ceptibility to oxidation[9]. Thus, sd-LDL particles possess elevated atherogenic potential. Furthermore, elevated concentrations of sd-LDL can be found in patients with type 2 diabetes, metabolic syndrome, chronic kidney disease, and familial combined hyperlipidemia[10-14], all of which have been found as highly atherogenic conditions. Although Hirano *et al.* showed that sd-LDL-C is significantly higher in patients with coronary artery disease (CAD) in a cross-sectional study[14], no prospective study has addressed whether sd-LDL-C can predict a risk for CVD in non-Western populations. Recently, the Québec Cardiovascular Study has shown prospectively that men with an elevated proportion of LDL with a diameter less than 25.5 nm had a 3.6-fold increased risk of CAD compared with men with relatively normal LDL[15], indicating the strong link of sd-LDL to CVD as a biomarker of cardiovascular disease. Due to its atherogenic properties it is useful to measure sd-LDL for risk assessment; however, a reliable routine method is lacking.

sd-LDL has been measured by ultracentrifugation[16] or gradient gel electrophoresis[17]; however, these methods are both unsuitable for routine analysis, because each requires expensive equipment, complicated techniques, and long assay times. Hirano *et al.* have recently developed a simple precipitation method for sd-LDL-C quantification consisting of 2 steps: removal of apolipoprotein B-containing sd-LDL-free lipoproteins by precipitation with heparin and magnesium, followed by LDL-C measurement by the homogeneous method[18, 19]. This assay allowed us to screen sd-LDL-C in a large cohort. Using this assay, Ai *et al.* recently performed a case control study using samples from the Framingham Offspring Study and found significantly higher sd-LDL-C in women with CAD[20]. Koba *et al.* also showed that sd-LDL-C is more powerful than LDL-C for the determination of CAD[21]; however, these are cross-sectional studies and a prospective study is required to determine whether sd-LDL is an independent predictor of CVD. Therefore, the aim of this study was to address the role of sd-LDL-C for incident CVD in a large cohort study in Japan, the Suita study.

Methods

Population

The Suita study, a cohort study on CVD of urban residents, was established in 1989. The details of this study have been described elsewhere[22]. Briefly, 6485 men and women aged 30-79 years underwent a baseline survey at the National Cerebral and Cardiovascular Center between September 1989 and March 1994, and received medical examinations every 2 years. For these participants, we set the baseline of the present study at medical examinations held between April 1994 and February 1995, since at that time serum samples were collected and stored at −80℃. During this period, 2,437 participants attended the medical examination and were followed until the end of 2007. Of these, 403 participants were excluded due to the following reasons: history of CAD or stroke (n=106), lost to follow-up (n=132), and other reasons such as missing data (n=165). Data from the remaining 2,034 participants (968 men and 1,066 women) were included in the analysis. Informed consent was obtained from all participants. This cohort study was approved by the Institutional Review Board of the National Cerebral and Cardiovascular Center.

Baseline Examination

Blood samples were collected after the participants had fasted for at least 10 hours. The samples were centrifuged immediately. Blood pressure was measured in triplicate on the right arm after 5 min of rest by well-trained physicians using a standard mercury sphygmomanometer. The average of the second and third measurements was used for analysis. At baseline examination, subjects were classified into one of the 5 blood pressure categories based on the criteria of ESH-ESC 2007: optimal (SBP < 120 mmHg and DBP < 80 mmHg), normal (SBP 120-129 mmHg or DBP 80-84 mmHg), high-normal blood pressure (SBP 130-139 mmHg or DBP 85-89 mmHg), hypertension grade 1 (SBP 140-159 mmHg or DBP 90-99 mmHg), or hypertension grade ≥2 (SBP ≥160 mmHg or DBP ≥100 mmHg). Antihypertensive drug users were classified according to their blood pressure at the baseline survey. Diabetes was defined as fasting serum glucose ≥7.0 mmol/L (126 mg/dL) or current use of medications for diabetes. Body mass index (BMI) was calculated as weight (kilograms) divided by height (meters) squared. Well-trained health nurses obtained information on smoking, drinking, and medical histories.

Laboratory Measurements

Serum total cholesterol, triglyceride, and HDL cholesterol (HDL-C) were determined by standard enzymatic methods. Serum glucose was also measured. For the purposes of this study, we used archived plasma samples that had been frozen at −80℃ and never previously thawed for the assessment of direct LDL-C and sd-LDL-C by homogeneous methods on a Hitachi 7180 automated analyzer (Hitachi, Tokyo, Japan)[18, 19]. The kits used for these tests (LDL-C and sd-LDL-C) were provided by Denka Seiken (Tokyo, Japan). Assays

for direct LDL-C and sd-LDL-C were previously calibrated and directly compared with concentrations obtained after isolation of LDL and sd-LDL by ultracentrifugation.

Endpoint Determination

As previously reported, the endpoints of the present study were (1) date of first CAD or stroke event; (2) date of death; (3) date of leaving Suita city; and (4) the end of December 2007. The first step in the survey for CAD and stroke involved checking the health status of all participants by repeated clinical visits every two years and yearly questionnaires by mail or telephone. In the second step, in-hospital medical records of participants who were suspected of having CAD were reviewed by registered hospital physicians or research physicians who were blinded to the baseline information. The criteria for a diagnosis of CAD included first-ever acute myocardial infarction, sudden cardiac death within 24 h after the onset of acute illness, or coronary artery disease followed by coronary artery bypass surgery or angioplasty. The criteria for definite and probable MI were defined according to the criteria of the MONICA (Monitoring Trends and Determinants of Cardiovascular Disease) project[23]. The criteria for stroke were defined according to the US National Survey of Stroke criteria[24]. Classification of patients into stroke subtypes was based on examination of computed tomography, magnetic resonance imaging, or autopsy.

Statistical Analysis

Continuous variables between groups were compared by analysis of variance and categorical variables were compared by a chi-square test. Triglyceride levels were logarithmically transformed to improve the skewed distribution. The hazard ratio (HR) for MI or stroke was calculated using a proportional hazards model adjusted for age, sex, hypertension (dichotomous variable), diabetes, HDL-C, BMI, smoking (never-smoked; ex-smoker; current smoker) and drinking (never-drank; ex-drinker; regular drinker). All confidence intervals were estimated at the 95% level and significance was set at $p<0.05$. All statistical analyses were conducted using the SAS statistical software package (release version 8.2; SAS Institute, Cary, NC, USA).

Results

Baseline Clinical Characteristics According to sd-LDL-C Quartiles

To study the role of sd-LDL in the incidence of CVD, we divided the cohort into quartiles according to the basal level of sd-LDL-C. **Table 1** shows the clinical characteristics and cardiovascular risk factors of the study population according to the quartiles of sd-LDL-C. BMI, total cholesterol, LDL-C, and triglyceride significantly increased across the sd-LDL-C quartiles both in men and women, while HDL-C decreased in both genders. A significant trend was observed across the quartiles for the severity of high blood pressure, lipid-lowering drug use, and prevalence of diabetes at baseline both in men and women; however, a significant trend for age was only found in women, not in men.

Incidence of CVD According to sd-LDL-C Quartiles

To confirm our previous study, the association between LDL-C and CAD was examined by dividing the cohort into quartiles according to the baseline LDL-C. It was found that age- and multivariable-adjusted HRs for CAD were statistically significant only in men, not in women or the total cohort. The HR of the 4th quartile in men was 3.53 (95% confidence intervals (CIs): 1.31-9.54) in an age-adjusted model and 3.56 (95% CIs: 1.28-9.86) in a multivariable-adjusted model, consistent with our previous report[1]. We then performed analysis to examine the effect of sd-LDL-C. During the observation period, 116 cases of CVD, 53 cases of stroke, 36 cases of cerebral infarction, and 63 cases of CAD were reported. As shown in **Table 2**, increasing quartiles of sd-LDL-C were significantly associated with increased risks of CVD (stroke + CAD), stroke, cerebral infarction, and CAD after age and multivariable adjustment. Age and sex-adjusted HRs per 10 mg/dL of sd-LDL-C for CVD, stroke, cerebral infarction, and CAD were 1.21 (95% CI: 1.12-1.31), 1.17 (95% CI: 1.05-1.30), 1.15 (95% CI: 1.00-1.33), and 1.29 (95% CI: 1.14-1.45), respectively. HRs after multivariable adjustment were almost the same. When we analyzed each gender, age-adjusted HRs per 10 mg/dL of sd-LDL-C for CVD, stroke, cerebral infarction, and CAD were significant in women, while those for CVD and CAD were significant in men. HR for CAD of the fourth quartile was almost 4 after age and multivariable adjustment in men.

After putting LDL-C into the multivariable adjusted-models (Model A), sd-LDL-C was still associated with increased risk for CVD, stroke, cerebral infarction, and CAD in the total cohort, for CVD in men, and CVD, stroke, and cerebral infarction in women. After further putting logarithmically transformed triglyceride and HDL-C variables into Model A (Model B), sd-LDL-C was still associated with

Table 1. Baseline characteristics of cardiovascular risk factors according to small dense LDL cholesterol quartiles

	Small dense LDL Cholesterol				*p* value for Trend
	Q1	Q2	Q3	Q4	
Men					
Number of subjects	241	243	242	242	
Small dense LDL, range (mean), mg/dL	6.3-27.8 (21.1)	27.9-38.2 (32.7)	38.3-53.4 (45.3)	53.5-119.6 (67.3)	
Age, year	60.9 ± 13.1	59.7 ± 12.5	59.1 ± 12.3	59.4 ± 11.3	0.421
Body mass index, kg/m^2	21.5 ± 2.5	22.4 ± 2.8	23.4 ± 2.4	24.0 ± 2.7	<0.001
TC, mg/dL	170 ± 25	189 ± 24	199 ± 25	220 ± 27	<0.001
HDL-C, mg/dL	60 ± 15	57 ± 14	51 ± 11	48 ± 11	<0.001
LDL-C, mg/dL	86 ± 20	111 ± 21	124 ± 23	140 ± 26	<0.001
Triglyceride, (median) mg/dL	66	87	112	167	<0.001
Large-LDL-C, mg/dL	65 ± 17	78 ± 21	79 ± 22	72 ± 24	<0.001
Sd-LDL-C/LDL-C ratio	0.25 ± 0.05	0.31 ± 0.07	0.38 ± 0.08	0.50 ± 0.11	<0.001
Blood pressure category, %					0.002
Optimal blood pressure	31	26	25	19	
Normal blood pressure	30	24	19	26	
High-normal blood pressure	16	30	25	29	
Hypertension grade 1-3	19	26	29	28	
Antilipidemic drug use, %	1	4	5	8	0.003
Diabetes, %	3	5	7	9	0.023
Current Smoking, %	44	41	41	44	0.021
Current Drinking, %	66	71	72	74	0.577
Women					
Number of subjects	266	267	266	267	
Small dense LDL, range (mean), mg/dL	7.5-23.9 (18.7)	24.0-33.0 (28.6)	33.1-44.6 (38.5)	44.7-136.6 (59.7)	
Age, year	51.7 ± 13.0	57.3 ± 11.9	60.2 ± 11.2	60.4 ± 9.1	<0.001
Body mass index, kg/m^2	21.0 ± 2.5	21.8 ± 3.2	22.5 ± 3.1	23.2 ± 2.8	<0.001
TC, mg/dL	175 ± 23	200 ± 22	216 ± 25	234 ± 32	<0.001
HDL-C, mg/dL	67 ± 13	64 ± 12	60 ± 13	54 ± 12	<0.001
LDL-C, mg/dL	83 ± 17	109 ± 17	130 ± 18	153 ± 30	<0.001
Triglyceride, (median) mg/dL	61	78	97	140	<0.001
Large-LDL-C, mg/dL	64 ± 14	81 ± 15	92 ± 17	93 ± 25	<0.001
Sd-LDL-C/LDL-C ratio	0.23 ± 0.04	0.27 ± 0.04	0.30 ± 0.05	0.40 ± 0.08	<0.001
Blood pressure category, %					<0.001
Optimal blood pressure	34	27	22	17	
Normal blood pressure	25	24	26	25	
High-normal blood pressure	16	29	20	35	
Hypertension grade 1-3	16	21	31	32	
Antilipidemic drug use, %	4	5	6	12	0.002
Diabetes, %	0	1	3	6	<0.001
Current Smoking, %	13	10	6	7	0.056
Current Drinking, %	34	30	22	23	0.014

TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol. Large LDL-C, LDL-C-Sd-LDL-C. Hypertension was defined as described in methods. Diabetes was defined as fasting serum glucose ≥7.0mmol/L (126 mg/dL), the use of anti-diabetic agents, or both.

Table 2. Age- and multivariable-adjusted hazard ratios and 95% confidence intervals for the incidence of cardiovascular disease according to small dense LDL cholesterol quartiles

	Small dense LDL Cholesterol, mg/dL				
	Q1 (Lower)	Q2	Q3	Q4 (Higher)	per 10 mg/dL
Men and women, range (mean)	6.3-25.5 (19.7)	25.6-35.3 (30.5)	35.4-49.0 (41.4)	49.1-136.6 (63.9)	
Person-years	5,576	5,789	5,527	5,741	
Cardiovascular disease					
Case	21	23	29	43	
Age and sex-adjusted HR	1	0.75 (0.43-1.29)	1.11 (0.68-1.83)	**1.64 (1.04-2.60)**	**1.21 (1.12-1.31)**
Model 1-adjusted HR	1	0.81 (0.45-1.42)	1.08 (0.65-1.81)	1.60 (0.99-2.60)	**1.21 (1.11-1.32)**
Stroke					
Case	14	13	10	16	
Age and sex-adjusted HR	1	0.58 (0.30-1.14)	0.80 (0.43-1.48)	1.21 (0.69-2.12)	**1.17 (1.05-1.30)**
Model 1-adjusted HR	1	0.63 (0.32-1.23)	0.79 (0.41-1.50)	1.19 (0.65-2.16)	**1.18 (1.04-1.33)**
Cerebral infarction					
Case	8	10	6	12	
Age and sex-adjusted HR	1	1.08 (0.45-2.57)	1.14 (0.47-2.73)	1.74 (0.77-3.90)	**1.15 (1.00-1.33)**
Model 1-adjusted HR	1	1.18 (0.48-2.88)	1.16 (0.46-2.89)	1.85 (0.77-4.40)	**1.18 (1.00-1.39)**
Coronary artery disease					
Case	7	10	19	27	
Age and sex-adjusted HR	1	1.36 (0.49-3.77)	2.26 (0.89-5.73)	**3.35 (1.38-8.13)**	**1.29 (1.14-1.45)**
Model 1-adjusted HR	1	1.44 (0.51-4.08)	2.17 (0.83-5.66)	**3.26 (1.29-8.20)**	**1.28 (1.13-1.46)**
Men, range (mean)	6.3-27.8 (21.1)	27.9-38.2 (32.7)	38.3-53.4 (45.3)	53.5-119.6 (67.3)	
Person-years	2,499	2,615	2,519	2,608	
Cardiovascular disease					
Case	19	19	22	36	
Age-adjusted HR	1	1.06 (0.56-2.01)	1.31 (0.70-2.44)	**2.03 (1.16-3.57)**	**1.15 (1.04-1.28)**
Model 1-adjusted HR	1	1.17 (0.61-2.24)	1.36 (0.70-2.62)	**2.12 (1.16-3.86)**	**1.16 (1.04-1.30)**
Stroke					
Case	14	13	10	16	
Age-adjusted HR	1	1.03 (0.48-2.21)	0.87 (0.38-1.99)	1.43 (0.69-2.97)	1.06 (0.92-1.23)
Model 1-adjusted HR	1	1.13 (0.51-2.47)	0.98 (0.40-2.38)	1.55 (0.70-3.41)	1.08 (0.92-1.28)
Cerebral infarction					
Case	8	10	6	12	
Age-adjusted HR	1	1.33 (0.52-3.39)	0.85 (0.29-2.48)	1.81 (0.73-4.48)	1.08 (0.91-1.29)
Model 1-adjusted HR	1	1.43 (0.54-3.78)	0.90 (0.29-2.80)	1.93 (0.70-5.29)	1.10 (0.90-1.36)
Coronary artery disease					
Case	5	6	12	20	
Age-adjusted HR	1	1.24 (0.37-4.07)	2.48 (0.87-7.07)	**3.89 (1.45-10.42)**	**1.27 (1.10-1.47)**
Model 1-adjusted HR	1	1.27 (0.38-4.29)	2.34 (0.78-6.97)	**4.03 (1.42-11.40)**	**1.28 (1.09-1.50)**
Women, range (mean)	7.5-23.9 (18.7)	24.0-33.0 (28.6)	33.1-44.6 (38.5)	44.7-136.6 (59.7)	
Person-years	3,077	3,174	3,008	3,133	
Cardiovascular disease					
Case	7	12	13	23	
Age-adjusted HR	1	1.01 (0.39-2.60)	0.99 (0.39-2.50)	1.73 (0.74-4.06)	**1.31 (1.16-1.47)**
Model 1-adjusted HR	1	1.04 (0.40-2.72)	0.91 (0.35-2.35)	1.52 (0.63-3.68)	**1.29 (1.13-1.48)**
Stroke					
Case	5	8	6	16	
Age-adjusted HR	1	0.95 (0.30-2.94)	0.64 (0.19-2.11)	1.72 (0.62-4.74)	**1.31 (1.13-1.52)**
Model 1-adjusted HR	1	0.98 (0.31-3.14)	0.64 (0.18-2.19)	1.66 (0.58-4.76)	**1.33 (1.12-1.59)**

(Cont Table 2)

	Small dense LDL Cholesterol, mg/dL				
	Q1 (Lower)	Q2	Q3	Q4 (Higher)	per 10 mg/dL
Cerebral infarction					
Case	0	5	4	7	
Age-adjusted HR	1	–	–	–	**1.31 (1.05-1.63)**
Model 1-adjusted HR	1	–	–	–	**1.37 (1.05-1.80)**
Coronary artery disease					
Case	2	4	7	7	
Age-adjusted HR	1	1.22 (0.22-7.76)	1.90 (0.39-9.24)	1.84 (0.38-8.91)	**1.32 (1.08-1.61)**
Model 1-adjusted HR	1	1.27 (0.22-7.33)	1.83 (0.35-9.45)	1.54 (0.30-7.83)	1.23 (0.99-1.53)

Model 1: adjusted for age, (sex), body mass index, smoking, drinking, blood pressure category (optimal, normal, and high-normal blood pressure, hypertension grade 1 and 2 + 3), diabetes, and lipid-lowering drug user
Bold numbers: statistically significant

increased risks of CVD and stroke in the total cohort and in women, but not in men (**Table 3**).

Discussion

This study clearly indicates an increased risk of CVD, stroke, cerebral infarction, and CAD attributed to elevated sd-LDL-C concentrations in a Japanese population without a previous history of CVD. We also showed that HR was significant after multivariable adjustment and by analysis including LDL-C, log-transformed triglyceride, and HDL-C in the same model. Thus, sd-LDL-C measurement with the new test is promising as a new biomarker to predict the risk of CVD.

In addition to traditional risk factors for CVD, such as hypertension, diabetes, and dyslipidemia, other biomarkers are required to better define the risk and refine therapeutic decisions. There is scientific evidence that sd-LDL particles are highly atherogenic and can be a biomarker of CVD [15, 20, 21]. Our data provide additional evidence to show the role of sd-LDL-C as a CVD risk in the general population. Furthermore, measuring sd-LDL-C with this test has an advantage because it is more user-friendly and more applicable than specialized tests such as gradient gel electrophoresis, nuclear magnetic resonance, and gradient ultracentrifugation.

Until now, there have been no target goals of sd-LDL-C to prevent CAD. In this study the HR of the 4th quartile was statistically significant, suggesting that the cutoff of sd-LDL-C is approximately 50 mg/dL, although significance was not obtained in women probably due to the low event rate; therefore, a larger study should be performed to define an appropriate cutoff for sd-LDL-C. Because statins, fibrates, and ezetimibe have been shown to reduce the amount of sd-LDL [25-28], a randomized control study is required to address whether lowering sd-LDL-C to a certain goal by these drugs can prevent the development of CAD.

In this study we found that sd-LDL-C was significantly associated with traditional risk factors, such as hypertension and diabetes. BMI and the prevalence of diabetes increased and HDL-C decreased across the sd-LDL-C quartiles, and more hypertensive subjects were found in second to fourth quartiles than in the first quartile in both genders. We also found that age-adjusted partial correlation coefficients between sd-LDL-C and BMI, log-transformed triglyceride, LDL-C, and HDL-C (Pearson) were 0.305, 0.636, 0.554, and −0.346 ($p<0.0001$), respectively. Thus these data suggest that increased concentrations of sd-LDL-C may be associated with metabolic disorders and that lifestyle modification, such as exercise and weight control, would be effective to reduce sd-LDL in patients with diabetes and metabolic syndrome. Furthermore, we should address whether sd-LDL-C can be used to identify a very high-risk patient with type 2 diabetes, metabolic syndrome, and other metabolic disorders. In contrast to the association with metabolic disorders, an age-related change in sd-LDL-C was found only in women, consistent with the trend of increased atherogenic dyslipidemia in postmenopausal women. Ai *et al.* also found that postmenopausal women had higher levels of sd-LDL-C than premenopausal women in the Framingham Offspring Study [20].

In addition to type 2 diabetes and metabolic syn-

Table 3. Relationship between major lipid variables and cardiovascular disease

	Cardiovascular disase	Stroke	Cerebral infarction	Coronary artery disease
Men and women				
Age and sex-adjusted	**1.21 (1.12-1.31)**	**1.17 (1.05-1.30)**	**1.15 (1.00-1.33)**	**1.29 (1.14-1.45)**
Multivariable-adjusted Sd-LDL-C/10 mg/dL	**1.21 (1.11-1.32)**	**1.18 (1.04-1.33)**	**1.18 (1.00-1.39)**	**1.28 (1.13-1.46)**
Model A				
Sd-LDL-C/10 mg/dL	**1.26 (1.11-1.43)**	**1.26 (1.06-1.50)**	**1.29 (1.02-1.62)**	**1.29 (1.07-1.55)**
LDL-C/10 mg/dL	0.96 (0.89-1.04)	0.94 (0.85-1.04)	0.93 (0.81-1.06)	0.99 (0.88-1.11)
Model B				
Sd-LDL-C/10 mg/dL	**1.20 (1.01-1.42)**	**1.35 (1.07-1.71)**	1.31 (0.96-1.78)	1.05 (0.81-1.36)
LDL-C/10 mg/dL	0.98 (0.90-1.06)	0.93 (0.83-1.03)	0.92 (0.80-1.07)	1.05 (0.93-1.19)
ln_TG	1.15 (0.71-1.86)	0.76 (0.40-1.46)	0.86 (0.37-1.96)	1.82 (0.87-3.81)
HDL-C/10 mg/dL	0.94 (0.81-1.08)	1.00 (0.84-1.20)	0.93 (0.73-1.18)	0.80 (0.61-1.04)
Men				
Age-adjusted	**1.15 (1.04-1.28)**	1.06 (0.92-1.23)	1.08 (0.91-1.29)	**1.27 (1.10-1.47)**
Multivariable-adjusted Sd-LDL-C/10 mg/dL	**1.16 (1.04-1.30)**	1.08 (0.92-1.28)	1.10 (0.90-1.36)	**1.28 (1.09-1.50)**
Model A				
Sd-LDL-C/10 mg/dL	**1.17 (1.00-1.38)**	1.17 (0.92-1.48)	1.20 (0.90-1.60)	1.18 (0.94-1.48)
LDL-C/10 mg/dL	0.99 (0.89-1.09)	0.94 (0.82-1.08)	0.93 (0.79-1.09)	1.07 (0.93-1.24)
Model B				
Sd-LDL-C/10 mg/dL	1.10 (0.88-1.38)	1.28 (0.92-1.77)	1.28 (0.87-1.90)	0.96 (0.70-1.31)
LDL-C/10 mg/dL	1.01 (0.90-1.13)	0.92 (0.78-1.07)	0.91 (0.76-1.10)	1.14 (0.97-1.33)
ln_TG	1.23 (0.66-2.26)	0.75 (0.32-1.76)	0.86 (0.31-2.38)	1.87 (0.75-4.62)
HDL-C/10 mg/dL	0.96 (0.80-1.14)	1.05 (0.85-1.28)	1.08 (0.94-1.40)	0.72 (0.50-1.03)
Women				
Age-adjusted	**1.31 (1.16-1.47)**	**1.31 (1.13-1.52)**	**1.31 (1.05-1.63)**	**1.32 (1.08-1.61)**
Multivariable-adjusted Sd-LDL-C/10 mg/dL	**1.29 (1.13-1.48)**	**1.33 (1.12-1.59)**	**1.37 (1.05-1.80)**	1.23 (0.99-1.53)
Model A				
Sd-LDL-C/10 mg/dL	**1.44 (1.17-1.77)**	**1.48 (1.13-1.94)**	**1.62 (1.08-2.43)**	1.33 (0.94-1.89)
LDL-C/10 mg/dL	0.92 (0.81-1.04)	0.92 (0.79-1.08)	0.88 (0.69-1.11)	0.94 (0.75-1.16)
Model B				
Sd-LDL-C/10 mg/dL	**1.35 (1.03-1.77)**	**1.47 (1.04-2.08)**	1.33 (0.78-2.29)	1.12 (0.70-1.79)
LDL-C/10 mg/dL	0.93 (0.81-1.07)	0.92 (0.78-1.09)	0.92 (0.72-1.19)	0.98 (0.78-1.24)
ln_TG	1.19 (0.53-2.69)	0.91 (0.31-2.68)	0.86 (0.17-4.25)	1.84 (0.47-7.15)
HDL-C/10 mg/dL	0.92 (0.72-1.19)	0.92 (0.67-1.26)	0.56 (0.31-1.00)	0.92 (0.60-1.41)

Multivariable adjusted for age, sex, body mass index, smoking, drinking, blood pressure category (optimal, normal, and high-normal bloodpressure, hypertension grade 1 and 2+3), diabetes, and antilipidemic drug user
Model A: sd-LDL-C per 10 mg/dL and LDL-C per 10 mg/dL in the same model
Model B: sd-LDL-C per 10 mg/dL, LDL-C per 10 mg/dL, ln(TG), and HDL-C per 10 mg/dL in the same model
Sd-LDL-C, small dense LDL cholesterol; ln_TG, logarithmical transformed TG
Bold numbers: statistically significant

drome, sd-LDL-C is increased in familial combined hyperlipidemia and postprandial hyperlipidemia[29, 30]. Hirano *et al.* demonstrated that sd-LDL-C determined by this simple precipitation method is useful for screening familial combined hyperlipidemia in large populations[13]. Because the prevalence of familial combined hyperlipidemia is high in the general population and the increase of sd-LDL particles as well as large VLDL particles is a characteristic feature of familial combined hyperlipidemia, this assay would be quite useful for its diagnosis. Although sd-LDL is decreased by lipid-lowering drugs, such as statins and fibrates, the effect of adequate combination therapy on sd-LDL-C has not yet been confirmed; therefore, this assay would be also useful in determining the therapeutic strategy for patients with a high serum level of sd-LDL-C.

There are some limitations in our study. First, we

used plasma stored at −80℃, and there is no guarantee that we would have obtained the same results if we had used fresh serum; however, our results are consistent with those reported by Hirano *et al.*, who measured sd-LDL-C in a Japanese general population with the same method, and comparison studies performed in Japan indicate virtually identical results with the use of fresh vs. frozen plasma for sd-LDL-C[13]. Second, the single measurement of sd-LDL-C at the baseline survey and the fact we did not evaluate the longitudinal trend for each risk factor including lipid-lowering agents may have caused us to underestimate the relationship between these conditions and CAD due to regression dilution bias, although we statistically adjusted for the use of lipid-lowering agents at the baseline survey. Third, serum LDL-C was measured by the direct homogeneous assay, which failed to meet the National Cholesterol Education Program total error goals for diseased individuals, although it met these goals in non-diseased individuals[31]. However, the present study is a cohort study of community-dwelling citizens without a history of CVD. Furthermore, the serum levels of LDL-C determined by direct homogeneous assay are almost consistent with those calculated by the Friedewald formula in a large Japanese cohort.

Conclusions

In this large urban cohort study conducted in Japan, we demonstrated that sd-LDL-C is significantly associated with the development of CVD, providing evidence of sd-LDL-C as an important biomarker to predict CVD. A large intervention study is required to determine the appropriate target level of sd-LDL-C.

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Special Report

Management of Type IIb Dyslipidemia

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Although the Japan Atherosclerosis Society guideline for the diagnosis and prevention of atherosclerosis cardiovascular diseases for the Japanese population provides targets for low-density lipoprotein (LDL) cholesterol, triglycerides, and high-density lipoprotein (HDL) cholesterol to prevent cardiovascular disease in patients with dyslipidemia, there is no guideline specifically targeting the treatment of type IIb dyslipidemia, which is one of the most common types of dyslipidemia, along with type IIa and type IV dyslipidemia. Type IIb dyslipidemia is important because it sometimes accompanies atherogenic lipid profiles, such as small, dense LDL, remnants, low HDL cholesterolemia. It is also associated with type 2 diabetes mellitus, metabolic syndrome, and chronic kidney disease (CKD), and most patients with familial combined hyperlipidemia (FCHL) show this phenotype; therefore, it is assumed that patients with type IIb dyslipidemia have a high risk for cardiovascular disease. Thus, the management of type IIb dyslipidemia is very important for the prevention of cardiovascular disease, so we have attempted to provide a guideline for the management of type IIb dyslipidemia.

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Key words; Atherogenic dyslipidemia, Cardiovascular disease, Diabetes, Metabolic syndrome, Lifestyle modification

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Epidemiology

Atherosclerotic Disease and Combined Dyslipidemia

Type IIb dyslipidemia is defined by elevated low-density lipoprotein (LDL) cholesterol and triglycer-

Table 1. Prevalence of type IIb dyslipidemia in 3 Japanese cohorts: NIPPON DATA90, Serum Lipid Survey in 2000, and Hisayama study

	NIPPON DATA90		Serum Lipid Survey		Hisayama Study	
	men	women	men	women	men	women
n	485	592	168	67	149	172
prevalence (%)	13.8	12.1	8.8	5.0	10.8	9.4
mean age ± SD	51.0 ± 12.3	58.9 ± 11.4	47.8 ± 10.1	57.9 ± 11.2	56 ± 11	62 ± 10
BMI	25.0 ± 2.9	24.5 ± 3.1	25.3 ± 2.8	24.9 ± 3.4	24.7 ± 2.5	24.6 ± 3.8
SBP (mmHg)	140.4 ± 17.7	143.3 ± 20.3	129.8 ± 19.7	128.0 ± 18.3	140.3 ± 18.0	138.7 ± 23.0
DBP (mmHg)	87.1 ± 11.5	84.2 ± 12.1	81.1 ± 12.6	77.70 ± 11.0	85.6 ± 10.6	81.0 ± 13.3
HbA1c (%)	5.2 ± 1.0	5.3 ± 1.1	5.1 ± 0.9	5.2 ± 0.8	5.3 ± 0.9	5.4 ± 0.9
T-Cho (mg/dL)	249.0 ± 27.5	254.7 ± 30.9	252.1 ± 24.2	255.5 ± 31.0	246.1 ± 32.2	248.5 ± 34.1
TG (mg/dL)	269.7 ± 150.9	235.8 ± 104.4	272.5 ± 221.2	190.3 ± 48.4	288.9 ± 223.6	214.0 ± 95.7
HDL-c (mg/dL)	44.4 ± 13.1	44.8 ± 13.0	47.0 ± 10.1	53.1 ± 11.3	53.2 ± 11.5	57.0 ± 12.8
LDL-c (mg/dL)	150.7 ± 34.0	158.8 ± 32.4	163.3 ± 20.8	164.4 ± 24.7	135.1 ± 50.3	148.8 ± 34.6
non-HDL-c (mg/dL)	204.6 ± 29.0	206.0 ± 33.0	205.1 ± 23.3	202.5 ± 27.8	192.9 ± 33.0	191.6 ± 35.1
diabetes (%)	13.0	15.0	3.6	9.0	34.9	25.0
hypertension (%)	60.0	65.5	10.7	25.4	57.7	51.7
metabolic syndrome (%)	38.6	33.5	32.7	14.9	51.7	30.8
smoking (%)	68.0	6.6	55.4	7.5	44.3	9.3
coronary artery disease (%)	2.5	4.2	3.6	3.0	2.0	1.2
stroke (%)	1.7	2.0	1.2	0.0	2.0	1.2

ides. In Japan, hypercholesterolemia and hypertriglyceridemia are defined as LDL cholesterol ≥140 mg/dL and triglycerides ≥150 mg/dL, respectively[1]. Type IIb dyslipidemia is frequently associated with type 2 diabetes mellitus, metabolic syndrome, and chronic kidney disease (CKD)[2-4]. Among primary dyslipidemia, familial combined hyperlipidemia (FCHL) often shows this type of dyslipidemia[5]. Type IIb dyslipidemia is also associated with the development of small, dense LDL, low high-density lipoprotein (HDL) cholesterolemia along with high triglycerides and LDL cholesterol. These features of small, dense LDL, low HDL cholesterolemia, high triglycerides are called the atherogenic lipid triad, implying their role in atherogenesis.

Although many lines of evidence indicate that LDL cholesterol is an important risk factor for cardiovascular disease (CVD)[6], it is still controversial whether plasma triglyceride levels are associated with the development of cardiovascular disease; however, recent reports have shown that plasma triglyceride levels are an independent risk for coronary artery disease (CAD)[7-10]. In addition, non-fasting triglyceride levels have been shown to be associated with CAD and stroke[11, 12]. In spite of the accumulating evidence against LDL cholesterol and triglycerides, few reports have addressed the effect of type IIb dyslipidemia on cardiovascular disease. Therefore, considering that elevated LDL cholesterol and triglyceride along with an increase of atherogenic lipoproteins, such as small, dense LDL and remnants, are found in type IIb dyslipidemia, and that this type of dyslipidemia is often associated with type 2 diabetes, metabolic syndrome, CKD, and FCHL, we should recognize that type IIb dyslipidemia is a high-risk condition for CVD and should establish a guideline for its management.

Epidemiology of Type IIb Dyslipidemia

To show the prevalence of type IIb dyslipidemia in the Japanese population, we studied the prevalence of type IIb dyslipidemia in three Japanese cohorts (NIPPON DATA90, Serum Lipid Survey in 2000, and the Hisayama study). NIPPON DATA90 is a cohort study of the National Survey on Circulatory Disorders of Japan, including the National Integrated Project for Prospective Observation of Non-communicable Disease and Its Trends in the Aged (NIPPON DATA)[13]. Baseline surveys of this cohort were performed in 1990. The Serum Lipid Level Survey 2000 was designed to produce representative data of serum lipid levels in the civilian Japanese population in 2000[14]. The total number of this cohort is 12,839. The Hisayama study is a cohort study that started in 1961 in a small town near Fukuoka[15]. **Table 1** shows

Table 2. Prevalence of type IIb dyslipidemia in each age group in the Serum Lipid Survey in 2000

age	30-39		40-49		50-59		60-69		70-79	
gender	men	women	men	women	men	women	men	women	men	women
prevalence (%)	9.73	1.54	9.21	2.31	9.36	8.24	6.99	11.01	5.49	6.41

the prevalence in three different cohorts: NIPPON DATA90, Serum Lipid Survey in 2000, and Hisayama study (from The Research Committee report for Primary Hyperlipidemia, Research on Measures against Intractable Diseases by the Ministry of Health, Labor, and Welfare in 2007, http://mhlw-grants.niph.go.jp/niph/search/NIDD00.do). The prevalence of type IIb dyslipidemia in men was 8.8 to 13.8%, and 5.0 to 12.1% in women. In all cohorts, the prevalence of type IIb dyslipidemia was higher in men than in women. The mean age at onset of type IIb dyslipidemia was higher in women than in men in all cohorts, indicating that women tend to develop type IIb dyslipidemia after menopause. In terms of the comorbidities, 3.6 to 34.9% of men had type 2 diabetes, while 9.0 to 25.0% of women had type 2 diabetes, showing no significant difference between men and women in the three cohorts; however, the prevalence of metabolic syndrome in type IIb dyslipidemia was higher in men (32.7 to 51.7%) than in women (14.9 to 33.5%).

Table 2 shows the prevalence of type IIb dyslipidemia in each age group of men and women according to the data from the Serum Lipid Survey in 2000. In men, the prevalence of type IIb dyslipidemia was approximately 20%, similar from their 30s to 50s, while in women, the prevalence was increased from their 50s. In their 60s, the prevalence was higher in women than in men.

Familial Combined Hyperlipidemia (FCHL)

FCHL, the most common genetic dyslipidemia in man, affects 1%-2% of the population and occurs in 10%-20% of premature myocardial-infarction survivors[16, 17]. It is believed that the primary metabolic defect in FCHL leads to hepatic overproduction of very-low-density lipoprotein (VLDL), resulting in elevated plasma cholesterol and triglyceride levels; type II b dyslipidemia. The existence of small, dense LDL particles is another characteristic feature of FCHL, and the combination of small, dense LDL with increased triglycerides and apolipoprotein B (apoB) concentrations and reduced HDL cholesterol levels is termed "atherogenic lipid triad". This atherogenic lipid triad is independently associated with an increased risk for CAD[18]. Moreover, it has been suggested that hyper-apoB, defined as an increased apoB:LDL cholesterol (apoB:LDL-C) ratio, reflects the presence of FCHL in affected individuals. Delayed clearance of triglyceride-rich remnant particles, such as intermediate density lipoproteins (IDL) and chylomicron remnants, leads to an increased residence time of these atherogenic particles within the circulation[16]. These changes also contribute to the insulin resistance often accompanying FCHL. FCHL shares considerable phenotypic overlap with type 2 diabetes as well as with metabolic syndrome, and therapeutic lifestyle change is effective to decrease LDL cholesterol and triglycerides in FCHL patients.

FCHL was first postulated to segregate as an autosomal dominant trait[19], but there is increasing evidence that it is an oligogenic disorder with a complex pattern of inheritance[20, 21]. FCHL family members exhibit varying degrees of dyslipidemia with either isolated high triglycerides, LDL cholesterol, or both, which is another feature of FCHL. Several candidate genes have been reported, such as upstream stimulatory factor 1 (USF1)[21], apoA5[22], Retinoid X receptor-γ[23], hepatic lipase[24], and so forth.

The diagnostic criteria for FCHL in Japan are as follows:

1) Increased levels of LDL cholesterol $\geq$140 mg/dL and/or triglycerides $\geq$150 mg/dL (mainly type IIb dyslipidemia, but sometimes type IIa or IV dyslipidemia)

2) ApoB/LDL cholesterol ratio $>$1.0 and/or presence of small, dense LDL (LDL particle size $<$25.5 nm)

3) Rule out familial hypercholesterolemia and secondary dyslipidemia, such as dyslipidemia accompanied with type 2 diabetes

4) Family history of dyslipidemia, such as IIa, IIb, or IV 1)-4): definite FCHL, 1)-3): probable FCHL

Management of Type IIb Dyslipidemia

Treatment Goals

On the basis of large outcome trials with statins and epidemiological data, the Japan Atherosclerosis Society[25] guideline for the diagnosis and prevention

Table 3. LDL-Cholesterol and non-HDL-Cholesterol Goals for Therapeutic Lifestyle Changes and Drug Therapy in Different Risk Categories[24]

treatment	risk category		goals (mg/dL)		
		coronary risk factors other than LDL-C	primary LDL-C	secondary non-HDL-C	HDL-C
Primary prevention improving lifestyle as the first line, followed by drug therapy	I (low)	0	<160	<190	≥40
	II (intermediate)	1-2	<140	<170	
	III (high)	3 or more	<120	<150	
Secondary prevention improving lifestyle and drug therapy	past history of CAD		<100	<130	

of atherosclerosis cardiovascular diseases for the Japanese population recommends reducing LDL cholesterol to a goal according to the number of coronary risks in patients with dyslipidemia[6]. Therefore, the same goals should be applied to the patients with type IIb dyslipidemia. In terms of the treatment goals for triglycerides, the guideline recommends reducing them to <150 mg/dL. In spite of the recommended goal for triglycerides, there is no clear consensus on the benefits of targeting hypertriglyceridemia. Because type IIb dyslipidemia is associated with atherogenic lipoprotein particles, such as remnants, IDL, small, dense LDL, and non-HDL cholesterol levels are considered as secondary targets after the goal for LDL cholesterol is achieved (**Table 3**)[26, 27]. Non-HDL cholesterol is calculated by subtracting HDL cholesterol from total cholesterol. It thus includes not only cholesterol from lipoprotein (a), LDL cholesterol, and IDL- cholesterol, which are traditionally included in the LDL cholesterol values calculated by the Friedewald formula, but also cholesterol from potentially atherogenic triglyceride-rich lipoproteins, such as VLDL remnants. Non-HDL cholesterol also has advantages, because fasting or additional tests are not required for the calculation. Several studies have reported that the level of non-HDL cholesterol is a better indicator of future cardiovascular events than LDL cholesterol[28, 29]. According to previous studies, the goal for non-HDL cholesterol is set at 30 mg/dL higher than that for LDL cholesterol. HDL cholesterol levels >40 mg/dL are the tertiary goal. Among all the lipid parameters, triglycerides are most responsive to lifestyle interventions such as diet and exercise; therefore, for the management of type IIb dyslipidemia we should begin with therapeutic lifestyle changes and should treat type 2 diabetes, metabolic syndrome, and CKD when dyslipidemia is caused by these disorders. We should also treat hypothyroidism or other disorders in cases of secondary dyslipidemia.

Therapeutic Lifestyle Changes

Therapeutic lifestyle changes, including those to diet and exercise, constitute the cornerstone of management in patients with type IIb dyslipidemia. Restriction of dietary cholesterol (less than 300 mg/day) and saturated fat, and increasing dietary fiber and plant sterols can lower LDL cholesterol, and restriction of alcohol, sugar, saturated fat and high intake of omega-3 fatty acids can reduce serum triglycerides[30]. Because weight reduction can further lower LDL cholesterol and triglycerides and raise HDL cholesterol levels, maximal improvement in dyslipidemia should be attempted with lifestyle intervention before prescribing lipid-lowering medications. For example, the total calorie intake should be approximately 30 kcal x standard body weight (kg) and fat intake should be 25 to 35% of the total calorie intake in type IIb dyslipidemia[31, 32].

Exercise, primarily aerobic exercise such as walking, is effective for the improvement of dyslipidemia. Appropriate daily exercise should be performed for 30 min or longer per day and more than 3 times per week. In obese patients, more daily exercise should be recommended; however, there is a risk of musculoskeletal injuries during exercise, particularly if the person is not used to exercise or has knee joint pain or back pain; therefore, consideration should be given to the physical fitness of the patient. In terms of the exercise intensity, 11 to 13 according to Borg's scale for the rating of perceived exertion is recommended. The details of therapeutic lifestyle changes are described in the JAS guideline[33].

Drug Therapy

Statin Monotherapy

A number of clinical trials have demonstrated that event reduction occurred in patients who had elevated triglycerides as well as LDL cholesterol levels, although all these studies were not specifically

designed to examine the effect of statins in type IIb dyslipidemia. Stronger statins, such as atorvastatin, pitavastatin, and rosuvastatin can lower LDL cholesterol more than so-called standard statins, such as pravastatin, simvastatin, and fluvastatin. Greater triglyceride-lowering effects can be obtained by stronger statins; therefore, if the target LDL and non-HDL cholesterol goals are not achieved by standard statins, changing to stronger statins is a reasonable option.

Many studies have been performed to show the effect of statins on cardiovascular disease. In the primary prevention West of Scotland Coronary Prevention Study, for example, CAD event reduction with pravastatin was 29% in hypercholesterolemic men with triglyceride below the median of 148 mg/dL and 32% in those with triglycerides at or above the median[34]. In a post hoc analysis of the Scandinavian Simvastatin Survival Study, the hypercholesterolemic patients in the highest quartile for triglycerides (>159 mg/dL) and the lowest quartile for HDL cholesterol (<39 mg/dL) had the greatest event reduction (52%) by simvastatin treatment[35]. In the CARDS trial, atorvastatin caused a significant event reduction in the subgroup of triglycerides >150 mg/dL, but not in the group of triglycerides <150 mg/dL[36], while the Prospective Pravastatin Pooling Project showed a non-significant event reduction in the subgroup of triglycerides >220 mg/dL[37]. Overall, meta-analysis by Cholesterol Treatment Trialist showed a significant event reduction by statins in patients with triglycerides 124-178 mg/dL and >178 mg/dL[38]; therefore, statin monotherapy is recommended as the first-line therapy in patients with type IIb dyslipidemia.

Fibrate Monotherapy

Fibrates can reduce triglycerides by 20% to 50% and increase HDL cholesterol by 10% to 35%. Their effect on LDL cholesterol is variable, yet fibrates can increase the size of LDL particles. Although the clinical data on fibrate therapy are less consistent than those on statins, post-hoc analyses of fibrate trials have demonstrated the greatest clinical benefit in patients with combined dyslipidemia. In the Helsinki Heart Study, cardiac events were reduced by 34% with gemfibrozil, yet in the high-risk subgroup defined by triglycerides >205 mg/dL and LDL cholesterol/HDL cholesterol ratio >5, cardiac events were reduced by 71% with gemfibrozil[39]. Similarly, in the Bezafibrate Infarction Prevention (BIP) study, the subgroup with triglycerides ≥200 mg/dL had a significant 39.5% reduction in cardiac events, compared with a nonsignificant 7.3% reduction in the study overall[40]. In these trials, LDL cholesterol was reduced only modestly, whereas triglycerides were decreased by 35% and 21%, respectively, and HDL cholesterol was increased by 8% and 18%, respectively.

The Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) study reported a nonsignificant 11% reduction in the primary endpoint of coronary heart disease (CHD) events in type 2 diabetics treated with fenofibrate (200 mg daily) compared with those on a placebo; however, a significant reduction in total cardiovascular events was achieved with fenofibrate therapy[41]. Consistent with other fibrate trials, the highest risk and greatest benefits of fenofibrate were seen among those with marked hypertriglyceridemia >203 mg/dL[42]. In the FIELD trial, fenofibrate was shown to decrease diabetic microangiopathy, such as nephropathy and retinopathy[43, 44]; however, there was no benefit of fibrates for all-cause mortality, cardiovascular mortality, sudden death, or stroke.

Therefore, fibrate monotherapy is recommended for patients with type IIb dyslipidemia, especially diabetic patients with microangiopathy. If target LDL cholesterol and non-HDL cholesterol goals are not achieved combination with ezetimibe, probucol, or statin would be optional.

Niacin Monotherapy

Niacin can favorably modify all major lipid fractions. It is the only lipid-lowering agent that decreases lipoprotein (a), and is the most effective in increasing HDL cholesterol. Niacin can reduce LDL cholesterol by 5% to 25%, reduce triglycerides by 20% to 50%, and increase HDL cholesterol by 15% to 35%; therefore, niacin monotherapy is a reasonable option for type IIb dyslipidemia.

In the Coronary Drug Project, conducted in patients with previous myocardial infarction, niacin therapy at a dose of 3 g per day decreased total cholesterol by 10% and triglycerides by 26% compared with a placebo, and it decreased recurrent nonfatal myocardial infarction by 27%[43].

However, these data were obtained by the once-daily extended-release formula of niacin, which has improved tolerability and hepatic safety compared with the regular formula. Unfortunately, the once-daily formula of niacin is not available in Japan. Further, Japanese patients are generally less tolerable to niacin than Caucasians, and the maximum dose of niacin is 1.5 g per day. Hepatic toxicity, glucose intolerance, and an increase in uric acid should be carefully monitored when niacin is used to treat patients with type IIb dyslipidemia.

Ezetimibe

Ezetimibe is a new option in the management of type IIb dyslipidemia as monotherapy or in combination. Ezetimibe is a selective cholesterol absorption inhibitor that preferentially blocks the absorption of cholesterol, while not affecting triglycerides and fat-soluble vitamins[44]. Although it has produced fair reductions in LDL cholesterol as monotherapy (on average 18%), it has also some favorable effects on triglycerides (−17%) and HDL cholesterol (+17%) in combined dyslipidemia. It also has important complementary effects in combination with statins and fibrates[45, 46]. Ezetimibe added to statin therapy provided an additional 25% reduction in LDL cholesterol and 11% reduction in triglycerides compared with a placebo. Recent studies showed that ezetimibe could ameliorate fatty liver and postprandial hyperlipidemia[47, 48]; therefore, ezetimibe can be recommended for type IIb dyslipidemic patients with fatty liver or postprandial hyperlipidemia.

Statin-Fibrate Combination Therapy

Statin-fibrate combination has been shown to provide benefits in patients with type IIb dyslipidemia. Several trials have examined the safety and efficacy of combination therapy with statins and fibrates. In a study of 389 patients with familial combined hyperlipidemia randomized to receive pravastatin 20 mg plus gemfibrozil 1,200 mg, or simvastatin 20 mg plus gemfibrozil 1,200 mg, LDL cholesterol was decreased by 35% and 39%, triglycerides was decreased by 48% and 54%, and HDL cholesterol was increased by 14% and 25%, respectively[49]. In a more recent study conducted in 120 patients with type 2 diabetes mellitus and combined hyperlipidemia, the combination of atorvastatin 20 mg and fenofibrate 200 mg decreased LDL cholesterol by 46%, triglycerides by 50%, and increased HDL cholesterol by 22%[50]. The Fluvastatin Alone and in Combination Treatment (FACT) study was a multicenter, prospective, double-blind study of 333 patients with CAD and combined dyslipidemia. The combination of fluvastatin 40 mg plus bezafibrate 400 mg was very effective for all lipid parameters, decreasing LDL cholesterol by 24% and triglycerides by 38% and increasing HDL cholesterol by 22%[51].

Action to Control Cardiovascular Risk in Diabetes (ACCORD) was designed to address the effect of add-on therapy of fenofibrate to simvastatin in type 2 diabetes patients; however, the combination of fenofibrate and simvastatin did not reduce the rate of fatal cardiovascular events, nonfatal myocardial infarction, or nonfatal stroke, as compared with simvastatin alone[52]. Therefore, the routine use of combination therapy with fenofibrate and simvastatin to reduce cardiovascular risk in the majority of high-risk patients with type 2 diabetes is not recommended. In subgroup analysis of the ACCORD trial, patients with high triglycerides (>204 mg/dL) and low HDL cholesterol (<34 mg/dL) had a significant event reduction by fenofibrate; thus this combination regimen is only recommended in type IIb dyslipidemia with low HDL cholesterolemia.

Thus, in spite of its powerful lipid-lowering effects, combination therapy with statins and fibrates requires careful selection and monitoring of patients. Because of the potential increased risk for myopathy with the statin-fibrate combination, patients with muscle symptoms may be advised to halt therapy immediately. If creatinine kinase is markedly elevated, assessment of renal function and serum potassium is required along with increased fluid intake to prevent acute renal failure associated with rhabdomyolysis.

Statin-Niacin Combination

The combination of statins with niacin can be an option because both have excellent outcomes of improving cardiovascular disease. In a review of 9 clinical trials of combination therapy with statins plus niacin, LDL cholesterol was reduced by 25% to 57% and HDL cholesterol was increased by 13% to 36%[53]. Fluvastatin 20 mg per day plus niacin 3 g per day has been reported to reduce lipoprotein (a) levels by 37%[54], and pravastatin 20 mg per day plus niacin 3 g per day was reported to decrease levels of small, dense LDL by 43%[55].

Combination therapy was also studied in the HDL-Atherosclerosis Treatment Study, in which simvastatin plus niacin significantly reduced the risk for a composite cardiovascular endpoint by 90% compared with a placebo[56]. Further, patients with coronary disease, normal LDL cholesterol levels, and low HDL cholesterol levels were treated with simvastatin plus niacin for three years. As a result, the rate of major clinical events was 90% lower in the combination group than in the placebo group. Further, the Atherothrombosis Intervention in Metabolic Syndrome with Low HDL/High Triglycerides and Impact on Global Health Outcomes (AIM-HIGH) study is on-going to investigate the effect of combination therapy of niacin and statin on cardiovascular event reduction; however, as described for niacin monotherapy, treatment with niacin should proceed with caution.

Statin-Eicosapentaenoic Acid Combination Therapy

Epidemiological and clinical evidence has consis-

tently demonstrated the triglyceride-lowering effect of eicosapentaenoic acid (EPA)[57]. At doses of 1.8 g per day, EPA reduces triglyceride levels by approximately 20%. The triglyceride-lowering effect is believed to be primarily the result of a reduction in hepatic triglyceride synthesis and hence diminished secretion of triglyceride-rich lipoproteins from the liver into the circulation. Observational and clinical trial data suggest that omega-3 fatty acid can reduce the risk of CAD-related death and reduce nonfatal coronary events[58-60].

Few intervention studies have reported the combination of statin and EPA. The Japan Eicosapentaenoic Acid Lipid Intervention Study (JELIS) was designed to study the effects of EPA on major coronary events in patients with hypercholesterolemia[61]. A total of 18,645 patients were randomized to receive either 1.8 g EPA plus statin or statin alone. A 19% relative reduction of the primary endpoint of major coronary events was shown in the study conducted in Japan. The mean triglyceride levels were 154 mg/dL in the EPA plus statin arm and 153 mg/dL in the statin arm, indicating that this study cohort included a significant number of patients with type IIb dyslipidemia. In this study, triglyceride levels were significantly decreased by 9% from baseline in the EPA group and by 4% in controls ($p<0.0001$ between groups). A greatest reduction of coronary events by EPA was found in secondary prevention; however, the OMEGA study did not support the effect of omega-3 fatty acids on cardiovascular events in secondary prevention[62]. Therefore, the potential of omega-3 fatty acids may vary, depending on individual clinical conditions. Currently, the JAS guideline recommends adding EPA for statin-treated high-risk patients and statin-EPA combination would be an option for patients with type IIb dyslipidemia.

Conclusions

Type IIb dyslipidemia is often associated with type 2 diabetes, metabolic syndrome, CKD, and FCHL, and atherogenic lipoproteins, such as small, dense LDL, remnants, and IDL; therefore, patients with this type of dyslipidemia have a high risk for CAD and need risk management, including hypertension, diabetes, and so forth. Data from prospective epidemiological as well as interventional studies indicate that a significant proportion of high-risk patients may benefit not only from LDL cholesterol lowering, but also from treatment of other lipid parameters, such as non-HDL cholesterol, triglycerides, and HDL cholesterol. Achievement of LDL cholesterol and non-HDL cholesterol targets in patients with type IIb dyslipidemia sometimes requires either higher doses of statins or increased use of combination therapy. Optimal management requires a targeted strategy to correct the underlying lipid abnormalities to reduce the risk for CAD events, while minimizing adverse effects. Future clinical trials are needed to determine both the risks and benefits of monotherapy versus combination regimens for the treatment of patients with type IIb dyslipidemia.

Conflict of Interest

Dr. Arai has received unrestricted grants from Otsuka Pharmaceutical Co., Ltd., received honoraria from MSD, and is an advisor of Kowa Pharmaceutical Co. Ltd. Dr. Ishibashi has received unrestricted grants from Takeda Pharmaceutical Co. Ltd. and is an advisor of Kowa Pharmaceutical Co. Ltd. Dr. Oikawa has received unrestricted grants from Daiichi-Sankyo Co. Ltd. Dr. Harada-Shiba has received unrestricted grants from MSD. Dr. Yamashita has received unrestricted grants from MSD, Otsuka Pharmaceutical Co., Ltd., Astellas Pharma Inc., and JT, collaborative research grants from Shionogi & Co., Ltd., Otsuka Pharmaceutical Co., Ltd., and National Institute of of Biomedical Innovation, honoraria for lectures from MSD, Bayer Yakuhin, Ltd., and Kowa Pharmaceutical Co., Ltd., and is an advisory of Skylight Biotech Co. Dr. Eto is an advisor of MSD. The other authors declare that they have no conflict of interest.

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Original Article

Multicenter Study to Determine the Diagnosis Criteria of Heterozygous Familial Hypercholesterolemia in Japan

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Aim: **Heterozygous patients of familial hypercholesterolemia (FH) are known to have a high risk of coronary artery disease (CAD). Early diagnosis and prompt treatment are necessary to prevent their CAD. In this study we tried to amend the Japanese diagnostic criteria of FH for general practitioners by examining each component of the current criteria.**
Methods: **A multicenter study was performed, which included 1356 dyslipidemic patients at 6 centers. Pretreatment demographic information including LDL-cholesterol (LDL-C), Achilles tendon thickness (ATT), family history of FH and premature CAD and the result of genetic analysis were analyzed.**
Results: **Of 1356 patients, 419 were diagnosed with FH by criteria in 1988, which were used as a golden standard. We tried to define FH according to 3 conventional major items, i.e., 1) LDL-C, 2) ATT and/or cutaneous nodular xanthomas (CX), 3) family history of FH and/or family history of premature CAD. We then determined the cutoff of LDL-C using the new criteria. When we used 180 mg/dL as the cutoff of LDL-C, 94.3% of FH patients and 0.85% of non-FH satisfied 2 or more criteria. When we used 190 mg/dL, 92.1% of FH and 0.85% of non-FH satisfied 2 or more criteria; therefore, we chose 180 mg/dL for the cutoff of LDL-C in the new criteria and proposed that the diagnosis of definite FH can be made if 2 or more criteria are satisfied.**
Conclusions: **We examined each component for the diagnosis of heterozygous FH in a multicenter study in Japan.**

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Key words; Diagnosis criteria, Familial hypercholesterolemia, LDL cholesterol, Achilles tendon thickness, LDL receptor

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Introduction

Familial hypercholesterolemia (FH) is a genetic disease caused by a mutation in genes related to low-density lipoprotein (LDL) metabolism. Heterozygous FH patients manifest high LDL cholesterol (LDL-C)

levels, skin and/or tendon xanthomas, and increased risk of premature coronary artery disease (CAD)[1]. High LDL-C levels are the first symptom that appears even from birth, while xanthomas on the Achilles tendon usually appear during or after the late 10s and CAD that determines the prognosis of FH patients appears during or after the third decade of life in men and the fifth decade in women[2-4]. Because morbidity and mortality of CAD in heterozygous FH are much higher than in the general population[1, 5-7], special attention should be paid to screen these patients and to prevent their atherosclerotic complications. For the diagnosis of FH, several criteria have been published throughout the world, including ours, reported in 1988[8]; however, appropriate diagnosis of FH by primary care physicians is not performed in general practice in Japan[9]. Therefore, it is very important to establish useful diagnostic criteria for primary care physicians to diagnose FH with high specificity and sensitivity.

Because FH patients are estimated to be more than 250,000, primary care physicians need to take care of most of them; therefore, the criteria should be as simple as possible for clinical usefulness and have high sensitivity and specificity. We have used diagnosis criteria for FH published in 1988 in Japan[8], which include hypercholesterolemia, presence of skin/tendon xanthoma and reduced LDL receptor activity as major items; however, it is difficult to measure LDL receptor activity in routine clinical practice and even lipid specialists do not measure its activity. Furthermore, it is not covered by Japan's health insurance system; therefore, it is necessary to make the current diagnostic criteria easy to use for general practitioners. Toward this end, we performed a multicenter collaborative study of 1397 patients with dyslipidemia.

Methods

Subjects

A total of 1397 patients with dyslipidemia, referred to the outpatient clinic of 6 hospitals (Kyoto University Hospital, Osaka University Hospital, Nippon Medical School Hospital, Chiba University Hospital, Kanazawa University Hospital, and National Cerebral and Cardiovascular Center Hospital), were the subjects to this study. Among these patients, 41 were excluded due to missing data. Consequently, 1356 patients with dyslipidemia were eligible for the present study. Most had been diagnosed with or without FH by lipid specialists at each hospital according to the criteria for FH reported in 1988, and genetic analysis was performed in 223 patients, some of whom were diagnosed with FH based only on mutations of the LDL receptor or PCSK9. The criteria were as follows: Major items included 3 items, (1) IIa or IIb phenotype at serum cholesterol level of 260 mg/dL or above; (2) Tendinous xanthoma or xanthoma tuberosum is present; (3) Reduced or abnormal receptor activity. Minor items included 3 items: (1) Xanthoma palpebratum; (2) Arcus juvenalis (<50 years); (3) Juvenile (<50 years) ischemic heart disease.

Table 1. Clinical characteristics of non-FH and FH patients in this cohort

	non-FH	FH	*p*
N	937	419	
Male (n, %)	453 (48.3%)	170 (42.7%)	<0.01
Age (y.o.)	58.3±16.3	52.9±18.6	<0.01
TC (mg/dL)	236±53	339±72	<0.01
LDL-C (mg/dL)	146±46	257±67	<0.01

Determination of Conventional Criteria for FH

In this study we tried to amend the current criteria. For the primary care setting, three major items, i.e. serum level of LDL-C, family history and specific physical findings of FH, were chosen as diagnostic items because all are easily assessed by general practitioners. Family history and specific physical findings were also separated in more detail. Finally, we set 5 items, (1) LDL-C, (2) specific physical findings: a) ATT, b) cutaneous nodular xanthomas (CX), (3) family history: a) family history of FH in 1st or 2nd degree relatives, b) family history of premature CAD in 1st or 2nd degree relatives. A family history of premature CAD was defined as having CAD before the age of 55 in males and 65 in females. First, we assessed the prediction for FH by the combination of physical findings and family history, and then we determined the cutoff point of LDL-C with the combination of the above-mentioned two items. LDL-C levels were calculated by the Friedewald formula. ATT levels were measured by X-ray according to the method previously described and determined as positive with 9 mm or more[10].

The data in the medical records of the patients were sent to the National Cerebral and Cardiovascular Center and examined. The study protocol was approved by the ethics committee of the National Cerebral and Cardiovascular Center (D#M20-25-2 for the multicenter trial and ID#M17-56-4 for the genetic analysis). The ethics committee of each hospital also approved the study protocol.