. 3). Similarly, there was no evidence for direct causal effect of genetically predicted AAM, ANM, and pregnancy loss on IS and its subtypes (Fig. 3). The MVMR-Egger method provided consistent results and did not suggest horizontal pleiotropy (Table S10–S13). Notably, MVMR showed a significant direct effect of genetically predicted EA on IS and LAS after controlling BMI and each women's reproductive trait (Table S10 and S12) and

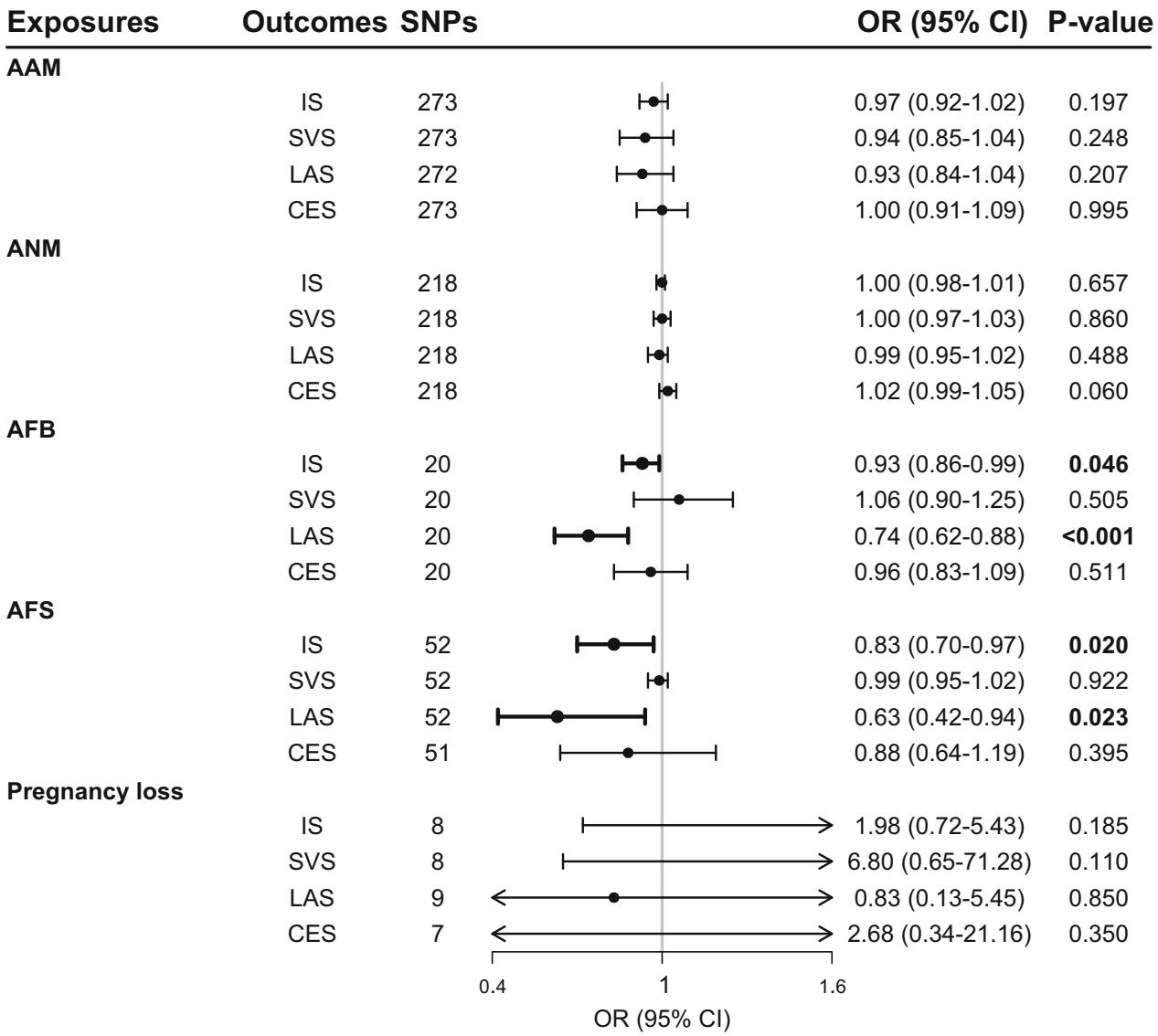

Figure 2. The effect of genetically determined women's reproductive traits on IS and its subtypes using UVMR. AAM, age at menarche; AFB, age at first birth; AFS, age at first sexual intercourse; ANM, age at natural menopause; CES, cardioembolic stroke; IS, ischemic stroke; LAS, large-artery atherosclerotic stroke; SVS, small vessel stroke.

significant direct effect of BMI on LAS after controlling EA and AFS (Table S12). MVMR also showed the causal associations of AFS with IS and LAS attenuated to the null after adjusting for AFB while AFB was significantly associated with IS and LAS (Table S14), which suggested that the associations between AFS and IS and LAS could be fully explained by AFB.

Results of MR analyses on the common cause and mediator

First, we investigated the role of common mechanism using a series of UVMR methods. Primary IVW methods showed elevated BMI was causally associated with earlier AFS (β, −0.148, 95% CI, −0.180 to −0.116; $p < 0.001$), higher risk of IS (OR, 1.13, 95% CI, 1.05–1.22; $p = 0.002$), and higher risk of LAS (OR, 1.30, 95% CI, 1.08–1.56; $p = 0.001$; Table 2) without evidence of horizontal pleiotropy detecting by MR-PRESSO method (Table S15). Although IVW method showed BMI associated with AFB, there was evidence of horizontal pleiotropy ($p < 0.001$; Table S15). Thus, result from MR-Egger showing no significant causal effect of BMI on AFB was considered as the main result (Table 2). The findings support the role of BMI as a common cause for AFS and IS and LAS due to the evidence for the effect of BMI on

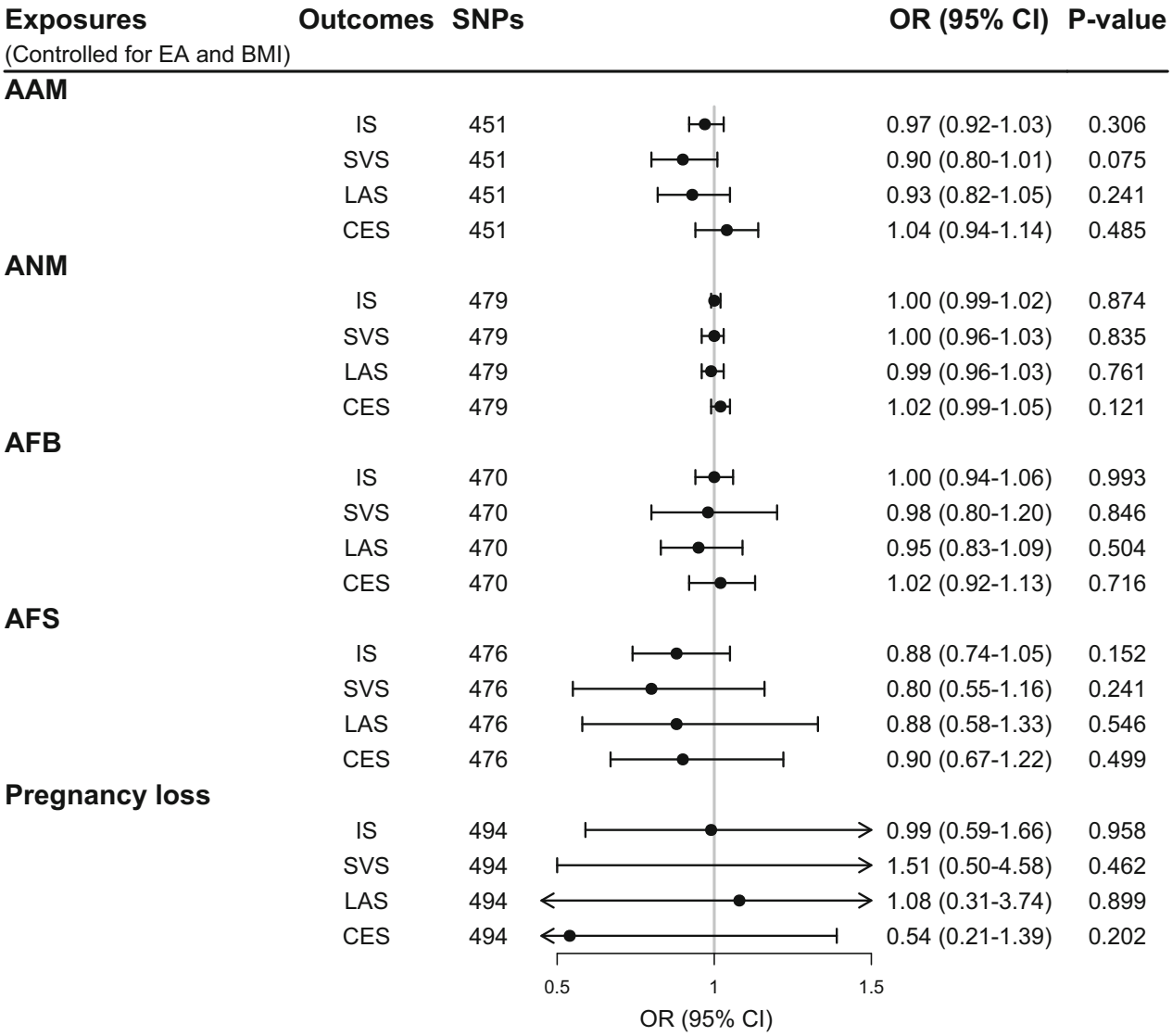

Figure 3. The direct effect of genetically determined women's reproductive traits on IS and its subtypes using MVMR controlled for EA and BMI. AAM, age at menarche; AFB, age at first birth; AFS, age at first sexual intercourse; ANM, age at natural menopause; CES, cardioembolic stroke; IS, ischemic stroke; LAS, large-artery atherosclerotic stroke; SVS, small vessel stroke.

both AFS and IS or AFS and LAS. Similarly, the results showed EA was the potential shared role in the relationships of AFB and AFS with IS and LAS (Table 2). Second, we conducted MR mediation analyses to investigate whether the effects of AFS on IS and LAS were mediated by EA, BMI, and AFB and also explored the effect of AFB on IS and LAS whether mediated by BMI. The effect of AFS was partially mediated by EA for IS (proportion mediated, 55.50%, 95% CI, 25.13%–85.86%; $p < 0.001$), and LAS (proportion mediated, 43.26%, 95% CI, 14.13%–72.17%; $p = 0.003$) (Table 3). Likewise, the effect of AFS on IS and LAS was partially mediated by AFB with proportions of 86.39% (95% CI 5.24%–168.54%; $p = 0.037$) and 79.57% (95% CI 0.87%–158.26%; $p = 0.048$), respectively (Table 3). However, there was no evidence for BMI played mediated role in the relationships of AFB and AFS with IS and LAS (Table 3).

In order to evaluate the impact of the sex heterogeneity of instruments on conducting sex-specific two-sample MR studies, we repeatedly UVMR and MVMR analyses using sex-combined genetic instruments for AFB and AFS. Results were also similar to the primary analysis using women-specific SNPs for AFB and AFS. For UVMR, we also observed significantly causal effect of AFB and AFS on IS and LAS in IVW methods (Table S16 and S17). MVMR showed no evidence for the direct effect

Table 2. Results of shared mechanism for EA and BMI in the relationships of AFB and AFS with IS and LAS.

Exposures	Outcomes	Beta (95% CI)	*p*-value
BMI			
	AFS	−0.148 (−0.180, −0.116)	<0.001
	AFB	−0.186 (−0.479, 0.107)	0.149
EA			
	AFS	0.415 (0.377, 0.454)	<0.001
	AFB	2.021 (1.908, 2.134)	<0.001
		OR (95% CI)	*p*-value
BMI			
	IS	1.132 (1.047, 1.224)	0.002
	LAS	1.299 (1.083, 1.558)	0.001
EA			
	IS	0.716 (0.653, 0.786)	<0.001
	LAS	0.523 (0.416, 0.659)	<0.001

Beta (95% CI) for AFB represents change in year of first birth per year increase in EA or per SD increase in BMI. Beta (95% CI) for AFS represents change in SD of first sexual intercourse per year increase in EA or per SD increase in BMI. OR (95% CI) represents change in odds ratio of IS and LAS per year increase in EA or per SD increase in BMI.
AFB, age at first birth; AFS, age at first sexual intercourse; BMI, body mass index; EA, educational attainment; IS, ischemic stroke; LAS, large-artery atherosclerotic stroke.

of AFB and AFS on IS and its subtypes, while EA had significant direct effect on IS and LAS after controlling AFB and BMI or AFS and BMI (Table S16 and S17).

Discussion

Leveraging large-scale GWAS data in UVMR, MVMR, and two-step MVMR analyses, we first investigated the causal associations between women's reproductive traits and ischemic stroke. And second, we examined whether there is evidence to support that BMI and EA may be a shared causal mechanism or mediator for AFS/AFB and IS/LAS. Using our primary MR analysis method, we found consistent and statistically significant associations across female and sex-combined analyses for earlier AFB and AFS with a higher risk of IS and LAS. Meanwhile, no evidence supported the causal associations of AAM, ANM, and pregnancy loss with IS or any subtypes. Further analyses yield a shared function of BMI on AFS and IS/LAS while EA was also shown to be a shared role of AFS/AFB and IS/LAS as well as a mediator in the pathway from AFS to IS/LAS. Additionally, the causal effects of AFS on IS and LAS also could be explained by the mediated effect from AFB.

Causal associations of each women's reproductive trait with IS and its subtypes

Consistent with previous studies, our results reported a relatively consistent association between early AFB and

Table 3. Results of mediation analysis using two-step MR.

Exposures	Mediators	Outcomes	Total effect	Direct effect A	Direct effect B	Mediation effect		Mediated proportion
			Effect size (95% CI)	Effect size (95% CI)	Effect size (95% CI)	Effect size (95% CI)	*p*	(%) (95% CI)
AFB								
	BMI	IS	−0.073 (−0.137, −0.010)	−0.077 (−0.113, −0.040)	0.081 (−0.006, 0.168)	−0.006 (−0.013, 0.001)	0.096	\
	BMI	LAS	−0.303 (−0.479, −0.126)	−0.077 (−0.113, −0.040)	0.134 (−0.063, 0.332)	−0.010 (−0.026, 0.006)	0.203	\
AFS								
	EA	IS	−0.191 (−0.352, −0.031)	0.366 (0.273, 0.458)	−0.290 (−0.429, −0.150)	−0.106 (−0.164, −0.048)	<0.001	55.50 (25.13, 85.86)
	BMI	IS	−0.191 (−0.352, −0.031)	−0.176 (−0.348, −0.005)	0.103 (0.018, 0.189)	−0.018 (−0.041, 0.005)	0.124	\
	AFB	IS	−0.191 (−0.352, −0.031)	2.144 (1.903, 2.384)	−0.077 (−0.005, −0.149)	−0.165 (−0.320, −0.010)	0.037	86.39 (5.24, 168.54)
AFS								
	EA	LAS	−0.460 (−0.065, −0.856)	0.366 (0.273, 0.458)	−0.543 (−0.880, −0.206)	−0.199 (−0.332, −0.065)	0.003	43.26 (14.13, 72.17)
	BMI	LAS	−0.460 (−0.065, −0.856)	−0.176 (−0.348, −0.005)	0.230 (0.030, 0.430)	−0.041 (−0.093, 0.012)	0.132	\
	AFB	LAS	−0.460 (−0.065, −0.856)	2.144 (1.903, 2.384)	−0.171 (−0.339, −0.003)	−0.366 (−0.728, −0.004)	0.048	79.57 (0.87, 158.26)

Total effect: the effect of exposure on the outcome. Direct effect A: the effect of exposure on the mediator. Direct effect B: the effect of mediator on the outcome after controlling for exposure. Mediation effect: the effect of exposure on the outcome acting through the mediator.
AFB, age at first birth; AFS, age at first sexual intercourse; BMI, body mass index; EA, educational attainment; IS, ischemic stroke; LAS, large-artery atherosclerotic stroke.

greater CVD risk.[11,40] It is also applicable to AFS since younger AFS may put girls at risk for earlier AFB. The two traits represent the timing of onset of women's reproductive behavior and have implications for reproductive health, adolescent development, and evolutionary fitness. Since the onset of reproductive behavior generally occurs in adolescence to early adulthood, it is often linked to externalizing behaviors such as psychiatric and substance use disorders (e.g. smoking and alcohol abuse) and is often influenced by a combination of biological and environmental factors.[16] These modifiable and non-modifiable risk factors contribute to atherosclerotic diseases that can eventually lead to IS as well. Furthermore, we also found evidence for the causal effects of AFB, AFS on IS subtypes to be specific for LAS which is the most frequent subtype of IS and may have worse outcomes than SVS. On this point, multiple lines of evidence suggested that atherosclerosis is a progressive disease caused by the accumulation of lipids and fibrous elements in large arteries and may start as early as childhood.[41,42]

As for AAM and ANM, they typically mark lifetime hormone estrogen exposure and are reported as independent risk factors for CVD,[5,43] although few studies have examined their effects on IS, particularly in European population. For example, a multicenter case–control study in postmenopausal women indicated that a very early AAM onset was independently related to a higher risk of IS[6] while a U-shaped association between AAM and IS was observed among Japanese women.[44] For ANM, some studies have reported that early menopause increases the risk of IS which may be ascribed to the postmenopausal decrease in endogenous estrogens,[45] while a meta-analysis provided inconsistent results that there were no statistically significant associations between earlier natural menopause and IS.[8] These conflicting findings observed in conventional observational studies are prone to confounders (such as obesity or oral contraceptive use) and reverse causation.[46] In this study, we observed no indirect or direct causal effects of AAM, ANM on IS, or its subtypes, which was consistent across all MR analyses. Nonetheless, further research is needed to elucidate hormonal effects on the two traits and unique sex-specific disease mechanisms.

Finally, our MR findings partially corroborated the results from an up-to-date study showing a null association between self-reported miscarriage and stroke,[11] yet some studies found a positive association between recurrent pregnancy loss and IS confined to younger women.[47] Given that pregnancy loss is a complicated trait shaped by both genetic and environmental factors, we performed an MVMR to control for the effect of BMI and education, and the negative result was consistent with the UVMR analysis.

EA and BMI as mediators or common causes in AFS/AFB and IS/LAS

One primary mechanism related to the health consequences of early sexual intercourse or childbirth was that teenage mothers are less likely to complete schooling and obtain a good education.[48] Prior research has suggested that EA plays an important role in health by shaping opportunities, income, employment, income healthy behaviors, and other socioeconomic factors.[49] Therefore, the explanation for why EA protected against IS might relate to the broad benefits of education. For example, higher education is correlated with healthier lifestyles, occupations with safer working conditions, and easier access to healthcare.[20] Our results implied that EA is a mediator and common cause of AFS and AFB, interpretating the hypothesized models for AFS/AFB to IS/LAS. Another possible mechanism for the correlations between AFS and IS/LAS emerged from the link between BMI and sexual behavior and adverse sexual health outcomes.[50] According to a Japan Public Health Center-based prospective Study, cumulative average BMI showed a positive linear effect on sub-distribution hazards of LAS in both sexes.[51] Although the causality between BMI with IS and its subtypes have not been well established, estimates generated from our MR analysis suggested that AFS to IS/LAS association is generally sensitive to the shared role of BMI. This might be due to some unmeasured stroke risk factors with obesity in women, such as low-grade systemic inflammation[52] and prothrombotic factors.[53] In this case, avoidance of obesity offers the potential of reducing IS and LAS in women. By contrast, the social and behavioral pathways regarding EA seems to be more powerful to support the mechanism of the causal relationship between AFB/AFS with IS/LAS.

Strengths and limitations

Our study has several advantages. We used data from up-to-date largest GWAS for each exposure or outcome in our study, and we explored a wide range of women's reproductive traits, including AAM, ANM, AFB, AFS, and pregnancy loss. The strong strength of the genetic instrumental variables allowed us to comprehensively explore the causal relationships of women's reproductive traits with the risk of IS and its subtypes using a series of MR methods, including UVMR, MVMR, and MR mediation analyses. This is an innovative attempt of considerable importance to understand whether women's reproductive traits can cause the risk of IS and its subtypes changes and whether EA and BMI mediated the effect of reproductive traits on IS or played a shared role for the associations, which were not explored by the previous MR studies.

Limitations need to be considered when interpreting our findings. First, the summary-level data for women's reproductive traits only involved women, whereas IS and its subtypes were assessed in both men and women. Thus, our results might be biased if the effects of the genetic variants are different between two sexes. However, we obtained results similar to the main results when using SNPs for AFB and AFS from summary-level data involving both men and women, showed the bias should be negligible. Second, although we used less stringent threshold ($p < 5 \times 10^{-6}$) to select SNPs as genetic instruments for pregnancy loss, the mean *F*-statistic in UVMR was more than 10 and the weak instrument bias from the threshold had been proved very small.[12] Nevertheless, a conditional *F*-statistic for pregnancy loss of 6.71 may also indicate risk of weak instrument, which may cause imprecise estimation of direct effect on pregnancy loss on IS and its subtypes. Whether pregnancy loss exerts on IS and its subtypes independent of EA and BMI needs further investigation. Third, although we cannot rule out the possibility of pleiotropy, we have conducted multiple sensitivity analyses (e.g., MR-Egger, weighted median, and GSMR) with different underlying assumptions, which gave similar conclusions. Forth, MR is not perfectly analogous to a Randomized Controlled Trial (RCT). Therefore, causal relationships of women's reproductive traits with IS and its subtypes derived from MR analyses may differ in magnitude from those seen or anticipated in an RCT and could be explained as life-course effects. Fifth, our study only included participants of European descent, which could limit the generalizability of our results to other ethnicities. More studies are needed to understand how the reproductive traits of women affect the risk of IS and its subtypes.

Conclusion

These results support that early AFB or AFS are risk factors for IS and LAS, in which EA or BMI may play mediator or shared roles in these causal relationships. Based on these findings, increasing the availability and accessibility of education and prevention of obesity might have the potential of substantially reducing the burden of IS attributable to altered certain women's reproductive traits. However, future studies are warranted to explore other factors related to EA which are responsible for the slice of the causal pie.

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Conflicts of Interest

The authors declare no conflicts of interest.

Author Contributions

ZQW, JWL, and JZ contributed to the research questions and study design. ZQW, JWL, and JZ contributed to the data curation. ZQW, JWL, and JZ contributed to methodology development. ZQW, JWL, and JZ conducted statistical analyses. ZQW, JWL, WPW, LZ, and JZ helped validation and performed sensitivity analyses. ZQW, JWL, WPW, JZ, and LZ interpreted the results and wrote the original draft of the manuscript. JZ, ZQW, JWL, WPW, and LZ helped review and edit the final draft of the manuscript. The author read and approved the final manuscript.

Data Availability Statement

Only publicly available data were used in this study, and data sources and handling of these data are described in the Materials and Methods and in the Supplementary Tables. Further information is available from the corresponding author upon request.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Data S1. The potential mechanisms of AFB, BMI, and EA play in the association between AFS and IS.
Data S2. The potential mechanisms of BMI and EA play in the association between AFB and IS.
Table S1. Genetic information of SNPs associated with age at menarche in UVMR and MVMR.
Table S2. Genetic information of SNPs associated with age at natural menopause in UVMR and MVMR.
Table S3. Genetic information of SNPs associated with age at first birth for women in UVMR and MVMR.
Table S4. Genetic information of SNPs associated with age at first sexual intercourse for women in UVMR and MVMR.
Table S5. Genetic information of SNPs associated with pregnancy loss in UVMR and MVMR.
Table S6. Genetic information of SNPs associated with BMI in MVMR.
Table S7. Genetic information of SNPs associated with educational attainment in MVMR.
Table S8. *F*-statistics for SNPs using univariable Mendelian randomization and multivariable Mendelian randomization.
Table S9. Associations of women's reproductive traits with ischemic stroke and its subtypes using univariable Mendelian randomization.
Table S10. Associations of women's reproductive traits with ischemic stroke controlled for EA and BMI using multivariable Mendelian randomization.
Table S11. Associations of women's reproductive traits with SVS controlled for EA and BMI using multivariable Mendelian randomization.

Table S12. Associations of women's reproductive traits with LAS controlled for EA and BMI using multivariable Mendelian randomization.
Table S13. Associations of women's reproductive traits with CES controlled for EA and BMI using multivariable Mendelian randomization.
Table S14. The effect of AFB and AFS on IS and LAS using multivariable Mendelian randomization including both AFB and AFS as exposures.
Table S15. The effect of genetically predicted BMI and EA on AFS, AFB, IS, and LAS
Table S16. Associations of sex-combined AFB with ischemic stroke and its subtypes using univariable and multivariable Mendelian randomization.
Table S17. Associations of sex-combined AFS with ischemic stroke and its subtypes using univariable and multivariable Mendelian randomization.

