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Association of triglyceride-rich lipoproteins-related markers and low-density lipoprotein heterogeneity with cardiovascular risk: Effectiveness of polyacrylamide-gel electrophoresis as a method of determining low-density lipoprotein particle size

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ABSTRACT

Background: Despite well-controlled low-density lipoprotein cholesterol (LDL-C), hypertriglyceridemia is an independent predictor of coronary events. We investigated the risk of atherosclerotic cardiovascular disease through examining the relation between triglyceride (TG) metabolism and LDL-heterogeneity as assessed by polyacrylamide-gel electrophoresis (PAGE).

Methods and results: Estimated LDL-particle size [relative LDL migration (LDL-Rm value)] measured by PAGE with the LipoPhor system (Joko, Tokyo, Japan) was evaluated in 645 consecutive patients with one additional risk factor for atherosclerotic cardiovascular disease.Multivariate regression analysis after adjustments for traditional risk factors revealed an elevated triglyceride-rich lipoproteins (TRLs)-related markers [TG, remnant-like particle cholesterol (RLP-C), very LDL (VLDL) fraction, apolipoprotein (apo) C-II, and apo C-III] level to be an independent predictor of smaller-size LDL-particle size, both in the overall population, and in a subset of patients with serum LDL-C <100 mg/dL. Even among the patients with LDL-C levels <100 mg/dL, the serum levels of atherogenic lipid markers in those with a LDL-Rm value ≥0.40, suggesting the presence of large amounts of small-dense LDL and upper limit (mean + 2 standard deviation) in this population, were significantly higher than in those with a LDL-Rm value <0.40. Moreover, the serum levels of TRLs-related markers showed high accurate area under the receiver-operating characteristic curve (TG, 0.896; RLP-C, 0.875; VLDL fraction, 0.803; apo C-II, 0.778; and apo C-III, 0.804, respectively) in terms of evaluation of the indicators of LDL-Rm value ≥0.40.

Conclusion: To further reduce the risk of atherosclerotic cardiovascular disease, it may be of particular importance to pay attention not only to the quantitative change in the serum LDL-C, but also TG-metabolism associated with LDL-heterogeneity. Combined evaluation of TRLs-related markers and LDL-Rm value may be useful for assessing the risk of atherosclerotic cardiovascular disease.

Introduction

Large observational studies clearly show that elevated triglyceride (TG) levels are associated with increased coronary artery disease (CAD) risk [1]. Moreover, in recent years evidence of the importance of the substances associated with hypertriglyceridemia as residual risks of CAD has been increasing even in successful low-density lipoprotein cholesterol (LDL-C) reduction trials [2]. TG metabolites, i.e. chylomicrons, very low density lipoprotein (VLDL), and remnant-like particle cholesterol (RLP-C), which are TG-rich lipoproteins (TRLs), and, apolipoprotein (apo) CII and apo CII which are involved in the metabolic process, etc. have been demonstrated to be involved in the progression of atherosclerosis [3].

In addition, conversion of LDL to small particles (small dense LDL, sd-LDL), which hypertriglyceridemia causes, is a powerful promoter of atherosclerotic cardiovascular disease, especially CAD, and has also been reported to be a predictor of ischemic cardiac events [4].

Density gradient ultracentrifugation, nondenaturing gradient gel electrophoresis, and nuclear magnetic resonance spectroscopy are the methods that are usually employed to measure LDL-particle size; but they are difficult to apply in clinical settings due to

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their cost and complexity [5]. On the other hand, estimation of LDL-particle size based on relative LDL migration (LDL-Rm) during polyacrylamide-gel electrophoresis (PAGE) has been reported. Currently, the Lipoprint system, also based on the PAGE system, is used for estimation of the LDL particle size by a simple procedure in the clinical setting, and has been shown to carry high diagnostic accuracy [6]. In Japan, however, the Lipoprint system is not commercially available at present. We used the LipoPhor system (Joko, Tokyo, Japan) for the evaluation of the LDL-particle size in this study. High correlation of the assay results between the two methods has been reported [5,7,8].

The purpose of this study was to evaluate the usefulness of measuring LDL-Rm value as an index of the LDL-particle size by examining the relation between the serum levels of TRLs-related markers (TG, VLDL fraction, RLP-C, apo C-II, and apo C-III) and the LDL-Rm value by the hospital-based cross-sectional study method, and furthermore, to reevaluate the risk of CAD through examining the relations between TG-metabolism and LDL-particle size.

Methods

Study design and populations

This study was designed as a hospital-based cross-sectional study to investigate the relationships between the serum levels of TRLs-related markers as indicators of the risk of atherosclerotic cardiovascular disease and LDL-heterogeneity in patients with the presence of one or more risk factors for atherosclerosis.

The study was conducted on a sample of 700 consecutive outpatients who had undergone regular examinations and blood examinations at Cardiovascular Center, Surugadai Nihon University Hospital between April 2009 and October 2009.

The criterion for patient registration in the cross-sectional study was the presence of one or more risk factors for atherosclerosis. The diagnostic criteria for the coronary risk factors that we used in this study analysis were: a diagnosis of hypertension was made when systolic pressure was 140 mmHg or diastolic pressure was 90 mmHg, or above, or taking medication. Diabetes was defined as a fasting plasma glucose concentrations ≥126 mg/dL and hemoglobin (Hb) A1c ≥6.5% [according to the National Glycohemoglobin Standardization Program (NGSP)], or current treatment with anti-diabetic agents. A diagnosis of dyslipidemia was made when the LDL-C level was 140 mg/dL or above, the TG level was 150 mg/dL or above, or the high-density lipoprotein cholesterol (HDL-C) level was less than 40 mg/dL, or if the patient was already on lipid-lowering medication. The severity of chronic kidney disease (CKD) was determined on the basis of the estimated glomerular filtration rate (GFR) using the abbreviated Modification of Diet in Renal Disease (MDRD) Study equation modified by a Japanese coefficient [9].

The Surugadai Nihon University Hospital Ethics Committee approved all study design and purpose.

Measurement of laboratory parameters

Fasting blood samples were collected early in the morning after a 12-h fast. The serum total cholesterol (TC), HDL-C, and TG levels were measured by the standard methods. LDL-C levels were estimated by using the Friedewald formula [10]. The RLP-C level was measured by an immunoadsorption assay (SRL Co., Ltd., Tokyo, Japan). The VLDL fraction was measured by performing PAGE electrophoresis using the LipoPhor system. The serum apo level was determined by turbidimetric latex agglutination assays (Daiichi Pure Chemicals Co., Ltd., Tokyo, Japan). The malondialdehyde-modified LDL (MDA-LDL) level was measured by an enzyme-linked immunosorbent assay (SRL Co., Ltd.). The high sensitivity C-reactive protein (hs-CRP) level was measured by a nephelometric assay (Behring Diagnostic, Marburg, Germany).

Measurement of LDL-Rm value

LDL-Rm value, an indicator of LDL-particle size, was measured relative to the mobility value of LDL by performing PAGE with the LipoPhor system. LDL-Rm value was calculated as the distance between the VLDL peak and the LDL peak divided by the distance between the VLDL peak and the HDL peak (Fig. 1). Several studies have reported that an LDL-Rm value of 0.40 or more suggests the presence of a large amount of sd-LDL in the LDL fraction [11–13]. The subjects of this study were not healthy persons in the general population, but the upper limit of the reference interval of the subjects' LDL-Rm values (mean ± 2 Standard deviation [covering 95% of the population: 0.350 ± 0.058]) was 0.408, and it was approximately the same as the 0.40 reported above. Accordingly, we conducted this study on the assumption that a large amount of sd-LDL was present in the LDL fraction when the LDL-Rm value was 0.40 in the present study as well.

Statistical analysis

Data are expressed as the mean ± standard deviation for continuous variables and as percentages for discrete variables. Univariate and multivariate regression analyses were performed to identify independent predictors of LDL-Rm value. All variables correlated with the LDL-Rm values at $p < 0.05$ in the univariate regression analysis were entered into the multivariate model. A receiver-operating characteristic (ROC) analysis was performed to determine the TRLs-related marker cut-off values that indicated an LDL-Rm value ≥0.40. Univariate and multivariate logistic regression analyses of variables affecting patient characteristics with LDL-Rm values were ≥0.40 were performed in patients with LDL-C level <100 mg/dL. A p-value less than 0.05 was considered to indicate statistical significance. All statistical analyses were performed with the SPSS software program (SPSS Inc., Chicago, IL, USA) for Windows (version 12.0.1).

Results

Patients

We excluded 55 subjects from the analysis because of missing laboratory data. Therefore, finally, 645 subjects were included in

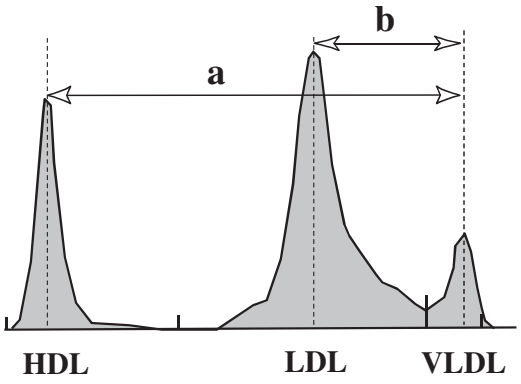

Fig. 1. Measurement of LDL-Rm value by lipoprotein polyacrylamide gel disk electrophoresis. LDL-Rm, relative low-density lipoprotein migration; LDL, low-density lipoprotein; HDL, high-density lipoprotein; VLDL, very-low-density lipoprotein; LDL-Rm value calculated from densitometer analysis of polyacrylamide disk gel electrophoresis; LDL-Rm value = b/a.

the analysis. The patient characteristics and laboratory profile are shown in Table 1. The mean LDL-Rm value was 0.350 ± 0.029.

Univariate and multivariate regression analyses of indicators of estimated LDL-particle size

No correlations were found between LDL-Rm value values and age CKD ≥ stage 3, or statin use. Male gender, presence of smoking, hypertension, and diabetes mellitus were associated with high LDL-Rm values. LDL-Rm values were significantly positively correlated with body mass index (BMI), number of risk factors, serum LDL-C, TRLs-related markers, and CRP levels, and significantly negatively correlated with HDL-C levels. These variables which showed $p < 0.05$ were entered into the multivariate regression model.

Table 1
Patient characteristics and laboratory profile.

	N = 645
Male/female, *n* (%)	445 (69)/200 (31)
Age (years)	62 ± 14
BMI (kg/m^2)	24.0 ± 3.9
Hypertension, *n* (%)	458 (71)
Diabetes mellitus, *n* (%)	181 (28)
HbA1c (%)	5.97 ± 0.76
Current smoking, *n* (%)	174 (27)
Dyslipidemia, *n* (%)	400 (62)
eGFR (ml/min/1.73 m^2)	70.8 ± 18.4
CKD≥ stage 3, *n* (%)	135 (21)
Number of risk factors	3.5 ± 1.5
Coronary artery disease, *n* (%)	142 (22)
Concomitant drug, n(%)	
Anti-plateletes	174 (27)
ACE inhibitors	52 (8)
ARBs	258 (40)
β blockers	135 (21)
Calcium channel blockers	297 (46)
Statins	297 (46)
Fibrate	12 (1.9)
Ezetimibe	11(1.7)
Colestimide	3 (0.5)
	N = 645
Lipids and apolipoproeins	
TC (mg/dL)	195 ± 38
LDL-C (mg/dL)	109 ± 31
HDL-C (mg/dL)	58 ± 17
Non-HDL-C (mg/dL)	137 ± 34
apo A-1 (mg/dL)	146 ± 30
apo B (mg/dL)	90 ± 21
MDA-LDL (U/L)	110 ± 44
TRLs-related markers	
TG (mg/dL)	120 (87–176)
VLDL (%)	11 (8.0–16.0)
RLP-C (mg/dL)	5.3 (3.8–7.4)
apo C-II (mg/dL)	4.6 ± 2.1
apo C-III (mg/dL)	10.2 ± 3.8
Inflammatory markers	
WBC count (cell/μl)	6096 ± 1630
CRP (mg/dl)	0.05 (0.03–0.12)
Estimated LDL-particle size	
LDL-Rm value	0.350 ± 0.029

BMI: body mass index; Hb: hemoglobin; eGFR: estimated glomerular filtration rate; CKD: chronic kidney disease; In this analysis, risk factors were defined as: age ≥65 years, male, BMI ≥25 kg/m^2, current smoker, hypertension, diabetes mellitus, CKD ≥stage 3, and dyslipidemia. We calculated the mean number of risk factors of the subjects of this study on the basis of the total numbers of risk factors that were present. ACE: angiotensin-converting enzyme; ARB: angiotensin receptor broker; TC: total cholesterol; LDL: low-density lipoprotein; HDL: high-density lipoprotein; apo: apolipoprotein; MDA-LDL: malondialdehyde-modified LDL; TG: triglyceride; VLDL: very low-density lipoprotein; RLP: remnant-like particle; WBC: white blood cell; CRP: C-reactive protein; LDL-Rm value: relative LDL migration measured by polyacrylamide-gel elecrophoresis.

As the TRLs-related markers constituted mutually confounding factors, five multivariate regression models incorporating the respective variables were established to carry out the analysis. All multivariate regression analysis models showed that the serum TRLs-related markers, HDL-C, and LDL-C levels were independent and significant indicators of LDL-Rm value in all of the subjects (Table 2a).

Accordingly, we performed univariate and multivariate regression analyses to identify indicators of LDL-Rm value in the subjects whose LDL-C level was <100 mg/dL (Table 2b). The results showed that male gender and presence of hypertension were significantly associated with high values of LDL-Rm. The LDL-Rm values were significantly positively correlated with BMI, number of risk factors, the serum TRLs-related markers, and CRP levels, and significantly negatively correlated with the HDL-C levels. As a result, all multivariate regression analysis models showed the serum TRLs-related markers, and HDL-C levels were identified as independent indicators in LDL-Rm value.

Comparison of the serum TRLs-related, and atherogenic markers values of the patients with LDL-C values <100 mg/dL according to whether their LDL-Rm values were ≥ 0.40 or < 0.40

We compared the TRLs-related marker and atherogenic marker levels of the subjects with LDL-C values <100 mg/dL according to whether they had LDL-Rm values ≥0.40 or <0.40. The difference in LDL-C values between the group that had LDL-Rm values ≥0.40 and the group that had LDL-Rm <0.40 was not significant [79 (66–94) mg/dL vs. 85 (75–92) mg/dL]. The serum TG [278 (205–331) mg/dL vs. 107 (874–145) mg/dL], RLP-C [9.4 (7.9–13.7) mg/dL vs. 4.3 (3.3–5.7) mg/dL], %VLDL [24.0 (17.8–29.2)% vs. 11.0 (7.0–15.0)%], apo CII [5.7 (5.1–8.3) mg/dL vs. 3.6 (2.8–4.7) mg/dL], and apo C III [14.3 (11.2–17.4) mg/dL vs. 8.6 (7.2–10.3) mg/dL] values were significantly higher in the LDL-Rm ≥0.40 group than in the LDL-Rm <0.40 group. The serum HDL-C levels were significantly lower in the LDL-Rm value ≥0.40 group than in the LDL-Rm value <0.40 group [43 (39–54) mg/dL vs. 59 (46–70) mg/dL]. Significantly higher apo B [85 (80–91) mg/dL vs. 71 (64–78) mg/dL], MDA-LDL [108 (102–127) U/L vs. 79 (66–95) U/L], and non-HDL-C [122 (113–138) mg/dL vs. 107 (97–116) mg/dL] levels were found in the LDL-Rm ≥0.40 group than in the LDL-Rm <0.40 group.

As a result, a lower LDL-C/apoB ratio, a rough indicator of LDL-particle size, [0.92 (0.85–1.05) vs. 1.17 (1.08–1.25)], and higher MDA-LDL/apoB ratio, extent of oxidative modification of LDL-particles [5,14,15] [1.34 (1.16–1.42) vs. 1.14 (0.98–1.30)], were found in the LDL-Rm value ≥0.40 group than in the LDL-Rm value <0.40 group (Fig. 2). Fig. 3 shows a case of atherogenic dyslipidemia-pattern with a serum LDL-C of <100 mg/dL and LDL-Rm of ≥0.40 on PAGE.

Characteristics of patients with LDL-C level <100 mg/dL according to whether their LDL-Rm value was ≥0.40 or <0.40

The presence of TG levels ≥150 mg/dL and presence of HDL-C levels <40 mg/dL were significantly higher in the LDL-Rm value ≥0.40 group than in the LDL-Rm value <0.40 group, but there were no significant differences between the two groups according to gender, age, BMI, whether hypertension was present, whether diabetes mellitus was present, HbA1c values, whether a current smoker, whether CKD ≥ stage 3, whether CAD was present, or statin use (Table 3a).

Since the univariate logistic regression analysis showed that the presence of TG levels ≥150 mg/dL and the presence of HDL-C levels of <40 mg/dL were significantly associated with LDL-Rm values ≥0.40, these variables were entered into the multivariate logistic

Table 2a
Univariate and multivariate regression analyses of indicators estimated LDL-particle size (all cases).

All cases												n = 645
Variables	Univariate		Multivariate 1		Multivariate 2		Multivariate 3		Multivariate 4		Multivariate 5	
	r	p	β	p	β	p	β	p	β	p	β	p
Age	0.024	0.547										
Male	0.128	0.001										
BMI	0.191	<0.0001										
Smoking	0.078	0.049										
Hypertension	0.102	0.0095										
Diabetes mellitus	0.077	0.0049										
CKD ≥ stage 3	0.059	0.130										
Numbers of risk factor	0.210	<0.0001										
LDL-C	0.195	0.0001	0.123	0.0005	0.209	<0.0001	0.090	0.015	0.094	0.010	0.089	0.0079
HDL-C	−0.396	<0.0001	−0.229	<0.0001	−0.268	<0.0001	−0.331	<0.0001	−0.407	<0.0001	−0.555	<0.0001
TRLs-related markers												
TG*	0.531	<0.0001	0.418	<0.0001	–	–	–	–				
VLDL*	0.347	<0.0001	–	0.200	<0.0001	–	–	–				
RLP-C*	0.449	<0.0001	–	–		0.364	<0.0001	–	–			
apo C-II	0.374	<0.0001	–	–	–				0.374	<0.0001	–	
apo C-III	0.385	<0.0001	–	–		–			–		0.517	<0.0001
CRP*	0.142	0.0003										
Statins use	0.077	0.059										

Table 2b
Univariate and multivariate regression analyses of indicators estimated LDL-particle size (LDL-C <100 mg/dL).

LDL-C < 100mg dL												n = 283
Variables	Univariate		Multivariate 1		Multivariate 2		Multivariate 3		Multivariate 4		Multivariate 5	
	r	p	β	p	β	p	β	p	β	p	β	p
Age	0.057	0.369										
Male	0.181	0.0043										
BMI	0.161	0.017										
Smoking	0.088	0.171										
Hypertension	0.161	0.011										
Diabetes mellitus	0.063	0.405										
CKD ≥ stage 3	0.114	0.074										
Numbers of risk factor	0.248	<0.0001										
LDL-C	0.053	0.405										
HDL-C	−0.375	<0.0001	−0.180	0.0074	−0.202	0.009	−0.302	<0.0001	−0.412	<0.0001	−0.535	<0.0001
TRLs -related markers												
TG*	0.557	<0.0001	0.490	<0.0001	–		–		–		–	
VLDL*	0.403	<0.0001	–		0.287	<0.0001	–		–		–	
RLP-C*	0.444	<0.0001	–		–		0.399	<0.0001	–		–	
apo C-II	0.369	<0.0001	–		–		–		0.410	<0.0001	–	
apo C-III	0.388	<0.0001	–		–		–		–		0.517	<0.0001
CRP*	0.131	0.041										
Statins use	0.077	0.227										

The abbreviations are the same as in Table 1; r: correlation coefficient; β: standard partial regression coefficient; *log-transformed value was used; male (0: female, 1: male); smoking (0: no, 1: yes); hypertension (0: no, 1: yes); diabetes mellitus (0: no, 1: yes); CKD ≥ stage 3 (0: no, 1: yes); statin use (0: no, 1: yes). Non-HDL-C, apo B, and MDA-LDL were excluded as independent variables because of their multicolinearity with the LDL-C values. apo A-1 was also excluded as independent variables because of their multicolinearity with the HDL-C values. Only variables with a *p*-value of <0.05 as obtained by multivariate analyses were included.

regression model. The presence of a TG level ≥150 mg/dL was a significant and independent predictor of an LDL-Rm value ≥0.40 in patients with an LDL-C level <100 md/dL (Table 3b).

Receiver-operating characteristic analysis of indicators of LDL-Rm value ≥0.40

An ROC analysis was performed to determine the TRLs-related marker cut-off values that indicated an LDL-Rm value ≥0.40, and a good area under the curve (AUC) was obtained for both of them (Fig. 4). It was particularly noteworthy that all of the TRLs-related markers cut-off values for LDL-Rm value ≥0.40 were close to the upper limit of the normal values that are used in routine clinical practice [16].

Discussion

The results of this cross-sectional study showed that: 1) TRLs, i.e. RLP-C and VLDL, which are related to TG-metabolism, and high apoC-II and apoC-III values, as well as TG levels are independent indicators of LDL conversion to small particles, and 2) the combined evaluation of TRLs-related markers and LDL-Rm value may become a significantly important tool in the risk stratification of patients with CAD, due to the simplicity of the measurement and calculation in clinical settings.

Although statins significantly decrease the incidence of CAD, residual CAD risk remains high. A Cholesterol Treatment Trialists' (CTT) meta-analysis of 14 large-scale clinical trials that verified the efficacy of statins showed that statins reduced CAD mortality by approximately 20% [17]. Naturally, involvement by other risk

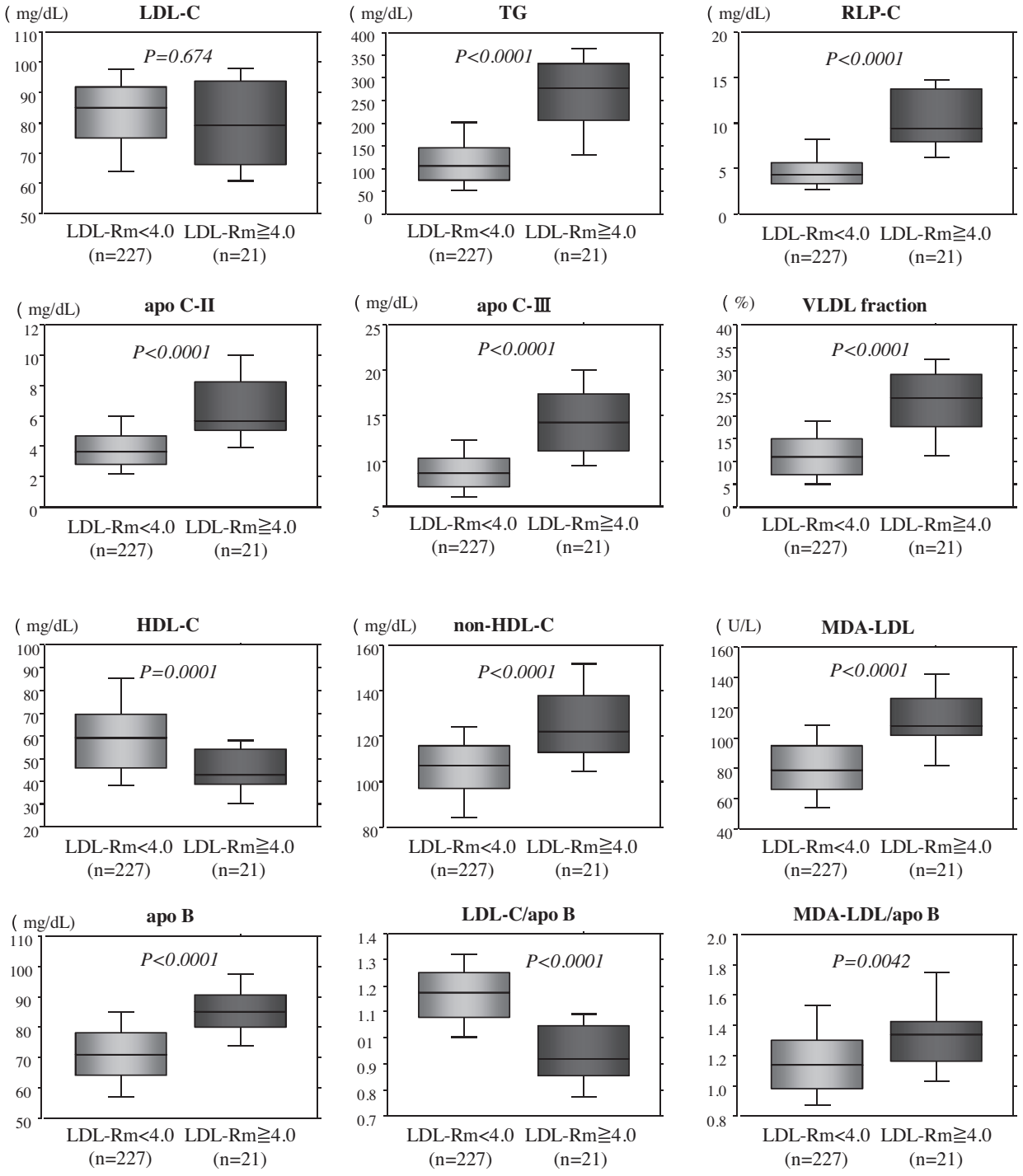

Fig. 2. Comparison of the serum TRLs-related, and atherogenic markers values of the patients with LDL-C levels <100 mg/dL according to whether their LDL-Rm values were ≥0.40 or <0.40. TRLs, triglyceride-rich lipoproteins; LDL-Rm, relative low-density lipoprotein migration; LDL, low-density lipoprotein; TG, triglyceride; RLP-C, remnant-like particle cholesterol; apo, apolipoprotein; VLDL, very LDL; HDL, high-density lipoprotein; MDA, malondialdehyde-modified.

factors besides LDL-C must be taken into consideration, but when viewed from the standpoint of lipid metabolism abnormalities, the results suggested a large involvement by atherosclerosis-inducing conversion of LDL to small particles. This may partly be due to uncorrected atherogenic dyslipidemia. The driving force behind atherogenic dyslipidemia is hypertriglyceridemia, which results from hepatic oversecretion and/or hypocatabolism of TRLs [18]. The Québec Cardiovascular Study demonstrated that in patients with LDL-particle sizes of 25.5 nm or smaller, the CAD incidence increased significantly as the serum LDL-C level increased, while in patients having large LDL-particle sizes of 26.0 nm or greater, no significant difference in the CAD incidence was observed depending on the absolute serum LDL-C level [19].

Now that the advent of statins has made it easy to manage LDL-C, perhaps what we should do to further improve the outcome of CAD is monitor LDL-particle size and attempt to reduce the residual CAD risk. Estimation of the LDL-particle size may be essential for prevention of CAD, especially in high-risk patients with multiple risk factors. The noteworthy result of this study is having shown that LDL-Rm value, which can be easily measured in

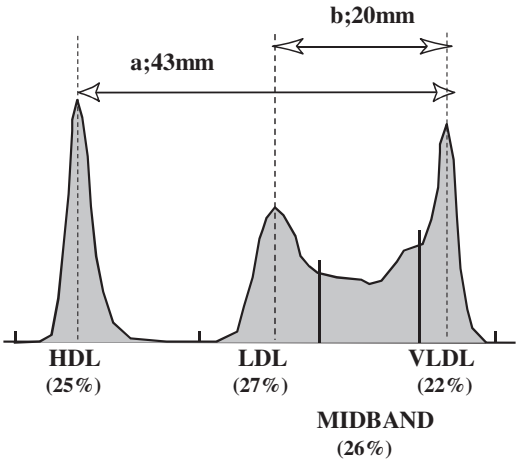

Fig. 3. A case of atherogenic dyslipidemia-pattern with a serum LDL-C of <100 mg/dL and LDL-Rm value ≥4.0 on lipoprotein polyacrylamide gel disk electrophoresis LDL, low-density lipoprotein; VLDL, very LDL; HDL, high-density lipoprotein; LDL-Rm, relative LDL migration. We present the case of a 57-year-old female metabolic syndrome patient who had a waist circumference of 92 cm, hypertension, a low HDL cholesterol level, hypertriglyceridemia, and no history of atherosclerotic cardiovascular disease. The patient's hypertension is being treated with antihypertensive medication, and we are trying to decrease the hypertriglyceridemia by improving the patient's lifestyle. The patient's atherosclerosis-related marker values are as follows: b/a = LDL-Rm value, 0.47; total cholesterol, 166 mg/dL; LDL-cholesterol, 73 mg/dL; HDL-cholesterol, 35 mg/dL; triglycerides, 279 mg/dL; VLDL fraction, 22%; remnant-like particle cholesterol, 16.1 mg/dL; apolipoprotein (apo) C-II, 4.2 mg/dL; apoC-III, 12.7 mg/dL; malondialdehyde-modified (MDA)-LDL, 99 U/L; apoB, 85 mg/dL; LDL-cholesterol/apoB, 0.86; MDA-LDL/apoB, 1.16.

Table 3a
Characteristics of patients with LDL-C level <100 mg/dL according to whether their LDL-Rm value was ≥0.40 or <0.40.

	LDL-Rm value ≥0.40 $n=21$	LDL-Rm value <0.40 $n=227$	p-Value
Male/female, n (%)	17(81)/4 (19)	159 (70)/68 (30)	0.292
Age (years)	61 ± 9.5	64 ± 14.4	0.390
BMI (kg/m^2)	23.7 ± 2.8	23.5 ± 4.1	0.807
Hypertension, n (%)	18 (86)	163 (72)	0.170
Diabetes mellitus, n (%)	8 (38)	66 (29)	0.387
HbA1c (%)	5.7 ± 0.6	5.5 ± 0.7	0.350
Current smoking (%)	5 (24)	30 (13)	0.155
CKD stage 3 ≥, n (%)	7 (33)	69 (30)	0.568
Coronary artery disease, n (%)	5 (24)	77 (34)	0.346
TG 150 mg/dL ≥, n (%)	18 (86)	52 (23)	<0.0001
HDL-C 40 mg/dL<, (%)	6 (29)	25 (11)	0.020
Statin use	12 (57)	129 (57)	0.978

The abbreviations are the same as in Table 1.

clinical settings, is capable of serving as an indicator of LDL-particle size. That was corroborated by the fact that good TRLs-related marker AUCs were obtained in an ROC analysis that stipulated LDL-Rm values ≥0.40, and that their cut-off values were shown to be similar to the upper limits of the standard values used in clinical settings [16].

Moreover, surprisingly, there was a group of subjects whose LDL-C values were well controlled, i.e. <100 mg/dL, but whose LDL-Rm values were ≥0.40, in which a larger amount of sd-LDL was suggested than in the group with LDL-Rm values <0.40, which occur as a result of high levels of atherogenic markers [20–24], which have strong atherosclerotic cardiovascular disease-inducing activity. This seems to mean that even when LDL-C has been aggressively reduced by high-dose statin therapy, the fact that TG is an independent predictor of CAD should be taken into account as a contributing factor. In recent years a subanalysis of the PROVE IT-TIMI 22 Trial [25] and post hoc analysis of the IDEAL and TNT Trials [26] showed that hypertriglyceridemia is an independent predictor of coronary events even in cases in which the target LDL-C level has been reached as a result of statin therapy. The above observations may strongly support our present results.

Study limitations/clinical implications

The first limitation of this study was that the subjects of the study had risk factors for atherosclerotic cardiovascular disease, and further study will be necessary to investigate whether the findings can be applied to the entire population. The second limitation is that LDL-Rm value should always be viewed as the mean size of aggregates of molecules having different LDL-particle sizes, and since its relation to the absolute amount of sd-LDL is unknown, the relationship between them should be clarified in the future.

This study focused on TG-metabolism, which is difficult to control by drug therapy, in a hospital-based cross-sectional study that closely reflected clinical practice, and demonstrated markers for evaluating risk with a view to lipid management of atherosclerosis patients. Thus, it seems that the finding that measurements of LDL-Rm value, which can be conveniently performed in clinical practice, are useful as a method of evaluating LDL-particle size will make a major contribution to further prevention of atherosclerotic cardiovascular disease.

Because this was a cross-sectional study, it is impossible to draw categorical conclusions in regard to causal relationships with the individual results. Nevertheless, changes in LDL-particle size and TRLs-related markers as a result of reducing the serum TG levels are important as a means of evaluating the risk of atherosclerotic diseases. Fig. 5 shows a case in which there was a simultaneous increase in LDL-particle size and improvement in atherogenic

Table 3b
Univariate and multivariate logistic regression analyses of variables affecting patient characteristics with LDL-Rm values were ≥0.40.

Variables	Univariate			Multivariate		
	OR	95% CI	p-Value	OR	95% CI	p-Value
Male/female	1.818	0.590–5.603	0.298			
Age ≥65 years	0.462	0.180–1.187	0.109			
BMI ≥25 kg/m^2	0.893	0.332–2.399	0.822			
Hypertension	2.370	0.675–8.325	0.178			
Diabetes mellitus	1.501	0.595–3.795	0.390			
Current smoking	2.154	0.732–6.341	0.164			
CKD ≥ stage 3	1.145	0.443–2.962	0.760			
Coronary artery disease	0.609	0.215–1.724	0.350			
TG ≥ 150 mg/dL	20.192	5.722–71.255	<0.0001	18.763	5.285–66.620	<0.0001
HDL-C < 40 mg/dL	3.232	1.149–9.090	0.026	2.200	0.699–6.929	0.178
Statin use	1.013	0.410–2.500	0.978			

The abbreviations are the same as in Table 1. OR: odds ratio; CI: confidence interval.

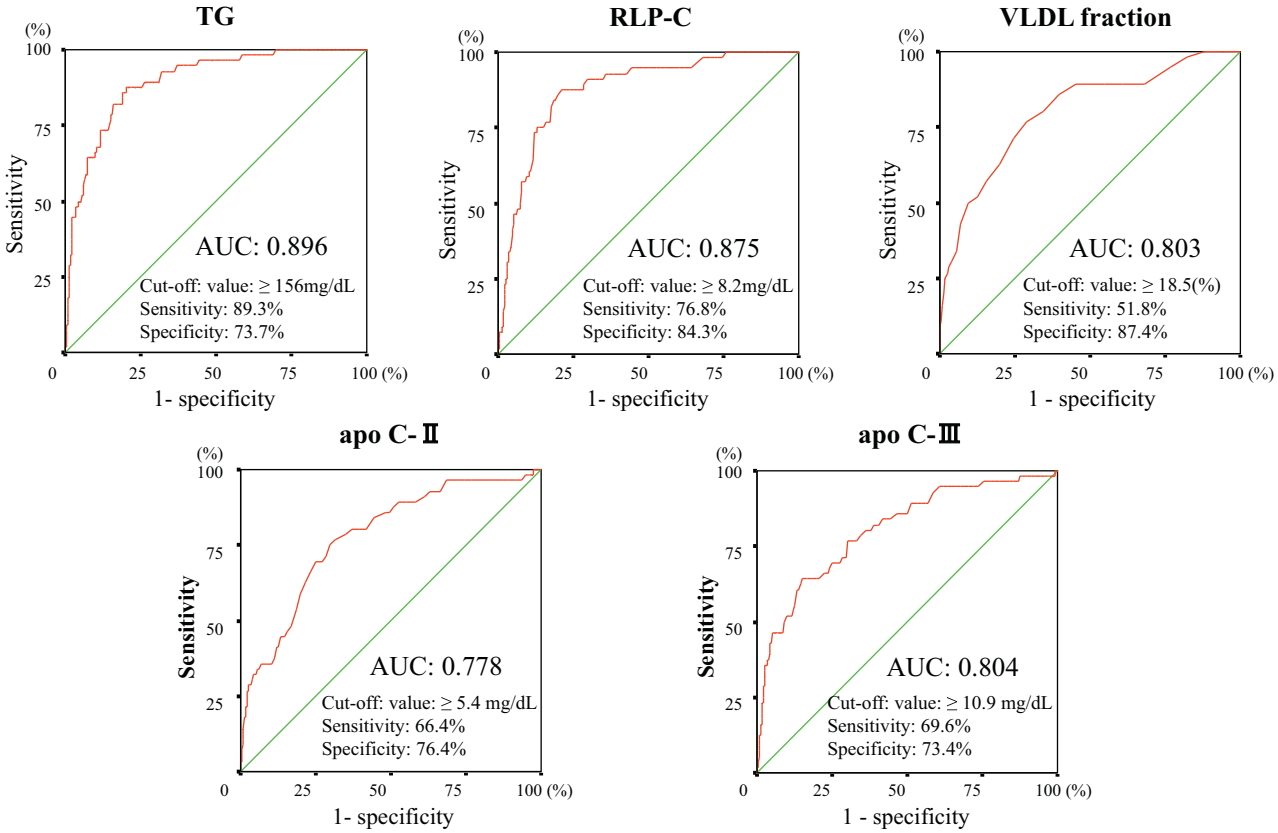

Fig. 4. Receiving-operating characteristics analysis of predictors of LDL-Rm value ≥0.40. LDL-Rm, relative low-density lipoprotein migration; TG, triglyceride; RLP-C, remnant-like particle cholesterol; VLDL, very low-density lipoprotein; apo, apolipoprotein; AUC, area under the curve.

	Baseline	6 month
Lipids (mg/dL)		
TC	206	182
LDL-C	103	94
HDL-C	48	59
non-HDL-C	158	123
TRL-related (mg/dL)		
TG	190	99
VLDL (%)	20	9
RLP-C	5.5	2.7
apoC-II	6.6	3.7
apoC-III	15.2	9
Atherogenic marker		
apoB (mg/dL)	114	87
MDA-LDL (U/L)	207	88
LDL-C/apoB	0.90	1.08
MDA-LDL/apoB	1.82	1.01

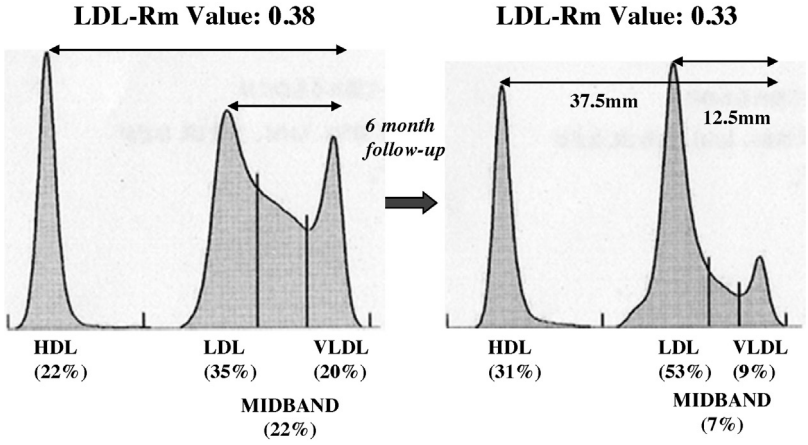

Fig. 5. Effect of TG-lowering on LDL-Rm value. We encountered a 60-year-old male hypertension patient with hypertriglyceridemia who was being treated for hypertension. We recommended a 6-month lifestyle improvement program that included therapeutic diet modification according to the American Heart Association/American College of Cardiology guideline [27]. Because elevated fasting serum TG levels ≥150 mg/dL persisted despite the lifestyle improvement, we prescribed fenofibrate 80 mg/day. After 6 months of fenofibrate therapy, the LDL-Rm value had decreased, and there were corresponding improvements in atherogenic and TRLs-related markers. TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides; VLDL, very LDL; RLP-C, remnant-like particle cholesterol; apo, apolipoprotein; MDA, malondialdehyde-modified; LDL-Rm, relative low-density lipoprotein migration; TRLs, triglyceride-rich lipoproteins.

markers as a result of reducing the serum TG levels of a hypertriglyceridemic patient.

Conclusions

The results suggest that the association between TG-metabolism and LDL-heterogeneity may be closely associated with of the risk of atherosclerotic cardiovascular disease. Combined evaluation of TRLs-related markers and LDL-Rm value may become a significantly important tool in the risk stratification of patients with atherosclerotic cardiovascular disease especially CAD, due to the simplicity of the measurement and calculation in clinical settings.

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