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Material and methods
We conducted Mendelian randomization (MR) analysis via summary statistics from genome-wide association studies (GWAS), including PW adjusted for sex, 21 candidate mediators and atherosclerotic cardiovascular disease (ASCVD), which includes coronary heart disease (CHD) and ischemic stroke (IS).Two-step MR was employed to identify and assess the mediation proportion of potential mediators in the association between PW and ASCVD. Additionally, we conducted a repeated analysis using PW adjusted for gestational age and sex.

Results
Univariable MR (UVMR) analysis revealed that for each 1-SD decrease in fetal genotype-determined PW adjusted for sex only, the risk of CHD increased by 24% (95% CI: 1.05–1.46) and the risk of large artery stroke (LAS) increased by 46% (95% CI: 1.13–1.89). Similar results were obtained in repeated analyses. The mediation MR analysis revealed that the causal relationship between fetal genotype-determined PW and CHD risk was primarily mediated by birthweight, type 2 diabetes, and education, each mediating 3.66% to 40.80% of the total effect. The causal relationship between fetal genotype-determined PW and LAS risk was mediated mainly by type 2 diabetes, which accounted for 22.11% of the total effect.

Conclusions
This study elucidated a unidirectional causal relationship between lower PW and a greater ASCVD risk, with factors such as birthweight, type 2 diabetes, and education mediating the association between PW and ASCVD.

The causal effects of placental weight on atherosclerotic cardiovascular disease: a Mendelian randomization and mediation analysis

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Keywords: Atherosclerotic cardiovascular disease, Placental weight, Coronary heart d isease, Ischemic stroke, Mediation analysis, Mendelian randomization

Background

Atherosclerotic cardiovascular disease (ASCVD), which encompasses two primary subtypes—coronary heart disease (CHD) and ischemic stroke (IS)— constitutes the f oremost cause of mortality globally[1,2]. Consequently, the associated global burden o f ASCVD remains a critical public health priority on an international scale[3]. The devel opment of ASCAD involves complex pathophysiologic processes involving genetic and environmental factors and their interactions over decades[4,5]. Improved prevention and intervention strategies for ASCAD need to comprehensively summarize the impa cts of environmental and genetic factors. The "Developmental Origins of Health and Disease" (DOHaD) hypothesis proposes that adverse developmental experiences earl y in life increase the risk of cardiovascular disease and metabolic comorbidities later i n life[6,7,8]. During the vulnerable period of intrauterine development, disturbances in gene expression or cell proliferation and differentiation caused by nutritional and oth

er environmental influences can alter the structure and function of life's major organ systems, a process known as developmental programming. These effects set the stag e for many diseases that manifest many years later, often in response to secondary e nvironmental stressors[9].

The placenta, a temporary organ in mammalian gestation, comprises the chorion ic villi, the placental mesenchyme, and the placental membrane. The chorionic villi re present the fetal component, whereas the placental mesenchyme constitutes the ma ternal component[10,11]. At the end of pregnancy, the placenta is expelled by the moth er. The primary functions of the placenta include providing nutrients and facilitating g as exchange, as well as secreting various hormones essential for maintaining a norma l pregnancy. Furthermore, the placenta serves as a selective barrier, permitting the p assage of nutrients and gases while obstructing potentially harmful substances from r eaching the fetus[12]. Failure of the placenta to protect or nourish the fetus may have severe consequences for the fetus, both before birth and throughout its postnatal life [9,13,14]. Previous observational studies have demonstrated that placental health is ass ociated with the risk of cardiovascular disease and metabolic comorbidities later in lif e[15–17,18]. Several animal experiments have also provided evidence that the placenta plays a vital role in regulating the development of the cardiovascular system[19–21,22].

Placental weight (PW) is easily measured and is considered an indicator for asses sing placental growth and function. It is often used in epidemiologic studies to repres ent placental growth and function[15,23,24]. Previous research has indicated a correlati

on between PW and cardiovascular disease; however, the existence of a causal associ ation remains uncertain[25–27,28]. A comprehensive investigation of the causal relation ship between PW and cardiovascular disease, as well as the mediating factors involve d in this association, could enhance our understanding of the etiological developmen t of cardiovascular disease and offer valuable insights into its prevention and interven tion strategies.

In this study, we employed the Mendelian randomization (MR) method to investi gate the potential causal relationship and mediating factors between PW and ASCVD. MR methods apply genetic variation as an instrumental variable (IV) to infer causal r elationships between related traits[29]. We used several extension methods of basic MR principles, including two-sample MR[30], two-step MR[31], and multivariate MR[32,3 3], and then integrated the results into mediation analyses. For mediation analysis, th e two-step MR strategy is sensitive to causal mediation effects and is less susceptible to measurement error[31].

Methods

Study design

Using summary data from genome-wide association studies (GWAS) of PW, CHD, IS and 21 candidate mediators, we established a workflow to examine the role of the se traits as plausible mediators of the effects of PW on CHD and IS risk. Specifically, th is MR study consisted of two phases of analysis. In the first phase, we used bidirectio

nal two-sample univariate MR (UVMR) to assess the causal effect of PW on ASCVD (Figure 1-A). In the second phase, we screened 21 candidate mediators that may mediate the association between PW and ASCVD and calculated the proportion of mediators for each eligible mediator using two-step MR (Figure 1-B).

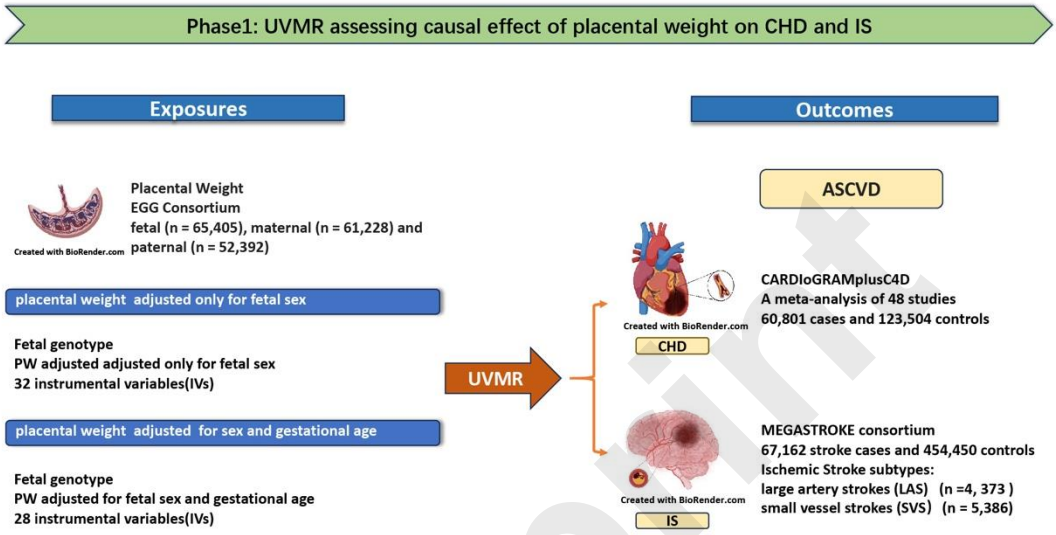

Figure 1-A: Overview of the MR study design. This MR study consisted of two analysis phases. In Phase 1, the causal associations of PW with CHD and IS were estimated using bidirectional UVMR.*UVMR*: univariable mendelian randomization; *MVMR*: multivariable mendelian randomization; *PW*: placental weight; *EGG*: early growth genetics; *CHD*: coronary heart disease; *IS*: ischemic stroke; *ASCVD*: atherosclerotic cardiovascular disease; IVs: instrumental variables.

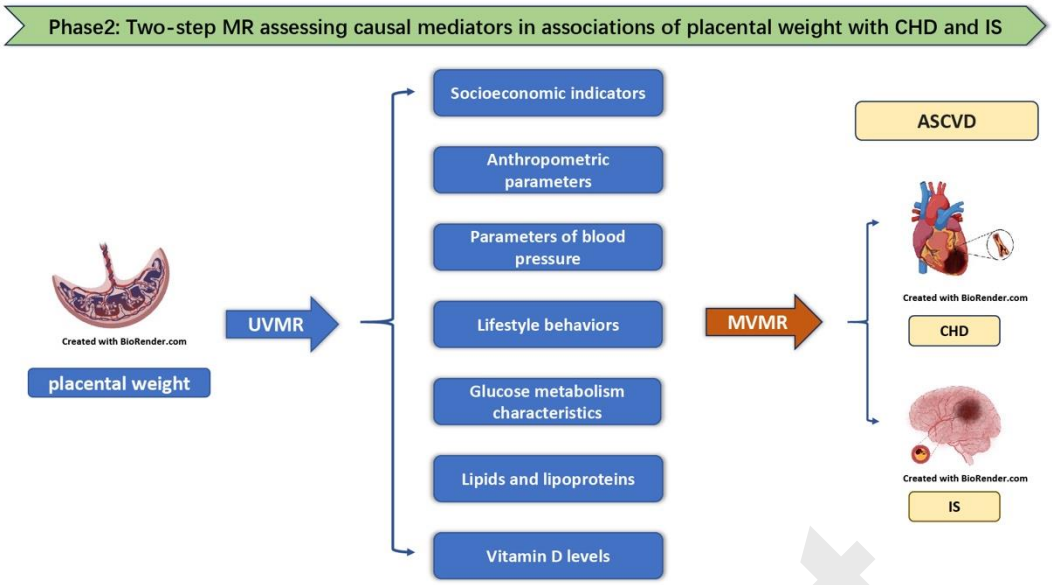

Figure 1-B: In Phase 2, two-step MR was applied to screen for 21 candidate mediators that may mediate the association between PW and ASCVD and quantify the mediation proportions for qualified mediators. *UVMR*: univariable mendelian randomization; *MVMR*: multivariable mendelian randomization; *CHD*: coronary heart disease; *IS*: ischemic stroke; *ASCVD*: atherosclerotic cardiovascular disease.

We used multiple approaches to fulfill the three core assumptions of MR as follows[29]. First, the IVs are strongly associated with at least one of the exposures (i.e., PW) in UVMR analyses or at least one of the multiple exposures in multivariable MR (MVMR) analyses. Second, IV was independent of confounders of the relationship between exposure and outcome (i.e., CHD and IS). Third, IVs affect outcomes only through exposure, not through any direct or indirect pathways. This study used summary statistics from publicly available GWAS from large-scale studies of individuals of predominantly European ancestry. This MR study was reported according to the Guidelines

for Strengthening the Reporting of Observational Studies in Epidemiology Using Mendelian Randomization (STROBE-MR)[34].

The dataset containing all the data used in this study is publicly accessible on the database website. The Institutional Review Board's Ethics Committee duly authorized written informed consent from all participants. Therefore, no additional ethical approval or informed consent was needed.

Data sources for and selection of genetic instrumental variable

The sources of exposure, mediator factors, and outcome data used in this study are provided in the supplementary materials (Additional file: Supplementary Table S2).

Placental weight

Summary statistics for PW are available from the Early Growth Genetics (EGG) Consortium's latest GWAS for PW. This study conducted a GWAS meta-analysis of fetal genotype (n=65,405), maternal genotype (n= 61,228) and paternal genotype (n=52,392) data from 21, 16, and 6 European studies according to fetal sex and gestation duration, respectively[35]. PW information was collected from medical records, researcher measurements, records at delivery, and birth registries. In the EGG consortium, PW data were converted to z scores for analysis via the within-cohort, unadjusted PW mean and standard deviation to calculate z scores for each individual. Individuals reportin

g multiple pregnancies, congenital anomalies, gestational age < 37 weeks and > 43 w eeks, PW < 200 and > 1500 g, and z scores ±5 SDs were excluded[35].

The study assessed the association between each genetic variant and PW using li near regression or linear mixed models, adjusted for fetal sex and identified 45 autos omal single-nucleotide polymorphisms (SNPs) independently associated with PW ($p < 5 \times 10^{-8}$). Of these, 39 association loci were identified by fetal GWA meta-analysis ($p < 5 \times 10^{-8}$), 4 loci by maternal analysis, and 2 loci by paternal analysis[35]. In addition, the study conducted a GWAS meta-analysis adjusted for fetal sex and gestational age. A total of 41 loci reached genome-wide significance ($p < 5 \times 10^{-8}$), of which 32 associ ation loci were identified by fetal GWA meta-analysis ($p < 5 \times 10^{-8}$), 4 loci by maternal analysis, and 2 loci by paternal analysis[35]. We performed more stringent linkage dis equilibrium clumping with a threshold $r^2 < 0.001$ cut-off using a 1,000-genome refer ence panel within a 10,000 kb window to select independent genetic variants. Finally, in analyses adjusted for fetal sex, we identified 32 IVs of fetal origin, 3 IVs of materna l origin, and 1 IV of paternal origin. And there were 28 fetal-origin IVs, 3 maternal-ori gin IVs, and 2 paternal-origin IVs in the analyses adjusted for fetal sex and gestational age. Considering that the small number of validated IVs for paternal and maternal so urces may lead to low study power in Mendelian randomization studies, in the prese nt study we performed Mendelian randomization analyses using 32 IVs of fetal origin adjusted for fetal sex. As a supplement, we used 28 IVs of fetal origin adjusted against

fetal sex and gestational age for repeated analyses (Additional file: Supplementary Ta bleS3).

Candidate mediators

Based on a literature review of previous observational and MR studies, we focus ed on 21 candidate regulatory mediators that may be present in the association betw een PW and ASCVD (Additional file: Supplementary Table S4), all of which can be fou nd in available GWAS genetic tools. The 21 candidate mediators were clustered into s even groups as follows: (1) socioeconomic indicators including education and househ old income; (2) anthropometric parameters including birthweight, body mass index (BMI), and waist-to-hip ratio (WHR) ; (3) blood pressure parameters including Hypert ension, systolic blood pressure (SBP) and diastolic blood pressure (DBP); (4) lifestyle b ehaviors including cigarettes smoked per day, alcoholic drinks per week and coffee co nsumption; (5) glucose metabolism parameter including Type 2 diabetes, glycated he moglobin (HbA1c) ; (6) lipids and lipoproteins including Total cholesterol， high-densi ty lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), Triglyc erides, Apolipoprotein B, Omega-3 fatty acids, Omega-6 fatty acids ; (7) Vitamin D lev els (Additional file: Supplementary Table S4).

In UVMR analyses, genetic IVs for each candidate mediator were genome-wide si gnificant ($p < 5 \times 10^{-8}$) and independent of each other (LD $r^2 < 0.001$ within 10,000 k b). In MVMR analyses, genetic IV was a combination of SNPs that were genome-wide

significant ($p < 5 \times 10^{-8}$) and independent of each other (LD $r^2 < 0.001$ within 10,000 kb) in either the GWAS for birthweight or the GWAS for each candidate mediator variable. If there were no SNPs for exposures in the GWAS summary statistics for CHD or IS, we used SNPs with chain disequilibrium ($r^2 > 0.8$) as proxies.

We screened for mediators of the causal associations of PW with CHD and IS according to the following criteria: [1] PW should be causally related to the mediator variables; [2] the mediator variables should have a direct causal effect on the outcome, independent of PW; and [3] the mediating effect of the mediator variables should be in the same direction as the total effect of PW on the outcome.

Outcomes

GWAS summary statistics for CHD were obtained from the UK Biobank and the Coronary Artery Disease Genome-wide Replication and Meta-analysis Consortium (CARDIoGRAM). The CARDIoGRAMplusC4D 1000 genome-based GWAS is a meta-analysis of 48 studies, including data from 60,801 cases of coronary artery disease and 123,504 controls of white European ancestry. Coronary heart disease was diagnosed according to the International Classification of Diseases (ICD-10: I20, I21, I22)[36].

Unlike CHD, the etiology of IS is diverse, and in addition to atherosclerotic embolization, other etiologies include cardiac embolization, small-artery atherosclerosis, and other mechanisms. The ischemic stroke subtypes associated with atherosclerosis include two main types: large artery stroke (LAS) and small vessel stroke (SVS). The GWAS summary statistics for IS and its subtypes used in this study were obtained from th

e MEGASTROKE Consortium, with a total of 34,217 cases of any ischemic stroke of European ancestry and 406,111 controls of European ancestry, including 4,373 cases of LAS and 5,386 cases of SVS[37]. Ischemic stroke and its two subtypes (large-artery stroke and small-vessel stroke) were classified according to the Org 10172 trial in the Treatment of Acute Stroke (TOAST) system[38].

Statistical Analysis

UVMR and MVMR analyses

We used the inverse variance weighted (IVW) method as the primary analysis for UVMR and multivariate inverse variance weighted (MV-IVW) as the primary analysis for MVMR. The IVW method uses a random-effects meta-analysis to combine the Wald ratio estimates for each SNP in the IVs group into a single causal estimate[39]. MR causal estimates were provided as odds ratio (ORs) (95% CIs) for binary outcomes and as β coefficients (95% CIs) for continuous outcomes.

Total Effects of PW on CHD and IS

We performed bidirectional UVMR to assess the overall causal effects of placental weight on CHD and IS, an approach that excludes confounding effects due to reverse causal associations. In addition, we performed leave-one-out analyses to assess the effects of individual variants on these associations. This approach examined whether specific genetic variants dominated estimates of causal effects by excluding one valid SNP at a time in IVW analyses.

Mediation MR analysis

We applied two-step MR analysis to assess whether mediators could mediate th e associations between PW and ASCVD, respectively[40]. The first step was to estimate the causal effect of genetically determined PW on each potential mediator (β1) usin g UVMR. The second step was to estimate the causal effect of each potential mediato r on CHD or IS (β2) using MVMR with adjustment for PW. Where there was evidence t hat PW affected the mediator variable, which in turn affected the outcome, we utilize d a 'product of coefficients' approach to assess PW's mediating effect on ASCVD via e ach mediator variable (β1 × β2). The mediation proportion for each mediating variabl e was calculated by dividing the mediated effect by the total effect. The standard err ors of the mediation effects were calculated via the delta method. We set the lower li mit of the negative mediation proportion to 0% because this is the minimum threshol d for determining the mediation proportion.

MR sensitivity analysis

For UVMR analysis, we used the inverse variance weighting (IVW) method as the primary analysis and adopted the MR-Egger, weighted median, simple mode, weighte d mode, and MR polytomous residual sums and outliers (PRESSO) methods as sensiti vity analyses of UVMR to assess the robustness of IVW estimates under different ass umptions. The MR-Egger method allows for the free estimation of the intercept as an indicator of polytropy to identify and adjust for potential directional polytropy bias b ut with limited precision[41]. The weighted median method selects the median MR est

imate as the causal estimate and provides a consistent causal estimate if more than 50% of the weights in the analysis are derived from valid IVs[42]. Simple and weighted mode methods cluster SNPs based on the similarity of causal effects and estimate causal effects on the basis of the maximum clustering of SNPs[43]. The presence of pleiotropy was also assessed by applying the MR-PRESSO method, which detects and corrects for any peripheral SNPs reflecting possible pleiotropy bias in MR causal estimates and assesses whether excluding peripheral SNPs affects causal estimates[44]. In addition, we used the cML-MA method for additional sensitivity analyses. cML-MA is an MR method based on constrained maximum likelihood and model averaging that has been used to control for both correlated and uncorrelated multicollinearity effects and is considered much more powerful than MR Egger[45].

For the MVMR analysis, we implemented the MVMR Egger method to verify the robustness of the MV-IVW results. The multivariate MR-Egger method has advantages over univariate methods in terms of the plausibility of the assumption required for consistent causal estimation and the ability to detect causal effects when the assumption is met[46]. We used the F-statistic to assess the validity of the IV and applied Cochran's Q-statistic to assess heterogeneity. In addition, MR Egger's intercept was used to assess the pleiotropy of the IVW estimates.

Given that multiple candidate mediators were tested in the analyses, we used the Benjamini-Hochberg method for false discovery rate (FDR) correction to account for multiple testing.IVW estimates with $p < 0.05$ and FDR q-values < 0.05 were regarded

as evidence of causality, whereas IVW estimates with $p < 0.05$ but FDR q-values ≥ 0.0 5 were regarded as suggestive causal evidence.

All the statistical analyses and data visualizations were performed using the R p ackages "TwoSample MR," "MRPRESSO," and "MRcML." These packages are readily a vailable in R software (version 4.3.2).

Results

Causal associations of PW with CHD and IS

In the UVMR analysis of PW with CHD (PW adjusted for sex only), a lower fetal ge notype-determined PW was positively associated with a higher risk of CHD(IVW-OR: 1.24; 95% CI: 1.05–1.46; p=1.10 ×10^{-2}). The same conclusion was reached in the repe at UVMR analysis of PW with CHD(PW adjusted for gestational duration and sex) (IV W-OR: 1.20; 95% CI: 1.00–1.43; p = 4.90×10^{-2})(Figure 2-A; additional file: Supplement ary Table S5).

In the UVMR analysis of PW with IS (PW adjusted for sex only), a lower fetal gen otype-determined PW was positively correlated with a higher risk of LAS (IVW-OR: 1. 46; 95% CI: 1.13–1.89; p = 3.9 × 10^{-3}). The same conclusion was reached in the repea ted UVMR analysis of PW with IS (PW adjusted for gestational duration and sex) (IVW -OR: 1.38; 95% CI: 1.06–1.80; p = 1.6 × 10^{-2}) (Figure 2-B; a Additional file: Supplement ary Table S7). In contrast, no correlation was found between PW and SVS risk in the U VMR analysis of PW with IS (Figure 2-B; a Additional file: Supplementary Table S7).

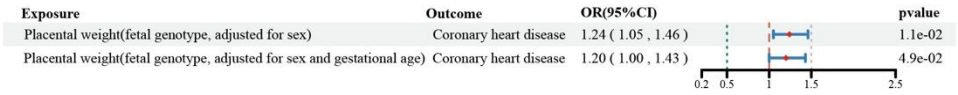

Figure 2-A. Causal association between lower placental weight with coronary heart disease

Figure 2-A: UVMR estimates (IVW) for the causal associations of PW with CHD and IS. The causal associations of PW with CHD. ORs (95% CIs) represent the risk for CHD or IS associated with each 1-SD lower PW. *UVMR*: univariable mendelian randomization; *IVW*: inverse variance weighted; *PW*: placental weight; *CHD*: coronary heart disease; *IS*: ischemic stroke; *OR*: odds ratio; *SD*: standard deviation.

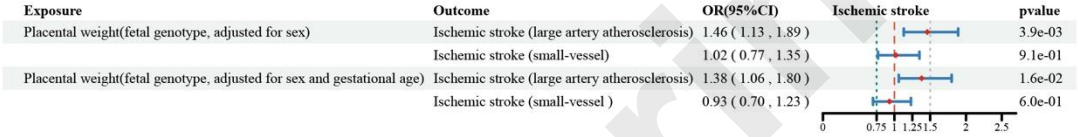

Figure 2-B. Causal association between lower placental weight with ischemic stroke

Figure 2-B: The causal associations of PW with IS. ORs (95% CIs) represent the risk for CHD or IS associated with each 1-SD lower PW. *IS*: ischemic stroke; *OR*: odds ratio; *SD*: standard deviation.

In the reverse MR analysis, no causal associations were found between CHD and PW or between IS and PW (Additional File: Supplementary Tables S6, S8).

The above IVW estimates have been confirmed by at least one sensitivity analysis. MR-Egger intercept analysis did not reveal evidence of horizontal pleiotropy (all P intercepts ≥0.05; Additional File: Supplementary Tables S9, S10). Cochran's Q test indicated potential heterogeneity, but IV validity revealed that instrument strength was sufficient (all SNP F-statistics ≥10). Leave-one-out analysis revealed that no individual

SNP significantly altered causal associations (Additional File: Figure S1 and S2).

Two-step MR assessing causal mediators in associations of PW with CHD and LAS

In the mediation analysis of PW with CHD (PW adjusted for sex only), out of 21 c andidate mediators, birthweight, education and type 2 diabetes met all screening crit eria, qualifying as mediators between fetal genotype-determined PW and CHD. In the repeated mediation analysis of PW with CHD (PW adjusted for gestational age and s ex), birthweight, education, Omega-6 fatty acid levels, and type 2 diabetes met all scr eening criteria, qualifying as mediators between fetal genotype-determined PW and CHD (Figure 3-A).

In the mediation analysis of PW with LAS (PW adjusted for sex only), among 21 c andidate mediators, type 2 diabetes met all screening criteria. It was eligible as a me diator between fetal genotype-determined PW and LAS. In the repeated mediation a nalysis of PW with LAS (PW adjusted for gestational age and sex), education and type 2 diabetes met all screening criteria and were eligible as mediators between fetal gen otype-determined PW and LAS (Figure 3-B).

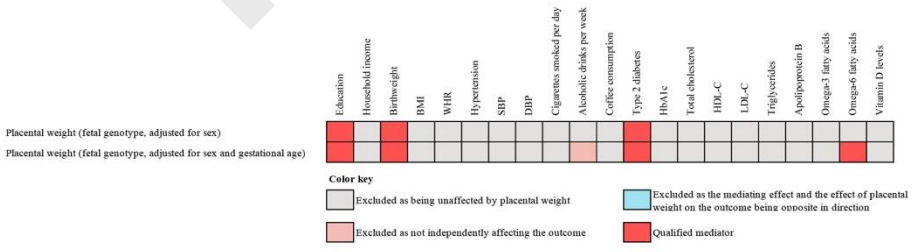

Figure 3-A. The qualified mediators in the mediation analysis of PW with CHD.

Figure 3-A: Selection process for mediators in the causal associations of PW with CHD and IS. The qualified mediators in the mediation analysis of PW with CHD. [1] PW sho

uld be causally related to the mediator variables; [2] the mediator variables should ha ve a direct causal effect on the outcome, independent of PW; and [3] the mediating e ffect of the mediator variables should be in the same direction as the total effect of P W on the outcome. *BMI*:body mass index,*WHR*:waist-to-hip ratio,*SBP*:systolic blood p ressure,*DBP*:diastolic blood pressure,*HbA1c*:glycated hemoglobin,*HDL-C*:high-density lipoprotein cholesterol,*PW*: placental weight, *CHD*: coronary heart disease.

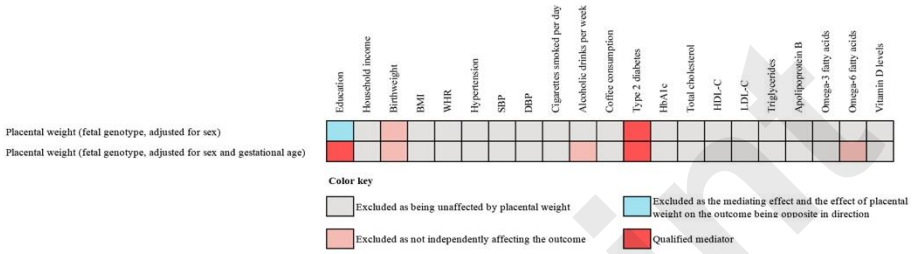

Figure 3-B.The qualified mediators in the mediation analysis of PW with IS.

Figure 3-B: The qualified mediators in the mediation analysis of PW with LAS. Three c riteria were applied to screen for the mediators in the causal associations of PW with CHD and IS. *BMI*:body mass index,*WHR*:waist-to-hip ratio,*SBP*:systolic blood pressure, *DBP*:diastolic blood pressure,*HbA1c*:glycated hemoglobin,*HDL-C*:high-density lipoprot ein cholesterol,*PW*: placental weight, *IS*: ischemic stroke.

Causal associations of PW with candidate mediators

In UVMR analyses of PW with mediators (PW adjusted for sex only), after false di scovery rate (FDR) correction, a higher fetal genotype-determined PW was found to b e causally associated with higher birthweight and educational attainment, as well as l ower risk of type 2 diabetes. (Additional file: Supplementary Table S11).

In the repeated UVMR analyses of PW with mediators (PW adjusted for gestational age and sex), higher fetal genotype-determined PW was found to be causally associated with higher alcohol intake, higher birthweight, and education attainment, as well as lower risk of type 2 diabetes, and lower levels of Omega-6 fatty acid levels, after correction for FDR (Additional file: Supplementary Table S12).

Causal associations of mediators with CHD and LAS

In MVMR analyses of PW with CHD (PW adjusted for sex only), with adjustment for fetal genotype-determined PW, each 1-unit increase in genetically determined education and birthweight was associated with a 35% and 22% lower risk of CHD, respectively. In contrast, with each 1-unit increase in genetically determined type 2 diabetes, the CHD risk increased by 1025% (Additional File: Supplementary Table S13). In the repeated analysis of PW with CHD (PW adjusted for gestational age and sex), with adjustment for fetal genotype-determined PW, each 1-unit increase in genetically determined education and birthweight was associated with a 36% and 24% lower risk of CHD, respectively. In contrast, each 1-unit increase in genetically determined Omega-6 fatty acids and type 2 diabetes was associated with a 28% and 1108% higher risk of CHD, respectively (Additional File: Supplementary Table S13).

In MVMR analysis of PW with LAS (PW adjusted for sex), with adjustment for fetal genotype-determined PW, each 1-unit increase in genetically determined education, the risk of LAS decreased by 50%, and a causal relationship between genetically determined type 2 diabetes and increased risk of LAS is also present. (Additional file: Su

pplementary Table S14). In the repeated analysis of PW with LAS, with adjustment for fetal genotype-determined PW, each 1-unit increase in genetically determined education, the risk of LAS decreased by 50%, and genetically determined type 2 diabetes was similarly shown to be causally associated with a higher risk of LAS (Additional file: Supplementary Table S14).

Mediators in the associations of PW with CHD and LAS

In the mediation analysis of PW with CHD (PW adjusted for sex), birthweight, type 2 diabetes, and education mediated 40.80%, 11.94%, and 3.66%, respectively, of the total effect of fetal genotype-determined PW on CHD. In the repeated analysis of PW with CHD (PW adjusted for gestational age and sex), birthweight, type 2 diabetes, Omega-6 fatty acids, and education mediated 27.03%, 13.68%, 8.54%, and 8.33%, respectively, of the total effect of fetal genotype-determined PW on CHD (Figure 4; Additional file: Supplementary Table S15).

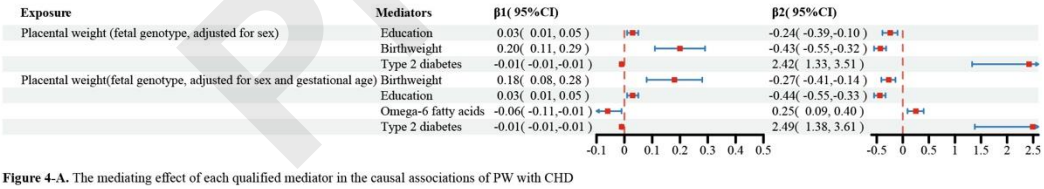

Figure 4-A. The mediating effect of each qualified mediator in the causal associations of PW with CHD

Figure 4-A: The mediating role of each mediator in the causal associations of placental weight with coronary heart disease. The mediating effect (β1 × β2) of each qualified mediator in the causal associations of PW with CHD. β1: Effect of placental weight on the mediator. β2: Effect of the mediator on coronary heart disease. *PW*: placental weight; *CHD*: coronary heart disease.

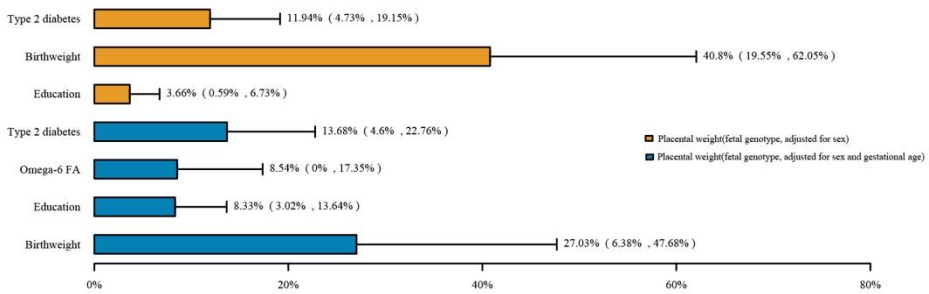

Figure 4-B. Proportion of mediation mediated by each qualified mediator for coronary heart disease

Figure 4-B: Proportion of mediation mediated by each qualified mediator for corona ry heart disease. Two-step MR was used to evaluate the mediating role of each medi ator in the causal associations of placental weight with coronary heart disease.

In the mediation MR analysis of PW with LAS, type 2 diabetes mediated 22.11% of the total effect of fetal genotype-determined PW on LAS. In the repeated analysis of PW with LAS, type 2 diabetes and education each mediated 14.19% and 7.41% of t he total effect of fetal genotype-determined PW on LAS (Figure 5; Additional file: Sup plementary Table S16).

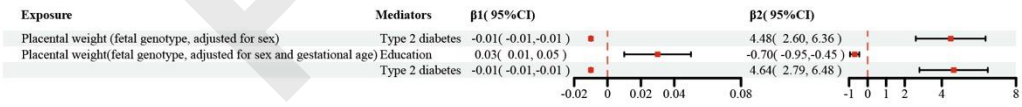

Figure 5-A. The mediating effect of each qualified mediator in the causal associations of PW with LAS(large artery atherosclerosis)

Figure 5-A: The mediating role of each mediator in the causal associations of placenta l weight with large artery stroke. The mediating effect (β1 × β2) of each qualified med iator in the causal associations of PW with large artery stroke. β1: Effect of placental weight on the mediator. β2: Effect of the mediator on large artery stroke. *PW*: placent al weight; *IS*: ischemic stroke

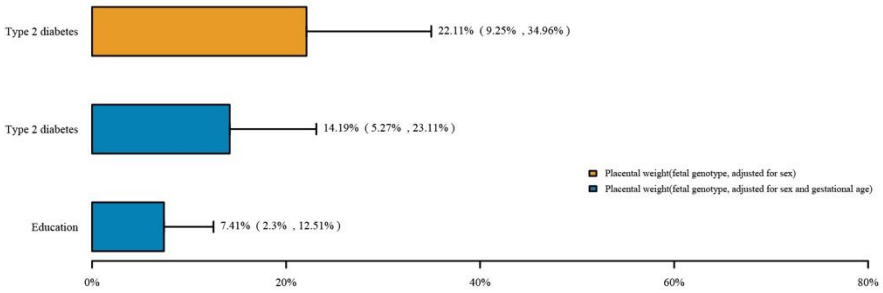

Figure 5-B. Proportion of mediation mediated by each qualified mediator for large artery atherosclerosis

Figure 5-B: Proportion of mediation mediated by each qualified mediator for large artery stroke. Two-step MR was used to evaluate the mediating role of each mediator in the causal associations of placental weight with large artery stroke.

Discussion

This MR study first evaluated the independent causal effect of low PW on ASCVD risk, identified potential mediators, and quantified their mediating role in this relationship. Specifically, in the UVMR analysis of PW with CHD, for every 1-SD decrease in fetal genotype-determined PW, the risk of CHD increased by 24%. Similar results were obtained in the repeated analysis (for every 1-SD decrease in PW, CHD risk increased by 20%). In the UVMR analysis of PW and IS, for every 1-SD decrease in fetal genotype-determined PW, the risk of large artery stroke (LAS) increased by 46%. Similar results were obtained in the repeated analysis of PW and IS (for every 1-SD decrease in fetal genotype-determined PW, the LAS risk increased by 38%).

In the UVMR analysis of PW and IS and the repeated analysis, no correlation was found between PW and the risk of small vessel stroke (SVS). We believe that this is be

cause the pathological features of small vessel stroke differ from those of large artery stroke (LAS)[47]. Atherosclerosis is not a major pathological feature of small-vessel stroke. Notably, the risk factors for small-vessel stroke as a whole also differ from the typical proatherogenic risk factors for large-artery stroke[48].

The mediation MR analysis and repeated analyses revealed that the causal association between lower placental weight and CHD risk was substantially mediated by birth weight, type 2 diabetes, and education. On the other hand, type 2 diabetes played a primary mediating role in the association between lower placental weight and LAS.

The intrauterine development stage is critical for lifelong health because the foundation of body planning and major organ systems is laid during this period. Anatomically, the placenta connects the fetus to the mother, transfers nutrients from the mother to the fetus, and creates a stable environment for fetal development, ensuring isolation from maternal and environmental stressors[9]. However, if normal placental function is compromised or exceeds the organ's adaptive capacity, the fetal environment may be disrupted, significantly impacting the lifelong health of the offspring[9]. Like low birthweight, low placental weight represents intrauterine malnutrition[9]. Low placental weight indicates poor placental growth during pregnancy, which can restrict the flow of nutrients and contribute to fetal malnutrition[9]. Previous studies have shown that low birth weight is associated with an increased risk of cardiometabolic disease later in life[9,28,49,50,51]. However, research on the causal relationship between low placental weight and the subsequent development of cardiovascular disease in later lif

e is still lacking. Our MR study elucidates the causal relationship between low placental weight and atherosclerotic cardiovascular disease. Our findings can be interpreted by the thrifty phenotype hypothesis. The adaptation of individuals to a nutrient-deficient intrauterine environment may result in modifications to insulin signaling pathways, epigenetic alterations, and variations in the proliferation and differentiation of cells within essential organs. These changes can subsequently lead to disturbances in the metabolism of glucose, lipids and other metabolites, ultimately predisposing individuals to cardiometabolic diseases as adults[6,52]. From a clinical perspective, our study indicates that individuals with low placental weight have a higher risk of developing ASCVD, encouraging further consideration of PW in risk stratification for ASCVD.

This MR study offered novel and reliable evidence on the causal link between low PW and late-life ASCVD risk, highlighting birthweight, type 2 diabetes, and education level as potential mediators. The strengths of this study included the rigorously designed MR analysis framework to investigate the causal relationship and potential mediating factors between low PW and ASCVD, and we utilized a substantial amount of GWAS summary statistics data. However, this study also has several limitations. First, this MR study was almost entirely restricted to individuals of European descent to ensure consistency in genetic background. The use of GWAS summary data mainly composed of individuals of European descent may introduce biased estimates and limit generalizability to different racial populations. Second, based on a two-sample MR design, we assumed that the relationship between the PW and outcomes in UVMR and

MVMR analyses is linear. Future research is necessary to investigate potential non-linear causal relationships using individual-level data. Third, potential exposure-mediator interactions cannot be modeled in the current two-sample MR setting. Nonetheless, the MR approach could largely alleviate the potential bias caused by the exposure-mediator interaction[50,53]. Fourth, to facilitate clinical practice, we focused on theoretically common and important candidate mediators associated with ASCVD as candidate mediators. However, this study cannot fully capture all the mediating factors, especially non-genetic factors. Finally, PW measured postpartum only roughly represents placental growth status and cannot entirely directly represent placental injury or placental dysfunction; future research needs to introduce more relevant indicators.

Conclusions

Our study revealed a causal relationship between genetically predicted lower placental weight and a greater risk of ASCVD. Mediators such as birth weight, type 2 diabetes, and education partially mediated the association between low placental weight and ASCVD. This finding underscores the significance of low PW as a predictor and a preventive factor for late-life ASCVD risk and offers substantial insights into the Developmental Origins of Health and Disease (DOHaD) hypothesis.

Abbreviations

PW Placental weight

ASCVD Atherosclerotic cardiovascular disease

MR Mendelian randomization

GWAS Genome-wide association studies

CHD Coronary heart disease

IS Ischemic stroke

LAS Large artery stroke

SVS Small vessel stroke

UVMR Univariable Mendelian randomization

MVMR Multivariable Mendelian randomization

IV Instrumental variable

IVW Inverse variance weighted

OR Odds ratio

PRESSO Polytomous residual sums and outliers

EGG Early Growth Genetics

FDR False discovery rate

SD Standard deviation

Declarations

Ethics approval and consent to participate:

Ethical approval and informed consent had been obtained in all original studies cited in this work.

Consent for publication:

Not applicable.

Availability of data and material :

All the summary-level GWAS data used in the analyses are publicly available as show n in Supplementary Table 1. The GWAS data can be obtained through the UK-bioban k, the EGG Consortium (http://egg-consortium.org), the IEU OpenGWAS project (http s://gwas.mrcieu.ac.uk), the SSGAC data portal, the GIANT consortium, the MEGASTRI KE Consortium and the International Consortium of Blood Pressure. The analytical sc ript of the MR analyses conducted in this study is available via the GitHub repository of the "TwoSampleMR" R package (https://github.com/MRCIEU/TwoSampleMR). All data generated or analysed during this study are included in this published article [an d its supplementary files].

Competing interests

The authors declared no conflict of interests.

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Authors' contributions

QL, DC, JS and SF contributed to the conception and design of the study. JS and SF co ntributed to statistical analysis. DC contributed to the drafting of the manuscript. QL, PM, and SS supervised the study and performed the quality assessment. All authors

contributed to the acquisition or interpretation of data, proof reading of the manuscript for important intellectual content, and the final approval of the version to be published.

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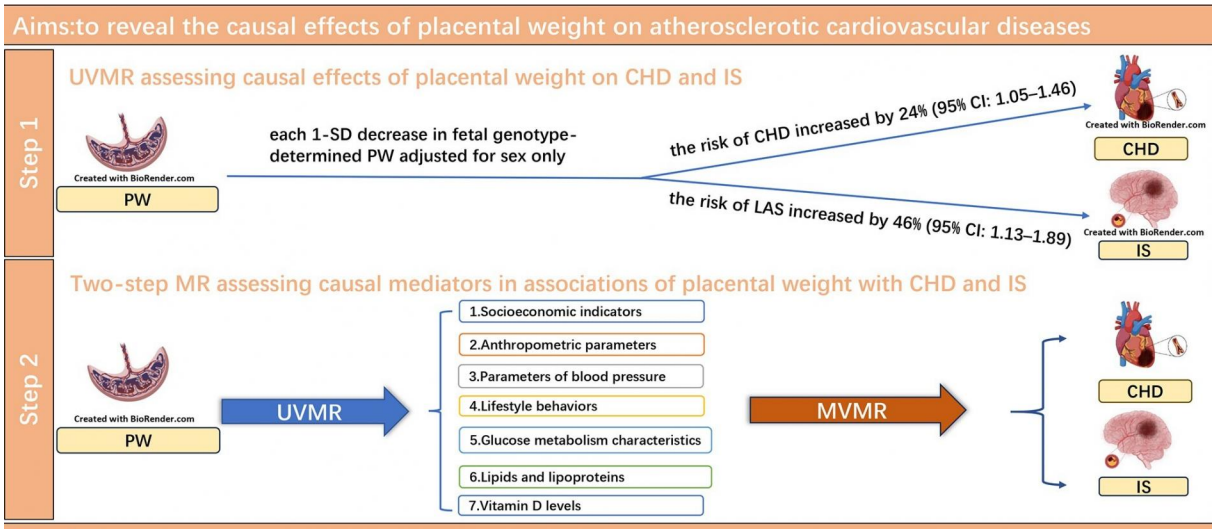
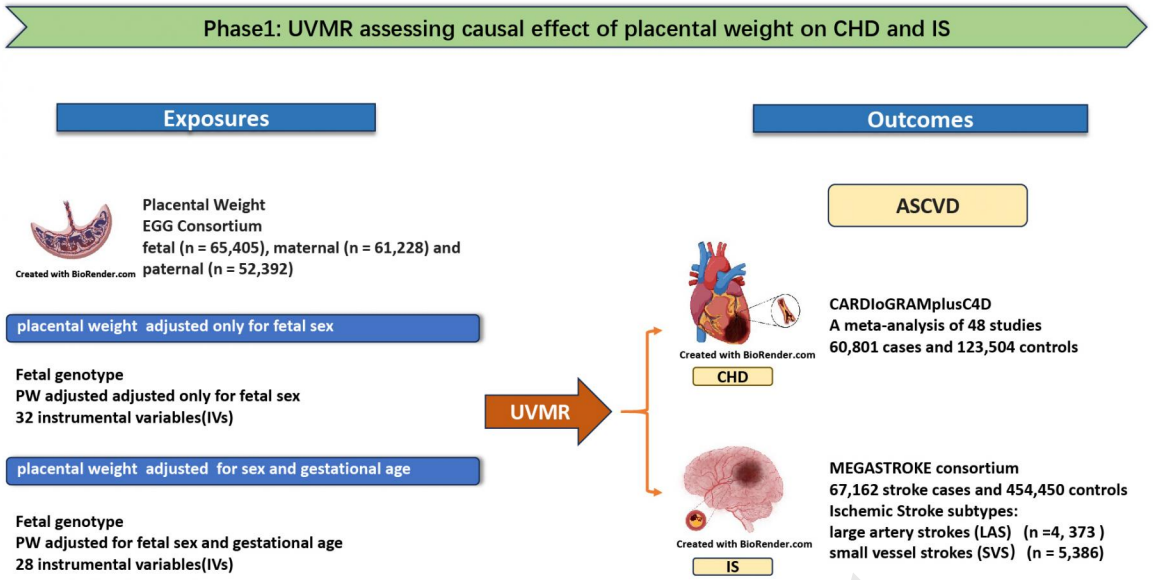
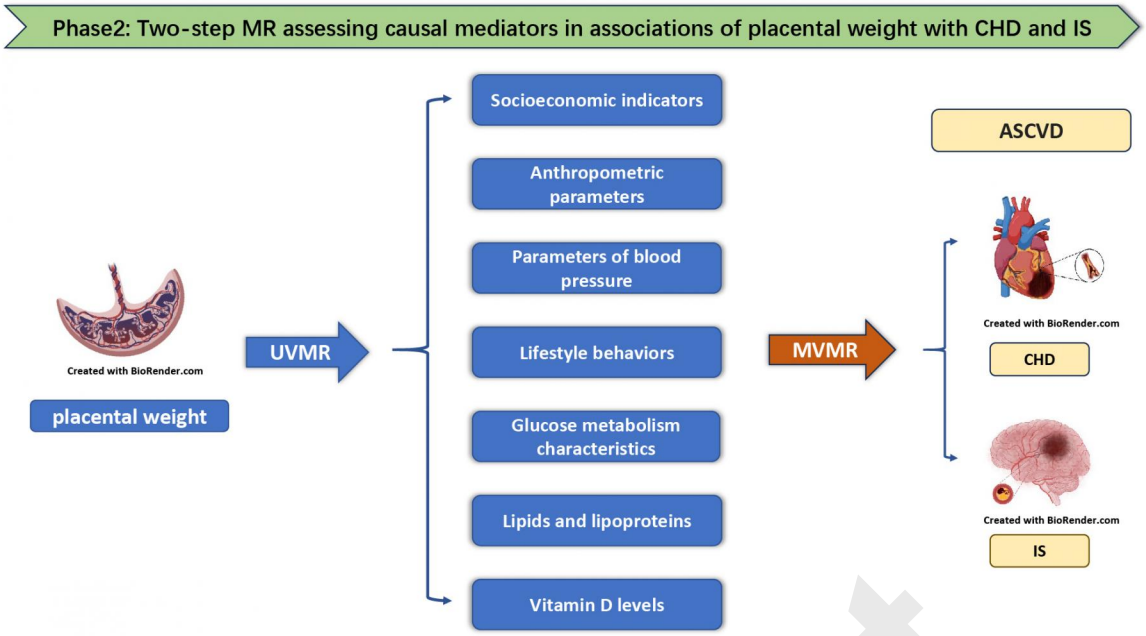


Conclusions

This study elucidates a unidirectional causal relationship between lower PW and higher ASCVD risk, with factors such as birthweight, type 2 diabetes, and education mediating the association between PW and ASCVD.

Overview of the MR study design. In Step 1, the causal effects of PW with CHD and IS were estimated using UVMR. In Step 2, two-step MR was applied to screen for 21 candidate mediators between PW and CHD or IS. *UVMR: Univariable Mendelian randomization; PW: Placental weight; CHD: Coronary heart disease; IS: Ischemic stroke; LSA :Large artery stroke; MVMR: Multivariable Mendelian randomization*


Phase1: UVMR assessing causal effect of placental weight on CHD and IS
Exposures
Outcomes
Created with BioRender.com
Placental Weight
EGG Consortium
fetal (n = 65,405), maternal (n = 61,228) and paternal (n = 52,392)
placental weight adjusted only for fetal sex
Fetal genotype
PW adjusted adjusted only for fetal sex
32 instrumental variables(IVs)
placental weight adjusted for sex and gestational age
Fetal genotype
PW adjusted for fetal sex and gestational age
28 instrumental variables(IVs)
UVMR
ASCVD
Created with BioRender.com
CHD
CARDIoGRAMplusC4D
A meta-analysis of 48 studies
60,801 cases and 123,504 controls
Created with BioRender.com
IS
MEGASTROKE consortium
67,162 stroke cases and 454,450 controls
Ischemic Stroke subtypes:
large artery strokes (LAS) (n =4, 373)
small vessel strokes (SVS) (n = 5,386)


Phase2: Two-step MR assessing causal mediators in associations of placental weight with CHD and IS
Created with BioRender.com
placental weight
UVMR
Socioeconomic indicators
Anthropometric parameters
Parameters of blood pressure
Lifestyle behaviors
Glucose metabolism characteristics
Lipids and lipoproteins
Vitamin D levels
MVMR
ASCVD
Created with BioRender.com
CHD
Created with BioRender.com
IS

Exposure	Outcome	OR(95%CI)	pvalue
Placental weight(fetal genotype, adjusted for sex)	Coronary heart disease	1.24 (1.05 , 1.46)	1.1e-02
Placental weight(fetal genotype, adjusted for sex and gestational age)	Coronary heart disease	1.20 (1.00 , 1.43)	4.9e-02

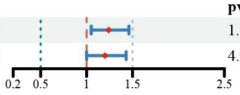


Figure 2-A. Causal association between lower placental weight with coronary heart disease

Exposure	Outcome	OR(95%CI)	pvalue
Placental weight(fetal genotype, adjusted for sex)	Ischemic stroke (large artery atherosclerosis)	1.46 (1.13 , 1.89)	3.9e-03
	Ischemic stroke (small-vessel)	1.02 (0.77 , 1.35)	9.1e-01
Placental weight(fetal genotype, adjusted for sex and gestational age)	Ischemic stroke (large artery atherosclerosis)	1.38 (1.06 , 1.80)	1.6e-02
	Ischemic stroke (small-vessel)	0.93 (0.70 , 1.23)	6.0e-01

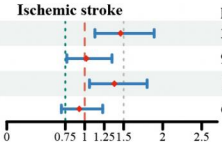


Figure 2-B. Causal association between lower placental weight with ischemic stroke

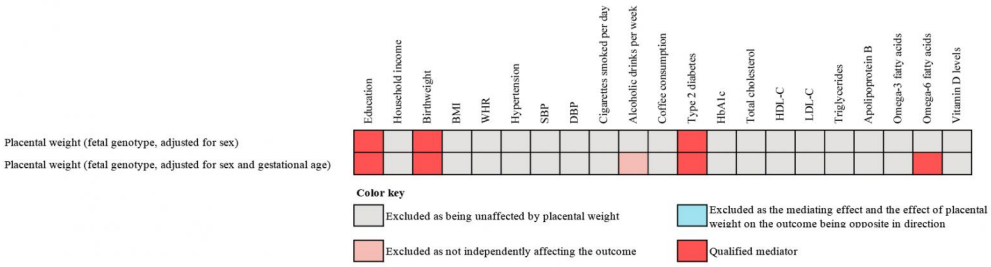


Figure 3-A. The qualified mediators in the mediation analysis of PW with CHD.

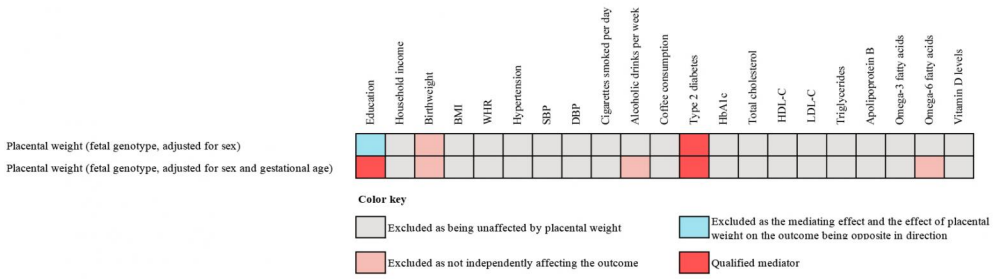


Figure 3-B.The qualified mediators in the mediation analysis of PW with IS.

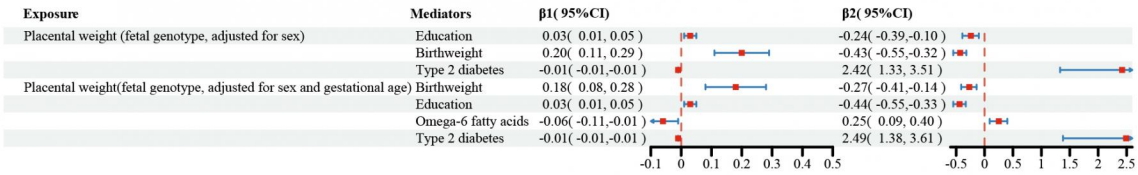


Figure 4-A. The mediating effect of each qualified mediator in the causal associations of PW with CHD

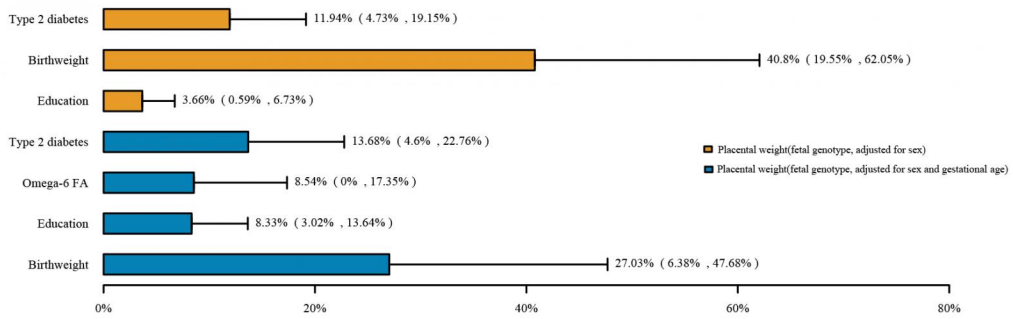


Figure 4-B. Proportion of mediation mediated by each qualified mediator for coronary heart disease

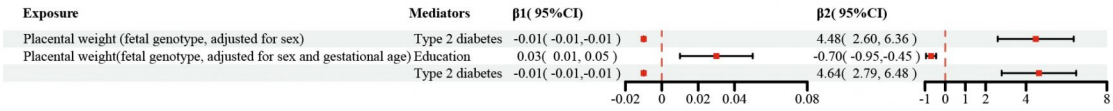


Figure 5-A. The mediating effect of each qualified mediator in the causal associations of PW with LAS(large artery atherosclerosis)

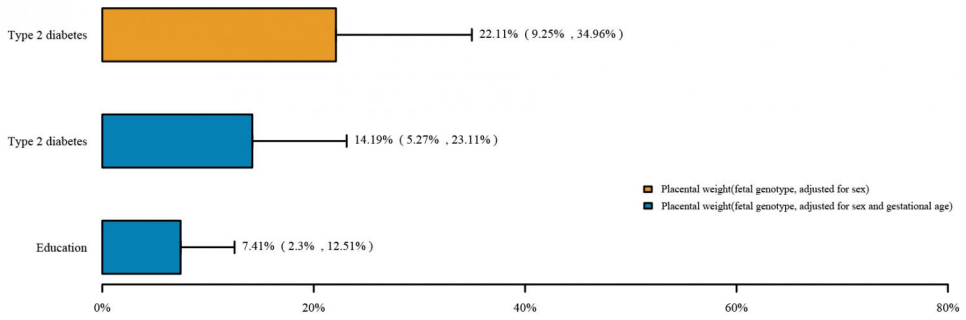


Figure 5-B. Proportion of mediation mediated by each qualified mediator for large artery atherosclerosis

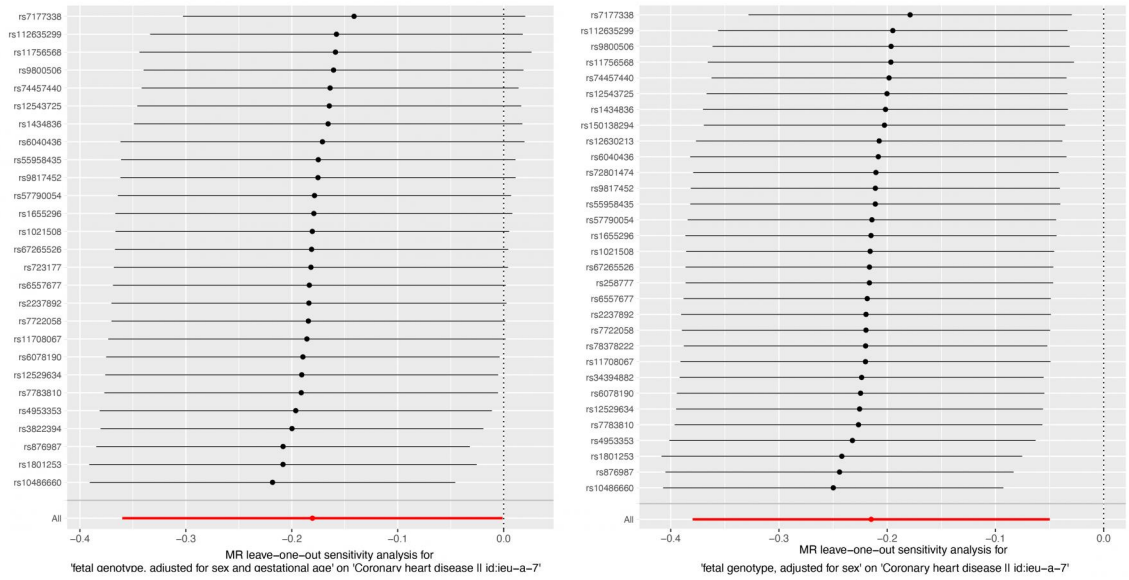


Fig. S1. Leave-one-out analysis for the associations of placental weight with coronary heart disease

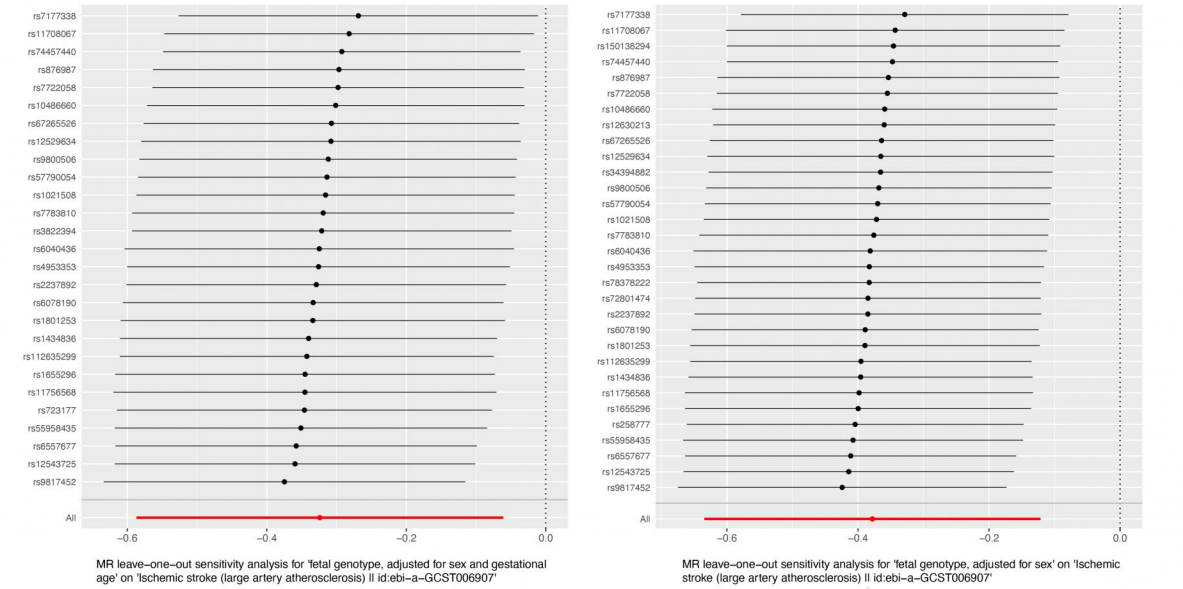


Fig. S2. Leave-one-out analysis for the associations of placental weight with ischemic stroke (large artery atherosclerosis)