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ORIGINAL RESEARCH

Lipid Transporter ABCA1 in Diabetic Polyneuropathy: Potential Mediation by Circulating Metabolites

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Background: Diabetic polyneuropathy (DPN) is a complication of diabetes characterized by peripheral sensory deficits or neuropathic pain. Emerging evidence suggests that DPN is associated with dyslipidemia. Probucol has shown promise in the treatment of diabetes and alleviation of neuropathic pain. However, the influence of probucol on DPN or dyslipidemia incidence is unclear. The study examined the mediating effects of circulating metabolites on the relationship between probucol target gene ATP-binding cassette subfamily A member 1 (ABCA1) and DPN.

Methods: A positive control analysis was done on low density lipoprotein (LDL) to validate genetic instruments. A two-step Mendelian randomization (MR) study was conducted to assess the correlation between the probucol target gene ABCA1 and DPN, focusing on the mediation effects of circulating metabolites connecting them. MR analysis was mainly estimated using Inverse-variance weighted (IVW) and MR-Egger regression conducting SNP heterogeneity and sensitivity analysis.

Results: Predicted based on genetics, the ABCA1 gene is associated with increased risk of LDL and four circulating metabolites. The direct effect of the Probucol target gene ABCA1 on DPN was 0.3006, and the mean diameter for high-density lipoprotein (HDL) particles, total cholesterol in medium LDL, triglycerides in medium very low-density lipoprotein (VLDL), and free cholesterol in very large HDL had partial mediating effects on the association between ABCA1 and DPN incidence.

Conclusion: This study supports an association between genetically predicted Probucol target ABCA1, circulating metabolites, and DPN. Our observations indicate a direct effect of ABCA1 on DPN and a mediating pathway through four blood circulation metabolites. Our findings offer genetic evidence for the mechanisms of Probucol in preventing, alleviating, and treating DPN pain.

Keywords: diabetic polyneuropathy, probucol, ATP-binding cassette subfamily A member 1, circulating metabolites, mendelian randomization

Introduction

Diabetic polyneuropathy (DPN), the most common neurologic complication in diabetic patients, involves a wide range of neurological injuries including allodynia, pain, paralysis and affects millions of patients worldwide.[1,2] Despite its high incidence, the pathogenesis of DPN remains incompletely understood, and current treatment strategies primarily focus on glycemic control, lifestyle modifications, and symptom-relieving medications.[3,4] Therefore, additional studies on the pathogenesis of DPN are needed to explore effective therapeutic targets to overcome this DPN treatment dilemma.[2,5]

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In recent years, compelling evidence has shown that the duration of diabetes and hemoglobin A1c (HbA1c) levels are important risk factors for DPN.[6–8] In addition, emerging evidence suggests that dyslipidemia treatment (drugs to reduce triglycerides, cholesterol, etc.) is also very effective at improving symptoms in patients with clinical DPN.[9,10] High triglyceridemia, abdominal obesity, and low high-density lipoprotein (HDL) levels have all been found to be strongly associated with the development of DPN,[1] but the underlying metabolic mechanism remains unclear.

Probucol, a widely used lipid-lowering agent, has demonstrated beneficial effects beyond lipid regulation, including improvements in neurological outcomes, diabetes, diabetic retinopathy, and DPN.[11,12] Oxidative stress is believed to play a key role in DPN pathogenesis, and probucol may exert its protective effects by attenuating this oxidative stress response. Emerging reports have suggested that Probucol can relieve hyperalgesia and allodynia in neuropathic pain via nuclear factor kappa-B (NF-dB)/ The NOD-like receptor pyrin domain-containing 3 (NLRP3) signaling. Probucol targets the intracellular inhibition of ATP-binding cassette subfamily A member 1 (ABCA1), which is a lipid transport protein that regulates lipid metabolism, to exert therapeutic effects.[13,14] ATP-binding cassette (ABC) transporters are found primarily in the plasma membrane and serve as a group of lipid transporters. ABCA1 is highly expressed in hepatocytes, macrophages, and smooth muscle cells. It functions as a membrane protein responsible for transporting cholesterol and facilitating the efflux of intracellular cholesterol, and has been widely acknowledged and utilized in the treatment of atherosclerosis.[15] This process helps to reduce excess cholesterol levels in peripheral tissues and the liver. Although probucol reduces high-density lipoprotein cholesterol (HDL-C) levels by acting on ABCA1, it improves HDL functionality.[16] Studies have shown that probucol significantly upregulates ABCA1 expression in macrophages, enhancing cholesterol efflux capacity.[17] It also increases the expression of scavenger receptor class B type I (SR-BI) in hepatocytes, promoting HDL binding to liver cells and accelerating cholesterol clearance.[16] Moreover, probucol reduces the activity of myeloperoxidase (MPO), thereby limiting the oxidative modification of HDL-C. This enhancement of HDL-C functionality contributes to the protection of neural cells against peroxide-induced damage.[18] These findings provide a theoretical foundation for investigating the innovative mechanism by which Probucol alleviates DPN-induced neuropathic pain via ABCA1. At present, the association between probucol treatment and DPN incidence is mostly based on cross-sectional analyses, such as observational cohort studies, which have certain limitations in inferring causality.

Mendelian randomization (MR) is a widely used method for inferring causality, in which genetic variants associated with an exposure are utilized to investigate potential causal relationships between that exposure and the selected outcome(s).[19,20] Genetic variants are transmitted randomly from parent to offspring during meiosis, become fixed at conception, and are less susceptible to confounding or reverse causality.[21] The recent development of genome-wide association studies (GWASs) and the vigorous advancement of molecular mechanism identification have provided a powerful basis for MR research. In addition, MR can be used to explore the effect of long-term regulation of drug targets on disease risk. Moreover, it can be used to simulate the effects of pharmacological regulation of drug targets in clinical trials and predict the clinical benefits and adverse effects of therapeutic interventions.[22] According to our previous literature search, no studies have comprehensively investigated the causal association between probucol treatment and DPN via MR.

In this study, we investigated whether circulating metabolites might mediate the effect of the probucol target gene ABCA1 on DPN. To substantiate these findings, we utilized GWAS data and conducted an MR analysis to explore the relationship between probucol treatment and DPN. Additionally, we conducted a two-step MR investigation to elucidate the potential metabolic pathway connecting ABCA1 to DPN via circulating metabolites, specifically blood lipids. Finally, through MR, we systematically explored circulating metabolites that have a significant causal relationship with ABCA1 and DPN.

Materials and Methods

Study Design

We systematically applied a two-sample MR design to assess the causal association among the lipid-lowering drug target ABCA1, human circulating metabolites, and DPN. The study design and analysis pipeline are illustrated in Figure 1. As shown in Figure 1A, ABCA1 was identified as a target of Probucol. Genetic instruments and metabolites were selected as

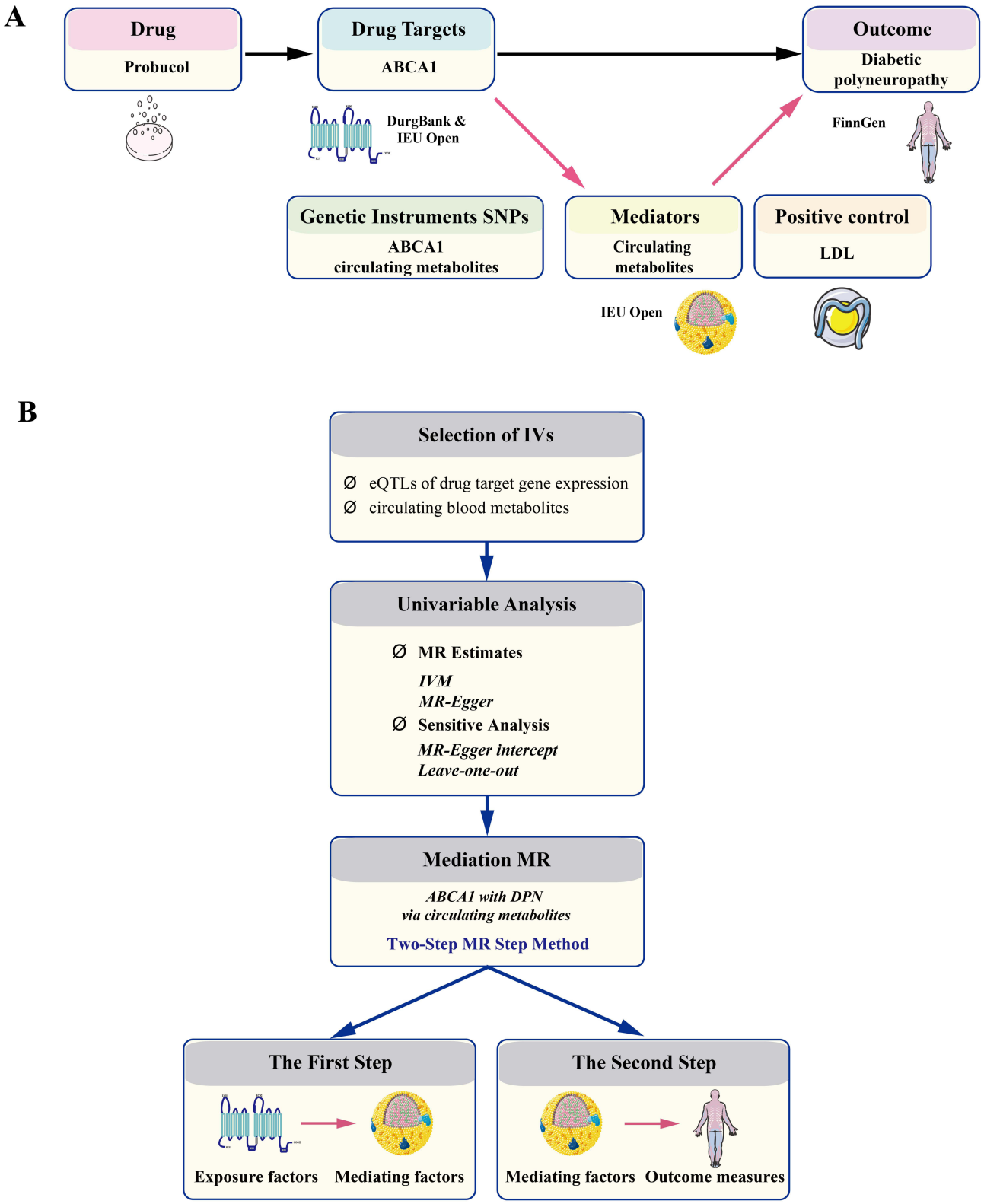

Figure 1 Study design of the MR analysis linking ABCA1 to DPN. (**A**) Conceptual framework: The drug Probucol targets ABCA1, which may influence DPN risk via circulating metabolites. (**B**) Analytical workflow: Instrumental variables were selected from eQTLs and metabolite GWAS. Univariable MR analyses (IVW, MR-Egger) were followed by sensitivity tests. A two-step MR mediation analysis evaluated the indirect effect of ABCA1 on DPN through circulating metabolites.

Abbreviations: ABCA1, ATP-binding cassette subfamily A member 1; IEU, Integrative Epidemiology Unit; SNP, single-nucleotide polymorphism; LDL, low-density lipoprotein; IVs, instrumental variables; eQTL, quantitative trait loci; MR, Mendelian randomization; IVW, Inverse-variance weighted. Symbols: "Ø" indicates processes performed in the analysis pipeline.

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exposures and mediators. Figure 1B outlines the MR workflow, including univariable and two-step mediation analyses to assess the direct and indirect effects of ABCA1 on DPN. To increase the validity of an MR study, the analyses need to meet four core assumptions:[23] (1) instrumental variables (IVs) exhibit a strong correlation with the exposure, with $P < 5\times10^{-8}$ indicating a statistically significant correlation (correlation hypothesis); (2) IVs are not directly related to the outcome and are only affected through exposure, indicating that there is no genetic pleiotropy (exclusivity hypothesis); (3) The independent variable must be free from any influence of the confounding variables, in accordance with the assumption of independence; and (4) target correlation should be satisfied when selecting IVs in quantitative trait loci (eQTL), ie, IVs should be within the range of cis-acting target genes.

Data Sources

The details of the contributing GWAS consortia can be found in Supplementary Table 1. The target gene ABCA1 of the lipid-lowering drug probucol was identified via a search of the DrugBank database (https://go.drugbank.com/drugs/DB01599). The eQTLs (eqtl-a-ENSG00000165029) of the probucol target gene ABCA1 were acquired from the Integrative Epidemiology Unit (IEU) OpenGWAS project website (https://gwas.mrcieu.ac.uk/). GWAS data for 123 circulating blood metabolites and LDL (ebi-a-GCST90025954 and prot-a-1780) were obtained from the IEU OpenGWAS project website. GWAS data for DPN (DM_POLYNEURO) were obtained from the FinnGen website (https://www.finngen.fi/fi). The data were obtained from European populations. Given that all the databases and GWAS data used were publicly available and approved by the appropriate ethical review board, no ethical approval was required for the current analysis.

Selection of IVs

Selection of IVs for eQTLs of Drug Target Gene Expression

The selection of IVs for the analysis of drug target gene expression in this MR study was based on four fundamental assumptions. First, in order to extract single-nucleotide polymorphisms (SNPs), a significance threshold of P-value < 5 × 10^−8 was utilized as the screening criterion to ensure stronger associations between the selected SNPs and the target phenotype. Second, independent variants were identified using R software, with a linkage disequilibrium threshold of R^2=0.3 and a linkage disequilibrium region width within a 100 kilobase (kb) distance. We set the minor allele frequency (MAF) > 0.01 to ensure the independence of each SNP and remove the influence of linkage disequilibrium on the results. Third, PhenoScanner (http://www.phenoscanner.medschl.cam.ac.uk/) was used to eliminate confounding factors and outcome-related SNPs, and drug target genes of eQTL data were used to extract SNPs within ±100 kb of the cis region of the target gene. Finally, the relevant SNPs were extracted from the GWAS summary data of the outcome variables (DPN, low-density lipoprotein (LDL), and circulating blood metabolites). To quantitatively assess the strength of the selected SNPs as instruments, we calculated the F-statistics for each metabolite. Typically, a threshold of F > 10 indicates that a SNP is suitable for use in MR analysis.[24]

Selection of IVs for Circulating Blood Metabolites

MR analysis was conducted based on 3 fundamental assumptions. First, for extracting SNPs, P-value < 5 × 10^−8 was used as the screening criterion to ensure a stronger association between the selected SNPs and the target phenotype. Second, independent variants were identified using R software. The linkage disequilibrium threshold was set at $R^2 < 0.001$ and the width of the linkage disequilibrium region was limited to a distance of 10,000 kb. The SNPs associated with the confounders or outcomes were eliminated via PhenoScanner. Finally, the relevant SNPs were extracted from the aggregated GWAS data for the outcome variable (DPN). To quantitatively assess the strength of the selected SNPs as instruments, we calculated the F statistics of the SNPs for each metabolite.

Statistical Analyses

MR Analysis Between the Probucol Target Gene and LDL (Positive Control)

MR analysis was mainly estimated using random-effect inverse-variance weighted (IVW) and MR-Egger regression. IVW was the main analysis method, and MR-Egger regression was the complementary method. We conducted the

following analysis accordingly: (1) SNP heterogeneity was determined by Cochran's Q test. A P-value < 0.05 indicated heterogeneity. In addition, the I^2 statistic was calculated to determine the proportion of the total variation that was due to heterogeneity. An I^2 value of ≥ 50% indicated substantial heterogeneity. (2) Pleiotropy analysis was conducted using the MR-Egger intercept. A MR-Egger regression intercept term that was not significantly different from zero ($P > 0.05$) was considered to indicate no evidence of pleiotropy among the SNPs. (3) The leave-one-out method was used for the sensitivity analysis. We conducted individual SNP analysis and leave-one-out analysis to evaluate the possibility of any associations being influenced by a single SNP.

Mediation Analysis Linking ABCA1 with DPN via Circulating Metabolites

The total effect of any exposure on an outcome can be divided into direct effects and indirect (intermediary) effects. The direct effect of the probucol target gene ABCA1 on DPN and the intermediary effect mediated by circulating blood metabolites were obtained by two-step multivariable MR. A two-step MR analysis was employed to assess the mediating role of circulating metabolites in the relationship between ABCA1 and DPN. In the first step, the SNPs of the exposure factors were used to estimate their causal effects on the mediating factors. The second step used the SNPs of the mediating factors to assess their causal effect on the outcome measures. Later, the direct effect of exposure factors on DPN incidence was estimated, and the indirect effect of the exposure factors on outcome variables was determined through the use of mediating factors.

We estimated the proportion of mediated effects (E%) using the following formula:

$$E\,(\%) = \frac{\beta 1 \times \beta 2}{\beta 3 + \beta 1 \times \beta 2}$$

The effect of ABCA1 on DPN is determined by β1 × β2, where β1 is the MR effect of ABCA1 on circulating blood metabolites and β2 is the MR effect of circulating blood metabolites on DPN. β3 is the MR effect of ABCA1 on DPN, ie, the direct effect. All MR analyses were performed using the "TwoSampleMR" package in R software (version 4.1.0).

Results

After checking for validity, we extracted 25, 27 and 5,658 SNPs associated with DPN, LDL and circulating metabolites, respectively, from the eQTL data of the drug target genes. Furthermore, 1,108 SNPs associated with DPN were obtained from circulating metabolites. The details are shown in Supplementary Tables 2–5.

Causal Association Analysis Between the Probucol Target Gene ABCA1 and LDL (Positive Control)

Based on LDL dataset prot-a-1780, MR analysis was performed on Probucol target gene ABCA1 and the positive control LDL. The IVW results showed that the ABCA1 gene was associated with a greater risk of increased LDL in Table 1 (odds ratio [OR] = 1.283, 95% CI = 1.174–1.402, $P < 0.001$). There was no evidence of heterogeneity observed in the IVW analysis (I^2 = 0%, Cochran's Q = 15.1039, P = 0.9177) and no evidence of horizontal pleiotropy (MR-Egger analysis: Egger intercept = −0.0111, P = 0.5617) in the associations between the drug target gene ABCA1 and the SNPs associated with LDL, indicating that the MR results of this study were robust (Table 1).

Causal Association Analysis of ABCA1 and DPN Based on eQTLs Data

Based on the Finnish dataset DM_POLYNEURO, which contained data on DPN, the eQTL of the probucol target gene ABCA1 was used as an IV, and the association between the probucol target gene ABCA1 and DPN was analyzed by MR analysis. The IVW results showed that a SNP associated with ABCA1 gene expression was associated with an increased risk of DPN (OR = 1.351, 95% CI = 1.153–1.582, P = 0.0002) (Table 2). There was no evidence of heterogeneity observed in the IVW analysis (I^2= 0%, Cochran's Q = 16.3995, P = 0.8731) and no evidence of horizontal pleiotropy (MR-Egger analysis: Egger intercept = −0.0089, P = 0.7939) in the associations between the drug target gene ABCA1 and the SNPs associated with DPN, indicating that the MR results of this study were robust (Table 2).

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Table 1 MR Estimates of the Causal Association Between ABCA1 Expression and LDL Levels

Data source	Phenotype	Sample Size	Cases	Population	Adjustment
IEU Open GWAS project	ABCA1	31684	–	European	Males and Females
	Circulating metabolites	7824	–	European	Males and Females
	Direct LDL cholesterol levels	437068	–	European	–
	LDL receptor-related protein 8	3301	–	European	Males and Females
FinnGen	DPN	375482	1048	European	–

Abbreviations: IEU, Integrative Epidemiology Unit; LDL, low density lipoprotein; DPN, Diabetic polyneuropathy.

Table 2 MR Estimates of the Causal Association Between ABCA1 Expression and DPN

Exposure	Outcome	Method	Nsnp	MR		Heterogeneity			Horizontal Pleiotropy		
				OR(95% CI)	*P* value	I^2(%)	Cochran's Q	*P* value	Egger Intercept	SE	*P* value
ABCA1	DPN	IVW	25	1.351(1.153–1.582)	0.0002	0	16.3995	0.8731			
ABCA1	DPN	MR-Egger	25	1.433(0.897–2.290)	0.1457	0	16.3296	0.8408	−0.0089	0.0335	0.7939

Abbreviations: ABCA1, ATP-binding cassette subfamily A member 1; DPN, Diabetic polyneuropathy; IVW, Inverse-variance weighted; Nsnp, Number of single-nucleotide polymorphism; MR, Mendelian randomization; SE, standard error.

Mediation MR of the Probucol Target Gene ABCA1, Circulating Metabolites, and DPN (Two-Step MR Analysis)

Circulatory metabolites may mediate the pathogenesis of DPN induced by the probucol target gene ABCA1. Two-step MR analysis was performed to investigate the pathway through which ABCA1 mediates DPN through circulatory metabolites. In the first step, eQTLs of the probucol target gene ABCA1 were used to estimate its causal effect on circulating metabolites. We observed significant associations between 32 circulating metabolites and the Probucol target gene ABCA1 using IVW analysis. The overall results and effect estimates are summarized in Figure 2, and detailed statistical outputs are provided in Table 3. Among these results, ABCA1 was shown to be a risk factor for a greater mean diameter of HDL particles (OR = 1.095, 95% CI = 1.064–1.127; $P < 0.0001$). No heterogeneity was observed in the IVW analysis ($I^2 = 5\%$, Cochran's Q = 47.1633, $P = 0.3842$), and there was no evidence of horizontal pleiotropy (MR-Egger analysis: Egger intercept = 0.0024, $P = 0.7100$) for the associations between the SNPs related to the probucol target gene ABCA1 and the mean diameter of the HDL particles. These findings indicate that the MR results of this study are robust (Table 3). As shown in Table 3, ABCA1 was a risk factor for increased total cholesterol in medium LDL (OR = 1.028, 95% CI = 1.001–1.057; $P = 0.0459$). There was no evidence of heterogeneity observed in the IVW analysis ($I^2 = 0\%$, Cochran's Q = 30.2372, $P = 0.9551$), and no evidence of horizontal pleiotropy (MR-Egger analysis: Egger intercept = −0.0091, $P = 0.1455$) for the associations between the SNPs related to the probucol target gene ABCA1 and total cholesterol in medium LDL, which indicated that the MR results of this study were robust. Moreover, ABCA1 was a risk factor for increased free cholesterol in very large HDL (OR = 1.084, 95% CI = 1.053–1.115, $P < 0.0001$). No heterogeneity was observed in the IVW analysis ($I^2 = 9\%$, Cochran's Q = 49.4522, $P = 0.3000$), and there was no evidence of horizontal pleiotropy (MR-Egger analysis: Egger intercept = −0.0021, $P = 0.7501$), supporting the robustness of the MR results (Table 3). Moreover, ABCA1 was a protective factor against increased triglycerides in medium very low-density lipoprotein (VLDL) (OR = 0.971, 95% CI = 0.944–0.998; $P = 0.0363$). No heterogeneity was detected in the IVW analysis ($I^2 = 0\%$, Cochran's Q = 37.5227, $P = 0.7779$), and there was no indication of horizontal pleiotropy (MR-Egger analysis: Egger intercept = 0.0023, $P = 0.7087$), indicating that the MR results were robust (Table 3). The other full results of the sensitivity and pleiotropy analyses listed in Supplementary Figure 1 were consistent with the above results.

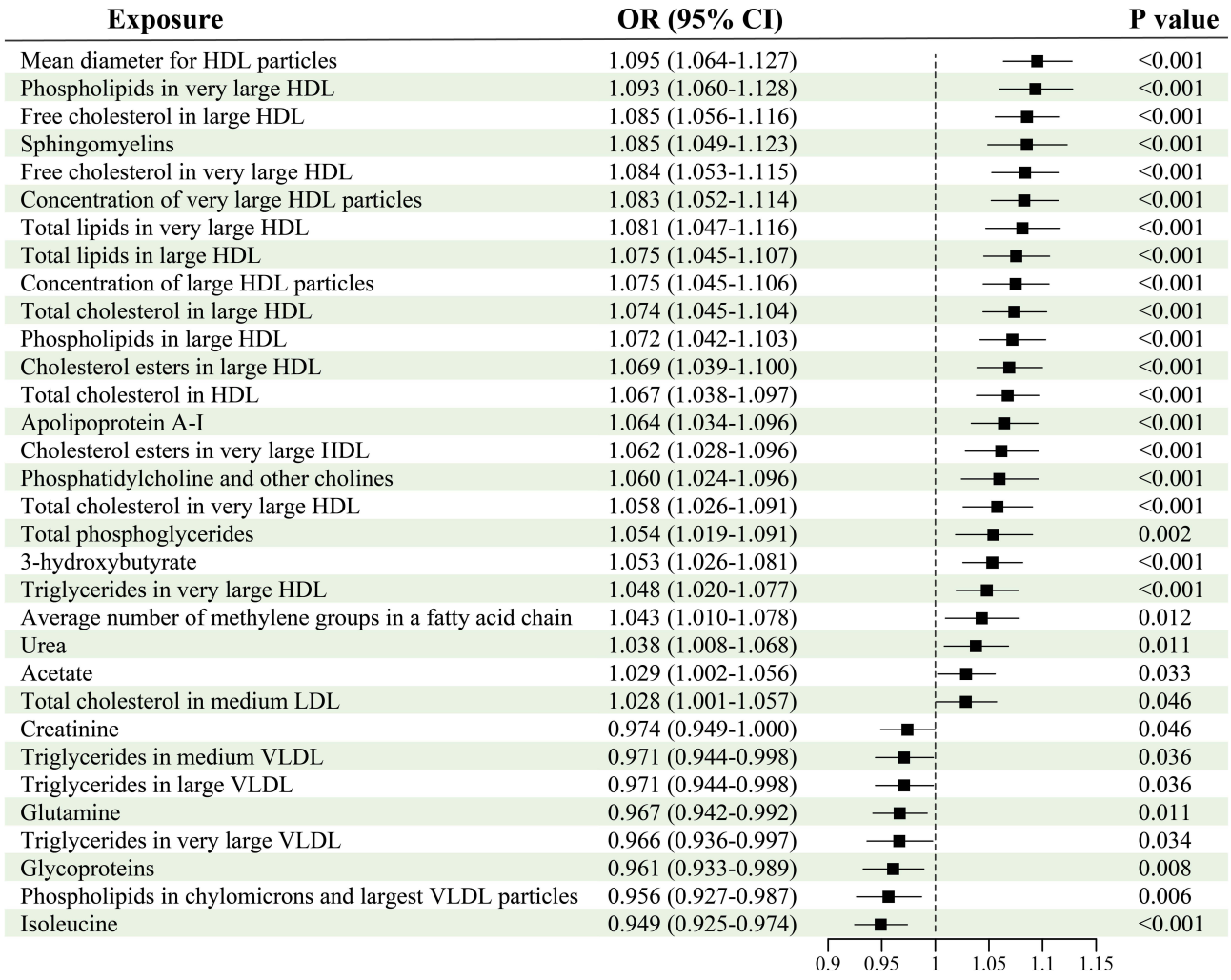

Figure 2 Forest plot of the causal associations of the probucol target gene ABCA1 with circulating metabolites. The effects of the probucol target gene ABCA1 on the 32 circulating metabolites that were significantly associated with ABCA1.
Abbreviations: OR, odds ratio; CI, confidence interval; HDL, high-density lipoprotein; LDL, low-density lipoprotein; VLDL, very low-density lipoprotein.

In the second step, the causal effect on the risk of DPN was assessed using the SNPs of circulating metabolites. Sixteen circulating metabolites were found to be significantly associated with DPN based on IVW analysis. These associations are presented in Figure 3, with corresponding estimates detailed in Table 4. Among these factors, a greater mean diameter of HDL particles was a risk factor for DPN (OR = 1.240, 95% CI = 1.015–1.515; $P = 0.0350$). No evidence of heterogeneity was observed in the IVW analysis ($I^2 = 0\%$, Cochran's Q = 10.0043, $P = 0.4401$) and no evidence of horizontal pleiotropy (MR-Egger analysis: Egger intercept = −0.0070, $P = 0.8724$), indicating robust MR results (Table 4). As shown in Table 4, greater total cholesterol in medium LDL was a risk factor for DPN (OR = 1.274, 95% CI = 1.012–1.603, $P = 0.0392$). No evidence of heterogeneity was observed in the IVW analysis ($I^2 = 10\%$, Cochran's Q = 21.1250, $P = 0.3299$), and no pleiotropy was detected (MR-Egger analysis: Egger intercept = 0.0012, $P = 0.9659$), supporting the robustness of the results (Table 4). Moreover, free cholesterol in very large HDL-C was a risk factor for DPN (OR = 1.354, 95% CI = 1.066–1.720, $P = 0.0129$). There was no evidence of heterogeneity observed in the IVW analysis ($I^2 = 4\%$, Cochran's Q = 13.5306, $P = 0.4077$) and no evidence of horizontal pleiotropy (MR-Egger analysis: Egger intercept = 0.0084, $P = 0.8368$) for the associations between the SNPs related to DPN and free cholesterol in very large HDL, which indicated that the MR results of this study were robust (Table 4). Finally, we found that increased triglycerides in medium VLDL were a protective factor against DPN (OR = 0.614, 95% CI = 0.405–0.930; $P = 0.0213$). There was no evidence of heterogeneity observed in the IVW analysis ($I^2 = 0\%$, Cochran's Q = 4.7632, $P = 0.6888$) and no evidence of horizontal pleiotropy (MR-Egger analysis: Egger intercept = 0.0297,

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Table 3 MR Estimates of the Causal Associations Between ABCA1 and Circulating Metabolites

Outcome	Method	Nsnp	MR		Heterogeneity			Horizontal Pleiotropy		
			OR (95% CI)	*P* value	I^2(%)	Cochran's Q	*P* value	Egger Intercept	SE	*P* value
Acetate	Inverse variance weighted	46	1.029(1.002–1.056)	0.0334	0	31.4641	0.9369			
	MR Egger	46	1.038(0.955–1.128)	0.3853	0	31.4149	0.9227	−0.0013	0.0058	0.8256
Apolipoprotein A-I	Inverse variance weighted	46	1.064(1.034–1.096)	0.0000	0	35.9598	0.8299			
	MR Egger	46	1.109(1.012–1.216)	0.0321	0	35.0816	0.8293	−0.0060	0.0064	0.3538
3-hydroxybutyrate	Inverse variance weighted	46	1.053(1.026–1.081)	0.0001	0	39.2822	0.7120			
	MR Egger	46	1.090(1.002–1.186)	0.0504	0	38.5577	0.7033	−0.0050	0.0059	0.3993
Average number of methylene groups in a fatty acid chain	Inverse variance weighted	46	1.043(1.010–1.078)	0.0116	11	50.8103	0.2554			
	MR Egger	46	0.975(0.879–1.081)	0.6350	10	48.7866	0.2866	0.0097	0.0072	0.1836
Creatinine	Inverse variance weighted	46	0.974(0.949–1.000)	0.0462	0	29.9666	0.9585			
	MR Egger	46	0.996(0.917–1.082)	0.9340	0	29.6417	0.9521	−0.0033	0.0058	0.5716
Glutamine	Inverse variance weighted	46	0.967(0.942–0.992)	0.0113	0	36.0409	0.8274			
	MR Egger	46	0.957(0.880–1.041)	0.3101	0	35.9801	0.7998	0.0014	0.0058	0.8064
Glycoproteins	Inverse variance weighted	46	0.961(0.933–0.989)	0.0075	0	35.6967	0.8380			
	MR Egger	46	0.990(0.902–1.086)	0.8273	0	35.2597	0.8236	−0.0043	0.0065	0.5120
Total cholesterol in HDL	Inverse variance weighted	46	1.067(1.038–1.097)	0.0000	0	35.9969	0.8288			
	MR Egger	46	1.100(1.008–1.201)	0.0384	0	35.4902	0.8162	−0.0044	0.0061	0.4803
Mean diameter for HDL particles	Inverse variance weighted	46	1.095(1.064–1.127)	0.0000	5	47.1633	0.3842			
	MR Egger	46	1.077(0.981–1.182)	0.1259	6	47.0137	0.3501	0.0024	0.0065	0.7100
Isoleucine	Inverse variance weighted	46	0.949(0.925–0.974)	0.0001	0	37.1326	0.7916			
	MR Egger	46	0.975(0.897–1.058)	0.5441	0	36.6918	0.7748	−0.0038	0.0058	0.5102
Total cholesterol in large HDL	Inverse variance weighted	46	1.074(1.045–1.104)	0.0000	0	35.9813	0.8293			
	MR Egger	46	1.072(0.982–1.170)	0.1277	0	35.9799	0.7998	0.0002	0.0061	0.9702
Cholesterol esters in large HDL	Inverse variance weighted	46	1.069(1.039–1.100)	0.0000	0	31.9336	0.9288			
	MR Egger	46	1.058(0.967–1.158)	0.2237	0	31.8805	0.9133	0.0015	0.0063	0.8187

Free cholesterol in large HDL	Inverse variance weighted	46	1.085(1.056–1.116)	0.0000	0	43.2097	0.5480			
	MR Egger	46	1.095(1.003–1.195)	0.0489	0	43.1682	0.5072	−0.0012	0.0061	0.8396
Total lipids in large HDL	Inverse variance weighted	46	1.075(1.045–1.107)	0.0000	0	32.1231	0.9253			
	MR Egger	46	1.069(0.977–1.170)	0.1528	0	32.1042	0.9085	0.0009	0.0063	0.8911
Concentration of large HDL particles	Inverse variance weighted	46	1.075(1.045–1.106)	0.0000	0	31.4908	0.9365			
	MR Egger	46	1.065(0.974–1.166)	0.1751	0	31.4479	0.9220	0.0013	0.0063	0.8369
Phospholipids in large HDL	Inverse variance weighted	46	1.072(1.042–1.103)	0.0000	0	29.7139	0.9615			
	MR Egger	46	1.063(0.972–1.163)	0.1879	0	29.6796	0.9515	0.0012	0.0063	0.8539
Triglycerides in large VLDL	Inverse variance weighted	46	0.971(0.944–0.998)	0.0361	0	41.8061	0.6080			
	MR Egger	46	0.908(0.831–0.992)	0.0379	0	39.3706	0.6701	0.0096	0.0062	0.1258
Total cholesterol in medium LDL	Inverse variance weighted	46	1.028(1.001–1.057)	0.0459	0	30.2372	0.9551			
	MR Egger	46	1.095(1.003–1.196)	0.0480	0	28.0410	0.9708	−0.0091	0.0061	0.1455
Triglycerides in medium VLDL	Inverse variance weighted	46	0.971(0.944–0.998)	0.0363	0	37.5227	0.7779			
	MR Egger	46	0.955(0.874–1.044)	0.3157	0	37.3813	0.7493	0.0023	0.0062	0.7087
Phosphatidylcholine and other cholines	Inverse variance weighted	46	1.060(1.024–1.096)	0.0008	0	30.2748	0.9546			
	MR Egger	46	1.074(0.965–1.195)	0.2002	0	30.2121	0.9437	−0.0019	0.0075	0.8034
Sphingomyelins	Inverse variance weighted	46	1.085(1.049–1.123)	0.0000	0	35.5579	0.8421			
	MR Egger	46	1.112(0.999–1.238)	0.0590	0	35.3400	0.8211	−0.0035	0.0075	0.6429
Total phosphoglycerides	Inverse variance weighted	46	1.054(1.019–1.091)	0.0024	0	33.4127	0.8986			
	MR Egger	46	1.067(0.958–1.187)	0.2444	0	33.3616	0.8787	−0.0017	0.0075	0.8223
Urea	Inverse variance weighted	46	1.038(1.008–1.068)	0.0114	0	28.2083	0.9763			
	MR Egger	46	1.038(0.948–1.136)	0.4291	0	28.2083	0.9691	0.0000	0.0064	0.9970
Total cholesterol in very large HDL	Inverse variance weighted	46	1.058(1.026–1.091)	0.0003	18	55.1415	0.1430			
	MR Egger	46	1.073(0.972–1.183)	0.1679	20	55.0331	0.1231	−0.0020	0.0069	0.7698

(*Continued*)

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Table 3 (Continued).

Outcome	Method	Nsnp	MR		Heterogeneity			Horizontal Pleiotropy		
			OR (95% CI)	*P* value	I^2(%)	Cochran's Q	*P* value	Egger Intercept	SE	*P* value
Cholesterol esters in very large HDL	Inverse variance weighted	46	1.062(1.028–1.096)	0.0003	21	56.7988	0.1116			
	MR Egger	46	1.080(0.976–1.196)	0.1447	22	56.6353	0.0958	−0.0025	0.0072	0.7232
Free cholesterol in very large HDL	Inverse variance weighted	46	1.084(1.053–1.115)	0.0000	9	49.4522	0.3000			
	MR Egger	46	1.099(1.002–1.207)	0.0519	11	49.3369	0.2683	−0.0021	0.0065	0.7501
Total lipids in very large HDL	Inverse variance weighted	46	1.081(1.047–1.116)	0.0000	21	56.8479	0.1108			
	MR Egger	46	1.093(0.987–1.210)	0.0951	23	56.7861	0.0935	−0.0016	0.0072	0.8278
Concentration of very large HDL particles	Inverse variance weighted	46	1.083(1.052–1.114)	0.0000	1	45.6304	0.4457			
	MR Egger	46	1.082(0.987–1.186)	0.1005	4	45.6297	0.4042	0.0002	0.0064	0.9793
Phospholipids in very large HDL	Inverse variance weighted	46	1.093(1.060–1.128)	0.0000	17	54.0276	0.1676			
	MR Egger	46	1.096(0.992–1.211)	0.0774	19	54.0238	0.1431	−0.0004	0.0070	0.9557
Triglycerides in very large HDL	Inverse variance weighted	46	1.048(1.020–1.077)	0.0008	0	41.7462	0.6106			
	MR Egger	46	1.017(0.932–1.110)	0.7078	0	41.2459	0.5903	0.0043	0.0061	0.4831
Triglycerides in very large VLDL	Inverse variance weighted	46	0.966(0.936–0.997)	0.0343	24	59.2713	0.0752			
	MR Egger	46	0.904(0.819–0.999)	0.0546	23	56.8378	0.0928	0.0096	0.0070	0.1769
Phospholipids in chylomicrons and largest VLDL particles	Inverse variance weighted	46	0.956(0.927–0.987)	0.0057	24	59.0706	0.0777			
	MR Egger	46	0.876(0.794–0.967)	0.0115	20	54.8612	0.1263	0.0126	0.0069	0.0729

Abbreviations: ABCA1, ATP-binding cassette subfamily A member 1; HDL, high-density lipoprotein; VLDL, very low-density lipoprotein; LDL, low density lipoprotein; Nsnp, Number of single-nucleotide polymorphism; MR, Mendelian randomization; OR, odds ratio; SE, standard error.

Exposure	Nsnp	OR (95% CI)	P value
Total lipids in very large VLDL	3	2.026 (1.012-4.059)	0.046
Free cholesterol to esterified cholesterol ratio	12	1.356 (1.007-1.827)	0.045
Free cholesterol in very large HDL	14	1.354 (1.066-1.720)	0.013
Phospholipids in medium LDL	16	1.329 (1.026-1.722)	0.031
Cholesterol esters in large VLDL	21	1.322 (1.059-1.651)	0.014
Cholesterol esters in medium LDL	21	1.318 (1.054-1.648)	0.015
Total cholesterol in large LDL	21	1.299 (1.021-1.654)	0.033
Total cholesterol in medium LDL	20	1.274 (1.012-1.603)	0.039
Total lipids in large LDL	22	1.273 (1.008-1.608)	0.042
Total cholesterol in LDL	20	1.271 (1.010-1.599)	0.041
Total lipids in medium LDL	20	1.265 (1.006-1.590)	0.044
Concentration of medium LDL particles	20	1.262 (1.002-1.590)	0.048
Mean diameter for HDL particles	11	1.240 (1.015-1.515)	0.035
Triglycerides in medium VLDL	8	0.614 (0.405-0.930)	0.021
Total lipids in small HDL	6	0.597 (0.411-0.867)	0.007
Concentration of small HDL particles	4	0.577 (0.382-0.872)	0.009

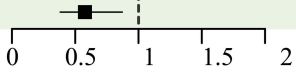

Figure 3 Forest plot of the causal associations between circulating metabolites and DPN. The causal relationships between the levels of 16 circulating blood metabolites and DPN were statistically significant.
Abbreviations: OR, odds ratio; CI, confidence interval; HDL, high-density lipoprotein; LDL, low-density lipoprotein; VLDL, very low-density lipoprotein.

$P = 0.6248$). The other full results of the sensitivity and pleiotropy analyses listed in Supplementary Figure 2 were consistent with the above results.

As shown in Figure 4A, we initially assessed the causal impact of ABCA1 on 32 circulating metabolites. Subsequently, we evaluated the effects of these metabolites on DPN risk (Figure 4B). By integrating results from both steps, we identified four overlapping metabolites (mean diameter for HDL particles, total cholesterol in medium LDL, triglycerides in medium VLDL and free cholesterol in very large HDL) that were causally linked to both ABCA1 and DPN. These metabolites were subsequently included in a two-step MR mediation analysis. The direct effect of ABCA1 on DPN and the indirect effects mediated by these circulating metabolites are illustrated in Figure 5.

The direct effect of the probucol target gene ABCA1 on DPN was 0.3006. The total effect of the ABCA1 target gene probucol on DPN, estimated through the mean diameter of HDL particles, was 0.3201, with a mediating effect of 0.0196, accounting for a mediation proportion of 6.1083%. The total effect of ABCA1 on DPN, estimated through the circulating metabolite total cholesterol in medium LDL, was 0.3073, with a mediating effect of 0.0068, accounting for a mediation proportion of 2.2057%. We also observed that the total effect of ABCA1 on DPN, estimated through the circulating metabolite triglycerides in medium VLDL, was 0.3150, with a mediating effect of 0.0145, accounting for a mediation proportion of 4.5955%. Moreover, the total effect of the probucol target gene ABCA1 on DPN, estimated through free cholesterol in very large HDL, was 0.3249, with a mediating effect of 0.0244, accounting for a mediation proportion of 7.5000%. The results are shown in Figure 5. No indication of heterogeneity or horizontal pleiotropy was detected in these associations.

Discussion

We conducted an investigation into the impact of genetic variation in the lipid-lowering drug target ABCA1 on the risk of DPN using genetic datasets on four circulating blood metabolites (mean diameter for HDL particles, total cholesterol in medium LDL, triglycerides in medium VLDL and free cholesterol in very large HDL). We discovered evidence indicating that several genetic variations of probucol targets were linked to a reduced risk of DPN. The strength of this approach lies in its ability to rapidly and cost-effectively prioritize candidate drugs for clinical trials on the basis of preclinical experiments based on patient data and MR analysis.

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Table 4 MR Estimates of the Causal Associations Between Circulating Metabolites and DPN

Exposure	Outcome	Method	Nsnp	MR		Heterogeneity			Horizontal Pleiotropy		
				OR (95% CI)	*P* value	I^2(%)	Cochran's Q	*P* value	Egger Intercept	SE	*P* value
Free cholesterol to esterified cholesterol ratio	DPN	Inverse variance weighted	12	1.356(1.007–1.827)	0.0447	28	15.3493	0.1671			
		MR Egger	12	1.071(0.621–1.847)	0.8113	28	13.9161	0.1769	0.0397	0.0391	0.3341
Mean diameter for HDL particles		Inverse variance weighted	11	1.240(1.015–1.515)	0.0350	0	10.0043	0.4401			
		MR Egger	11	1.292(0.761–2.193)	0.3673	10	9.9740	0.3526	−0.0070	0.0426	0.8724
Total cholesterol in large LDL	DPN	Inverse variance weighted	21	1.299(1.021–1.654)	0.0334	22	25.5401	0.1815			
		MR Egger	21	1.246(0.806–1.926)	0.3350	25	25.4689	0.1457	0.0065	0.0281	0.8202
Cholesterol esters in large VLDL		Inverse variance weighted	21	1.322(1.059–1.651)	0.0138	10	22.1733	0.3312			
		MR Egger	21	1.246(0.828–1.874)	0.3039	14	22.0374	0.2824	0.0090	0.0263	0.7359
Total lipids in large LDL	DPN	Inverse variance weighted	22	1.273(1.008–1.608)	0.0424	21	26.5076	0.1878			
		MR Egger	22	1.290(0.839–1.984)	0.2597	25	26.5008	0.1499	−0.0019	0.0272	0.9437
Total cholesterol in LDL		Inverse variance weighted	20	1.271(1.010–1.599)	0.0408	12	21.5872	0.3053			
		MR Egger	20	1.271(0.839–1.927)	0.2733	17	21.5872	0.2508	0.0000	0.0285	0.9999
Total cholesterol in medium LDL	DON	Inverse variance weighted	20	1.274(1.012–1.603)	0.0392	10	21.1250	0.3299			
		MR Egger	20	1.264(0.836–1.910)	0.2809	15	21.1228	0.2733	0.0012	0.0276	0.9659
Cholesterol esters in medium LDL		Inverse variance weighted	21	1.318(1.054–1.648)	0.0153	10	22.1387	0.3330			
		MR Egger	21	1.137(0.756–1.712)	0.5448	11	21.3346	0.3186	0.0232	0.0274	0.4079
Total lipids in medium LDL	DPN	Inverse variance weighted	20	1.265(1.006–1.590)	0.0444	11	21.4472	0.3126			
		MR Egger	20	1.228(0.801–1.882)	0.3581	16	21.4160	0.2589	0.0047	0.0290	0.8732
Concentration of medium LDL particles		Inverse variance weighted	20	1.262(1.002–1.590)	0.0478	12	21.5861	0.3053			
		MR Egger	20	1.215(0.789–1.872)	0.3873	16	21.5354	0.2533	0.0060	0.0292	0.8392
Phospholipids in medium LDL	DPN	Inverse variance weighted	16	1.329(1.026–1.722)	0.0314	11	16.8701	0.3267			
		MR Egger	16	1.020(0.639–1.628)	0.9344	7	15.0043	0.3779	0.0444	0.0336	0.2082

Triglycerides in medium VLDL		Inverse variance weighted	8	0.614(0.405–0.930)	0.0213	0	4.7632	0.6888			
		MR Egger	8	0.466(0.151–1.439)	0.2326	0	4.4978	0.6096	0.0297	0.0577	0.6248
Total lipids in small HDL	DPN	Inverse variance weighted	6	0.597(0.411–0.867)	0.0068	0	2.2302	0.8165			
		MR Egger	6	0.981(0.389–2.475)	0.9691	0	0.9099	0.9231	−0.0769	0.0669	0.3146
Concentration of small HDL particles		Inverse variance weighted	4	0.577(0.382–0.872)	0.0090	0	1.9235	0.5884			
		MR Egger	4	1.013(0.317–3.241)	0.9846	0	0.8920	0.6402	−0.0801	0.0789	0.4167
Free cholesterol in very large HDL	DPN	Inverse variance weighted	14	1.354(1.066–1.720)	0.0129	4	13.5306	0.4077			
		MR Egger	14	1.268(0.652–2.463)	0.4973	11	13.4808	0.3351	0.0084	0.0399	0.8368
Total lipids in very large VLDL		Inverse variance weighted	3	2.026(1.012–4.059)	0.0463	0	1.9049	0.3858			
		MR Egger	3	7.668 (0.858–68.529)	0.3194	0	0.3278	0.5670	−0.1490	0.1186	0.4281

Abbreviations: HDL, high-density lipoprotein; LDL, low density lipoprotein; VLDL, very low-density lipoprotein; DPN, Diabetic polyneuropathy; Nsnp, Number of single-nucleotide polymorphism; MR, Mendelian randomization; OR, odds ratio; SE, standard error.

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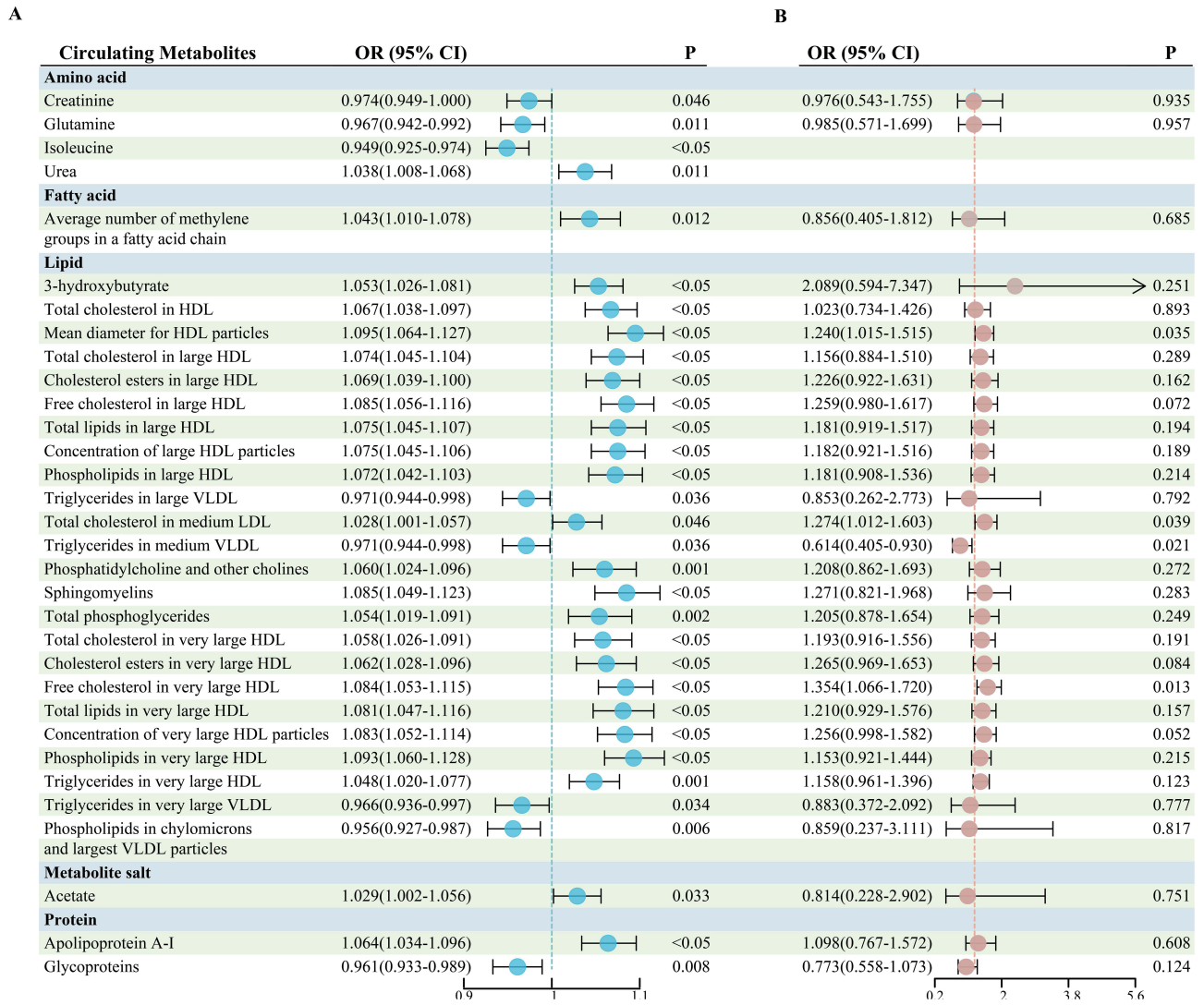

Figure 4 The forest plot shows the impact of ABCA1 on circulating metabolites and the influence of metabolites on DPN. (**A**) The impact of ABCA1 on the remaining 32 circulating metabolites. (**B**) The influence of the aforementioned 32 metabolites on DPN. Only one SNP associated with DPN was affected by the circulating blood metabolites 3-hydroxybutyrate and acetate; therefore, those metabolites could not be analyzed using the IVW and MR-Egger methods. The results of the causal association analysis shown in the figure were analyzed using the Wald ratio method.
Abbreviations: CI, confidence interval; HDL, high-density lipoprotein; LDL, low-density lipoprotein; VLDL, very low-density lipoprotein; OR, odds ratio.

The prevalence of diabetes is steadily increasing each year, and based on the latest data from 2021, it is predicted that approximately 783.2 million individuals worldwide (12.2% of the global population) will be affected by diabetes by 2045.[25] As the most common vascular complication, DPN significantly compromises the quality of life of diabetic patients. The patients often experience symmetrical limb pain, especially in the distal limbs, with various paresthesia, including numbness, coldness, burning, and the most serious is painful neuropathy. Up to half of patients with DPN experience severe neuropathic pain with allodynia or hyperalgesia, which significantly impacts their quality of life, sleep, and mood. The associated medical costs are also substantial. Due to the complex pathogenesis and numerous influencing factors associated with DPN, there is currently a lack of effective clinical treatment options available for this condition. In the current investigation, we assessed the relationships between genetically predicted gene expression of the probucol target gene ABCA1, LDL, and DPN. Additionally, we conducted an investigation into the mediating role of circulating metabolites in the association between ABCA1 and DPN. Our study indicated that there was a genetic variation in ABCA1 which was associated with an increased risk of elevated LDL levels (OR = 1.283, 95% CI=1.174–1.402, $P < 0.001$) and a greater risk of developing DPN (OR = 1.351, 95% CI=1.153–1.582, $P = 0.0002$). The direct effect of the probucol target gene ABCA1 on DPN was 0.3006. Finally, we

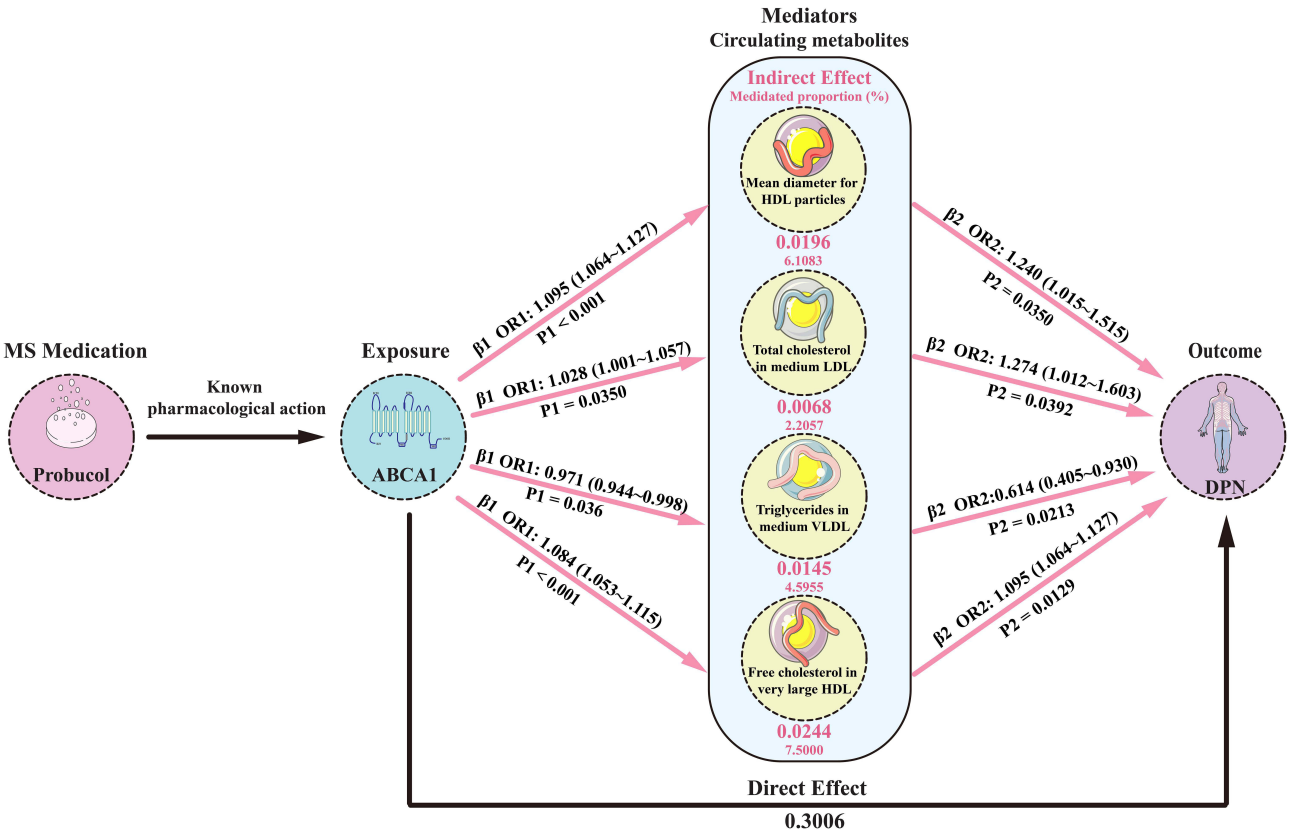

Figure 5 Mediating effects of circulating blood metabolites on the association between ABCA1 and DPN. The potential causal effects of the following factors included in the MR analysis are summarized: mean diameter for HDL particles, total cholesterol in medium LDL, triglycerides in medium VLDL and free cholesterol in very large HDL. Red arrows indicate the involvement of mediating effects.

Abbreviations: ABCA1, ATP-binding cassette subfamily A member 1; HDL, high-density lipoprotein; LDL, low-density lipoprotein; VLDL, very low-density lipoprotein; OR, odds ratio; DPN, Diabetic polyneuropathy.

observed that four circulating blood metabolites (mean diameter of HDL particles, total cholesterol in medium LDL, triglycerides in medium VLDL and free cholesterol in very large HDL) had significant causal relationships with both ABCA1 expression and DPN.

Previous research has highlighted the potential contributions of metabolic syndrome (MetS) and its components, especially dyslipidemia, which frequently coexist with diabetes and its associated complications, to DPN.[26,27] It has been demonstrated that the presence of saturated fatty acid (SFA) palmitate and other forms of dyslipidemia can lead to a reduction in mitochondrial ATP production and a decrease in mitochondrial abundance. This ultimately contributes to the development of distal-to-proximal nerve damage, as clinically observed in DPN.[28,29] Long-chain fatty acids have also been demonstrated to traverse the blood-nerve barrier and induce neurogenic inflammation, a phenomenon substantiated by augmented expression of tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) in animal models of DPN.[30] The generation of oxidized cholesterol (oxysterol) triggers tissue injury by binding to receptors, thereby initiating inflammatory and immune responses that are manifested in endothelial and Schwann cells, ultimately contributing to vascular injury in DPN patients.[31] Other researchers have used the QUADAS-2 tool to assess the correlation between LDL and DPN incidence. These authors suggested that LDL status could be associated with an increased risk of DPN.[32] Consistent with these findings, liver fat content (LFC) is believed to be positively correlated with the severity of chronic complications in type 2 diabetes (T2DM) patients.[33] Overall, given the risk of dyslipidemia in patients with DPN, controlling blood lipid levels may have strong potential in the treatment of DPN. Therefore, studies have addressed the application of lipid-lowering drugs in patients with DPN.

According to a report on 531 patients with T2DM who were treated with statins or fibrates for lipid-lowering therapy over a period of 5 years, it was found that fenofibrate treatment was significantly associated with a reduction in

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neuropathy as measured by the Michigan Neuropathy Scoring instrument.[34] Mechanistic studies have proved that fenofibrate significantly activates the peroxisome proliferator-activated receptor α (PPARα), adenosine monophosphate-activated protein kinase (AMPK), and peroxisome proliferator-activated receptor γ coactivator 1 (PGC1) pathway in the sciatic nerve.[35] Supplementation with fish oil containing docosahexaenoic acid (DHA) could prevent the development of DPN and could maintain the nerve conduction velocity and Na+/K+-ATPase activity in the sural nerve,[36,37] which provides evidence that the anti-inflammatory properties of omega-3s may oppose DPN. Statins are inhibitors of 3-hydroxy-3-methylglutaryl-coenzyme A reductase, the key enzyme involved in cholesterol synthesis. The effects of statins on DPN have yielded inconsistent findings, potentially due to the detrimental impact of insufficient cholesterol (the fundamental constituent of myelin) on neurological well-being.[38,39] Overall, the therapeutic effect of statins is unlikely to prevent DPN, but this finding provides new insights into alternative lipid-lowering or multitargeting strategies for DPN treatment.

In our study, we focused on the lipid-lowering agent probucol, which exerts its lipid-lowering effect by inhibiting the target gene ABCA1. Recent findings in probucol-treated mice showed that reduced hepatic ABCA1 activity unexpectedly enhanced in vivo reverse cholesterol transport (RCT).[40] This may be due to increased hepatic cholesterol uptake via SR-BI, redirecting cholesterol into intrahepatic compartments that are also accessible for ABCA1-mediated resecretion into plasma to form new HDL particles. However, the identity and function of these intrahepatic cholesterol pools remain unclear. Future studies using pulse-chase experiments in polarized hepatocyte models are needed to trace intracellular cholesterol trafficking and quantify fluxes, which was a limitation of the current study. Other studies have shown that ABCA1 is also closely associated with cancer resistance mechanisms.[41] Remarkably, ABCA1 was found to be associated with elevated levels of neuroactive steroids, which can affect Na+/K+-ATPase activity, nerve conduction velocity, and thermal nociceptive activity.[42] Moreover, another study recruited a total of 158 Emirati patients with T2DM.[43] The authors found that the presence of rs2230806 in the ABCA1 gene was significantly associated with the prevalence of multiple complications, including DPN, in patients with diabetes. Consistent with this conclusion, other scholars have shown that ABCA1 deficiency contributes to glomerular endothelium injury and dysfunction, suggesting that the targeting of ABCA1 could be a potential therapeutic strategy for early-stage diabetic kidney disease.[44] Based on the results of our research, we presented compelling evidence of the detrimental impact of the expression of the probucol target gene ABCA1 on DPN in the general population, utilizing a robust set of genetic instruments for ABCA1 as instrumental variables and a large GWAS for DPN. To our knowledge, this study represents the first utilization of MR analysis to investigate the association among ABCA1, a target gene of probucol, circulating metabolites and DPN in a population-based setting, providing theoretical support for multiple uses of the drug probucol. This study also demonstrated the potential of MR analysis as a valuable tool for identifying new indications for approved drugs.

Although the four identified circulating metabolites—mean diameter for HDL particles, total cholesterol in medium LDL, triglycerides in medium VLDL and free cholesterol in very large HDL—showed relatively modest mediation effects (2.2–7.5%) in the association between ABCA1 and DPN, their consistent identification across analyses suggests potential biological relevance. Small effect sizes are common in studies of complex traits, as individual metabolites often act as part of a broader network of interacting factors. These modest effects may indicate that the metabolites function not as independent drivers, but as contributors within larger pathophysiological processes. For example, alterations in HDL particle size may impair endothelial function,[45] exacerbating microvascular dysfunction in DPN. Cholesterol in medium LDL is more prone to oxidative modification,[46] which can induce endothelial inflammation and compromise the blood–nerve barrier. Triglycerides enrichment in medium VLDL may contribute to systemic inflammation or insulin resistance,[47] thereby indirectly accelerating neural injury. In addition, elevated free cholesterol in very large HDL may reflect impaired cholesterol efflux,[48] which could disrupt membrane fluidity and ion channel function (eg, Na+/K+-ATPase), ultimately impairing neuronal conduction. Importantly, these metabolites may act synergistically, amplifying their overall impact beyond that suggested by individual effect sizes. Future multi-omics studies—integrating metabolomics with proteomics or transcriptomics—will be essential to determine whether these metabolites exert independent effects or participate in coordinated regulatory networks linking ABCA1 to DPN.

The strength of our study lies in the utilization of participants from exclusively European populations for analysis, thereby mitigating potential bias arising from population heterogeneity. The MR design minimizes the potential biases caused by

reverse causation and confounding factors, enhancing the validity of the observed associations. In our study, we employed MR-Egger and penalty-weighted median methods to account for different assumptions regarding direct pleiotropy while conducting sensitivity analysis. The consistent findings obtained from these complementary approaches demonstrate the robustness of the estimated causal effects. These findings provide a theoretical basis for probucol as a potential therapeutic agent for DPN. However, it is important to note that our study has several limitations. Firstly, the genetic variants used to mimic the inhibition of ABCA1 may only reflect the long-term effects of ABCA1 inhibitors. It is crucial to recognize that these effects may not accurately represent the short-term impact of ABCA1 inhibitors. Therefore, MR analysis is more valuable in determining the direction rather than quantifying the magnitude of the potential causal effect. Secondly, further investigation is required to generalize these findings to other populations, as the data used in this study were exclusively derived from individuals of European ancestry. Moreover, while some associations did not meet stringent multiple testing thresholds, this exploratory study prioritized biological relevance over strict correction. We highlighted findings with $P < 0.01$ as more robust, while treating nominal associations ($P < 0.05$) with caution, consistent with prior guidance.[49] These results offer hypotheses for future validation. Finally, given the frequent coexistence of dyslipidemia and various vascular diseases in patients with diabetes and its complications, combination therapy involving hypoglycemic drugs and lipid-lowering agents is commonly employed. This article solely focused on probucol and its target, but further investigation into the potential application of other medications could yield additional clinical benefits for patients. In our future work, we will build on the results of this study and explore the mechanism of specific regulation of DPN by ABCA1 targets to provide a theoretical basis for identifying clinical treatment targets for DPN.

Conclusions

In conclusion, this study offers compelling evidence that the genetic variability in probucol targets, by modulating ABCA1, is linked to a reduced susceptibility to DPN. Specifically, we observed the direct effect of ABCA1 on DPN, as well as mediating effects of four circulating blood metabolites (mean diameter for HDL particles, total cholesterol in medium LDL, triglycerides in medium VLDL and free cholesterol in very large HDL) on that association. These findings provide genetic evidence supporting the mechanism by which probucol reduces DPN risk and may offer valuable insights for future mechanistic and clinical studies.

Abbreviations

DPN, Diabetic polyneuropathy; HbA1c, hemoglobin A1c; HDL, high-density lipoprotein; NF-κB, nuclear factor kappa-B; NLRP3, the NOD-like receptor pyrin domain-containing 3; ABCA1, ATP-binding cassette subfamily A member 1; ABC, ATP-binding cassette; HDL-C, high-density lipoprotein cholesterol; SR-BI, scavenger receptor class B type I; MPO, myeloperoxidase; MR, Mendelian randomization; GWASs, genome-wide association studies; IVs, instrumental variables; eQTL, quantitative trait loci; IEU, Integrative Epidemiology Unit; SNPs, single-nucleotide polymorphisms; LDL, low density lipoprotein; OR, odds ratio; MAF, the minor allele frequency; IVW, Inverse-variance weighted; VLDL, very low-density lipoprotein; MetS, metabolic syndrome; SFA, the presence of saturated fatty acid; TNF-α, tumor necrosis factor-α; IL-6, interleukin-6; LFC, liver fat content; T2DM, Type 2 diabetes; PPARα, peroxisome proliferator-activated receptor α; AMPK, adenosine monophosphate-activated protein kinase; PGC1, peroxisome proliferator-activated receptor γ coactivator 1; DHA, docosahexaenoic acid; CAD, coronary artery disease; RCT, reverse cholesterol transport.

Data Sharing Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request. The GWAS Summary statistics used in this study were publicly accessed from the DrugBank database (https://go.drugbank.com/drugs/DB01599), the IEU OpenGWAS project website (https://gwas.mrcieu.ac.uk/) and FinnGen website (https://www.finngen.fi/fi). The raw data of the manuscript were uploaded onto the Research Data Deposit (RDD) (https://www.researchdata.org.cn/default.aspx) with an RDD number of RDDB2024440999.

Ethics Approval and Consent to Participate

Pursuant to Items 1 and 2 of Article 32 of the Measures for Ethical Review of Life Science and Medical Research Involving Human Subjects (effective February 18, 2023), the present study is exempt from ethical review, as it exclusively uses publicly available, anonymized data obtained through lawful means.

Acknowledgments

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

All authors declare that they have no competing interests related to this work.

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