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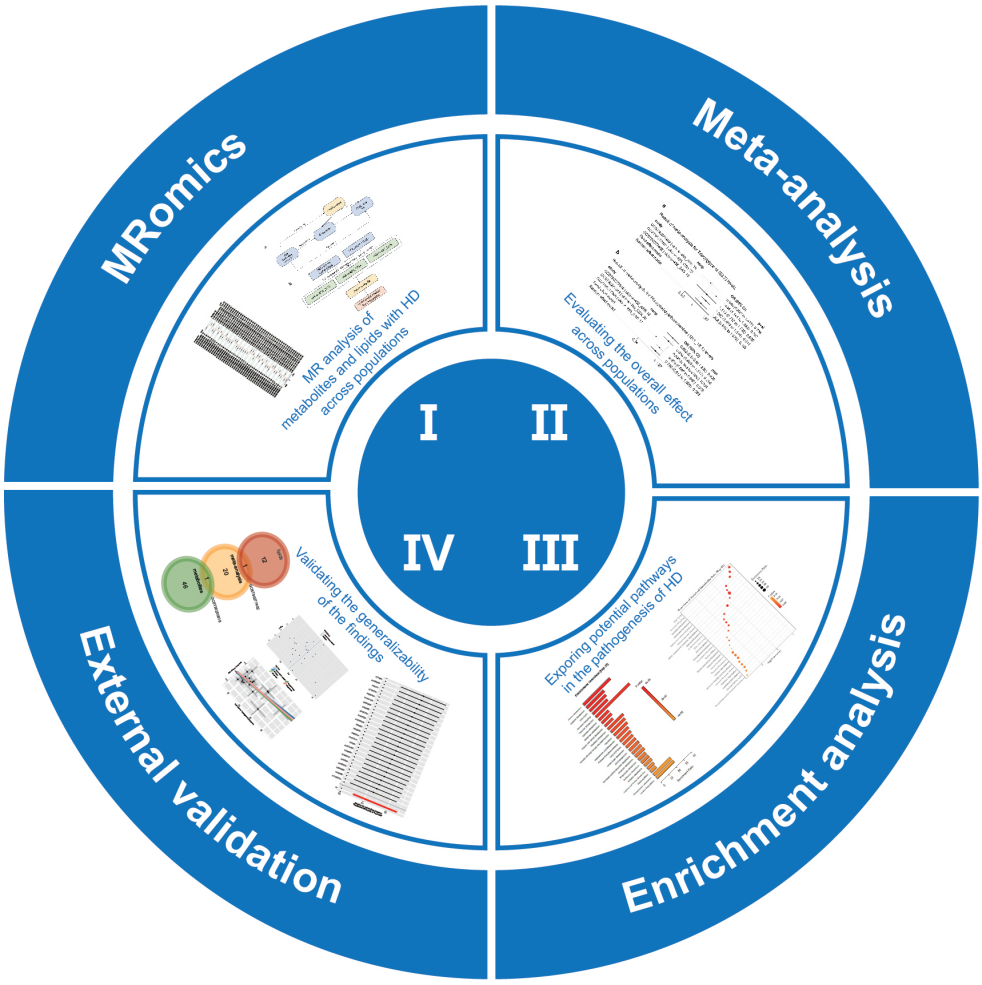
Graphical Abstract



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Causal Relationships between 1400 Serum Metabolite Traits, 179 Plasma Lipids, and Hemorrhoids: A Mendelian Randomization Study and Meta-analysis

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Abstract

Background: Hemorrhoids is a common anorectal disorder that significantly impacts patients' quality of life over the long term and imposes a substantial economic burden. However, the potential link between HD and serum metabolites and lipids has been scarcely studied, and its pathogenesis remains unclear.

Methods: The causal relationships between serum metabolite traits, plasma lipids, and HD were evaluated using Mendelian Randomization. Inverse Variance Weighted was the most important analysis approach. Sensitivity analysis was used to assess the robustness of the results. Enrichment analysis was applied to the metabolites obtained from the results of MR analysis and meta-analysis.

Results: The risk factors included Citramalate levels (OR = 1.487, 95%CI = 1.172-1.888, P = 0.001), P-cresol sulfate levels (OR = 1.476, 95%CI = 1.111-1.961, P = 0.007), and Triacylglycerol (53:3) levels (OR = 1.262, 95%CI = 1.049-1.518, P = 0.014). The protective factors included Imidazole lactate levels (OR = 0.865, 95%CI = 0.756-0.989, P = 0.034), 1-(1-enyl-stearoyl)-GPE (p-18:0) levels (OR = 0.722, 95%CI = 0.552-0.944, P = 0.017), and Phosphatidylethanolamine (18:1_18:1) levels (OR = 0.803, 95%CI = 0.666-0.969, P = 0.022). Enrichment analysis showed that risk factors were enriched in Alanine Metabolism (Holm P = 0.0039), Cysteine Metabolism (Holm P = 0.0146), Urea Cycle (Holm P = 0.0181), and Ammonia Recycling (Holm P = 0.0245). Protective factors were enriched in the Urea Cycle (Holm P = 0.00754), Ammonia Recycling (Holm P = 0.0102), Aspartate Metabolism (Holm P = 0.0147), Glutamate Metabolism (Holm P = 0.038), Arginine and Proline Metabolism (Holm P = 0.0479), and Malate-Aspartate Shuttle (Holm P = 0.0495).

Conclusion: Previous studies often focused on the causal relationships between a few potential risk factors and HD in a single population. Our study is the first to address this gap by integrating a broad range of 1400 metabolites and 179 lipids with HD across diverse populations, and identifying specific metabolic pathways involved in HD development.

Keywords: Serum Metabolites; Plasma Lipids; Hemorrhoids; Mendelian Randomization; Meta-analysis.

Introduction

Hemorrhoids (HD) is one of the most common types of anorectal disorders. Epidemiological studies indicate that over 50% of individuals experience at least one episode of HD before the age of 50 [1]. Furthermore, studies have suggested that HD may be associated with an increased risk of colorectal cancer [2]. The pathogenesis of HD is complex and involves multiple factors, including dietary habits, lifestyle, local inflammation, and abnormalities in local anatomical structures [3, 4]. HD is prevalent worldwide and has a long-term and significant negative impact on patients' quality of daily life. However, the scientific community has yet to fully understand the specific pathophysiological processes of HD and its association with risk factors.

Numerous studies have indicated significant correlations between serum metabolites and HD triggers, such as constipation, diarrhea, and varicose veins. For example, C4 and fibroblast growth factor 19 (FGF19) in serum are considered biomarkers for bile acid diarrhoea [5], highlighting the importance of serum metabolites in the pathogenesis of diarrhea, which itself may trigger HD. Notably, many studies have discovered

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complex interactions between serum metabolites and the gut microbiota [6-8]. Evidence from Mendelian Randomization studies supports a causal correlation between the gut microbiota and the occurrence of HD [9]. Although existing evidence suggests a potential link between serum metabolites and HD, the direct causal relationship between the two requires further research to be clarified.

Lipids are important components of cell membranes and are involved in many physiological functions, including energy storage, signal transduction, and immune responses [10-12]. Studies indicate that plasma lipids may be associated with various inflammatory diseases [13], and HD, as an inflammatory anorectal disease, may be related to vascular inflammation and tissue damage. For instance, oxidized low-density lipoprotein (ox-LDL) can activate endothelial cells and smooth muscle cells, promoting the release of inflammatory factors [14, 15], which may exacerbate the local inflammatory state in HD patients. Additionally, dyslipidemia is related to an imbalance in the gut microbiota [16], which has been proven to be associated with various intestinal diseases, including HD [9, 17]. Therefore, regulating plasma lipid levels and improving dyslipidemia may have positive significance in the prevention and treatment of HD. However, further in-depth research is needed to clarify the specific mechanisms between dyslipidemia and HD.

Mendelian Randomization (MR), as an innovative method for causal inference, has been widely applied in epidemiological and genetic research in recent years. MR utilizes single nucleotide polymorphisms (SNPs) as naturally occurring instrumental variables (IVs). By analyzing the association between genetic variants and diseases, MR infers the potential causal relationship between exposure factors and outcomes. Since genetic variations are determined before an individual's birth and are not directly influenced by environmental factors, MR analysis can simulate the conditions of randomized controlled trials, effectively reducing confounding factors and reverse causation, thereby providing more reliable causal inference evidence.

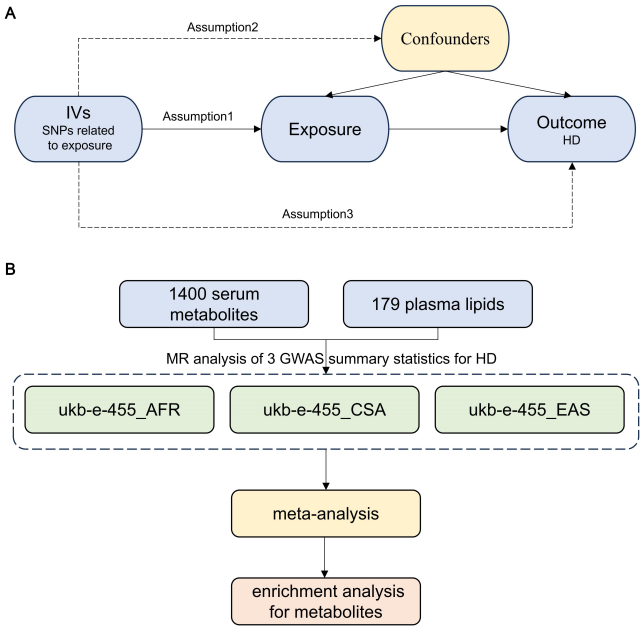
Previous MR studies have identified obesity and plasma low-density lipoprotein (LDL) levels as risk factors for HD [18, 19], but these studies often focused on only a few potential risk factors. For the first time, this study conducted a comprehensive scan of the causal relationship between HD and metabolites and lipids in blood, using 1,400 serum metabolites and 179 plasma lipids as exposure factors. Moreover, previous MR studies often focused on a single population, raising concerns about the generalizability of their conclusions to other populations. To address this issue, we obtained GWAS data from individuals of African American or Afro-Caribbean, South Asian, and East Asian descent, and performed a meta-analysis of MR results across these populations to obtain robust conclusions. We also conducted external validation using GWAS data on HD from individuals of European ancestry.

Materials and methods

Study design

The three assumptions underlying the causal interpretation of MR analysis and the design of this study are displayed in Figure 1. The IVs used in the analysis need to satisfy three assumptions: (1) IVs must be strongly associated with the exposure variable; (2) IVs must follow the principle of random assignment and not be influenced by confounding factors; (3) IVs should not be directly related to the outcome but should influence the outcome only through the potential causal relationship between the exposure and the outcome [20].

Figure 1: Flowchart of study design. (**A**) Three assumptions of MR analysis. (**B**) Study workflow.



GWAS data for serum metabolites and HD

The data used in this study were all obtained from publicly available GWAS datasets. GWAS summary statistics for 1,400 serum metabolite traits were acquired from the genome-wide association study by Chen et al. (SNPs = 1,540,000,000 approximately). The study cohort included 8, 299 individuals of European ancestry [21]. GWAS summary statistics for plasma lipids were obtained from the genome-wide association analysis by Ottensmann et al. [22] (SNPs = 11,318,730). The study sample comprised 7,147 Finnish individuals. HD data were retrieved from the IEU Open GWAS [23], sourced from the UK Biobank. The three HD datasets used in this study have the following GWAS IDs: ukb-e-455_AFR (SNPs = 15,526,533), ukb-e-455_CSA (SNPs = 981,032), and ukb-e-455_EAS (SNPs = 8,191,759). The ukb-e-455_AFR dataset includes samples from 6,458 African American or Afro-Caribbean individuals. The ukb-e-455_CSA dataset includes samples from 8,640 South Asian individuals. The ukb-e-455_EAS dataset includes samples from 2,658 East Asian individuals. Table S1 presents the baseline characteristics of the GWAS summary statistics used in preliminary analysis. In external validation, we selected ebi-a-GCST90086078 as the GWAS dataset for HD. The ebi-a-GCST90086078 data were derived from 56,637 individuals of European ancestry.

Selection of instrumental variables

The selected IVs must meet three criteria: First, SNPs associated with serum metabolite traits and plasma metabolites must have a genome-wide significance threshold of 1×10^{-5}. Second, to ensure the independence of selected SNPs, the

linkage disequilibrium (LD) threshold is set to $r^2 \leq 0.001$ with a physical distance greater than 10,000 kb to eliminate LD. Third, the strength of the IVs is evaluated using the F-statistic, and SNPs with an F-statistic less than 10 are excluded.

Mendelian randomization analysis

MR analysis was performed using the "TwoSampleMR" package (version 0.6.4) in R (version 4.3.1). Statistical significance was defined as a P < 0.05. The causal relationship between exposure and HD was estimated using three methods: Inverse Variance Weighted (IVW), MR-Egger, and Weighted Median. IVW was the primary analysis method, as it employs weighted linear regression to assess the association between IVs and the outcome. When the genetic variants satisfy the three assumptions of instrumental variables and are not affected by pleiotropy, IVW provides a consistent estimate of the causal effect between exposure and outcome.MR-Egger accounts for potential heterogeneity in IVs and can detect and correct for bias, thus being used to evaluate horizontal pleiotropy [24]. When the results of these three methods were inconsistent, the IVW results were used as the primary evaluation, while the “forest plot” packages were employed for graphical representations. We followed the STROBE-MR (Strengthening the reporting of observational studies in epidemiology using mendelian randomization) guidelines for MR results reporting [25].

Sensitivity analysis

Sensitivity analyses included heterogeneity testing, horizontal pleiotropy testing, and leave-one-out testing. Heterogeneity was assessed using MR-Egger and IVW methods, quantified by Cochran’s Q statistic. A P > 0.05 indicated that heterogeneity was not statistically significant. Horizontal pleiotropy was evaluated using the MR-Egger regression intercept and MR-PRESSO. For the MR-Egger regression intercept, a P > 0.05 suggested that horizontal pleiotropy could be ignored [26]. For MR-PRESSO, a P > 0.05 after the Global Test indicated that horizontal pleiotropy could be ignored. The stability of the results was assessed using the leave-one-out method; if the remaining results showed no significant changes after removing a single SNP, the results were considered stable.MR-PRESSO was performed using the MR-PRESSO package (version 1.0) in R (version 4.3.1), while the “forest plot” packages were employed for graphical representations. Funnel plots were used to assess whether there were biases in the results.

Enrichment analysis

Enrichment analysis for metabolites was conducted using MetaboAnalyst 6.0 [27]. Metabolites involved in serum metabolite traits with causal relationships to HD were classified into risk factor groups and protective factor groups according to the following rules: First, for serum metabolite levels, metabolites were classified as either risk factors or protective factors based on the sign of the beta value. For serum metabolite ratios, if the beta value was positive, the numerator component was classified as a risk factor, and the denominator component was classified as a protective factor; if the beta value was negative, the classification was reversed. Enrichment analysis was conducted separately for the two groups. The data were sourced from The Small Molecule Pathway Database (SMPDB). A significance threshold of P < 0.05 was set for this study.

Results

Causal effects of serum metabolite traits on HD

Results of African American or Afro-Caribbean population

After rigorous and comprehensive sensitivity analysis and excluding all results that failed the sensitivity analysis, 23 serum metabolite traits were identified as having a reliable causal relationship with HD, with 13 serum metabolite levels and ratios being risk factors for HD and 10 being protective factors (Figure S1-4). Among the risk factors, the top three with the most significant causal relationship with HD were: Cortolone glucuronide (1) levels (OR = 1.544, 95%CI = 1.124-2.123, P = 0.007), 3-methyl-2-oxobutyrate levels (OR = 2.079, 95%CI = 1.189-3.635, P = 0.010), and Glycocholate levels (OR = 1.405, 95%CI = 1.063-1.857, P = 0.017). Among the protective factors, the top three with the most significant causal relationship with HD were: 1-(1-enyl-palmitoyl)-2-oleoyl-GPE (p-16:0/18:1) levels (OR = 0.566, 95%CI = 0.413-0.776, P = 0.0004), X-11299 levels (OR = 0.439, 95%CI = 0.257-0.747, P = 0.002), and Aspartate to phosphate ratio (OR = 0.422, 95%CI = 0.226-0.791, P = 0.007). All MR results and sensitivity analysis outcomes are listed in Table S2.

Results of South Asian population

After conducting rigorous and comprehensive sensitivity analysis and excluding results that did not pass the sensitivity test, 32 results were considered reliable. 17 serum metabolite traits were identified as risk factors for HD (Figure S1, 5-7), with the three most significant causal effects being: Gamma-glutamyltyrosine levels (OR = 1.748, 95%CI = 1.258-2.427, P = 0.001), Adenosine 5'-monophosphate (AMP) to phosphate ratio (OR = 1.910, 95%CI = 1.267-2.879, P = 0.002), and Guaiacol sulfate levels (OR = 1.851, 95%CI = 1.245-2.750, P = 0.002). 15 serum metabolite traits were identified as protective factors for HD, with the top three significant causal effects being: 1-(1-enyl-stearoyl)-GPE (p-18:0) levels (OR = 0.623, 95%CI = 0.447-0.867, P = 0.005), Sphingomyelin (d18:2/24:1, d18:1/24:2) levels (OR = 0.570, 95%CI = 0.385-0.845, P = 0.005), and Glycerol to mannitol to sorbitol ratio (OR = 0.704, 95%CI = 0.541-0.914, P = 0.009). All MR results and their sensitivity analysis results are shown in Table S3.

Results of East Asian population

34 serum metabolite traits with causal relationship with HD was identified by MR analysis and sensitivity analysis, including 14 risk factors and 20 protective factors (Figure S1, 8-10). The top three risk factors with the most significant causal relationship with HD are: X-25271 levels (OR = 4.134, 95%CI = 1.798-9.507, P = 0.001), 5-hydroxyindole sulfate levels (OR = 2.610, 95%CI = 1.469-4.638, P = 0.001), and Spermidine to pyruvate ratio (OR = 2.405, 95%CI = 1.347-4.294, P = 0.003). The top three protective factors with the most significant causal relationship with HD are: Xanthurenate levels (OR = 0.409, 95%CI = 0.220-0.759, P = 0.005), Homostachydrine levels (OR = 0.318, 95%CI = 0.140-0.723, P = 0.006), and X-21796 levels (OR = 0.402, 95%CI = 0.203-0.799, P = 0.009). All MR results that passed the sensitivity analysis and their sensitivity analysis results are shown in Table S4.

Causal effects of plasma lipids on HD

Results of African American or Afro-Caribbean population

After MR analysis and comprehensive sensitivity analysis, 4 lipids were identified to have a causal relationship with HD, 2 of which are risk factors and 2 are protective factors for HD (Figure S11-14). Phosphatidylcholine (18:2_20:4) levels (OR = 1.588, 95%CI = 1.097-2.299, P = 0.014)and Phosphatidylcholine (O-16:1_16:0) levels (OR = 1.676, 95%CI = 1.109-2.533, P = 0.014) were identified as increasing the risk of HD. Phosphatidylcholine (O-16:0_20:3) levels (OR = 0.462, 95%CI = 0.293-0.727, P = 0.001) and Phosphatidylethanolamine (O-18:1_18:2) levels (OR = 0.569, 95%CI = 0.384-0.842, P = 0.005) were identified as protective factors. Details of the results of MR analysis and sensitivity analysis were shown in Table S5.

Results of South Asian population

After a rigorous and comprehensive sensitivity analysis, 10 plasma lipids were considered to have a reliable causal relationship with HD (Figure S11-14). Among them, 8 plasma lipids were identified as risk factors for HD: Triacylglycerol (53:4) levels (OR = 1.247, 95%CI = 1.021-1.523, P = 0.030), Triacylglycerol (52:3) levels (OR = 1.332, 95%CI = 1.062-1.672, P = 0.013), Triacylglycerol (56:7) levels (OR = 1.340, 95%CI = 1.038-1.730, P = 0.024), Triacylglycerol (56:4) levels (OR = 1.373, 95%CI = 1.019-1.850, P = 0.037), Triacylglycerol (56:8) levels (OR = 1.389, 95%CI = 1.095-1.761, P = 0.007), Triacylglycerol (53:2) levels (OR = 1.399, 95%CI = 1.045-1.874, P = 0.024), Triacylglycerol (53:3) levels (OR = 1.444, 95%CI = 1.141-1.828, P = 0.002), Ceramide (d42:1) levels (OR = 1.462, 95%CI = 1.102-1.939, P = 0.008). Additionally, 2 plasma lipids were identified as protective factors against HD: Sphingomyelin (d36:2) levels (OR = 0.730, 95%CI = 0.540-0.986, P = 0.040), Sterol ester (27:1/18:1) levels (OR = 0.772, 95%CI = 0.609-0.979, P = 0.033). All MR results and their sensitivity analysis results are shown in Table S6.

Results of East Asian population

After MR analysis and sensitivity analysis, 7 plasma lipids were considered to have a reliable causal relationship with HD, and all were identified as protective factors for HD (Figure S11-14): Sterol ester (27:1/20:3) levels (OR = 0.541, 95%CI = 0.331-0.885, P = 0.014), Ceramide (d42:1) levels (OR = 0.610, 95%CI = 0.387-0.960, P = 0.033), Phosphatidylcholine (16:0_16:1) levels (OR = 0.468, 95%CI = 0.243-0.901, P = 0.023), Phosphatidylcholine (18:0_18:3) levels (OR = 0.400, 95%CI = 0.164-0.977, P = 0.044), Phosphatidylcholine (O-16:0_16:0) levels (OR = 0.352, 95%CI = 0.160-0.770, P = 0.009), Phosphatidylethanolamine (18:1_18:1) levels (OR = 0.540, 95%CI = 0.343-0.850, P = 0.008), Triacylglycerol (54:7) levels (OR = 0.600, 95%CI = 0.365-0.987, P = 0.044). Details of the results of MR analysis and sensitivity analysis were shown in Table S7.

Meta-analysis of the results of MR

A meta-analysis will be conducted on the MR analysis results of serum metabolite traits and plasma lipids whose results of MR analysis were positive in at least one population, to evaluate the overall effect of serum metabolite traits and plasma lipids on HD across samples from different populations.

Meta-analysis of the causal relationships between serum metabolite traits and HD

Figure 2 shows the meta-analysis results of the effects of serum metabolites on HD. The heterogeneity test results indicate that a fixed-effect model should be chosen (P values are all greater than 0.05). The factors that increase the risk of HD are: Citramalate levels (OR = 1.487, 95%CI = 1.172-1.888, P = 0.001), P-cresol sulfate levels (OR = 1.476, 95%CI = 1.111-1.961, P = 0.007), Guaiacol sulfate levels (OR = 1.481, 95%CI = 1.113-1.971, P = 0.007), Gamma-glutamyltyrosine levels (OR = 1.472, 95%CI = 1.127-1.923, P = 0.005), Glycocholate levels (OR = 1.202, 95%CI = 1.018-1.420, P = 0.030), X-12839 levels (OR = 1.205, 95%CI = 1.007-1.442, P = 0.042), X-21258 levels (OR = 1.287, 95%CI = 1.007-1.645, P = 0.044), Adenosine 5'-monophosphate (AMP)to phosphate ratio (OR = 1.665, 95%CI = 1.219-2.275, P = 0.001), Pyruvate to 3-methyl-2-oxobutyrate ratio (OR = 1.341, 95%CI = 1.094-1.643, P = 0.005) (Figure 2A). The factors that reduce the risk of HD are: Imidazole lactate levels (OR = 0.865, 95%CI = 0.756-0.989, P = 0.034), 1- (1-enylstearoyl)-GPE (p-18:0) levels (OR = 0.722, 95%CI = 0.552-0.944, P = 0.017), Lanthionine levels (OR = 0.767, 95%CI = 0.597-0.983, P = 0.037), Sphingomyelin (d18:2/24:1, d18:1/24:2) levels (OR = 0.735, 95%CI = 0.548-0.985, P = 0.040), Dihomo-linolenoylcarnitine (C20:3n3 or 6) levels (OR = 0.827, 95%CI = 0.705-0.971, P = 0.020), X-17325 levels (OR = 0.803, 95%CI = 0.656-0.984, P = 0.034), X-17351 levels (OR = 0.786, 95%CI = 0.622-0.992, P = 0.043), X-24306 levels (OR = 0.765, 95%CI = 0.609-0.960, P = 0.021), Alpha-ketoglutarate to kynurenine ratio (OR = 0.817, 95%CI = 0.686-0.973, P = 0.024), Aspartate to phosphate ratio (OR = 0.665, 95%CI = 0.460-0.962, P = 0.030), Salicylate to oxalate (ethanedioate) ratio (OR = 0.705, 95%CI = 0.537-0.925, P = 0.012) (Figure 2B).

Meta-analysis of the causal relationships between plasma lipids and HD

Figure 3 shows the meta-analysis results of the effects of plasma lipids on HD. The heterogeneity test results indicate that a fixed-effect model should be chosen (P values are all greater than 0.05). Triacylglycerol (53:3) levels have been identified as a risk factor for HD (OR = 1.262, 95%CI = 1.049-1.518, P = 0.014) (Figure 3A). Phosphatidylethanolamine (18:1_18:1) levels are a protective factor against HD (OR = 0.803, 95%CI = 0.666-0.969, P = 0.022) (Figure 3B).

Enrichment analysis of serum metabolites

According to the rules proposed in the Methods section, the serum metabolite characteristics with positive meta-analysis results were classified into risk factor groups and protective factor groups. Serum metabolites that could not be retrieved in HMDB, PubChem, and KEGG were excluded (refer to Table S8 for metabolite information in different databases).Metabolite enrichment analysis showed that the enriched metabolic pathways for the risk factor group were: Alanine Metabolism (Holm P = 0.0039), Cysteine Metabolism (Holm P = 0.0146), Urea Cycle (Holm P = 0.0181), Ammonia Recycling (Holm P = 0.0245) (Figure 4A, Table S9).The enriched metabolic pathways for the protective factor group included: Urea Cycle (Holm P = 0.00754), Ammonia Recycling (Holm P = 0.0102), Aspartate Metabolism (Holm P = 0.0147), Glutamate Metabolism (Holm P = 0.038), Arginine and Proline Metabolism (Holm P = 0.0479), Malate-Aspartate Shuttle (Holm P = 0.0495) (Figure 4B, Table S10).

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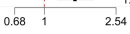
Figure 2: Meta-analysis results of serum metabolite traits. (**A**) The meta-analysis identified 9 serum metabolite traits as risk factors for HD. (**B**) The meta-analysis identified 11 serum metabolite traits as protective factors for HD.

A

Result of meta-analysis for Citramalate levels

study	nsnp	OR (95% CI)	pval
GCST90199692 | ukb-e-455_AFR	13	1.445 (0.842 to 2.482)	0.182
GCST90199692 | ukb-e-455_CSA	19	1.555 (1.158 to 2.086)	0.003
GCST90199692 | ukb-e-455_EAS	16	1.270 (0.683 to 2.361)	0.450
Fixed effect model		1.487 (1.172 to 1.888)	0.001
Random effect model		1.487 (1.172 to 1.888)	0.001



Result of meta-analysis for P-cresol sulfate levels

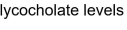
study	nsnp	OR (95% CI)	pval
GCST90199740 | ukb-e-455_AFR	13	1.290 (0.812 to 2.051)	0.281
GCST90199740 | ukb-e-455_CSA	17	1.555 (1.045 to 2.314)	0.030
GCST90199740 | ukb-e-455_EAS	12	1.823 (0.782 to 4.249)	0.164
Fixed effect model		1.476 (1.111 to 1.961)	0.007
Random effect model		1.476 (1.111 to 1.961)	0.007

Result of meta-analysis for Guaiacol sulfate levels

study	nsnp	OR (95% CI)	pval
GCST90199940 | ukb-e-455_AFR	14	1.279 (0.752 to 2.176)	0.364
GCST90199940 | ukb-e-455_CSA	18	1.851 (1.245 to 2.750)	0.002
GCST90199940 | ukb-e-455_EAS	14	1.005 (0.521 to 1.936)	0.989
Fixed effect model		1.481 (1.113 to 1.971)	0.007
Random effect model		1.430 (0.998 to 2.049)	0.051

Result of meta-analysis for Gamma-glutamyltyrosine levels

study	nsnp	OR (95% CI)	pval
GCST90200295 | ukb-e-455_AFR	14	0.943 (0.524 to 1.697)	0.844
GCST90200295 | ukb-e-455_CSA	21	1.748 (1.258 to 2.427)	0.001
GCST90200295 | ukb-e-455_EAS	15	1.254 (0.601 to 2.617)	0.547
Fixed effect model		1.472 (1.127 to 1.923)	0.005
Random effect model		1.361 (0.906 to 2.044)	0.138



Result of meta-analysis for Glycocholate levels

study	nsnp	OR (95% CI)	pval
GCST90200387 | ukb-e-455_AFR	25	1.405 (1.063 to 1.857)	0.017
GCST90200387 | ukb-e-455_CSA	28	1.026 (0.810 to 1.301)	0.829
GCST90200387 | ukb-e-455_EAS	23	1.396 (0.907 to 2.148)	0.130
Fixed effect model		1.202 (1.018 to 1.420)	0.030
Random effect model		1.227 (0.974 to 1.547)	0.083

Result of meta-analysis for X-12839 levels

study	nsnp	OR (95% CI)	pval
GCST90200518 | ukb-e-455_AFR	18	0.908 (0.642 to 1.285)	0.587
GCST90200518 | ukb-e-455_CSA	22	1.373 (1.090 to 1.728)	0.007
GCST90200518 | ukb-e-455_EAS	19	1.173 (0.706 to 1.949)	0.538
Fixed effect model		1.205 (1.007 to 1.442)	0.042
Random effect model		1.160 (0.880 to 1.529)	0.293

Result of meta-analysis for X-21258 levels

study	nsnp	OR (95% CI)	pval
GCST90200565 | ukb-e-455_AFR	12	1.189 (0.766 to 1.847)	0.441
GCST90200565 | ukb-e-455_CSA	16	1.462 (1.067 to 2.005)	0.018
GCST90200565 | ukb-e-455_EAS	12	0.681 (0.290 to 1.599)	0.378
Fixed effect model		1.287 (1.007 to 1.645)	0.044
Random effect model		1.265 (0.961 to 1.666)	0.094

Result of meta-analysis for Adenosine 5\'-monophosphate (AMP) to phosphate ratio

study	nsnp	OR (95% CI)	pval
GCST90200747 | ukb-e-455_AFR	12	1.039 (0.563 to 1.921)	0.902
GCST90200747 | ukb-e-455_CSA	18	1.910 (1.267 to 2.879)	0.002
GCST90200747 | ukb-e-455_EAS	9	2.162 (0.997 to 4.687)	0.051
Fixed effect model		1.665 (1.219 to 2.275)	0.001
Random effect model		1.632 (1.079 to 2.468)	0.020

Result of meta-analysis for Pyruvate to 3-methyl-2-oxobutyrate ratio

study	nsnp	OR (95% CI)	pval
GCST90200778 | ukb-e-455_AFR	15	1.259 (0.782 to 2.025)	0.343
GCST90200778 | ukb-e-455_CSA	19	1.320 (1.004 to 1.736)	0.047
GCST90200778 | ukb-e-455_EAS	16	1.445 (0.975 to 2.141)	0.067
Fixed effect model		1.341 (1.094 to 1.643)	0.005
Random effect model		1.341 (1.094 to 1.643)	0.005

B

Result of meta-analysis for Imidazole lactate levels

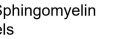
study	nsnp	OR (95% CI)	pval
GCST90199639 | ukb-e-455_AFR	16	0.900 (0.695 to 1.164)	0.422
GCST90199639 | ukb-e-455_CSA	21	0.837 (0.704 to 0.995)	0.044
GCST90199639 | ukb-e-455_EAS	17	0.930 (0.639 to 1.354)	0.707
Fixed effect model		0.865 (0.756 to 0.989)	0.034
Random effect model		0.865 (0.756 to 0.989)	0.034

Result of meta-analysis for 1-(1-enyl-stearoyl)-GPE (p-18:0) levels

study	nsnp	OR (95% CI)	pval
GCST90199910 | ukb-e-455_AFR	16	0.705 (0.359 to 1.384)	0.310
GCST90199910 | ukb-e-455_CSA	22	0.623 (0.447 to 0.867)	0.005
GCST90199910 | ukb-e-455_EAS	15	1.226 (0.663 to 2.267)	0.516
Fixed effect model		0.722 (0.552 to 0.944)	0.017
Random effect model		0.772 (0.514 to 1.159)	0.211

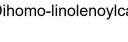
Result of meta-analysis for Lanthionine levels

study	nsnp	OR (95% CI)	pval
GCST90199931 | ukb-e-455_AFR	15	0.572 (0.355 to 0.920)	0.021
GCST90199931 | ukb-e-455_CSA	21	0.812 (0.584 to 1.129)	0.215
GCST90199931 | ukb-e-455_EAS	16	1.046 (0.554 to 1.975)	0.889
Fixed effect model		0.767 (0.597 to 0.983)	0.037
Random effect model		0.766 (0.589 to 0.996)	0.047



Result of meta-analysis for Sphingomyelin (d18:2/24:1, d18:1/24:2) levels

study	nsnp	OR (95% CI)	pval
GCST90200040 | ukb-e-455_AFR	18	0.951 (0.559 to 1.621)	0.855
GCST90200040 | ukb-e-455_CSA	25	0.570 (0.385 to 0.845)	0.005
GCST90200040 | ukb-e-455_EAS	21	1.158 (0.524 to 2.559)	0.717
Fixed effect model		0.735 (0.548 to 0.985)	0.040
Random effect model		0.789 (0.510 to 1.221)	0.288



Result of meta-analysis for Dihomo-linolenoylcarnitine (C20:3n3 or 6) levels

study	nsnp	OR (95% CI)	pval
GCST90200139 | ukb-e-455_AFR	25	0.790 (0.587 to 1.063)	0.119
GCST90200139 | ukb-e-455_CSA	27	0.901 (0.731 to 1.109)	0.324
GCST90200139 | ukb-e-455_EAS	22	0.612 (0.386 to 0.970)	0.037
Fixed effect model		0.827 (0.705 to 0.971)	0.020
Random effect model		0.823 (0.694 to 0.975)	0.025

Result of meta-analysis for X-17325 levels

study	nsnp	OR (95% CI)	pval
GCST90200535 | ukb-e-455_AFR	14	0.823 (0.578 to 1.171)	0.278
GCST90200535 | ukb-e-455_CSA	19	0.881 (0.663 to 1.172)	0.386
GCST90200535 | ukb-e-455_EAS	14	0.573 (0.347 to 0.948)	0.030
Fixed effect model		0.803 (0.656 to 0.984)	0.034
Random effect model		0.803 (0.656 to 0.984)	0.034

Result of meta-analysis for X-17351 levels

study	nsnp	OR (95% CI)	pval
GCST90200560 | ukb-e-455_AFR	11	0.560 (0.360 to 0.873)	0.010
GCST90200560 | ukb-e-455_CSA	16	0.838 (0.612 to 1.147)	0.269
GCST90200560 | ukb-e-455_EAS	14	1.110 (0.629 to 1.960)	0.719
Fixed effect model		0.786 (0.622 to 0.992)	0.043
Random effect model		0.787 (0.561 to 1.105)	0.166

Result of meta-analysis for X-24306 levels

study	nsnp	OR (95% CI)	pval
GCST90200631 | ukb-e-455_AFR	16	0.764 (0.505 to 1.156)	0.202
GCST90200631 | ukb-e-455_CSA	20	0.724 (0.538 to 0.975)	0.033
GCST90200631 | ukb-e-455_EAS	15	1.025 (0.517 to 2.035)	0.943
Fixed effect model		0.765 (0.609 to 0.960)	0.021
Random effect model		0.765 (0.609 to 0.960)	0.021

Result of meta-analysis for Alpha-ketoglutarate to kynurenine ratio

study	nsnp	OR (95% CI)	pval
GCST90200810 | ukb-e-455_AFR	17	0.966 (0.756 to 1.236)	0.785
GCST90200810 | ukb-e-455_CSA	18	0.708 (0.517 to 0.971)	0.032
GCST90200810 | ukb-e-455_EAS	18	0.654 (0.435 to 0.983)	0.041
Fixed effect model		0.817 (0.686 to 0.973)	0.024
Random effect model		0.791 (0.616 to 1.017)	0.067

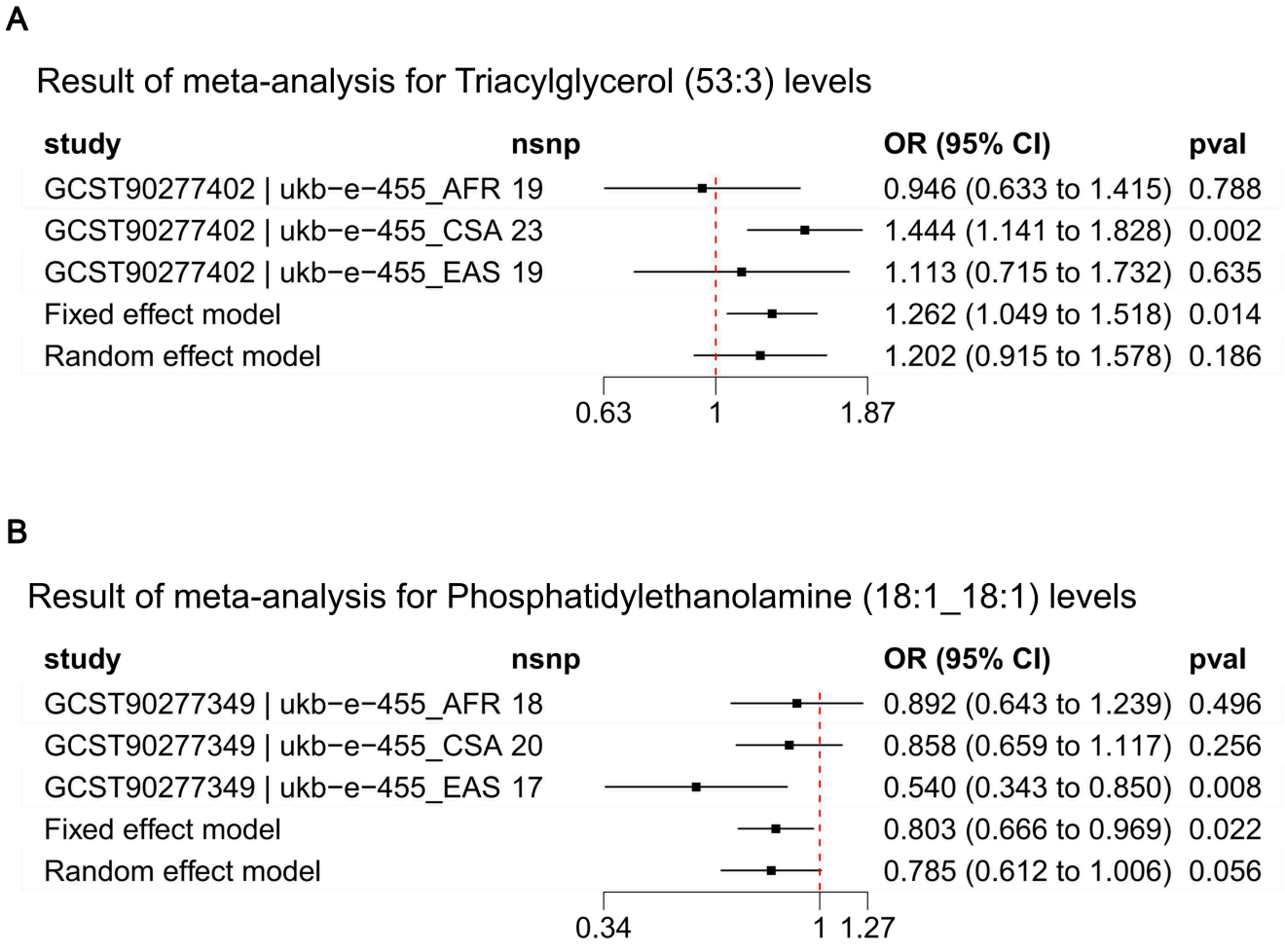
Result of meta-analysis for Aspartate to phosphate ratio

study	nsnp	OR (95% CI)	pval
GCST90200844 | ukb-e-455_AFR	9	0.422 (0.226 to 0.791)	0.007
GCST90200844 | ukb-e-455_CSA	15	0.757 (0.450 to 1.274)	0.294
GCST90200844 | ukb-e-455_EAS	9	1.231 (0.475 to 3.190)	0.668
Fixed effect model		0.665 (0.460 to 0.962)	0.030
Random effect model		0.683 (0.400 to 1.167)	0.163

Result of meta-analysis for Salicylate to oxalate (ethanedioate) ratio

study	nsnp	OR (95% CI)	pval
GCST90200993 | ukb-e-455_AFR	10	0.712 (0.478 to 1.059)	0.094
GCST90200993 | ukb-e-455_CSA	14	0.604 (0.392 to 0.929)	0.022
GCST90200993 | ukb-e-455_EAS	9	1.083 (0.514 to 2.283)	0.833
Fixed effect model		0.705 (0.537 to 0.925)	0.012
Random effect model		0.705 (0.537 to 0.925)	0.012

Figure 3: Meta-analysis results of plasma lipids. (**A**) The meta-analysis identified Triacylglycerol (53:3) levels as risk factors for HD. (**B**) The meta-analysis identified Phosphatidylethanolamine (18:1_18:1) levels as protective factors for HD.



Discussion

In the development of HD, the dilation and congestion of hemorrhoidal veins are the main pathological features. Inflammation and damage of local vascular endothelium play a crucial role in the occurrence of HD [3]. However, it remains unclear whether local inflammation in hemorrhoid patients causes changes in blood composition or if abnormal blood components trigger local inflammation, leading to the development of HD. In this study, we combined metabolomics, lipidomics, and genomics for the first time to explore the causal relationships between serum metabolites, plasma lipids, and HD using MR analysis, and employed meta-analysis to investigate the overall effects of MR results across different population samples.

It was observed that many lipids or lipoids and their metabolites are included among the protective factors for HD. Lipoids such as ceramides can maintain the integrity of the intestinal barrier by enhancing tight junctions in intestinal epithelial cells [28], thereby preventing harmful substances and bacteria from entering the bloodstream [29]. Additionally, fatty acids, especially unsaturated fatty acids, play an important role in maintaining vascular health. Polyunsaturated fatty acids (PUFAs) such as eicosapentaenoic acid (EPA) can reduce the risk of HD by inhibiting platelet aggregation and decreasing inflammation and damage in the vascular endothelium [30].

Metabolite enrichment analysis results indicate that Alanine metabolism and Cysteine metabolism may play key roles in the pathogenesis of HD. Abnormal activation of Alanine metabolism may influence the tumor necrosis factor-alpha (TNF-α) signaling pathway, thereby modulating the inflammatory response and lipid metabolism processes [31, 32]. Additionally, enhanced Cysteine metabolism is closely associated with the inflammatory response in venous tissue [33]. This inflammatory response may lead to endothelial damage in the venous wall. Furthermore, enhanced Cysteine metabolism is associated with increased production of matrix metalloproteinases (MMPs) [34-36]. The upregulation of MMPs expression promotes collagen degradation and extracellular matrix (ECM) remodeling, leading to significant changes in the structure and function of the venous wall. These changes may ultimately result in the development of varicose veins [37, 38]. When these pathological changes occur in the veins of the anorectal region, they may trigger the formation of HD.

Metabolite enrichment analysis revealed a series of metabolic

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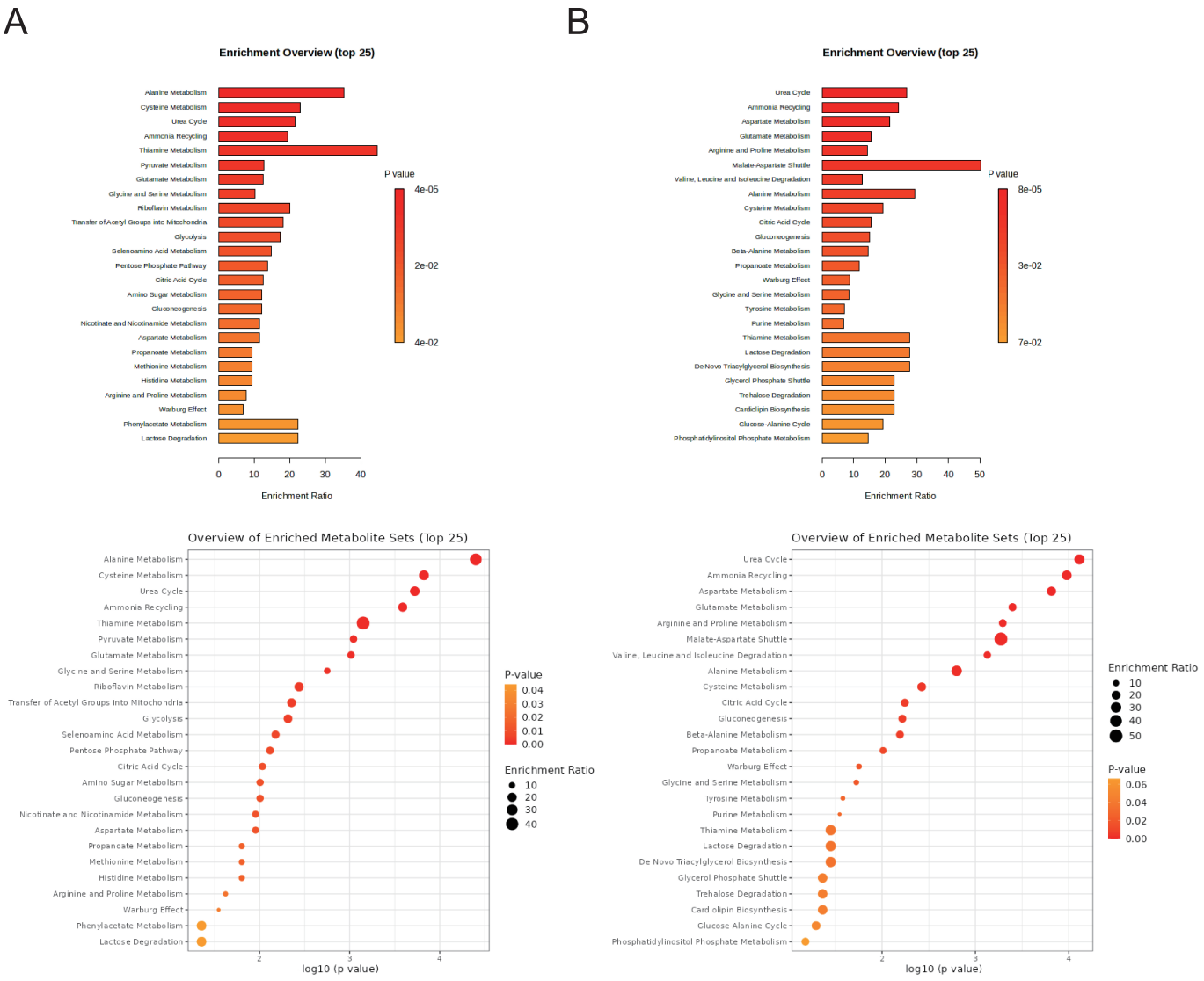
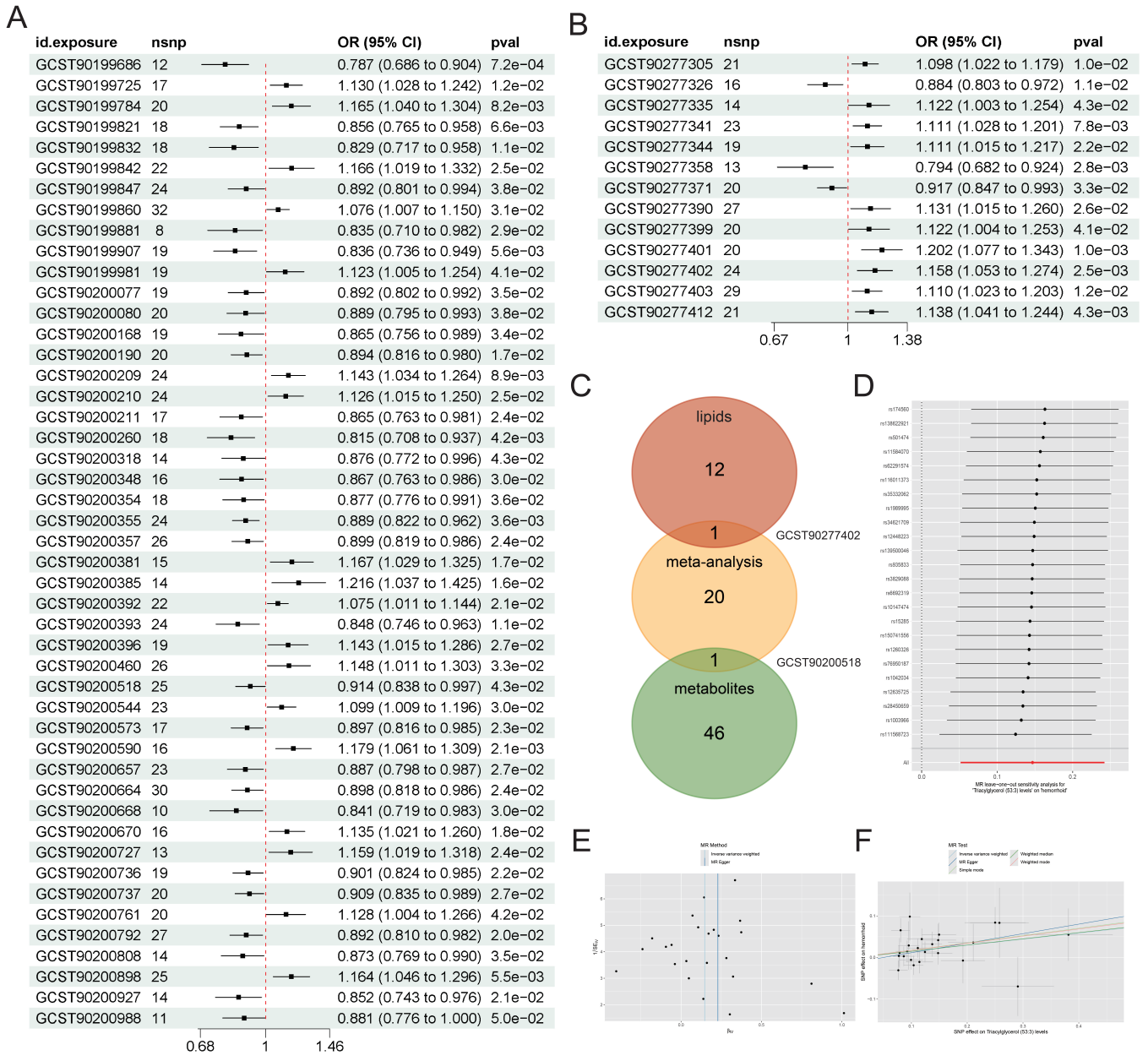
Figure 4: Serum metabolites enrichment analysis results. (**A**) Enrichment analysis results of serum metabolites in the risk factor group. (**B**) Enrichment analysis results of serum metabolites in the protective factor group.



pathways with potential protective effects. Specifically, Aspartate metabolism, Glutamate metabolism, Arginine and Proline metabolism, and the Malate-Aspartate shuttle play key roles in regulating the pathophysiological processes related to HD. Aspartate metabolism reduces oxidative stress by affecting intracellular metabolic processes such as the TCA cycle and pyruvate metabolism, thereby improving endothelial function [39-41]. Regulation of Glutamate metabolism may have antithrombotic effects by modulating the expression of nitric oxide synthase 3 (NOS3) and the metabolism of Arginine, Glutamate, and Glutamine [42-45]. The metabolic product of Arginine, nitric oxide (NO), has been shown to suppress inflammation, reduce inflammatory responses, and minimize tissue damage [46]. Proline metabolism reduces inflammation-induced tissue damage by activating immune cells such as T cells and natural killer (NK) cells [47]. The Malate-Aspartate shuttle plays a crucial role in maintaining intracellular NADH balance by transporting NADH to the mitochondria, reducing cytoplasmic NADH concentration, and thus lowering oxidative stress production. Excess accumulation of NADH in the cytoplasm may provide substrates for NAD (P) H oxidase, thereby promoting oxidative stress and inflammatory responses [48, 49]. The Malate-Aspartate shuttle may alleviate inflammation in the anorectal region by inhibiting this pathway, thereby preventing the occurrence of HD. In summary, the regulation of these metabolic pathways may offer new insights into the prevention and treatment of HD.

Interestingly, urea cycle and ammonia recycling were observed to exhibit both promoting and inhibiting effects in the development of HD. Urea cycle and ammonia recycling are key pathways in the metabolism, utilization, and excretion of ammonia, and are crucial for maintaining ammonia homeostasis in the internal environment [50-53]. On the one hand, high ammonia concentrations have been shown to promote inflammatory responses [54, 55], which may enhance the release of inflammatory mediators and, in turn, contribute to the development of HD. On the other hand, moderate ammonia levels have been found to mitigate the damage of inflammatory responses to

Figure 5: Results of external validation. (**A**) Forest plot shows the causal associations between HD and serum metabolites in the European ancestry population. (**B**) Forest plot shows the causal associations between HD and plasma lipids in the European ancestry population. (**C**) Venn diagram illustrates the overlap between the MR analysis in the European ancestry population and the previous meta-analysis results. (**D**) Leave-one-out analysis shows the influence of each individual SNP on the overall causal effect between GCST90277402 and HD, used to assess horizontal pleiotropy in the MR analysis. (**E**) Funnel plot indicates no apparent bias among SNPs in the MR analysis between GCST90277402 and HD. (**F**) Scatter plot exhibits the beta estimates of the MR results between GCST90277402 and HD using different analytical methods.



pancreatic β-cells through the regulation of glutamine metabolism [56, 57], suggesting that ammonia may have anti-inflammatory effects at certain concentrations. Therefore, urea cycle and ammonia recycling play a critical role in maintaining ammonia balance in the internal environment, and subtle changes in ammonia levels can influence the development of HD by modulating inflammatory responses. This finding not only reveals the complex roles of the urea cycle and ammonia recycling in the pathophysiology of HD but also provides new perspectives for further research, suggesting that regulating ammonia metabolism pathways may offer potential strategies for the prevention and treatment of HD.

Triacylglycerol (53:3) levels were found to be positively associated with HD risk, while Phosphatidylethanolamine (18:1_18:1) levels were negatively correlated with HD risk. Research suggests that Triacylglycerol (53:3) may increase the risk of thrombosis [58], as well as induce inflammatory responses such as leukocyte adhesion and exudation, which in turn may

promote the development of varicose veins [59, 60]. When these pathological changes are localized to the anorectal region, they may lead to the formation of HD. Phosphatidylethanolamine (18:1_18:1) is an important phospholipid compound with various physiological functions in the human body, but research on this specific phospholipid remains limited. These findings provide new insights into the role of plasma lipids in the pathogenesis of HD and may offer a scientific basis for developing new therapeutic strategies.

This study reports for the first time the causal relationship between serum metabolites, plasma lipids, and the occurrence of HD. It identifies risk factors for HD and provides new insights into the mechanisms underlying HD. Through MR analysis, we identified a series of metabolites and lipids associated with the risk of HD occurrence, and explored their overall causal relationships with HD in different population samples through meta-analysis. These findings provide a new theoretical foundation for the clinical diagnosis and treatment strategies of HD.

We must acknowledge that, while the results showed consistency across diverse populations, the genetic and environmental backgrounds of these specific populations may influence the generalizability of the findings. Therefore, we conducted external validation in a European ancestry population. We selected ebi-a-GCST90086078 as the GWAS dataset for HD and conducted MR analysis using 1,400 serum metabolites and 179 plasma lipids as outcomes. The ebi-a-GCST90086078 data were derived from 56,637 individuals of European ancestry. SNPs were selected with a genome-wide significance threshold of 1×10^{-5} . Based on the MR analysis results, in the European ancestry population, the causal relationships between HD and metabolites and lipids are illustrated in Figure 5. By intersecting the MR analysis results with those of the previous meta-analysis, we observed that Triacylglycerol (53:3) levels (GCST90277402) showed a significant positive association with HD in the results of European ancestry population, in line with the meta-analysis results of African American or Afro-Caribbean, South Asian, and East Asian populations. This indicated the generalizability of this finding. GCST90200518 also demonstrated a causal relationship with HD in both the external validation and meta-analysis; however, the direction of the effect was opposite. For detailed results, please refer to the Raw Data. Nevertheless, we observed that the results in the European ancestry population differ markedly from those in the other three populations, reminding us that the population limitations still exist.

Besides, since we only used summary statistics for estimation, we did not evaluate the causal association in different groups, such as different gender or age groups. Another major limitation of this study is the lack of consideration of potential confounding factors, including BMI, smoking status, diet, environmental exposures and so on. These confounders may also contribute to the development of HD and thereby compromise the accuracy and reliability of the analytical results.

However, despite these preliminary results providing valuable clues for the pathophysiological research of HD, there is still a lack of in-depth understanding regarding the precise connection between these metabolites and lipids and HD on a multi-omics level. Therefore, future research needs to adopt multi-omics approaches, such as proteomics, metabolomics, and genomics, to comprehensively explore the interactions and potential molecular mechanisms between these biomarkers and HD. Through this interdisciplinary research approach, we aim to further uncover the complex pathological processes of HD and provide a scientific basis for developing more effective prevention and treatment strategies.

Conclusion

In-depth MR analysis and meta-analysis were conducted in this study to reveal the potential causal relationship between serum metabolites, plasma lipids, and the occurrence of HD. Specific metabolites and lipid markers were found significantly associated with the risk of HD and identified metabolite-enriched pathways, providing new insights into its pathophysiology. Our findings offer new insights into the clinical diagnosis and treatment of HD and provide a reference for future multi-omics studies to uncover deeper molecular mechanisms, laying the foundation for the development of effective prevention and treatment strategies.

Abbreviations

Extracellular matrix: ECM; Eicosapentaenoic acid: EPA; Genome-wide association study: GWAS; Hemorrhoids: HD; Instrumental variable: IV; Inverse Variance Weighted: IV; Low-density lipoprotein: LDL; Matrix metalloproteinase: MMP; Mendelian randomization: MR; Natural killer: NK; Nitric oxide: NO; Nitric oxide synthase 3: NOS3; Polyunsaturated fatty acid: PUFA; Single nucleotide polymorphism: SNP; Strengthening the reporting of observational studies in epidemiology using mendelian randomization: STROBE-MR; Tumor necrosis factor-alpha: TNF-α.

Author Contributions

Lu Hu and Danyang Wang made equal contributions to this manuscript. Lu Hu: Writing - original draft, Investigation, Data curation, Project administration. Danyang Wang: Formal analysis, Methodology, Supervision, Writing - review & editing. Chang You: Writing - review & editing. Ziyan Zhao: Writing - review & editing. Wanlin Zheng: Writing - review & editing. Luyao Wang: Writing - review & editing. Yutong Li: Visualization. Liangzhe Dai: Visualization. Hongkai Yu: Writing - original draft, Investigation, Data curation, Project administration. All authors read and approved the final manuscript.

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Ethicals Approval and Consent to Participate

Not Applicable.

Competing Interests

The authors declare that they have no existing or potential commercial or financial relationships that could create a conflict of interest at the time of conducting this study.

Data availability

The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding authors.

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