Clin. Lab. 2027;73:XXX-XXX

ORIGINAL ARTICLE

Potential Role of HDL-C in the Association between Diabetic Nephropathy and Atherosclerosis: a Mendelian Randomization Study

Junjie Zhu [1], Qiaonan Ge [2], Xiaoling Hong [2], Yangjin Chen [3], Danhua Wang [4]

[1] *Department of Nephrology, Pan'an People's Hospital, Jinhua, Zhejiang, China*
[2] *Department of Hemodialysis, Pan'an People's Hospital, Jinhua, Zhejiang, China*
[3] *Department of Pediatrics, Pan'an People's Hospital, Jinhua, Zhejiang, China*
[4] *Department of Endocrinology, Pan'an People's Hospital, Jinhua, Zhejiang, China*

ABSTRACT

Background: **This study aimed to investigate the potential causal relationship between diabetic nephropathy (DN) and atherosclerosis using Mendelian randomization (MR) analysis and to further evaluate the associations between DN and lipid-related traits. In addition, mediation MR analysis was performed to determine whether high-density lipoprotein cholesterol (HDL-C) mediates the association between DN and atherosclerosis.**

Methods: **Genome-Wide Association Study (GWAS) summary statistics for DN, atherosclerosis, and lipid-related traits were obtained from the IEU Open GWAS Project. Two-sample MR analyses were conducted using DN as the exposure and atherosclerosis, HDL-C, low-density lipoprotein cholesterol (LDL-C), apolipoprotein A (ApoA), and apolipoprotein B (ApoB) as outcomes. Causal effects were evaluated using five complementary MR methods, including inverse variance weighted (IVW), MR-Egger, weighted median, MR-robust adjusted profile score (RAPS), and MR-PRESSO. Sensitivity analyses were performed using Cochran's Q test and the MR-Egger intercept test. Mediation MR analysis was subsequently conducted using HDL-C as the mediator, with indirect effects estimated using the product-of-coefficients method.**

Results: **A total of 28 DN-associated single-nucleotide polymorphisms (SNPs) were selected as instrumental variables. MR analyses demonstrated that genetically predicted DN was causally associated with an increased risk of atherosclerosis. The IVW, weighted median, MR-RAPS, and MR-PRESSO methods yielded p-values of 0.0185, 0.0306, 0.0175, and 0.0185, respectively, with corresponding odds ratios (ORs) [95% confidence intervals (CIs)] of 1.0447 (1.0116 - 1.0788), 1.0500 (CI: 1.0063 - 1.0955), 1.0494 (1.0159 - 1.0839), and 1.0449 (1.0138 - 1.077). Further analyses revealed a significant association between DN and reduced HDL-C levels. The IVW, MR-RAPS, and MR-PRESSO methods produced p-values of 0.0020, 0.0046, and 0.0046, respectively, with betas of -0.0065 (-0.0106, -0.0024), -0.0061 (-0.0104, -0.0019), and -0.0054 (-0.0088, -0.0021). Mediation MR analysis suggests HDL-C may partially account for the association between DN and atherosclerosis. The estimated indirect effect was β = 0.0018 (95% CI: 0.0004 - 0.0039), corresponding to a mediated proportion of 4.22% (95% CI: 0.92% - 8.94%), with an overall mediation p-value of 0.0058.**

Conclusions: **This MR study provides evidence supporting a potential causal association between DN and an increased risk of atherosclerosis. Mediation MR analysis further suggests HDL-C may partially mediate this association, although this finding requires cautious interpretation and further validation. These findings indicate that dysregulated lipid metabolism, particularly reduced HDL-C levels, may be involved in the development of vascular complications in patients with DN.**

(Clin. Lab. 2027;73:xx-xx. DOI: 10.7754/Clin.Lab.2026.260610)

Correspondence:
Danhua Wang
Department of Endocrinology
Pan'an People's Hospital
Jinhua, 322399, Zhejiang
China
Phone: + 86 57984652423
Email: wangdanhuapa@outlook.com

Manuscript accepted July 9, 2026

KEYWORDS

diabetic nephropathy, atherosclerosis, HDL-C, genome-wide association study, Mendelian randomization

LIST OF ABBREVIATIONS

DN - Diabetic nephropathy
HDL-C - High-density lipoprotein cholesterol
GWAS - Genome-Wide Association Study
LDL-C - Low-density lipoprotein cholesterol
ApoA - Apolipoprotein A
IVW - Inverse variance weighted
SNPs - Single-nucleotide polymorphisms
OR - Odds ratio
MR - Mendelian randomization
CI - Confidence interval
SE - Standard error
RAPS - Robust Adjusted Profile Score
IEU - Integrative Epidemiology Unit (OpenGWAS)
MR-PRESSO - Mendelian Randomization Pleiotropy RESidual Sum and Outlier

INTRODUCTION

The global prevalence of diabetes mellitus has increased substantially over recent decades, making it one of the leading chronic metabolic disorders and a major public health challenge worldwide [1-3]. Beyond persistent hyperglycemia and metabolic dysregulation, diabetes is frequently accompanied by a range of chronic complications, including diabetic nephropathy (DN), diabetic peripheral neuropathy, and diabetic foot disease. Among these, DN is one of the most common and severe microvascular complications and remains a leading cause of end-stage kidney disease worldwide [4-6]. As the disease progresses, DN not only accelerates renal function decline but also markedly increases the risks of cardiovascular events and all-cause mortality.

Accumulating evidence indicates that patients with DN experience a substantially greater burden of cardiovascular disease than both the general population and individuals with diabetes without nephropathy. The risks of heart failure, hypertension, and atherosclerosis are consistently elevated in this population [7-10]. Clinical studies have further demonstrated that DN is associated with endothelial dysfunction, increased arterial stiffness, and greater carotid intima–media thickness, all of which are established markers of atherosclerotic vascular injury [11,12]. These observations suggest that renal impairment and vascular disease may share common pathophysiological mechanisms. Nevertheless, the boilogical pathways through which DN contributes to the development of atherosclerosis remain incompletely understood.

Several mechanisms have been proposed to explain the increased vascular risk associated with DN, including chronic inflammation, oxidative stress, endothelial dysfunction, and abnormalities in lipid metabolism. For example, Dimas et al. reported that matrix metalloproteinases are involved not only in the progression of renal fibrosis but also in vascular remodeling and plaque instability [12]. El-Asrar and colleagues observed that elevated levels of fatty acid-binding proteins were associated with increased carotid intima–media thickness, suggesting a potential role in the development of atherosclerosis among patients with DN [13]. In addition, clinical studies have shown that improving lipid metabolic profiles may reduce the risk of atherosclerotic complications in individuals with DN [14]. Collectively, these findings support the hypothesis that dysregulated lipid metabolism may represent a key biological link between DN and atherosclerosis.

Among lipid-related biomarkers, high-density lipoprotein cholesterol (HDL-C) has attracted particular attention because of its anti-inflammatory, antioxidant, and vasculoprotective properties. HDL-C plays a central role in reverse cholesterol transport and contributes to the maintenance of endothelial homeostasis. Previous studies have shown that patients with DN frequently exhibit reduced HDL-C levels and impaired HDL functionality, both of which have been implicated in endothelial injury, foam cell formation, and the progression of atherosclerosis [13,14]. These observations raise the possibility that HDL-C may act as an intermediate factor linking DN to atherosclerotic disease.

Despite growing evidence supporting an association between DN and atherosclerosis, most existing studies are observational in nature or focused on mechanistic exploration, making them susceptible to residual confounding and reverse causation. Consequently, whether DN exerts a causal effect on atherosclerosis and whether lipid abnormalities, particularly reduced HDL-C levels, mediate this relationship remain uncertain. Genetic studies have demonstrated that susceptibility to DN is influenced by inherited factors [15-17]. Mendelian randomization (MR), which uses genetic variants as instrumental variables, provides a powerful approach for causal inference by minimizing confounding and reverse causation bias [18-20]. This methodology has been increasingly applied in studies of metabolic and cardiovascular diseases.

In the present study, we performed a two-sample MR analysis to investigate the potential causal associations between DN, atherosclerosis, and four lipid-related traits. We further conducted mediation MR analyses to evaluate whether HDL-C mediates the relationship between DN and atherosclerosis. By leveraging large-scale Genome-Wide Association Study (GWAS) data, this study aimed to provide genetic evidence for the role of lipid metabolism in DN-related vascular disease and to offer insights into cardiovascular risk management in patients with DN.

MATERIALS AND METHODS

Study design

A two-sample Mendelian randomization (MR) framework was employed to investigate the potential causal relationship between diabetic nephropathy (DN) and atherosclerosis and to further evaluate whether lipid-related traits mediate this association. MR analysis relies on three fundamental assumptions: 1) instrumental variables (IVs) are robustly associated with exposure; 2) IVs are independent of potential confounding factors; and 3) IVs influence the outcome exclusively through exposure rather than through alternative biological pathways (Figure 1). Based on these assumptions, the study was conducted in three sequential stages. First, the causal effect of DN on atherosclerosis was evaluated. Second, the associations between DN and lipid-related traits were assessed. Finally, mediation MR analyses were performed to determine whether high-density lipoprotein cholesterol (HDL-C) mediates the relationship between DN and atherosclerosis.

Data sources

All Genome-Wide Association Study (GWAS) summary statistics used in this study were obtained from the IEU Open GWAS Project database (https://gwas.mrcieu.ac.uk/) [21,22]. To minimize potential bias arising from population stratification, all datasets were restricted to individuals of European ancestry. GWAS summary statistics for DN were identified using the keyword "diabetic nephropathy." The dataset with GWAS ID ebi-a-GCST90018832 was selected for analysis [23]. This dataset included 452,280 European participants, comprising 1,032 DN cases and 451,248 controls, and contained information on 24,190,738 single-nucleotide polymorphisms (SNPs). GWAS summary statistics for atherosclerosis were retrieved using the keyword "atherosclerosis." The dataset with GWAS ID finn-b-I9_CORATHER was included as the outcome dataset [24,25]. This dataset comprised 211,203 European individuals, including 23,363 cases and 187,840 controls, with genotype information for 16,380,426 SNPs.

To further investigate the relationship between DN and lipid metabolism, four lipid-related traits were included: high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), apolipoprotein A (ApoA), and apolipoprotein B (ApoB). The HDL-C dataset (GWAS ID: ebi-a-GCST90014007) included 357,810 European participants and 10,783,660 SNPs [26]. The LDL-C dataset (GWAS ID: ebi-a-GCST90002412) comprised 431,167 participants and 16,293,344 SNPs [27]. GWAS summary statistics for ApoA (GWAS ID: ukb-d-30630_irnt) and ApoB (GWAS ID: ukb-d-30640_irnt) were derived from the UK Biobank [28-30] and contained 13,585,234 and 13,585,958 SNPs, respectively.

Selection of instrumental variables

Instrumental variables (IVs) were selected according to established MR principles. Single-nucleotide polymorphisms (SNPs) significantly associated with diabetic nephropathy (DN) were initially identified as candidate IVs. Because the conventional genome-wide significance threshold ($p < 5 \times 10^{-8}$) yielded a limited number of eligible SNPs, a less stringent threshold ($p < 1 \times 10^{-5}$) was adopted based on previous MR studies [31] to increase the number of available instruments and improve the stability of subsequent analyses. To evaluate the robustness of this instrument-selection strategy, we additionally assessed the number and characteristics of eligible SNPs across a series of significance thresholds. The results are summarized in Supplementary Table 1. Because only one SNP met the conventional genome-wide significance threshold ($p < 5 \times 10^{-8}$), a standard multi-instrument MR analysis could not be performed under this criterion. To ensure the independence of selected genetic instruments, linkage disequilibrium (LD) clumping was performed using PLINK with an LD threshold of $r^2 < 0.001$ and a clumping window of 10,000 kb. This procedure minimized potential bias arising from correlated variants. In addition, F-statistics were calculated for all selected SNPs to evaluate instrument strength. SNPs with F-statistics < 10 were excluded to reduce the risk of weak instrument bias and ensure adequate explanatory power of the retained instruments. In addition, when evidence of horizontal pleiotropy was detected, outlier SNPs identified by the MR-PRESSO outlier test were excluded, and the corresponding MR analyses were repeated using the refined instrumental variable set.

Mendelian randomization analysis

Two-sample MR analyses were conducted to evaluate the causal effects of DN on atherosclerosis and lipid-related traits. Five complementary MR methods were applied, including inverse variance weighted (IVW), MR-Egger, MR-robust adjusted profile score (RAPS), weighted median, and MR-PRESSO. The IVW method was considered the primary analytical approach for estimating overall causal effects. The weighted median method was used to assess the robustness of causal estimates, whereas MR-Egger regression was employed to detect and account for potential horizontal pleiotropy. MR-RAPS was additionally applied to provide robust causal estimates in the presence of weak instruments and potential participant overlap between the exposure and outcome GWAS datasets. MR-PRESSO was applied to identify and correct for the influence of outlier variants that might distort causal estimates. To further evaluate the validity of the MR findings, heterogeneity among instrumental variables was assessed using Cochran's Q statistic. For analyses showing significant heterogeneity, the multiplicative random-effects inverse variance weighted model (IVW-mre) was used instead of the fixed-effects IVW model to obtain more robust causal estimates. Horizontal pleiotropy was examined

using the MR-Egger intercept test. Steiger directionality tests were performed to evaluate the inferred causal direction between exposure and outcome. In addition, bidirectional MR analyses were conducted for DN and atherosclerosis as well as DN and HDL-C to further assess potential reverse causality. To account for multiple comparisons, p-values were adjusted using the Benjamini-Hochberg (BH) procedure. All statistical analyses were performed using R software (version 4.5.3), primarily through the TwoSampleMR package (version 0.7.9) and the MRPRESSO package (version 1.0). No prospective analysis protocol was preregistered, because this study was based on a secondary analysis of publicly available GWAS summary statistics.

Mediation Mendelian randomization analysis

Based on the primary MR findings, significant associations were observed between DN and HDL-C as well as between DN and atherosclerosis. Therefore, HDL-C was further evaluated as a potential mediator of the relationship between DN and atherosclerosis. Mediation MR analyses were conducted using DN as the exposure, HDL-C as the mediator, and atherosclerosis as the outcome. Following previously established approaches [32], the indirect effect was estimated using the product-of-coefficients method, while standard errors (SEs) and 95% confidence intervals (CIs) were calculated using the Delta method. The mediation effect was quantified by the indirect-effect β estimate, the proportion mediated, and the corresponding p-value. Given that mediation MR analyses may still be influenced by residual pleiotropy and instrument-selection characteristics, the mediation results should be interpreted primarily as statistical evidence supporting a potential mediating pathway. Further experimental and clinical studies are required to validate the underlying biological mechanisms.

Ethics statement

All GWAS summary statistics used in this study were obtained from publicly available databases. Ethical approval and informed consent had been obtained in the original studies by their respective investigators. As the present study was based exclusively on de-identified, publicly accessible summary-level data and did not involve individual participant data, no additional ethical approval or informed consent was required.

RESULTS

Mendelian Randomization analysis of diabetic nephropathy and atherosclerosis risk

Using the predefined instrumental variable selection criteria, 28 SNPs significantly associated with diabetic nephropathy (DN) were identified from the GWAS dataset ebi-a-GCST90018832.

After linkage disequilibrium clumping ($r^2 < 0.001$, window size = 10,000 kb), all retained SNPs exhibited F-statistics greater than 10, indicating sufficient instrument strength and minimizing the likelihood of weak instrument bias. Because evidence of horizontal pleiotropy was detected during subsequent analyses, the MR-PRESSO outlier test was performed. Five outlier SNPs (rs12661002, rs2237897, rs28564801, rs35978445, and rs76441909) were identified and excluded, leaving 23 SNPs for the final analyses. All subsequent MR analyses were repeated using the refined instrumental variable set. Following harmonization with the atherosclerosis GWAS dataset (finn-b-I9_CORATHER), 18 DN-associated SNPs were initially matched. After excluding one palindromic SNP with ambiguous allele frequency (rs11629844) during harmonization, 17 SNPs were retained for subsequent MR analyses (Table 1, Figure 2).

The causal association between DN and atherosclerosis was evaluated using five MR methods (Table 1). Significant associations were observed using the inverse variance weighted (IVW), weighted median, MR-RAPS, and MR-PRESSO approaches. The corresponding p-values were 0.0185, 0.0306, 0.0175, and 0.0185, respectively, with odds ratios (ORs) of 1.0447 (95% CI: 1.0116 - 1.0788), 1.0500 (95% CI: 1.0063 - 1.0955), 1.0494 (95% CI: 1.0159 - 1.0839), and 1.0449 (95% CI: 1.0138 - 1.077). Sensitivity analyses revealed evidence of heterogeneity among instrumental variables, with Cochran's Q test p-values of 0.3443 and 0.2844 for the MR-Egger and IVW methods, respectively. However, the MR-Egger intercept test did not indicate significant horizontal pleiotropy ($p = 0.1866$) (Table 2, Figure 2). Steiger directionality analysis further supported the inferred causal direction from DN to atherosclerosis ($p < 0.05$). Reverse MR analysis did not identify a significant causal effect of atherosclerosis on DN (all $p > 0.05$; Supplementary Table 2). Collectively, these findings support a potential causal association between genetically predicted DN and an increased risk of atherosclerosis.

Mendelian randomization analysis of diabetic nephropathy and lipid-related traits

To further investigate the relationship between DN and lipid metabolism, MR analyses were performed for four lipid-related traits, including HDL-C, LDL-C, ApoA, and ApoB.

For HDL-C, 22 DN-associated SNPs were initially harmonized with the outcome dataset. After quality-control procedures, including harmonization, six SNPs (rs1162 9844, rs116739291, rs12187498, rs55853916, rs9111 56, and rs9623240) were excluded because they were palindromic SNPs with ambiguous allele frequencies or failed harmonization, leaving 16 SNPs for analysis (Table 3, Figure 3). The IVW, MR-RAPS, and MR-PRESSO methods consistently demonstrated significant associations between genetically predicted DN and lower HDL-C levels. The corresponding p-values were 0.0020, 0.0046, and 0.0046, respectively, with betas of -0.0065 (95% CI: -0.0106, -0.0024), -0.0061 (95% CI: -0.0104, -0.0019), and -0.0054 (95% CI: -0.0088, -

Table 1. Mendelian randomization analysis of the development of atherosclerosis in diabetic nephropathy.

MR info							
Outcomes	**SNP (n)**	**Method**	**Beta**	**SE**	**OR [95% CI]**	**p-value**	**p-adjusted**
Coronary atherosclerosis	**17**	**MR Egger**	**0.0024**	**0.0338**	**1.0024 [0.9382 - 1.0711]**	**0.9438**	**0.9438**
		IVW	**0.0437**	**0.0164**	**1.0447 [1.0116 - 1.0788]**	**0.0077**	**0.0185**
		Weighted median	**0.0487**	**0.0217**	**1.0500 [1.0063 - 1.0955]**	**0.0245**	**0.0306**
		MR-RAPS	**0.0482**	**0.0165**	**1.0494 [1.0159 - 1.0839]**	**0.0035**	**0.0175**
		MR-PRESSO	**0.0439**	**0.0154**	**1.0449 [1.0138 - 1.077]**	**0.0111**	**0.0185**

Table 2. The Cochran's Q and horizontal pleiotropy result of the occurrence of four cholesterol indicators in diabetic nephropathy.

Exposure	**Method**	**Cochran's Q**			**Horizontal pleiotropy**		
		Q	**Q _df**	**Q_pval**	**Intercept**	**SE**	**p**
Coronary atherosclerosis	**MR Egger**	**16.5845**	**15**	**0.3443**	**0.0150**	**0.0109**	**0.1866**
	IVW	**18.7027**	**16**	**0.2844**	**-**	**-**	**-**
HDL-C	**MR Egger**	**18.7106**	**14**	**0.1763**	**-0.0026**	**0.0016**	**0.1164**
	IVW	**22.4546**	**15**	**0.0964**	**-**	**-**	**-**
LDL-C	**MR Egger**	**31.9155**	**20**	**0.0442**	**0.0003**	**0.0012**	**0.8309**
	IVW-mre	**31.9902**	**21**	**0.0587**	**-**	**-**	**-**
Apoliprotein A	**MR Egger**	**26.43480**	**19**	**0.1185**	**-0.0011**	**0.0014**	**0.4625**
	IVW	**27.2173**	**20**	**0.1292**	**-**	**-**	**-**
Apoliprotein B	**MR Egger**	**36.3933**	**19**	**0.0094**	**0.0011**	**0.0017**	**0.5051**
	IVW-mre	**37.2772**	**20**	**0.0108**	**-**	**-**	**-**

0.0021) (Table 3, Figure 4A). Cochran's Q tests indicated no heterogeneity (MR-Egger: $p = 0.1763$; IVW: $p = 0.0964$). In addition, the MR-Egger intercept test suggested the presence of horizontal pleiotropy ($p = 0.1164$) (Table 2). Steiger directionality analysis supported the inferred causal direction from DN to HDL-C ($p < 0.05$). Reverse MR analysis showed no significant causal association between HDL-C and DN, although heterogeneity and horizontal pleiotropy were observed among the selected instrumental variables (Supplementary Table 2).

For ApoA, 21 DN-associated SNPs were successfully harmonized and retained after quality control (Table 3, Figure 3). Because Cochran's Q tests indicated heterogeneity, the IVW-mre model was applied instead of the fixed-effect IVW model (Table 2). A nominal association between DN and reduced ApoA levels was observed using the IVW method ($p = 0.0219$; beta = -0.0045, 95% CI: -0.0084, -0.0007), whereas no significant associations were detected using the remaining MR approaches (Table 2, Figure 4C). Cochran's Q tests indicated heterogeneity, while the MR-Egger intercept test showed no evidence of horizontal pleiotropy ($p = 0.4625$).

For LDL-C and ApoB, 22 and 21 DN-associated SNPs were harmonized with the corresponding outcome datasets, respectively. No SNPs were excluded during quality control (Table 3, Figure 3). Because heterogeneity was detected by Cochran's Q tests, the IVW-mre model was used for causal estimation (Table 2). Across all five MR methods, no significant associations were identified between DN and either LDL-C or ApoB (all $p > 0.05$) (Table 3, Figure 4B and 4D). The horizontal pleiotropy tests showed no evidence of horizontal pleiotropy for LDL-C ($p = 0.8309$) or ApoB ($p = 0.5051$, Table 2). Overall, the most consistent evidence was observed for an association between genetically predicted DN and reduced HDL-C levels, whereas associations with the oth-

Table 3. Mendelian randomization analysis of the occurrence of four cholesterol indicators in diabetic nephropathy.

MR info							
Outcomes	**SNP (n)**	**Method**	**Beta**	**SE**	**Beta [95% CI]**	**p-value**	**p-adjusted**
HDL-C	**16**	**MR Egger**	**-0.0023**	**0.0032**	**-0.0023 [-0.0085, 0.004]**	**0.4908**	**0.9816**
		IVW	**-0.0065**	**0.0021**	**-0.0065 [-0.0106, -0.0024]**	**0.0020**	**0.0306**
		Weighted median	**-0.0036**	**0.0027**	**-0.0036 [-0.0089, 0.0018]**	**0.1959**	**0.4897**
		MR-RAPS	**-0.0061**	**0.0022**	**-0.0061 [-0.0104, -0.0019]**	**0.0046**	**0.0306**
		MR-PRESSO	**-0.0054**	**0.0017**	**-0.0054 [-0.0088, -0.0021]**	**0.0046**	**0.0306**
LDL-C	**22**	**MR Egger**	**-0.0004**	**0.0027**	**-4e-04 [-0.0056, 0.0048]**	**0.8807**	**0.9864**
		IVW-mre	**2.97e-05**	**0.0017**	**2.97e-05 [-0.0033, 0.0034]**	**0.9862**	**0.9864**
		Weighted median	**9.93e-05**	**0.0021**	**9.93e-05 [-0.004, 0.0042]**	**0.9624**	**0.9864**
		MR-RAPS	**-0.0004**	**0.0017**	**-0.0004 [-0.0038, 0.003]**	**0.8114**	**0.9864**
		MR-PRESSO	**2.97e-05**	**0.0017**	**2.97e-05 [-0.0033, 0.0034]**	**0.9864**	**0.9864**
Apoliprotein A	**21**	**MR Egger**	**-0.0029**	**0.0030**	**-0.0029 [-0.0088, 0.0031]**	**0.3558**	**0.7906**
		IVW	**-0.0045**	**0.0020**	**-0.0045 [-0.0084, -0.0007]**	**0.0219**	**0.1095**
		Weighted median	**-0.0046**	**0.0026**	**-0.0046 [-0.0097, 0.0004]**	**0.0759**	**0.2530**
		MR-RAPS	**-0.0048**	**0.0020**	**-0.0048 [-0.0087, -0.001]**	**0.1373**	**0.3923**
		MR-PRESSO	**-0.0045**	**0.0020**	**-0.0045 [-0.0084, -0.0007]**	**0.0616**	**0.2464**
Apoliprotein B	**21**	**MR Egger**	**-0.0004**	**0.0027**	**-0.0004 [-0.0056, 0.0048]**	**0.8807**	**0.9864**
		IVW-mre	**2.90e-05**	**0.0017**	**2.90e-05 [-0.0033, 0.0034]**	**0.9862**	**0.9864**
		Weighted median	**0.0001**	**0.0021**	**0.0001 [-0.0040, 0.0042]**	**0.9624**	**0.9864**
		MR-RAPS	**-0.0004**	**0.0017**	**-0.0004 [-0.0038, 0.003]**	**0.8114**	**0.9864**
		MR-PRESSO	**2.97e-05**	**0.0017**	**2.97e-05 [-0.0033, 0.0034]**	**0.9864**	**0.9864**

er lipid-related traits were limited.

Mediation Mendelian randomization analysis of HDL-C in the association between diabetic nephropathy and atherosclerosis

Given the observed associations of DN with both reduced HDL-C levels and increased atherosclerosis risk, mediation MR analyses were subsequently performed to evaluate whether HDL-C may mediate the relationship between DN and atherosclerosis. MR analysis demonstrated a significant inverse association between HDL-C and atherosclerosis risk. Using the IVW method, beta was -0.2845 and the estimated OR was 0.7523 (95% CI: 0.6869 - 0.8241; $p = 8.99e^{-10}$), indicating that higher HDL-C levels were associated with a lower risk of atherosclerosis. Subsequent mediation MR analysis showed that HDL-C accounted for part of the association between DN and atherosclerosis. The estimated indirect effect (β) [95% CI] was 0.0018 [0.0004, 0.0039], corresponding to a proportion mediated of 4.22 [0.92, 8.94] %, with an overall mediation p-value of 0.0058 (Table 4, Figure 5). These findings suggest the possibil-

Table 4. Mediated Mendelian randomization analysis of the impact of diabetic nephropathy on HDL-mediated atherosclerosis.

Mediator	Exposure	Outcome	SNPs	Beta	SE	p-value	Beta of mediation effect [95% CI]	Proportion of mediation effect [95% CI]	p-value
HDL	diabetic nephropathy	HDL	16	-0.0065	0.0021	0.0020	0.0018 [0.0004, 0.0039]	4.22 [0.92, 8.94] %	0.0058
	HDL	coronary atherosclerosis	294	-0.2845	0.0464	8.99e-10			
	diabetic nephropathy	coronary atherosclerosis	23	0.0437	0.0164	0.0077			

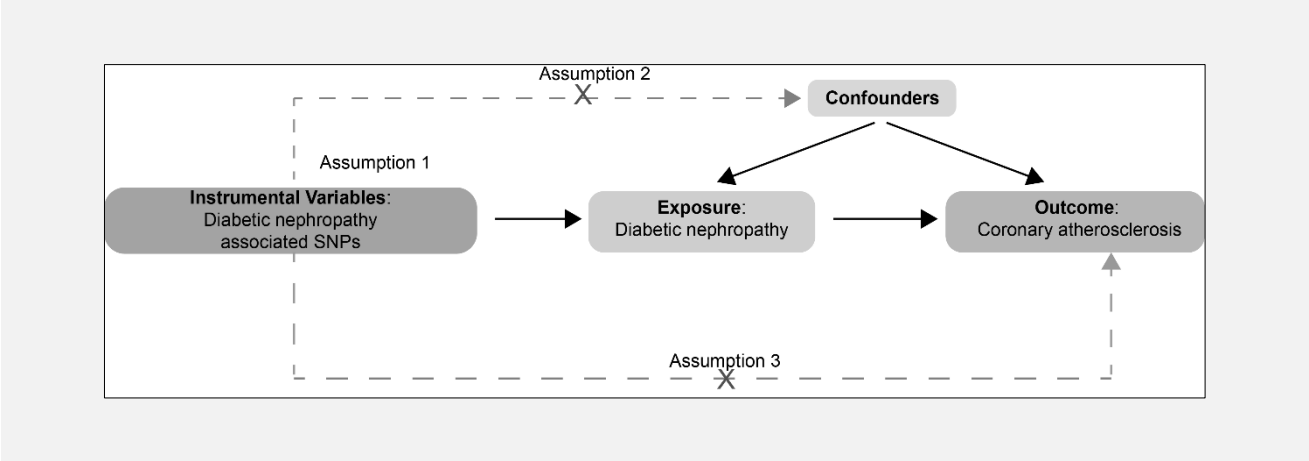

Figure 1. Three main assumptions regarding Mendelian randomization analysis of diabetic nephropathy and atherosclerosis.

ity that HDL-C may partially mediate the association between DN and atherosclerosis. However, given the evidence of heterogeneity and potential horizontal pleiotropy observed in the HDL-C analyses, this potential mediation effect should be interpreted with caution and requires further validation.

DISCUSSION

By using large-scale GWAS datasets and a two-sample Mendelian randomization (MR) framework, this study systematically evaluated the potential causal relationship between diabetic nephropathy (DN) and atherosclerosis. The results indicate that genetically predicted DN is associated with an increased risk of atherosclerosis. Among the lipid-related traits examined, DN showed the most consistent association with reduced high-density lipoprotein cholesterol (HDL-C) levels. Furthermore, mediation MR analysis suggests that HDL-C may partially account for the association between DN and atherosclerosis. Collectively, these findings provide genetic evidence supporting a link between DN and cardiovascular injury and raise the possibility that lipid metabolic dysregulation, particularly reduced HDL-C levels, may be involved in the development of DN-related vascular disease.

Previous clinical and epidemiological studies have consistently reported an increased burden of cardiovascular disease among patients with DN. Feingold et al. [33] highlighted that dyslipidemia remains highly prevalent in patients with type 2 diabetes mellitus, even in the setting of adequate glycemic control, and is commonly characterized by elevated triglycerides, increased non-HDL cholesterol, reduced HDL-C levels, and a predominance of small dense LDL particles. In addition, DN has been associated with increased arterial stiffness, greater carotid intima-media thickness, and a higher prevalence of atherosclerotic cardiovascular disease [34-36]. However, most of the available evidence originates from observational studies and may be influenced by residual confounding factors, including age, obesity, blood pressure, medication use, and lifestyle characteristics. Consequently, whether DN directly contributes to atherosclerotic disease progression has remained uncertain.

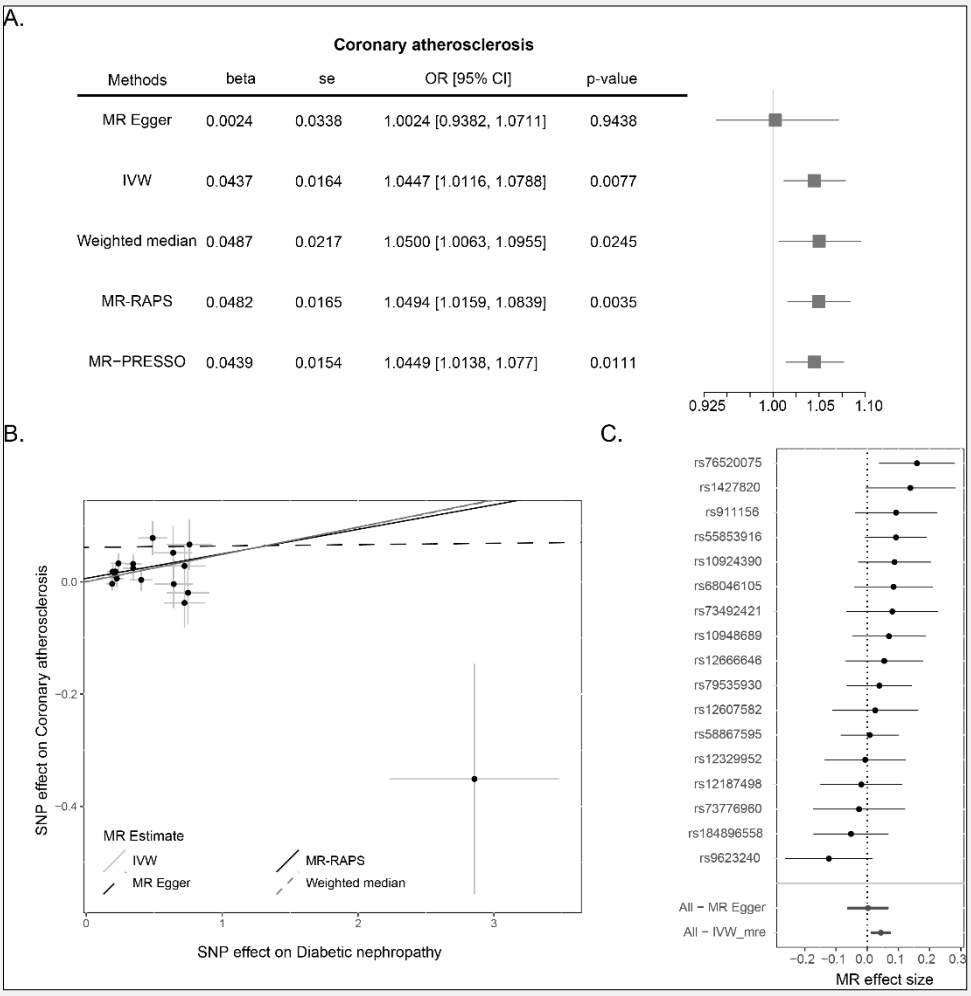

Figure 2. Mendelian randomization analysis results of diabetic nephropathy on the development of atherosclerosis.

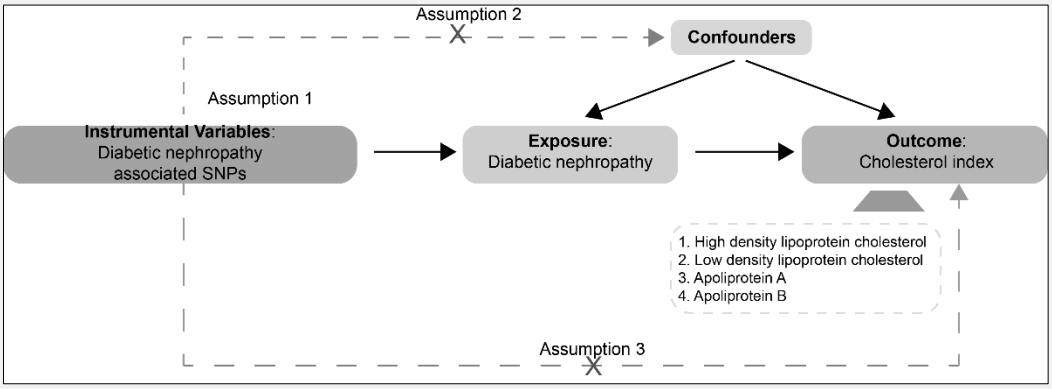

Figure 3. Three main assumptions regarding Mendelian randomization analysis of diabetic nephropathy and four cholesterol indicators.

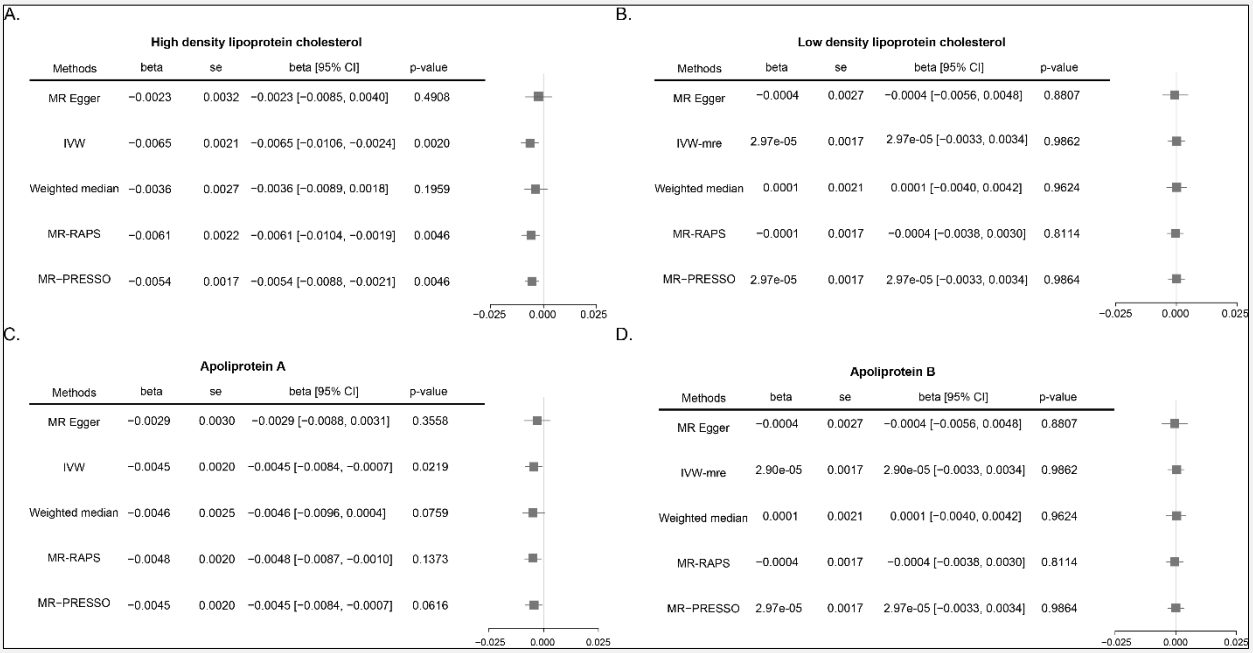

Figure 4. Results of Mendelian randomization analysis of four cholesterol indicators in diabetic nephropathy.

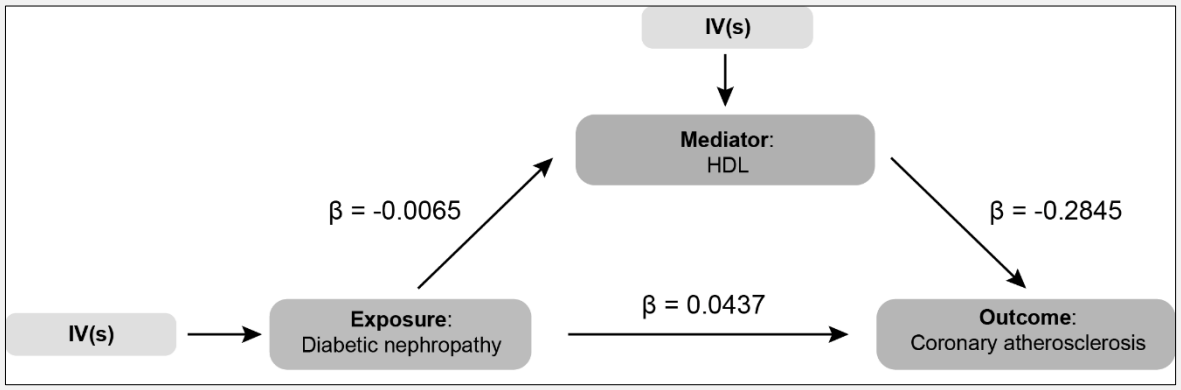

Figure 5. Mediating Mendelian randomization analysis hypothesis that diabetic nephropathy affects HDL-mediated atherosclerosis.

In the present study, MR analysis revealed a significant association between genetically predicted DN and an increased risk of atherosclerosis. This finding suggests that DN may represent more than a manifestation of renal microvascular injury and may also participate in systemic vascular pathology. The inferred causal direction is further supported by directionality testing, and reverse MR analyses did not provide evidence for reverse causation. The observed association is broadly consistent with previous clinical observations demon-

strating a high cardiovascular risk profile among patients with DN and further supports the hypothesis that renal dysfunction and vascular disease share common pathophysiological pathways.

A notable finding of this study was the relatively consistent association between DN and reduced HDL-C levels. In contrast, no robust associations were observed for LDL-C or apolipoprotein B. This pattern suggests that alterations in HDL-C may play a more prominent role in DN-related vascular complications than traditional atherogenic lipoprotein markers. HDL exerts multiple vasculoprotective effects beyond reverse cholesterol transport, including anti-inflammatory, antioxidant, endothelial-protective, and anti-atherogenic functions [37]. Therefore, reductions in HDL-C levels or impairments in HDL functionality may exacerbate endothelial injury and chronic inflammation, thereby facilitating atherosclerotic progression in patients with DN. Several previous studies support the potential involvement of HDL-C in DN-associated vascular disease. Gao et al. [38] reported significantly higher lipoprotein(a) concentrations and lower HDL-C levels in patients with type 2 diabetes and DN compared with diabetic patients without nephropathy. Similarly, a randomized controlled trial conducted by Elbarbary and colleagues demonstrated that six months of omega-3 fatty acid supplementation in children with type 1 diabetes and DN significantly improved HDL-C levels while simultaneously reducing multiple metabolic and vascular risk markers, including triglycerides, LDL cholesterol, urinary albumin-to-creatinine ratio, kidney injury molecule-1, and carotid intima-media thickness [14]. Although these studies were observational or interventional in nature and cannot directly establish causality, their findings are broadly consistent with the genetic associations observed in the present study.

An additional contribution of this work is the mediation MR analysis, which suggests that HDL-C may partially account for the association between DN and atherosclerosis. The estimated mediated proportion was approximately 4.22%, indicating that disturbances in lipid metabolism may represent one biological pathway through which DN contributes to vascular injury. At the same time, the relatively limited mediation proportion suggests that additional mechanisms are likely involved. Chronic inflammation, oxidative stress, endothelial dysfunction, advanced glycation end-product accumulation, and activation of the renin-angiotensin-aldosterone system have all been implicated in the pathogenesis of both DN and atherosclerosis and may jointly contribute to disease progression. Further mechanistic studies are therefore warranted to clarify the relative contributions of these pathways.

This study possesses several strengths. By leveraging large-scale GWAS datasets and genetically determined instrumental variables, MR analysis can reduce bias arising from residual confounding and reverse causation, limitations that commonly affect conventional observational studies. Moreover, the incorporation of mediation MR analysis together with directionality and reverse MR analyses enabled a more comprehensive evaluation of the potential relationship linking DN, HDL-C, and atherosclerosis.

Several limitations should also be acknowledged. First, although a range of sensitivity analyses was performed to improve the robustness of the findings, the use of a relaxed instrumental variable selection threshold ($p < 1 \times 10^{-5}$) may have increased the possibility of invalid instruments being included. Second, although horizontal pleiotropy in the HDL-C analysis was substantially reduced after outlier removal, residual pleiotropic effects cannot be completely excluded.

Therefore, the observed mediation effect of HDL-C should be interpreted with caution. Third, all GWAS datasets were derived from individuals of European ancestry, which may limit the generalizability of the findings to other populations. Finally, this study provides genetic epidemiological evidence based on publicly available GWAS summary statistics, and the findings require further validation in independent prospective cohorts and experimental studies.

CONCLUSION

In conclusion, this study provides genetic evidence supporting a potential association between DN and an increased risk of atherosclerosis. Mediation MR analysis suggests that HDL-C may partially mediate this association, although this finding requires further validation. These results indicate that, in addition to monitoring renal function decline, greater attention should be paid to lipid metabolic abnormalities, particularly reductions in HDL-C levels, during the long-term management of patients with DN. Future studies integrating mechanistic experiments and prospective clinical investigations are needed to elucidate how DN influences vascular pathology through HDL-related pathways and to determine whether targeting these pathways may contribute to the prevention of cardiovascular complications in patients with DN.

Data Availability Statement:
The R scripts used for all statistical analyses are publicly available at https://github.com/1wangdanhua/MR_DN_Atherosclerosis/. All GWAS summary statistics analyzed in this study are publicly available from the IEU OpenGWAS Project under the accession IDs reported in the Methods section.

Declaration of Generative AI in Scientific Writing:
During the preparation of this manuscript, the authors used ChatGPT (OpenAI) to assist with language editing, improving the clarity and readability of the text. After using this tool, the authors reviewed, revised, and edited the content as necessary, and they take full re-

sponsibility for the scientific content, interpretation, and conclusions presented in the article.

Declaration of Interest:
The authors declare no conflicts of interest.

References:

1. Li C, Qi J, Yin P, et al. The burden of type 2 diabetes attributable to air pollution across China and its provinces, 1990-2021: an analysis for the Global Burden of Disease Study 2021. Lancet Reg Health West Pac 2024;53:101246. (PMID: 39655197)

2. American Diabetes Association Professional Practice Committee. 2. Diagnosis and Classification of Diabetes: Standards of Care in Diabetes-2025. Diabetes Care 2025;48:S27-s49. (PMID: 39651986)

3. Gloyn AL, Drucker DJ. Precision medicine in the management of type 2 diabetes. Lancet Diabetes Endocrinol 2018;6:891-900. (PMID: 29699867)

4. de Boer IH, Khunti K, Sadusky T, et al. Diabetes Management in Chronic Kidney Disease: A Consensus Report by the American Diabetes Association (ADA) and Kidney Disease: Improving Global Outcomes (KDIGO). Diabetes Care 2022;45:3075-90. (PMID: 36189689)

5. Armstrong DG, Tan TW, Boulton AJM, Bus SA. Diabetic Foot Ulcers: A Review. JAMA 2023;330:62-75. (PMID: 37395769)

6. Røikjer J, Mørch CD, Ejskjaer N. Diabetic Peripheral Neuropathy: Diagnosis and Treatment. Curr Drug Saf 2021;16:2-16. (PMID: 32735526)

7. Ng ACT, Delgado V, Borlaug BA, Bax JJ. Diabesity: the combined burden of obesity and diabetes on heart disease and the role of imaging. Nat Rev Cardiol 2021;18:291-304. (PMID: 33188304)

8. Lehrke M, Marx N. Diabetes Mellitus and Heart Failure. Am J Med 2017;130:S40-s50. (PMID: 28526183)

9. Cryer MJ, Horani T, DiPette DJ. Diabetes and Hypertension: A Comparative Review of Current Guidelines. J Clin Hypertens (Greenwich) 2016;18:95-100. (PMID: 26234374)

10. Poznyak A, Grechko AV, Poggio P, Myasoedova VA, Alfieri V, Orekhov AN. The Diabetes Mellitus-Atherosclerosis Connection: The Role of Lipid and Glucose Metabolism and Chronic Inflammation. Int J Mol Sci 2020;21(5):1835. (PMID: 32155866)

11. Sharma A, Choi J, Sim L, et al. Ameliorating diabetes-associated atherosclerosis and diabetic nephropathy through modulation of soluble guanylate cyclase. Front Cardiovasc Med 2023;10: 1220095. (PMID: 37502180)

12. Dimas GG, Didangelos TP, Grekas DM. Matrix Gelatinases in Atherosclerosis and Diabetic Nephropathy: Progress and Challenges. Curr Vasc Pharmacol 2017;15:557-65. (PMID: 28155628)

13. Abo El-Asrar M, Ismail EAR, Elnhrawy AM, Thabet RA. Fatty acid binding protein 1 (FABP1) and fatty acid binding protein 2 (FABP2) as a link between diabetic nephropathy and subclinical atherosclerosis in children and adolescents with type 1 diabetes. J Diabetes Complications 2023;37:108414. (PMID: 36758511)

14. Elbarbary NS, Ismail EAR, Mohamed SA. Omega-3 fatty acids supplementation improves early-stage diabetic nephropathy and subclinical atherosclerosis in pediatric patients with type 1 diabetes: A randomized controlled trial. Clin Nutr 2023;42:2372-80. (PMID: 37862823)

15. Naing C, Htet NH, Basavaraj AK, Nalliah S. An association between IL-10 promoter polymorphisms and diabetic nephropathy: a meta-analysis of case-control studies. J Diabetes Metab Disord 2018;17:333-43. (PMID: 30918869)

16. Mirabedini S, Musavi H, Makhlough A, Hashemi-Sooteh MB, Zargari M. Association of S19W polymorphism in APOA5 gene and serum lipid levels in patients with type 2 diabetic nephropathy. Horm Mol Biol Clin Investig 2023;44:243-9. (PMID: 36855913)

17. Sultan S, Alharbi M, Alrayes N, et al. Association of a single nucleotide polymorphism in SOD2 with susceptibility for the development of diabetic nephropathy in patients with type 2 diabetes: A Saudi population study. Endocrinol Diabetes Metab 2023; 6:e449. (PMID: 37698290)

18. Birney E. Mendelian Randomization. Cold Spring Harb Perspect Med 2022;12(4):a041302. (PMID: 34872952)

19. Davey Smith G, Hemani G. Mendelian randomization: genetic anchors for causal inference in epidemiological studies. Hum Mol Genet 2014;23:R89-98. (PMID: 25064373)

20. Nordestgaard AT. Causal relationship from coffee consumption to diseases and mortality: a review of observational and Mendelian randomization studies including cardiometabolic diseases, cancer, gallstones and other diseases. Eur J Nutr 2022;61:573-87. (PMID: 34319429)

21. Zheng H, Shi YZ, Liang JT, Lu LL, Chen M. Modifiable factors for migraine prophylaxis: A mendelian randomization analysis. Front Pharmacol 2023;14:1010996. (PMID: 36713835)

22. Hemani G, Zheng J, Elsworth B, et al. The MR-Base platform supports systematic causal inference across the human phenome. Elife 2018;7:e34408. (PMID: 29846171)

23. Sakaue S, Kanai M, Tanigawa Y, et al. A cross-population atlas of genetic associations for 220 human phenotypes. Nat Genet 2021;53:1415-24. (PMID: 34594039)

24. Uusimaa J, Kettunen J, Varilo T, et al. The Finnish genetic heritage in 2022 - from diagnosis to translational research. Dis Model Mech 2022;15(10):dmm049490. (PMID: 36285626)

25. Liu Z, Wang H, Yang Z, Lu Y, Zou C. Causal associations between type 1 diabetes mellitus and cardiovascular diseases: a Mendelian randomization study. Cardiovasc Diabetol 2023;22: 236. (PMID: 37659996)

26. Mbatchou J, Barnard L, Backman J, et al. Computationally efficient whole-genome regression for quantitative and binary traits. Nat Genet 2021;53:1097-103. (PMID: 34017140)

27. Klimentidis YC, Arora A, Newell M, et al. Phenotypic and Genetic Characterization of Lower LDL Cholesterol and Increased Type 2 Diabetes Risk in the UK Biobank. Diabetes 2020;69: 2194-205. (PMID: 32493714)

28. Rusk N. The UK Biobank. Nat Methods 2018;15:1001. (PMID: 30504882)

29. Conroy MC, Lacey B, Bešević J, et al. UK Biobank: a globally important resource for cancer research. Br J Cancer 2023;128: 519-27. (PMID: 36402876)

30. Fry A, Littlejohns TJ, Sudlow C, et al. Comparison of Sociodemographic and Health-Related Characteristics of UK Biobank Participants With Those of the General Population. Am J Epidemiol 2017;186:1026-34. (PMID: 28641372)

31. Ma J, Li J, Jin C, et al. Association of gut microbiome and primary liver cancer: A two-sample Mendelian randomization and case-control study. Liver Int 2023;43:221-33. (PMID: 36300678)

32. Chen L, Peters JE, Prins B, et al. Systematic Mendelian randomization using the human plasma proteome to discover potential therapeutic targets for stroke. Nat Commun 2022;13:6143. (PMID: 36253349)

33. Feingold KR. Role of Glucose and Lipids in the Atherosclerotic Cardiovascular Disease in Patients with Diabetes, in Endotext, K.R. Feingold, et al., Editors. 2000, MDText.com, Inc. Copyright © 2000-2026, MDText.com, Inc.: South Dartmouth (MA).

34. Bell DSH, McGill JB, Jerkins T. Management of the 'wicked' combination of heart failure and chronic kidney disease in the patient with diabetes. Diabetes Obes Metab 2023;25:2795-804. (PMID: 37409564)

35. Van Buren PN, Toto R. Hypertension in diabetic nephropathy: epidemiology, mechanisms, and management. Adv Chronic Kidney Dis 2011;18:28-41. (PMID: 21224028)

36. Menini S, Iacobini C, Fantauzzi CB, Pugliese G. L-carnosine and its Derivatives as New Therapeutic Agents for the Prevention and Treatment of Vascular Complications of Diabetes. Curr Med Chem 2020;27:1744-63. (PMID: 31296153)

37. Nagao M, Nakajima H, Toh R, Hirata KI, Ishida T. Cardioprotective Effects of High-Density Lipoprotein Beyond its Anti-Atherogenic Action. J Atheroscler Thromb 2018;25:985-93. (PMID: 30146614)

38. Gao RH, Liu B, Yang Y, Ran R, Zhou Y, Liu SM. Serum Lipoprotein(a) and High-Density Lipoprotein Cholesterol Associate with Diabetic Nephropathy: Evidence from Machine Learning Perspectives. Diabetes Metab Syndr Obes 2023;16:1847-58. (PMID: 37378072)

Additional material can be found online at:
http://supplementary.clin-lab-publications.com/260610/