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Research Report

Remnant cholesterol traits and risk of stroke: A multivariable Mendelian randomization study

Zhiyuan Wu[a,b,1], Yue Jiang[a,1], Zheng Guo[b], Pingan Li[a], Yulu Zheng[b], Yutao Wang[c], Haiping Zhang[a], Lois Balmer[b], Xingang Li[b], Lixin Tao[a], Qi Zhang[d,*], Bo Gao[a,*] and Xiuhua Guo[a,*]

[a]Beijing Municipal Key Laboratory of Clinical Epidemiology, School of Public Health, Capital Medical University, Beijing 100069, China
[b]Centre for Precision Health, School of Medical and Health Sciences, Edith Cowan University, Joondalup, WA 6027, Australia
[c]Centre of Xunshu, Shanghai Fufan Information Technology Co., Ltd, Shanghai 200433, China
[d]Department of Informatics, Huashan Hospital, Fudan University, Shanghai 200040, China
*To whom correspondence should be addressed: Email: statguo@ccmu.edu.cn (X.G.); Email: spencerzq@huashan.org.cn (Q.Z.); Email: gaobo19877@126.com (B.G.)
[1]First coauthors: Z.W. and Y.J. analyzed the data and drafted the manuscript together.
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Abstract

Observational epidemiological studies have reported a relationship between remnant cholesterol and stroke. However, the results are inconclusive, and causality remains unclear due to confounding or reverse causality. Our objective in this study was to investigate the causal relevance of remnant cholesterol and the risk of stroke and its subtypes using the Mendelian randomization (MR) approach. Genome-wide association studies (GWASs) including 115,082 European individuals (UK Biobank) were used to identify instruments for remnant cholesterol, including intermediate-density lipoprotein (IDL) cholesterol and very-low-density lipoprotein (VLDL) cholesterol. Summary-level data for total stroke, intracerebral hemorrhage, subarachnoid hemorrhage, ischemic stroke (IS), and IS subtypes were obtained from GWAS meta-analyses conducted by the MEGASTROKE consortium. Univariable and multivariable MR analyses were performed. The GWAS identified multiple single-nucleotide polymorphisms after clumping for remnant cholesterol ($n = 52$), IDL cholesterol ($n = 62$), and VLDL cholesterol ($n = 67$). Assessed individually using MR, remnant cholesterol (weighted median: odds ratio [OR] 1.32 per 1-SD higher trait; 95% CI: 1.04–1.67; $P = 0.024$) had effect estimates consistent with a higher risk of LAS-IS, driven by IDL cholesterol (OR 1.32; 95% CI: 1.04–1.68; $P = 0.022$). In multivariable MR, IDL cholesterol (OR 1.46; 95% CI: 1.10–1.93; $P = 0.009$) retained a robust effect on LAS-IS after controlling for VLDL cholesterol and high-density lipoprotein cholesterol. The MR analysis did not indicate causal associations between remnant cholesterol and other stroke subtypes. This study suggests that remnant cholesterol is causally associated with the risk of LAS-IS driven by IDL cholesterol.

Significance Statement

Our study highlighted that remnant cholesterol is causally associated with ischemic stroke (IS) but not with intracerebral hemorrhage and subarachnoid hemorrhage. The causal effect of remnant cholesterol on IS is mainly driven by intermediate-density lipoprotein (IDL) cholesterol. Among IS subtypes, remnant cholesterol and IDL cholesterol are most significantly associated with large artery atherosclerosis stroke. Additionally, further studies are needed to clarify the mechanism underlying the effect of remnant cholesterol on large artery atherosclerosis stroke and the benefits of extra lowering of remnant cholesterol on the primary prevention of stroke.

Introduction

Stroke is the second-leading cause of death and adult-acquired disability worldwide (1). In past decades, the prevalence and incidence of stroke saw an increase due to population aging and improved survival rates, with 101 million cases and 12.2 million new stroke onsets reported in 2019 (2). Ischemic stroke (IS) accounts for ~80% of all strokes, and the major etiological subtypes of IS are large artery atherosclerosis stroke (LAS), small vessel stroke, and cardioembolic stroke (3). The remaining 20% are intracerebral hemorrhage and subarachnoid hemorrhage (4). Hypertension, obesity, smoking, diabetes, and lipid metabolism are well-established risk factors for stroke (5).

Remnant cholesterol has been suggested in population-based studies to increase the incidence of cardiovascular diseases, including stroke (6, 7). Remnant cholesterol is mainly the cholesterol content of triglyceride-rich lipoproteins (TRLs), i.e. the intermediate-density lipoprotein (IDL), very-low-density lipoprotein (VLDL), and chylomicron remnants (8). Remnant cholesterol concentration was calculated using low-density lipoprotein (LDL) cholesterol and high-density lipoprotein (HDL) cholesterol subtracted from the total cholesterol in most previous

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observational epidemiological studies. In addition, unmeasured confounding bias and reverse causality are inherent in traditional epidemiological designs. Mendelian randomization (MR) is an epidemiological study design using genetic variants associated with exposures as proxy indicators to infer the causal effect on outcomes, which could reduce confounding by environmental factors (due to alleles being randomly allocated at conception) and avoid reverse causation, as disease phenotype cannot affect genotype (9). Of note, previous studies have indicated the causal effect of remnant cholesterol on heart diseases, such as ischemic heart disease (10), myocardial infarction (11), and aortic valve stenosis (12), based on MR design. However, no causal evidence between remnant cholesterol and stroke risk could be claimed yet.

In the present study, we aimed to investigate the causal effect of remnant cholesterol on stroke and its subtypes using a two-sample MR approach. In addition, we explored which component trait of remnant cholesterol could be causally associated with total stroke or any stroke subtype using univariable and multivariable MR.

Methods

Study design and data sources

This is a two-sample MR study following STROBE-MR guidelines (Strengthening the Reporting of Mendelian Randomization Studies) (13). The study is based on publicly available summary statistics from genome-wide association study (GWAS) consortia. All studies obtained relevant ethical approval and participant consent. An overview of the study design is shown in Fig. 1. As this study was based on published studies and summary-level data, ethical approval was exempted by the Ethics Committee of Edith Cowan University (grant number: 2021-03164-WU).

The genetic data for total stroke, IS, and its subtypes were obtained from a publicly available database released by the MEGASTROKE project launched by the International Stroke Genetics Consortium (14). MEGASTROKE project is a multiancestry genome-wide-association meta-analysis of 521,612 individuals from 29 studies with genome-wide genotypes imputed to 1000 Genomes Project (1000G) phase 1v3 or similar. The MEGASTROKE consortium defined stroke using standard diagnostic criteria based on clinical and imaging findings, including total stroke comprising IS, intracerebral hemorrhage, and stroke of unknown or undetermined type ($n = 67,162$) with up to 454,450 controls. ISs ($n = 60,341$) were further classified into subtypes using the Trial of ORG 10172 in Acute Stroke Treatment (TOAST) criteria (3), including LAS ($n = 6,688$), small vessel stroke ($n = 11,710$), and cardioembolic stroke ($n = 9,006$). The TOAST criteria are based on clinical manifestations found by history and examination, brain imaging findings, and ancillary findings, including vascular and cardiac imaging. Patients are categorized into five major subtypes: LAS, small artery occlusion (lacune), cardioembolism, other demonstrated causes, and undetermined causes, which represent the most common causes.

In addition, MEGASTROKE project released the meta-analysis results for the subset of Europeans of 406,111 controls and 40,585 stroke cases, including IS ($n = 34,217$), LAS ($n = 4,373$), small vessel stroke ($n = 5,386$), and cardioembolic stroke ($n = 7,193$). Due to the requirement of a homogeneous population using a two-sample MR design, summary statistics data from the European ancestry were used in the primary analysis. Summary statistics data for lacunar stroke were available from another pooled analysis of European ancestry (15). Genotype data were obtained from a total of 6,030 cases confirmed by MRI or standard phenotyping and 248,929 controls.

Summary statistics data for intracerebral hemorrhage were available from a genome-wide association meta-analysis of 3,026 European-descent individuals, including 1,545 (664 lobar intracerebral hemorrhage and 881 nonlobar intracerebral hemorrhage) cases and 1,481 noncases (16). Hemorrhage cases were defined as those with compatible brain imaging (computed tomography or MRI) showing the presence of intraparenchymal bleeding.

We obtained summary-level GWAS data of subarachnoid hemorrhage from European ancestry after excluding the UK Biobank participants (to eliminate overlap between exposure and outcome GWASs). This study (stage 1) consisted of individual-level genotypes from 23 different cohorts, including 5,425 aneurysm subarachnoid hemorrhage cases confirmed using imaging and 71,934 controls (17). GWAS summary-level data regarding aneurysmal subarachnoid hemorrhage can be obtained from the Cerebrovascular Disease Knowledge Portal (www.cerebrovascularportal.org).

Genetic instrument selection

We selected single-nucleotide polymorphisms (SNPs) previously shown to be associated with the remnant cholesterol traits (remnant cholesterol, IDL cholesterol, and VLDL cholesterol) at the level of genome-wide significance ($P < 5 \times 10^{-8}$) among 115,082 UK Biobank participants of European ancestry (18). The remnant cholesterol traits were measured by using the targeted high-throughput NMR platform. Information (chromosome, position, effect allele, effect size, and mapped gene) on SNPs used as instrumental variables is available in Additional File S1: Data S1. In addition, we selected SNPs ($n = 88$) associated with HDL cholesterol for genetic instruments in the same population.

Statistical analysis

This study was based on the two-sample MR framework, using the summary statistics of effect sizes of each instrumental SNP with exposure (one unit: per SD higher trait) and outcome (dichotomous), and the harmonization of the direction of estimates by effect alleles. The effect for each instrument was calculated with the Wald estimator and the SEs with Delta method. We then pooled individual MR estimates to infer the causal effect of exposure on outcome using inverse-variance-weighted (IVW) meta-analysis, weighted median method, and MR-Egger regression. The IVW method involves weighting multiple estimates of the effect of individual genetic variants on an outcome to produce an overall estimate of the causal effect of the outcome, provided that the instrumental variables satisfy the core assumptions (19). The weighted median method involves median-weighting the effects of multiple genetic variants on the outcome, where these effects are arranged and the middle value is taken as the estimated value. Compared with other methods, the weighted median approach is more flexible as it can provide robust estimates even when some of the genetic variants do not adhere to the core assumptions (20). The MR-Egger method utilizes Egger regression to fit a linear regression model, which involves weighting the impact of individual genetic variants on the outcome. The MR-Egger method allows for a consideration of potential asymmetry between the magnitude and the level of effects within genetic variations, which can detect and correct for biases resulting from potential level-effect pleiotropy between genetic variants and the outcome (20).

No horizontal pleiotropy is a key assumption of the MR framework. First, we examined the heterogeneity between causal

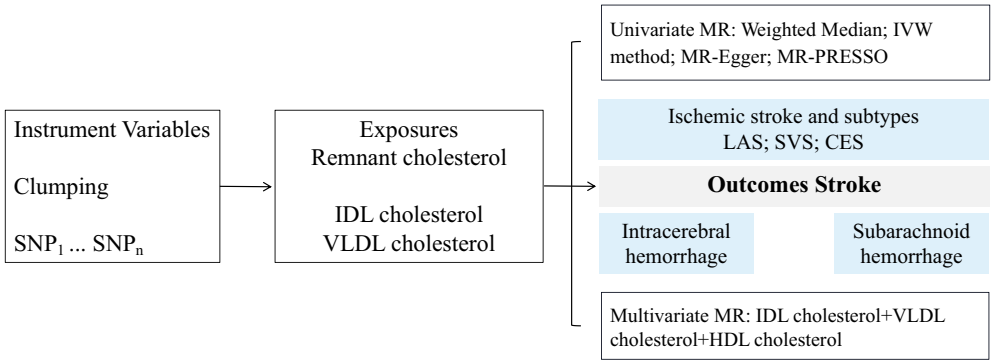


Fig. 1. An overview of the current MR study design. IDL, intermediate-density lipoprotein; VLDL, very-low-density lipoprotein; HDL, high-density lipoprotein; IVW, inverse variance weighted; MR, Mendelian randomization; PRESSO, Pleiotropy RESidual Sum and Outlier; SNP, single-nucleotide polymorphism; LAS, large artery atherosclerosis stroke; SVS, small vessel stroke; CES, cardioembolic stroke.

Table 1. Univariable MR analysis effect estimates for associations between remnant cholesterol and stroke.

Outcome	Methods	SNPs, *n*	OR	95% CI	P_{effect}
Total stroke	MR-Egger	48	1.202	1.037–1.393	0.018
	Weighted median	48	1.142	1.043–1.251	0.004
	IVW	48	1.086	0.989–1.192	0.083
Ischemic stroke	MR-Egger	48	1.222	1.038–1.437	0.020
	Weighted median	48	1.137	1.031–1.255	0.010
	IVW	48	1.089	0.983–1.207	0.104
Large artery stroke	MR-Egger	48	1.453	1.020–2.071	0.044
	Weighted median	48	1.316	1.038–1.669	0.024
	IVW	48	1.287	1.040–1.594	0.021
Small vessel stroke	MR-Egger	49	0.976	0.749–1.272	0.859
	Weighted median	49	1.073	0.876–1.315	0.493
	IVW	49	1.106	0.940–1.302	0.226
Cardioembolic stroke	MR-Egger	48	1.066	0.863–1.318	0.554
	Weighted median	48	0.889	0.743–1.065	0.202
	IVW	48	0.995	0.876–1.131	0.940
Lacunar stroke	MR-Egger	37	1.152	0.851–1.561	0.366
	Weighted median	37	1.159	0.953–1.411	0.139
	IVW	37	1.157	0.968–1.383	0.109
Intracerebral	MR-Egger	26	1.242	0.285–5.418	0.776
	Weighted median	26	1.403	0.710–2.769	0.330
	IVW	26	0.965	0.524–1.775	0.908
Hemorrhage	MR-Egger	25	1.274	0.204–7.954	0.798
Lobar ICH	Weighted median	25	0.962	0.402–2.300	0.930
	IVW	25	1.160	0.550–2.445	0.697
Nonlobar ICH	MR-Egger	26	1.008	0.244–4.165	0.991
	Weighted median	26	1.292	0.562–2.967	0.547
	IVW	26	0.801	0.445–1.443	0.460
Subarachnoid hemorrhage	MR-Egger	20	0.677	0.303–1.511	0.353
	Weighted median	20	1.068	0.749–1.523	0.718
	IVW	20	0.954	0.634–1.433	0.819

ICH, intracerebral hemorrhage; IVW, inverse variance weighted; MR, Mendelian randomization; OR, odds ratio; SNP, single-nucleotide polymorphism.

estimates from different genetic variants using Cochran's Q statistic (statistical significance set at $P < 0.05$) (21). Second, we used MR-Egger regression to test the intercept term for average pleiotropic effect. Third, the MR Pleiotropy RESidual Sum and Outlier (PRESSO) test was used to assess the robustness of the causal effect after removing the outliers (22). Outliers are detected by sequentially removing each variant from the analyses and comparing the observed residual sum of squares to the expected residual sum of squares as a global measure of heterogeneity. We calculated the *F*-statistic and proportion of variance explained (R^2 statistic) to test the weak instrumental bias as previous studies (23).

To further account for potential horizontal pleiotropy, we performed a multivariable MR analysis for IDL cholesterol and VLDL cholesterol because of the fact that 10 SNPs were selected as the genetic instruments of both IDL cholesterol and VLDL cholesterol (24). The pleiotropy effect on HDL cholesterol was also adjusted, given (i) the association of elevated levels of remnant cholesterol traits with reduced HDL cholesterol (10) and (ii) the inconsistent effects of selected SNPs on atherogenic and antiatherosclerotic lipoprotein lipids (25).

We further performed a sensitivity analysis to test the reliability of the causal association estimation using the multiancestry GWAS meta-analysis statistics released by the MEGASTROKE consortium. In addition, we also checked the effect of remnant cholesterol using GIGASTROKE data (26).

All analyses were performed in R (v4.2.0; The R Foundation for Statistical Computing) using the two-sample MR, MVMR, MendelianRandomization, and MR-PRESSO packages.

Results

As shown in Additional File S1: Data S1, there were 52 SNPs selected for remnant cholesterol, 62 SNPs for IDL cholesterol, and 67 for VLDL cholesterol. In this two-sample MR analysis, there was no population overlap between the exposure and outcome samples, as the summary statistics of exposure from UK Biobank and summary statistics of stroke from several stroke consortiums. Assessed using univariable MR, genetically predicted remnant cholesterol was consistently associated with LAS-IS using three MR methods. The odds ratios (ORs) of per SD higher trait were 1.316 (95% CI: 1.038–1.669) of weighted median, 1.287 (1.040–1.594) of IVW method, and 1.453 (1.020–2.071) of MR-Egger regression (Table 1). Remnant cholesterol was causally associated with total stroke and IS when assessed using the weighted median and MR-Egger methods.

We found that genetically predicted IDL cholesterol was consistently associated with LAS-IS using three MR methods (Table 2). The ORs of the per SD higher trait were 1.320 (1.041–1.675), 1.297 (1.056–1.592), and 1.512 (1.105–2.069), respectively. IDL cholesterol was causally associated with total stroke and IS when assessed using the weighted median and MR-Egger methods, while VLDL cholesterol was causally associated with total stroke and IS by IVW method. Figure 2 summarizes the main results of univariable MR analyses.

The heterogeneity tests and pleiotropy tests are shown in Additional File S2: Tables S1 and S2. The intercept from the MR-Egger regression analysis did not reach statistical significance when assessing LAS-IS, suggesting no apparent evidence of overall directional pleiotropy. In addition, remnant cholesterol and IDL cholesterol were causally associated with LAS-IS using PRESSO method after removing the potential outliers (Additional File S2: Table S3). Additional File S2: Table S4 summarizes the results using the GWAS statistics of stroke from the multiancestry population, and the observed results were more significant and robust. The forest plots and funnel plots regarding remnant cholesterol traits and stroke subtypes were presented (Additional File S3: Figs. S1–S6). The *F*-statistics indicated the absence of a potential weak instrument bias (Additional File S1: Data S2).

In multivariable MR analysis, IDL cholesterol (OR 1.457; 95% CI: 1.100–1.931; $P = 0.009$) was causally associated with a higher risk of LAS-IS, and HDL cholesterol was causally associated with a lower risk (OR 0.724; 95% CI: 0.569–0.922; $P = 0.009$). The *F*-statistics for IDL cholesterol, VLDL cholesterol, and HDL cholesterol were 23.5, 18.3, and 13.8, respectively. Genetically predicted VLDL cholesterol was not significantly associated with IS or any IS subtypes, as shown in Fig. 3. The causal effect of IDL cholesterol on LAS-IS retained consistency when assessed using the GWAS statistics of stroke from the multiancestry population. The SNP information used in multivariable MR was summarized in Additional File S1: Data S3. Using GIGASTROKE summary GWAS data, the causal effect of IDL cholesterol on LAS-IS remained (OR 1.261; 95% CI: 1.009–1.577; $P = 0.042$) based on 157 instrumental variables.

Discussion

In this study, we investigated the causal association of remnant cholesterol traits with the risk of stroke, including IS and subtypes, intracerebral hemorrhage, subarachnoid hemorrhage, and cerebral small vessel disease using the univariable and

Table 2. Univariable MR analysis effect estimates for associations of IDL cholesterol and VLDL cholesterol with stroke.

Outcome	Methods	IDL cholesterol			VLDL cholesterol		
		SNP	OR (95% CI)	P_{effect}	SNP	OR (95% CI)	P_{effect}
Total stroke	MR-Egger	58	1.205 (1.064–1.365)	0.005	64	0.953 (0.84–1.082)	0.463
	Weighted median	58	1.133 (1.043–1.231)	0.003	64	0.961 (0.879–1.05)	0.379
	IVW	58	1.084 (0.995–1.181)	0.065	64	1.074 (1.004–1.149)	0.039
Ischemic stroke	MR-Egger	58	1.222 (1.068–1.397)	0.005	64	0.935 (0.82–1.067)	0.322
	Weighted median	58	1.128 (1.031–1.233)	0.008	64	0.955 (0.873–1.045)	0.318
	IVW	58	1.095 (0.999–1.201)	0.052	64	1.092 (1.017–1.173)	0.015
Large artery stroke	MR-Egger	58	1.512 (1.105–2.069)	0.012	63	0.899 (0.628–1.285)	0.560
	Weighted median	58	1.320 (1.041–1.675)	0.022	63	1.027 (0.797–1.323)	0.839
	IVW	58	1.297 (1.056–1.592)	0.013	63	1.171 (0.972–1.412)	0.097
Small vessel stroke	MR-Egger	58	1.125 (0.842–1.504)	0.429	64	0.865 (0.661–1.132)	0.295
	Weighted median	58	1.066 (0.866–1.312)	0.547	64	0.93 (0.757–1.143)	0.491
	IVW	58	1.038 (0.858–1.256)	0.703	64	1.116 (0.968–1.286)	0.131
Cardioembolic stroke	MR-Egger	58	1.031 (0.863–1.232)	0.735	63	0.8 (0.638–1.004)	0.058
	Weighted median	58	0.984 (0.821–1.178)	0.857	63	0.897 (0.754–1.068)	0.223
	IVW	58	1.017 (0.906–1.142)	0.772	63	0.993 (0.883–1.116)	0.904
Lacunar stroke	MR-Egger	51	1.070 (0.829–1.380)	0.605	51	1.122 (0.848–1.485)	0.425
	Weighted median	51	1.020 (0.854–1.218)	0.827	51	1.104 (0.899–1.357)	0.346
	IVW	51	1.020 (0.859–1.211)	0.822	51	1.171 (1.020–1.345)	0.025
Intracerebral hemorrhage	MR-Egger	35	0.887 (0.230–3.422)	0.862	37	1.405 (0.632–3.126)	0.410
	Weighted median	35	0.989 (0.481–2.036)	0.976	37	1.271 (0.697–2.318)	0.434
	IVW	35	0.711 (0.388–1.301)	0.268	37	0.997 (0.664–1.499)	0.990
Lobar ICH	MR-Egger	34	0.985 (0.204–4.757)	0.986	37	2.616 (0.96–7.132)	0.069
	Weighted median	34	0.665 (0.293–1.510)	0.330	37	1.277 (0.608–2.681)	0.518
	IVW	34	0.786 (0.394–1.569)	0.495	37	1.145 (0.672–1.951)	0.618
Non-lobar ICH	MR-Egger	34	0.855 (0.213–3.431)	0.827	37	0.964 (0.381–2.442)	0.939
	Weighted median	34	0.389 (0.172–0.880)	0.023	37	1.224 (0.625–2.397)	0.555
	IVW	34	0.586 (0.316–1.085)	0.089	37	1.121 (0.693–1.813)	0.642
Subarachnoid hemorrhage	MR-Egger	28	0.653 (0.326–1.304)	0.238	32	1.042 (0.654–1.659)	0.863
	Weighted median	28	1.030 (0.689–1.540)	0.886	32	0.945 (0.678–1.318)	0.741
	IVW	28	0.794 (0.555–1.135)	0.205	32	0.962 (0.763–1.212)	0.743

IDL, intermediate-density lipoprotein; VLDL, very-low-density lipoprotein; ICH, Intracerebral hemorrhage; IVW, inverse variance weighted; MR, Mendelian randomization; OR, odds ratio; SNP, single-nucleotide polymorphism.

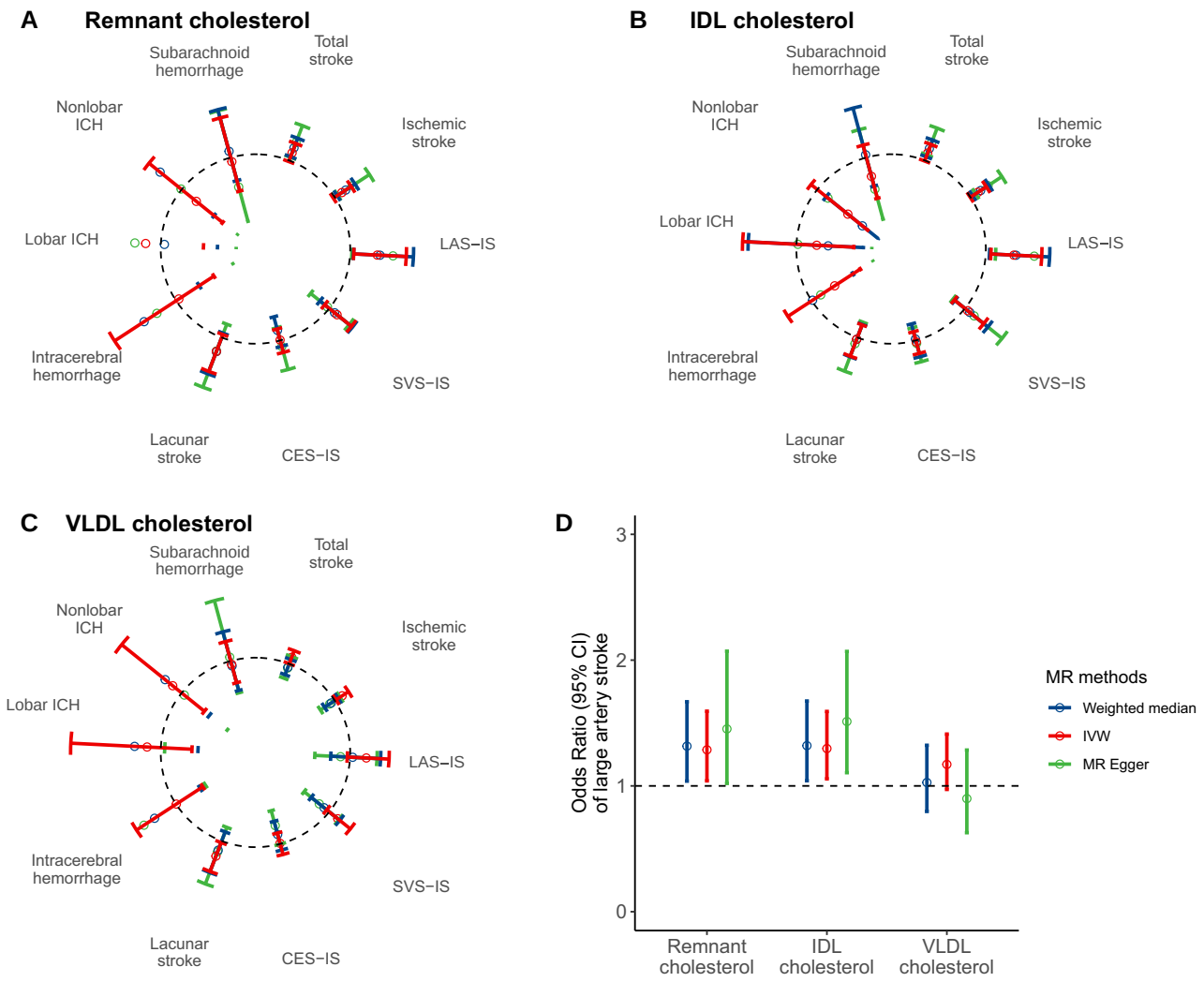
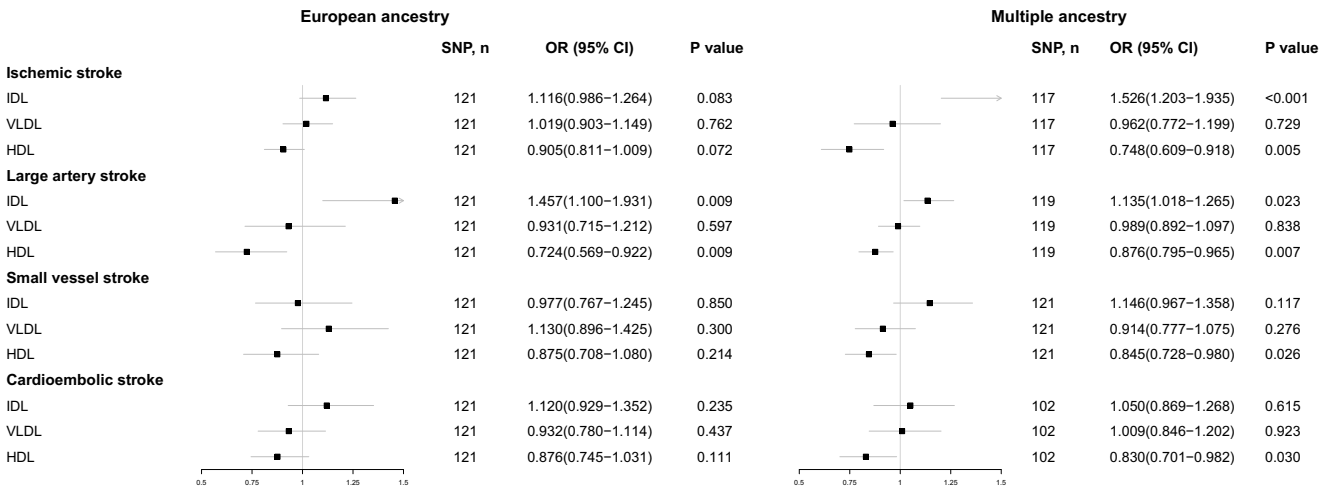


Fig. 2. Causal effect of remnant cholesterol traits on LAS and other stroke subtypes assessed by using a univariable MR analysis. A) Total remnant cholesterol and the risk of stroke; B) IDL cholesterol and the risk of stroke; C) VLDL cholesterol and the risk of stroke; D) remnant cholesterol traits on LAS. IDL, intermediate-density lipoprotein; VLDL, very-low-density lipoprotein; IVW, inverse variance weighted; MR, Mendelian randomization; IS, ischemic stroke; LAS, large artery atherosclerosis stroke; SVS, small vessel stroke; CES, cardioembolic stroke; ICH, intracerebral hemorrhage.



	European ancestry			Multiple ancestry		
	SNP, n	OR (95% CI)	P value	SNP, n	OR (95% CI)	P value
Ischemic stroke						
IDL	121	1.116(0.986−1.264)	0.083	117	1.526(1.203−1.935)	<0.001
VLDL	121	1.019(0.903−1.149)	0.762	117	0.962(0.772−1.199)	0.729
HDL	121	0.905(0.811−1.009)	0.072	117	0.748(0.609−0.918)	0.005
Large artery stroke						
IDL	121	1.457(1.100−1.931)	0.009	119	1.135(1.018−1.265)	0.023
VLDL	121	0.931(0.715−1.212)	0.597	119	0.989(0.892−1.097)	0.838
HDL	121	0.724(0.569−0.922)	0.009	119	0.876(0.795−0.965)	0.007
Small vessel stroke						
IDL	121	0.977(0.767−1.245)	0.850	121	1.146(0.967−1.358)	0.117
VLDL	121	1.130(0.896−1.425)	0.300	121	0.914(0.777−1.075)	0.276
HDL	121	0.875(0.708−1.080)	0.214	121	0.845(0.728−0.980)	0.026
Cardioembolic stroke						
IDL	121	1.120(0.929−1.352)	0.235	102	1.050(0.869−1.268)	0.615
VLDL	121	0.932(0.780−1.114)	0.437	102	1.009(0.846−1.202)	0.923
HDL	121	0.876(0.745−1.031)	0.111	102	0.830(0.701−0.982)	0.030

Fig. 3. Multivariable MR analysis effect estimates of IDL cholesterol and VLDL cholesterol on IS and its subtypes using the GWAS statistics from European (left) and multiple ancestry (right). IDL, intermediate-density lipoprotein; VLDL, very-low-density lipoprotein; HDL, high-density lipoprotein; OR, odds ratio; CI, confidence interval; SNP, single-nucleotide polymorphism.

multivariable MR approach. Beyond the association reported in observational studies, this current analysis applied the MR approach to evaluate the causal effect of remnant cholesterol traits on the risk of stroke, which supports a causal association of remnant cholesterol and IDL cholesterol levels with LAS in the European population.

Previous observational studies have examined the association of remnant cholesterol and stroke particularly for IS. In the Copenhagen General Population Study of 102,964 individuals, individuals with high remnant cholesterol concentrations had a higher risk of IS up to 14 years of follow-up (7). A cohort study from the Copenhagen General Population Study and the Copenhagen City Heart Study also confirmed the association between elevated remnant cholesterol levels and a higher risk of IS (6). In a post hoc analysis from the Kailuan Study cohort, remnant cholesterol variability was also associated with a higher risk of IS in the general population (27). The fact is that remnant cholesterol concentration was calculated using a standard lipid profile in these cohort studies. And the causality of remnant cholesterol with the risk of stroke could not be claimed due to unmeasured bias in the traditional epidemiological designs. In this current study, the summary statistics of remnant cholesterol traits were based on a targeted high-throughput NMR metabolomics platform (18). Using the MR design, we quantified the causal association of remnant cholesterol with IS consistent with previous cohort studies. Moreover, the exposure effect estimation in the observational study is potentially attenuated or amplified due to unmeasured bias. The MR framework utilizes the GWAS data as instrumental variables to infer the effect of exposures, which could supplement the causal evidence between risk factors and outcomes. This current study, together with previous epidemiological studies, could confirm the causal relationship between IDL cholesterol and IS, particular for LAS-IS.

Due to the different pathogenesis and pathophysiology among the subtypes of stroke, remnant cholesterol may have a different effect on the risk of stroke subtypes. There is no evidence of associations between remnant cholesterol and the subtypes of stroke. In our MR analysis, findings indicated a causal association between remnant cholesterol and LAS-IS, but not intracerebral hemorrhage, subarachnoid hemorrhage, and other IS subtypes. IDL cholesterol and VLDL cholesterol are the main components of remnant cholesterol in the fasting state (28). VLDL triglyceride is metabolized in muscle and adipose tissue forming IDL, which is taken up by the liver or further metabolized to LDL. In this context, we found a strong and consistent causal effect of IDL cholesterol on the risk of LAS-IS. The underlying mechanism most likely involves lipoprotein remnants penetrating the arterial wall, where the cholesterol content is easily accumulated and thereby leads to atherosclerosis and low-grade inflammation in the large cerebral artery (29). A cohort study of 90,438 UK Biobank participants found that there was no significant association of IDL cholesterol and VLDL cholesterol with IS (30), among which the adverse effect of IDL cholesterol could be concealed by subtypes of stroke. On the contrary, other studies reported that VLDL and IDL particles were significantly associated with IS (31, 32). The effects of common risk factors for stroke may be stratified by different subtypes, as reported from the previous studies (33–36). In addition to LDL particles, small VLDL and IDL particles are also able to enter the arterial intima and be retained within the subendothelial extracellular matrix (37). Sphingomyelinase (SMase) as a small VLDL- and IDL-modifying enzyme may be another mechanism of remnant particle accumulation in atherogenesis, namely enhanced retention of atherogenic TRL particles in intimal areas expressing extracellular SMase activity.

Our results indicated that randomized clinical trials (RCTs) with remnant cholesterol lowering in individuals with high concentrations with the aim of preventing IS and LAS-IS are needed. RCTs with statins have shown beneficial effects on lowering the risk of stroke, mainly by lowering LDL cholesterol (38). The fact is that statins provide a significant reduction in remnant lipoprotein cholesterol (39). Thus, stroke-reducing effect of statins could be attributed to the lowering of both LDL cholesterol and remnant cholesterol traits and the concomitantly enhanced cellular cholesterol efflux (40). A recent RCT study in type 2 diabetes patients showed that Pemafibrate could significantly reduce the levels of remnant cholesterol and VLDL cholesterol, but without lowering the primary efficacy endpoint of a composite of nonfatal myocardial infarction, IS, coronary revascularization, or death from cardiovascular causes (41). More evidence is needed for the effect of lowering remnant cholesterol and IDL cholesterol on IS and IS subtypes. In addition, the partial inconsistency of population heterogeneity should be acknowledged. In the sensitivity analysis (Fig. 3), we found that the effect of IDL cholesterol on IS was altered to be significant when using GWAS data of multiple ancestries. But the two-sample MR design is generally based on two nonoverlapped homogenous populations. Thus, the primary conclusion should largely rely on the data on European ancestry. The most important point is that the causal effect of IDL cholesterol on LAS-IS was repeatedly confirmed using data of European and multiple ancestries from METASTROKE and GIGASTROKE consortiums.

There are potential limitations to this study. First, the selected instrument SNPs are probably associated with multiple factors or pathways. In this study, we used MR-Egger to test for potential pleiotropy. No pleiotropy was observed for the causal association of remnant cholesterol and IDL cholesterol with the risk of LAS-IS. In addition, we performed multivariable MR analysis to evaluate the independent causal effects of IDL cholesterol and VLDL cholesterol on the risk of stroke subtypes. Second, the MR method assumes a linear relationship between exposure and outcome, and we cannot rule out the possibility of a nonlinear causal relationship between remnant cholesterol traits and risk of stroke or subtypes. For example, a cohort study suggested a U-shaped relationship between HDL cholesterol and stroke (42). Third, the MR method examines only the effects of lifetime exposure to remnant cholesterol traits on the risk of stroke, which might differ from the effects of real-world situations (e.g. lipid-lowering therapy, age-specific effect of exposure, and interaction of dietary factors). Due to the use of summary-level data from publicly available databases in this study, the calculation of a polygenic risk score was not feasible. The causal effect of remnant cholesterol on IS warrants further exploration and comparison using a one-sample MR design. Fourth, our analyses were primarily based on datasets involving individuals of European ancestry and might thus not be applicable to other ancestries. Nevertheless, the results of sensitivity analysis using summary GWAS statistics of stroke from multiancestry meta-analysis showed a consistent causal effect estimation of remnant cholesterol traits.

Conclusion

Our study indicated the causal associations of genetically predicted remnant cholesterol with the risk of LAS-IS driven by IDL cholesterol. Further studies are warranted to elucidate the

possible clinical effect of remnant cholesterol–lowering therapy on the risk of large artery atherosclerosis-IS, as well as its underlying mechanisms.

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Supplementary Material

Supplementary material is available at *PNAS Nexus* online.

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Author Contributions

X.G., B.G., and Q.Z. had full access to all the data in the study and took responsibility for ensuring the integrity of the data and the accuracy of the data analysis. Concept and design: Z.W., B.G., and X.G.; acquisition, analysis, or interpretation of the data: Z.W., Z.G., Y.Z., and X.L.; drafting of the manuscript: Z.W., Y.J., H.Z., and L.T.; critical revision of the manuscript for important intellectual content: Z.W., P.L., L.B., and Y.W. All authors approved the submitted version and agreed to its publication.

Data Availability

GWAS summary-level data are publicly available. The data and code are available upon reasonable request.

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