Gut microbiota and hypertensive disorders in pregnancy: evidence from the Mendelian randomization study

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ABSTRACT

Background: Recent studies have shown that gut microbiota (GM) is related to hypertensive disorders in pregnancy (HDP). However, the causal relationship needs to be treated with caution due to confounding factors and reverse causation.

Methods: We obtained genetic variants from genome-wide association studies including GM (N = 18,340) in MiBioGen Consortium as well as HDP (7,686 cases/115,893 controls) and specific subtypes in FinnGen Consortium. Then, Inverse variance weighted, maximum likelihood, weighted median, MR-Egger, and MR.RAPS methods were applied to examine the causal association. Reverse Mendelian randomization (RMR) and multivariable MR were performed to confirm the causal direction and adjust the potential confounders, respectively. Furthermore, sensitivity analyses including Cochran's Q statistics, MR-Egger intercept, MR-PRESSO global test, and the leave-one-out analysis were conducted to detect the potential heterogeneity and horizontal pleiotropy.

Results: The present study found causalities between eight gut microbial genera and HDP. The HDP-associated gut microbial genera identified by MR analyses varied in different subtypes. Specifically, our study found causal associations of *LachnospiraceaeUCG010, Olsenella, RuminococcaceaeUCG009, Ruminococcus2, Anaerotruncus, Bifidobacterium*, and *Intestinibacter* with GH, of *Eubacterium* (*ruminantium group*), *Eubacterium* (*ventriosum group*), *Methanobrevibacter, RuminococcaceaeUCG002*, and *Tyzzerella3* with PE, and of *Dorea* and *RuminococcaceaeUCG010* with eclampsia, respectively.

Conclusions: This study first applied the MR approach to detect the causal relationships between GM and specific HDP subtypes. Our findings may promote the prevention and treatment of HDP targeted on GM and provide valuable insights to understand the mechanism of HDP in different subtypes from the perspective of GM.

INTRODUCTION

Hypertensive disorders in pregnancy (HDP), defined as elevated blood pressure that occurs for the first time during pregnancy, are serious complications that affect 4.1-19.4% of pregnant women globally [1]. After years of prevention and intervention, HDP remains the second leading cause of maternal and prenatal mortality [2]. Although various mechanisms including oxidative stress [3], chronic uterine placental ischemia [4], immune dysregulation [5], and vascular endothelial dysfunction [6] have been studied, the explicit pathogenesis of HDP has not been fully elucidated.

Gut microbiota (GM) has been observed to change significantly during gestation and is crucial for maintaining host physiology and homeostasis [7]. Mounting evidence demonstrated the gut microbiota

dysbiosis in HDP patients. For example, Chen et al. reported that PE (pre-eclampsia) patients have a lower diversity of GM with some beneficial genera reduced such as *Faecalibacterium* and *Akkermansia* [8]. Another nested case-control study demonstrated the difference in GM composition in early pregnancy between HDP patients and healthy controls [9]. However, these findings had some limitations. Firstly, the environment of the human intestine is very complex and often affected by various factors, some covariates that cannot be measured may cause confounders. Secondly, most existing results came from observational studies, the timing of exposure and outcome remains unclear and it's easy to cause reverse causal association. Furthermore, the previous studies are mainly focused on PE patients, ignoring other subtypes which have different pathogenesis and degree of organ damage. Therefore, it is crucial to explore the possible causal association between GM and HDP in specific subtypes after confounders and reverse causation well controlled.

Mendelian randomization (MR) is a useful method for causal inference using genetic variants (e.g. Single Nucleotide Polymorphisms, SNP) as instrumental variables (IVs) [10]. Because the alleles from parents to offspring are randomly assigned, freely combined and the genotypes remain stable after birth. MR is regarded as the "most natural" randomized controlled trial (RCT), and its advantages such as reducing confounding factors as well as excluding reverse causality provide an effective way for causal inference based on observational studies [11, 12]. Furthermore,

the ability and accuracy of genetic variants detection in genome-wide association studies (GWAS) have been greatly improved, and the measurement error has been reduced compared to conventional research [13]. Many studies have used MR analysis to explore the correlation between GM and some complex human diseases [14–16]. Therefore, our study performed a bidirectional multivariable MR analysis using the GWAS summary statistics to detect the causal relationship between GM and different subtypes of HDP, which may provide novel insights to understand the mechanism of HDP.

RESULTS

A total of 7,121 SNPs associated with 119 bacterial genera were included for GM instruments. The characters of selected IVs were shown in Supplementary Table 1.

Forward MR analyses

HDP
Results at a significant threshold of $P < 0.05$ by using the inverse-variance weighted (IVW) method were shown in Figure 1. We found a causal association of increase in *RuminococcaceaeUCG009* ($OR = 1.18$, $95\%CI$: 1.03-1.34, $P = 0.015$) and higher risk of HDP, while genetically increased in *Bifidobacterium* ($OR = 0.81$, $95\%CI$: 0.68-0.97, $P = 0.022$), *Eubacterium* (*ruminantium group*) ($OR = 0.81$, $95\%CI$: 0.69-0.96, $P = 0.012$), *Intestinibacter* ($OR = 0.83$, $95\%CI$: 0.72-0.96, $P = 0.011$), *Parabacteroides* ($OR = 0.75$, $95\%CI$:
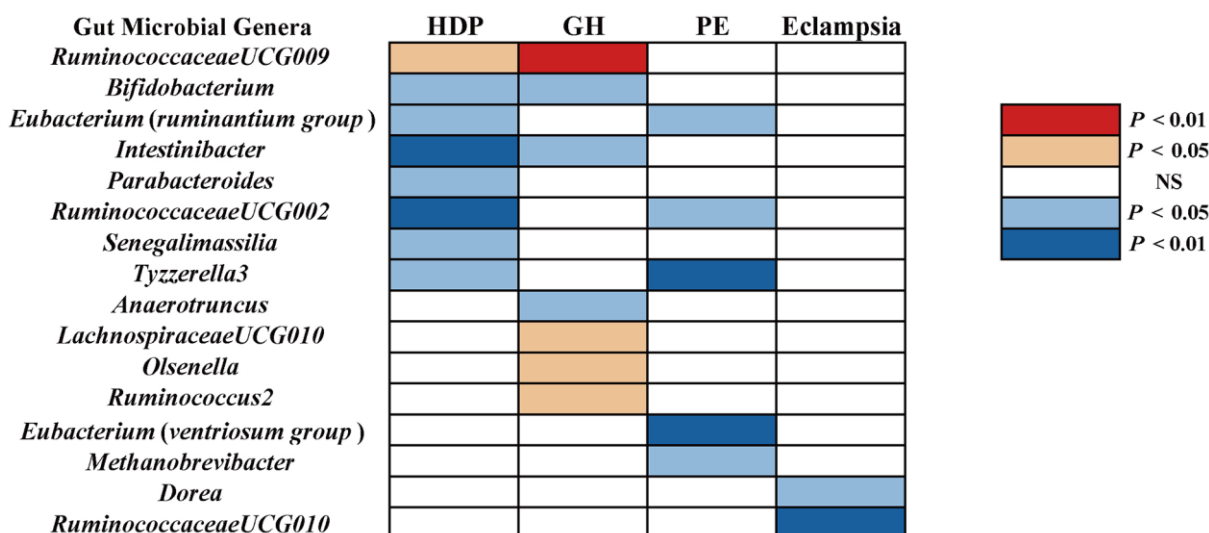

Figure 1. The causal effect of gut microbial genera on RDP (GR, PE, and Eclampsia) identified at the nominal significance by using the IVW method ($P < 0.05 / 0.01$). Red represents the risk factors for RDP, blue represents the protective factors for RDP, and white represents no causal association. RDP, hypertensive disorders in pregnancy; GR, gestational hypertension; PE, pre-eclampsia; NS, No significant association.

(*OR* = 0.75, 95%*CI*: 0.57-0.99, *P* = 0.047), *RuminococcaceaeUCG002* (*OR* = 0.84, 95%*CI*: 0.74-0.96, *P* = 0.011), *Senegalimassilia* (*OR* = 0.80, 95%*CI*: 0.65-0.98, P = 0.033), and *Tyzzerella3* (*OR* = 0.87, 95%*CI*: 0.77-0.99, *P* = 0.039) were associated with protective effects on HDP. These causal associations, however, lost their significance when multiple comparisons were adjusted (*q* > 0.1). The *F*-statistics ranged from 142.45 to 242.46 among all the results above, excluding the weak IVs bias. Details of all the IVW results were shown in Supplementary Table 2.

GH
Using the IVW method, we found suggestive causal associations of increases in *LachnospiraceaeUCG010* (*OR* = 1.29, 95%*CI*: 1.00-1.66, *P* = 0.049), *Olsenella* (*OR* = 1.15, 95%*CI*: 1.02-1.31, *P* = 0.028), *RuminococcaceaeUCG009* (*OR* = 1.27, 95%*CI*: 1.08-1.49, *P* = 0.005), and *Ruminococcus2* (*OR* = 1.25, 95%*CI*: 1.03-1.51, *P* = 0.022) and higher risk of GH, while genetically increased in *Anaerotruncus* (*OR* = 0.74, 95%*CI*: 0.55-0.99, *P* = 0.047), *Bifidobacterium* (*OR* = 0.80, 95%*CI*: 0.65-0.98, *P* = 0.030), and *Intestinibacter* (*OR* = 0.80, 95%*CI*: 0.67-0.97, *P* = 0.023) were related to protective effects on GH (Figure 1). However, causal associations lost their significance when multiple comparisons were adjusted. Details of all the IVW results were shown in Supplementary Table 3. The *F*-statistics ranged from 144.51 to 205.87 among all the results above. Additionally, causal associations between GM and GH risk were found in more than three MR methods (Table 1 and Figure 2), including IVW, Maximum Likelihood (MaxLik), Weighted Median (WM), MR-Egger regression, and MR robust adjusted profile score (MR.RAPS).

PE
We found five suggestive causal effects of GM on PE (*P* < 0.05, *q* > 0.1; Figure 1). Specifically, *Eubacterium* (*ruminantium group*) (*OR* = 0.86, 95%*CI*: 0.75-0.99, *P* = 0.045), *Eubacterium* (*ventriosum group*) (*OR* = 0.74, 95%*CI*: 0.59-0.93, *P* = 0.011), *Methanobrevibacter* (*OR* = 0.79, 95%*CI*: 0.65-0.96, *P* = 0.019), *RuminococcaceaeUCG002* (*OR* = 0.80, 0.65-0.98, *P* = 0.029), and *Tyzzerella3* (*OR* = 0.80, 95%*CI*: 0.68-0.93, *P* = 0.003) were negatively associated with the risk of PE. Details of all the IVW results were shown in Supplementary Table 4. The *F*-statistics ranged from 147.81 to 179.04 among all the results above. Furthermore, causal associations between GM and PE risk were found in more than three MR methods (Table 1 and Figure 3).

Eclampsia
We found suggestive causal effects of GM on Eclampsia in two microbial genera, including *Dorea*

(*OR* = 0.27, 95%*CI*: 0.09-0.76, *P* = 0.014) and *RuminococcaceaeUCG010* (*OR* = 0.20, 95%*CI*: 0.07-0.58, *P* = 0.003), which were negatively associated with the risk of eclampsia (Figure 1). Details of all the IVW results were shown in Supplementary Table 5. The *F*-statistics were 174.09 and 151.49, respectively, Furthermore, causal associations between GM and eclampsia risk were found in more than two of the MR methods (Table 1 and Figure 3).

Sensitivity analyses

Cochran's Q statistics showed no significant heterogeneity in selected IVs (*P* > 0.05 in IVW and MR-Egger methods, Supplementary Table 6). Both the MR-Egger intercept and the MR-PRESSO global test confirmed there is no significant directional horizontal pleiotropy (*P* > 0.05, Supplementary Table 6). Additionally, the leave-one-out analysis revealed that there are no outlier IVs that would have a significant impact on the result if retained (Supplementary Figures 1–3).

Reverse MR analyses

We performed the reverse MR analysis to assess whether specific HDP subtypes causally affect gut microbiota to confirm the causal direction. However, all methods showed no causal relationship except for the genus *Bifidobacterium* (*P* > 0.05, Supplementary Table 7). The sensitivity analyses including Cochran's Q test, MR-Egger regression intercept, MR-PRESSO global test, and the leave-one-out sensitivity analysis confirmed the robustness of the reverse MR results (Supplementary Table 8 and Supplementary Figures 4–6).

Multivariable MR analyses

MVMR analysis was performed to assess the causal effect of GM on GH, PE, and eclampsia, respectively after confounding factors were adjusted (BMI, alcohol drinking, smoking, and T2D). For the genus *Intestinibacter*, after adjusting for BMI (*OR* = 0.77, 95%*CI*: 0.67-0.88, *P* < 0.001), alcohol drinking (*OR* = 0.76, 95%*CI*: 0.67-0.85, *P* < 0.001), smoking (*OR* = 0.84, 95%*CI*: 0.70-0.99, *P* = 0.049), and T2D (*OR* = 0.81, 95%*CI*: 0.72-0.92, *P* = 0.001), *Intestinibacter* remained causally associated with GH risk (Figure 4). Detailed MVMR results of other suggestive association GM on HDP subtypes were shown in Table 2.

DISCUSSION

In this multivariable MR study, we detected causal associations between eight particular bacterial genera and the risk of HDP, then replicated

Table 1. MR analyses of gut microbiota on HDP subtypes by different methods.

Exposure	Outcome	F-Stat	Inverse variance weighted		Maximum likelihood		Weighted median		MR.RAPS		MR Egger	
			OR (95%CI)	P	OR (95%CI)	P	OR (95%CI)	P	OR (95%CI)	P	OR (95%CI)	P
Anaerotruncus	GH	166.34	0.74 (0.55,0.99)	0.047	0.74 (0.58,0.95)	0.019	0.86 (0.61,1.23)	0.410	0.68 (0.54,0.86)	0.001	0.54 (0.23,1.31)	0.203
Bifidobacterium	GH	139.63	0.80 (0.65,0.98)	0.030	0.80 (0.66,0.97)	0.026	0.88 (0.65,1.19)	0.401	0.79 (0.66,0.95)	0.014	0.53 (0.32,0.86)	0.027
Intestinibacter	GH	159.39	0.80 (0.67,0.97)	0.023	0.81 (0.66,0.98)	0.027	0.76 (0.59,0.97)	0.028	0.80 (0.66,0.98)	0.031	0.62 (0.34,1.14)	0.150
Lachnospiraceae UCG010	GH	144.51	1.29 (1.00,1.66)	0.049	1.31 (1.01,1.69)	0.046	1.28 (0.90,1.83)	0.168	1.18 (0.92,1.50)	0.188	1.15 (0.53,2.51)	0.731
Olsenella	GH	205.87	1.15 (1.02,1.31)	0.028	1.16 (1.02,1.32)	0.028	1.20 (1.01,1.42)	0.035	1.16 (1.01,1.32)	0.038	1.05 (0.70,1.58)	0.810
Ruminococcaceae UCG009	GH	177.26	1.27 (1.08,1.49)	0.005	1.28 (1.08,1.52)	0.005	1.22 (0.97,1.54)	0.085	1.20 (1.03,1.41)	0.023	1.70 (0.88,3.29)	0.143
Ruminococcus2	GH	156.71	1.25 (1.03,1.51)	0.022	1.25 (1.03,1.52)	0.022	1.21 (0.91,1.62)	0.190	1.26 (1.03,1.54)	0.026	1.50 (0.95,2.37)	0.106
Eubacterium (*ruminantium group*)	PE	157.34	0.86 (0.75,0.99)	0.045	0.86 (0.74,1.00)	0.049	0.90 (0.74,1.10)	0.314	0.89 (0.77,1.03)	0.118	1.24 (0.77,2.01)	0.391
Eubacterium (*ventriosum group*)	PE	160.92	0.74 (0.59,0.93)	0.011	0.75 (0.59,0.94)	0.014	0.72 (0.53,0.98)	0.034	0.75 (0.59,0.94)	0.015	0.68 (0.24,1.90)	0.473
Methanobrevibacter	PE	179.04	0.79 (0.65,0.96)	0.019	0.79 (0.65,0.96)	0.020	0.80 (0.63,1.02)	0.070	0.79 (0.66,0.95)	0.014	0.55 (0.26,1.13)	0.180
Ruminococcaceae UCG002	PE	166.26	0.80 (0.65,0.98)	0.029	0.80 (0.66,0.97)	0.021	0.80 (0.61,1.05)	0.105	0.85 (0.71,1.02)	0.075	0.90 (0.52,1.55)	0.702
Tyzzerella3	PE	147.81	0.80 (0.68,0.93)	0.003	0.79 (0.68,0.93)	0.004	0.77 (0.63,0.95)	0.015	0.79 (0.68,0.92)	0.003	0.73 (0.29,1.80)	0.506
Dorea	Eclampsia	174.09	0.27 (0.09,0.76)	0.014	0.26 (0.09,0.75)	0.014	0.46 (0.10,2.14)	0.323	0.16 (0.06,0.46)	0.001	1.07 (0.06,20.03)	0.963
Ruminococcaceae UCG010	Eclampsia	151.49	0.20 (0.07,0.58)	0.003	0.20 (0.07,0.60)	0.004	0.24 (0.06,1.00)	0.050	0.30 (0.11,0.83)	0.020	0.20 (0.01,3.67)	0.340

GH, gestational hypertension; PE, pre-eclampsia; *F*-stat, *F* statistics to detect weak instrumental variable bias; MR.RAPS, Mendelian randomization robust adjusted profile score; *OR*, odds ratio; *CI*, confidence interval; *P*, *P* value.

the analyses in specific subtypes (GH, PE, and eclampsia). Specifically, we identified suggestive causal associations of *LachnospiraceaeUCG010, Olsenella, RuminococcaceaeUCG009, Ruminococcus2, Anaerotruncus, Bifidobacterium*, and *Intestinibacter* with GH, of *Eubacterium* (*ruminantium group*), *Eubacterium* (*ventriosum group*), *Methanobrevibacter, RuminococcaceaeUCG002*, and *Tyzzerella3* with PE, as well as of *Dorea* and *RuminococcaceaeUCG010* with eclampsia. For example, our MR analyses revealed a protective effect of *Bifidobacterium* on GH. A case-control study including 170 women in early pregnancy found that the relative abundance of *Bifidobacterium* significantly decreases in HDP patients compared with the control group [17], which was consistent with the previous studies on hypertension patients in Tangshan and Henan [18, 19]. It has been reported that *Bifidobacterium* can restore intestinal barrier function by stimulating the expression of Mucins 3 [20]. The Mice infection model also supported the role of *Bifidobacterium* in maintaining barrier permeability by reducing the concentration of Shiga toxin in *enterohemorrhagic E. coli* strains [21]. Furthermore, treatment with *Bifidobacterium bifidum* significantly lowered the rates of bacterial translocation [22], and stopped the entry of GM-derived lipopolysaccharide (LPS) into blood [23], thereby reducing placental inflammation and maintaining normal blood pressure. All the evidence above supported the protective role of *Bifidobacterium* on GH.

In addition, we also found the genus *Intestinibacter* to be associated with a lower risk of GH. There have been relatively few previous studies on *Intestinibacter*, but observational study and animal model have both reported the role of *Intestinibacter* in producing butyrate [24, 25], which is a short chain fatty acid (SCFA) metabolized by GM. Placental inflammation and angiogenic factors played the central role in affecting blood pressure in pregnancy, and macrophages are the key regulator [26]. *In vivo* and *in vitro* experiments found that butyrate significantly reduces the effects of LPS to promote macrophage 1 polarization and inhibit macrophage 2 polarization, thereby reducing blood pressure [27, 28]. Furthermore, Jin et al. reported that butyrate promotes the effect on macrophage autophagy by decreasing autophagy
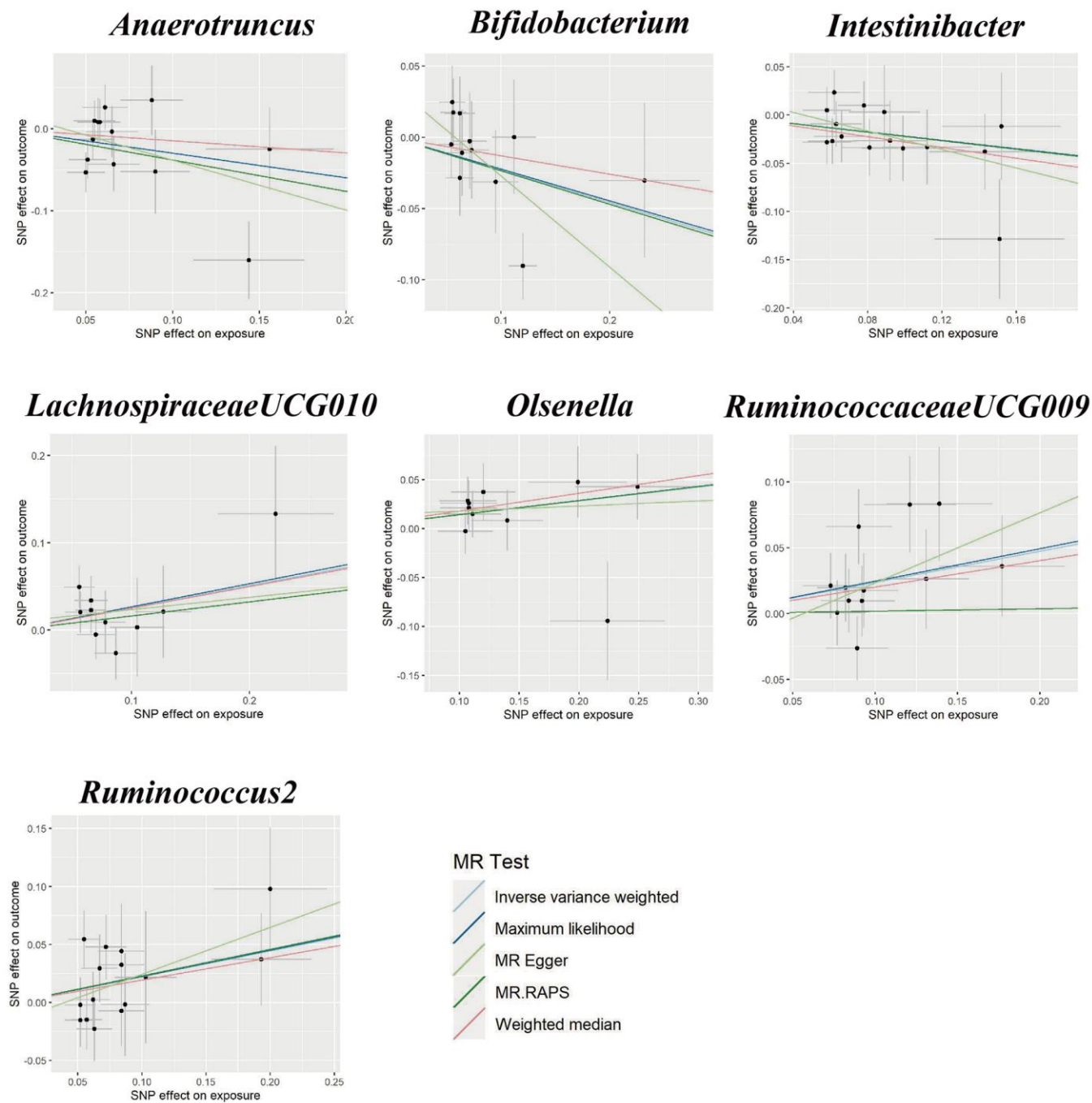

Figure 2. Scatter plots for the causal relationship between gut microbiota and gestational hypertension.

receptors like P62 level and elevating LC3-II/LC3-I ratio, thus alleviating PE symptoms in rats [29]. The higher abundance of butyrate has been reported to decrease the risk of insulin resistance and type 2 diabetes(T2D) [30] and T2D is positively associated with blood pressure, suggesting that the effect of *Intestinibacter* on GH may be biased by T2D. But our multivariable MR analysis demonstrated that after adjusting T2D, the protective effect remained, which excluding the influence of confounding factors. Meanwhile, *Bifidobacterium* [9], *Eubacterium* (*ruminantium group*) [31], *Tyzzerella3* [32], and *Dorea* [33] have also been reported to produce
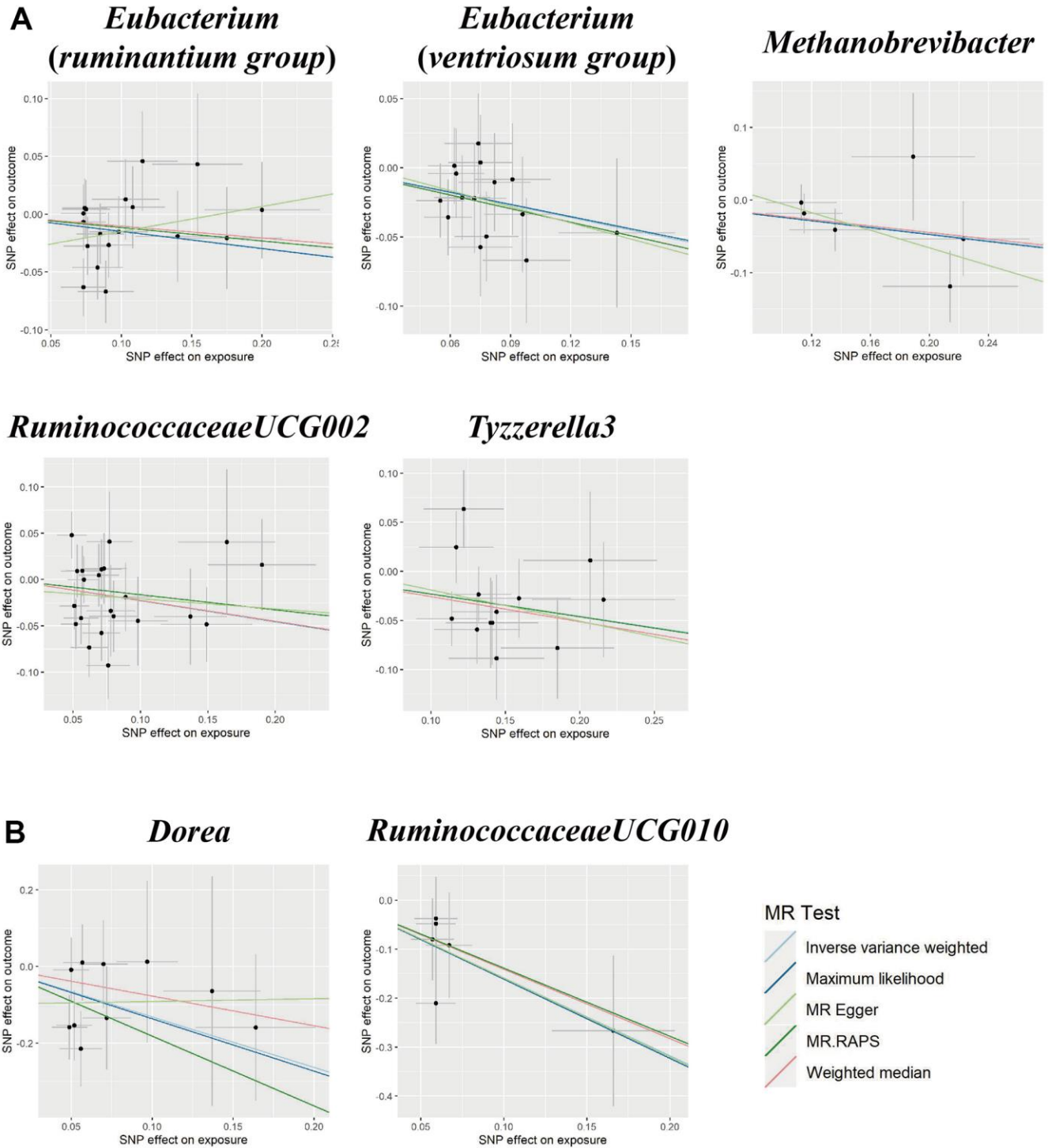
Figure 3. Scatter plots for the causal association between gut microbiota and (**A**) pre-eclampsia or (**B**) eclampsia.

SCFA with the function to effectively reduce blood pressure [34, 35], which consistent with our MR results that the increased abundance of those gut microbiota were related to the lower risk of HDP.

Interestingly, the HDP-associated gut microbial genera identified by our MR analyses varied in different subtypes. For example, we didn't find the relationships between *Bifidobacterium* and other HDP subtypes except for GH, which was consistent with another MR analysis on the gut microbiota and adverse pregnancy outcomes [16], while Li et al. reported the opposite result [36]. It may, because of the different pathogenesis, biochemical index, and degree of organ damage in GH, PE, and eclampsia. Additionally, our study demonstrated that *RuminococcaceaeUCG009* increases the risk of GH, while *Rumino-coccaceaeUCG002* and *RuminococcaceaeUCG010* were found to be protective factors to PE and eclampsia, respectively. Some genera of *Ruminococcaceae* are beneficial SCFA-producing bacteria that could not only power the intestinal epithelial cells [37] but also reduce proinflammatory cytokine by monocytes [38]. For example, in population-based studies, the abundance of genus *RuminococcaceaeUCG002* was found to have benefi-cial implications for host glucose homeostasis and lipid metabolism [39, 40] as well as *Ruminococcaceae UCG010* was found to be fewer in hypertension patients compared with health group [41]. However, *RuminococcaceaeUCG009* was found to be positively correlated with the production of inflammatory factors and LPS in serum thus may cooperatively contribute to HDP, which supported our result [42]. Thus, our findings opened up new possibilities for understanding the differences in gut microbial genera mediating mechanisms in various subtypes of HDP. Considering the different effects of the same gut microbial genera (e.g., *Ruminococcaceae*) on human blood pressure, further RCTs at a more specific species level are needed to support this finding.

The present study has some strengths. It was the first multivariable MR analysis to explore the causal relationship between GM and HDP subtypes and find the difference of causal-related GM in GH, PE, and eclampsia. The findings would facilitate the targeted prevention and treatment of different subtypes. Secondly, our study was based on the largest GWAS summary datasets to date, along with bidirectional MR, multivariable MR analyses, and several sensitivity analyses, which indicates the robustness of our findings. Thirdly, confounding variables and reverse causation were less likely to have an impact on the causal inference by using the MR design.

Our analysis still has several limitations. Firstly, the significance threshold of exposure IVs was set at 1e-05 because of insufficient IVs under genome-wide significance. However, IVs with F-statistics < 10 were excluded to avoid the weak instrumental bias. Secondly, MR analyses could only conduct at the bacterial genus level rather than at a more specific species level because of limited 16S rRNA sequencing resolution. Thirdly, our research was unable to provide further mechanisms for the distinct gut microbiota taxa associated with GH, PE, and eclampsia which need subsequent functional studies to elucidate.
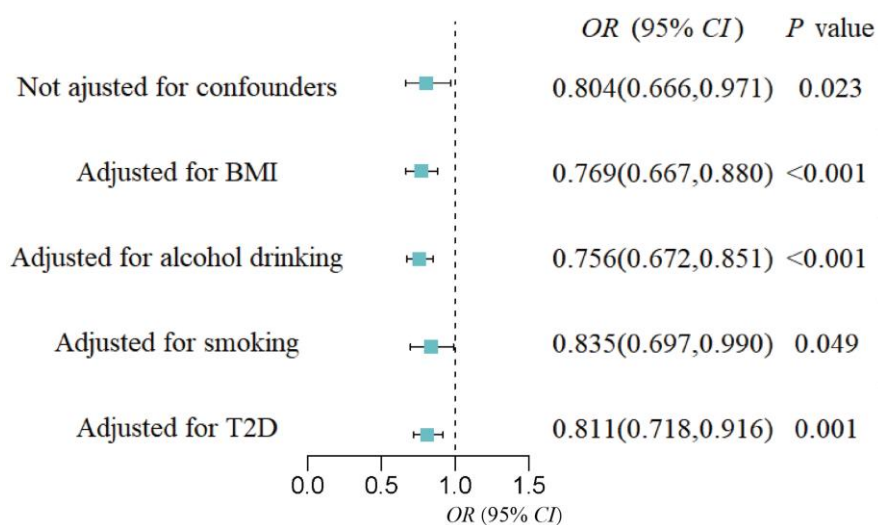

Figure 4. Forest plot of the causal effect of the genus *Intestinibacter* on gestational hypertension after adjusting for confounders.

Table 2. Multivariable MR analyses of gut microbiota on HDP subtypes after adjusting confounding factors.

Exposure	Outcome	BMI		Alcohol drinking		Smoking		T2D	
		OR (95%CI)	*P*	*OR (95%CI)*	*P*	*OR (95%CI)*	*P*	*OR (95%CI)*	*P*
Anaerotruncus	GH	0.71(0.43,1.18)	0.187	0.76(0.52,1.11)	0.159	0.70(0.50,0.98)	0.035	0.91(0.71,1.15)	0.419
Bifidobacterium	GH	0.79(0.68,0.93)	0.004	0.76(0.63,0.91)	0.004	0.74(0.62,0.89)	0.001	0.77(0.54,1.08)	0.128
Intestinibacter	GH	0.77(0.67,0.88)	<0.001	0.76(0.67,0.85)	<0.001	0.84(0.70,0.99)	0.049	0.81(0.72,0.92)	0.001
Lachnospiraceae UCG010	GH	1.59(1.21,2.08)	0.001	1.15(0.84,1.56)	0.388	1.33(1.10,1.59)	0.003	1.13(1.06,1.19)	0.025
Olsenella	GH	1.14(0.93,1.42)	0.215	1.13(1.00,1.28)	0.049	1.12(1.01,1.25)	0.041	1.17(1.06,1.289)	0.003
Ruminococcaceae UCG009	GH	1.40(1.11,1.77)	0.005	1.28(1.04,1.57)	0.020	1.49(1.28,1.74)	<0.001	1.12(1.06,1.19)	<0.001
Ruminococcus2	GH	1.25(1.04,1.51)	0.021	1.18(0.94,1.47)	0.154	1.29(1.09,1.54)	0.004	1.24(0.84,1.84)	0.285
Eubacterium (*ruminantium group*)	PE	0.85(0.71,1.02)	0.082	0.89(0.77,1.03)	0.119	0.87(0.74,1.02)	0.085	0.81(0.65,1.02)	0.070
Eubacterium (*ventriosum group*)	PE	0.79(0.64,0.98)	0.026	0.73(0.63,0.85)	<0.001	0.76(0.63,0.93)	0.006	0.82(0.71,0.95)	0.007
Methanobrevibacter	PE	0.77(0.59,1.02)	0.067	0.77(0.66,0.91)	0.002	0.65(0.50,0.86)	0.002	0.76(0.64,0.90)	0.001
RuminococcaceaeUCG002	PE	1.13(0.87,1.48)	0.360	0.79(0.63,0.98)	0.034	0.80(0.65,0.98)	0.003	0.67(0.50,0.90)	0.008
Tyzzerella3	PE	0.76(0.69,0.83)	<0.001	0.88(0.76,1.02)	0.089	0.76(0.65,0.90)	0.001	0.87(0.21,1.20)	0.394
Dorea	Eclampsia	0.53(0.25,1.14)	0.106	0.30(0.12,0.72)	0.007	0.29(0.11,0.74)	0.010	0.22(0.06,0.82)	0.024
RuminococcaceaeUCG010	Eclampsia	0.01(0.00,0.01)	<0.001	0.31(0.09,1.03)	0.057	0.40(0.17,0.90)	0.027	20.61(1.71,247.96)	0.017

GH, gestational hypertension; PE, pre-eclampsia; *F*-stat, *F* statistics to detect weak instrumental variable bias; *OR*, odds ratio; *CI*, confidence interval; *P*, *P* value; BMI, body mass index; T2D, type 2 diabetes.

In conclusion, by performing bidirectional multivariable MR analyses on GWAS summary data, this study explored the causal relationship between GM and different subtypes of HDP. Our findings may offer a new strategy for prevention and treatment in different HDP subtypes by targeting the gut microbiota and provide novel insights to understand the mechanism of HDP.

MATERIALS AND METHODS

Data sources

GWAS summary statistics for GM were obtained from the Microbiome Genome (MiBioGen) Consortium which consisted of 24 multiple ancestry cohorts including 18,340 participants [43]. After extracting DNA from fecal samples, data were generated by 16S rRNA gene sequencing in the Illumina platform. Setting SILVA as the reference, all the data were annotated to genus and higher levels to profile the microbial composition [44].

According to the pathogenesis, biochemical index, and degree of organ damage, HDP could be divided into

five subtypes including gestational hypertension (GH), pre-eclampsia (PE), eclampsia and so on [45]. In this study, firstly we tested the whole HDP group and then primarily focused on GH, PE, and eclampsia patients because they are major or most serious HDP subtypes. GWAS summary statistics for HDP were extracted from the FinnGen Consortium and updated in 2023 [46]. Briefly, the study for HDP included 123,579 female subjects (7,686 cases and 115,893 controls) covering a total of 16,379,784 SNPs. The genetic association datasets consisted of 118,990 pregnant women (4,255 cases and 114,735 controls) with GH, 118,291 pregnant women (3,556 cases and 114,735 controls) with PE, and 115,025 pregnant women (290 cases and 114,735 controls) with eclampsia, respectively. Association analysis was conducted with sex, age, genotyping batch, and 10 principal components corrected as covariates. Detailed information on exposure and outcome GWAS datasets were summarized in Supplementary Table 9.

Instrumental variables

To satisfy the three key assumptions of MR analysis (Figure 5), five steps were applied to select the optimal

IVs: 1) SNPs under a locus-wide significance threshold of $P < 1e-05$ were obtained as potential IVs related to exposure [14]. 2) PLINK clumping method ($r^2 < 0.001$, clump window $< 10,000$ kb) was performed to ensure the IVs were independent [47]. 3) SNPs with minor allele frequency < 0.01 and palindromic SNPs were excluded. 4) The proxy SNPs ($r^2 > 0.8$) were selected based on European population data in the 1000 Genome project after removing the SNPs closely related to the outcome phenotype ($P < 5e-08$) [48]. 5) SNPs with F-statistics < 10 were eliminated to avoid weak IV bias [49].

Statistical analyses

We used the inverse-variance weighted (IVW) method as the primary MR analysis to detect the causal associations between exposure (GM) and outcomes (HDP, GH, PE, and eclampsia). The IVW method calculates the total causal effect by using the weighted linear regression model combined with the weight coefficient, under the condition that the intercept is zero [50]. IVW results were corrected for multiple comparisons applying the q-value procedure ($q < 0.1$), while $P < 0.05$ but $q > 0.1$ was considered to have a suggestive association [51].

Several MR methods including Maximum Likelihood (MaxLik), Weighted Median (WM), MR-Egger regression, and MR robust adjusted profile score (MR.RAPS) were also conducted to test the robustness of our study. MaxLik estimates the parameter values that have the greatest likelihood of leading to a particular outcome by using the known sample. Its standard error would be lower than IVW when heterogeneity and horizontal pleiotropy do not exist [52]. WM improves the power of causal effect

detection based on the assumption that up to 50% of IVs are valid [53]. MR-Egger regression method could identify and correct pleiotropy, but the estimation accuracy will be very low unless using a larger sample size [54]. MR.RAPS applies robust estimates to correct for systematic and idiosyncratic pleiotropy, the results of which are unbiased even though weak IVs exist [55].

Cochran's IVW Q statistics and leave-one-out analysis were used to identify potential heterogeneous IVs. MR-Egger intercept and MR Pleiotropy RESidual Sum and Outlier (MR-PRESSO) global test were conducted to test whether directional horizontal pleiotropy is driving the results of MR analyses [56, 57].

Reverse MR analysis was used to confirm the causal direction. The methods were similar to those of forward MR except for setting exposures as HDP subtypes and outcome as GM. Finally, we conducted multivariable MR (MVMR) analyses considering the possible confounders which may affect the outcome. The confounders including BMI (IEU number: ukb-b-19953), alcohol drinking (IEU number: ukb-b-5779), smoking (IEU number: ieu-b-4877), and T2D (IEU number: ebi-a-GCST006867).

The flowchart of this study was shown in Figure 6. All MR analyses were performed by the packages "TwoSampleMR", "MRPRESSO", and "qvalue" in R software.

Consent for publication

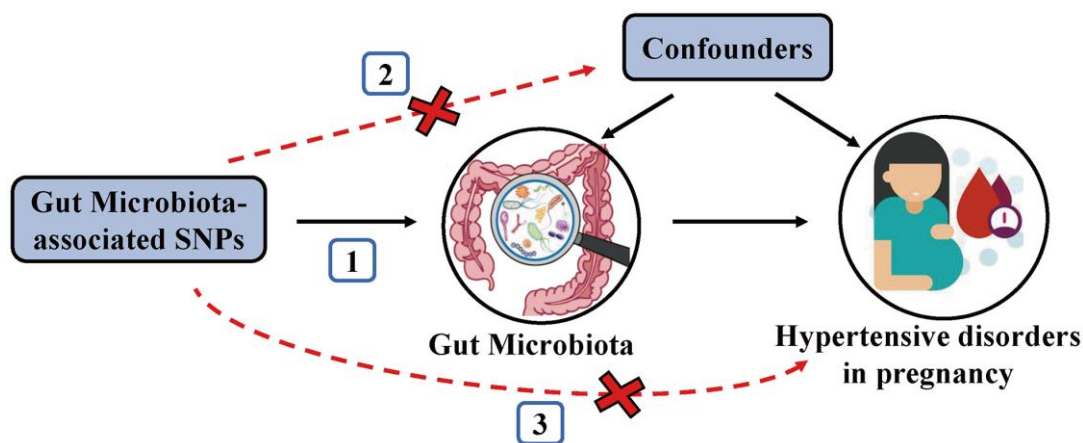All the authors endorsed the publication of the manuscript.

Figure 5. Schematic representation of the MR analysis. The three assumptions of MR are as follows: (1) Instrumental variables must be associated with gut microbiota, (2) Instrumental variables must not be associated with confounders; and (3) Instrumental variables must influence hypertensive disorders in pregnancy only through gut microbiota, not through other pathways.
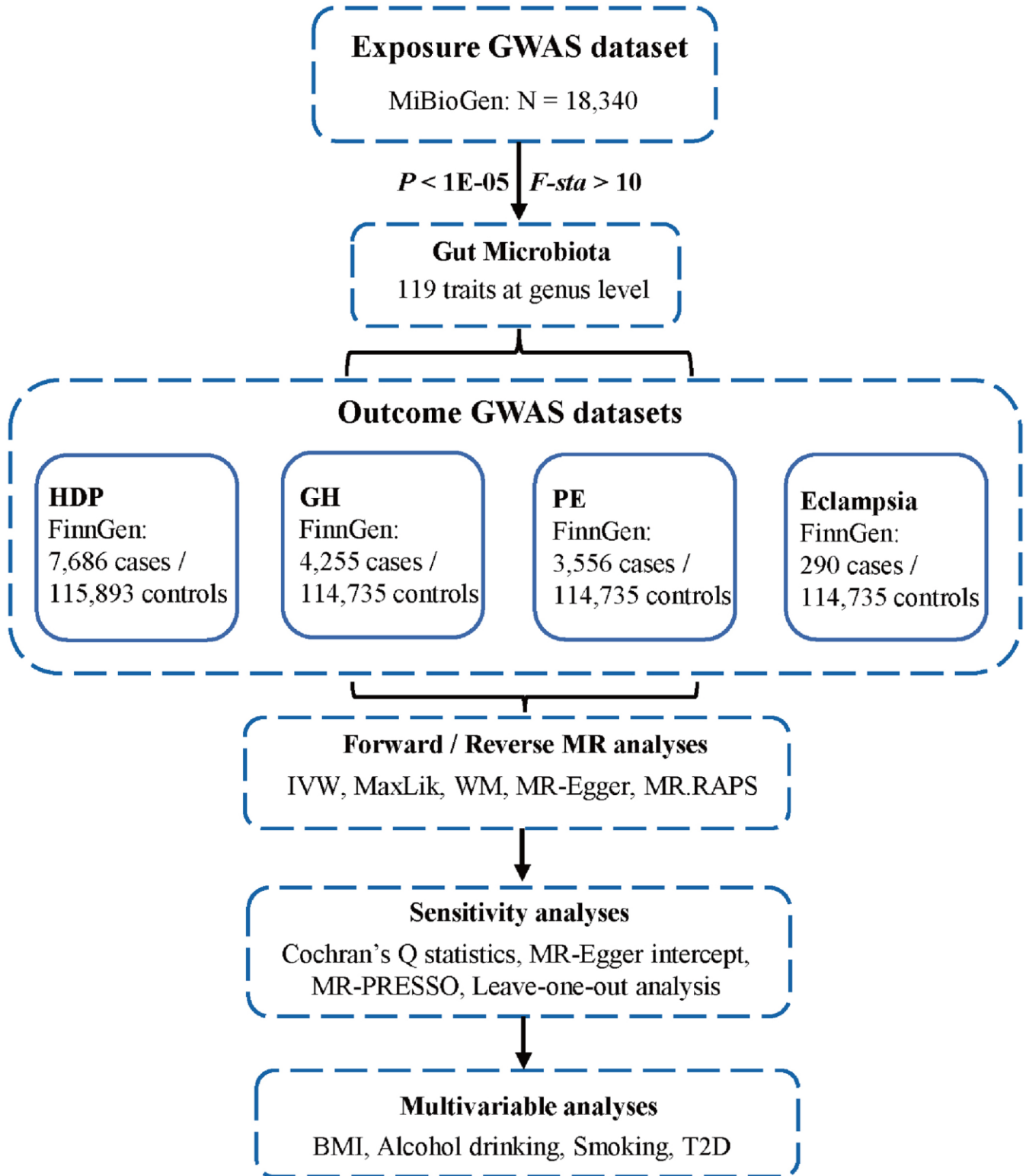
Figure 6. Flowchart of this study. GWAS, genome-wide association studies; HDP, hypertensive disorders in pregnancy; GH, gestational hypertension; PE, pre-eclampsia; MR, Mendelian randomization; IVW, inverse-variance weighted; MaxLik, maximum likelihood; WM, weighted median; MR.RAPS, MR robust adjusted profile score; MR-PRESSO, MR Pleiotropy RESidual Sum and Outlier; BMI, body mass index; T2D, type 2 diabetes.

AUTHOR CONTRIBUTIONS

XW conceived the study, obtained the data. XW and QL composed the article. QL and DL performed the statistical analysis. JC and HH prepared the figures and tables. HT conceived the study and reviewed the article. All authors approved the submitted version.

CONFLICTS OF INTEREST

The authors declared there are no conflicts of interest exist.

ETHICAL STATEMENT

No ethical statement is required because the study was based on GWAS publicly available data.

FUNDING

REFERENCES

1. Umesawa M, Kobashi G. Epidemiology of hypertensive disorders in pregnancy: prevalence, risk factors, predictors and prognosis. Hypertens Res. 2017; 40:213–20.
https://doi.org/10.1038/hr.2016.126
PMID:27682655

2. Vogel JP, Souza JP, Mori R, Morisaki N, Lumbiganon P, Laopaiboon M, Ortiz-Panozo E, Hernandez B, Pérez-Cuevas R, Roy M, Mittal S, Cecatti JG, Tunçalp Ö, Gülmezoglu AM, and WHO Multicountry Survey on Maternal and Newborn Health Research Network. Maternal complications and perinatal mortality: findings of the World Health Organization Multicountry Survey on Maternal and Newborn Health. BJOG. 2014 (Suppl 1); 121:76–88.
https://doi.org/10.1111/1471-0528.12633
PMID:24641538

3. Grum T, Seifu A, Abay M, Angesom T, Tsegay L. Determinants of pre-eclampsia/Eclampsia among women attending delivery Services in Selected Public Hospitals of Addis Ababa, Ethiopia: a case control study. BMC Pregnancy Childbirth. 2017; 17:307.
https://doi.org/10.1186/s12884-017-1507-1
PMID:28915802

4. Dekker GA, Sibai BM. Etiology and pathogenesis of preeclampsia: current concepts. Am J Obstet Gynecol. 1998; 179:1359–75.
https://doi.org/10.1016/s0002-9378(98)70160-7
PMID:9822529

5. Saito S, Shiozaki A, Nakashima A, Sakai M, Sasaki Y. The role of the immune system in preeclampsia. Mol Aspects Med. 2007; 28:192–209.
https://doi.org/10.1016/j.mam.2007.02.006
PMID:17433431

6. Levine RJ, Lam C, Qian C, Yu KF, Maynard SE, Sachs BP, Sibai BM, Epstein FH, Romero R, Thadhani R, Karumanchi SA, and CPEP Study Group. Soluble endoglin and other circulating antiangiogenic factors in preeclampsia. N Engl J Med. 2006; 355:992–1005.
https://doi.org/10.1056/NEJMoa055352
PMID:16957146

7. Goltsman DSA, Sun CL, Proctor DM, DiGiulio DB, Robaczewska A, Thomas BC, Shaw GM, Stevenson DK, Holmes SP, Banfield JF, Relman DA. Metagenomic analysis with strain-level resolution reveals fine-scale variation in the human pregnancy microbiome. Genome Res. 2018; 28:1467–80.
https://doi.org/10.1101/gr.236000.118
PMID:30232199

8. Chen X, Li P, Liu M, Zheng H, He Y, Chen MX, Tang W, Yue X, Huang Y, Zhuang L, Wang Z, Zhong M, Ke G, et al. Gut dysbiosis induces the development of pre-eclampsia through bacterial translocation. Gut. 2020; 69:513–22.
https://doi.org/10.1136/gutjnl-2019-319101
PMID:31900289

9. González-Rodríguez I, Gaspar P, Sánchez B, Gueimonde M, Margolles A, Neves AR. Catabolism of glucose and lactose in Bifidobacterium animalis subsp. lactis, studied by 13C Nuclear Magnetic Resonance. Appl Environ Microbiol. 2013; 79:7628–38.
https://doi.org/10.1128/AEM.02529-13
PMID:24077711

10. Smith GD, Ebrahim S. 'Mendelian randomization': can genetic epidemiology contribute to understanding environmental determinants of disease? Int J Epidemiol. 2003; 32:1–22.
https://doi.org/10.1093/ije/dyg070
PMID:12689998

11. Lawlor DA, Harbord RM, Sterne JA, Timpson N, Davey Smith G. Mendelian randomization: using genes as instruments for making causal inferences in epidemiology. Stat Med. 2008; 27:1133–63.
https://doi.org/10.1002/sim.3034
PMID:17886233

12. Didelez V, Sheehan N. Mendelian randomization as an instrumental variable approach to causal inference. Stat Methods Med Res. 2007; 16:309–30. https://doi.org/10.1177/0962280206077743 PMID:17715159

13. Evans DM, Davey Smith G. Mendelian Randomization: New Applications in the Coming Age of Hypothesis-Free Causality. Annu Rev Genomics Hum Genet. 2015; 16:327–50. https://doi.org/10.1146/annurev-genom-090314-050016 PMID:25939054

14. Sanna S, van Zuydam NR, Mahajan A, Kurilshikov A, Vich Vila A, Võsa U, Mujagic Z, Masclee AAM, Jonkers DM, Oosting M, Joosten LAB, Netea MG, Franke L, et al. Causal relationships among the gut microbiome, short-chain fatty acids and metabolic diseases. Nat Genet. 2019; 51:600–5. https://doi.org/10.1038/s41588-019-0350-x PMID:30778224

15. Jia J, Dou P, Gao M, Kong X, Li C, Liu Z, Huang T. Assessment of Causal Direction Between Gut Microbiota-Dependent Metabolites and Cardiometabolic Health: A Bidirectional Mendelian Randomization Analysis. Diabetes. 2019; 68:1747–55. https://doi.org/10.2337/db19-0153 PMID:31167879

16. Li C, Liu C, Li N. Causal associations between gut microbiota and adverse pregnancy outcomes: A two-sample Mendelian randomization study. Front Microbiol. 2022; 13:1059281. https://doi.org/10.3389/fmicb.2022.1059281 PMID:36590417

17. Yu J, Zhang B, Miao T, Hu H, Sun Y. Dietary Nutrition and Gut Microbiota Composition in Patients With Hypertensive Disorders of Pregnancy. Front Nutr. 2022; 9:862892. https://doi.org/10.3389/fnut.2022.862892 PMID:35464021

18. Li J, Zhao F, Wang Y, Chen J, Tao J, Tian G, Wu S, Liu W, Cui Q, Geng B, Zhang W, Weldon R, Auguste K, et al. Gut microbiota dysbiosis contributes to the development of hypertension. Microbiome. 2017; 5:14. https://doi.org/10.1186/s40168-016-0222-x PMID:28143587

19. Miao T, Yu Y, Sun J, Ma A, Yu J, Cui M, Yang L, Wang H. Decrease in abundance of bacteria of the genus *Bifidobacterium* in gut microbiota may be related to pre-eclampsia progression in women from East China. Food Nutr Res. 2021; 65. https://doi.org/10.29219/fnr.v65.5781 PMID:34262418

20. Bron PA, Kleerebezem M, Brummer RJ, Cani PD, Mercenier A, MacDonald TT, Garcia-Ródenas CL, Wells JM. Can probiotics modulate human disease by impacting intestinal barrier function? Br J Nutr. 2017; 117:93–107. https://doi.org/10.1017/S0007114516004037 PMID:28102115

21. Asahara T, Shimizu K, Nomoto K, Hamabata T, Ozawa A, Takeda Y. Probiotic bifidobacteria protect mice from lethal infection with Shiga toxin-producing Escherichia coli O157:H7. Infect Immun. 2004; 72:2240–7. https://doi.org/10.1128/IAI.72.4.2240-2247.2004 PMID:15039348

22. Johansson ME, Jakobsson HE, Holmén-Larsson J, Schütte A, Ermund A, Rodríguez-Piñeiro AM, Arike L, Wising C, Svensson F, Bäckhed F, Hansson GC. Normalization of Host Intestinal Mucus Layers Requires Long-Term Microbial Colonization. Cell Host Microbe. 2015; 18:582–92. https://doi.org/10.1016/j.chom.2015.10.007 PMID:26526499

23. Fan M, Li X, Gao X, Dong L, Xin G, Chen L, Qiu J, Xu Y. LPS Induces Preeclampsia-Like Phenotype in Rats and HTR8/SVneo Cells Dysfunction Through TLR4/p38 MAPK Pathway. Front Physiol. 2019; 10:1030. https://doi.org/10.3389/fphys.2019.01030 PMID:31507429

24. Zhang L, Wang Z, Zhang X, Zhao L, Chu J, Li H, Sun W, Yang C, Wang H, Dai W, Yan S, Chen X, Xu D. Alterations of the Gut Microbiota in Patients with Diabetic Nephropathy. Microbiol Spectr. 2022; 10:e0032422. https://doi.org/10.1128/spectrum.00324-22 PMID:35863004

25. Uddin MK, Mahmud MR, Hasan S, Peltoniemi O, Oliviero C. Dietary micro-fibrillated cellulose improves growth, reduces diarrhea, modulates gut microbiota, and increases butyrate production in post-weaning piglets. Sci Rep. 2023; 13:6194. https://doi.org/10.1038/s41598-023-33291-z PMID:37062780

26. Jung E, Romero R, Yeo L, Gomez-Lopez N, Chaemsaithong P, Jaovisidha A, Gotsch F, Erez O. The etiology of preeclampsia. Am J Obstet Gynecol. 2022; 226:S844–66. https://doi.org/10.1016/j.ajog.2021.11.1356 PMID:35177222

27. Ji J, Shu D, Zheng M, Wang J, Luo C, Wang Y, Guo F, Zou X, Lv X, Li Y, Liu T, Qu H. Microbial metabolite butyrate facilitates M2 macrophage polarization and function. Sci Rep. 2016; 6:24838. https://doi.org/10.1038/srep24838 PMID:27094081

28. Jiang X, Huang X, Tong Y, Gao H. Butyrate improves cardiac function and sympathetic neural remodeling

following myocardial infarction in rats. Can J Physiol Pharmacol. 2020; 98:391–9.
https://doi.org/10.1139/cjpp-2019-0531
PMID:31999473

29. Jin J, Gao L, Zou X, Zhang Y, Zheng Z, Zhang X, Li J, Tian Z, Wang X, Gu J, Zhang C, Wu T, Wang Z, Zhang Q. Gut Dysbiosis Promotes Preeclampsia by Regulating Macrophages and Trophoblasts. Circ Res. 2022; 131:492–506.
https://doi.org/10.1161/CIRCRESAHA.122.320771
PMID:35950704

30. Chen Z, Radjabzadeh D, Chen L, Kurilshikov A, Kavousi M, Ahmadizar F, Ikram MA, Uitterlinden AG, Zhernakova A, Fu J, Kraaij R, Voortman T. Association of Insulin Resistance and Type 2 Diabetes With Gut Microbial Diversity: A Microbiome-Wide Analysis From Population Studies. JAMA Netw Open. 2021; 4:e2118811.
https://doi.org/10.1001/jamanetworkopen.2021.18811 PMID:34323983

31. Barcenilla A, Pryde SE, Martin JC, Duncan SH, Stewart CS, Henderson C, Flint HJ. Phylogenetic relationships of butyrate-producing bacteria from the human gut. Appl Environ Microbiol. 2000; 66:1654–61.
https://doi.org/10.1128/AEM.66.4.1654-1661.2000
PMID:10742256

32. Püngel D, Treveil A, Dalby MJ, Caim S, Colquhoun IJ, Booth C, Ketskemety J, Korcsmaros T, van Sinderen D, Lawson MA, Hall LJ. *Bifidobacterium breve* UCC2003 Exopolysaccharide Modulates the Early Life Microbiota by Acting as a Potential Dietary Substrate. Nutrients. 2020; 12:948.
https://doi.org/10.3390/nu12040948
PMID:32235410

33. Xu HM, Xie SW, Liu TY, Zhou X, Feng ZZ, He X. Microbiota alteration of Chinese young male adults with high-status negative cognitive processing bias. Front Microbiol. 2023; 14:989162.
https://doi.org/10.3389/fmicb.2023.989162
PMID:36937259

34. Verhaar BJH, Collard D, Prodan A, Levels JHM, Zwinderman AH, Bäckhed F, Vogt L, Peters MJL, Muller M, Nieuwdorp M, van den Born BH. Associations between gut microbiota, faecal short-chain fatty acids, and blood pressure across ethnic groups: the HELIUS study. Eur Heart J. 2020; 41:4259–67.
https://doi.org/10.1093/eurheartj/ehaa704
PMID:32869053

35. Chen L, He FJ, Dong Y, Huang Y, Wang C, Harshfield GA, Zhu H. Modest Sodium Reduction Increases Circulating Short-Chain Fatty Acids in Untreated Hypertensives: A Randomized, Double-Blind, Placebo-Controlled Trial. Hypertension. 2020; 76:73–9.

https://doi.org/10.1161/HYPERTENSIONAHA.120.14800
PMID:32475312

36. Li P, Wang H, Guo L, Gou X, Chen G, Lin D, Fan D, Guo X, Liu Z. Association between gut microbiota and preeclampsia-eclampsia: a two-sample Mendelian randomization study. BMC Med. 2022; 20:443.
https://doi.org/10.1186/s12916-022-02657-x
PMID:36380372

37. Morgan XC, Tickle TL, Sokol H, Gevers D, Devaney KL, Ward DV, Reyes JA, Shah SA, LeLeiko N, Snapper SB, Bousvaros A, Korzenik J, Sands BE, et al. Dysfunction of the intestinal microbiome in inflammatory bowel disease and treatment. Genome Biol. 2012; 13:R79.
https://doi.org/10.1186/gb-2012-13-9-r79
PMID:23013615

38. Nastasi C, Candela M, Bonefeld CM, Geisler C, Hansen M, Krejsgaard T, Biagi E, Andersen MH, Brigidi P, Ødum N, Litman T, Woetmann A. The effect of short-chain fatty acids on human monocyte-derived dendritic cells. Sci Rep. 2015; 5:16148.
https://doi.org/10.1038/srep16148 PMID:26541096

39. Eetemadi A, Tagkopoulos I. Methane and fatty acid metabolism pathways are predictive of Low-FODMAP diet efficacy for patients with irritable bowel syndrome. Clin Nutr. 2021; 40:4414–21.
https://doi.org/10.1016/j.clnu.2020.12.041
PMID:33504454

40. Lüll K, Arffman RK, Sola-Leyva A, Molina NM, Aasmets O, Herzig KH, Plaza-Díaz J, Franks S, Morin-Papunen L, Tapanainen JS, Salumets A, Altmäe S, Piltonen TT, Org E. The Gut Microbiome in Polycystic Ovary Syndrome and Its Association with Metabolic Traits. J Clin Endocrinol Metab. 2021; 106:858–71.
https://doi.org/10.1210/clinem/dgaa848
PMID:33205157

41. Calderón-Pérez L, Gosalbes MJ, Yuste S, Valls RM, Pedret A, Llauradó E, Jimenez-Hernandez N, Artacho A, Pla-Pagà L, Companys J, Ludwig I, Romero MP, Rubió L, Solà R. Gut metagenomic and short chain fatty acids signature in hypertension: a cross-sectional study. Sci Rep. 2020; 10:6436.
https://doi.org/10.1038/s41598-020-63475-w
PMID:32296109

42. Sun Y, Wu D, Zeng W, Chen Y, Guo M, Lu B, Li H, Sun C, Yang L, Jiang X, Gao Q. The Role of Intestinal Dysbacteriosis Induced Arachidonic Acid Metabolism Disorder in Inflammaging in Atherosclerosis. Front Cell Infect Microbiol. 2021; 11:618265.
https://doi.org/10.3389/fcimb.2021.618265
PMID:33816331

43. Kurilshikov A, Medina-Gomez C, Bacigalupe R, Radjabzadeh D, Wang J, Demirkan A, Le Roy CI,

Raygoza Garay JA, Finnicum CT, Liu X, Zhernakova DV, Bonder MJ, Hansen TH, et al. Large-scale association analyses identify host factors influencing human gut microbiome composition. Nat Genet. 2021; 53:156–65.
https://doi.org/10.1038/s41588-020-00763-1
PMID:33462485

44. Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glöckner FO. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. 2013; 41:D590–6.
https://doi.org/10.1093/nar/gks1219
PMID:23193283

45. Brown MA, Magee LA, Kenny LC, Karumanchi SA, McCarthy FP, Saito S, Hall DR, Warren CE, Adoyi G, Ishaku S, and International Society for the Study of Hypertension in Pregnancy (ISSHP). Hypertensive Disorders of Pregnancy: ISSHP Classification, Diagnosis, and Management Recommendations for International Practice. Hypertension. 2018; 72:24–43.
https://doi.org/10.1161/HYPERTENSIONAHA.117.10803
PMID:29899139

46. Kurki MI, Karjalainen J, Palta P, Sipilä TP, Kristiansson K, Donner K, Reeve MP, Laivuori H, Aavikko M, Kaunisto MA, Loukola A, Lahtela E, Mattsson H, et al. FinnGen: unique genetic insights from combining isolated population and national health register data. medRxiv. 2022.

47. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, Maller J, Sklar P, de Bakker PI, Daly MJ, Sham PC. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet. 2007; 81:559–75.
https://doi.org/10.1086/519795
PMID:17701901

48. Johnson AD, Handsaker RE, Pulit SL, Nizzari MM, O'Donnell CJ, de Bakker PI. SNAP: a web-based tool for identification and annotation of proxy SNPs using HapMap. Bioinformatics. 2008; 24:2938–9.
https://doi.org/10.1093/bioinformatics/btn564
PMID:18974171

49. Burgess S, Thompson SG, and CRP CHD Genetics Collaboration. Avoiding bias from weak instruments in Mendelian randomization studies. Int J Epidemiol. 2011; 40:755–64.
https://doi.org/10.1093/ije/dyr036
PMID:21414999

50. Burgess S, Butterworth A, Thompson SG. Mendelian randomization analysis with multiple genetic variants using summarized data. Genet Epidemiol. 2013; 37:658–65.
https://doi.org/10.1002/gepi.21758
PMID:24114802

51. Storey JD, Tibshirani R. Statistical significance for genomewide studies. Proc Natl Acad Sci USA. 2003; 100:9440–5.
https://doi.org/10.1073/pnas.1530509100
PMID:12883005

52. Luque-Fernandez MA, Schomaker M, Rachet B, Schnitzer ME. Targeted maximum likelihood estimation for a binary treatment: A tutorial. Stat Med. 2018; 37:2530–46.
https://doi.org/10.1002/sim.7628
PMID:29687470

53. Bowden J, Davey Smith G, Haycock PC, Burgess S. Consistent Estimation in Mendelian Randomization with Some Invalid Instruments Using a Weighted Median Estimator. Genet Epidemiol. 2016; 40:304–14.
https://doi.org/10.1002/gepi.21965
PMID:27061298

54. Bowden J, Davey Smith G, Burgess S. Mendelian randomization with invalid instruments: effect estimation and bias detection through Egger regression. Int J Epidemiol. 2015; 44:512–25.
https://doi.org/10.1093/ije/dyv080
PMID:26050253

55. Zhao Q, Wang J, Hemani G, Bowden J, Small DS. Statistical inference in two-sample summary-data Mendelian randomization using robust adjusted profile score. The Annals of Statistics. 2020; 48.

56. Verbanck M, Chen CY, Neale B, Do R. Detection of widespread horizontal pleiotropy in causal relationships inferred from Mendelian randomization between complex traits and diseases. Nat Genet. 2018; 50:693–8.
https://doi.org/10.1038/s41588-018-0099-7
PMID:29686387

57. Rees JMB, Wood AM, Burgess S. Extending the MR-Egger method for multivariable Mendelian randomization to correct for both measured and unmeasured pleiotropy. Stat Med. 2017; 36:4705–18.
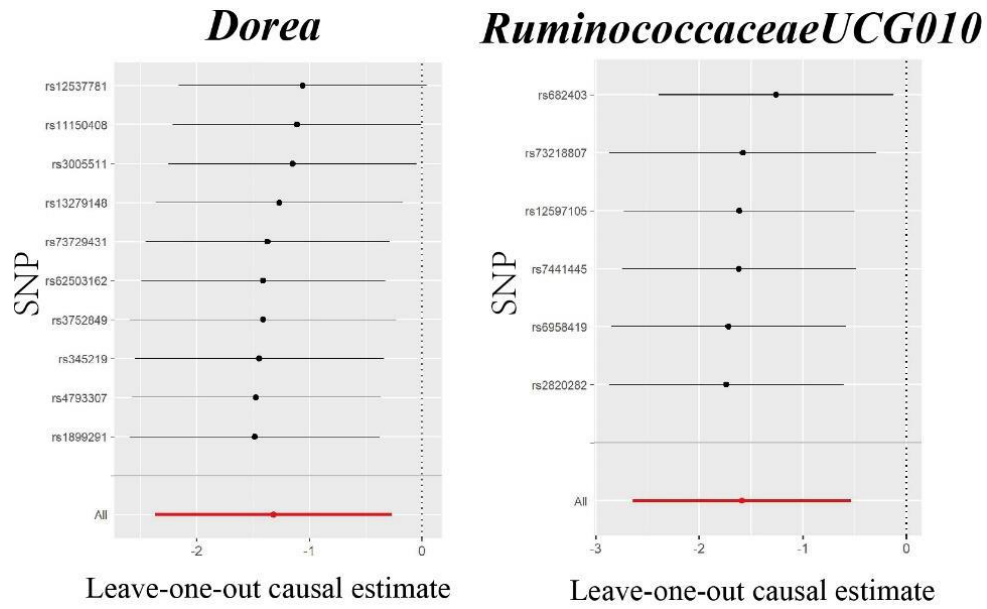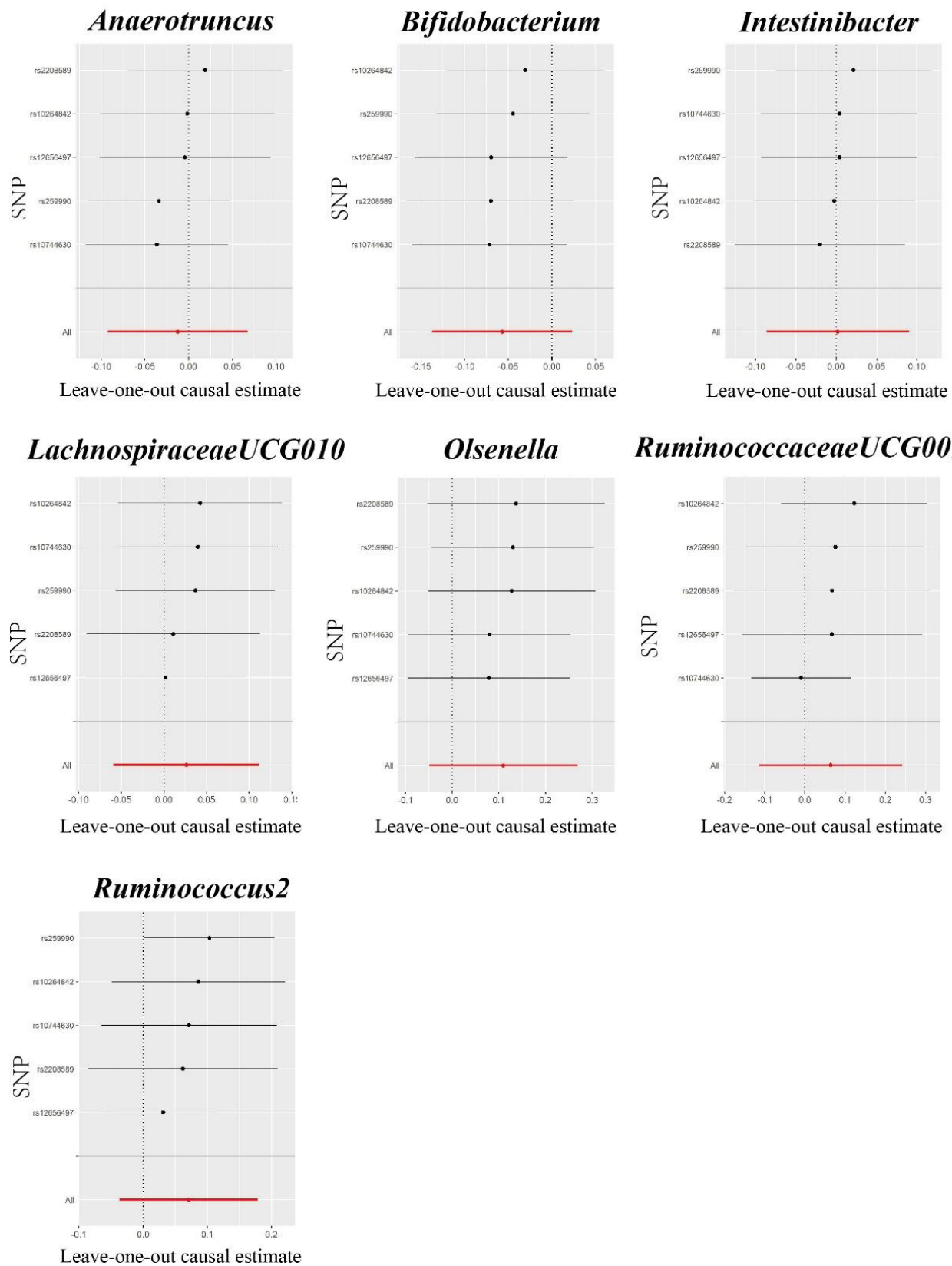https://doi.org/10.1002/sim.7492
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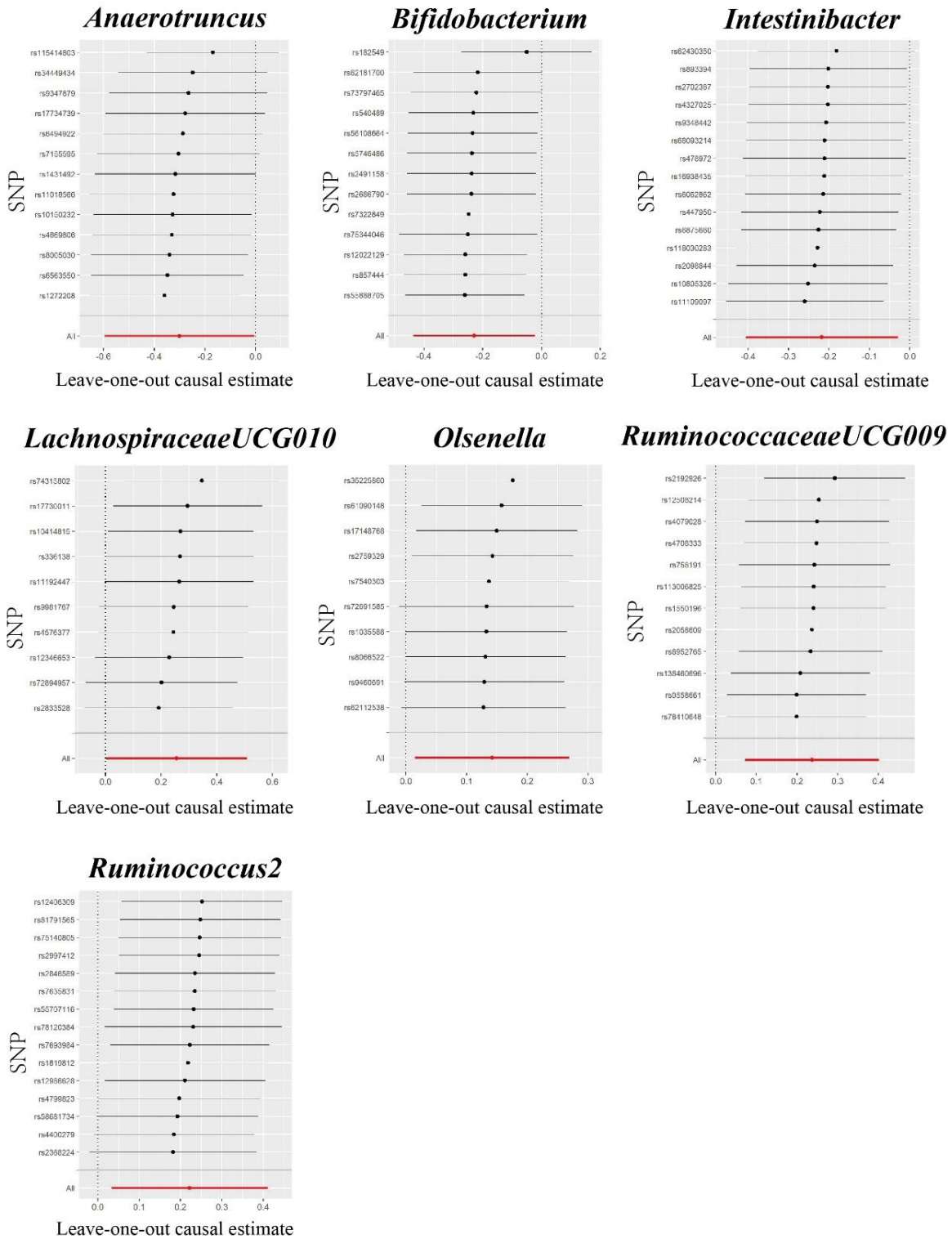
Supplementary Figure 1. Leave-one-out plots for the causal association between gut microbiota and GH in forward MR analyses.
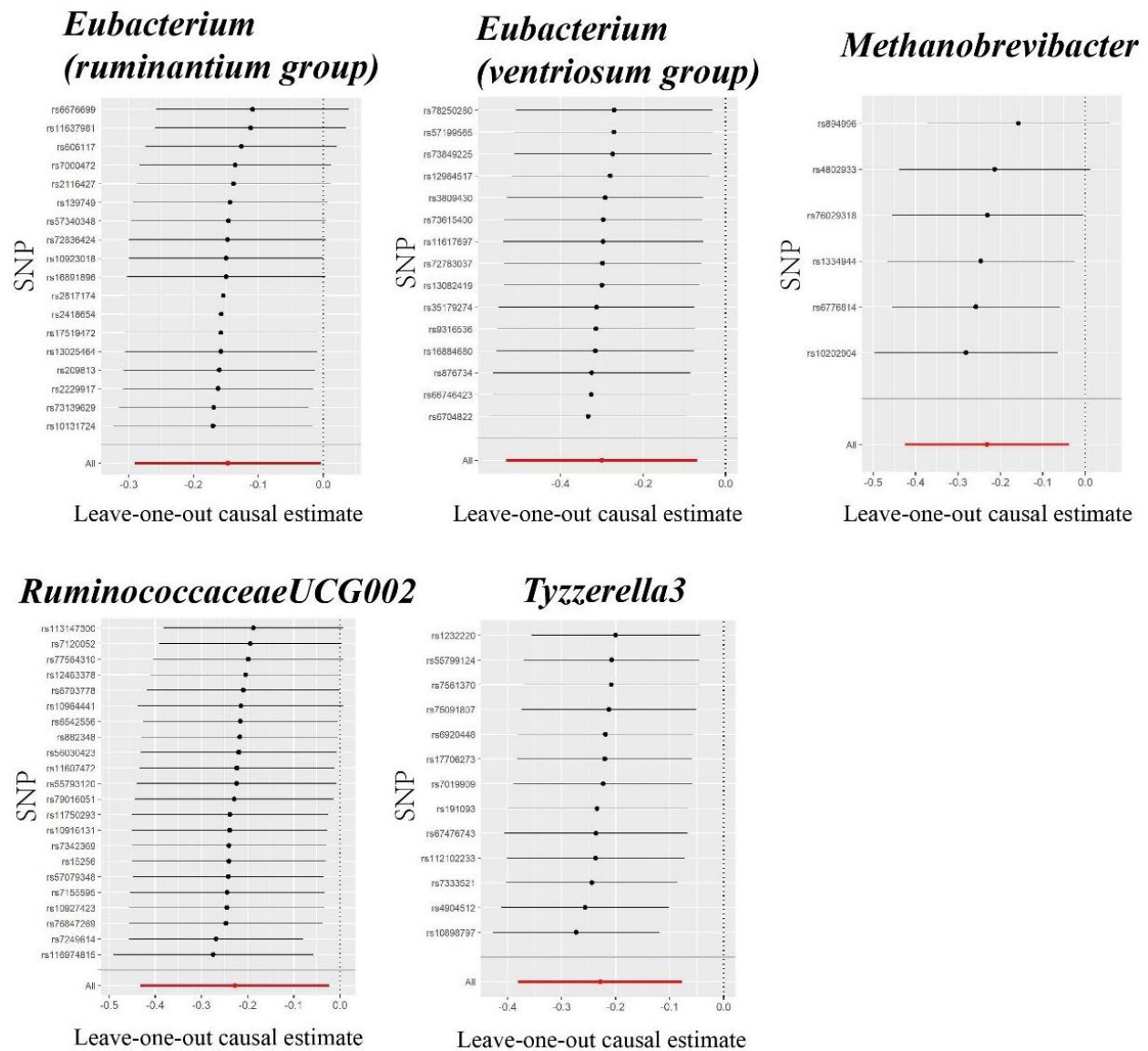
Supplementary Figure 2. Leave-one-out plots for the causal association between gut microbiota and PE in forward MR analyses.
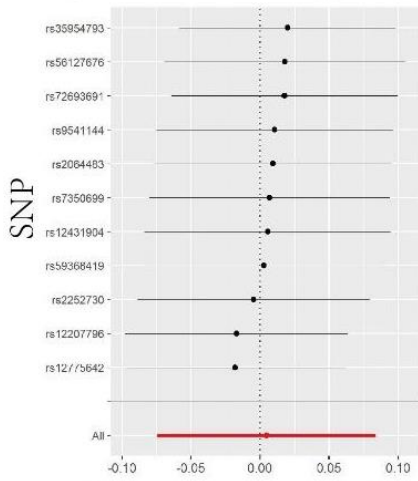
Supplementary Figure 3. Leave-one-out plots for the causal association between gut microbiota and eclampsia in forward MR analyses.
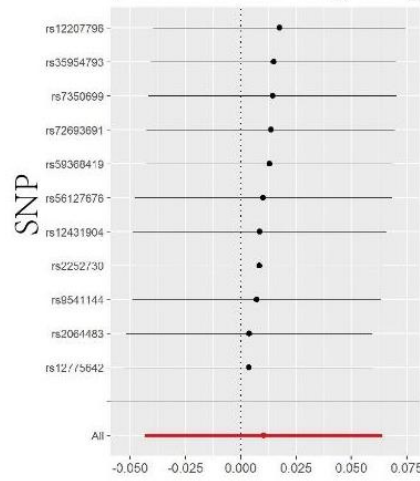
Supplementary Figure 4. Leave-one-out plots for the causal association between gut microbiota and GH in reverse MR analyses.
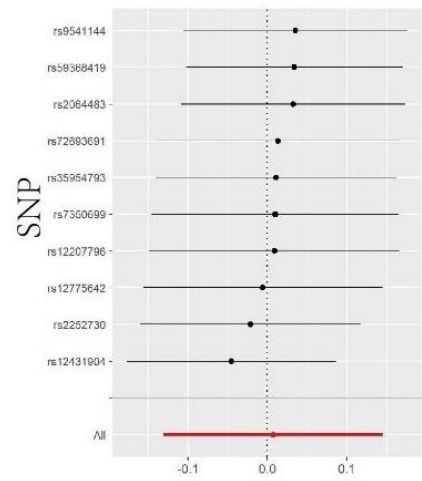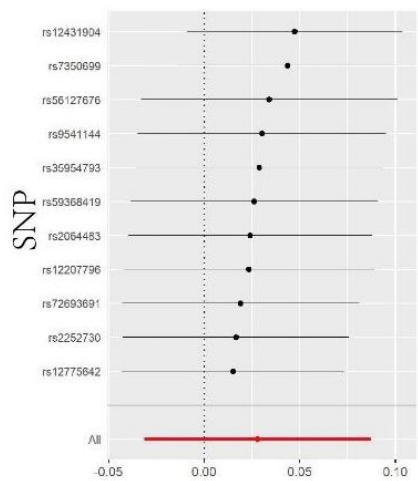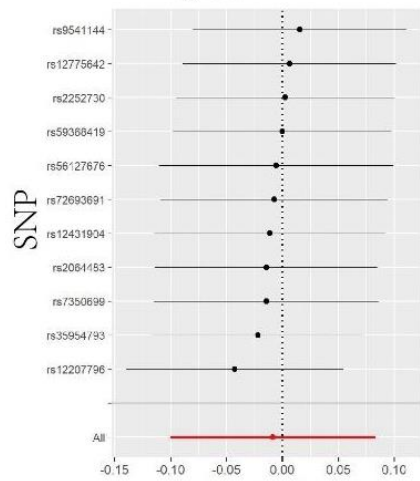
Supplementary Figure 5. Leave-one-out plots for the causal association between gut microbiota and PE in reverse MR analyses.
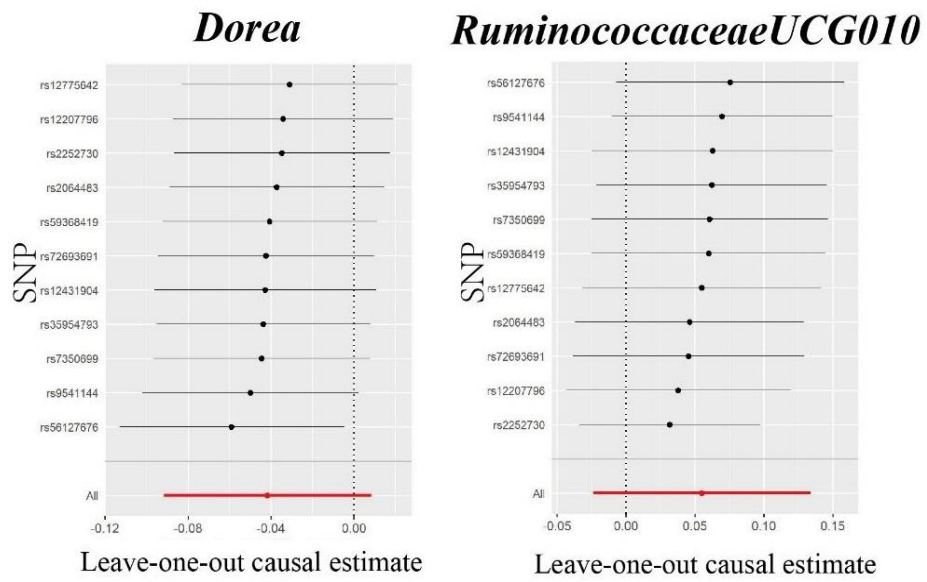
Supplementary Figure 6. Leave-one-out plots for the causal association between gut microbiota and eclampsia in reverse MR analyses.

Supplementary Tables

Please browse Full Text version to see the data of Supplementary Tables 1–5.

Supplementary Table 1. Genome-wide significant and independent SNPs that were used as instruments for gut microbiota.

Supplementary Table 2. Odds ratio for association of genetically predicted gut microbiota with HDP using the method of Inverse variance weighted (IVW).

Supplementary Table 3. Odds ratio for association of genetically predicted gut microbiota with GH using the method of Inverse variance weighted (IVW).

Supplementary Table 4. Odds ratio for association of genetically predicted gut microbiota with PE using the method of Inverse variance weighted (IVW).

Supplementary Table 5. Odds ratio for association of genetically predicted gut microbiota with eclampsia using the method of Inverse variance weighted (IVW).

Supplementary Table 6. Tests for detecting horizontal and directional pleiotropy in forward MR analysis.

Exposure		MR-PRESSO global test		MR-Egger intercept pEgger			Cochran's Q test	
		MR-PRESSO RSSobs	*P* value	Egger-intercept	Standard Error	*P* value	IVW (*P*)	MR-Egger (*P*)
GH	*RuminococcaceaeUCG009*	12.846	0.490	-0.298	0.033	0.384	0.457	0.440
GH	*Ruminococcus2*	13.843	0.624	-0.016	0.018	0.405	0.589	0.571
GH	*Intestinibacter*	9.643	0.880	0.022	0.025	0.396	0.865	0.864
GH	*Olsenella*	7.423	0.773	0.013	0.028	0.660	0.696	0.622
GH	*Bifidobacterium*	20.512	0.201	0.037	0.021	0.102	0.307	0.467
GH	*Anaerotruncus*	22.391	0.108	0.022	0.031	0.481	0.011	0.093
GH	*LachnospiraceaeUCG010*	9.269	0.572	0.009	0.030	0.769	0.613	0.521
PE	*Tyzzerella3*	14.042	0.493	0.013	0.065	0.847	0.422	0.344
PE	*Eubacteriumventriosumgroup*	6.553	0.978	0.007	0.039	0.865	0.972	0.956
PE	*Methanobrevibacter*	6.128	0.555	0.055	0.053	0.357	0.493	0.505
PE	*RuminococcaceaeUCG002*	28.348	0.221	-0.009	0.021	0.650	0.206	0.174
PE	*Eubacteriumruminantiumgroup*	18.405	0.507	-0.036	0.023	0.140	0.484	0.578
Eclampsia	*RuminococcaceaeUCG010*	3.664	0.801	0.001	0.105	0.991	0.746	0.609
Eclampsia	*Dorea*	8.411	0.679	-0.098	0.098	0.348	0.649	0.660

Supplementary Table 7. Reverse MR analyses of gut microbiota on HDP subtypes by different methods.

Exposure	Outcome	Inverse variance weighted		Maximum likelihood		Weighted median		MR.RAPS		MR Egger	
		β (95%*CI*)	*P*	β (95%*CI*)	*P*	β (95%*CI*)	*P*	β (95%*CI*)	*P*	β (95%*CI*)	*P*
GH	*Anaerotruncus*	0.006 (-0.041,0.053)	0.795	0.006 (-0.037,0.050)	0.773	-0.038 (-0.097,0.021)	0.207	0.011 (-0.031,0.054)	0.61	-0.099 (-0.283,0.085)	0.31
GH	*Bifidobacterium*	-0.083 (-0.160,-0.007)	0.033	-0.926 (-0.141,-0.044)	<0.001	-0.027 (-0.089,0.036)	0.405	-0.085 (-0.130,-0.041)	<0.001	0.140 (-0.151,0.432)	0.359
GH	*Intestinibacter*	0.003 (-0.046,0.053)	0.893	0.004 (-0.047,0.054)	0.891	-0.012 (-0.077,0.052)	0.731	-0.002 (-0.051,0.048)	0.939	-0.031 (-0.226,0.163)	0.755
GH	*Lachnospiraceae*	0.002 (-0.047,0.051)	0.943	0.002 (-0.047,0.051)	0.941	-0.015 (-0.084,0.055)	0.674	-0.003 (-0.051,0.045)	0.896	-0.039 (-0.236,0.158)	0.706
GH	*Olsenella*	-0.013 (-0.012,0.090)	0.798	-0.014 (-0.106,0.078)	0.762	0.043 (-0.086,0.171)	0.514	-0.006 (-0.094,0.083)	0.901	0.221 (-0.182,0.624)	0.296
GH	*Ruminococcaceae*	0.004 (-0.060,0.068)	0.901	0.004 (-0.060,0.069)	0.899	-0.002 (-0.090,0.087)	0.973	0.005 (-0.059,0.069)	0.871	-0.063 (-0.322,0.195)	0.637
GH	*Ruminococcus2*	0.017 (-0.038,0.072)	0.546	0.018 (-0.028,0.064)	0.435	-0.026 (-0.091,0.039)	0.43	0.012 (-0.032,0.055)	0.603	-0.046 (-0.267,0.176)	0.692
PE	*Eubacterium (ruminantium group)*	0.005 (-0.074,0.084)	0.909	0.005 (-0.073,0.082)	0.903	-0.018 (-0.124,0.088)	0.783	0.003 (-0.074,0.081)	0.81	-0.047 (-0.305,0.211)	0.73
PE	*Eubacterium (ventriosum group)*	0.010 (-0.043,0.064)	0.703	0.011 (-0.044,0.065)	0.703	0.013 (-0.054,0.081)	0.703	0.001 (-0.054,0.057)	0.969	-0.040 (-0.208,0.129)	0.657
PE	*Methanobrevibacter*	0.007 (-0.131,0.146)	0.918	0.008 (-0.118,0.133)	0.902	-0.010 (-0.185,0.165)	0.909	0.003 (-0.120,0.125)	0.967	0.218 (-0.324,0.761)	0.453
PE	*Ruminococcaceae UCG002*	0.028 (-0.031,0.087)	0.354	0.029 (-0.024,0.081)	0.283	0.022 (-0.048,0.093)	0.54	0.003 (-0.048,0.055)	0.195	-0.082 (-0.262,0.097)	0.392
PE	*Tyzzerella3*	-0.008 (-0.100,0.083)	0.856	-0.009 (-0.102,0.085)	0.851	-0.019 (-0.143,0.105)	0.764	-0.019 (-0.112,0.074)	0.692	0.121 (-0.171,0.412)	0.439
Eclampsia	*Dorea*	-0.002 (-0.021,-0.017)	0.821	-0.002 (-0.022,0.017)	0.817	0.002 (-0.024,0.029)	0.859	-0.002 (-0.023,0.018)	0.825	0.006 (-0.053,0.065)	0.847
Eclampsia	*Ruminococcaceae UCG010*	-0.003 (-0.025,0.019)	0.78	-0.003 (-0.027,0.019)	0.78	0.003 (-0.023,0.030)	0.808	-0.003 (-0.027,0.021)	0.795	0.010 (-0.055,0.076)	0.767

Supplementary Table 8. Tests for detecting horizontal and directional pleiotropy in reverse MR analysis.

Exposure	Outcome	MR-PRESSO global test		MR-Egger intercept pEgger			Cochran's Q test	
		MR-PRESSO RSSobs	P value	Egger-intercept	Standard Error	P value	IVW (P)	MR-Egger (P)
GH	*RuminococcaceaeUCG009*	14.827	0.075	0.059	0.081	0.521	0.041	0.038
GH	*Ruminococcus2*	10.731	0.183	0.057	0.042	0.265	0.114	0.204
GH	*Intestinibacter*	1.994	0.881	-0.014	0.038	0.733	0.874	0.781
GH	*Olsenella*	2.715	0.786	0.082	0.068	0.314	0.771	0.945
GH	*Bifidobacterium*	4.230	0.618	0.027	0.034	0.483	0.601	0.550
GH	*Anaerotruncus*	7.333	0.364	0.019	0.037	0.636	0.329	0.238
GH	*LachnospiraceaeUCG010*	3.951	0.673	0.023	0.036	0.575	0.623	0.526
PE	*Tyzzerella3*	12.231	0.449	-0.022	0.024	0.384	0.432	0.415
PE	*Eubacteriumventriosumgroup*	3.393	0.987	0.008	0.014	0.555	0.985	0.981
PE	*Methanobrevibacter*	14.515	0.255	-0.033	0.042	0.453	0.237	0.215
PE	*RuminococcaceaeUCG002*	16.654	0.203	0.019	0.015	0.236	0.192	0.240
PE	*Eubacteriumruminantiumgroup*	13.034	0.397	0.009	0.021	0.690	0.370	0.301
Eclampsia	*RuminococcaceaeUCG010*	22.031	0.064	0.019	0.020	0.368	0.053	0.057
Eclampsia	*Dorea*	8.979	0.688	-0.018	0.013	0.185	0.709	0.824

Supplementary Table 9. Detailed information for genome-wide association studies involved in the present Mendelian randomization study.

Variable	Consortium or study	Sample size	Journal	Year	Cohort	Nation of cohort	Number of samples
Gut microbiota	MiBioGen	18,340	Nat Genet.	2021	BSPSPC	Germany	721
					CARDIAw	USA	257
					COPSAC	Denmark	380
					DanFunD16	Denmark	2,396
					FGFP	Belgian	2,259
					FOCUS	Germany	960
					GEM_HCE_v12	Canada	378
					GEM_HCE_v24	Canada	203
					GEM_ICHIP_HCE	Canada	662
					GenR	The Netherlands	1,328
					HCHS/SOL	USA	1,097
					KSCS	South Korea	811
					LLD	The Netherlands	875
					METSIM	Finland	522
					MIBS	The Netherlands	80
					NGRC	USA	77
					NTR	The Netherlands	279
					PNP	Israel	481
					POPCOL	Sweden	134
					RS3	The Netherlands	1,220
					SHIP	Germany	996
					SHIP-TREND	Germany	905
					TwinsUK	UK	1,205
HDP	FinnGen	1,22,421		2021	FinnGen	European	7,686 cases/114,735 controls
GH	FinnGen	1,18,990		2021	FinnGen	European	4,255 cases/114,735 controls
PE	FinnGen	1,18,291		2021	FinnGen	European	3,556 cases/114,735 controls
Eclampsia	FinnGen	1,15,025		2021	FinnGen	European	290 cases/114,735 controls