

The Association of Small Dense Low-Density Lipoprotein Cholesterol and Coronary Heart Disease in Subjects at High Cardiovascular Risk

Mayu Higashioka^{1,2}, Satoko Sakata^{1,3,4}, Takanori Honda¹, Jun Hata^{1,3,4}, Mao Shibata^{1,3}, Daigo Yoshida¹, Kenichi Goto⁴, Takanari Kitazono^{3,4}, Haruhiko Osawa² and Toshiharu Ninomiya^{1,3}

¹Department of Epidemiology and Public Health, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan.

²Department of Diabetes and Molecular Genetics, Graduate School of Medicine, Ehime, Japan.

³Center for Cohort Studies, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan.

⁴Department of Medicine and Clinical Science, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan.

Aim: The present study aims to investigate the association between serum small dense low-density lipoprotein (sdLDL) cholesterol level and the development of coronary heart disease (CHD) in subjects at high cardiovascular risk.

Methods: A total of 3,080 participants without prior cardiovascular disease (CVD), aged ≥ 40 years, were followed up for a median of 8.3 years, which were divided into two groups, those with serum sdLDL cholesterol levels of < 35 mg/dL or ≥ 35 mg/dL. Then, subjects were stratified by the status of diabetes, CVD-related comorbidities (defined as the presence of diabetes, chronic kidney disease, or peripheral artery disease), and the CVD risk assessment according to the Japan Atherosclerosis Society Guidelines. The hazard ratios (HRs) and 95% confidence intervals (CIs) were computed using a Cox proportional hazards model.

Results: During the follow-up, 79 subjects developed CHD. The risk for incident CHD was higher in subjects with serum sdLDL cholesterol of ≥ 35 mg/dL than those with sdLDL cholesterol of < 35 mg/dL (HR 2.09, 95%CI 1.26–3.45) after adjusting for traditional risk factors. In the subgroup analyses, the multivariable-adjusted HR for incident CHD increased significantly in those with serum sdLDL cholesterol of ≥ 35 mg/dL among subjects with diabetes (HR 2.76, 95%CI 1.09–7.01), subjects with CVD-related comorbidities (HR 2.60, 95%CI 1.21–5.58), and high-risk category defined as the presence of CVD-related comorbidities or a Suita score of ≥ 56 points (HR 1.93, 95%CI 1.02–3.65).

Conclusions: Elevated serum sdLDL cholesterol was associated with the development of CHD even in subjects at high cardiovascular risk.

Key words: Small dense LDL cholesterol, Coronary heart disease, Risk assessment, Prospective study, High-risk population

Abbreviations: BMI, body mass index; CHD, coronary heart disease; CI, confidence interval; CVD, cardiovascular disease; ECG, electrocardiogram; eGFR, estimated glomerular filtration rate; HbA1c, hemoglobin A1c; HDL, high-density lipoprotein; HR, hazard ratio; LDL, low-density lipoprotein; MESA, Multi-Ethnic Study of Atherosclerosis; sdLDL, small dense low-density lipoprotein.

Introduction

Coronary heart disease (CHD) is a common noncommunicable disease and one of the leading

causes of mortality worldwide. According to a report from the Global Burden of Diseases study, in 2017, there were 8.93 million deaths from CHD, and CHD was the leading cause of years of life lost¹. In Japan,

Address for correspondence: Satoko Sakata, Department of Epidemiology and Public Health, Graduate School of Medical Sciences, Kyushu University 3-1-1 Maidashi, Higashi-ku, Fukuoka City, Fukuoka, 812-8582, Japan E-mail: ssakata@intmed2.med.kyushu-u.ac.jp

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heart disease is the second leading cause of death in 2017, with CHD accounting for over 30% of heart disease deaths²). Hence, preventing the development of CHD is a highly important issue in contemporary and future public health on a global scale.

Previous observational studies and clinical trials have revealed that serum low-density lipoprotein (LDL) cholesterol is a significant risk factor for the development of CHD³). The Guidelines for the Prevention of Atherosclerotic Cardiovascular Disease 2017 from the Japan Atherosclerosis Society recommend monitoring and intervention on LDL cholesterol for primary prevention of atherosclerotic cardiovascular disease (CVD), of which target levels of LDL cholesterol-lowering treatment are categorized according to the absolute risk of CHD estimated by the Suita score, a risk index used to estimate the 10-year risk of CHD for the Japanese population⁴⁻⁵).

Recently, the difference in the atherogenic effect across differently sized LDL particles has attracted attention. In particular, small dense LDL (sdLDL), which is small in size and highly dense, has been shown to have more atherogenic effect than other LDL particles⁶⁻⁸). Several prospective cohort studies have reported the significant association between serum sdLDL cholesterol and the development of CHD regardless of serum LDL cholesterol levels⁹⁻¹¹). Our study group also reported a significant association between serum sdLDL cholesterol and the development of CHD in a general Japanese population; the cutoff value of sdLDL cholesterol for discriminating the risk of developing CHD was around 35 mg/dL¹²). However, it is still unknown whether the association between sdLDL cholesterol and CHD is observed even in individuals at high cardiovascular risk. It is of particular importance to investigate the association in subpopulations with varied cardiovascular risks to evaluate clinical usefulness of measuring sdLDL in routine care settings.

Aim

The present study aims to clarify the association between sdLDL cholesterol and CHD among Japanese general population stratified by predefined CHD risks using the risk stratification algorithm of the Japan Atherosclerosis Society 2017 Guidelines.

Methods

Study Population

A detailed description of the Hisayama study was published previously¹³). Briefly, a total of 3,384 residents aged 40 years or older (78.2% of the total popu-

lation of this age group) underwent the screening survey for the present study in 2007 and 2008. After excluding 8 subjects who did not consent to participate in the study, 223 subjects who had past history of stroke or CHD, and 73 subjects for whom serum sdLDL cholesterol data were lacking, the remaining 3,080 subjects (1,290 men and 1,790 women) were enrolled in the present study.

The study was approved by the Kyushu University Institutional Review Board for Clinical Research, and written informed consent was obtained from all participants.

Follow-Up Survey

The subjects were followed up prospectively until November 2015 or their death (median, 8.3 years) by annual health examinations or by mail or telephone for any subject who did not undergo the examination or who moved out of the town as described previously¹⁴). The development of CHD was also checked by a daily monitoring system organized by the study team, local physicians, and members of the Health and Welfare Office of the town. Subjects with suspected CHD events were evaluated on the basis of all available clinical information, and their diagnoses were adjudicated by study team physicians. When a subject died, an autopsy was performed at the Department of Pathology of Kyushu University, if consent for autopsy was obtained. During the follow-up period, autopsy examination was performed for 192 (57.8%) of 332 deceased subjects. In addition to the deceased cases, four subjects were lost to follow up, all of whom were subjects who moved out of the town.

Small Dense Low-Density Lipoprotein Cholesterol Measurement

At the screening examination, portions of the plasma specimens were stored at -80°C until serum sdLDL cholesterol concentrations were measured in 2014 on a Hitachi 7180 automated chemistry analyzer using a homogeneous assay (sdLDL-EX "SEIKEN"; Denka Seiken, Tokyo)¹⁵). The subjects were divided into two groups, those with serum sdLDL cholesterol of <35 and of ≥ 35 mg/dL, which is the cutoff value reported previously¹²).

Outcomes

The primary outcome of the present study was CHD. The criteria for the diagnosis of CHD included first-ever fatal and nonfatal myocardial infarction, silent myocardial infarction, sudden cardiac death within 1 hour after the onset of acute illness, coronary angioplasty, and bypass grafting. The diagnosis of myocardial infarction was based on detailed clinical

information and at least two of the following findings: typical clinical symptoms, electrocardiogram (ECG) evidence of myocardial infarction, elevated cardiac enzymes, or morphologic findings, including echocardiographic, scintigraphic, or angiographic abnormalities compatible with myocardial injury, or myocardial necrosis or scars more than 1 cm in diameter at autopsy^{14, 16}.

Other Variables

Information on medical history including the presence of peripheral artery disease; medication for hypertension, diabetes, and dyslipidemia; smoking habits; alcohol intake; and physical activity at baseline was obtained using a standard self-administered questionnaire and was checked by trained interviewers. Smoking and drinking habits were classified as either current use or not. Current smoking was defined as smoking at least one cigarette per day. Current drinking was defined as drinking at least one alcoholic beverage per month. The subjects engaging in sports or other forms of exertion ≥ 3 times a week during their leisure time made up a regular exercise group. Body height and weight were measured with the subjects in light clothing without shoes, and body mass index (BMI) was calculated (kg/m^2). Blood pressure was measured three times in a sitting position using an automated sphygmomanometer (BP-203 RVIII; Omron Healthcare), and the mean of three measurements was used in the present analysis. Hypertension was defined as blood pressure $\geq 140/90$ mmHg and/or current use of antihypertensive agents. ECG abnormalities were defined as left ventricular hypertrophy (Minnesota code 3-1), ST depression (4-1, 4-2, or 4-3), or atrial fibrillation/flutter (8-3). Blood samples were collected from an antecubital vein. Plasma glucose levels were measured by the hexokinase method. Diabetes mellitus was defined as a fasting plasma glucose level ≥ 126 mg/dL, 2-hour post load or casual glucose level of ≥ 200 mg/dL, or current use of glucose-lowering agents (i.e., oral glucose-lowering agents or insulin). Prediabetes was defined as a fasting plasma glucose level of 110 to 125 mg/dL and 2-hour post load glucose level of <200 mg/dL or fasting plasma glucose level of <126 mg/dL and 2-hour post load glucose level of 140 to 199 mg/dL. Hemoglobin A1c (HbA1c) was measured by latex aggregation immunoassay using a Determiner HbA1c kit (Kyowa Medex, Tokyo). Serum LDL cholesterol and high-density lipoprotein (HDL) cholesterol were measured enzymatically, and serum triglycerides were measured with colorimetric method. The estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration equation

with a Japanese coefficient of 0.813, for which serum creatinine was measured enzymatically¹⁷. Chronic kidney disease was defined as eGFR <60 mL/min/ 1.73 m² or proteinuria ($\geq 1+$ on dipstick) according to the Kidney Disease Improving Global Outcomes guideline¹⁸.

Risk Assessments for Cardiovascular Disease

We performed the risk assessment for CVD according to the Guidelines for Prevention of Atherosclerotic Cardiovascular Disease 2017 from the Japan Atherosclerosis Society⁴ (**Supplementary Fig. 1**). CVD-related comorbidities were defined as the presence of diabetes, chronic kidney disease, and/or peripheral artery disease. First, subjects with CVD-related comorbidities were classified as a high-risk category. Next, the Suita scores were calculated with the variables of age, sex, smoking habits, blood pressure level, serum LDL and HDL cholesterol levels, and prediabetes among subjects without CVD-related comorbidities. Because data on family history of premature CHD were not available, 0 point was assigned to all participants. Subjects were classified into categories based on the total Suita score as follows: low-risk category (≤ 40 points), moderate-risk category (41–55 points), and high-risk category (≥ 56 points)⁴.

Statistical Analysis

The significance of differences in the mean values or frequencies of risk factors between subjects with sdLDL cholesterol of <35 mg/dL and those with sdLDL cholesterol of ≥ 35 mg/dL was tested using Student's *t* test or the χ^2 test, respectively. Serum triglyceride level was shown as median and interquartile range in the baseline characteristics, because of the skewed distribution. The incidence rate of CHD was calculated using the person-year method. The Cox proportional hazards model was used to estimate the hazard ratio (HR) and its 95% confidence intervals (CIs) for the development of CHD by subpopulations stratified by the status of (1) diabetes, (2) the CVD-related comorbidities, and (3) the CVD risk assessment. In the multivariable analysis, the risk estimates were adjusted for potential confounding factors at baseline—namely, age, sex, systolic blood pressure, use of antihypertensive agents, HbA1c, use of glucose-lowering agents, serum HDL cholesterol, lipid-modifying agents, BMI, eGFR, ECG abnormalities, current smoking, current drinking, and regular exercise. The heterogeneities in the association between subgroups were tested by adding multiplicative interaction terms to the relevant Cox model. A two-sided value of $p < 0.05$ was considered statistically significant in all analyses. Statistical analyses were conducted using Statisti-

Table 1. Baseline characteristics of overall subjects and characteristics according to small dense LDL cholesterol level.

Variables	Overall subjects	Serum sdLDL cholesterol level		<i>p</i> value ^a
		< 35 mg/dL	≥ 35 mg/dL	
<i>N</i>	3,080	1,728	1,352	-
Age (years)	63 (13)	64 (14)	62 (11)	< 0.001
Men (%)	41.9	36.1	49.3	< 0.001
Systolic blood pressure (mmHg)	132 (19)	129 (20)	136 (18)	< 0.001
Use of antihypertensive agents (%)	29.5	29.4	29.7	0.87
Diabetes (%)	15.0	11.3	19.7	< 0.001
Prediabetes (%)	23.1	18.5	29.1	< 0.001
HbA1c (%)	5.5 (0.8)	5.4 (0.7)	5.6 (0.8)	< 0.001
Use of glucose-lowering agents (%)	6.5	6.0	7.1	0.23
Serum LDL cholesterol (mg/dL)	122 (32)	107 (24)	141 (30)	< 0.001
Serum non-HDL cholesterol (mg/dL)	142 (36)	123 (26)	167 (31)	< 0.001
Serum HDL cholesterol (mg/dL)	67 (18)	72 (18)	61 (16)	< 0.001
Serum triglycerides (mg/dL) ^b	100 (73–143)	82 (62–107)	136 (101–192)	< 0.001
Use of lipid-modifying agents (%)	13.9	14.7	12.9	0.14
BMI (kg/m ²)	23 (4)	22 (3)	24 (3)	0.68
eGFR (ml/min/1.73 m ²)	75 (13)	75 (13)	76 (12)	< 0.001
Urinary protein (%)	5.9	5.2	6.9	0.046
Chronic kidney disease (%)	13.7	14.0	13.5	0.71
Electrocardiogram abnormalities (%)	15.3	14.2	16.8	0.046
History of peripheral artery disease	0.2	0.3	0.2	0.41
Current smoking (%)	19.9	16.6	24.3	< 0.001
Current drinking (%)	47.6	42.8	53.9	< 0.001
Regular exercise (%)	11.9	11.8	11.9	0.94

Abbreviations: BMI, body mass index; eGFR, estimated glomerular filtration rate; HbA1c, hemoglobin A1c; HDL, high-density lipoprotein; LDL, low-density lipoprotein; sdLDL, small dense low-density lipoprotein.

Data are presented as the mean values (standard deviation), percentages, or median (interquartile range).

^aThe significance of differences in mean values and frequencies of risk factors between the level of sdLDL cholesterol were tested by Student's *t* test and chi-square test, respectively.

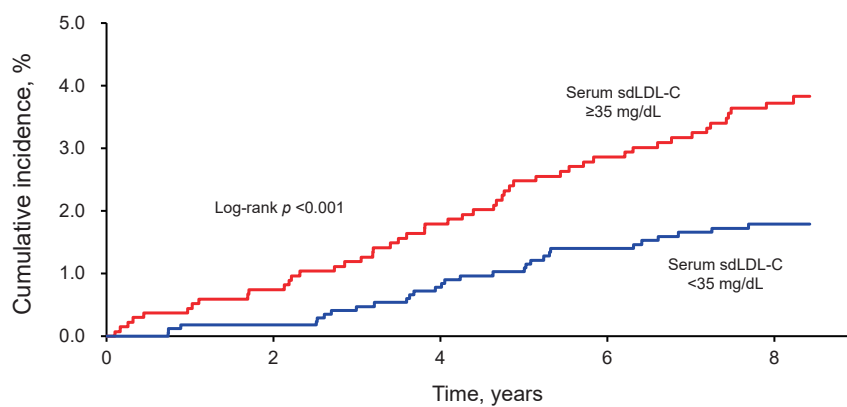
^bOnly subjects with available fasting blood samples were included in the analysis using serum triglyceride (*n* = 3,056).

cal Analysis Software version 9.4 (SAS Institute, Cary, NC).

Results

The baseline characteristics of the total study population and those with serum sdLDL cholesterol levels of less than 35 mg/dL and of 35 mg/dL or more are shown in **Table 1**. The mean values of systolic blood pressure, HbA1c, serum LDL cholesterol, serum non-HDL cholesterol, and eGFR; the median value of serum triglycerides; and the frequencies of male sex, diabetes, prediabetes, urinary protein, ECG abnormalities, current smoking, and current drinking were significantly higher in subjects with serum sdLDL cholesterol levels of ≥ 35 mg/dL compared to serum sdLDL cholesterol levels of < 35 mg/dL, whereas the mean values of age and serum HDL cholesterol were significantly lower.

During the follow-up period, 79 subjects experienced a first-ever CHD event. The crude cumulative incidence of CHD was significantly higher in subjects with serum sdLDL cholesterol levels of ≥ 35 mg/dL than < 35 mg/dL (log-rank *p* < 0.001, **Fig. 1**). **Table 2** demonstrates the age- and sex-adjusted and multivariable-adjusted HRs (95% CIs) of serum sdLDL cholesterol of ≥ 35 mg/dL against < 35 mg/dL for the development of CHD, in overall subjects and in the subgroups according to diabetic status, the presence or absence of CVD-related comorbidities (diabetes, chronic kidney disease, and/or peripheral artery disease), and the risk assessment status based on the guidelines from the Japan Atherosclerosis Society. In overall subjects, the age- and sex-adjusted HR for the development of CHD was 2.44 (95% CI, 1.52–3.90) for serum sdLDL cholesterol of ≥ 35 mg/dL as compared with serum sdLDL cholesterol of < 35 mg/dL. This association remained significant even after adjust-



	sdLDL-C <35.0 mg/dL n=1728	sdLDL-C ≥35.0 mg/dL n=1352
Number of events/person year	29/13325	50/10692
Incidence rate per 1,000 person year (95%CI)	2.18 (1.51 - 3.13)	4.68 (3.55 - 6.16)

Fig. 1. Crude cumulative incidence of coronary heart disease according to serum small dense low-density lipoprotein cholesterol

Abbreviations: sdLDL-C, small dense low-density lipoprotein cholesterol.

ment for age, sex, systolic blood pressure, use of anti-hypertensive agents, HbA1c, use of glucose-lowering agents, BMI, eGFR, ECG abnormalities, current smoking, current drinking, and regular exercise (HR 2.09, 95%CI 1.26–3.45). In the subgroup analysis, the multivariable-adjusted HRs for the development of CHD increased significantly in those with serum sdLDL cholesterol of ≥ 35 mg/dL compared to those with serum sdLDL cholesterol of < 35 mg/dL in both subjects with and without diabetes (without diabetes: HR 1.89, 95%CI 1.01–3.52; with diabetes: HR 2.76, 95%CI 1.09–7.01), in subjects with CVD-related comorbidities (HR 2.60, 95%CI 1.21–5.58), and in subjects in the high-risk category (HR 1.93, 95%CI 1.02–3.65). There was no evidence of significant heterogeneities in the association between these subgroups (all p for heterogeneity > 0.5). We also divided subjects into low-risk category and moderate-/high-risk category for the risk assessment status, and the multivariable-adjusted HR of the development of CHD was 5.77 (95%CI 0.25–133.23) among subjects in the low-risk category and 1.70 (95%CI 1.05–2.76) among subjects in the moderate-/high-risk category, without any evidence of heterogeneity between subgroups (p for heterogeneity = 0.77). When we focus on the results of the subjects in the high-risk category for the risk assessment status, the age- and sex-adjusted cumulative incidence of CHD was higher in subjects with serum sdLDL cholesterol levels of ≥ 35 mg/dL than those with < 35 mg/dL ($p = 0.03$, [Supplementary Fig. 2](#)).

Fig. 2 shows the joint influence of serum sdLDL cholesterol levels and the risk assessment status on the development for CHD. As compared to subjects with serum sdLDL cholesterol of < 35 mg/dL and at low-/moderate-risk category, the multivariable-adjusted HRs for the development of CHD increased significantly in subjects with serum sdLDL cholesterol of < 35 mg/dL and at high-risk category (HR 2.67, 95%CI 1.26–5.66) or in subjects with serum sdLDL cholesterol of ≥ 35 mg/dL and at low-/moderate-risk category (HR 2.05, 95%CI 1.01–4.16). Moreover, subjects with serum sdLDL cholesterol of ≥ 35 mg/dL and in the high-risk category had an additive excess risk of CHD events (HR 5.13, 95%CI 2.64–9.98). Similar joint excess risks of CHD events were observed according to the presence or absence of diabetes or CVD-related comorbidities ([Supplementary Fig. 3](#)).

Finally, we examined the influence of serum sdLDL cholesterol on the development of CHD between subgroups of other lipid parameters—namely, serum LDL cholesterol, serum non-HDL cholesterol, serum HDL cholesterol, and serum triglycerides—in subjects in the high-risk category. The correlation between serum sdLDL cholesterol and other lipid parameters in those subjects were relatively strong ([Supplementary Table 1](#)). The multivariable-adjusted risk of CHD tended to be higher in subjects with serum sdLDL cholesterol of ≥ 35 mg/dL than in those with serum sdLDL cholesterol of < 35 mg/dL regardless of whether the levels of the other lipid parameters were higher or lower, although the HRs did not reach

Table 2. Hazard ratios for the development of coronary heart disease according to serum small dense low-density lipoprotein cholesterol levels in subgroups of the status of cardiovascular risk

	No. of events/subjects		Crude incidence rate (per 1,000 person-years)		HR (95%CI) of serum sdLDL-C ≥ 35 mg/dL vs. <35 mg/dL.	
	Serum sdLDL-C (mg/dL)		Serum sdLDL-C (mg/dL)		Age- and sex-adjusted	Multivariable-adjusted
	<35	≥ 35	<35	≥ 35		
Overall subjects ^a	29/1728	50/1352	2.18	4.68	2.44 (1.52–3.90)	2.09 (1.26–3.45)
Diabetes ^b						
Absence	21/1533	29/1086	1.77	3.35	2.19 (1.23–3.90)	1.89 (1.01–3.52)
Presence	8/195	21/266	5.43	10.32	2.30 (0.999–5.29)	2.76 (1.09–7.01)
<i>p</i> for heterogeneity					0.81	0.87
CVD-related comorbidities ^c						
Absence	17/1340	25/967	1.61	3.22	2.35 (1.25–4.43)	1.86 (0.94–3.69)
Presence	12/388	25/385	4.32	8.5	2.21 (1.08–4.52)	2.60 (1.21–5.58)
<i>p</i> for heterogeneity					0.60	0.57
Risk assessment status ^d						
Low-/moderate-risk category	14/1268	18/829	1.40	2.69	2.66 (1.28–5.55)	2.02 (0.98–4.16)
high-risk category	15/460	32/523	4.54	7.99	1.99 (1.05–3.76)	1.93 (1.02–3.65)
<i>p</i> for heterogeneity					0.90	0.89
Low-risk category	1/707	1/282	0.17	0.43	4.02 (0.21–75.58)	5.77 (0.25–133.23)
Moderate-/high-risk category	28/1021	49/1070	3.71	5.85	2.04 (1.25–3.31)	1.70 (1.05–2.76)
<i>p</i> for heterogeneity					0.86	0.77

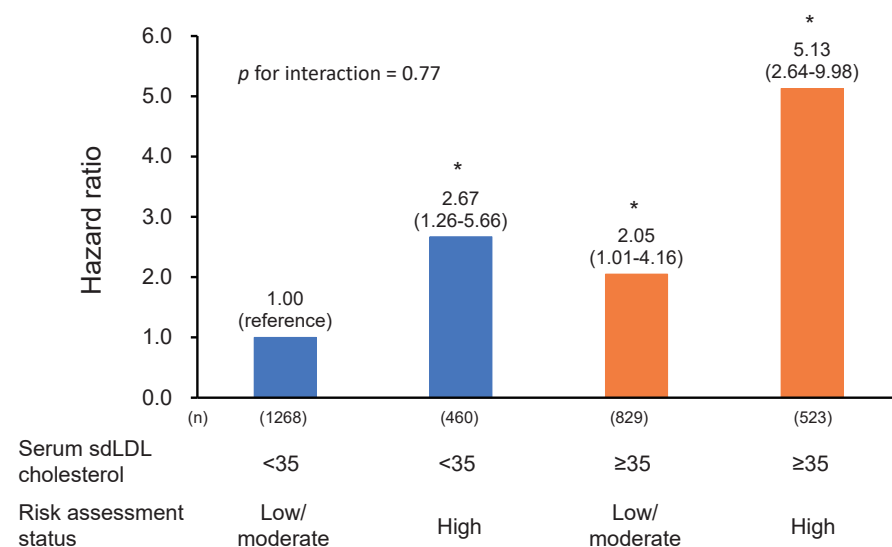
Abbreviations: CI, confidence interval; HR, hazard ratio; sdLDL-C, small dense low-density lipoprotein cholesterol

^aFor the analysis in the overall subjects, age, sex, systolic blood pressure, use of antihypertensive agents, hemoglobin A1c, use of glucose-lowering agents, serum high-density lipoprotein cholesterol, use of lipid-modifying agents, body mass index, estimated glomerular filtration rate, electrocardiogram abnormalities, current smoking, current drinking, and regular exercise were included in the relevant Cox model as covariates.

^bFor the subgroup analysis of diabetic status, age, sex, systolic blood pressure, use of antihypertensive agents, serum high-density lipoprotein cholesterol, use of lipid-modifying agents, body mass index, estimated glomerular filtration rate, electrocardiogram abnormalities, current smoking, current drinking, and regular exercise were included in the relevant Cox model as covariates.

^cCVD-related comorbidities were defined as the presence of diabetes, chronic kidney disease, and/or peripheral artery disease. For the subgroup analysis of the status of CVD-related comorbidities, age, sex, systolic blood pressure, use of antihypertensive agents, serum high-density lipoprotein cholesterol, use of lipid-modifying agents, body mass index, electrocardiogram abnormalities, current smoking, current drinking, and regular exercise were included in the relevant Cox model as covariates.

^dFor the risk assessment status were classified according to the guideline from Japan Atherosclerosis Society. For the subgroup analysis of the risk assessment status, use of antihypertensive agents, use of lipid-modifying agents, body mass index, electrocardiogram abnormalities, current drinking, and regular exercise were included as covariates.

**Fig. 2.** Multivariable-adjusted hazard ratios for the development of coronary heart disease according to serum small dense low-density lipoprotein cholesterol and the risk assessment status

Abbreviations: sdLDL, small dense low-density lipoprotein.

* $p < 0.05$ vs. reference.

Adjusted for use of antihypertensive agents, use of lipid-modifying agents, body mass index, ECG abnormalities, current drinking, and regular exercise.

the level of statistical significance in subjects with lower levels of serum LDL and HDL cholesterol (**Supplementary Fig. 4**).

Discussion

The present study clearly demonstrated that a higher serum sdLDL cholesterol level was associated with increased risk for the development of CHD not only among the participants overall but also among subjects in the high-risk category for CVD, which was defined as recommended in the guidelines of the Japan Atherosclerosis Society. Subjects with higher cardiovascular risk and a higher serum sdLDL cholesterol level had an additively greater risk of CHD events than those with low/moderate cardiovascular risk and a lower serum sdLDL cholesterol level. Moreover, it is noteworthy that the excess risk of a higher serum sdLDL cholesterol level on CHD events was observed among subjects in the high-risk category for CVD regardless of the serum levels of other lipid parameters. These findings provide evidence that the measurement of serum sdLDL cholesterol is clinically valuable for estimating the future onset of CHD beyond the predefined cardiovascular risks.

There have been a few studies addressing the association between serum sdLDL cholesterol and CHD risk in the high-risk population for CVD. In the Multi-Ethnic Study of Atherosclerosis (MESA), a higher serum sdLDL cholesterol level was associated with the development of CHD among overall study participants, but the association was not statistically significant among subjects with type 2 diabetes¹¹. Our result was inconsistent with this finding. The reasons for this discrepancy are not entirely clear, but it may be related to the accuracy of the follow-up data or statistical power of the MESA study, as the MESA study failed to reveal a significant association between serum LDL cholesterol and CHD, even though serum LDL cholesterol is an established risk factor for CHD in patients with diabetes¹⁹.

The strengths of the present study were its longitudinal population-based design, almost perfect follow-up of subjects, and accuracy in the diagnosis of CHD on the basis of medical records and autopsy findings. However, some potential limitations of this study should be noted. First, we were able to measure the serum sdLDL cholesterol level and other risk factors only once at baseline. We were also unable to obtain information about medical treatments during the follow-up period. In addition, information on the family history of early onset of CHD was not available, likely leading to an underestimation of the absolute risk based on the Suita score. This limitation

could lead to misclassification of these variables, which in turn would weaken the association found in the current study, biasing the results toward the null hypothesis. Second, we could not conduct the subgroup analyses among subjects with or without chronic kidney disease or peripheral artery disease, because of the limited number of subjects with either disease. Third, we measured serum sdLDL cholesterol levels using frozen serum samples stored at -80°C for seven years, while serum sdLDL cholesterol levels were unlikely to be affected by the frozen storage²⁰. Finally, since these analyses were conducted only in one cohort of Japanese, the generalizability of our findings might be limited. Therefore, our findings should be validated in other cohorts of various ethnic populations.

Conclusions

Elevated sdLDL cholesterol level was associated with the development of CHD among subjects at high-risk category as estimated according to the guidelines of the Japan Atherosclerosis Society. High-risk approaches for preventing CHD using serum sdLDL cholesterol measurement adhering with the existing guideline are likely to provide additional information against the burden of CHD in Japan. Further investigations are required to clarify whether serum sdLDL cholesterol would be an interventional target for reducing the risk of CHD events among subjects at high cardiovascular risk.

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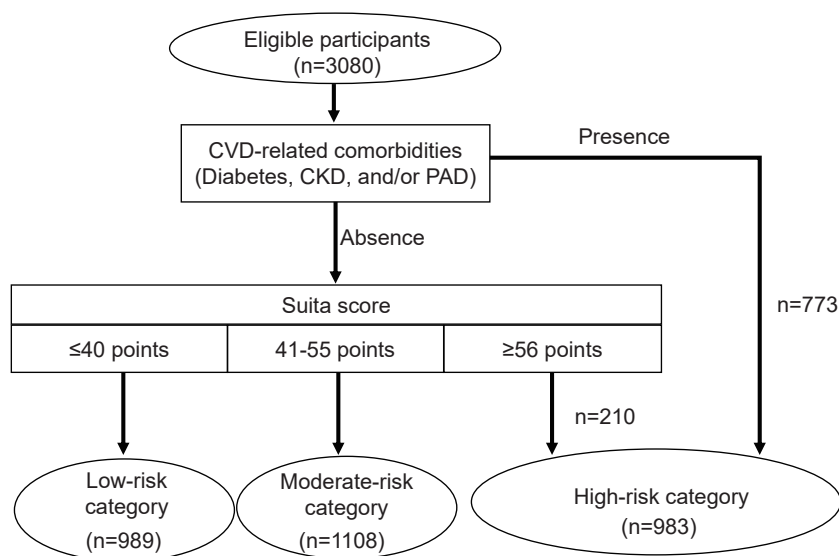
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Conflict of Interest

Toshiharu Ninomiya received research funding from DENKA SEIKEN Co., Ltd. The other authors declare that they have no conflicts of interest.

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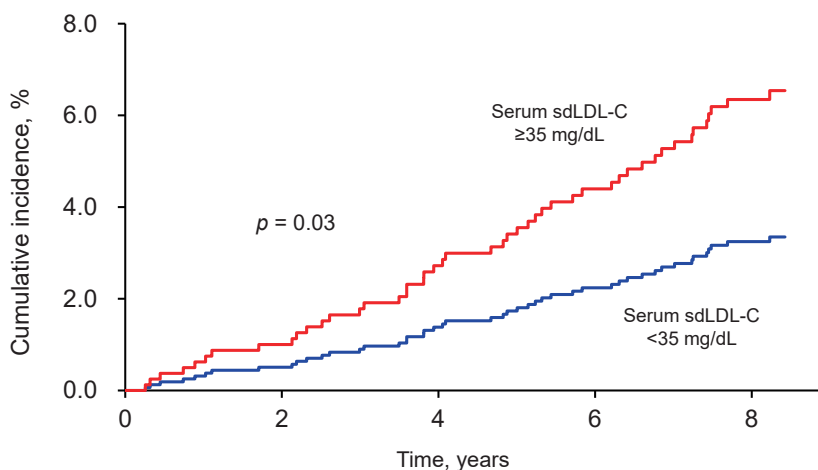
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Supplementary Fig. 1. Flowchart of the risk assessment according to the guidelines from the Japan Atherosclerosis Society

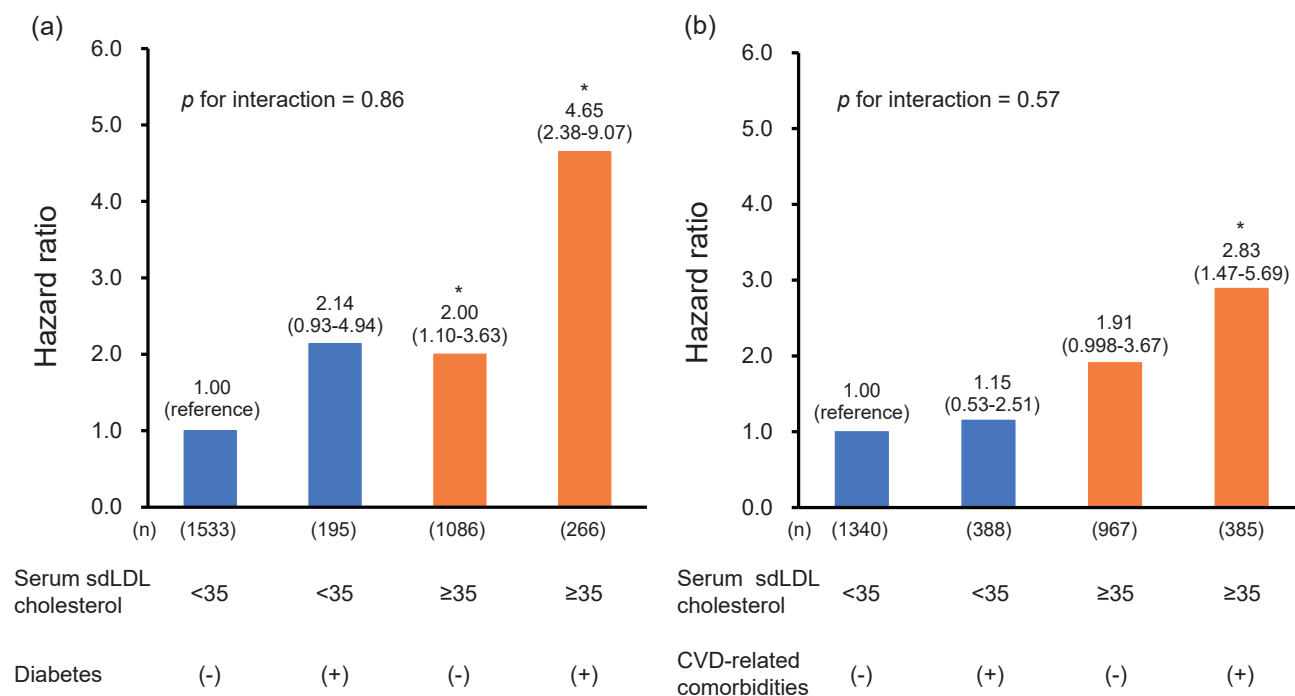
Abbreviations: CKD, chronic kidney disease; CVD, cardiovascular disease; PAD, peripheral artery disease.

Suita score was calculated on the basis of the following risk factors at baseline for each participant: age, sex, smoking habits, blood pressure level, serum high-density lipoprotein cholesterol, serum low-density lipoprotein cholesterol, and prediabetes among subjects without CVD-related comorbidities. Eligible participants ($n=3,080$) were selected from 3,384 study population, after excluding 8 subjects who did not consent to participate in the study, 223 subjects who had past history of CVD, and 73 subjects for whom serum sdLDL cholesterol data were lacking.



Supplementary Fig. 2. Age- and sex-adjusted cumulative incidence of coronary heart disease according to serum small dense low-density lipoprotein cholesterol among subjects in the high-risk category ($n=983$)

Abbreviations: sdLDL-C, small dense low-density lipoprotein cholesterol.



Supplementary Fig. 3. Multivariable-adjusted hazard ratios for the development of coronary heart disease according to serum small dense low-density lipoprotein cholesterol and presence or absence of (a) diabetes or (b) CVD-related comorbidities

Abbreviations: CVD, cardiovascular disease; sdLDL, small dense low-density lipoprotein.

**p* < 0.05 vs. reference.

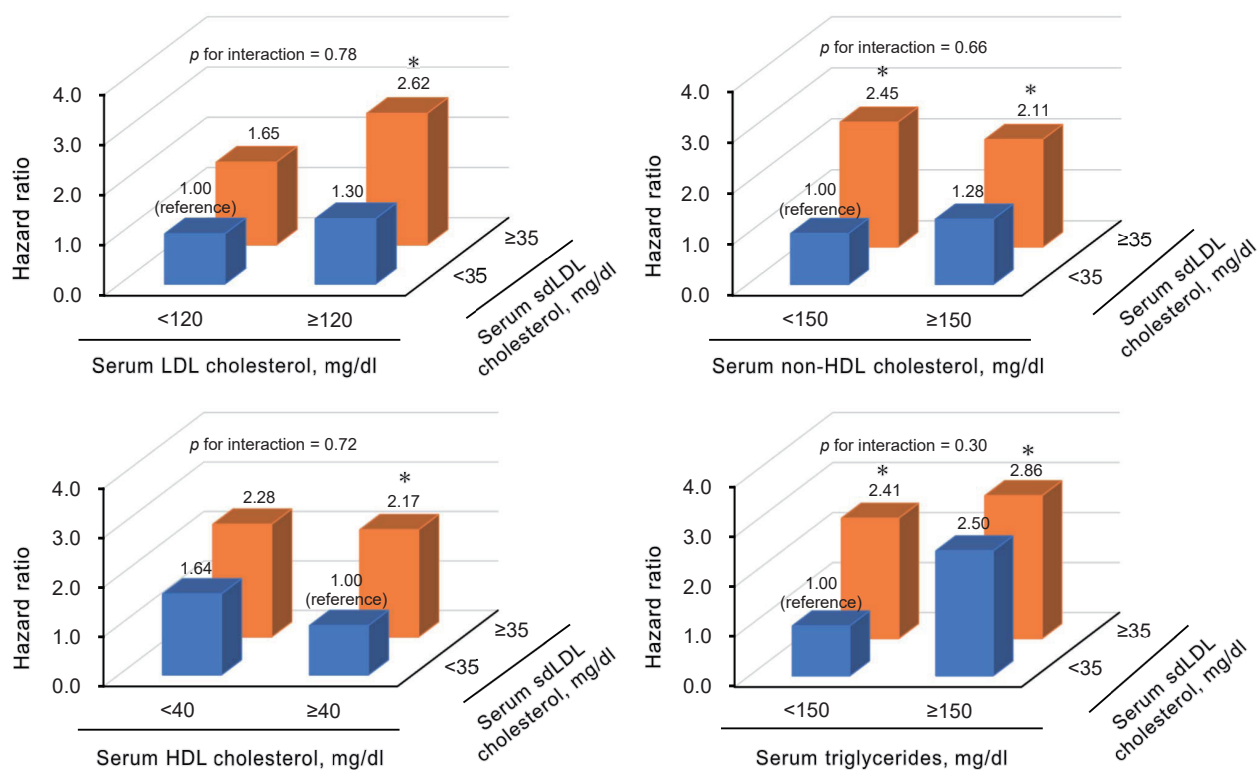
Adjusted for age, sex, systolic blood pressure, use of antihypertensive agents, high-density lipoprotein cholesterol, use of lipid-modifying agents, body mass index, estimated glomerular filtration rate, electrocardiogram abnormalities, current smoking, current drinking, and regular exercise. In the analysis for (b), the estimated glomerular filtration rate was excluded from the adjusted factors.

Supplementary Table 1. Correlation between serum small dense low-density lipoprotein cholesterol level and other lipid parameters among subjects in the high-risk category

Parameter	<i>n</i>	Spearman's rank correlation coefficient	<i>p</i> value
Serum LDL cholesterol	983	0.65	<0.001
Serum non-HDL cholesterol	983	0.76	<0.001
Serum HDL cholesterol	983	-0.30	<0.001
Serum triglycerides	974 ^a	0.62	<0.001

Abbreviations: HDL, high-density lipoprotein; LDL, low-density lipoprotein.

^aOnly subjects with available fasting blood samples were included in the analysis using serum triglycerides



Supplementary Fig. 4. Multivariable-adjusted hazard ratios for the development of coronary heart disease according to serum sdLDL cholesterol and other lipid parameters among subjects in the high-risk category ($n=983$)

Abbreviations: HDL, high-density lipoprotein; LDL, low-density lipoprotein; sdLDL, small dense low-density lipoprotein.

* $p < 0.05$ vs. reference.

Hazard ratios were adjusted for age, sex, systolic blood pressure, antihypertensive drugs, hemoglobin A1c, antidiabetic medication, HDL cholesterol, lipidlowering drugs, body mass index, estimated glomerular filtration rate, electrocardiogram abnormalities, current smoking, current drinking, and regular exercise.

In the analysis of subgroups of serum HDL cholesterol, serum HDL cholesterol was excluded from the adjusted factors.

In the analysis of serum triglycerides, only subjects with available fasting blood samples were included in the analysis using serum triglyceride ($n=974$).