Effects of remnant cholesterol on the efficacy of genotype-guided dual antiplatelet in *CYP2C19* loss-of -function carriers with minor stroke or transient ischaemic attack: a post-hoc analysis of the CHANCE-2 trial

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eClinicalMedicine
2024;67: 102357

Published Online xxx
https://doi.org/10.1016/j.eclinm.2023.102357

Summary

Background The atherogenicity of remnant cholesterol (RC), a contributor to residual risk of cardiovascular events, has been underlined by recent guidelines. We aimed to evaluate the relationship between RC levels and the efficacy and safety of genotype-guided dual antiplatelet therapy in the CHANCE-2 trial.

Methods This post-hoc study used data from the CHANCE-2 trial, which was a randomised, double-blind, placebo-controlled trial of 6412 patients (aged >40 years) enrolled from 202 hospitals in China, between Sept 23, 2019, and March 22, 2021, who carried *CYP2C19* loss-of-function alleles and had either an acute minor stroke or high-risk transient ischaemic attack (TIA), and could start treatment within 24 h of symptom onset. Participants received either (1:1) ticagrelor plus aspirin (control) or clopidogrel plus aspirin (intervention) and the effect of reducing the 3-month risk of any new stroke was assessed (ischemic or haemorrhagic, primary outcome). From the CHANCE-2 study population, we enrolled 5890 patients with complete data on RC. The cutoff point of RC for distinguishing patients with greater benefit from ticagrelor-aspirin versus clopidogrel-aspirin was determined with subpopulation treatment effect pattern plot. The primary efficacy and safety outcome was recurrent stroke and severe or moderate bleeding within 90 days, respectively. CHANCE-2 is registered with ClinicalTrials.gov, NCT04078737.

Findings The cutoff to define elevated RC was 0.91 mmol/L. Ticagrelor-aspirin versus clopidogrel-aspirin was associated with a reduced risk of recurrent stroke in patients with non-elevated RC levels (122 [5.3%] versus 179 [7.8%]; hazard ratio [HR], 0.68; 95% confidence interval [CI], 0.54–0.85), but this benefit was absent in those with elevated RC levels (58 [8.4%] versus 48 [7.3%]; HR, 1.10; 95% CI, 0.73–1.65; *P*-interaction = 0.03). When analyzed as a continuous variable, the benefit of ticagrelor-aspirin on recurrent stroke decreased as RC levels increased. The rates of severe or moderate bleeding between treatment groups were similar across RC categories (0.3% versus 0.3%, *P*-interaction = 0.95).

Interpretation Our post-hoc findings suggest that RC could be a potential biomarker to discriminate patients who received more benefits from ticagrelor-aspirin versus clopidogrel-aspirin therapy in *CYP2C19* loss-of-function carriers with minor stroke or TIA. These findings need to be validated in an independent study.

Funding The National Key Research and Development Program of China, Beijing Natural Science Foundation Haidian original innovation joint fund, Fund for Young Talents of Beijing Medical Management Center, the high-level public health talents, Training Fund for Open Projects at Clinical Institutes and Departments of Capital Medical University; and Salubris.

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Keywords: Remnant cholesterol; Ticagrelor-aspirin; Clopidogrel-aspirin; Recurrent stroke; CHANCE-2 trial

Research in context

Evidence before this study

We conducted a literature search of Web of Science and PubMed using the search term "(remnant cholesterol) AND (dual antiplatelet therapy or antiplatelet therapy or Ticagrelor or Clopidogrel or Aspirin) AND (stroke) AND (*CYP2C19*)" for the time period of database inception up to Oct 23, 2023, without any language restrictions. No studies were found that met these criteria. However, when we broadened the search to include "platelet activity or platelet function" we identified one original study that aimed to test the interaction between lipid parameters and platelet function in patients with coronary artery disease (CAD) on dual anti-platelet therapy. The results showed that remnant cholesterol was not significantly associated with platelet function in patients with dual antiplatelet therapy of aspirin in combination of *P2Y12* inhibitor of clopidogrel, prasugrel, or ticagrelor.

Added value of this study

To the best of our knowledge, no study has investigated the relationship of remnant cholesterol levels with the efficacy and safety of genotype-guided dual antiplatelet therapy in *CYP2C19* loss-of-function carriers with minor stroke or transient ischemic attack (TIA). Our post-hoc study of the CHANCE-2 trial found that ticagrelor-aspirin versus clopidogrel-aspirin was associated with a reduced risk of recurrent stroke in patients with non-elevated remnant cholesterol levels, but this benefit was absent in those with elevated remnant cholesterol levels. When analysed as a continuous variable, the benefit of ticagrelor-aspirin on recurrent stroke decreased as remnant cholesterol levels increased. The rates of severe or moderate bleeding between treatment groups were similar across remnant cholesterol categories.

Implications of all the available evidence

The findings of our secondary analysis of the CHANCE-2 trial indicate that baseline remnant cholesterol level may discriminate the benefits of genotype-guided dual antiplatelet therapy in *CYP2C19* LOF carriers with minor stroke or TIA. Patients with non-elevated remnant cholesterol received more benefits from ticagrelor-aspirin therapy without increasing in bleeding events, compared to clopidogrel-aspirin therapy. The findings suggested that remnant cholesterol, as a convenient parameter, may be applied to guide individualised antiplatelet therapy in clinical practice. These findings need to be validated in an independent study.

Introduction

Antithrombotic trials have proved the efficacy of dual antiplatelet therapy with clopidogrel-aspirin or ticagrelor-aspirin for treating patients with minor stroke or transient ischemic attack (TIA).[1–4] Whereas, a considerable proportion of patients experience recurrent stroke despite receiving appropriate antiplatelet treatments. The high residual risk of recurrent stroke may be attributable to the poor individual response to antiplatelet therapy. Thus, early identification and management of these patients is of greatly importance for the secondary prevention of stroke.

Atherogenic dyslipidemia, characterised by high triglyceride-rich lipoproteins and normal low-density lipoprotein (LDL) cholesterol, is a common lipid disorder associated with increased cardiovascular disease risk.[5] It is considered as one of the main causes of lipid-dependent residual risk, regardless of LDL cholesterol concentration.[5–9] Recent investigations revealed that the residual risk might be attributable to the role of remnant cholesterol (RC).[5,10–12] As the cholesterol content of triglyceride-rich lipoproteins, RC, composed of very-low and intermediate density lipoproteins (VLDL and IDL) in the fasting state and chylomicron remnants in the nonfasting state,[13] is increasingly acknowledged as an important causal risk factor in the development of atherosclerotic cardiovascular disease in various studies.[5,13–17] It has been reported that the components of RC were highly atherogenic and acted by enhancing system inflammation, platelet activation, coagulation, thrombus formation, and macrophage foam cell formation, as a consequence, inadequate platelet inhibition.[18,19] However, current evidence regarding the effects of RC on platelet activation was limited and inconsistent.[20–23] Additionally, little is known about whether the benefits of different dual antiplatelet strategies of aspirin in combination with different P2Y12-receptor inhibitors varied with residual risk measured by RC levels in patients with a history of stroke.

In this secondary analysis, we aimed to investigate the effect of RC levels on the efficacy and safety of ticagrelor-aspirin versus clopidogrel-aspirin in patients with minor ischemic stroke or TIA who carried *CYP2C19* loss-of-function (LOF) alleles, based on data from the CHANCE-2 (Ticagrelor or Clopidogrel with Aspirin in High-Risk Patients with Acute Nondisabling Cerebrovascular Events II) trial.

Methods

Study design and participants

This post-hoc study was conducted based on the CHANCE-2 trial, which was a randomised, double-blind, placebo-controlled trial enrolling 6412 patients from 202 hospitals in China between Sept 23, 2019, and March 22, 2021. Details on the study design, protocol, and primary results of the CHANCE-2 trial have been published elsewhere.[4,24] Included patients were aged 40 or older, had either an acute minor stroke (defined by a National Institutes of Health Stroke Scale score ≤3) or high-risk TIA (defined by a ABCD^2 [age, blood pressure, clinical features, duration of TIA, and the presence or absence of diabetes mellitus] score ≥4), carried *CYP2C19* LOF alleles identified using a rapid point-of-care genotyping system, and could started the study drug treatment within 24 h of symptom onset. We excluded 522 patients with missing data on RC (Fig. 1).

The trial was approved by the ethics committee at Beijing Tiantan Hospital (IRB approval number: KY2019-035-02) and all participating centres. Written informed consent was provided by all the patients or their representatives before enrollment. CHANCE-2 is registered with ClinicalTrials.gov, NCT04078737.

Randomisation, masking, and treatment

Within 24 h after symptom onset, patients were randomly assigned in a 1:1 ratio to receive 90-days of ticagrelor (180 mg loading dose on day 1 followed by 90 mg twice daily on days 2–90 plus placebo clopidogrel) or clopidogrel (300 mg loading dose on day 1 followed by 75 mg daily on days 2–90 plus placebo ticagrelor). All patients received 21-days of aspirin (75–300 mg loading dose on day 1 followed by 75 mg daily for 21 days). To ensure blinding, all individuals involved, including participants, investigators, clinical research associates, data analysts, and laboratory staff, were unaware of the group allocation. Within CHANCE-2, as per the study protocol, placebos for clopidogrel or ticagrelor were identical to the active drugs in appearance and taste. Minor side effects are unusual with the medication, so it was not anticipated that either participants or clinicians would be able to differentiate the placebos from the active drugs.

Measurements of RC

Fasting venous blood samples were drawn in serum separator tubes after randomisation for the biochemical measurements. Serum lipids were measured by enzymatically with the Hitachi automated analyser 7600 by laboratory personnel unaware of the clinical data. The collection, preservation, and processing of the blood samples were performed in accordance with laboratory's policies and procedures in each study centre. RC was calculated as total cholesterol minus measured LDL cholesterol minus measured high density lipoprotein (HDL) cholesterol.[5]

Outcomes

The primary efficacy outcome was new ischemic or hemorrhagic stroke within 90 days. The secondary efficacy outcomes included new stroke within 30 days, a vascular event (a composite of stroke, TIA, myocardial infarction or vascular death) within 90 days, and ischemic stroke within 90 days.

The primary safety outcome was severe or moderate bleeding which was defined using the Global Utilization of Streptokinase and Tissue Plasminogen Activator for

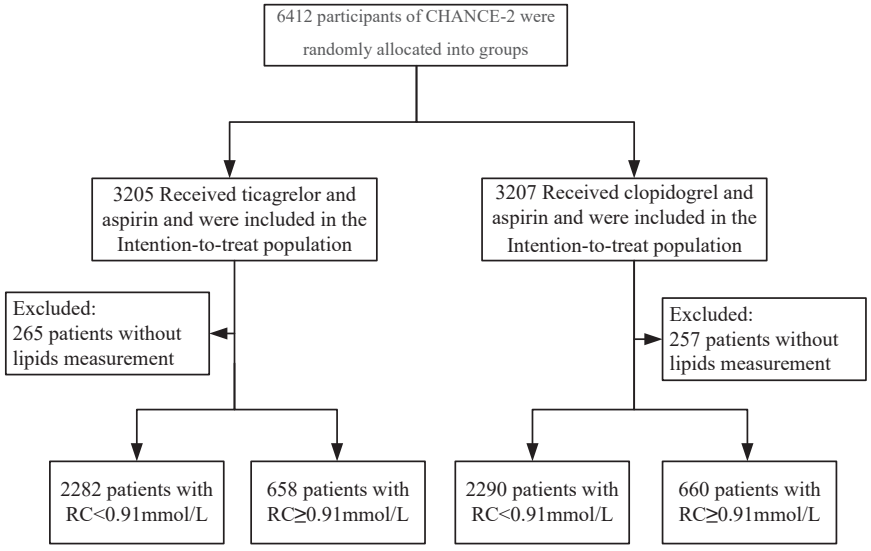

***Fig. 1:* Flowchart of the study**. RC denotes remnant cholesterol.

Occluded Coronary Arteries (GUSTO) criteria within 90 days.[25] Secondary safety outcome was any bleeding through 90 days of follow-up, details of bleeding events have been reported previously.[26] All efficacy and safety outcomes were confirmed by an independent clinical event adjudication committee, whose members were unaware of the trial group assignments.

Statistical analysis

Since the threshold of RC level to predict recurrent stroke remained undetermined, we used the subpopulation treatment effect pattern plot (STEPP) methodology to determine the cutoff. The STEPP was a common method performed to evaluate treatment-effect heterogeneity when the hazard ratio (HR) expression was measured on a continuous scale.[27–30] To determine the cutoff of RC for distinguishing patients with greater benefit from this treatment arm, the change point of the subpopulation RC, making the beginning of greater divergence, was selected, and serial interactions between treatment groups and RC were explored.

Categorical variables were presented as percentages and continuous variables as medians with interquartile ranges (IQR). Baseline characteristics were analysed by the $\chi 2$ test for the categorical variables and Wilcoxon test for the continuous variables. Kaplan–Meier product limit method was used to generate survival plots on the primary efficacy outcome. Differences in the outcomes during the 90-day follow-up period were assessed using a Cox proportional hazard regression model with study centres set as a random effect, and HRs with 95% confidence intervals (CIs) were reported. When there were multiple events of the same type, the time to the first event was used in the model. Data from patients who had no event during 90-day follow-up were censored at termination of the trial or nonvascular death. Interactions of treatments with RC categories were evaluated with the addition of treatment by RC categories in the Cox models. We also evaluated the impact of RC levels as continuous variables on the effect of dual-antiplatelet therapy in preventing recurrent stroke assuming a linear relationship. To test the robustness of the findings, two sensitivity analyses were further performed. First, we excluded patients with lipid-lowering treatment to avoid the effects of lipid-lowering on the lipid parameters and recurrent stroke. Second, the analyses were repeated in patients with a low LDL cholesterol concentration (<100 mg/dL), as suggested by most guidelines for the secondary prevention of cardiovascular events.

All statistical analyses were performed with SAS statistical software, version 9.4 (SAS Institute Inc) and R software (version 4.2.2). All tests were 2-sided, and $P < 0.05$ was considered statistically significant.

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing the report.

Results

Determination of the RC cutoff

In the STEPP, the event rate curve in the HR of the primary efficacy outcome in the ticagrelor-aspirin group and that in the clopidogrel-aspirin group gradually narrowed with increasing RC levels. Notably, the two curves intersected at the subpopulation of 0.91 mmol/L for RC in terms of survival rate and the difference in HR (Fig. 2). Additionally, in the subpopulation of 0.91 mmol/L for RC, the difference in the HR was markedly over zero, and the HR was below 1 (Fig. 2). Accordingly, the cutoff point of RC determined in our study was 0.91 mmol/L.

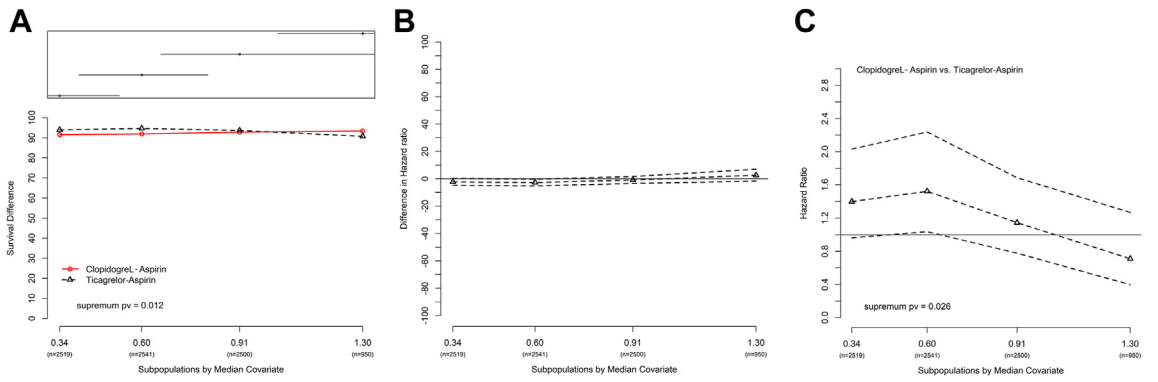

Fig. 2: **Subpopulation treatment effect pattern plot for treatment groups and remnant cholesterol**. The treatment effect is defined as the primary outcome of new stroke within 3 months. Plots showed that treatment effects in subpopulations denoted by median remnant cholesterol on the X-axis. Treatment effects were shown as a (A) differences in survival rate; (B) differences in hazard ratio, and (C) hazard ratios with 95% confidence interval. The supremum *P* value denotes the interaction term derived from subpopulation treatment effect pattern plot analysis. x-axis: subpopulations by median remnant cholesterol.

Baseline characteristics

From CHANCE-2, 5890 patients with complete data on RC were included. Baseline characteristics between patients included and excluded were well balanced, except that the patients included were more likely to have a higher level of diastolic blood pressure, a higher proportion of ischemic stroke history and current smokers. There was no difference in the rate of recurrent stroke within 90 days between the two groups of patients (Table S1).

Among the patients in this study, the median RC levels were 0.60 mmol/L (IQR, 0.38–0.87 mmol/L), 4572 patients (77.6%) with RC <0.91 mmol/L, and 1318 patients (22.4%) with elevated RC (RC ≥0.91 mmol/L). Baseline characteristics were not significantly different between ticagrelor-aspirin and clopidogrel-aspirin groups within RC categories (Table 1).

Efficacy and safety outcomes

The proportional hazards assumption was met in all analyses. As presented in Table 2 and Fig. 3, ticagrelor-aspirin significantly reduced the risk of recurrent stroke within 90 days in patients with non-elevated RC

Characteristics	RC <0.91 mmol/L (N = 4572)		RC ≥0.91 mmol/L (N = 1318)	
	Ticagrelor-aspirin (N = 2282)	Clopidogrel-aspirin (N = 2290)	Ticagrelor-aspirin (N = 658)	Clopidogrel-aspirin (N = 660)
Median age (IQR)–yr	65.4 (57.2–71.7)	64.9 (57.2–71.8)	63.4 (56.1–70.1)	63.5 (55.6–69.5)
Female sex–no. (%)	717 (31.4)	746 (32.6)	263 (40.0)	248 (37.6)
Han ethnicity–no. (%)	2236 (98.0)	2236 (97.6)	650 (98.8)	651 (98.6)
Median blood pressure (IQR)–mm Hg				
Systolic	148 (135–162)	148 (135–160)	150 (138–163)	150 (137–164)
Diastolic	86 (80–94.5)	86 (80–95)	88 (80–96)	86.5 (80–96)
Medical history–no. (%)				
Hypertension	1384 (60.6)	1421 (62.1)	421 (64.0)	409 (62.0)
Diabetes mellitus	539 (23.6)	508 (22.2)	205 (31.2)	186 (28.2)
Dyslipidemia	224 (9.8)	213 (9.3)	68 (10.3)	54 (8.2)
Previous ischemic stroke	506 (22.2)	513 (22.4)	116 (17.6)	124 (18.8)
Previous TIA	35 (1.5)	36 (1.6)	7 (1.1)	2 (0.3)
Myocardial infarction	34 (1.5)	24 (1.0)	12 (1.8)	14 (2.1)
Current smoking–no. (%)	730 (32.0)	710 (31.0)	205 (31.2)	202 (30.6)
CYP2C19 LOF allele carriers–no. (%)				
Intermediate metabolizers	1774 (77.7)	1811 (79.1)	513 (78.0)	500 (75.8)
Poor metabolizers	508 (22.3)	479 (20.9)	145 (22.0)	160 (24.2)
Median time from symptom onset to randomization, n (%)				
<12 h	963 (42.2)	919 (40.1)	250 (38.0)	261 (39.5)
≥12 h	1319 (57.8)	1371 (59.9)	408 (62.0)	399 (60.5)
Qualifying event–no. (%)				
Ischemic stroke	1850 (81.1)	1834 (80.1)	513 (78.0)	541 (82.0)
TIA	432 (18.9)	456 (19.9)	145 (22.0)	119 (18.0)
Median NIHSS score in patients with qualifying ischemic stroke (IQR)[a]	2 (1–3)	2 (1–3)	2 (1–3)	2 (1–3)
Median ABCD^2 score in patients with qualifying TIA (IQR)[b]	4 (4–5)	4 (4–5)	4 (4–5)	4 (4–5)
Previous antiplatelet therapy–no. (%)[c]	277 (12.1)	268 (11.7)	73 (11.1)	69 (10.5)
Previous lipid-lowering therapy–no. (%)[c]	182 (8.0)	188 (8.2)	50 (7.6)	35 (5.3)
Antihypertensive therapy during the treatment–no. (%)	2182 (95.6)	2175 (95.0)	638 (97.0)	627 (95.0)
Lipid-lowering therapy during the treatment–no. (%)	1279 (56.0)	1274 (55.6)	400 (60.8)	371 (56.2)
Symptomatic ICAS, n (%)	857 (40.5)	868 (40.8)	264 (42.6)	224 (37.1)
Symptomatic ECAS, n (%)	192 (9.1)	177 (8.3)	60 (9.7)	57 (9.4)
Total cholesterol, mmol/L	4.4 (3.8–5.1)	4.4 (3.8–5.1)	5.3 (4.7–6.0)	5.3 (4.3–6.10)
Triglyceride, mmol/L	1.3 (0.9–1.7)	1.3 (0.9–1.7)	2.5 (1.7–3.4)	2.4 (1.6–3.5)
LDL-C, mmol/L	2.8 (2.2–3.4)	2.8 (2.2–3.4)	3.0 (2.4–3.6)	2.9 (2.3–3.6)
HDL-C, mmol/L	1.1 (1.0–1.4)	1.1 (1.0–1.3)	1.1 (0.9–1.3)	1.0 (0.9–1.3)

ECAS denotes extracranial artery stenosis. ICAS denotes intracranial artery stenosis. IQR denotes interquartile range. LOF denotes loss-of-function. LDL-C denotes low density lipoprotein cholesterol. HDL-C denotes high density lipoprotein cholesterol. RC denotes remnant cholesterol. TIA denotes transient ischemic attack. [a]National Institutes of Health Stroke Scale (NIHSS) scores range from 0 to 42, with higher scores indicating more severe stroke. [b]ABCD^2 score assesses the risk of stroke on the basis of age, blood pressure, clinical features, duration of TIA, and presence or absence of diabetes, with scores ranging from 0 to 7 and higher scores indicating greater risk. [c]Medication within 1 month before symptom onset.

Table 1: **Baseline characteristics according to RC and treatment.**

Outcomes	RC < 0.91 mmol/L				RC ≥0.91 mmol/L				P_{int}
	Ticagrelor-aspirin event rate (%)[a]	Clopidogrel-aspirin event rate (%)[a]	HR (95% CI)	*P* value	Ticagrelor-aspirin event rate (%)[a]	Clopidogrel-aspirin event rate (%)[a]	HR (95% CI)	*P* value	
Primary outcome									
Stroke	122 (5.3)	179 (7.8)	0.68 (0.54–0.85)	<0.001	55 (8.4)	48 (7.3)	1.10 (0.73–1.65)	0.64	0.03
Secondary outcome									
Stroke within 30 days	102 (4.5)	153 (6.7)	0.66 (0.51–0.85)	<0.001	41 (6.2)	39 (5.9)	1.02 (0.64–1.62)	0.95	0.11
Composite vascular events[b]	153 (6.7)	216 (9.4)	0.70 (0.57–0.86)	<0.001	59 (9.0)	56 (8.5)	1.05 (0.71–1.54)	0.81	0.07
Ischemic stroke	121 (5.3)	175 (7.6)	0.68 (0.54–0.86)	<0.001	54 (8.2)	47 (7.1)	1.11 (0.73–1.67)	0.63	0.04
Primary safety outcome									
Severe or moderate bleeding[c]	7 (0.3)	8 (0.3)	0.91 (0.33–2.54)	0.86	2 (0.3)	2 (0.3)	0.89 (0.12–6.31)	0.90	0.95
Secondary safety outcome									
Any bleeding	117 (5.1)	58 (2.5)	2.13 (1.54–2.94)	<0.001	41 (6.2)	18 (2.7)	2.13 (1.18–3.84)	0.01	0.99

CI denotes confidence interval. ESER denotes Essen Stroke Risk Score. HR denotes hazard ratio. mRS denotes modified Rankin Scale. TIA denotes transient ischemic attack. [a]Event rates for ordinal stroke or TIA are raw estimates, whereas event rates for other outcomes are Kaplan–Meier estimates of the percentage of patients with events at 90 days. [b]Composite vascular events include ischemic stroke, hemorrhagic stroke, TIA, myocardial infarction, vascular death. [c]Severe or moderate bleeding and mild bleeding were defined according to GUSTO (Global Utilization of Streptokinase and Tissue Plasminogen Activator for Occluded Coronary Arteries) criteria.

***Table 2*: Efficacy and Safety Outcomes of patients with different antiplatelet therapies stratified by RC.**

(RC <0.91 mmol/L) (122 [5.3%] versus 179 [7.8%]; HR, 0.68; 95% CI, 0.54–0.85; $P < 0.001$), while the benefit disappeared in patients with elevated RC (58 [8.4%] versus 48 [7.3%]; HR, 1.10; 95% CI, 0.73–1.65; $P = 0.64$) ($P = 0.03$ for interaction). When considered as a continuous variable, RC levels also significantly modulated the effect of ticagrelor-aspirin on the primary outcome when the relationship was evaluated assuming linearity. As RC levels increased, the risk of recurrent stroke within 90 days increased in patients receiving ticagrelor-aspirin compared with those receiving clopidogrel-aspirin (Fig. 4). Secondary outcomes yielded similar trends with the primary outcome (Table 2).

The primary safety outcome of severe or moderate bleeding occurred with similar frequency in the ticagrelor-aspirin group and clopidogrel-aspirin group independent of RC levels (0.3% versus 0.3% in both groups, *P* for interaction = 0.95). Similarly, the rate of any bleeding was similar in patients with different RC categories (Table 2).

Sensitivity analyses

After excluding 455 patients with previous lipid-lowering, the results did not change materially (Table S2). Ticagrelor-aspirin was associated with a reduced rate of recurrent stroke in patients with non-elevated RC (HR, 0.64; 95% CI, 0.50–0.82; $P < 0.001$), but not in patients with elevated RC (HR, 1.09; 95% CI, 0.72–1.65; $P = 0.69$) ($P = 0.03$ for interaction). Additionally, the trends persisted in patients with LDL cholesterol <100 mg/dL (2.6 mmol/L) (N = 2416), the benefit of ticagrelor-aspirin versus clopidogrel-aspirin

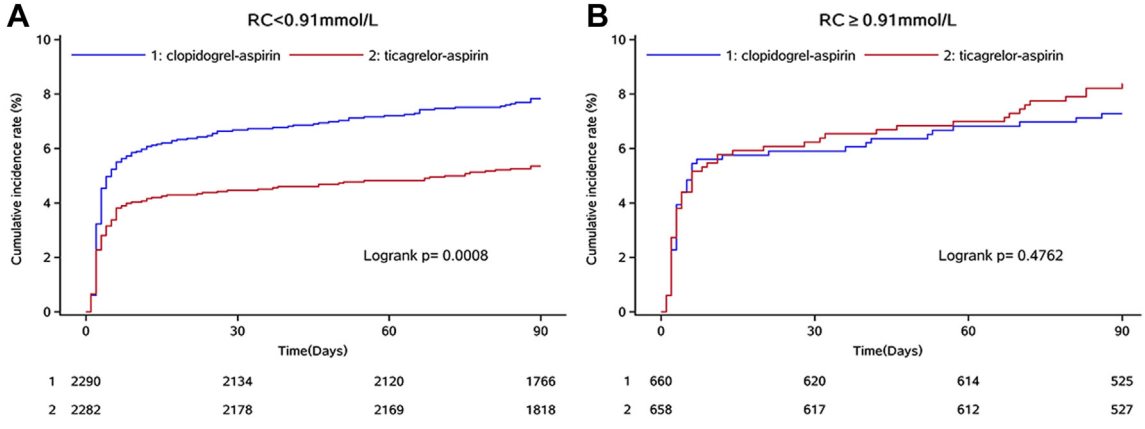

***Fig. 3*: Cumulative probability of stroke according to RC levels (A. RC < 0.91 mmol/L and B. RC ≥ 0.91 mmol/L) and dual-antiplatelet treatments**. RC denotes remnant cholesterol.

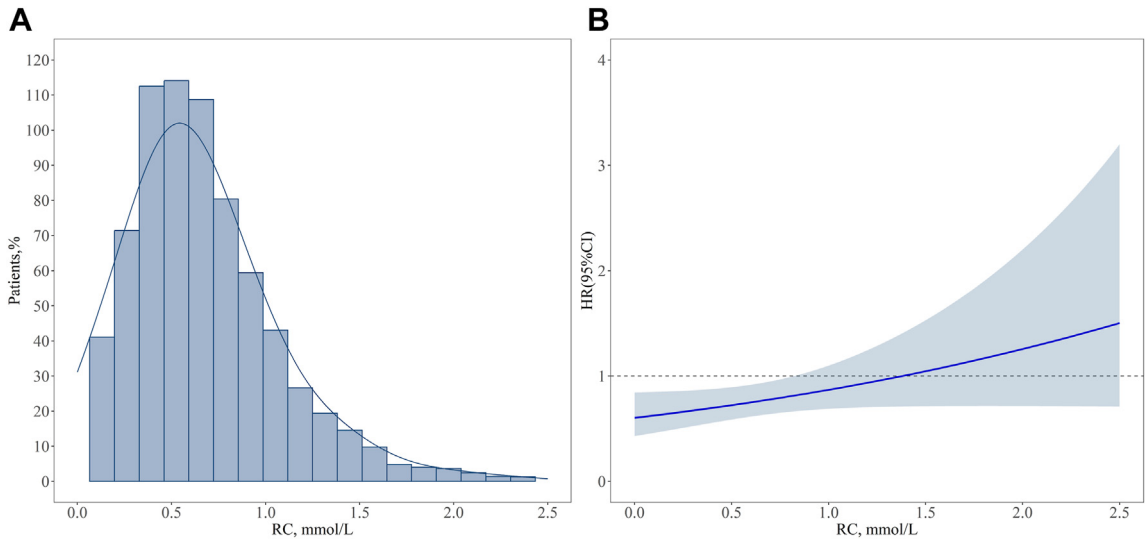

Fig. 4: **Distribution of RC and effects of ticagrelor-aspirin versus clopidogrel-aspirin on the primary efficacy outcome by RC as a continuous variable**. CI denotes confidence interval. RC denotes remnant cholesterol. HR denotes hazard ratio. Figure A shows the distribution of RC. Figure B shows the linear association between RC and hazard ratio for the primary efficacy outcome. The significant interaction test indicates that the slope is significantly different from zero.

was also observed in patients with non-elevated RC (P = 0.04 for interaction) (Table S3). No significant difference in safety outcomes across RC categories was observed in both sensitivity analyses.

Discussion

This post-hoc analysis of the CHANCE-2 trial found that RC discriminated the efficacy of ticagrelor-aspirin and clopidogrel-aspirin in *CYP2C19* LOF carriers with minor stroke or TIA. Specifically, with respect to patients with non-elevated RC, ticagrelor-aspirin therapy reduced the risk of recurrent stroke within 90 days by 32% and without increased bleeding events, compared with clopidogrel-aspirin therapy.

Although LDL-C and non-HDL-C are major atherogenic lipids, our exploratory results showed that as LDL-C levels increased, the risk of recurrent stroke within 90 days did not significantly increase in patients receiving ticagrelor-aspirin compared with those receiving clopidogrel-aspirin. However, there was no significant interaction between LDL-C levels and dual-antiplatelet therapy in terms of recurrent stroke within 90 days (P for interaction = 0.36) (Figure S1). As previously reported by the CHANCE trial, the efficacy of dual-antiplatelet therapies did not differ by LDL-C levels, thus we needed to explore other potential lipids biomarkers to determine patients who would receive more benefits from dual-antiplatelet therapy. In terms of non-HDL, it was equivalent to the combined LDL-C, remnant cholesterol, and lipoprotein(a) cholesterol. Considering the non-significant component of LDL-C, and the undermined role of remnant cholesterol and other components in non-HDL-C, we primarily investigate the role RC, rather non-HDL-C in our study. While the effects of non-HDL-C on the efficacy and safety of dual-antiplatelet deserved to be explored in our future investigations.

Our study used fasting blood samples to calculated RC. The composition of RC was different in the fasting and nonfasting state, with VLDL and IDL in the fasting state and of chylomicron remnants in the nonfasting state. Studies reported that compared with the fasting state, maximal mean changes in random, nonfasting RC was +8 mg/dL (0.2 mmol/L) in the general population. However, in most individuals, the differences in plasma fasting and nonfasting RC are small and therefore likely clinically insignificant. Additionally, the European Atherosclerosis Society recommends that nonfasting lipid profiling, including calculation of remnant cholesterol, can be used routinely in clinics given that both fasting and nonfasting conditions have been shown to be good predictors for CVD and for the evaluation of treatment response. We found the association of RC with the risk of stroke were consistent with fasting and nonfasting measurements in previous studies, indicating different states may not have an important effect on the associations of RC with the risk of stroke. Since no relevant evidence regarding the effects of RC on antiplatelet therapies among patients with minor stroke or TIA, whether the modified role of RC may be different for nonfasting state needed further investigations.

Notably, the cutoff points to determined elevated RC in the secondary prevention of atherosclerotic diseases were inconsistent without a commonly recognised

value. For example, the Copenhagen General Population Study showed that among individuals with baseline diagnoses of myocardial infarction/ischemic stroke, a lower remnant cholesterol of 0.8 mmol L–1 (32 mg dL–1) was estimated to reduce recurrent major cardiovascular event by 20% in secondary prevention.[31] While the Improving Care for Cardiovascular Disease in China (CCC) study defined elevated RC as ≥1.0 mmol/L among patients with acute coronary syndrome in the secondary prevention of atherosclerotic cardiovascular disease.[32] In our study, as RC levels increased, the risk of recurrent stroke within 90 days increased in patients receiving ticagrelor-aspirin compared with those receiving clopidogrel-aspirin, and elevated RC was defined as RC levels over 0.91 mmol/L by using the STEPP method. Additionally, using the cutoff points provided in previous studies, we still observed a significant interaction between RC levels and dual-antiplatelet therapy in terms of the risk of recurrent stroke within 3 months (P for interaction was 0.02 and 0.04, respectively, data were not shown). These results indicated that RC may be a potential biomarker to discriminate patients who received more benefits from ticagrelor-aspirin, although the precious cutoff point of RC still needed to be validated in other studies.

Experimental and human studies have illustrated the effects of RC and its components on platelet activity, generating inconsistent conclusions. A vitro experiment study explored the effects of remnant-like particles on shear-induced platelet activation and their inhibition by antiplatelet agents. The results showed that aspirin and cilostazol inhibited the enhancement of not only shear-induced platelet aggregation but also p-selectin expression and platelet-derived microparticle generation by remnant-like particles.[20] Orth et al. investigated the in-vitro effects of VLDL, chylomicrons, and chylomicron remnants from hypertriglyceridemic participants on the aggregation behavior of platelets from normolipemic donors, the results showed that triglyceride-rich lipoproteins could inhibit platelet aggregation in vitro.[22] Similarly, Mochizuki et al. also found that chylomicron remnant and VLDL remnant significantly enhanced the platelet aggregation in healthy persons.[23] However, human studies yielded inconsistent conclusions. A study enrolled 58 patients with coronary artery disease to investigate the interaction between lipid parameters and platelet function. The results showed that RC was not significantly associated with platelet function in patients with dual antiplatelet therapy of aspirin in combination of P2Y12 inhibitor of clopidogrel, prasugrel, or ticagrelor.[21] The discrepancies between experimental studies and human studies indicated that effect of RC on platelet activation may be influenced by antiplatelet therapy, however, this hypothesis was not confirmed yet. Additionally, none of these researches was conducted among patients with stroke. Our study added novelty evidence on this topic by comparing different antiplatelet strategies and the interaction of RC with antiplatelet therapy among patients with minor stroke or TIA. The results showed that ticagrelor-aspirin therapy was superior to clopidogrel-aspirin therapy in patients with non-elevated RC.

Alongside LDL cholesterol, triglyceride-rich lipoproteins (mainly RC and triglyceride) have been emerging as a new target for the secondary prevention of stroke. Several randomised clinical trials, such as the JELIS (Japan EPA Lipid Intervention Study),[33] REDUCE-IT (Reduction of Cardiovascular Events With Icosapent Ethyl–Intervention Trial),[33] STRENGTH (Long-Term Outcomes Study to Assess STatin Residual Risk Reduction With EpaNova in HiGh CV Risk PatienTs With Hypertriglyceridemia),[34] and PROMINENT (Pemafibrate to Reduce Cardiovascular OutcoMes by Reducing Triglycerides IN patiENts With diabesTes)[35] trials, have shown the potential benefits of lowering triglyceride-rich lipoproteins on reducing recurrent major cardiovascular events in patients with cardiovascular disease. Given the synergistic effects of lipid-lowering agents and antiplatelet therapies on preventing thrombotic disease, our study excluded patients who were treated with lipid-lowering agents, and the results showed that even in patients without lipid-lowering agents, the benefit of ticagrelor-aspirin on reducing recurrent stroke remained. Additionally, many studies have shown the discordance between LDL cholesterol and RC on the risk of ischemic events,[17,36,37] which persisted in the secondary prevention of stroke. Our results showed that even in patients with optimal LDL cholesterol, those with elevated RC did not receive the same benefit from ticagrelor-aspirin as those with non-elevated RC, indicating RC plays an independent role in discriminating the benefits of dual antiplatelet therapy.

Possible mechanisms underlying our findings that ticagrelor-aspirin was associated with a reduced risk of recurrent stroke in patients with non-elevated RC may be a synergistic relationship between thrombotic effects of elevated RC and the antithrombotic effects of dual antiplatelet treatment. The causal associations of RC and high risk of stroke have been widely confirmed in observational and genetic studies, which could be explained by the fact that RC causes atherosclerosis, either directly in the cerebral vessels or in more vessels like the carotid artery or in the heart, from where blood clots can cause an embolism to the cerebral arteries.[5,10–12] RC can be taken up directly by macrophages without any need for prior modification, thereby converting such cell into foam cells, which is a hallmark of atherosclerosis lesions.[38,39] Also, high RC concentrations are associated with low-grade inflammation, which could be caused by inflammation in the arterial wall and be associated with unstable plaques.[40] Studies demonstrated that individuals with high RC exhibited an increased bone marrow activity, higher monocyte counts with a higher

expression of integrins involved in adhesion to the arterial wall, and higher lipid accumulation on monocytes.[41] Therefore, high levels of platelet inhibition may not be sufficient for adequate protection against ischemic events in patients with elevated RC, and the benefit of ticagrelor over clopidogrel may not be evident in patients with elevated RC. While the more precise mechanisms underlying the results needed further investigations.

Several limitations of our study should be noted. First, RC concentration was not measured directly, it was obtained by calculation with the baseline lipid profiles, which may deviate from the actual level. However, the calculated RC is closely correlated with the directly measured RC and is convenient for routine clinical application with no extra expense, thus the calculated RC is more commonly used in population studies.[42] Second, the performance of RC as a biomarker is evaluated in the same data from which the cut-off was determined, which may leads to an overoptimistic assessment of the performance of the biomarker, thus the findings needed to be validated in an independent study. Third, this is a post hoc analysis to determine the effect of RC on the treatment efficacy, the RC groups obtained by post hoc analysis were not specifically powered for the primary or key secondary outcomes. Fifth, the RC used in our study was in the fasting state, considering the different components of RC in the fasting and nonfasting state, whether the results were available for nonfasting RC needed further explorations. Finally, the study was conducted in Chinese population, the findings needed to be validated in other ethnicities.

This post-hoc analysis of the CHANCE-2 trial found evidence to suggest that baseline RC level may discriminate the benefits of genotype-guided dual antiplatelet therapy in *CYP2C19* LOF carriers with minor stroke or TIA. Patients with non-elevated RC received more benefits from ticagrelor-aspirin therapy without increasing in bleeding events, compared to clopidogrel-aspirin therapy. The findings suggested that RC, as a convenient parameter, may be applied to guide individualised antiplatelet therapy in clinical practice, while the findings still needed to be validated in an independent study.

Contributors

AW and XM accessed and verified the underlying data. XM was responsible for the conceptualisation and design of the study. AXW, XT, and XM were responsible for acquisition, analysis and interpretation of data. XWX, HL, JJ, JXL, YLW, XQZ, ZXL, LPL and YJW were responsible for data verification. AXW and XT were responsible for writing the original draft of the manuscript. AXW and XT were responsible for review and editing of the manuscript. AW, XT, YW, and XM had access to the database and XM had final responsibility for the decision to submit for publication. All authors commented on drafts of the manuscript. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Data sharing statement

Data are available to researchers on request for purposes of reproducing the results or replicating the procedure by directly contacting the corresponding author.

Declaration of interests

We declare no competing interests.

Acknowledgements

This work was supported by the National Key Research and Development Program of China (2022YFC3600600 and 2022YFC3600400), Beijing Natural Science Foundation Haidian original innovation joint fund (L222123), Fund for Young Talents of Beijing Medical Management Center (QML20230505), the high-level public health talents (xuekegugan-02-47), and Training Fund for Open Projects at Clinical Institutes and Departments of Capital Medical University (CCMU2022ZKYXZ009). Salubris contributes ticagrelor, clopidogrel and its placebo at no cost and with no restrictions. Chongqing Jingyin Bioscience Co., Ltd. provides GMEX point-of-care genotyping system and technical supports for CHANCE-2 at no cost and with no restrictions. The funding has no role in the writing of the manuscript or the decision to submit it for publication. We thank all study participants, their relatives, the members of the survey teams at the 202 centres of the CHANCE-2 study.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.eclinm.2023.102357.

References

1 Wang Y, Wang Y, Zhao X, et al. Clopidogrel with aspirin in acute minor stroke or transient ischemic attack. *N Engl J Med*. 2013;369(1):11–19.
2 Johnston SC, Easton JD, Farrant M, et al. Clopidogrel and aspirin in acute ischemic stroke and high-risk TIA. *N Engl J Med*. 2018;379(3):215–225.
3 Kaushik A, Deora S, Choudhary R. Ticagrelor and aspirin or aspirin alone in acute ischemic stroke or TIA. *N Engl J Med*. 2020;383(17):1692–1693.
4 Wang Y, Meng X, Wang A, et al. Ticagrelor versus clopidogrel in CYP2C19 loss-of-function carriers with stroke or TIA. *N Engl J Med*. 2021;385(27):2520–2530.
5 Castañer O, Pintó X, Subirana I, et al. Remnant cholesterol, not LDL cholesterol, is associated with incident cardiovascular disease. *J Am Coll Cardiol*. 2020;76(23):2712–2724.
6 Brunzell JD, Davidson M, Furberg CD, et al. Lipoprotein management in patients with cardiometabolic risk: consensus conference report from the American Diabetes Association and the American College of Cardiology Foundation. *J Am Coll Cardiol*. 2008;51(15):1512–1524.
7 Chapman MJ, Ginsberg HN, Amarenco P, et al. Triglyceride-rich lipoproteins and high-density lipoprotein cholesterol in patients at high risk of cardiovascular disease: evidence and guidance for management. *Eur Heart J*. 2011;32(11):1345–1361.
8 Joshi P, Khokhar A, Massaro J, et al. Remnant lipoprotein cholesterol and incident coronary heart disease: the jackson heart and framingham offspring cohort studies. *J Am Heart Assoc*. 2016;5(5):e002765.
9 Varbo A, Nordestgaard BG. Nonfasting triglycerides, low-density lipoprotein cholesterol, and heart failure risk: two cohort studies of 113 554 individuals. *Arterioscler Thromb Vasc Biol*. 2018;38(2):464–472.
10 Wadström BN, Pedersen KM, Wulff AB, Nordestgaard BG. Elevated remnant cholesterol, plasma triglycerides, and cardiovascular and non-cardiovascular mortality. *Eur Heart J*. 2023;44(16):1432–1445.
11 Varbo A, Nordestgaard B. Remnant cholesterol and ischemic heart disease. *Curr Opin Lipidol*. 2014;25(4):266–273.
12 Varbo A, Benn M, Tybjærg-Hansen A, Jørgensen A, Frikke-Schmidt R, Nordestgaard B. Remnant cholesterol as a causal risk factor for ischemic heart disease. *J Am Coll Cardiol*. 2013;61(4):427–436.

13 Twickler TB, Dallinga-Thie GM, Cohn JS, Chapman MJ. Elevated remnant-like particle cholesterol concentration: a characteristic feature of the atherogenic lipoprotein phenotype. *Circulation*. 2004;109(16):1918–1925.
14 Jørgensen AB, Frikke-Schmidt R, West AS, Grande P, Nordestgaard BG, Tybjærg-Hansen A. Genetically elevated non-fasting triglycerides and calculated remnant cholesterol as causal risk factors for myocardial infarction. *Eur Heart J*. 2013;34(24):1826–1833.
15 Zilversmit DB. Atherogenesis: a postprandial phenomenon. *Circulation*. 1979;60(3):473–485.
16 Navarese EP, Vine D, Proctor S, et al. Independent causal effect of remnant cholesterol on atherosclerotic cardiovascular outcomes: a mendelian randomization study. *Arterioscler Thromb Vasc Biol*. 2023;43(9):e373–e380.
17 Quispe R, Martin SS, Michos ED, et al. Remnant cholesterol predicts cardiovascular disease beyond LDL and ApoB: a primary prevention study. *Eur Heart J*. 2021;42(42):4324–4332.
18 Fujioka Y, Ishikawa Y. Remnant lipoproteins as strong key particles to atherogenesis. *J Atheroscler Thromb*. 2009;16(3):145–154.
19 Masuda D, Yamashita S. Postprandial hyperlipidemia and remnant lipoproteins. *J Atheroscler Thromb*. 2017;24(2):95–109.
20 Yamazaki M, Uchiyama S, Xiong Y, Nakano T, Nakamura T, Iwata M. Effect of remnant-like particle on shear-induced platelet activation and its inhibition by antiplatelet agents. *Thromb Res*. 2005;115(3):211–218.
21 Jäger B, Piackova E, Haller PM, et al. Increased platelet reactivity in dyslipidemic patients with coronary artery disease on dual antiplatelet therapy. *Arch Med Sci*. 2019;15(1):65–71.
22 Orth M, Luley C, Wieland H. Effects of VLDL, chylomicrons, and chylomicron remnants on platelet aggregability. *Thromb Res*. 1995;79(3):297–305.
23 Mochizuki M, Takada Y, Urano T, et al. The in vitro effects of chylomicron remnant and very low density lipoprotein remnant on platelet aggregation in blood obtained from healthy persons. *Thromb Res*. 1996;81(5):583–593.
24 Wang Y, Johnston C, Bath PM, et al. Clopidogrel with aspirin in High-risk patients with acute non-disabling cerebrovascular events II (CHANCE-2): rationale and design of a multicentre randomised trial. *Stroke Vasc Neurol*. 2021;6(2):280–285.
25 The GUSTO investigators. An international randomized trial comparing four thrombolytic strategies for acute myocardial infarction. *N Engl J Med*. 1993;329(10):673–682.
26 Wang A, Meng X, Tian X, et al. Bleeding risk of dual antiplatelet therapy after minor stroke or transient ischemic attack. *Ann Neurol*. 2022;91(3):380–388.
27 Bonetti M, Gelber RD. Patterns of treatment effects in subsets of patients in clinical trials. *Biostatistics*. 2004;5(3):465–481.
28 Bonetti M, Gelber RD. A graphical method to assess treatment-covariate interactions using the cox model on subsets of the data. *Stat Med*. 2000;19(19):2595–2609.
29 Bonetti M, Zahrieh D, Cole BF, Gelber RD. A small sample study of the STEPP approach to assessing treatment-covariate interactions in survival data. *Stat Med*. 2009;28(8):1255–1268.
30 Voors AA, Mulder H, Reyes E, et al. Renal function and the effects of vericiguat in patients with worsening heart failure with reduced ejection fraction: insights from the VICTORIA (Vericiguat Global Study in Subjects with HFrEF) trial. *Eur J Heart Fail*. 2021;23(8):1313–1321.
31 Langsted A, Madsen CM, Nordestgaard BG. Contribution of remnant cholesterol to cardiovascular risk. *J Intern Med*. 2020;288(1):116–127.
32 Yang N, Wang M, Liu J, et al. The level of remnant cholesterol and implications for lipid-lowering strategy in hospitalized patients with acute coronary syndrome in China: findings from the improving care for cardiovascular disease in China-acute coronary syndrome project. *Metabolites*. 2022;12(10):898.
33 Yokoyama M, Origasa H, Matsuzaki M, et al. Effects of eicosapentaenoic acid on major coronary events in hypercholesterolaemic patients (JELIS): a randomised open-label, blinded endpoint analysis. *Lancet*. 2007;369(9567):1090–1098.
34 Nicholls SJ, Lincoff AM, Garcia M, et al. Effect of high-dose omega-3 fatty acids vs corn oil on major adverse cardiovascular events in patients at high cardiovascular risk: the STRENGTH randomized clinical trial. *JAMA*. 2020;324(22):2268–2280.
35 Das Pradhan A, Glynn RJ, Fruchart JC, et al. Triglyceride lowering with pemafibrate to reduce cardiovascular risk. *N Engl J Med*. 2022;387(21):1923–1934.
36 Zafrir B, Khoury R, Saliba W. Remnant cholesterol and risk of myocardial infarction in patients with coronary artery disease undergoing revascularization. *J Clin Lipidol*. 2023;17(3):332–341.
37 Hao QY, Gao JW, Yuan ZM, et al. Remnant cholesterol and the risk of coronary artery calcium progression: insights from the CARDIA and MESA study. *Circ Cardiovasc Imaging*. 2022;15(7):e014116.
38 Nordestgaard BG. Triglyceride-rich lipoproteins and atherosclerotic cardiovascular disease: new insights from epidemiology, genetics, and biology. *Circ Res*. 2016;118(4):547–563.
39 Varbo A, Nordestgaard B. Remnant cholesterol and risk of ischemic stroke in 112,512 individuals from the general population. *Ann Neurol*. 2019;85(4):550–559.
40 Varbo A, Benn M, Tybjærg-Hansen A, Nordestgaard BG. Elevated remnant cholesterol causes both low-grade inflammation and ischemic heart disease, whereas elevated low-density lipoprotein cholesterol causes ischemic heart disease without inflammation. *Circulation*. 2013;128(12):1298–1309.
41 Bernelot Moens SJ, Verweij SL, Schnitzler JG, et al. Remnant cholesterol elicits arterial wall inflammation and a multilevel cellular immune response in humans. *Arterioscler Thromb Vasc Biol*. 2017;37(5):969–975.
42 Varbo A, Nordestgaard BG. Directly measured vs. calculated remnant cholesterol identifies additional overlooked individuals in the general population at higher risk of myocardial infarction. *Eur Heart J*. 2021;42(47):4833–4843.