Prognostic utility of triglyceride-rich lipoprotein-related markers in patients with coronary artery disease

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Abstract  TG-rich lipoprotein (TRL)-related biomarkers, including TRL-cholesterol (TRL-C), remnant-like lipoprotein particle-cholesterol (RLP-C), and apoC-III have been associated with atherosclerosis. However, their prognostic values have not been fully determined, especially in patients with previous CAD. This study aimed to examine the associations of TRL-C, RLP-C, and apoC-III with incident cardiovascular events (CVEs) in the setting of secondary prevention of CAD. Plasma TRL-C, RLP-C, and total apoC-III were directly measured. A total of 4,355 participants with angiographically confirmed CAD were followed up for the occurrence of CVEs. During a median follow-up period of 5.1 years (interquartile range: 3.9–6.4 years), 543 (12.5%) events occurred. Patients with incident CVEs had significantly higher levels of TRL-C, RLP-C, and apoC-III than those without events. Multivariable Cox analysis indicated that a log unit increase in TRL-C, RLP-C, and apoC-III increased the risk of CVEs by 49% (95% CI: 1.16–1.93), 21% (95% CI: 1.09–1.35), and 40% (95% CI: 1.11–1.77), respectively. High TRL-C, RLP-C, and apoC-III were also independent predictors of CVEs in individuals with LDL-C levels ≤1.8 mmol/L (n = 1,068). The addition of RLP-C level to a prediction model resulted in a significant increase in discrimination, and all three TRL biomarkers improved risk reclassification. Thus, TRL-C, RLP-C, and apoC-III levels were independently associated with incident CVEs in Chinese CAD patients undergoing statin therapy.

Supplementary key words  triglyceride-rich lipoprotein-cholesterol • remnant-like lipoprotein particle-cholesterol • apolipoprotein C-III • cardiovascular events

CAD persists as a leading cause of death in Western societies and in China (1). Elevated LDL-C is a well-established causal risk factor for CAD (2). However, there is still a considerable residual risk of atherosclerotic cardiovascular events (CVEs) even after LDL-C lowering to recommended goals (3). A growing amount of evidence supports the concept that elevated plasma TG-rich lipoproteins (TRLs) contribute to this residual risk (4, 5). The cholesterol content of partially lipolyzed TRLs, also called remnant cholesterol (RC), includes cholesterol from either CMs or VLDL remnants, as well as from IDLs. RC can be classified into TRL-cholesterol (TRL-C) or remnant-like lipoprotein particle-cholesterol (RLP-C) (6). In view of the fact that TRL can be catabolized by macrophages, there is evidence that it is RC that promotes formation and progression of atherosclerosis rather than their TG content per se (7). Indeed, experimental studies have shown that remnant lipoproteins can enter the intima of the arterial wall and be taken up by macrophages to induce foam cell formation (8). Several observational clinical studies, including ours, associated high RC concentrations with increased risk for CAD (4, 9). Furthermore, evidence from Mendelian randomization studies indicated a causal association of RC with CAD development in previously healthy individuals (10, 11). However, the prognostic value of plasma RC levels in secondary prevention settings is still undefined because previous studies showed inconsistent and controversial results (10, 12–15). Thus, further investigation on the association between RC levels and CVE risk is needed.

ApoC-III is a key regulator of TG metabolism that not only inhibits lipoprotein lipase-mediated hydrolysis of TG, but also stimulates proinflammatory molecule expression and enhances endothelial cell dysfunction (16–18). Previous nested case-control and cross-sectional studies have shown that elevated apoC-III levels were related to CVEs (19, 20). On the same token, Mendelian randomization studies have shown that loss-of-function variants on the APOC3 gene were associated with lower TG levels and reduced risk of CVEs (21, 22). However, no prospective data from a large sample size study is currently available concerning the association between plasma apoC-III and CVEs in patients with preexisting CAD. Furthermore, there is no evidence on how TRL-related biomarkers could modify CVE risk stratification in clinical practice on top of classical risk markers.

Therefore, the aim of this prospective multicenter investigation was to test the prognostic value of directly measured plasma TRL-C, RLP-C, and total apoC-III levels for incident
CVEs in a real-world setting of statin-treated Chinese patients with previous manifestation of CAD. Considering the heterogeneous nature of RLP-C, we used a simple and reliable method for its measurement as previously described (12, 23).

MATERIALS AND METHODS

Study design and populations
A total of 5,028 patients were consecutively enrolled due to angina-like chest pain that was confirmed as CAD by coronary angiography from three centers in China from May 2011 to February 2017. Participants were excluded from the study if they were aged <18 years, had familial hypercholesterolemia, or had severe chronic heart failure, thyroid dysfunction, severe liver and/or renal insufficiency, or malignant diseases. Patients without complete clinical data and blood samples were also excluded. All patients received guideline recommended pharmacological therapy for stable CAD (2). The study protocol was performed according to the Declaration of Helsinki and approved by the FuWai Hospital and National Center for Cardiovascular Diseases, Beijing, China review board. All study participants signed an informed consent.

Baseline data collection
Clinical data of each participant were obtained by experienced physicians and nurses when first enrolled. CAD was defined as the presence of coronary stenosis with luminal diameter narrowing of 50% or more in any major coronary vessels detected by coronary angiography and assessed by two experienced physicians. BMI was calculated by dividing weight in kilograms by height in square meters. A diagnosis of hypertension was made when systolic and diastolic blood pressure were respectively greater than 140/90 mmHg or if subjects were taking antihypertensive medications. Diabetes was self-reported or considered present if one of four criteria were met: fasting plasma glucose level \( \geq 7.0 \) mmol/l, 2 h plasma glucose of the oral glucose tolerance test \( \geq 11.1 \) mmol/l, hemoglobin A1c level \( \geq 6.5\% \), or use of oral antidiabetic medications or insulin injections. Information on other diseases, family history of CAD, and current therapy was self-reported or collected from medical record review as described previously (24).

Blood samples were obtained from each patient from the cubital vein in the early morning after at least 12 h overnight fasting and stored at \(-80^\circ\)C until analysis. The concentrations of plasma TG, total cholesterol (TC), HDL-C, apoA1, and apoB were directly measured using an automatic biochemistry analyzer (Hitachi 7150, Japan). The LDL-C concentration was analyzed by selective turbidimetric assay (Denka Seiken Co., Ltd.) and analyzed on a Hitachi 7180 automatic analyzer. Two intra- and inter-assay coefficients of variation for these assays were all \(<6\%\). At baseline, lipid profiles of each participant were measured in triplicate and the mean value was used in the final analysis.

Endpoint assignment
Patients were followed up semiannually by clinic visits or by telephone interviews conducted by trained nurses or doctors. The primary endpoint was a composite of incident MI, ischemic stroke, unstable angina pectoris (UAP) requiring hospitalization, coronary revascularization, and cardiovascular death. MI was defined according to medical records showing typical chest pain, typical changes on the electrocardiogram, and elevated cardiac necrosis biomarkers, such as creatine kinase-MB and troponin-I (25). Ischemic stroke was defined as a new-onset stroke diagnosed by computed tomography or magnetic resonance imaging during the follow-up period. A diagnosis of UAP was made by angiography at rest or new-onset severe angina pectoris, presenting or not presenting changes on the electrocardiogram, without troponin elevation but requiring hospitalization. Revascularization was defined as percutaneous coronary intervention or coronary artery bypass grafting when myocardial ischemia was confirmed beyond 90 days after discharge. Diagnosis of cardiovascular death was confirmed by hospital records, death certificates, and information provided by family members. All events were counted as separate and unique. Thirty-eight patients (0.76\%) were lost to follow-up, and therefore a total of 4,355 patients were included in the present study (supplemental Fig. S1).

Statistical analysis
Continuous variables were expressed as mean \( \pm \) SD or median with (interquartile range) when appropriate. Categorical variables were presented as number (percentage). Differences were assessed by Student’s t-test and the Mann-Whitney U test, ANOVA, \( \chi^2 \) analysis, and Fisher’s test as appropriate. The correlation between lipid, TRL-related biomarkers, and other biomarkers was evaluated by Pearson’s correlation coefficients. Kaplan-Meier survival curves with log-rank test were applied. The association of TRL-related biomarkers with study endpoints was tested in univariate and multivariate Cox proportional hazard models with hazard ratios (HRs) and 95% CIs; model 1 was adjusted for age and sex; model 2 was additionally adjusted for traditional risk factors (BMI, current smoking, diabetes, baseline statin use, and family history of CAD, TC, LDL-C, HDL-C, TG, and hsCRP). Restricted cubic splines were calculated to assess the potential linearity relationship between TRL-related biomarkers and events. Subgroup analyses were performed to test the robustness of our findings. The incremental discrimination value of the addition of TRL-C, RLP-C, and apoC-III levels to classical risk biomarkers in risk prediction models were assessed by Harrell’s C-statistic. Risk reclassification was tested by continuous net reclassification improvement (NRI) and integrated discrimination improvement (IDI) for survival data (26). All statistical analyses were performed with SPSS version 24.0 software (SPSS Inc., Chicago, IL) and R version 3.5.2 (R Foundation for Statistical Computing, Vienna, Austria). Statistical significance was set as \( P < 0.05 \).

RESULTS

Baseline characteristics
The baseline clinical characteristics of the studied population are shown in Table 1. The mean age of participants was \(58.21 \pm 9.72\) years, in which 71.1\% (n = 3,096) were
male. Compared with event-free patients, those with incident events were more likely to be older (59.57 ± 10.14 years vs. 58.01 ± 9.64 years, P = 0.001) and had higher levels of TG, TRL-C, RLP-C, apoC-III, and hsCRP at baseline, but did not differ with respect to sex and the prevalence of smoking, diabetes, and baseline statin use. There was no statistically significant difference in TC or LDL-C between the two groups.

Baseline clinical characteristics stratified by the quintiles of plasma TRL-C, RLP-C, and apoC-III concentrations are presented in supplemental Tables S1–S3. Patients in the upper quintiles were youngest in age, more likely to be female, and had higher levels of BMI. Prevalence of statin therapy at baseline was less frequent in patients with higher TRL-C and RLP-C concentrations, whereas there was no similar trend toward apoC-III levels. The highest baseline LDL-C levels were seen in those in the highest quintiles of plasma TRL-C, RLP-C, and apoC-III.

Correlations between plasma lipids and lipoproteins are presented in Fig. 1. There were moderate positive intercorrelations among TRL-C, RLP-C, and apoC-III. These three parameters were weakly correlated with LDL-C and apoB levels, but inversely correlated with HDL-C.

**TRL-related biomarker levels and outcomes**

During a median follow-up period of 5.1 years (interquartile range: 3.9–6.4 years), a total of 543 (12.5%) CVEs were observed, representing 24.7 (95% CI: 16.2–35.7) events per 1,000 person-years. Among the 543 patients with events, 132 (3.0%) had UAP requiring hospitalization, 44 (1.0%) had MI, 109 (2.5%) developed stroke, 183 (4.2%) underwent post-discharge percutaneous coronary intervention or coronary artery bypass grafting and 75 (1.7%) died. As shown in Fig. 2, Kaplan-Meier analysis demonstrated that patients in the highest quintiles of TRL-C, RLP-C, and apoC-III had a higher probability of developing incident CVEs compared with those in the lowest quintiles (all log-rank P < 0.005). Supplemental Fig. S2 also shows that patients with the highest levels of TG and non-HDL-C had the lowest total event-free survival rates.

**Table 1. Baseline characteristics of study patients presenting or not presenting CVEs during follow-up**

| Variables                          | Overall (n = 4,355) | With Event (n = 543) | Without Event (n = 3,812) | P    |
|-----------------------------------|--------------------|---------------------|--------------------------|------|
| Age, years                        | 58.21 ± 9.72       | 59.57 ± 10.14       | 58.01 ± 9.64             | 0.001|
| Male sex, n (%)                   | 3,096 (71.1)       | 370 (68.1)          | 2,726 (71.5)             | 0.105|
| BMI, kg/m²                        | 25.85 ± 3.10       | 25.85 ± 2.98        | 25.85 ± 3.12             | 0.998|
| Family history of CAD, n (%)      | 612 (14.1)         | 69 (12.7)           | 543 (14.2)               | 0.335|
| Currently smoker, n (%)           | 2,354 (54.1)       | 283 (52.1)          | 2,071 (54.3)             | 0.334|
| Alcohol consumption, n (%)        | 1,438 (33.0)       | 161 (29.7)          | 1,277 (33.5)             | 0.740|
| Hypertension, n (%)               | 2,834 (65.1)       | 372 (68.5)          | 2,462 (64.6)             | 0.073|
| Diabetes, n (%)                   | 2,392 (54.9)       | 290 (53.4)          | 2,102 (55.1)             | 0.447|
| TC, mmol/l                        | 4.08 ± 1.05        | 4.16 ± 1.00         | 4.07 ± 1.06              | 0.061|
| HDL-C, mmol/l                     | 1.06 ± 0.29        | 1.05 ± 0.28         | 1.06 ± 0.29              | 0.301|
| LDL-C, mmol/l                     | 2.44 ± 0.90        | 2.48 ± 0.86         | 2.44 ± 0.90              | 0.251|
| Non-HDL-C, mmol/l                 | 3.02 ± 1.00        | 3.11 ± 0.95         | 3.01 ± 1.01              | 0.038|
| TG, mmol/l                        | 1.46 (1.09–2.92)   | 1.54 (1.09–2.20)    | 1.45 (1.08–2.01)         | 0.008|
| TRL-C, mmol/l                     | 0.50 (0.36–0.69)   | 0.53 (0.38–0.74)    | 0.50 (0.35–0.69)         | 0.002|
| RLP-C, mmol/l                     | 0.28 (0.15–0.54)   | 0.33 (0.17–0.62)    | 0.28 (0.14–0.52)         | 0.001|
| Lp(a), mg/dl                      | 15.12 (6.74–36.12) | 15.37 (7.64–34.57)  | 15.03 (6.62–36.30)       | 0.394|
| ApoAI, mg/dl                      | 132.58 ± 28.93     | 134.76 ± 29.85      | 132.42 ± 28.79           | 0.325|
| ApoB, mg/dl                       | 90.84 ± 29.01      | 92.64 ± 27.62       | 90.58 ± 29.20            | 0.122|
| ApoC-III, mg/dl                   | 8.82 (6.68–11.6)   | 9.19 (6.95–12.29)   | 8.76 (6.65–11.48)        | 0.003|
| hsCRP, mg/l                       | 1.36 (0.73–2.87)   | 1.64 (0.81–3.22)    | 1.33 (0.72–2.82)         | 0.002|
| Glucose, mmol/l                   | 6.16 ± 2.00        | 6.27 ± 2.08         | 6.15 ± 1.99              | 0.193|
| Hemoglobin A1C, %                 | 6.64 ± 1.22        | 6.75 ± 1.36         | 6.63 ± 1.20              | 0.087|
| Previous medical history          |                    |                     |                          |      |
| Prior angina, n (%)               | 3,310 (76.0)       | 422 (77.7)          | 2,888 (75.8)             | 0.318|
| Prior MI, n (%)                   | 990 (22.7)         | 140 (25.8)          | 850 (22.3)               | 0.072|
| Prior revascularization, n (%)    | 586 (13.5)         | 75 (13.8)           | 511 (13.4)               | 0.795|
| Medication use, n (%)             |                    |                     |                          |      |
| ACEI or ARB, n (%)                | 1,088 (25.0)       | 126 (23.2)          | 962 (25.2)               | 0.306|
| Beta-blockers, n (%)              | 3,481 (79.9)       | 419 (77.2)          | 3,062 (80.3)             | 0.085|
| Aspirin, n (%)                    | 4,272 (98.1)       | 328 (97.4)          | 3,744 (98.2)             | 0.202|
| Statins at baseline, n (%)        | 2,647 (60.8)       | 310 (57.1)          | 2,337 (61.3)             | 0.600|
| High-intensity, n (%)             | 568 (13.0)         | 79 (14.5)           | 489 (12.8)               | 0.265|
| Statins at follow-up, n (%)       | 4,216 (96.8)       | 521 (95.2)          | 3,695 (96.9)             | 0.228|

Data are expressed as the mean value ± SD, median and range (25th and 75th percentiles), or number (percent) of patients.

*High-intensive statin refers to atorvastatin ≥40 mg or rosvastatin ≥20 mg.
log-transformed TRL-C, RLP-C, and apoC-III were associated with 39% (HR: 1.39; 95% CI: 1.15–1.67), 19% (HR: 1.19; 95% CI: 1.09–1.32), and 44% (HR: 1.44; 95% CI: 1.18–1.77) higher risk of CVEs in univariate Cox regression analysis. The increase in risk per log increment in TRL-C, RLP-C, and apoC-III concentration after multifactorial adjustment were 1.49 (95% CI: 1.16–1.93), 1.21 (95% CI: 1.09–1.35), and 1.40 (95% CI: 1.11–1.77), respectively. Age- and sex-adjusted restricted cubic splines showed a nonlinear relation between the three parameters on continuous scales and the risk of CVEs (Fig. 3).

Subgroup analyses were then performed according to clinical and laboratory characteristics (supplemental Fig. S3). When stratified by baseline median levels of hsCRP, multivariate Cox analysis revealed that only RLP-C and apoC-III tended to be positively associated with the risk of events in both hsCRP strata, while all three TRL-related biomarkers were significantly associated with CVEs in patients with LDL-C lower than 2.6 mmol/l. To further explore whether these three parameters were potential residual risk markers in patients with LDL-C ≥1.8 mmol/l (27), we conducted multivariate Cox regression analyses in 1,068 CAD patients with 123 events (Table 2). All three parameters were significantly associated with a higher rate of events when tested either as categorical or continuous variables.

### Incremental effect of TRL-related biomarkers on risk discrimination and reclassification

The Cox prediction model consisting of traditional cardiovascular risk factors presented a C-statistic of 0.729 (95% CI: 0.711–0.747; Table 3). Adding RLP-C to the risk prediction model significantly increased the C-statistic to 0.757 (95% CI: 0.749–0.774; P for difference = 0.001). On the other hand, the addition of TRL-C, apoC-III, TG, non-HDL-C, or hsCRP did not significantly improve risk discrimination (supplemental Table S5). The addition of all three TRL-related biomarkers resulted in modest increases in NRI and IDI. The addition of TG or non-HDL-C had no effect on NRI and IDI.

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**Fig. 1.** Correlations of TRL-related biomarkers, apolipoproteins, and plasma lipids. The text showed the correlation coefficient and significant correlations (P<0.05) are labeled with asterisks.

**Fig. 2.** Kaplan-Meier analyses for incident CVEs according to quartiles of TRL-related biomarkers.

**Table 2.** Multivariate Cox regression analyses in 1,068 CAD patients with 123 events.
DISCUSSION

In this prospective cohort study, TRL-related biomarkers were independently associated with incident CVEs in Chinese patients with previous manifestation of CAD. Results persisted even in a subgroup of patients with LDL-C ≤1.8 mmol/l. Of the tested biomarkers, only RLP-C increased risk discrimination on top of classical risk markers, though...
all three improved risk reclassification. Therefore, this study adds important information enhancing the previous evidence of an independent association of TRLs with development of atherosclerosis and its consequences.

The recurrence of CVEs following evidence-proven therapies, including intensive statin regimens in patients with CAD, is still a great challenge and TRLs have been highly considered as a major component of this so-called residual risk (28). Although there were several previous reports examining the prognostic utility of TRL-related biomarkers in CAD patients, they were limited by inconsistent results (10, 14, 15). Additionally, no integrated and comparative data concerning their prognostic implications in one cohort is currently available. To examine this important but unsolved issue, we performed this study and found that TRL-C, RLP-C, and apoC-III were independent predictors of incident CVEs.

Experimental studies showed that RC may not need to be oxidized and, thus, may be more easily taken up by macrophages in the arterial wall than LDL-C (29). Moreover, TRL remnants may carry up to 40 times more cholesterol per particle than LDL due to their larger size (11). Besides being a major component of TRLs, the independent pathogenic role of apoC-III in the development of atherosclerosis has been carefully investigated (16, 18). ApoC-III also promotes the production and secretion of hepatic VLDL, which may lead to increased circulating TRL levels (17).

Considering the possible atherogenic role of these TRL-related biomarkers, we aimed to evaluate their prognostic role in the present study. Previously, a small prospective study with 135 CAD patients followed up by a median of 2.2 years reported that fasting RLP-C independently predicted study endpoints, length of follow-up, and population studied. No uniform method for TRL-C or RLP-C measurement was widely accepted and the above-mentioned studies differed by using immunonephelometry, ultracentrifugation, or direct calculation (12, 14, 15, 30). Marked differences in median plasma TRL-C or RLP-C levels across studies were observed, from 0.08 to 0.90 mmol/l, suggesting different sensitivities and specificities among the used methods (12–14). In this study, we performed a simple and reliable assay for TRL-C and RLP-C measurement that was already validated in previous studies, which indicates that this fully automated assay might have an opportunity to be implemented as a routine diagnostic test in the future (12, 23).

Non-HDL-C is recommended by some guidelines as a secondary target in patients with high TG; however, in this study, patients had normal median TG levels (27). Moreover, non-HDL-C and apoB showed no association with CVEs in the univariate Cox analysis, while TRL-C and RLP-C still predicted future CVEs even after being adjusted for non-HDL-C or apoB. In addition, although TG concentrations could represent RC levels because they are correlated, RC may be more important than TG to explain the residual risk due to the involved biological mechanism (7). Recent studies and a guideline also reported that the prognostic value of RC is determined by apoB-containing particle concentration rather than by the TG itself (27, 31). In the present study, RC remained significantly associated with incident CVE risk after additionally adjusting for TG. The addition of RLP-C also had a greater incremental effect on the C-statistics of the variables in models compared with the addition of TG. Thus, the measurement of TRL-C and RLP-C in CAD patients might be more useful for the identification of those at high future cardiovascular risk.

The association of apoC-III levels with CVEs found in the present study was previously described in cross-sectional investigations (19, 20). In a prospective cohort study of 633

### Table 2: Multivariate Cox hazard analyses models for incident CVEs in CAD patients with LDL-C ≤ 1.8 mmol/l (n = 1,068)

| Variables               | HR (95% CI)   | P    |
|-------------------------|---------------|------|
| TRL-C                   |               |      |
| Per Log unit increase   | 1.95 (1.33–2.86) | 0.001|
| Tertile 3 versus tertile 1 | 1.89 (1.21–2.97) | 0.006|
| RLP-C                   |               |      |
| Per Log unit increase   | 1.30 (1.07–1.59) | 0.010|
| Tertile 3 versus tertile 1 | 1.71 (1.10–2.66) | 0.017|
| ApoC-III                |               |      |
| Per Log unit increase   | 1.57 (1.05–2.37) | 0.030|
| Tertile 3 versus tertile 1 | 1.54 (1.01–2.35) | 0.042|

Data are adjusted for age, sex, BMI, current smoking, diabetes, baseline statin use, family history of CAD, TC, LDL-C, HDL-C, TG, and hsCRP.

Non-HDL-C is recommended by some guidelines as a secondary target in patients with high TG; however, in this study, patients had normal median TG levels (27). Moreover, non-HDL-C and apoB showed no association with CVEs in the univariate Cox analysis, while TRL-C and RLP-C still predicted future CVEs even after being adjusted for non-HDL-C or apoB. In addition, although TG concentrations could represent RC levels because they are correlated, RC may be more important than TG to explain the residual risk due to the involved biological mechanism (7). Recent studies and a guideline also reported that the prognostic value of RC is determined by apoB-containing particle concentration rather than by the TG itself (27, 31). In the present study, RC remained significantly associated with incident CVE risk after additionally adjusting for TG. The addition of RLP-C also had a greater incremental effect on the C-statistics of the variables in models compared with the addition of TG. Thus, the measurement of TRL-C and RLP-C in CAD patients might be more useful for the identification of those at high future cardiovascular risk.

The association of apoC-III levels with CVEs found in this study was previously described in cross-sectional investigations (19, 20). In a prospective cohort study of 633 patients with previous MI, reported that higher RLP-C levels were associated with lower 2-year all-cause mortality despite rigorous adjustment for traditional risk factors.

In the present study, elevated TRL-C and RLP-C, either as categorical or continuous variables, were associated with a worse prognosis in CAD patients and the association persisted after adjustment for multiple CAD risk factors. Discrepancies among the above-cited studies might be due to differences in biochemical assays, definition of study endpoints, length of follow-up, and population studied. No uniform method for TRL-C or RLP-C measurement was widely accepted and the above-mentioned studies differed by using immunonephelometry, ultracentrifugation, or direct calculation (12, 14, 15, 30). Marked differences in median plasma TRL-C or RLP-C levels across studies were observed, from 0.08 to 0.90 mmol/l, suggesting different sensitivities and specificities among the used methods (12–14). In this study, we performed a simple and reliable assay for TRL-C and RLP-C measurement that was already validated in previous studies, which indicates that this fully automated assay might have an opportunity to be implemented as a routine diagnostic test in the future (12, 23).

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The association of apoC-III levels with CVEs found in this study was previously described in cross-sectional investigations (19, 20). In a prospective cohort study of 633

### Table 3: C-statistics, NRI, and IDI for the incremental predictive values of the combination of traditional risk factors and TRL-related markers

|                  | C-Index | P   | Continuous NRI | P   | IDI | P   |
|------------------|---------|-----|----------------|-----|-----|-----|
| Model 1: CAD risk factors          | 0.729  | 0.711–0.747 |     |     |     |     |
| Model 1 + TRL-C                 | 0.735  | 0.717–0.753 | 0.336 | vs. M1 | 0.13 | 0.07–0.19 | 0.020 | 0.04 | 0.02–0.06 | 0.030 |
| Model 1 + RLP-C                 | 0.757  | 0.749–0.774 | 0.001 | vs. M1 | 0.26 | 0.12–0.41 | 0.005 | 0.07 | 0.04–0.09 | 0.010 |
| Model 1 + apoC-III              | 0.749  | 0.722–0.757 | 0.122 | vs. M1 | 0.16 | 0.08–0.16 | 0.015 | 0.05 | 0.04–0.08 | 0.020 |

Model 1 included age, sex, current smoking, diabetes, hypertension, TC, HDL-C, and LDL-C.
patients with CAD, Olivier et al. (32) showed that patients with an apoC-III level exceeding 10.5 mg/dl had a 2.35-fold higher risk for cardiac mortality than those with lower levels. A nested case-control analysis from the Cholesterol and Recurrent Events (CARE) trial, including 418 MI patients, found that apoC-III was an independent predictor for recurrent events (33). In agreement with these studies, our study with a larger sample size and longer follow-up period confirmed the prognostic value of apoC-III. It should be noted that total apoC-III was measured in this study, and previous studies also showed that the apoC-III subfractions were important predictive biomarkers for CVEs (34, 35).

In this study, we assessed the incremental value of the addition of TRL-related biomarkers into a risk prediction model separately from established risk factors using C-statistics, continuous NRI, and IDI. Only RLP-C added to risk discrimination, though all three biomarkers improved risk reclassification. In fact, in vitro studies have shown that the atherothrombosis activity of RLP-C was 3- to 10-fold higher than LDL, VLDL, or CMs (36). Experimental studies also found that compared with other TRLs, the expression of endothelial proatherogenic molecules and severity of endothelial dysfunction induced by RLP-C were noticeably higher (37). Undoubtedly, more detailed mechanistic studies should be performed. In addition, RLP-C showed greater improvement in the C-statistics compared with other CAD biomarkers like hsCRP. Considering the relative importance of the measures of TRL-related biomarkers to further risk stratification, we suggest that the measurement of RLP-C in CAD patients might be useful for the identification of those at high future cardiovascular risk.

The prognostic value of TRL-related biomarkers was also revealed in subgroup analysis. In patients with LDL-C values ≤ 1.8 mmol/l, TRL-C and RLP-C were still independently associated with the risk of incident CVEs, suggesting that TRL-C and RLP-C might explain part of residual risk. This result was in agreement with a Japanese study that demonstrated that fasting RLP-C was a significant predictor of CVEs in 247 CAD patients with LDL-C ≤ 1.8 mmol/l (38). However, considering the limited population (n = 1,068), this association in patients with lower LDL-C levels needs further evaluation, especially nowadays with the ultra-low LDL-C levels attained with proprotein convertase subtilisin/kexin type 9 inhibitor/statin associations (39).

A potential implication of our study findings is that the development of strategies targeted against TRL-related markers is an important method for the future. Phase I clinical trial antisense inhibition of APOC3 showed decreased TG levels and phase III studies are anticipated (40). Whether proprotein convertase subtilisin/kexin type 9 inhibitor leads to further clinical benefit through RC is currently under investigation (41).

This study has several limitations. First, circulating TRL-related biomarkers were measured once at baseline, and we could not control for possible fluctuations during follow-up. Despite this, a robust association with CVE risk was encountered. Second, not all events involved in the study endpoint were considered as hard (MI, stroke, or death). However, recent investigations also added UAP and revascularization to their endpoints, especially in individuals undergoing statin therapy (39, 42). Third, this study only enrolled Chinese patients, and further data is necessary in individuals from other ethnicities. However, this is also a study strength because it expands previous evidence of TRL and CVEs to Chinese individuals. Finally, although this study was performed in a relatively large sample size with a 5 year follow-up, further larger and longitudinal studies are required to verify our results, especially in subgroups of patients with very low LDL-C.

In conclusion, the concentrations of TRL-related biomarkers were independently associated with incident CVEs and could explain part of the residual risk in Chinese CAD patients undergoing statin therapy. These novel findings support the notion that the TRL-related biomarkers could be useful tools to improve risk stratification and also for use as therapeutic targets to reduce this risk, as recently shown (43).

Data availability
All data generated or analyzed during this study are included in this published article and its supplementary information files.

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Conflict of interest
R.D.S. has received honoraria related to consulting, research, and or speaker activities from: Amgen, Aché, Astra Zeneca, Esperion, Kowa, Merck, Novo-Nordisk, PTC, Pfizer, and Sanofi/Regeneron. All other authors declare that they have no conflicts of interest with the contents of this article.

Abbreviations
CVE, cardiovascular event; HR, hazard ratio; hsCRP, high-sensitivity C-reactive protein; IDI, integrated discrimination improvement; Lp(a), lipoprotein (a); NRI, net reclassification improvement; RC, remnant cholesterol; RLP-C, remnant-like lipoprotein particle cholesterol; TC, total cholesterol; TRL, TG-rich lipoprotein; TRL-C, TG-rich lipoprotein-cholesterol; UAP, unstable angina pectoris.

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