

The Causal Effect of Serum Lipid Levels Mediated by Neuregulin 4 on the Risk of Four Atherosclerosis Subtypes: Evidence from Mendelian Randomization Analysis

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Background: Neuregulin 4 (NRG4) was known to be associated with serum lipid levels and atherosclerosis. However, it is unknown whether the role of NRG4 in lipid homeostasis is causal to atherosclerosis and whether the effect is beneficial across different atherosclerosis subtypes.

Methods: We investigated the causal role of the levels of serum low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol, and triglycerides regulated by NRG4 in subtypes of atherosclerosis through two sample Mendelian randomization. Aggregated genome-wide association study (GWAS) summary data for serum lipid level of 1.32 million individuals with European ancestry were obtained from the Global Lipids Genetics Consortium. GWAS summary data for four atherosclerosis subtypes (peripheral, coronary, cerebral and the other atherosclerosis) were obtained from FinnGen Consortium. Generalized inverse-variance-weighted Mendelian randomization and several sensitivity analyses were used to obtain the causal estimates.

Results: A 1-SD genetically elevated LDL-C level mediated by NRG4 was validated to be nominally associated with the risk of peripheral atherosclerosis (log (odds ratio)= 4.14, 95% confidence interval 0.11 to 8.17, P = 0.04), and the other associations were not significant or could not be validated by sensitivity analyses.

Conclusion: LDL-C lowering mediated by NRG4 is likely to prevent peripheral atherosclerosis.

Keywords: Mendelian randomization analysis, NRG4, atherosclerosis, lipid

Introduction

Atherosclerosis is a multifocal, smoldering, immunoinflammatory disease of the vascular intima, which is fueled by lipids.¹ It has always been a major cause of mortality in developed countries and has a global impact.² All the vascular system from aorta to coronary arteries can be involved in atherosclerosis. Genome-wide association studies (GWAS) in humans revealed that genetic loci responsible in different vascular locations were not overlapping, suggesting that distinct genetic mechanisms might be involved at different locations.^{3–6}

Lipid and lipoprotein biomarkers were proved to be associated with atherosclerosis by both observational studies and randomized controlled trials (RCTs).^{7,8} Nevertheless, the causal relationship with atherosclerosis for high-density lipoprotein cholesterol (HDL-C) and triglycerides (TG) seems to be less clear compared with low-density lipoprotein cholesterol (LDL-C), leading to a heated debate on the role of HDL-C and TG in cardiovascular disease.^{8–10}

Neuregulin 4 (NRG4) is a member of the secreted epidermal growth factor family, with the highest expression levels in brown adipose tissue (BAT) but found in very low abundance in other tissues such as skeletal muscle, liver, brain, heart and kidney.¹¹ As an endocrine factor released from BAT, NRG4 may link the activation of BAT to protection against diet-induced obesity, insulin resistance, and hepatic steatosis.¹² In addition, the functions of NRG4 in regulating atherosclerosis have been uncovered. NRG4 displays an anti-inflammatory role, which helps protect against atherosclerosis.¹³ Except for anti-inflammation, NRG4 might induce other cellular responses including glucose and lipid metabolism.¹⁴ NRG4 could activate ErbB3/4 signaling in hepatocytes and attenuates hepatic lipogenic signaling,¹¹ thereby preserving glucose and lipid homeostasis in obesity. Nevertheless, more evidence is needed to determine whether the role of NRG4 in lipid homeostasis is causal to atherosclerosis and whether the effect is beneficial across different atherosclerosis subtypes.

Mendelian randomization (MR) is an instrumental variable analysis to investigate the causal relationship between exposure and the outcome of interest in epidemiology. Recent GWAS have identified genetic variants that influence lipid metabolism, thus providing comprehensive data on the genetic determinants. Using genetic variants as instrumental variables for a trait, MR allows investigating the associations independent of the conventional biases in observational studies, including confounding, reverse causation and measurement errors.¹⁵

Given the connection between NRG4 and lipid metabolic disorder, it is interesting to explore the causal role of NRG4 in lipid homeostasis for different atherosclerosis subtypes and the potential diagnostic and therapeutic role of NRG4 for atherosclerosis patients with disordered lipid metabolism. In this study, we utilized large-scale and publicly available GWAS datasets to conduct a two-sample MR study, aiming to examine the causal relationship between lipid levels regulated by NRG4 and atherosclerosis subtypes.

Materials and Methods

Study Design

Figure 1 illustrates the design of this study. We aimed to evaluate the causal role of lipids mediated by NRG4 gene on atherosclerosis. The lipid-related exposures included HDL-C, LDL-C, and TG.

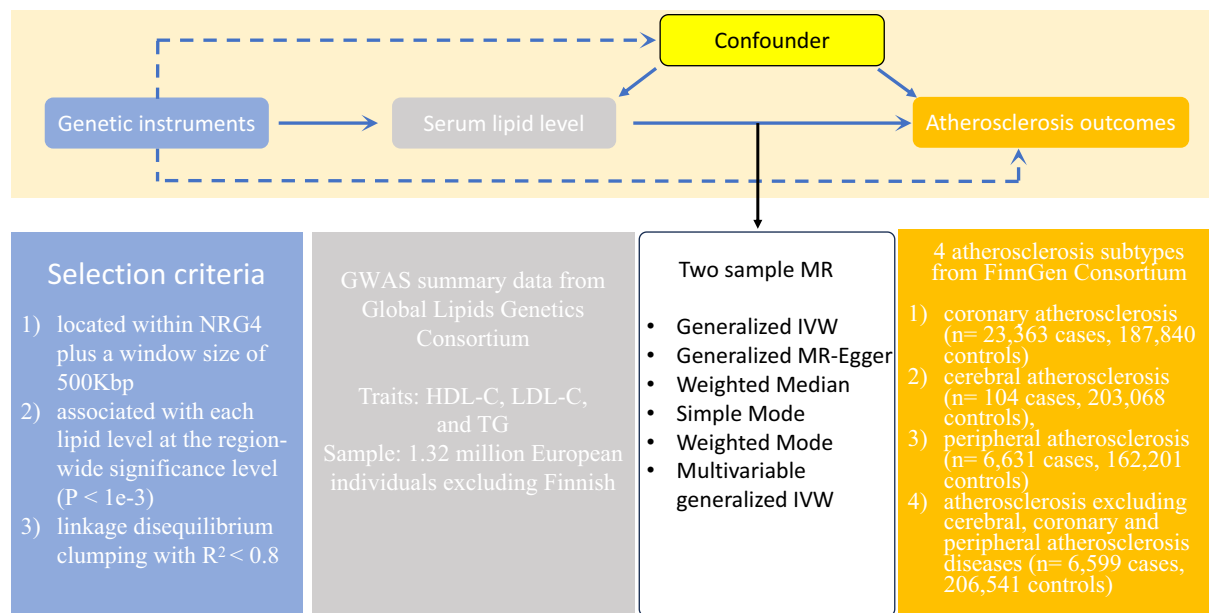


Figure 1 Study design.

Notes: We conducted two-sample MR analyses to answer whether the role of NRG4 in lipid homeostasis is causal to atherosclerosis and whether the effect is beneficial across four different atherosclerosis subtypes, including 1) coronary atherosclerosis, 2) cerebral atherosclerosis, 3) peripheral atherosclerosis, and 4) atherosclerosis excluding cerebral, coronary, and peripheral atherosclerosis diseases. Summary data of exposures and outcomes were obtained from related meta-analyses of GWAS. The generalized inverse variance-weighted approach was applied as the primary method to estimate the causal effect on selected outcomes. Several sensitivity analyses were conducted.

Abbreviations: IVW, inverse variance weighted; LD, linkage disequilibrium; SNP, single-nucleotide polymorphism; NRG4, Neuregulin 4; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides; GWAS, genome-wide association study; MR, Mendelian randomization.

Firstly, we selected four sets of genetic variants that were proxies of the effect of NRG4 on lipid levels. Four atherosclerosis outcomes were then selected. The generalized inverse variance-weighted (IVW) approach was the primary method. Sensitivity analyses, including generalized MR-Egger, weighted median, simple mode, and weighted mode method, were conducted for validation. Secondly, we tried to assess whether lipid exposure traits and atherosclerosis outcome traits were affected by the same or distinct causal variants using colocalization analysis.

Selection of Genetic Instruments

Aggregated GWAS summary data of 1.32 million individuals of European ancestry were conducted by the Global Lipids Genetics Consortium.¹⁶ We used the results excluding Finnish samples to avoid largely overlap with the cohort for outcome. The genetic instruments were chosen according to those GWAS datasets (Table S1) as follows. Firstly, SNPs located within NRG4 plus a window size of 500Kbp were selected. Secondly, SNPs associated with each lipid level at the region-wide significance level ($P < 1e-3$) were extracted, respectively, according to the Global Lipids Genetics Consortium results. Finally, a standard linkage disequilibrium clumping process was conducted with $R^2 < 0.8$ as the threshold to remove variants with very high correlation. F-statistics were calculated to estimate the strength of the genetic predictors. All selected GWASs from the Global Lipids Genetics Consortium obtained ethical approval from the steering committee and individuals provided informed consent.

Study Outcomes

GWAS summary statistics for four atherosclerosis subtypes were obtained from the FinnGen consortium,¹⁷ including 1) coronary atherosclerosis (CORATHER) (n = 23,363 cases, 187,840 controls), 2) cerebral atherosclerosis (CERATHER) (n = 104 cases, 203,068 controls), 3) peripheral atherosclerosis (PERIPHATHERO) (n = 6631 cases, 162,201 controls), and 4) atherosclerosis excluding cerebral, coronary and peripheral atherosclerosis diseases (ATHSCLE) (n = 6599 cases, 206,541 controls). These definitions of each disease could be found on the website of FinnGen consortium (<https://r9.risteys.finnngen.fi/>). All selected GWAS from the FinnGen consortium obtained ethical approval from the FinnGen Steering Committee and individuals provided informed consent.

Statistical Analysis

The generalized inverse variance weighted method¹⁸ was utilized to estimate the MR effect as the primary analysis. This model considers the correlation among instrument variables (IVs) and allows for a relaxed clumping threshold. All MR estimates were scaled to standard deviation (SD) unit to reflect the equivalent of one SD unit change.

This study was conducted according to the Strengthening the Reporting of Mendelian Randomization Studies guidelines.¹⁹ The three critical assumptions of MR were tested through several sensitivity analyses. The relevance assumption was validated by estimating the strength of the genetic predictors using F-statistics. F statistics ($F = \beta^2/se^2$) were calculated for each SNP, and all F statistics were >10 and considered with sufficient strength. MR-Egger regression, weighted median analysis, simple and weighted mode analyses were utilized for sensitivity analyses. Multivariable MR was used to adjust for pleiotropy. Cochran's Q test was used to estimate the heterogeneity of IVs. Two colocalization methods, Bayesian colocalization analysis and Sum of Single Effects (SuSiE) regression,²⁰ were used to verify whether the two traits may be causally influenced by distinct variants that happen to be correlated with each other.

All the analyses were performed with R platform (version 4.2.2). The “TwoSampleMR”, “MendelianRandomization”, and “coloc” packages were used in our statistical analyses.^{21,22} For all analyses, Bonferroni corrections were applied to establish adjusted thresholds for multiple testing, thereby protecting against Type 1 Error.

Results

Genetically proxied HDL-C level using NRG4 SNPs as the instrument showed little evidence of association with four types of atherosclerosis [CERATHER ($\beta = 6.64$, 95% CI -16.43 to 29.72 , $P = 0.57$), ATHSCLE ($\beta = -0.15$, 95% CI -3.13 to 3.43 , $P = 0.93$), CORATHER ($\beta = -1.56$, 95% CI -4.14 , 1.01 , $P = 0.824$), and PERIPHATHERO ($\beta = -1.14$, 95% CI -5.27 to 2.99 , $P = 0.58$) (β means log (odds ratio) in this study)] (Table 1). The sensitivity analyses including MR-Egger, weighted median, simple and weighted mode analyses also showed little evidence of the association (all $P > 0.003$).

Table 1 Causal Effect of Serum Lipid Levels Genetically Proxied by SNPs of NRG4 on the Risk of Atherosclerosis Subtypes

Exposure	Outcome	Beta	Standard Error	Lower CI	Upper CI	P	n of IV
HDL-C	PERIPHATHERO	-1.14036	2.108699156	-5.27334	2.992612	0.588653	22
	ATHSCLE	0.152251	1.674480183	-3.12967	3.434172	0.927553	22
	CERATHER	6.644805	11.77304766	-16.4299	29.71955	0.572476	22
	CORATHER	-1.56647	1.316547084	-4.14685	1.013917	0.234113	22
LDL-C	PERIPHATHERO	4.137396	2.056849254	0.106046	8.168747	0.04427	16
	ATHSCLE	3.429489	1.944125378	-0.38093	7.239905	0.077727	16
	CERATHER	11.88789	18.77830914	-24.9169	48.6927	0.526691	16
	CORATHER	0.422931	1.865835318	-3.23404	4.079901	0.82068	16
logTG	PERIPHATHERO	3.174145	2.118703518	-0.97844	7.326728	0.134093	13
	ATHSCLE	3.110228	2.00677474	-0.82298	7.043434	0.121174	13
	CERATHER	-12.1432	15.94296386	-43.3908	19.10446	0.446261	13
	CORATHER	2.921694	1.306035096	0.361913	5.481476	0.025282	13

Note: Data are presented as one standard deviation change of lipid levels via SNPs of NRG4 estimated by generalized inverse variance weighted method. **Abbreviations:** CI, confidence interval; IV, instrumental variable; NRG4, Neuregulin 4; SNP, single-nucleotide polymorphism; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; logTG, log transform of triglycerides; CORATHER, coronary atherosclerosis; CERATHER, cerebral atherosclerosis; PERIPHATHERO, peripheral atherosclerosis; ATHSCLE, atherosclerosis excluding cerebral, coronary, and peripheral atherosclerosis diseases.

The causal effect of genetically proxied LDL-C level mediated by NRG4 on PERIPHATHERO was nominal ($\beta=4.14$, 95% CI 0.11 to 8.17, $P=0.04$), and the result generated by weighted median method passed the multiple testing threshold ($P=3.92e-4$) (Table 1, Tables S2 and S3, Figure S1). Genetically proxied LDL-C level using NRG4 SNPs as the instrument showed little evidence of association with three subtypes of atherosclerosis [CERATHER ($\beta=11.89$, 95% CI -24.92 to 48.69, $P=0.53$), ATHSCLE ($\beta=3.42$, 95% CI -0.38 to 7.24, $P=0.08$), CORATHER ($\beta=0.42$, 95% CI -3.23 to 4.08, $P=0.82$)]. The other sensitivity analyses agreed with these results except that weighted median method showed significant causality between LDL-C level and CORATHER ($P=1.70e-3$).

Genetically proxied TG level using NRG4 SNPs as the instrument showed nominal evidence of association with CORATHER ($\beta=2.92$, 95% CI 0.36 to 5.48, $P=2.52e-2$), which was replicated by weighted median, weighted mode, and simple mode ($P<0.05$) (Table 1, Table S2). We did not find any other significant result for TG.

The heterogeneity test showed no heterogeneity for all significant results ($P>0.05$). The MR-Egger intercept term test showed little evidence of directional pleiotropy ($P\geq 0.003$). SuSiE colocalization analysis could not find credible sets and the result of Bayesian colocalization analysis suggested that the IVs were associated with only exposures but not outcomes. It is possibly caused by the lack of strong associations with the outcomes. The minimum of P value for the associations of IVs with all outcomes was 0.003, and all the other P values were greater than 0.01. Thus, colocalization was not an adequate method for a sensitivity analysis in our scenario.²³ In the multivariable MR analyses, we tested the significant associations of TG and LDL-C from single variable MR. LDL-C was associated with PERIPHATHERO ($P=4.82e-3$) in multivariable MR analysis. TG was not associated with CORATHER any more in the multivariable MR, implying potential pleiotropic effects of those IVs.

Discussion

In our study, we investigated the causal role of plasma lipid levels mediated by NRG4 in atherosclerosis subtypes through Mendelian randomization. NRG4 is associated with a lower risk of atherosclerosis, supported by its role in vascular inflammation and adhesion responses, endothelial dysfunction and apoptosis.¹³ In addition, it also plays crucial roles in

maintaining energy balance, regulating lipid metabolism by reducing lipogenesis in hepatocytes and activating BAT.^{12,14} However, whether NRG4 exerts its role in the risk of atherosclerosis through lipid mechanisms has not been explored before.

We found that LDL-C levels proxied by NRG4 IVs were nominally associated with only one specific atherosclerosis subtype. HDL-C showed no association with any of the four subtypes, and the nominal association of TG with CORATHER could not be validated by multivariable MR. Although numerous observational studies have found a consistent inverse association between HDL-C/TG and atherosclerosis, it is still in dispute whether HDL-C/TG is an actor or bystander in the risk of atherosclerosis.⁸ The first reason causing the disagreement is the pleiotropic bias.⁸ Many SNPs associated with LDL-C are also associated with HDL-C and TG. Unlike HDL-C and TG, the genetic determinants of serum LDL-C levels have been more clearly demonstrated to causally increase the risk of atherosclerosis, as evidenced by multiple MR studies and RCTs of LDL-C lowering medications.²⁴ Another factor contributing to disagreement is the heterogeneous nature of associated SNPs, which may operate through different mechanisms.¹⁰ For example, Mendelian randomization studies suggested that the majority of genetic variants influencing pathways leading to increased plasma HDL-C levels did not typically correlate with a reduced risk of cardiovascular disease.²⁵ However, there are exceptions, such as variants related to cholesteryl ester transfer protein (CETP).²⁶ Additionally, HDL of different sizes might exert inconsistent effects.²⁷

In our study, LDL-C was not associated with all four subtypes of atherosclerosis at different locations. Atherosclerosis affects various vascular sites, resulting in specific clinical outcomes like ischemic stroke and myocardial infarction. Human genome-wide association studies have shown that genetic factors contributing to carotid plaque formation are distinct from those associated with coronary artery disease. This suggests that separate genetic pathways may influence atherosclerosis development at different vascular locations.⁶ It was reported that LDL cholesterol lowering is likely to prevent large artery atherosclerosis compared with small artery occlusion.⁷ The various effects of LDL-C on atherosclerosis at different locations might be due to different vascular geometry, hemodynamics, and intrinsic factors, such as the developmental origin of each vascular bed.⁶

The main advantages of our study include the large number of atherosclerosis cases and the availability of data on atherosclerosis subtypes. The exposure and outcome dataset were obtained from non-overlapped samples of European ancestry indicating the genetic similarity among different datasets. In addition, we have used the most up-to-date summary-level genetic data on lipids and atherosclerosis traits. Finally, we have used several sensitivity analyses to correct for possible bias. However, our study still has several limitations. Firstly, the numbers of controls were relatively large, but the numbers of cases for atherosclerosis subtypes, especially cerebral atherosclerosis, were still relatively small. Thus, some null results in some of our MR analyses might be caused by insufficient statistical power. Secondly, we cannot rule out bias caused by subpopulation stratification. Thirdly, the mechanism underlying the effect of NRG4 specifically on peripheral atherosclerosis, while not affecting other types of atherosclerosis, remains unclear. Additionally, the potential relevance of NRG4 as a diagnostic tool or target for the treatment of atherosclerosis remains to be explored by other methods like randomized clinical trials and other ethics besides European population.

Conclusion

Among four atherosclerosis subtypes, we found that LDL-C level proxied by NRG4 was nominally associated with only peripheral atherosclerosis according to Mendelian randomization analyses and several sensitivity analyses.

Institutional Review Board Statement

All selected GWASs from the Global Lipids Genetics Consortium obtained ethical approval from the steering committee and individuals provided informed consent. All selected GWASs from the FinnGen consortium obtained ethical approval from the FinnGen Steering Committee and individuals provided informed consent. According to Article 32 of the Regulations on Ethical Review of Life Science and Medical Research Involving Human Subjects issued by the Chinese government, our MR study is exempt from approval by the local ethics review board.

Data Sharing Statement

All the data used can be downloaded according to the weblink in [Table S1](#).

Informed Consent Statement

Informed consent was obtained from all subjects involved in the study.

Author Contributions

All authors contributed to data analysis, drafting or revising the article, have agreed on the journal to which the article will be submitted, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

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Disclosure

Longyi Zheng, Chengjing Zhang, Shichang Bu, and Wencheng Guo are co-first authors for this study. The authors declare no conflicts of interest in this work.

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