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Original Article

Evaluating the Causal Effects of Low Density Lipoprotein Cholesterol Levels on Ischemic Stroke: A Mendelian Randomization Study

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Abstract

Background: Ischemic stroke (IS) is the leading cause of disability and mortality worldwide. Low-density lipoprotein cholesterol (LDL-C) levels hadno potential risk on ischemic stroke. However, higher LDL-C levels were closely related to IS. Based on two antagonistic viewpoints, a Mendelian randomization (MR) study was designed to evaluate the causal effects of LDL-C levels on IS.

Methods: Datasets of LDL-C levels and ischemic stroke were acquired from genome-wide association studies (GWAS). Weighted median method was conducted for main analysis, and MR-Egger and inverse-variance weighted (IVW) methods were performed for auxiliary analyses. Heterogeneity and pleiotropic tests were utilized to confirm the reliability of this study.

Results: A total of 359 single nucleotide polymorphisms (SNPs) were associated with LDL-C levels ($P < 5 \times 10^{-8}$) and 337 SNPs were available in ischemic stroke with eliminating outliers. LDL-C levels were significantly associated with ischemic stroke (OR = 1.104, 95%CI = 1.019 - 1.195, $P = 1.52 \times 10^{-2}$). MR-Egger and IVW showed directionally similar estimates (MR-Egger: OR = 1.120, 95%CI = 1.040 - 1.207, $P = 3.12 \times 10^{-3}$; IVW: OR = 1.120, 95%CI = 1.064 - 1.178, $P = 1.17 \times 10^{-5}$).

Conclusion: LDL-C levels had causal effects on IS, providing insights into the design of future interventions to reduce the burden of ischemic stroke.

Keywords: Low-density lipoprotein cholesterol; Ischemic stroke; Mendelian randomization; Causality

Introduction

Stroke has become the second leading cause of disability and death worldwide, with low - and middle-income countries bearing the highest burden of disease. In addition, ischemic stroke was considered the most common type of stroke, with about 80 percent prevalence (1). More im-

portantly, the number of patients with ischemic stroke is estimated to increase by 27% due to population growth and aging from 2017 to 2047 (2, 3).

Many behavioral and pathological conditions had a considerably wide range of risk of establishing a stroke, including the low physical activities, excessive alcohol, smoking, embolism, large-and-small vessel disease (4, 5). Simultaneously, the low-density lipoprotein cholesterol (LDL-C) levels were dependent risk factors for ischemic stroke (6). Despite growing evidence indicating that patients with LDL-C levels have no increased risk of ischemic stroke, owing to the reverse causation and potential biases from confounding effects, the specific association between LDL-C levels and ischemic stroke remains disputed. The summary suggested that LDL-C levels ≧ 130 mg/dl were closely related to ischemic stroke (7). Currently, the assessment of the genetics causal effects of LDL-C on ischemic stroke based on objective and bias-evading data was urgently required to sentence the controversy.

Nevertheless, there was no direct and presentable evidence of this association reported. Therefore, exploring the causal effects of LDL-C levels on risk of ischemic stroke is necessary and important, which may help to solve the subsequent social burden and be beneficial to the government public health policy.

Mendelian randomization (MR) is an innovative analysis utilizing hypothetical genetic variation that satisfy the hypothesis of instrumental variables to determine the causality in epidemiological studies (8). Since genetic variation has existed with birth and remains stable in the whole lifetime, MR achieves a dominant position in avoiding the relation inversion influence and the variation brought by confounding factors.

The present two-sample MR study was performed to explore the association between LDL-C levels and ischemic stroke.

Methods

Study design

Two different dataset summaries were selected for MR analysis from genome-wide association studies (GWAS) to explore the association between LDL-C levels and ischemic stroke. The MR analysis considered LDL-C levels as the exposure and ischemic stroke as the outcome to assess the impact of the former on the latter and summarize. As the analysis was based on published research datasets and did not contain individual identities, ethical approval was not required. A brief description of MR design between LDL-C levels and ischemic stroke was shown in Fig. 1.

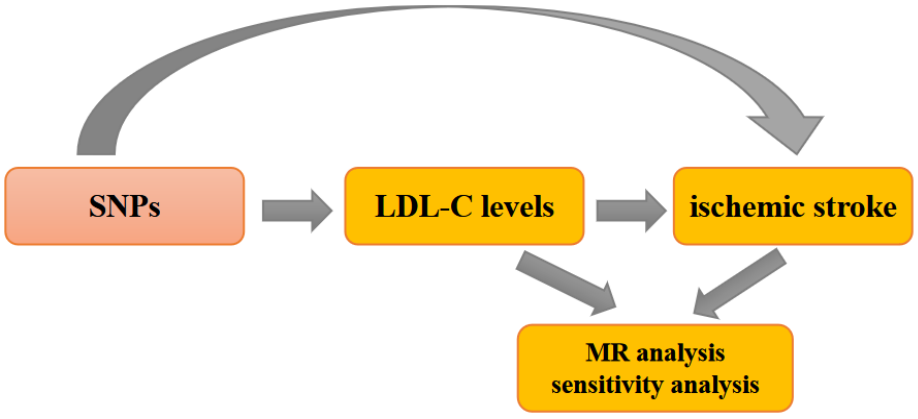

Fig. 1: Schematic representation of an MR analysis. We selected SNPs which associated with LDL-C levels and the corresponding effects for these SNPs was estimated based on the risk of ischemic stroke obtained from public GWAS summary datasets

Data sources

The recently developed analysis of two-sample MR analysis was utilized to assess the causal effects of LDL-C levels on ischemic stroke. Single nucleotide polymorphisms (SNPs) associated with LDL-C levels were acquired from public GWAS summary datasets, comprising 431,167 samples. A total of 359 SNPs, which reached the genome-wide significance threshold ($P < 5 \times 10^{-8}$) were selected as instrumental variables. Corresponding data for the 338 SNPs related to ischemic stroke were obtained from public GWAS summary datasets. The SNP datasets used for both LDL-C levels and ischemic stroke estimates were acquired from European studies with a similar population, which minimized the possibility of population stratification bias.

Statistical analysis

A two-sample MR analysis was employed to estimate the causal effects of LDL-C levels on ischemic stroke. The weighted median was the main statistical method to evaluate the association of genetically predicted LDL-C levels with risk of ischemic stroke. MR-Egger and inverse-variance weighted (IVW) are used as auxiliary analyses methods to evaluate the robustness of weighted median results. F-statistic was used to assess the correlation hypothesis between the IV assumptions and exposure factors. The weak instrument strength can be obtained with F<10. In addition, Mendelian randomization pleiotropy residual sum and outlier (MR - PRESSO) test were used to exclude potentially anomalous outlier SNPS and globally test the assessed level multiplexicity. As well, heterogeneity test and pleiotropy test were conducted, in which *P*-values were set at 0.1 for statistical significance for heterogeneity test and 0.05 for statistical significance for pleiotropy. Independence hypothesis and exclusivity hypothesis were used to ensure the instrumental variables are associated only by exposure factors and not by other factors in MR analysis.

Results

Selection of Instrumental Variables

In public GWAS summary datasets, 359 SNPs were associated with LDL-C levels ($P < 5 \times 10^{-8}$), 338 of which were available in the ischemic stroke GWAS. After removing SNPs, which were in outlier by MR-PRESSO, 337 were remaining for use in the MR analysis.

Independence hypothesis and exclusivity hypothesis

Three core assumptions are satisfied by the current MR analysis. Firstly, we excluded SNPs that did not meet the independence and exclusivity hypothesis before the experiment was conducted. After that, the F-statistic of all SNPs has been greater than 10, which is considered consistent with the correlation hypothesis. We defined confounding factors as hypertension, hyperlipidemia, atrial fibrillation, diabetes and atherosclerotic disease. No SNP associated to the confounding factors was found through querying all phenotypes of all SNPs on the PhenoScanner website.

MR analysis for the causal association

The weighted median between LDL-C levels and ischemic stroke provide strong evidence of an association (OR = 1.104, 95%CI = 1.019 - 1.195, $P = 1.52 \times 10^{-2}$). We performed a second MR-PRESSO analysis on the retained data after SNP elimination, and no abnormal outliers were found in this analysis. There were signs of strong association using the MR-Egger (OR = 1.120, 95%CI = 1.040 - 1.207, $P = 3.12 \times 10^{-3}$) and IVW (OR = 1.120, 95%CI = 1.064 - 1.178, $P = 1.17 \times 10^{-5}$), supporting the robustness of weighted median. The causal association between genetically predicted LDL-C levels and ischemic stroke was shown in Fig. 2. The scatter plot of the ischemic stroke associations against the LDL-C levels associations was shown in Fig. 3, which allowed visualization of the causal-effects estimate for each individual SNP on ischemic stroke.

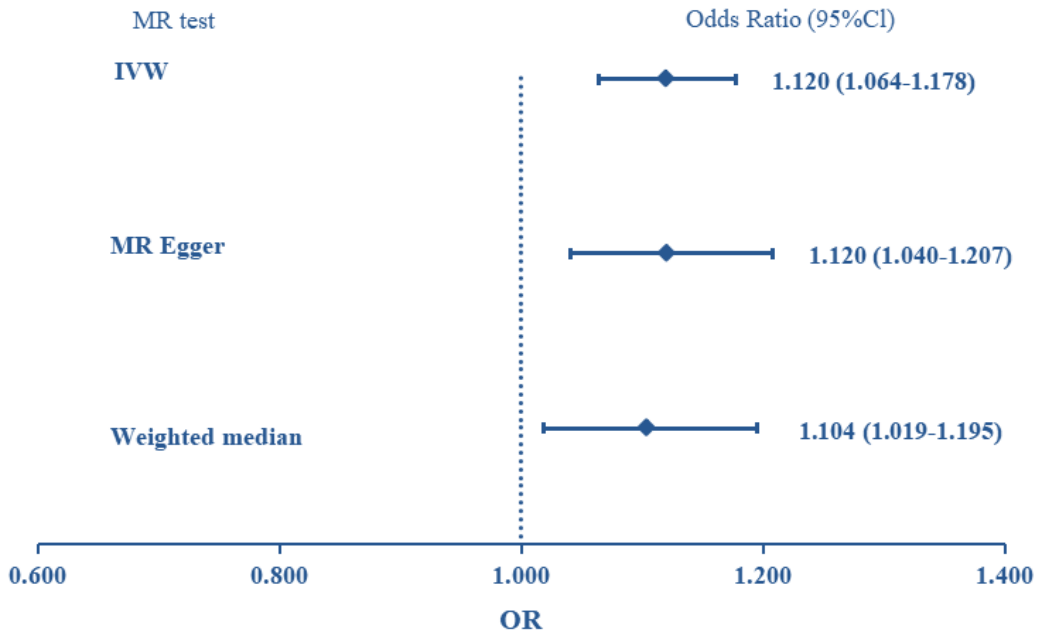

Fig. 2: Forest plot to visualize causal effects of LDL-C levels on the risk of ischemic stroke by three methods

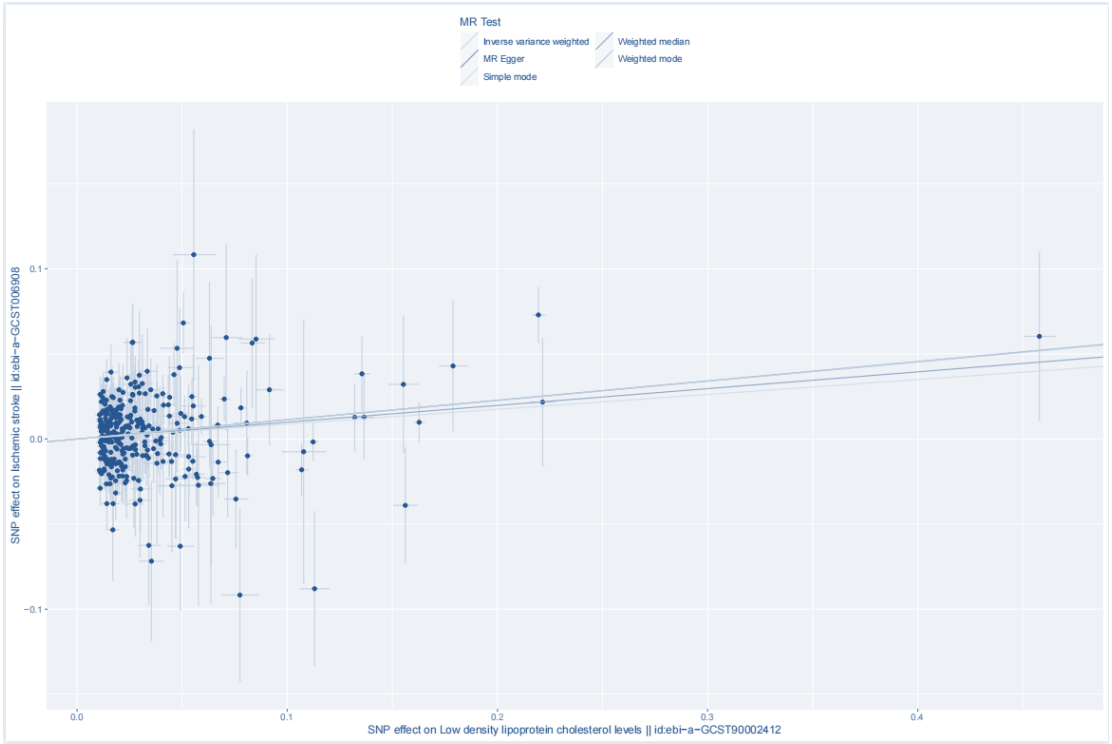

Fig. 3: Scatter plots in which the single-nucleotide polymorphism (SNP)-outcome associations are plotted against the SNP-LDL-C levels associations, allowing visualization of the causal-effects estimate for each individual SNP on ischemic stroke

Horizontal pleiotropy analysis

As shown in Fig. 4, the scatters nearly distributed symmetrically which found by human observation.

Heterogeneity test and pleiotropy test

As shown in Table 1, heterogeneity was found in this study, that resulted from MR-Egger (P = 4.12×10^{-6}) and IVW ($P = 4.90 \times 10^{-6}$). No pleiotropy (P = 0.985) was found, thus few confounding factors existed.

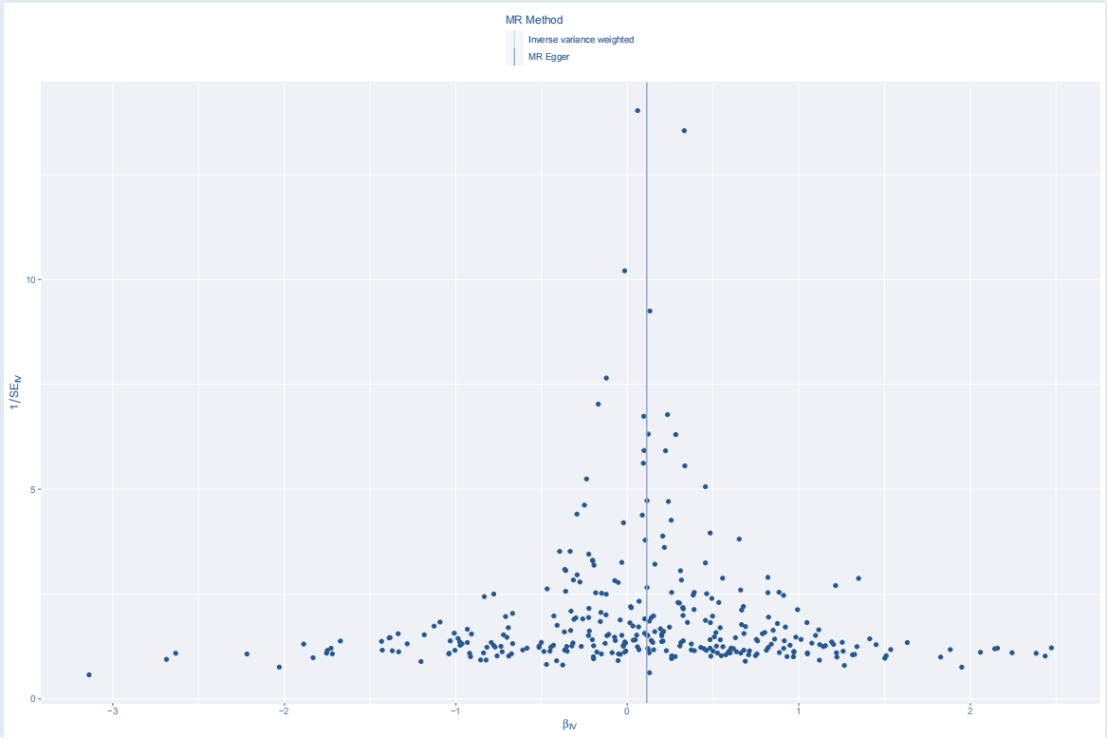

Fig. 4: Funnel plots to visualize overall heterogeneity of MR estimates for the effects of LDL-C levels on the risk of ischemic stroke

Table 1: Heterogeneity test in the causality of LDL-C levels and ischemic stroke

Method	*Number of SNPs*	*OR*	*Standard error*	*Association P value*	*Cochran Q statistic*	*Heterogeneity P value*
Inverse variance weighted	337	1.120	0.02579176	1.17×10^{-5}	451.4940	0.00000490
MR-Egger	337	1.120	0.03812522	3.12×10^{-3}	451.4936	0.00000412
Weighted median	337	1.104	0.04066792	1.53×10^{-2}		

LDL-C = Low density lipoprotein cholesterol.

Discussion

The main findings of this study were that LDL-C levels are likely to increase the risk of ischemic stroke. LDL-C, the main lipoprotein in fasting plasma, acts the main vehicle for transporting cholesterol to extrahepatic tissues. Lower LDL-C was associated with fewer cardiovascular events (9). Especially, with no timely LDL-C removal in plasma, the prevalence of the formation of inti-

mal atherosclerotic plaque has increased to a higher level than normal. Therefore, the level of LDL-C is related to the incidence and degree of cardiovascular disease, which is considered the main performance of atherosclerosis. Long-term arterial stiffness cause ischemia of the circulatory system, which is drastically lead to ischemic stroke.

For note, stroke has become the leading cause of long-term disability in adults (10). Although, not only intravenous thrombolysis, interventional thrombolysis and anticoagulation therapy have been taken as the main methods for the treatment of ischemic stroke, but also acute ischemic stroke can be treated effectively by timely and successful reperfusion (11), the intervention measures on controlling LDL-C levels are supposed to be supported immediately. The results of this study were consistent with previous studies, which indicated that intensive statins with the goal of lowering LDL-C levels took an important role in the treatment of ischemic stroke (9). Besides, PCSK9 inhibitors were deemed as a worthily considerate approach to treatment the patients who are high-risk for another cardiovascular event or recurrent stroke and have no acceptable LDL-cholesterol levels despite aggressive statin treatment (12). It has been supported an emerging direction for the intervention of LDL-C level to prevent ischemic stroke.

In this MR study, the causality of risk factor on LDL-C levels and ischemic stroke was comprehensively assesses by using 337 SNPs from >40,000 Europeans. Credible evidence for the causal effects of ischemic stroke risk factors was found among two-sample MR analysis. The results of our MR analysis made the supplement for the genetically causal association and mechanisms underlying the association of elevated LDL-C levels with increased risk of ischemic stroke. Compared with traditional observational epidemiological study, MR analysis has provided better evidence to assess the causal association between LDL-C levels and ischemic stroke. The chief strength of the study includes use of MR, which effectively avoids the influence of various confounding factors (13). In addition, the alleles random combination and the determine at conception had strong effects on MR analysis (14). Hence, our results reached the representation that the lifetime risk of ischemic stroke is increasing due to LDL-C levels. The causal associations between LDL-C levels and ischemic stroke GWAS were finished just in European ancestry populations using the MR analysis, which summary genetic association results were obtained in samples from the similar or comparable populations. This synchronism also increased the plausibility of the 2-sample MR assumption, reducing bias due to population stratification. Horizontal pleiotropy may have introduced bias if the SNPs were associated with other slight confounding lipids that had no availability on direct monitoring, through pathways not involving LDL-C levels. No negative control population availably assess this. However, results from MR-Egger and IVW, which are less susceptible to horizontal pleiotropy, have direction in accordance with the weighted median estimates. Overall, the studies included were of high quality and had a low risk of bias. Therefore, the statistical evidence for association in this data analysis had strongly high reliability.

Given the causal effects of increasing LDL-C levels has shown on the development of ischemic stroke, clinical guidelines ought to recommend dyslipidemia as an effective indicator of primary prevention. We suggest that the general population would be popularized the interrelated cognition to reduce the population-wide incidence of the ischemic stroke through more aggressive control of LDL-C levels earlier. In addition, LDL-C levels controlled effectively would as the useful prevention of cardiovascular disease (15). Risk factors for ischemic stroke often effect in the time-courses. Effective indicator therefore ought to be taken when monitoring the long-term LDL-C levels on ischemic stroke patients.

Healthy lifestyle can reduce the risk of stroke by 80% (16). Future investigation into more quantifiable measures for lifestyle-related risk factors with a MR study such as lipid intake of high-fat

foodstuffs would be profitable to examine the impact of various viands and diets on ischemic stroke or other hyperlipidemia diseases. Moreover, cohorts of other ethnicities that East Asians and mixed population, excepting for European had employed in MR studied on ischemic stroke from GWAS, which would provide the insight on different magnitudes of causal effects result from different genetic compositions from inter-ethnic diversity would have led to affirm genetic risk factors on the development of ischemic stroke.

Conclusion

We found potential evidence about the causal effects of LDL-C levels on increased risk of ischemic stroke by using MR analysis. This suggested that the mechanism risk factors mediate the effects on ischemic stroke should be further investigate. This will better define the potential role of LDL-C levels in preventing ischemic stroke onset and progression and inform the design of a randomized controlled trial with a LDL-based intervention.

Acknowledgements

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Competing interest

The authors have no relevant financial or non-financial interests to disclose.

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