Remnant Lipoproteins Are Residual Risk Factor for Future Cardiovascular Events in Patients With Stable Coronary Artery Disease and On-Statin Low-Density Lipoprotein Cholesterol Levels <70 mg/dL

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Background: This study examined the predictive value of remnant lipoprotein levels for cardiovascular events (CVEs) in patients with stable coronary artery disease (CAD) and low-density lipoprotein cholesterol (LDL-C) levels <70 mg/dL on statin treatment.

Methods and Results: Serum levels of remnant lipoproteins (remnant-like lipoprotein particles cholesterol: RLP-C) were measured by an immunoseparation method in 247 consecutive patients with CAD who had on-statin LDL-C levels <70 mg/dL. All the patients were followed prospectively for a period of ≤60 months or until the occurrence of the primary composite endpoint of cardiac death, nonfatal myocardial infarction, unstable angina requiring coronary revascularization, worsening heart failure, peripheral artery disease, aortic event, and ischemic stroke. During a mean follow-up period of 38 months, 33 CVEs occurred. Kaplan-Meier analysis demonstrated that higher RLP-C levels (≥3.9 mg/dL, determined by ROC curve) resulted in a significantly higher probability for the primary endpoint than did lower RLP-C levels (<3.9 mg/dL) (P<0.01 by log-rank test). Stepwise multivariate Cox proportional hazard analysis showed that RLP-C was a significant predictor of the primary endpoint after adjustment for known risk factors and lipid variables including triglycerides, and total apolipoprotein B (hazard ratio 1.62, 95% confidence interval 1.26–2.07, P<0.01).

Conclusions: RLP-C levels are a residual risk factor for future CVEs in patients with CAD and on-statin LDL-C <70 mg/dL.

Key Words: Cardiovascular events; Hypercholesterolemia; Prognosis; Risk factors; Statins

The European and AHA/ACC Guidelines recommend a lower target of low-density lipoprotein cholesterol (LDL-C) <70 mg/dL in very high risk patients.[1,2] Although LDL-C lowering by statin and/or non-statin therapies greatly reduces cardiovascular disease (CVD) risk, a considerable residual risk of CVD persists despite on-treatment LDL-C <70 mg/dL.[3–6] This residual risk can be classified into lipid-related and non-lipid-related. Part of the lipid-related residual risk is thought to be attributed to triglyceride (TG)-rich lipoproteins (TGLs),[7–11] which include chylomicrons, very low-density lipoproteins (VLDL), and the products of their hydrolysis, remnant lipoproteins. TGLs bind to arterial endothelium where lipoprotein lipase initiates TG hydrolysis, leading to the production of remnant lipoproteins. Remnant lipoproteins can enter the deeper structures of the arterial wall[12] and be taken up by macrophages, causing foam cell formation.[13] It is not yet established, however, as to how these specific lipoprotein fractions are responsible for the lipid-related residual risk in patients with on-statin LDL-C <70 mg/dL.

As TGLs are not measured readily in clinical practice, the NCEP Guidelines recommend non-high-density lipoprotein cholesterol (HDL-C) measurement as a secondary target of lipid-lowering therapy once the LDL-C goal has been achieved in patients with hypertriglyceridemia.[14] Non-HDL-C levels may vary as a function of cholesterol content per lipoprotein particle, and LDL particle numbers may still be high even in patients treated with statins.[15] As LDL, intermediate-density lipoproteins (IDL), VLDL, chylomicrons, and remnants of VLDL and chylomicron particles each carry only 1 apolipoprotein B (ApoB-100 or ApoB-48) molecule, the total ApoB (ApoB-100+ApoB-48) value represents the total number of potentially atherogenic lipoproteins, whereas non-HDL-C provides the cholesterol content of the same lipoproteins. In this context, total ApoB may be a better indicator of residual coronary artery disease (CAD) risk than non-HDL-C.[7,10]

In the past, it has been difficult to assay remnant lipoproteins because of their heterogeneous nature. However,

a simple and reliable technique for measuring remnant-like lipoprotein particle cholesterol (RLP-C) using an immunoseparation method has been developed.[16,17] We have previously shown that RLP-C levels are an important predictor of secondary CVD events in patients with hypertriglyceridemia.[18–21] The present study prospectively examined whether RLP-C levels predict the risk of future CVD events (CVEs) in patients with CAD who had achieved LDL-C levels <70 mg/dL during statin therapy.

Methods

Patients
This study enrolled 292 consecutive patients with stable CAD who had LDL-C levels <70 mg/dL on statin treatment during ≥3 months prior to enrollment. They were admitted to the cardiology section of Yamanashi University Hospital from January 1, 2008 to March 31, 2015, and had routine measurement of lipid parameters including RLP-C at enrollment. The exclusion criteria were: (1) acute coronary syndrome, stroke, cardiogenic shock, pulmonary edema, major surgery, trauma or serious infectious disease within 4 weeks prior to enrollment, (2) neoplasm, significant hepatic or inflammatory disease, (3) chronic renal failure or serum creatinine level >2.5 mg/dL, congestive heart failure (HF), or left main trunk disease, (4) other serious diseases, and (5) age >80 years. A total of 36 patients were excluded on the basis of these criteria, and the final study group comprised 256 patients. All the patients were ethnic Japanese and had stable CAD with no episodes of angina at rest and no change in the frequency of angina in response to sublingual nitroglycerin in the previous 3 months. All gave written informed consent at enrollment. The study was approved by the Ethics Committee of Yamanashi University Hospital. The investigation conformed to the principles outlined in the 1975 Declaration of Helsinki.

Follow-up Study
All study patients received statin treatment and the standard medical treatment for stable CAD,[22] as outlined in **Table 1**. Types and doses of statins depended on the attending doctors. The patients also made lifestyle modifications. During the follow-up period, all patients continued with statin treatment and other medications they had been taking at the time of final enrollment. After baseline data had been obtained at the study hospital, all patients were followed prospectively every month by the patients' primary physicians at a hospital for up to 60 months or until the occurrence of a CVE: cardiac death, nonfatal myocardial infarction (MI), unstable angina pectoris (UAP) with coronary revascularization, worsening HF, peripheral artery disease (PAD) requiring endovascular or surgical treatment or amputation, aortic dissection, aortic aneurysm requiring endovascular or surgical treatment, or ischemic stroke. The time to the first event was evaluated prospectively. The primary endpoint was a composite of these events. Cardiac death was confirmed by hospital records. MI and UAP were diagnosed according to the AHA/ACC Guidelines,[23,24] and their presence was confirmed by coronary angiography in all patients. HF was defined as resting dyspnea with progressive fluid retention requiring hospitalization and treatment with an intravenous diuretic. The diagnosis of ischemic stroke was based on medical history, physical examination, and brain computed tomography or magnetic resonance imaging. Data were obtained every 3 months from the patients' primary physicians and then collated by investigators who were blinded to each patient's status at enrollment. All endpoint data were checked for accuracy, consistency, and completeness by other investigators who also had no information on the baseline characteristics of the patients.

Laboratory Assays and Measurements
Venous blood was collected after a 12-h fast. Serum was stored at 4°C and used for the assays within 3 days of sampling. Fasting serum total cholesterol (TC) and TG concentrations were measured enzymatically, while serum HDL-C concentration was measured by heparin-Ca^{2+}/Ni^{2+} precipitation. Non-HDL-C was calculated TC minus HDL-C. LDL-C levels were calculated by the Friedewald formula. If TG levels were ≥400 mg/dL, LDL-C levels were measured by direct assay. Levels of total ApoB were determined by immuno-turbidimetric assays (Daiichi Chemicals, Tokyo, Japan). Apolipoprotein AI (ApoA-I) levels were measured by the turbidimetric immunoassay system of Daiichi Pure Chemicals (Tokyo, Japan). RLP-C levels were measured using an immunoseparation assay as previously described.[16–21] Intra- and interassay coefficients of variation of RLP-C levels were 2.3% and 3.6%, respectively. Left ventricular ejection fraction (LVEF) was measured by echocardiography using the Teicholz formula.[25]

Statistical Analysis
All data are expressed as mean±SD, median and interquartile range (25th and 75th percentiles), or frequency (%). The Shapiro-Wilk test showed that age, body mass index (BMI), hemoglobin A1c (HbA1c), C-reactive protein (CRP), B-type natriuretic peptide (BNP), LVEF, TC, TG, HDL-C, LDL-C, non-HDL-C, ApoA-I, ApoB, lipoprotein (a) [Lp(a)], and RLP-C were not distributed normally, and were therefore expressed as the median and interquartile range (25th and 75th percentiles). Continuous variables were compared between groups using an unpaired t-test or the Mann-Whitney U-test, as appropriate. Frequencies were compared using the Chi-square test or Fisher's exact test. Spearman's rank correlation test was used for correlations between variables that were not distributed normally. The predictive value of the variables was assessed by univariate or stepwise multivariate Cox proportional hazards analyses. Multivariate Cox proportional hazards analysis was performed using confounders that were significant in the univariate model. The univariate and multivariate Cox proportional hazards analyses examined 1-SD increases in continuous variables. The presence of dichotomous variables was coded as 1 and the absence as 0. Kaplan-Meier survival analysis was used to compare 2 groups using the cutoff level of RLP-C, non-HDL-C, or TG.

C-statistics that incorporated receiver-operating characteristic (ROC) curve analysis were used to examine the additive effects of RLP-C, total ApoB, non-HDL-C, or TG on the predictive value of the baseline model consisting of traditional CVD risk factors, which included age, male sex, hypertension (defined as ≥140/90 mmHg or use of antihypertensive medication), current smoking, diabetes mellitus (defined by the American Diabetes Association or use of antidiabetic medication),[26] and LDL-C, and HDL-C levels. We also performed category-free net reclassification improvement (NRI) and integrated discrimination improvement (IDI) to analyze the degree to which the addition of

Table 1. Baseline Characteristics of Study Patients

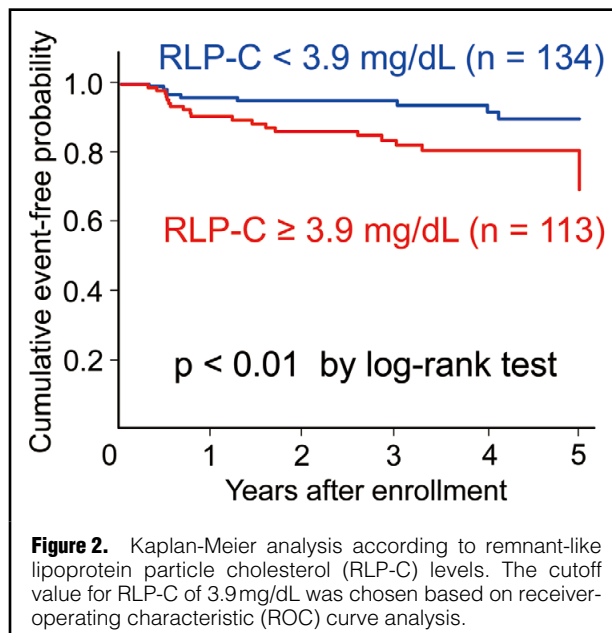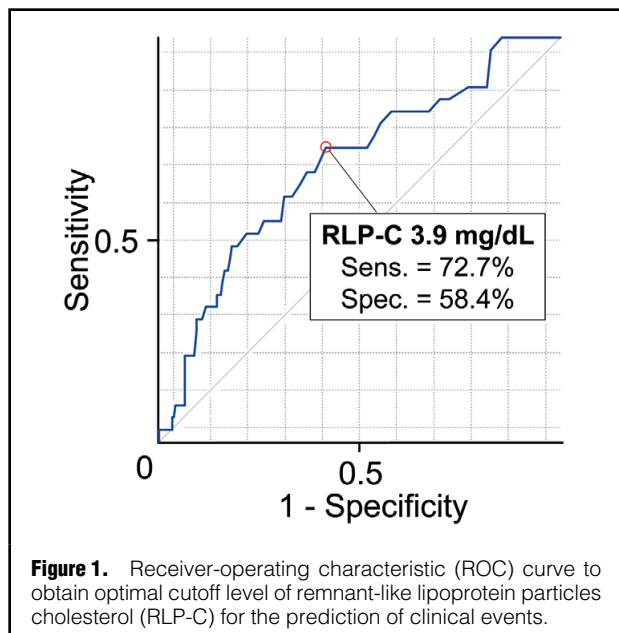|	All patients (n=247)	Without event (n=214)	With event (n=33)	P value
Age, years	67 (60–74)	67 (59–74)	70 (64–77)	0.11
Male sex, n (%)	225 (91)	195 (91)	30 (91)	1.00
Hypertension, n (%)	191 (77)	166 (78)	25 (76)	0.99
Current smoking, n (%)	63 (26)	48 (22)	15 (45)	<0.01
Diabetes mellitus, n (%)	107 (43)	89 (42)	18 (55)	0.23
Multivessel CAD, n (%)	152 (62)	131 (61)	21 (64)	0.94
Previous MI, n (%)	141 (57)	123 (57)	18 (55)	0.90
BMI, kg/m²	23.6 (22–26)	23.6 (22–26)	23.6 (21–25)	0.35
HbA1c, %	6.3 (5.8–6.9)	6.2 (5.8–6.7)	6.8 (6.1–7.5)	0.03
CRP, mg/dL	0.06 (0.02–0.17)	0.05 (0.02–0.18)	0.08 (0.04–0.16)	0.13
eGFR, mL/min/1.73m²	65.2±15.2	65.5±16.3	63.1±18.1	0.44
BNP, pg/mL	44 (18–107)	43 (16–101)	73 (27–171)	0.01
LVEF, %	62 (53–70)	63 (54–70)	57 (49–70)	0.30
TC, mg/dL	130 (120–143)	130 (120–142)	130 (122–150)	0.54
TG, mg/dL	118 (86–183)	109 (84–177)	159 (123–222)	<0.01
HDL-C, mg/dL	40 (35–50)	40 (35–51)	36 (32–47)	0.12
LDL-C, mg/dL	62 (56–67)	62 (56–67)	61 (54–66)	0.40
Non-HDL-C, mg/dL	86 (79–97)	85 (78–94)	91 (84–106)	0.03
ApoA-I, mg/dL	123 (109–140)	124 (109–140)	118 (109–139)	0.41
ApoB, mg/dL	64 (58–73)	63 (58–72)	71 (63–81)	0.01
Lp(a), mg/dL	15 (7.9–28)	15 (7.8–27)	17 (8.0–35)	0.35
RLP-C, mg/dL	3.6 (2.5–5.5)	3.4 (2.5–4.9)	5.5 (3.3–8.9)	<0.01
Medication, n (%)				
Aspirin	238 (96)	206 (96)	32 (97)	1.00
Thienopyridine	218 (88)	190 (89)	28 (85)	0.72
ACEI/ARB	165 (67)	148 (69)	17 (52)	0.07
β-blocker	95 (38)	82 (38)	13 (39)	1.00
Calcium antagonist	155 (63)	139 (65)	16 (48)	0.10
EPA	16 (6)	14 (7)	2 (6)	1.00
Ezetimibe	24 (10)	22 (10)	2 (6)	0.75
Fibrates	3 (1)	3 (1)	0 (0)	1.00
Metformin	30 (12)	27 (13)	3 (9)	0.78
Statin				
Atorvastatin	95 (38)	83 (39)	12 (36)	0.94
Rosuvastatin	116 (47)	101 (47)	15 (45)	1.00
Pravastatin	12 (5)	11 (5)	1 (3)	1.00
Pitavastatin	20 (8)	17 (8)	3 (9)	0.73
Simvastatin	4 (2)	2 (1)	2 (6)	0.09

Data are expressed as the mean value±SD, median and range (25th and 75th percentiles) or number (%) of patients. P values, comparison between patients with and without cardiovascular events. Hypertension, defined as blood pressure >140/90mmHg, or receiving antihypertensive therapy. Diabetes mellitus defined according to the American Diabetes Association criteria or taking antidiabetic medication. ACEI, angiotensin-converting enzyme inhibitor; ApoA-I, apolipoprotein A-I; ApoB, total apolipoprotein B; ARB, angiotensin II receptor blocker; BMI, body mass index; BNP, B-type natriuretic peptide; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; EPA, eicosapentaenoic acid; HbA1c, hemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; Lp(a), lipoprotein (a); LVEF, left ventricular ejection fraction; MI, myocardial infarction; RLP-C, remnant-like lipoprotein particle cholesterol; TC, total cholesterol; TG, triglyceride.

each lipid variable to the baseline model of traditional CVD risk factors improved its predictive ability.

All probabilities were expressed as two-tailed values, with statistical significance inferred at P<0.05. All confidence intervals (CI) were computed at the 95% level. The statistical analyses were performed using STATA 10.0 (StataCorp, College Station, TX, USA).

Calculation of the Sample Size

On the basis of our preliminary observations in patients with stable CAD and on-statin LDL-C <70mg/dL, the composite endpoint occurred in approximately 20% of patients with higher RLP-C levels (≥4.5mg/dL) during 5 years of follow-up, and in 5% of patients with lower RLP-C levels (<4.5mg/dL). To provide our two-sided statistical analyses with sufficient statistical power of 0.90 (β=0.10 and α=0.05), a total of 232 patients with stable

Figure 1. Receiver-operating characteristic (ROC) curve to obtain optimal cutoff level of remnant-like lipoprotein particles cholesterol (RLP-C) for the prediction of clinical events.

Figure 2. Kaplan-Meier analysis according to remnant-like lipoprotein particle cholesterol (RLP-C) levels. The cutoff value for RLP-C of 3.9 mg/dL was chosen based on receiver-operating characteristic (ROC) curve analysis.

CAD and on-statin LDL-C <70 mg/dL were required for the follow-up study. A total of 256 patients gave this prospective study sufficient statistical power.

Results

We withdrew 9 patients from the study after enrollment because they could no longer be contacted; thus, 247 patients completed the follow-up study. The duration of the follow-up period ranged from 2 to 60 months (mean 38±21 months). During the follow-up period, 33 patients had a CVE, which comprised 2 cardiac deaths, 1 AMI, 13 UAP requiring unplanned coronary revascularization, 9 worsening HF requiring hospital admission, 1 PAD requiring endovascular treatment, 3 aortic aneurysms requiring surgical treatment, and 4 ischemic strokes (**Supplementary Table**). The incidence of each event according to RLP-C levels is shown in **Supplementary Table**. The incidence of the primary composite endpoint and HF was higher in patients with high RLP-C levels than in those with low RLP-C levels (**Supplementary Table**).

Baseline Characteristics
The baseline characteristics of the study patients are summarized in **Table 1**. Compared with patients without CVE, those with an event had a higher prevalence of current smoking and higher levels of HbA1c, BNP, TG, non-HDL-C, total ApoB, and RLP-C at baseline (**Table 1**). There was no significant difference in LDL-C levels between the 2 groups. The types of statin administered were similar in patients with and without events (**Table 1**). RLP-C levels showed significant correlations with TG (ρ=0.80, P<0.01), TC (ρ=0.58, P<0.01), total ApoB (ρ=0.55, P<0.01), and non-HDL-C (ρ=0.70, P<0.01), but not with LDL-C (ρ=−0.09, P=0.17).

Predictive Value of RLP-C
ROC curve analysis showed that the cutoff value for RLP-C level was 3.9 mg/dL (**Figure 1**), with a sensitivity of 72.7% and specificity of 58.4% for predicting future CVEs. When the patients were stratified into 2 groups using the RLP-C cutoff value, Kaplan-Meier analysis showed that patients with high levels of RLP-C (≥3.9 mg/dL) (n=113) had a higher probability of future CVEs than those with lower levels (<3.9 mg/dL; n=134, P<0.01, log-rank test; **Figure 2**). Univariate Cox proportional hazard analysis showed current smoking (hazard ratio (HR): 2.50; 95% CI: 1.25–5.00), HbA1c (HR: 1.32; 95% CI: 1.01–1.73), TG (HR: 1.51; 95% CI 1.13–2.02), total ApoB (HR:1.47; 1.02–2.12), Lp(a) (HR: 1.31; 95% CI: 1.01–1.71), and RLP-C (HR: 1.54; 95% CI: 1.22–1.96) were significant predictors for CVEs (**Table 2**). As shown in **Table 2**, high levels of RLP-C (HR: 1.62; 95% CI: 1.26–2.07) and current smoking (HR: 2.39; 95% CI: 1.18–4.87) remained significant predictors of CVEs in the multivariate Cox proportional hazard analysis. In the Kaplan-Meier analysis, higher levels of TG (≥118 mg/dL, determined by ROC curve analysis) resulted in a higher probability of future CVEs than those with lower levels (<118 mg/dL) (**Supplementary Figures 1,2**), but higher non-HDL-C levels (≥86 mg/dL, determined by ROC curve) did not (**Supplementary Figures 3,4**).

Incremental Effect of RLP-C on Predictive Value of Traditional Risk Factors
The addition of RLP-C had a significant incremental effect on the area under the ROC curve (AUC) obtained with our baseline model that consisted of traditional risk factors including age, male sex, hypertension, current smoking, diabetes mellitus, LDL-C, and HDL-C (AUC; baseline model, 0.68 vs. baseline model+RLP-C, 0.77, P=0.02) (**Table 3**). The addition of total ApoB, but not non-HDL-C and TG, also had a significant incremental effect (**Table 3**). The addition of RLP-C level, total ApoB, non-HDL-C, or TG to the traditional risk factors enhanced Category-free NRI and IDI (**Table 4**). The addition of RLP-C but not TG to the traditional risk factors+non-HDL-C+ApoB enhanced Category-free NRI and IDI (**Table 4**).

Table 2. Univariate and Stepwise Multivariate Cox Hazard Analysis Models for Future Cardiovascular Events

	Univariate analysis			Multivariate analysis		
	HR	95% CI	P value	HR	95% CI	P value
Age, per 11 years	1.44	0.98–2.10	0.06		–	
Male sex	0.98	0.30–3.20	0.97		–	
Hypertension	0.83	0.38–1.85	0.65		–	
Current smoking	2.50	1.25–5.00	0.01	2.39	1.18–4.87	0.02
Diabetes mellitus	1.65	0.83–3.28	0.15		–	
Multivessel disease	1.10	0.54–2.23	0.80		–	
Previous MI	0.89	0.45–1.77	0.74		–	
BMI, per 3 kg/m^2	0.80	0.53–1.20	0.28		–	
eGFR, per 16 mL/min/1.73 m^2	0.84	0.60–1.18	0.30		–	
HbA1c, per 1%	1.32	1.01–1.73	0.04		Not selected	
BNP, per 122 pg/mL	1.19	0.96–1.48	0.12		–	
LVEF, per 12%	0.69	0.46–1.04	0.08		–	
LDL-C, per 8 mg/dL	0.92	0.60–1.41	0.70		–	
HDL-C, per 16 mg/dL	0.75	0.47–1.20	0.23		–	
Non-HDL-C, per 16 mg/dL	1.44	0.99–2.10	0.06		–	
TG, per 87 mg/dL	1.51	1.13–2.02	<0.01		Not selected	
ApoA-I, per 75 mg/dL	0.82	0.33–2.06	0.67		–	
ApoB, per 13 mg/dL	1.47	1.02–2.12	0.04		Not selected	
ApoB/ApoA-I, per 0.2	1.30	0.95–1.77	0.10		–	
Lp(a), per 21 mg/dL	1.31	1.01–1.71	0.04		Not selected	
RLP-C, per 3 mg/dL	1.54	1.22–1.96	<0.01	1.62	1.26–2.07	<0.01

The hazard ratio (HR) and 95% confidence interval (CI) for continuous variables were estimated by a 1-SD increase (per 1-SD). Abbreviations as in Table 1.

Table 3. C-Statistics for Discrimination Ability of ApoB, Non-HDL-C, TG, or RLP-C in Combination With Traditional Risk Factors to Predict Cardiovascular Events

	AUC	95% CI	P value
Traditional risk factors	0.68	0.58–0.78	–
+ApoB	0.76	0.67–0.86	0.03
+Non-HDL-C	0.74	0.65–0.85	0.09
+TG	0.75	0.65–0.85	0.06
+RLP-C	0.77	0.67–0.88	0.02

Our baseline model of traditional risk factors included age, male sex, hypertension, current smoking, diabetes mellitus, LDL-C and HDL-C. AUC, area under the receiver-operating characteristic curve. Other abbreviations as in Tables 1,2.

Table 4. NRI and IDI for the Incremental Predictive Values of the Combination of Traditional Risk Factors, ApoB, Non-HDL-C, TG and RLP-C

	Category-free NRI		IDI	
	Index	P value	Index	P value
Traditional risk factors	–	–	–	–
+ApoB	0.49	<0.01	0.03	0.04
+Non-HDL-C	0.62	<0.01	0.05	<0.01
+TG	0.54	<0.01	0.07	<0.01
+RLP-C	0.89	<0.01	0.10	<0.01
Traditional risk factors+non-HDL-C+ApoB	–	–	–	–
+RLP-C	0.52	<0.01	0.06	0.02
+TG	0.08	0.68	0.03	0.20
Traditional risk factors+non-HDL-C+RLP-C	–	–	–	–
+ApoB	0.43	0.02	0.005	0.50

The traditional risk factors consisted of age, male sex, hypertension, current smoking, diabetes mellitus, LDL-C and HDL-C. IDI, integrated discrimination improvement; NRI, net reclassification improvement. Other abbreviations as in Table 1.

Discussion

The present study found that RLP-C levels are an independent predictor of CVEs in patients with CAD and on-statin treatment LDL-C <70 mg/dL. The study also demonstrated that the addition of RLP-C had an additive prognostic value to models of traditional risk factors that include non-HDL-C and ApoB. Thus, RLP-C levels have an important role in the residual risk of future CVEs in patients with stable CAD and on-statin LDL-C <70 mg/dL. It remains to be determined whether RLP remains a risk factor for CVD in patients with very low levels of LDL-C (e.g., <30 mg/dL) after proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors. The very recent AHA/ACC Guidelines[2] recommend a modified equation[27] for calculation of LDL-C levels <70 mg/dL instead of the Friedewald formula. The re-calculated results using the novel equation[27] remained similar to those obtained using the Friedewald formula (data not shown).

Because the present study measured RLP from fasting serum, the fasting RLP are derived from VLDL.[28] Statins cause substantial falls in LDL and a relatively modest decrease in TGLs, including VLDL and RLP.[29,30] Our previous in vitro studies showed that RLP induced expression of endothelial proatherogenic molecules and endothelial vasomotor dysfunction to a several-fold greater extent compared with LDL or other TGLs.[31,32] As RLP had greater atherogenicity than other TGLs, RLP-C levels remained a predictor of CVEs despite the reduction in RLP-C levels on statin treatment.

Although the NCEP Guidelines recommend non-HDL-C measurement as a secondary target of lipid-lowering therapy after the LDL-C goal has been achieved,[14] non-HDL-C includes atherogenic TGLs when TG is >200 mg/dL.[14] As the present patients had a normal range of TG levels and low non-HDL-C levels, non-HDL lipoproteins may not have included a significant level of atherogenic TGLs. Median levels of RLP-C and non-HDL-C in the present patients were 3.6 mg/dL and 86 mg/dL, respectively. Thus, judging by their concentrations, the atherogenicity of RLP-C made little contribution to that of non-HDL-C.

Total ApoB levels were a predictor of the CVEs in the present study's univariate Cox proportional hazard analysis. The total ApoB concentration reflects the number of LDL and TGL particles, and the level of ApoB may not be reduced as much as expected from the reduction of LDL-C while on statin treatment.[33] In this sense, total ApoB may reflect atherogenic TGLs while on statin treatment.[7] However, total ApoB lost its predictability in the multivariate Cox proportional hazard analysis using covariates including RLP-C in the present study because total ApoB had a significant correlation with RLP-C. TG had significant predictability of CVEs in both the Kaplan-Meier curve analysis and univariate Cox proportional hazard analysis. And the addition of TG to the traditional risk factors enhanced Category-free NRI and IDI. However, TG lost its predictability in the multivariate Cox proportional hazard analysis when RLP-C was included in covariates because TG levels also had a strong correlation with RLP-C levels.

Previous reports have shown that fibrates and ezetimibe as well as statins effectively reduce RLP-C.[34,35] Therefore, an increased dose of statin alone or in combination with fibrates or ezetimibe may have been needed to reduce RLP-C in the present patients. Although PCSK9 inhibitors cause a relatively modest reduction of TGLs,[36] their effect on RLP-C levels remains unclear.

Study Limitations

First, although the current immunoseparation method for assaying RLP has been shown by us and other investigators to be useful for assessing and monitoring CVD risk in patients with hypertriglyceridemia,[16-21] the assay has economic and technical limitations for routine clinical practice in countries other than Japan and the USA. Second, the relatively small number of enrolled patients limited the statistical power of the study. Third, we did not have information regarding medication for CVD prior to enrollment in the study, which might have affected the frequency of CVEs during the follow-up period.

Conclusions

Our study showed evidence that RLP-C levels are a residual risk of future CVD in patients with stable CAD and on-statin LDL-C <70 mg/dL.

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Supplementary Files