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Original Article

Investigating the causative impact of metabolite function on preeclampsia through a Mendelian randomization approach

Senglim Choeng[1*], Vicheth Virak[3*], Siyou Choeng[4], Rayuth Lim[4], Pengkhun Nov[2], Shilei Pan[1]

[1]Department of Obstetrics and Gynecology, Zhujiang Hospital, Southern Medical University, No. 253, Middle Gongye Avenue, Haizhu District, Guangzhou 510282, Guangdong, The People's Republic of China; [2]Department of Radiation Oncology, Oncology Center, Zhujiang Hospital of Southern Medical University, No. 253, Middle Gongye Avenue, Haizhu District, Guangzhou 510282, Guangdong, The People's Republic of China; [3]Department of Cardiology, Laboratory of Heart Center, Zhujiang Hospital, Southern Medical University, No. 253, Middle Gongye Avenue, Haizhu District, Guangzhou 510282, Guangdong, The People's Republic of China; [4]Norton University, St Keo Chenda, Chroy Changvar District, Phnom Penh 12000, Cambodia. [*]Equal contributors.

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Abstract: Background: Preeclampsia (PE) is a pregnancy-related condition marked by high blood pressure, posing significant risks to both maternal and fetal health. While the precise causes of PE remain unclear, this study aims to investigate the causal relationship between cellular metabolites and the onset of PE using a Mendelian randomization (MR) approach. Despite the critical role of metabolite function in the development of PE, this area has been relatively underexplored. By employing MR methodology, this research seeks to analyze how metabolite function influences the risk of developing PE. Methods: This study used genetic variants associated with specific metabolite risk factors as instrumental variables (IVs) to assess their causal effects on PE. Comprehensive data from various cohorts, including genome-wide association studies (GWAS) and individuals with PE, were analyzed to investigate these relationships. Results: We identified 61 metabolites and uncovered five compelling links between metabolite function and PE. Our Mendelian randomization analysis revealed that elevated sphingomyelin levels were protective (OR: 0.7102), while increased levels of 1-linoleoyl-GPG (18:2), cis-3,4-methyleneheptanoate, and tetradecanedioate emerged as causal risk factors for PE. Conclusion: This MR study provides important insights into the impact of metabolite function on PE. Additional research is required to unveil the exact mechanisms by which these metabolite factors impact the development of this condition. This investigation has the potential to pave the way for targeted therapeutic approaches in the future.

Keywords: Metabolites function, preeclampsia, Mendelian randomization study

Introduction

Preeclampsia (PE) is a serious pregnancy complication characterized by high blood pressure (BP) and evidence of damage to other organs, especially the liver and kidneys [1]. It is a leading cause of maternal and fetal mortality worldwide, accounting for approximately 70,000 maternal deaths and 500,000 fetal deaths each year [2]. The condition is particularly prevalent among certain demographic groups, including nulliparous women and those of advanced maternal age, with a twofold increase in risk compared to women aged 20 to 29 years.

Despite decades of research, the underlying pathogenesis of PE remains poorly understood, hindering the development of effective therapeutic interventions [3, 4]. The PE pathogenesis involves a complex interplay of various mechanisms. For instance, abnormal placental development due to inadequate trophoblast invasion and compromised remodeling of spiral arteries can lead to reduced uteroplacental blood flow, resulting in placental ischemia, and systemic endothelial dysfunction [5]. Immune maladaptation, characterized by dysregulation of the maternal immune response to the developing fetus, contributes to the abnormal activa-

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tion of inflammatory pathways and insufficient immune tolerance towards the semi-allogeneic fetus. This results in systemic inflammation and endothelial dysfunction [6], which can further be exacerbated by reactive oxygen species (ROS)-mediated oxidative stress (OS) and impaired antioxidant defenses in PE [7]. Endothelial dysfunction, a hallmark of PE pathology, manifests as impaired vasodilation, heightened vascular tone, and altered endothelial cell function, contributing to hypertension, proteinuria, and even multi-organ failure [8]. Abnormal angiogenesis, characterized by dyshomeostatic interactions between pro-angiogenic and anti-angiogenic factors, further complicates placental vascular development and contributes to PE pathogenesis [9].

Metabolites have emerged as potential biomarkers and causal factors in the development of PE [10]. Aberrant levels of various metabolites have been observed especially in female PE patients, suggesting that altered cellular metabolism might play critical roles in PE pathogenesis. Furthermore, epigenetic modifications, such as DNA methylation, have been shown to regulate the expression of genes involved in metabolic pathways, potentially linking metabolic dysregulation to the onset of PE [11, 12]. Accumulating evidence indicates the potential role of metabolites in the development of PE [13]. Studies have identified associations between specific metabolites and the risk of PE, with one study revealing a causal connection between certain blood metabolites and the condition, suggesting their potential as personalized biomarkers and therapeutic targets. This offers new insights into the biological functions of these molecules and paves the way for advancements in diagnostic tools and treatment strategies for PE [14]. Another study demonstrates that metabolites play a causal role in the onset of PE [15].

However, actual underlying causal factors remain unexplored, as observational studies are susceptible to confounding and reverse causation [16-19]. Mendelian randomization (MR) studies can help address this challenge by using genetic variants as instrumental variables (IVs) to investigate the causal impact of metabolites on PE risk [13, 20-22]. This approach leverages the random assortment of genetic variants during meiosis to mimic a randomized controlled trial (RCT), thereby reducing the influence of confounding and reverse causation. This study aims to employ an MR approach to investigate the functional role of cellular metabolites in PE risks. By identifying specific metabolites that causally contribute to PE, this study provides valuable insights into the underlying pathogenesis of PE and informs the development of targeted interventions.

Methods and materials

Study design

Here, we explored the causative relationship between the cellular metabolite function and the incidence of PE by using an MR approach [23-25]. In this framework, genetic variations serve as instrumental variables (IVs) to identify potential risk factors, thereby enhancing the reliability of causal inferences. To validate these IVs, the selected genetic variations must meet three critical criteria: first, they must be directly associated with metabolite functions; second, they should not be linked to confounding variables that could influence the relationship between cellular metabolism and PE; and third, these variations should affect PE solely through their impact on metabolite function, without involving unrelated biological pathways. The research included in our analysis has received all necessary ethical approvals and obtained informed consent from all participants, ensuring strict adherence to ethical standards and the protection of participant data confidentiality. In summary, our study utilized an MR design to explore the causal connections between metabolite functions in cells and PE. By employing genetic variants as IVs, we aimed to fulfill the essential criteria for reliable causal inference in MR studies, with careful consideration given to ethical practices and participant consent (**Figure 1**).

Data sources

We obtained genome-wide association study (GWAS) data for metabolite traits using accession numbers GCST90199621-90201020 from publicly available databases, which enabled us to generate summary statistics for various metabolic cell types [26]. To identify relevant data for our research on PE, we searched

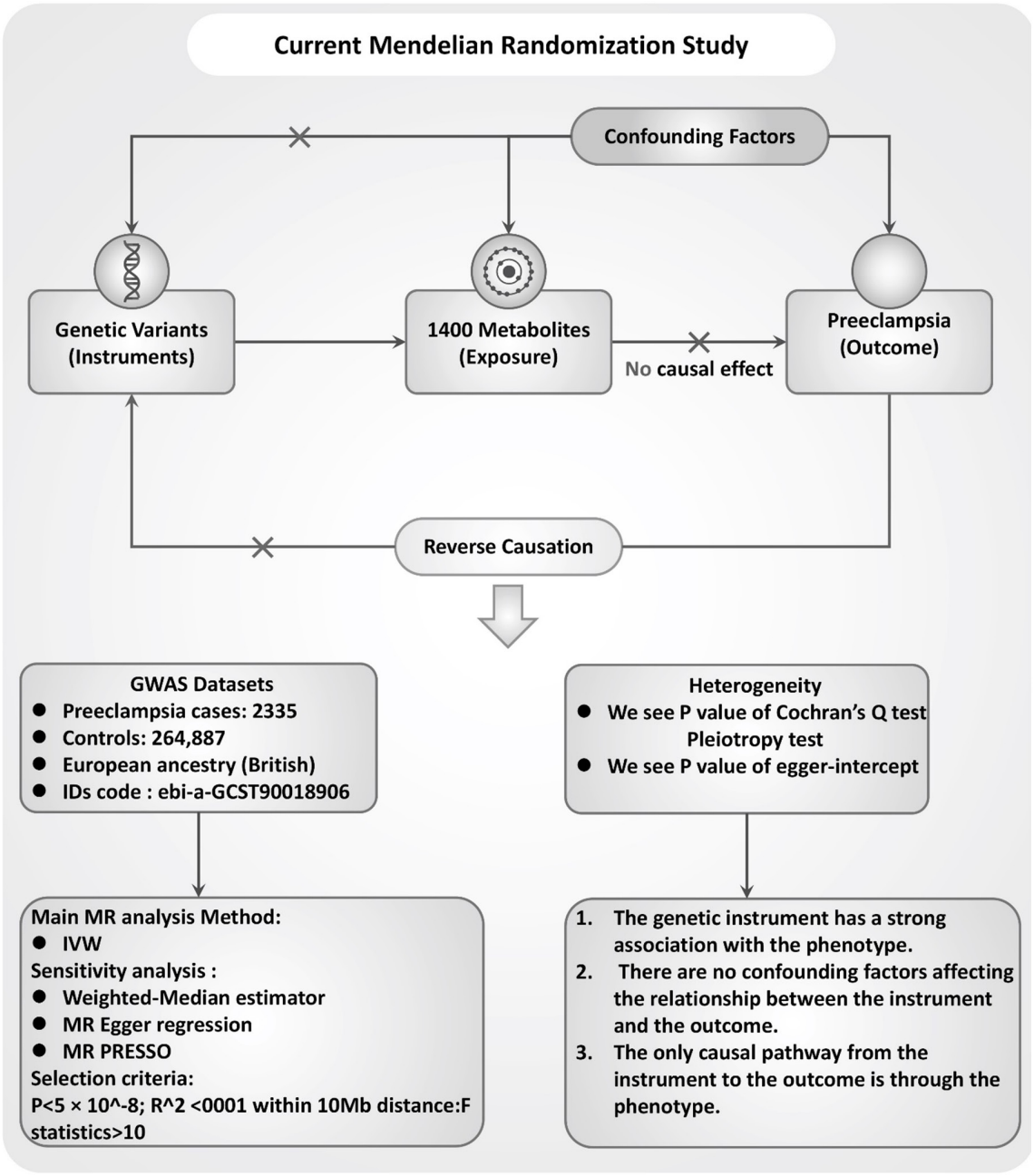

Figure 1. The flowchart of the study design.

the GWAS database at https://gwas.mrcieu.ac.uk/ using targeted keywords specific to PE. We specifically selected the dataset ebi-a-GCST90018906 for PE and retrieved pertinent information from https://www.ebi.ac.uk/gwas/ using the associated IDs for each case. This dataset facilitated our analysis of the relationship between 1,400 types of metabolites and PE. We estimated approximately 22 million single nucleotide polymorphisms (SNPs) using a reference panel derived from Sardinian sequences, which were genotyped utilizing high-density arrays [27]. Correlations were assessed after adjusting for covariates. GWAS is a powerful research method that scans the genomes of many individuals to identify genetic variations associated with specific traits or diseases. By comparing the DNA of individuals with a particular phenotype to those without, researchers can pinpoint SNPs and other

genetic markers that may contribute to the development of that phenotype. This approach enhances our understanding of the genetic basis of complex traits and can provide insights into disease mechanisms and potential treatments. Using the ID of each PE case, we accessed online data from the GWAS database, which included information on 267,242 European individuals, comprising 2,355 cases and 264,887 controls for PE. This data was instrumental in analyzing the relationship between 1,400 types of metabolites and each PE based on the provided IDs at https://www.ebi.ac.uk/gwas/.

Genetic instrument selection

Here, we established stringent criteria for selecting genetic IVs related to SNPs and metabolite traits. To address the challenge of numerous SNPs achieving genome-wide significance ($P < 5 \times 10^{-8}$), we applied even stricter criteria ($P < 5 \times 10^{-9}$) for IV selection [28]. Utilizing the linkage disequilibrium (LD) reference panel from the 1,000 Genomes Project, we classified IVs and set a constraint of $R^2 < 0.001$ within a 1,000-kilobase (kb) distance to identify the most relevant IVs for our analysis. For metabolite GWAS datasets, we maintained a significance threshold of 5×10^{-8}, along with a clustering limit of $R^2 < 0.1$ within a 500 kb window, allowing us to capture a sufficient number of IVs while ensuring statistical rigor [29]. To enhance the reliability of our genetic instruments, we selected those with F-statistics exceeding 10, ensuring the robustness of our analyses. These IVs were derived from summarized data related to PE outcomes. Following established protocols [30], we excluded any SNPs with potential pleiotropic effects on PE, applying a threshold of $P < 10^{-5}$. We also harmonized SNPs across exposure and outcome datasets to maintain consistency in effect size estimations, facilitating coherent comparisons based on identical genetic variants. SNPs with effect allele frequencies (EAFs) exceeding 0.42 or that were incongruous with harmonization criteria were excluded from the analysis, as outlined in [29]. This meticulous selection and harmonization of SNPs uphold the integrity and consistency of our MR investigation.

Statistical analysis

The analysis was conducted using R 4.3.1 software (http://www.Rproject.org). To investigate the causal link between 1,400 types of metabolites and PE, we employed three key methodologies: inverse variance weighting (IVW) [31], median-based weighting (MVW) [32], and pattern-based weighting (PBW) [33]. These analyses were performed using the "TwoSampleMR" software package (version 0.4.3) [34]. Cochran's Q statistical test, along with corresponding *p*-values, was utilized to assess heterogeneity among the selected IVs. In instances of significant heterogeneity (indicating rejection of the null hypothesis), we applied random effects IVW instead of fixed effects [31]. To address potential horizontal pleiotropy, we utilized the MR-Egger method, which could detect horizontal pleiotropy through a statistically significant intercept term [31]. Additionally, we employed the MR Pleiotropy Residual Sum and Outlier (MR-PRESSO) technique to identify and eliminate potential horizontal pleiotropic outliers that could significantly distort our estimation outcomes [35]. Validation of findings was performed using scatterplots and funnel plots to confirm the absence of bias from outliers and demonstrate the robustness of observed correlations, with no significant heterogeneity detected.

Results

In this study, we identified 61 metabolite phenotypes associated with the onset of PE (**Table 1**).

Focus on four potential metabolites

Among the metabolites identified, we found a significant protective effect associated with elevated levels of sphingomyelin against PE (OR: 0.7102; 95% CI: 0.5893-0.8559; P = 0.0003). Conversely, increased levels of 1-linoleoyl-GPG (18:2) emerged as a causal risk factor for PE (OR: 1.1626; 95% CI: 1.0380-1.3021; P = 0.0091). Additionally, dyshomeostasis in the levels of cis-3,4-methyleneheptanoate was linked to an enhanced risk of PE (OR: 1.1989; 95% CI: 1.0482-1.3712; P = 0.0081), indicating that deviations from optimal levels may heighten the risk of PE. Moreover, elevated levels of tetradecanedioate (C14-DC) were identified as a significant causal risk factor for PE (OR: 1.1399; 95% CI: 1.0364-1.2538; P = 0.0070) (**Figure 2**).

Table 1. The table shows 61 metabolite phenotypes associated with PE

Exposure	Method	nsnp	pval	or	or_lci95	or_uci95
Carnitine levels	IVW	26	0.001446	1.19969	1.07254	1.34191
Imidazole lactate levels	IVW	28	0.028272	1.11585	1.01174	1.23068
X-21733 levels	IVW	29	0.00397	0.78154	0.66087	0.92425
X-23655 levels	IVW	27	0.036421	0.83157	0.69961	0.98841
3-indoxyl sulfate levels	IVW	22	0.030552	1.20686	1.01779	1.43104
Isovalerylcarnitine (C5) levels	IVW	26	0.039302	1.18323	1.00828	1.38854
1-arachidonylglycerol (20:4) levels	IVW	24	0.03365	0.86334	0.75388	0.9887
Glutamine degradant levels	IVW	27	0.049262	1.15453	1.00046	1.33232
Beta-hydroxyisovaleroylcarnitine levels	IVW	36	0.035043	1.13837	1.00913	1.28416
Tetradecanedioate (C14-DC) levels	IVW	19	0.007017	1.13998	1.03641	1.2539
Hexadecanedioate (C16-DC) levels	IVW	23	0.005256	1.15611	1.04413	1.28009
Glycerophosphoethanolamine levels	IVW	24	0.041421	0.85098	0.72872	0.99375
Gamma-glutamylalanine levels	IVW	17	0.010699	1.26286	1.05566	1.51074
21-hydroxypregnenolone disulfate levels	IVW	38	0.034322	1.1284	1.00896	1.26198
Androstenediol (3beta,17beta) monosulfate (1) levels	IVW	33	0.048412	1.15421	1.001	1.33088
1-lignoceroyl-GPC (24:0) levels	IVW	16	0.047407	0.79881	0.63973	0.99744
2-oxoarginine levels	IVW	22	0.050088	0.82332	0.67781	1.00008
1-(1-enyl-stearoyl)-GPE (p-18:0) levels	IVW	23	0.049509	0.83994	0.70576	0.99963
N-oleoyltaurine levels	IVW	20	0.041109	1.15277	1.00575	1.32128
Imidazole propionate levels	IVW	25	0.016001	1.22743	1.03892	1.45013
Alliin levels	IVW	20	0.004166	0.82526	0.72367	0.94113
Margaroylcarnitine (C17) levels	IVW	26	0.007218	0.81626	0.70389	0.94657
(R)-3-hydroxybutyrylcarnitine levels	IVW	24	0.016678	1.24366	1.0403	1.48676
2-hydroxydecanoate levels	IVW	18	0.013847	0.78795	0.65174	0.95262
2-aminophenol sulfate levels	IVW	34	0.043673	1.14814	1.00393	1.31306
2-aminoheptanoate levels	IVW	27	0.015798	0.83306	0.71822	0.96626
17alpha-hydroxypregnanolone glucuronide levels	IVW	33	0.021658	1.13733	1.01902	1.26936
Octadecenedioylcarnitine (C18:1-DC) levels	IVW	17	0.017637	1.13494	1.02228	1.26002
Sphingomyelin (d18:2/14:0, d18:1/14:1) levels	IVW	22	0.000325	0.71026	0.58937	0.85593
Carnitine C14:1 levels	IVW	34	0.033096	0.8505	0.73281	0.9871
Octadecanedioylcarnitine (C18-DC) levels	IVW	27	0.035831	1.13386	1.00834	1.27499
Sphingomyelin (d18:1/20:1, d18:2/20:0) levels	IVW	29	0.026295	0.86666	0.76387	0.98328
Glycodeoxycholate 3-sulfate levels	IVW	31	0.005922	1.12108	1.03344	1.21615
1-(1-enyl-palmitoyl)-2-palmitoleoyl-GPC (P-16:0/16:1) levels	IVW	33	0.014955	0.88263	0.79819	0.976

1-linoleoyl-GPG (18:2) levels	IVW	25	0.009162	1.16265	1.03806	1.30218
1-palmitoyl-2-oleoyl-GPI (16:0/18:1) levels	IVW	26	0.005863	1.20241	1.05465	1.37088
1-(1-enyl-palmitoyl)-2-palmitoyl-GPC (P-16:0/16:0) levels	IVW	33	0.01439	0.88869	0.80854	0.97677
Linoleoyl-arachidonoyl-glycerol (18:2/20:4) [2] levels	IVW	35	0.021491	0.89148	0.80833	0.98319
Hexadecenedioate (C16:1-DC) levels	IVW	31	0.001809	1.15083	1.05361	1.25701
Perfluorooctanoate (PFOA) levels	IVW	23	0.0361	0.82143	0.6834	0.98734
Glucuronide of piperine metabolite C17H21NO3 (3) levels	IVW	21	0.011192	1.12435	1.027	1.23093
N-acetyl-isoputreanine levels	IVW	38	0.015813	1.134	1.0239	1.25595
N-lactoyl isoleucine levels	IVW	17	0.031321	0.79997	0.65288	0.9802
Glucuronide of piperine metabolite C17H21NO3 (4) levels	IVW	21	0.001924	1.17213	1.06021	1.29587
3-hydroxy-2-methylpyridine sulfate levels	IVW	21	0.026209	0.81256	0.67667	0.97573
4-acetylcatechol sulfate (1) levels	IVW	18	0.034041	1.11816	1.00844	1.2398
Eicosenedioate (C20:1-DC) levels	IVW	23	0.047558	1.12999	1.00131	1.2752
Cis 3,4-methyleneheptanoate levels	IVW	26	0.008102	1.19894	1.04826	1.37129
Metabolonic lactone sulfate levels	IVW	31	0.007636	1.09653	1.02475	1.17334
S-carboxyethylcysteine levels	IVW	23	0.004516	1.28177	1.07993	1.52133
5-oxoproline levels	IVW	25	0.038897	0.89321	0.80243	0.99426
N-acetyl-L-alanine levels	IVW	33	0.03339	0.85663	0.7428	0.9879
Creatine levels	IVW	25	0.046112	0.88895	0.79184	0.99797
4-acetaminophen sulfate levels	IVW	25	0.048816	1.12652	1.00062	1.26826
Linoleate (18:2n6) levels	IVW	22	0.039225	1.24362	1.01082	1.53003
Cysteinylglycine levels	IVW	20	0.042406	0.86486	0.75172	0.99504
Phenylalanine levels	IVW	21	0.045316	1.18638	1.00357	1.40249
1-methylnicotinamide levels	IVW	19	0.028478	1.26337	1.02492	1.5573
Xylose levels	IVW	26	0.03048	1.16199	1.01424	1.33126
Alanine levels	IVW	22	0.017537	0.81857	0.69392	0.96561
Mannose levels	IVW	26	0.039254	0.86323	0.75058	0.99278
Cysteine levels	IVW	15	0.021448	0.84612	0.73382	0.9756
X-07765 levels	IVW	25	0.034636	1.16919	1.01135	1.35166
X-12127 levels	IVW	30	0.033932	1.14585	1.01038	1.29948
X-13723 levels	IVW	14	0.001782	0.74995	0.62609	0.89831
X-15728 levels	IVW	32	0.03854	0.85852	0.74301	0.99199
X-17676 levels	IVW	28	0.036768	1.14273	1.00823	1.29517
X-21471 levels	IVW	28	0.013731	1.14843	1.02872	1.28207
X-21470 levels	IVW	18	0.005734	1.18702	1.05107	1.34055

X-23659 levels	IVW	28	0.031115	1.12281	1.01057	1.24753
X-24243 levels	IVW	20	0.009484	1.25285	1.05665	1.48549
X-24546 levels	IVW	27	0.003498	1.18348	1.05696	1.32516
Adenosine 5'-diphosphate (ADP) to creatine ratio	IVW	22	0.028968	1.14059	1.01358	1.28351
N-acetylputrescine to (N(1) + N(8))-acetylspermidine ratio	IVW	32	0.019076	1.11675	1.01826	1.22478
Arachidonate (20:4n6) to oleate to vaccenate (18:1) ratio	IVW	20	0.037643	0.88605	0.79053	0.99311
Arachidonate (20:4n6) to pyruvate ratio	IVW	16	0.037229	0.85239	0.73347	0.9906
Serine to pyruvate ratio	IVW	19	0.037136	0.83396	0.70306	0.98923
5-oxoproline to citrate ratio	IVW	16	0.015054	0.86305	0.76642	0.97186
Oleoyl-linoleoyl-glycerol (18:1 to 18:2) [2] to linoleoyl-arachidonoyl-glycerol (18:2 to 20:4) [1] ratio	IVW	25	0.005739	1.12493	1.03478	1.22294
Oleoyl-linoleoyl-glycerol (18:1 to 18:2) [2] to linoleoyl-arachidonoyl-glycerol (18:2 to 20:4) [2] ratio	IVW	28	0.028899	1.12772	1.01245	1.25613
Spermidine to N-acetylputrescine ratio	IVW	22	0.032946	0.87442	0.77297	0.98919
Adenosine 5'-monophosphate (AMP) to valine ratio	IVW	19	0.034289	0.80834	0.66378	0.98438
Adenosine 5'-monophosphate (AMP) to glutamate ratio	IVW	23	0.010418	0.77721	0.64089	0.94252
Salicylate to caprylate (8:0) ratio	IVW	23	0.007718	1.2258	1.05529	1.42386
Inosine to theophylline ratio	IVW	22	0.019586	0.85146	0.74392	0.97455
Glucose-to-mannose ratio	IVW	25	0.011125	1.1756	1.03757	1.33199
Phosphate to 5-oxoproline ratio	IVW	25	0.010714	1.16973	1.03703	1.31941
Fructose to maltose ratio	IVW	23	0.042986	1.1583	1.00465	1.33546

Exposure	Method	No.of SNP	OR(95% CI)	P
Sphingomyelin (d18:2/14:0, d18:1/14:1) levels	Inverse variance weighted	22	0.710 (0.589 to 0.856)	0.000
Sphingomyelin (d18:2/14:0, d18:1/14:1) levels	MR Egger	22	0.697 (0.485 to 1.000)	0.064
Sphingomyelin (d18:2/14:0, d18:1/14:1) levels	Weighted median	22	0.719 (0.551 to 0.938)	0.015
Sphingomyelin (d18:2/14:0, d18:1/14:1) levels	Weighted mode	22	0.735 (0.481 to 1.123)	0.170
1−linoleoyl−GPG (18:2) levels	Inverse variance weighted	25	1.163 (1.038 to 1.302)	0.009
1−linoleoyl−GPG (18:2) levels	MR Egger	25	1.234 (1.040 to 1.465)	0.025
1−linoleoyl−GPG (18:2) levels	Weighted median	25	1.274 (1.101 to 1.474)	0.001
1−linoleoyl−GPG (18:2) levels	Weighted mode	25	1.232 (1.079 to 1.406)	0.005
Cis 3,4−methyleneheptanoate levels	Inverse variance weighted	26	1.199 (1.048 to 1.371)	0.008
Cis 3,4−methyleneheptanoate levels	MR Egger	26	1.214 (0.975 to 1.511)	0.096
Cis 3,4−methyleneheptanoate levels	Weighted median	26	1.246 (1.021 to 1.520)	0.031
Cis 3,4−methyleneheptanoate levels	Weighted mode	26	1.243 (1.001 to 1.544)	0.060
Tetradecanedioate (C14−DC) levels	Inverse variance weighted	19	1.140 (1.036 to 1.254)	0.007
Tetradecanedioate (C14−DC) levels	MR Egger	19	1.135 (0.986 to 1.306)	0.096
Tetradecanedioate (C14−DC) levels	Weighted median	19	1.229 (1.074 to 1.406)	0.003
Tetradecanedioate (C14−DC) levels	Weighted mode	19	1.157 (1.033 to 1.296)	0.022

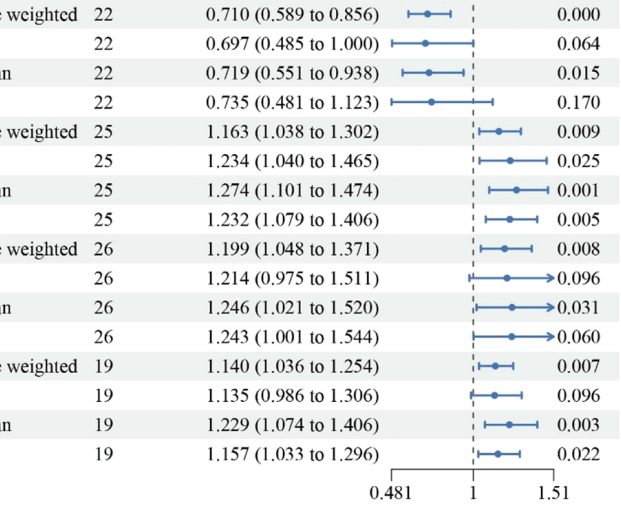

Figure 2. The causal role of metabolites and preeclampsia.

Sensitivity analysis outcomes

The sensitivity analysis results confirmed the robustness of our causal estimates, even in the presence of observed heterogeneity, as evidenced by the application of a random-effects IVW approach. Furthermore, MR-Egger intercept analysis revealed no significant pleiotropic effects (Table S1A and S1B). Data visualization techniques, including scatter plots (**Figure 3**), funnel plots (**Figure 4**), and leave-one-out (**Figure 5**) analyses, effectively ruled out the influence of outliers and horizontal pleiotropy on the identified hub metabolites.

Discussion

This study investigated the influence of metabolic cell function on the development of PE through an MR approach. The primary objective was to reveal causal associations between specific metabolic cell functions and the incidence of cardiovascular conditions, such as PE. By employing MR and utilizing genetic variants as instrumental factors, we sought to investigate the potential causal impacts of metabolic cell functions on PE. The findings from this research could enhance our understanding of the underlying mechanisms and identify promising targets for interventions aimed at preventing and managing this serious pregnancy complication.

Sphingomyelin, a vital sphingolipid component of cell membranes, particularly in the nervous system, plays a crucial role in maintaining structural integrity and is involved in cell signaling pathways [36]. As a regulator of these pathways, sphingomyelin may help mitigate the risk of pregnancy-related complications, including PE. Studies suggest that it contributes to the maintenance of vascular integrity, endothelial function, and inflammatory responses, which are all key factors involved in the pathogenesis of PE [37]. By investigating the genetic variants associated with altered sphingomyelin levels and its potential impact on PE risk through MR, we can elucidate the causal relationship between sphingomyelin and PE. Our findings indicate that elevated sphingomyelin levels are linked to a decreased risk of PE, implying a protective influence of this sphingolipid on pregnancy outcomes.

1-Linoleoyl-GPG (18:2), also known as 1-linoleoyl-sn-glycero-3-phosphoglycerol, is a specific phospholipid found in various biological systems, including cell membranes and tissues [38]. Characterized by a linoleoyl fatty acid chain esterified to the sn-1 position of the glycerol backbone, and a phosphogly-cerol headgroup attached to the sn-3 position [39]. It plays a critical role in cellular function and metabolism [40]. As a component of cell membranes, it contributes to membrane structure and fluidity, influencing the transport of molecules across the membrane [41]. Additionally, it is implicated in cell signaling pathways related to lipid metabolism and inflammatory process-

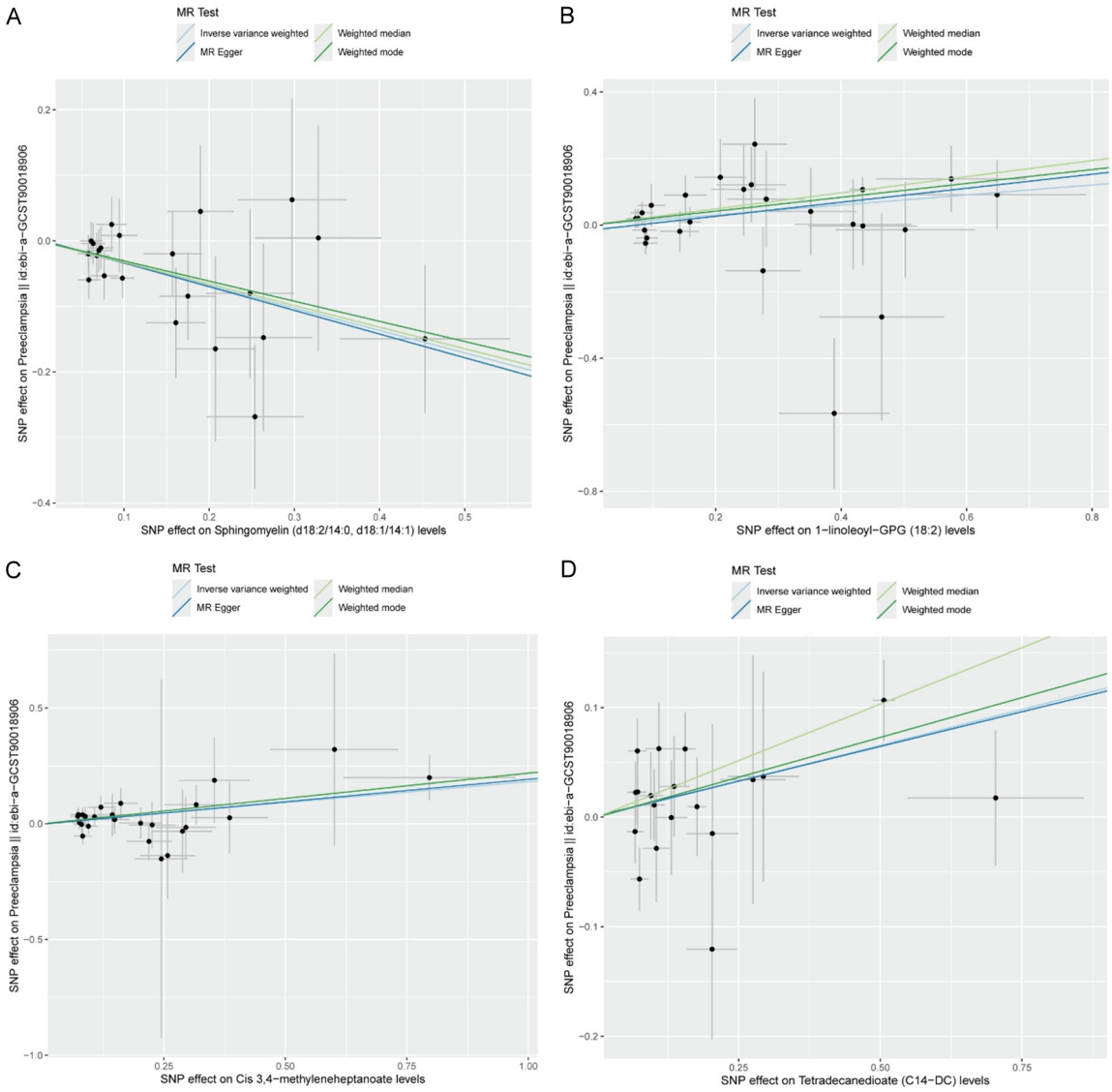

Figure 3. The scatter plot demonstrating the genetic associations of four metabolites and PE. A. Sphingomyelin in PE; B. Linoleoyl-GPG (18:2) in PE; C. Cis 3,4-methyleneheptanoate in PE; D. Tetradecanedioate (C14-DC) in PE.

es [42]. Recent studies have suggested a potential link between 1-linoleoyl-GPG levels and the development of PE, a serious pregnancy complication marked by high BP and damage to other organs [43, 44]. Pregnant women with PE have been found to have elevated levels of 1-linoleoyl-GPG compared to healthy counterparts [17], prompting further exploration of its role in PE pathophysiology and its potential as a biomarker for early detection or monitoring of the condition. Further research is essential to uncover the mechanisms behind this and determine how it affects clinical practice, as stated in [45]. It's been shown that the dysregulation of lipid metabolism in PE might have a significant association with elevated levels of 1-linoleoyl-GPG [46]. Another study analyzed the expression of enzymes involved in the biosynthesis and degradation of 1-linoleoyl-GPG in placental tissues from PE patients. The findings revealed dysregulated expression patterns, indicating a potential mechanism for the elevation of 1-Linoleoyl-GPG in the context of PE [45].

Cis 3,4-methyleneheptanoate is a crucial metabolite involved in energy metabolism and various physiological functions. It plays a significant role in lipid metabolism and energy generation while being implicated in signaling

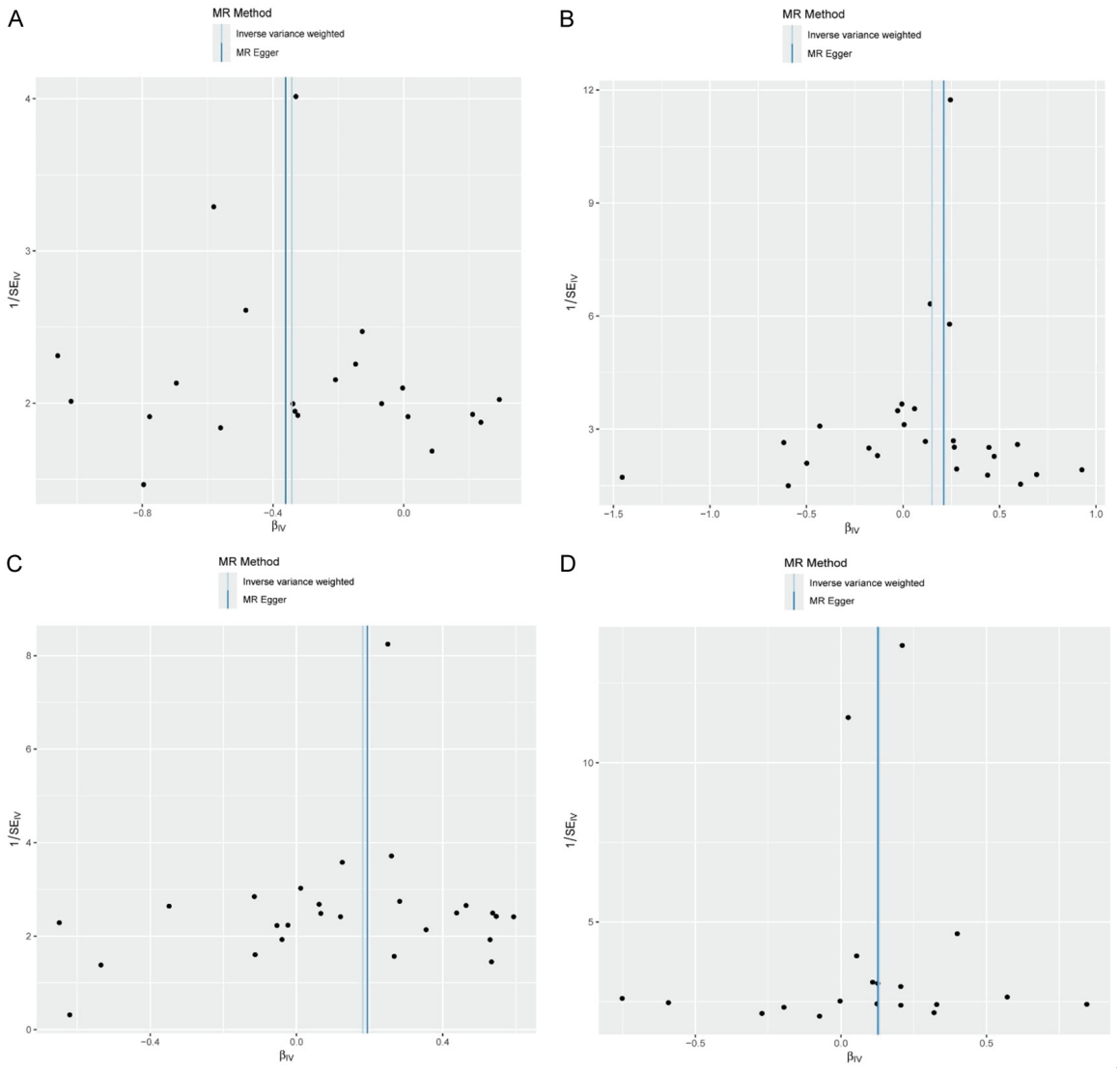

Figure 4. The funnel plot represents IVs for each significant causal relation between metabolites and PE. A. Sphingomyelin in PE; B. Linoleoyl-GPG (18:2) in PE; C. Cis 3,4-methyleneheptanoate in PE; D. Tetradecanedioate (C14-DC) in PE.

pathways related to inflammation and OS [47-49]. Alterations in cis 3,4-methyleneheptanoate levels have been linked to metabolic disorders such as obesity and cardiovascular disease [50]. In pregnancy, its levels are particularly relevant due to their potential impact on maternal and fetal health, with abnormal levels suggested to correlate with complications, including PE [51]. PE is a serious condition characterized by high blood pressure and damage to other organs, often occurring after the 20th week of pregnancy [52]. Further studies have indicated that elevated levels of cis-3,4-methyleneheptanoate may pose a risk factor for the development of PE during pregnancy. The potential relationship between the levels of this metabolic compound and the onset of PE signifies its importance in maternal health and requires a thorough investigation [17]. Our research has highlighted cis 3,4-methyleneheptanoate as a causal risk factor for PE, underscoring the need for further investigation into its role in pregnancy-related complications and its potential for developing diagnostic tools and targeted interventions.

Tetradecanedioate (C14-DC), a dicarboxylic acid, has gained substantial recognition within

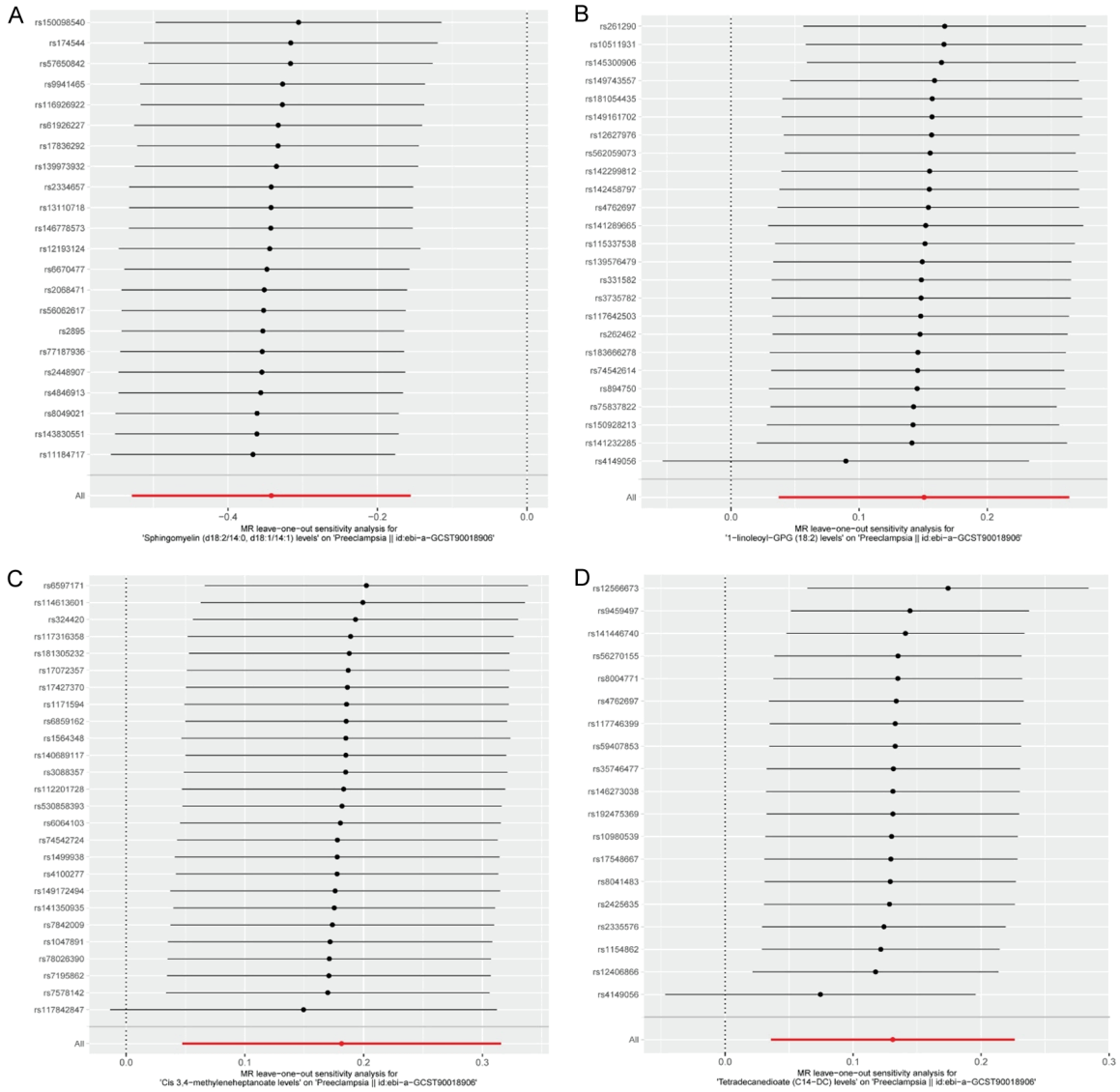

Figure 5. Leave-one-out showed causal relation between metabolites and PE. A. Sphingomyelin in PE; B. Linoleoyl-GPG (18:2) in PE; C. Cis 3,4-methyleneheptanoate in PE; D. Tetradecanedioate (C14-DC) in PE.

the scientific community for its potential impact on a variety of physiological and pathological processes [53]. C14-DC levels play a crucial role in energy metabolism and the beta-oxidation of fatty acids. It plays a role in breaking down long-chain fatty acids to generate power in the form of ATP. Additionally, C14-DC levels have been linked to certain metabolic disorders and diseases, making it an important biomarker for identifying and monitoring these conditions. Understanding the function of C14-DC levels can provide valuable insights into metabolic processes and contribute to the development of diagnostic and therapeutic approaches for associated medical conditions [54, 55]. C14-DC levels have been implicated as a potential causal risk factor in PE [56]. PE is a pregnancy complexity marked by elevated blood pressure and indications of harm to other organ systems, frequently affecting the liver and kidneys. One study discovered that increased levels of C14-DC were linked to a higher risk of developing PE [57]. The researchers suggested that the abnormal metabolism of long-chain fatty acids, possibly related to C14-DC levels, could contribute to the pathogenesis of PE [58, 59]. Our research has revealed that C14-DC levels are a causal risk

factor in PE. Higher concentrations of C14-DC have been associated with an elevated risk of developing this pregnancy complication, suggesting a possible involvement in the pathogenesis of PE. This discovery sheds light on the importance of understanding and monitoring C14-DC levels in the context of pregnancy-related complications.

While our study offers valuable insights, several limitations must be acknowledged. Firstly, despite conducting multiple sensitivity tests, the assessment of horizontal pleiotropy was not exhaustive. Secondly, the absence of individual-level data restricted further categorical analyses within the population. Thirdly, relying on a Eurocentric database may limit the applicability of our findings to diverse cultural groups. Lastly, employing a less strict threshold for evaluating results could lead to incorrect detections. Nonetheless, this research facilitates a deeper exploration of the strong relationship between metabolic cell analysis and the risk of developing PE.

Conclusions

Our comprehensive two-sample MR analysis has identified causal links between various metabolite cell phenotypes and the risk of PE. By meticulously controlling for confounding variables and addressing potential reverse causality, we have enhanced the robustness of our findings. This study paves the way for exploring the underlying mechanisms of PE and presents opportunities for early interventions and improved therapeutic strategies.

Disclosure of conflict of interest

None.

Abbreviations

EAFs, Effect allele frequencies; GWAS, Genome-wide association studies; IVW, Inverse variance weighting; IVs, Instrumental variables; LD, Linkage disequilibrium; MR, Mendelian randomization; MR-PRESSO, MR Pleiotropy Residual Sum and Outlier; PE, Preeclampsia; SNPs, Single-nucleotide polymorphisms.

Address correspondence to: Shilei Pan, Department of Obstetrics and Gynecology, Zhujiang Hospital, Southern Medical University, No. 253, Middle Gongye Avenue, Haizhu District, Guangzhou 510282, Guangdong, The People's Republic of China. E-mail: 13602882918@163.com; Pengkhun Nov, Department of Radiation Oncology, Oncology Center, Zhujiang Hospital of Southern Medical University, No. 253, Middle Gongye Avenue, Haizhu District, Guangzhou 510282, Guangdong, The People's Republic of China. ORCID: 0000-0002-7016-8285; E-mail: pengkhun01@gmail.com

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Table S1A. Heterogeneity of metabolites in preeclampsia

Exposure	egger_intercept	se	pval
Carnitine levels	-0.009913475	0.015426409	0.526556274
Imidazole lactate levels	0.010927799	0.01650048	0.51362526
3-indoxyl sulfate levels	-0.008304764	0.018765627	0.662839
Isovalerylcarnitine (C5) levels	-0.033772239	0.029698658	0.266696354
1-arachidonylglycerol (20:4) levels	0.037537461	0.019545765	0.067851747
Glutamine degradant levels	-0.005008903	0.015834282	0.754376068
Beta-hydroxyisovaleroylcarnitine levels	-0.015358134	0.018100583	0.402100403
Tetradecanedioate (C14-DC) levels	0.001191665	0.013978407	0.933058279
Hexadecanedioate (C16-DC) levels	-0.004684509	0.011495691	0.687764079
Glycerophosphoethanolamine levels	-0.030836678	0.018164993	0.103688446
Gamma-glutamylalanine levels	-0.016640162	0.024440313	0.506342021
21-hydroxypregnenolone disulfate levels	-0.009102141	0.017207438	0.600075964
Androstenediol (3beta,17beta) monosulfate (1) levels	0.002658048	0.013921243	0.849821797
1-lignoceroyl-GPC (24:0) levels	0.042636109	0.031503536	0.197385076
2-oxoarginine levels	-0.004982146	0.033528033	0.883360155
1-(1-enyl-stearoyl)-GPE (p-18:0) levels	0.013819761	0.033558386	0.684649937
N-oleoyltaurine levels	0.003417909	0.02587603	0.896380108
Imidazole propionate levels	-0.006031894	0.02381578	0.802307394
Alliin levels	-0.007259853	0.01940328	0.712659376
Margaroylcarnitine (C17) levels	0.023581264	0.018017718	0.203002646
(R)-3-hydroxybutyrylcarnitine levels	0.026014679	0.025249807	0.314067432
2-hydroxydecanoate levels	0.010833514	0.02292067	0.642844073
2-aminophenol sulfate levels	0.025067907	0.017642644	0.165026344
2-aminoheptanoate levels	-0.009658511	0.018779308	0.611547489
17alpha-hydroxypregnanolone glucuronide levels	-0.003206036	0.013288028	0.810932666
Octadecenedioylcarnitine (C18:1-DC) levels	-0.007820737	0.017501218	0.661354741
Sphingomyelin (d18:2/14:0, d18:1/14:1) levels	0.002240401	0.01837619	0.904180098
Carnitine C14:1 levels	0.013703025	0.018497951	0.46422461
Octadecanedioylcarnitine (C18-DC) levels	-0.01614085	0.018675656	0.395655489
Sphingomyelin (d18:1/20:1, d18:2/20:0) levels	-0.00831166	0.016388485	0.616156809
Glycodeoxycholate 3-sulfate levels	-0.01840586	0.014488413	0.214045515
1-(1-enyl-palmitoyl)-2-palmitoleoyl-GPC (P-16:0/16:1) levels	-0.00718557	0.012878107	0.580873928
1-linoleoyl-GPG (18:2) levels	-0.015667903	0.017165875	0.370850414

1-palmitoyl-2-oleoyl-GPI (16:0/18:1) levels	-0.004047221	0.021715378	0.853716488
1-(1-enyl-palmitoyl)-2-palmitoyl-GPC (P-16:0/16:0) levels	-0.012205457	0.013235387	0.363554778
Linoleoyl-arachidonoyl-glycerol (18:2/20:4) [2] levels	-0.013558288	0.016422811	0.414973009
Hexadecenedioate (C16:1-DC) levels	-0.005663838	0.011228574	0.617782458
Perfluorooctanoate (PFOA) levels	0.009165092	0.024618352	0.713409762
Glucuronide of piperine metabolite C17H21NO3 (3) levels	-0.00072463	0.013976012	0.959190934
N-acetyl-isoputreanine levels	0.006921981	0.015058254	0.648509903
N-lactoyl isoleucine levels	-0.02263498	0.029369292	0.452852587
Glucuronide of piperine metabolite C17H21NO3 (4) levels	-0.004161934	0.015182324	0.786942725
3-hydroxy-2-methylpyridine sulfate levels	-0.035239094	0.031935506	0.283613786
4-acetylcatechol sulfate (1) levels	-0.005656084	0.016989009	0.743512578
Eicosenedioate (C20:1-DC) levels	-0.009640829	0.018259159	0.60303487
Cis 3,4-methyleneheptanoate levels	-0.002211489	0.015845211	0.890165716
Metabolonic lactone sulfate levels	-0.00653795	0.010677385	0.545099372
S-carboxyethylcysteine levels	-0.015031805	0.021836906	0.498756191
5-oxoproline levels	0.012178462	0.01762109	0.496401014
N-acetyl-L-alanine levels	-0.012778413	0.019255845	0.511843959
Creatine levels	0.011577359	0.014109906	0.42034682
4-acetaminophen sulfate levels	-0.007678745	0.024711647	0.758801527
Linoleate (18:2n6) levels	-0.05388713	0.026131693	0.052427922
Cysteinylglycine levels	-0.033757423	0.020588031	0.118434566
Phenylalanine levels	0.017460598	0.02276722	0.452554976
1-methylnicotinamide levels	0.033192593	0.034377605	0.347815187
Xylose levels	-0.00569529	0.019728542	0.775302916
Alanine levels	-0.035424834	0.020292733	0.096209627
Mannose levels	0.006204624	0.018213709	0.736325926
Cysteine levels	0.001059828	0.019028077	0.95642923
X-07765 levels	0.015483715	0.019722054	0.440409655
X-12127 levels	0.01996676	0.021624598	0.363721275
X-13723 levels	-0.010879605	0.033079063	0.747902473
X-15728 levels	-0.001147479	0.019239108	0.952835473
X-17676 levels	0.00844784	0.015045433	0.579272615
X-21471 levels	0.000802947	0.018508164	0.965727393
X-21733 levels	0.028904488	0.025708628	0.270777884
X-21470 levels	-0.006866025	0.021849832	0.757401301

X-23655 levels	-0.062817478	0.029862309	0.045644113
X-23659 levels	0.012111756	0.013305413	0.371033001
X-24243 levels	0.004233661	0.026354805	0.874165334
X-24546 levels	-0.014751594	0.014706605	0.325442801
Adenosine 5'-diphosphate (ADP) to creatine ratio	0.002078585	0.018979957	0.913885633
N-acetylputrescine to (N(1) + N(8))-acetylspermidine ratio	-0.007215122	0.012762832	0.576057986
Arachidonate (20:4n6) to oleate to vaccenate (18:1) ratio	-0.010926413	0.018810387	0.568529613
Arachidonate (20:4n6) to pyruvate ratio	0.008081097	0.030926224	0.797664087
Serine to pyruvate ratio	-0.019645018	0.027633082	0.486771752
5-oxoproline to citrate ratio	-0.005174468	0.015207307	0.738710795
Oleoyl-linoleoyl-glycerol (18:1 to 18:2) [2] to linoleoyl-arachidonoyl-glycerol (18:2 to 20:4) [1] ratio	-0.006997047	0.014514322	0.634305817
Oleoyl-linoleoyl-glycerol (18:1 to 18:2) [2] to linoleoyl-arachidonoyl-glycerol (18:2 to 20:4) [2] ratio	-0.004199237	0.01469327	0.77730113
Spermidine to N-acetylputrescine ratio	8.12E-05	0.01985001	0.996775308
Adenosine 5'-monophosphate (AMP) to valine ratio	0.010714955	0.026034054	0.685793688
Adenosine 5'-monophosphate (AMP) to glutamate ratio	-0.018020755	0.02812502	0.528623961
Salicylate to caprylate (8:0) ratio	0.019721628	0.018203975	0.290927191
Inosine to theophylline ratio	0.017913958	0.02050227	0.392622704
Glucose-to-mannose ratio	0.005811479	0.018512824	0.756414056
Phosphate to 5-oxoproline ratio	-0.007348035	0.015297572	0.635522748
Fructose to maltose ratio	0.00941075	0.023583071	0.693887628

Table S1B. Pleiotropy of metabolites in preeclampsia

Exposure	Method	Q	Q_df	Q_pval
Carnitine levels	MR Egger	18.70048812	24	0.767768183
Carnitine levels	Inverse variance weighted	19.11346156	25	0.791648051
Imidazole lactate levels	MR Egger	37.67616381	26	0.064880143
Imidazole lactate levels	Inverse variance weighted	38.31173696	27	0.07306596
3-indoxyl sulfate levels	MR Egger	21.19304001	20	0.385849702
3-indoxyl sulfate levels	Inverse variance weighted	21.40057515	21	0.434726025
Isovalerylcarnitine (C5) levels	MR Egger	31.80043861	24	0.13201906
Isovalerylcarnitine (C5) levels	Inverse variance weighted	33.51387477	25	0.118713829
1-arachidonylglycerol (20:4) levels	MR Egger	23.15281577	22	0.393143966
1-arachidonylglycerol (20:4) levels	Inverse variance weighted	27.03436919	23	0.254507736
Glutamine degradant levels	MR Egger	26.26410651	25	0.393613897

Glutamine degradant levels	Inverse variance weighted	26.36923274	26	0.442959537
Beta-hydroxyisovaleroylcarnitine levels	MR Egger	36.5096309	34	0.352852895
Beta-hydroxyisovaleroylcarnitine levels	Inverse variance weighted	37.28270353	35	0.364488611
Tetradecanedioate (C14-DC) levels	MR Egger	19.14920619	17	0.320035411
Tetradecanedioate (C14-DC) levels	Inverse variance weighted	19.15739263	18	0.382192064
Hexadecanedioate (C16-DC) levels	MR Egger	20.65096616	21	0.480428896
Hexadecanedioate (C16-DC) levels	Inverse variance weighted	20.81702347	22	0.532069811
Glycerophosphoethanolamine levels	MR Egger	22.22031538	22	0.446806089
Glycerophosphoethanolamine levels	Inverse variance weighted	25.13098043	23	0.343567485
Gamma-glutamylalanine levels	MR Egger	9.457432108	15	0.852412109
Gamma-glutamylalanine levels	Inverse variance weighted	9.920987451	16	0.870721824
21-hydroxypregnenolone disulfate levels	MR Egger	25.4539976	36	0.904841552
21-hydroxypregnenolone disulfate levels	Inverse variance weighted	25.73380211	37	0.918186551
Androstenediol (3beta,17beta) monosulfate (1) levels	MR Egger	22.34940728	31	0.871733206
Androstenediol (3beta,17beta) monosulfate (1) levels	Inverse variance weighted	22.38586332	32	0.896741385
1-lignoceroyl-GPC (24:0) levels	MR Egger	21.42018058	14	0.091339453
1-lignoceroyl-GPC (24:0) levels	Inverse variance weighted	24.22259032	15	0.061408649
2-oxoarginine levels	MR Egger	27.7277834	20	0.116001542
2-oxoarginine levels	Inverse variance weighted	27.75839611	21	0.147137486
1-(1-enyl-stearoyl)-GPE (p-18:0) levels	MR Egger	26.10193763	21	0.202588802
1-(1-enyl-stearoyl)-GPE (p-18:0) levels	Inverse variance weighted	26.31272879	22	0.238497488
N-oleoyltaurine levels	MR Egger	18.03724729	18	0.453201385
N-oleoyltaurine levels	Inverse variance weighted	18.05473059	19	0.518785167
Imidazole propionate levels	MR Egger	27.96929556	23	0.21694494
Imidazole propionate levels	Inverse variance weighted	28.04730219	24	0.258049804
Alliin levels	MR Egger	15.54956847	18	0.623950766
Alliin levels	Inverse variance weighted	15.68956115	19	0.677879741
Margaroylcarnitine (C17) levels	MR Egger	20.94781035	24	0.64180213
Margaroylcarnitine (C17) levels	Inverse variance weighted	22.66072048	25	0.597367696
(R)-3-hydroxybutyrylcarnitine levels	MR Egger	25.32951557	22	0.281577925
(R)-3-hydroxybutyrylcarnitine levels	Inverse variance weighted	26.55166695	23	0.275520989
2-hydroxydecanoate levels	MR Egger	20.94266767	16	0.180724918
2-hydroxydecanoate levels	Inverse variance weighted	21.23508027	17	0.21595124
2-aminophenol sulfate levels	MR Egger	27.67748038	32	0.685215458
2-aminophenol sulfate levels	Inverse variance weighted	29.69635237	33	0.63239151

2-aminoheptanoate levels	MR Egger	17.94258609	25	0.844818237
2-aminoheptanoate levels	Inverse variance weighted	18.20710774	26	0.868108805
17alpha-hydroxypregnanolone glucuronide levels	MR Egger	35.86051456	31	0.250972354
17alpha-hydroxypregnanolone glucuronide levels	Inverse variance weighted	35.92785416	32	0.289495845
Octadecenedioylcarnitine (C18:1-DC) levels	MR Egger	15.78055903	15	0.39678392
Octadecenedioylcarnitine (C18:1-DC) levels	Inverse variance weighted	15.99064154	16	0.453614157
Sphingomyelin (d18:2/14:0, d18:1/14:1) levels	MR Egger	13.51198391	20	0.854353182
Sphingomyelin (d18:2/14:0, d18:1/14:1) levels	Inverse variance weighted	13.52684808	21	0.889053078
Carnitine C14:1 levels	MR Egger	38.15572837	32	0.209770713
Carnitine C14:1 levels	Inverse variance weighted	38.8100563	33	0.224200481
Octadecanedioylcarnitine (C18-DC) levels	MR Egger	38.39245265	25	0.042336773
Octadecanedioylcarnitine (C18-DC) levels	Inverse variance weighted	39.53956758	26	0.043261128
Sphingomyelin (d18:1/20:1, d18:2/20:0) levels	MR Egger	28.14129363	27	0.403716281
Sphingomyelin (d18:1/20:1, d18:2/20:0) levels	Inverse variance weighted	28.40938217	28	0.442920223
Glycodeoxycholate 3-sulfate levels	MR Egger	31.42384656	29	0.345721878
Glycodeoxycholate 3-sulfate levels	Inverse variance weighted	33.17261355	30	0.315100112
1-(1-enyl-palmitoyl)-2-palmitoleoyl-GPC (P-16:0/16:1) levels	MR Egger	30.88885479	31	0.471823446
1-(1-enyl-palmitoyl)-2-palmitoleoyl-GPC (P-16:0/16:1) levels	Inverse variance weighted	31.20018294	32	0.506858994
1-linoleoyl-GPG (18:2) levels	MR Egger	27.23809075	23	0.245969318
1-linoleoyl-GPG (18:2) levels	Inverse variance weighted	28.22468526	24	0.2506771
1-palmitoyl-2-oleoyl-GPI (16:0/18:1) levels	MR Egger	20.945745	24	0.641923813
1-palmitoyl-2-oleoyl-GPI (16:0/18:1) levels	Inverse variance weighted	20.98048094	25	0.693697562
1-(1-enyl-palmitoyl)-2-palmitoyl-GPC (P-16:0/16:0) levels	MR Egger	24.0481299	31	0.808575635
1-(1-enyl-palmitoyl)-2-palmitoyl-GPC (P-16:0/16:0) levels	Inverse variance weighted	24.89855248	32	0.810116542
Linoleoyl-arachidonoyl-glycerol (18:2/20:4) [2] levels	MR Egger	27.55008142	33	0.735139794
Linoleoyl-arachidonoyl-glycerol (18:2/20:4) [2] levels	Inverse variance weighted	28.23165812	34	0.745810249
Hexadecenedioate (C16:1-DC) levels	MR Egger	31.53439362	29	0.340677082
Hexadecenedioate (C16:1-DC) levels	Inverse variance weighted	31.81106167	30	0.376365909
Perfluorooctanoate (PFOA) levels	MR Egger	25.75847705	21	0.215808369
Perfluorooctanoate (PFOA) levels	Inverse variance weighted	25.92848006	22	0.254765408
Glucuronide of piperine metabolite C17H21NO3 (3) levels	MR Egger	15.43110006	19	0.694832269
Glucuronide of piperine metabolite C17H21NO3 (3) levels	Inverse variance weighted	15.43378829	20	0.751071583
N-acetyl-isoputreanine levels	MR Egger	38.90695246	36	0.340193609
N-acetyl-isoputreanine levels	Inverse variance weighted	39.13532099	37	0.374166543
N-lactoyl isoleucine levels	MR Egger	8.640415581	15	0.895556246
N-lactoyl isoleucine levels	Inverse variance weighted	9.234397572	16	0.903447421

Glucuronide of piperine metabolite C17H21NO3 (4) levels	MR Egger	22.00773344	19	0.283871545
Glucuronide of piperine metabolite C17H21NO3 (4) levels	Inverse variance weighted	22.09477678	20	0.335390027
3-hydroxy-2-methylpyridine sulfate levels	MR Egger	22.36171098	19	0.266625986
3-hydroxy-2-methylpyridine sulfate levels	Inverse variance weighted	23.79473443	20	0.251473626
4-acetylcatechol sulfate (1) levels	MR Egger	13.8827441	16	0.607448845
4-acetylcatechol sulfate (1) levels	Inverse variance weighted	13.99358387	17	0.667556062
Eicosenedioate (C20:1-DC) levels	MR Egger	17.18484974	21	0.699841713
Eicosenedioate (C20:1-DC) levels	Inverse variance weighted	17.46363332	22	0.737273727
Cis 3,4-methyleneheptanoate levels	MR Egger	13.56804143	24	0.955812662
Cis 3,4-methyleneheptanoate levels	Inverse variance weighted	13.58752074	25	0.968454992
Metabolonic lactone sulfate levels	MR Egger	24.37920794	29	0.71002103
Metabolonic lactone sulfate levels	Inverse variance weighted	24.75414073	30	0.736889844
S-carboxyethylcysteine levels	MR Egger	18.2777975	21	0.631362987
S-carboxyethylcysteine levels	Inverse variance weighted	18.75164658	22	0.660610178
5-oxoproline levels	MR Egger	24.67176013	23	0.367428519
5-oxoproline levels	Inverse variance weighted	25.1841395	24	0.39581006
N-acetyl-L-alanine levels	MR Egger	21.75309671	31	0.890615447
N-acetyl-L-alanine levels	Inverse variance weighted	22.19347783	32	0.902144035
Creatine levels	MR Egger	26.00604395	23	0.300580983
Creatine levels	Inverse variance weighted	26.76727634	24	0.315417402
4-acetaminophen sulfate levels	MR Egger	19.26465436	23	0.685761234
4-acetaminophen sulfate levels	Inverse variance weighted	19.36120989	24	0.732461689
Linoleate (18:2n6) levels	MR Egger	21.60288276	20	0.362442096
Linoleate (18:2n6) levels	Inverse variance weighted	26.19609708	21	0.199070346
Cysteinylglycine levels	MR Egger	13.01459174	18	0.790705444
Cysteinylglycine levels	Inverse variance weighted	15.70308488	19	0.676987324
Phenylalanine levels	MR Egger	13.98951782	19	0.784302024
Phenylalanine levels	Inverse variance weighted	14.57768155	20	0.800041412
1-methylnicotinamide levels	MR Egger	22.38588106	17	0.170330728
1-methylnicotinamide levels	Inverse variance weighted	23.61347972	18	0.168102967
Xylose levels	MR Egger	14.93933491	24	0.922521215
Xylose levels	Inverse variance weighted	15.02267263	25	0.940851004
Alanine levels	MR Egger	16.45647688	20	0.687932088
Alanine levels	Inverse variance weighted	19.50391276	21	0.552848584
Mannose levels	MR Egger	26.97413321	24	0.30566034

Mannose levels	Inverse variance weighted	27.1045611	25	0.350681145
Cysteine levels	MR Egger	11.28444521	13	0.587002018
Cysteine levels	Inverse variance weighted	11.28754749	14	0.663313747
X-07765 levels	MR Egger	14.78338507	23	0.902228724
X-07765 levels	Inverse variance weighted	15.39976153	24	0.908514723
X-12127 levels	MR Egger	26.06676222	28	0.569374701
X-12127 levels	Inverse variance weighted	26.91931073	29	0.576058615
X-13723 levels	MR Egger	11.33817009	12	0.500181206
X-13723 levels	Inverse variance weighted	11.44634333	13	0.573479484
X-15728 levels	MR Egger	33.68038211	30	0.293790268
X-15728 levels	Inverse variance weighted	33.68437581	31	0.338797204
X-17676 levels	MR Egger	15.61232355	26	0.945083504
X-17676 levels	Inverse variance weighted	15.92759305	27	0.954313253
X-21471 levels	MR Egger	33.6842142	26	0.143161032
X-21471 levels	Inverse variance weighted	33.68665257	27	0.175346181
X-21733 levels	MR Egger	45.20645737	27	0.015463698
X-21733 levels	Inverse variance weighted	47.32291449	28	0.012661222
X-21470 levels	MR Egger	9.255558887	16	0.902516973
X-21470 levels	Inverse variance weighted	9.354303762	17	0.928486574
X-23655 levels	MR Egger	33.28945371	25	0.12398894
X-23655 levels	Inverse variance weighted	39.18169992	26	0.046840096
X-23659 levels	MR Egger	23.47846788	26	0.605760068
X-23659 levels	Inverse variance weighted	24.30709178	27	0.613232516
X-24243 levels	MR Egger	21.15316177	18	0.271756407
X-24243 levels	Inverse variance weighted	21.18348778	19	0.326751379
X-24546 levels	MR Egger	23.81703711	25	0.529964625
X-24546 levels	Inverse variance weighted	24.82316478	26	0.529002092
Adenosine 5’-diphosphate (ADP) to creatine ratio	MR Egger	17.24106159	20	0.63726958
Adenosine 5’-diphosphate (ADP) to creatine ratio	Inverse variance weighted	17.25305507	21	0.69565686
N-acetylputrescine to (N(1) + N(8))-acetylspermidine ratio	MR Egger	35.6006332	30	0.221443089
N-acetylputrescine to (N(1) + N(8))-acetylspermidine ratio	Inverse variance weighted	35.9798868	31	0.246621827
Arachidonate (20:4n6) to oleate to vaccenate (18:1) ratio	MR Egger	21.75726951	18	0.24294012
Arachidonate (20:4n6) to oleate to vaccenate (18:1) ratio	Inverse variance weighted	22.16511123	19	0.276116156
Arachidonate (20:4n6) to pyruvate ratio	MR Egger	9.07589158	14	0.82615843
Arachidonate (20:4n6) to pyruvate ratio	Inverse variance weighted	9.144170537	15	0.869859287
Serine to pyruvate ratio	MR Egger	18.96824101	17	0.330358147

Serine to pyruvate ratio	Inverse variance weighted	19.53216989	18	0.359763983
5-oxoproline to citrate ratio	MR Egger	13.78062294	14	0.466179584
5-oxoproline to citrate ratio	Inverse variance weighted	13.89640113	15	0.53340042
Oleoyl-linoleoyl-glycerol (18:1 to 18:2) [2] to linoleoyl-arachidonoyl-glycerol (18:2 to 20:4) [1] ratio	MR Egger	22.42049622	23	0.495002895
Oleoyl-linoleoyl-glycerol (18:1 to 18:2) [2] to linoleoyl-arachidonoyl-glycerol (18:2 to 20:4) [1] ratio	Inverse variance weighted	22.65289618	24	0.540357942
Oleoyl-linoleoyl-glycerol (18:1 to 18:2) [2] to linoleoyl-arachidonoyl-glycerol (18:2 to 20:4) [2] ratio	MR Egger	36.76532887	26	0.078466235
Oleoyl-linoleoyl-glycerol (18:1 to 18:2) [2] to linoleoyl-arachidonoyl-glycerol (18:2 to 20:4) [2] ratio	Inverse variance weighted	36.88082542	27	0.097313473
Spermidine to N-acetylputrescine ratio	MR Egger	16.17173532	20	0.705914527
Spermidine to N-acetylputrescine ratio	Inverse variance weighted	16.17175207	21	0.759924616
Adenosine 5’-monophosphate (AMP) to valine ratio	MR Egger	19.08395672	17	0.323734469
Adenosine 5’-monophosphate (AMP) to valine ratio	Inverse variance weighted	19.2741156	18	0.375129316
Adenosine 5’-monophosphate (AMP) to glutamate ratio	MR Egger	24.66318206	21	0.262046447
Adenosine 5’-monophosphate (AMP) to glutamate ratio	Inverse variance weighted	25.14534086	22	0.290174927
Salicylate to caprylate (8:0) ratio	MR Egger	11.36261344	21	0.955251669
Salicylate to caprylate (8:0) ratio	Inverse variance weighted	12.53630251	22	0.945293044
Inosine to theophylline ratio	MR Egger	13.51080592	20	0.85440857
Inosine to theophylline ratio	Inverse variance weighted	14.27425344	21	0.857533114
Glucose-to-mannose ratio	MR Egger	24.23205262	23	0.391053344
Glucose-to-mannose ratio	Inverse variance weighted	24.33587485	24	0.442531227
Phosphate to 5-oxoproline ratio	MR Egger	26.32956258	23	0.285556211
Phosphate to 5-oxoproline ratio	Inverse variance weighted	26.59368987	24	0.323745259
Fructose to maltose ratio	MR Egger	21.24505336	21	0.444068555
Fructose to maltose ratio	Inverse variance weighted	21.40614993	22	0.495773296