

# Diabetes mellitus, but not small dense low-density lipoprotein, is predictive of cardiovascular disease: A Korean community-based prospective cohort study

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## ABSTRACT

**Aims/Introduction:** Small dense low-density lipoprotein (sdLDL) has been suggested to be a potential risk factor for cardiovascular diseases (CVD).

**Materials and Methods:** We carried out a prospective nested case–control study in the Korean Health and Genome Study. Participants were men and women aged 40–69 years who developed CVD ( $n = 313$ ), and were matched by age and sex to controls who remained free of CVD ( $n = 313$ ) during the 8-years follow-up period (from 2001 to 2009). LDL subfractions were analyzed in frozen samples collected from the 626 participants using polyacrylamide tube gel electrophoresis.

**Results:** Patients with CVD had a significantly higher glycated hemoglobin level compared with the controls (5.72 vs 5.56). The proportion of patients with diabetes mellitus (DM) was higher in those who developed CVD during follow up (8.0% vs 1.9%). The frequency of CVD according to each tertile of LDL particle size and the number of metabolic syndrome components did not differ significantly. In the multivariate analysis, DM (odds ratio 4.244, 95% confidence interval 1.693–10.640,  $P = 0.002$ ) was the only independent predictive factor of CVD. LDL particle size was not associated with the risk for future CVD.

**Conclusions:** Small dense LDL might not be a significant predictor of CVD in this Korean community-based prospective cohort study. (*J Diabetes Invest*, doi: 10.1111/jdi.12091, 2013)

**KEY WORDS:** Cardiovascular disease, Low-density lipoprotein particle size, Small dense low-density lipoprotein

## INTRODUCTION

Cardiovascular disease (CVD) is a leading cause of death and affects the majority of adults over the age of 60 years in many countries. A critical component of lowering the burden of CVD is the identification and aggressive treatment of high-risk individuals. The Adult Treatment Panel III of the Expert Panel of the National Cholesterol Education Program<sup>1</sup> has identified a group of risk factors associated with cardiovascular disease, including elevated low-density lipoprotein (LDL) cholesterol concentrations, cigarette smoking, hypertension, reduced high-density lipoprotein (HDL) cholesterol concentrations, family history of premature coronary heart disease and older

age. Current efforts have focused on determining whether additional diagnostic criteria could improve the accuracy of CVD estimation<sup>2–5</sup>. Several studies have suggested that small dense LDL (sdLDL) is associated with an increased risk of CVD<sup>6–8</sup>. However, the use of sdLDL as a potential risk factor has been refuted by other studies reporting opposing findings<sup>9,10</sup>. To the best of our knowledge, there has been no study analyzing the effects of LDL particle size on CVD in a Korean cohort study. The aim of the present study was to investigate whether LDL particle size is associated with CVD using data from a Korean community-based prospective cohort study.

## MATERIALS AND METHODS

### Participants

We carried out a nested case–control analysis of participants of the Korean Health and Genome Study (KHGS). A detailed description of the KHGS is reported elsewhere<sup>11</sup>. In brief, the Korean government funded a large community-based epidemiological study to investigate the trends in diabetes and the associated risk factors in 2001. Participants included residents of a rural community (Ansung), 70 km south of Seoul. The baseline

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examination was carried out in 2001–2002, and biennial follow-up examinations were continued through 2010. The KHGS is still ongoing. The age range for eligibility was 40–69 years. Of the 7,192 eligible individuals in Ansong, 5,018 were surveyed (70% response rate) using a cluster sampling method. Anthropometric parameters and blood pressure were measured by standard methods. Fasting plasma glucose, lipid profiles, insulin and proteinuria were measured in a central laboratory. Social factors (smoking, exercise, number of pregnancies and history of parents with validated premature coronary heart disease) were also assessed. Current smokers were defined as those having smoked at least one cigarette per day for at least 1 year. Metabolic Syndrome (MetS) was defined based on the National Cholesterol Education Program Adult Treatment Panel III criteria for Asian-Americans<sup>1</sup> recommended by the Korean Diabetes Association in patients presenting with at least three of the following components: (i) waist circumferences 90 cm or greater in men or 80 cm or greater in women; (ii) triglycerides 150 mg/dL or greater; (iii) HDL cholesterol less than 40 mg/dL in men or less than 50 mg/dL in women; (iv) blood pressure 130/85 mmHg or greater, or current use of antihypertensive medications; or (v) fasting plasma glucose 100 mg/dL or greater, or previously diagnosed type 2 diabetes or on oral antidiabetic agents or insulin. Coronary heart disease (CHD) was defined as definite myocardial infarction confirmed by electrocardiogram and/or enzyme changes or any angina diagnosis that went on to intervention after confirmation of coronary artery stenosis by coronary angiography. CVD included both CHD and stroke events. Complete data from the baseline investigation and frozen samples for further analysis were available for 1,371 participants who registered for the cohort study in the first year (2001). In order to select participants free from CVD at baseline, 366 men and women were excluded as a result of the presence of: (i) previous CHD or stroke; or (ii) Q or QS complexes, or left bundle branch block (Minnesota codes 1.1–1.3 or 7.1, respectively) in the baseline electrocardiogram. Among 1005 participants without a previous history of CVD at baseline, 313 developed CVD events during the 8-years follow-up period (from 2001 to 2009). The CVD group comprised all those who developed CVD during the follow-up period. A single control was matched to each case by the same sex and age using a statistical matching tool. Informed written consent from participants was obtained. The institutional review board at Samsung Medical Center approved the present study protocol.

### Laboratory Measurements

Samples were frozen at  $-70^{\circ}\text{C}$  and never thawed until they were moved to the Department of Laboratory Medicine and Genetics, Samsung Medical Center for analysis. The LDL subfraction was analyzed using polyacrylamide tube gel electrophoresis (Lipoprint™ LDL System; Quantimetrix, Redondo Beach, CA, USA)<sup>7</sup> of the sample. The samples were then categorized as either phenotype A or B based on the mean LDL particle

size. LDL subtypes 1–2 were predominantly large, buoyant LDLs; subtypes 3–7 were predominantly small dense LDLs. The mean LDL particle size for “phenotype A” was greater than 26.5 nm (265 Å), hence it was considered “large, buoyant LDL dominant”, whereas the mean particle size for “phenotype B” was less than 26.5 nm, and was therefore considered, “small, dense LDL dominant”. The proportion of total LDL comprised by sdLDL (subtypes 3–7) percentage was computed as follows:

$$\begin{aligned} \text{sdLDL (\%)} &= \frac{(\text{LDL3} + \text{LDL4} + \text{LDL5} + \text{LDL6} + \text{LDL7})}{(\text{LDL1} + \text{LDL2} + \text{LDL3} + \text{LDL4} + \text{LDL5} + \text{LDL6} + \text{LDL7})} \\ &\times 100. \end{aligned}$$

### Statistical Analysis

Statistical analyses were carried out using PASW Statistics 18.0 for Windows (SPSS Inc., Chicago, IL, USA). Descriptive statistics for continuous data are expressed as the mean  $\pm$  standard deviation if normally distributed, and the median (interquartile range [IQR]) if not. Discrete data was summarized as numbers with percentages. A paired *t*-test or the Wilcoxon signed-rank test was used to compare differences in continuous variables between the two groups. Differences in discrete variables between the two groups were analyzed using the Bhappkar test<sup>12</sup>. The asymptomatic marginal-homogeneity test was used to assess the distribution of CVD events in relation to each tertile of LDL particle size, and the number of MetS components. Conditional logistic regression analysis was undertaken to derive a model of factors associated with CVD. For all statistical analyses, a two-sided *P*-value  $<0.05$  was considered statistically significant.

### RESULTS

The clinical and metabolic characteristics of CVD cases and controls are summarized in Table 1. Because of the sex- and age-matched study design, the male/female ratio and mean age were identical. Patients with CVD had a significantly higher glycated hemoglobin (HbA<sub>1c</sub>) level compared with the controls (5.72 vs 5.56). The proportion of patients with diabetes mellitus (DM) was higher in those who developed CVD during follow up (8.0% vs 1.9%). Body mass index (BMI), waist circumference, blood pressure (systolic and diastolic), lipid profiles, LDL/HDL ratio, fasting plasma glucose, serum insulin, CRP and number of pregnancies did not differ significantly between the two groups. Differences in mean-LDL particle size, percent sdLDL of total LDL and the proportion of patients with sdLDL (phenotype B) or very sdLDL (LDL particle size  $<25.5$  nm) between the two groups did not reach the statistical significance. The prevalence of metabolic syndrome, being a current smoker, proteinuria, having a parent with validated premature CHD and persons engaging in at least 2–3 days/week of moderate to vigorous exercise did not differ significantly between

**Table 1** | Baseline characteristics of participants with or without cardiovascular disease

<i>n</i> = 626	Control group ( <i>n</i> = 313)	CVD group ( <i>n</i> = 313)	<i>P</i> -value
Age	56.64 ± 8.42	56.65 ± 8.55	NS
Sex (male/female)	136/177	136/177	NS
BMI (kg/m <sup>2</sup> )	23.83 ± 3.23	24.05 ± 3.35	NS
Waist circumference (cm)	84.66 ± 9.18	84.96 ± 8.62	NS
SBP (mmHg)	121.99 ± 15.94	124.42 ± 16.20	NS
DBP (mmHg)	75.00 ± 10.39	75.76 ± 10.05	NS
Total cholesterol (mg/dL)	179.85 ± 33.72	179.79 ± 31.54	NS
HDL (mg/dL)	45.45 ± 10.39	44.47 ± 10.03	NS
Triglyceride (mg/dL)	154.53 ± 81.32	160.83 ± 86.52	NS
LDL (mg/dL)	105.40 ± 31.31	105.56 ± 30.63	NS
LDL/HDL ratio	2.43 ± 0.86	2.49 ± 0.90	NS
LDL size (nm)	26.63 ± 0.59	26.61 ± 0.58	NS
sdLDL %	13.44 ± 14.13	13.72 ± 13.80	NS
Fasting glucose (mg/dL)	82.48 ± 12.87	84.80 ± 19.93	NS
HbA <sub>1c</sub> (%)	5.56 ± 0.75	5.72 ± 1.04	NS
Insulin (μIU/mL)	7.76 ± 8.53	8.23 ± 9.60	0.034
CRP (mg/dL)	0.18 ± 0.31	0.19 ± 0.26	NS
Pregnancy ( <i>n</i> )	2.89 ± 3.16	3.01 ± 3.19	NS
Diabetic patient	6 (1.9%)	25 (8.0%)	NS
MetS patient	94 (30.3%)	105 (33.5%)	0.002
Current smoker	82 (26.2%)	90 (28.7%)	NS
Proteinuria	5 (1.6%)	5 (1.6%)	NS
Exercise (≥2–3 days/week)	41 (13.1%)	39 (12.5%)	NS
Family history of premature CHD	36 (11.5%)	50 (16.0%)	NS
Phenotype B (sdLDL)	93 (29.7%)	91 (29.4%)	NS
Very sdLDL	13 (4.2%)	18 (5.8%)	NS

Data are means ± standard deviation except for the frequency data. BMI, body mass index; CHD, coronary heart disease; CRP, C-reactive protein; CVD, cardiovascular disease; DBP, diastolic blood pressure; HbA<sub>1c</sub>, glycated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MetS, metabolic syndrome; NS, not significant; SBP, systolic blood pressure; sdLDL, small dense low-density lipoprotein; very sdLDL, low-density lipoprotein particle size <25.5 nm.

**Table 2** | Small dense low-density lipoprotein parameters with increasing number of metabolic syndrome components

<i>n</i> = 626	Metabolic syndrome components			<i>P</i> -value
	0 ( <i>n</i> = 104)	1–2 ( <i>n</i> = 323)	≥3 ( <i>n</i> = 199)	
LDL size	26.92 ± 0.36	26.72 ± 0.52	26.30 ± 0.63	<0.001
sdLDL %	6.81 ± 6.98	11.10 ± 12.09	21.14 ± 16.12	<0.001
Phenotype B	8 (7.7%)	70 (21.7%)	46 (50.3%)	<0.001

Data are summarized as numbers with percentages. LDL, low-density lipoprotein; sdLDL, small dense low-density lipoprotein.

the groups. LDL particle size parameters (i.e., LDL size, sdLDL percentage and proportion of phenotype B) showed a significant correlation with an increasing number of MetS components (Table 2). However, the frequency of CVD according to

each tertile of the number of MetS components did not differ significantly (Table 3b). The risk of CVD was not inversely correlated with LDL particle size (Table 3). In addition, the rate of CVD events in participants with both MetS and sdLDL (phenotype B) was not significantly different from those of the others (with either MetS or sdLDL or without both, *P* = 0.828; data not shown). Multiple stepwise regression analyses of CVD and other risk factors were carried out as described in Table 4. DM (odds ratio [OR] 4.244, 95% confidence interval [CI]

**Table 3** | Frequency of cardiovascular disease according to the components of metabolic syndrome, and each tertile of low-density lipoprotein size

MetS components	Metabolic syndrome components				<i>P</i> -value
	0	1–2	≥3		
<b>(a)</b>					
Control group ( <i>n</i> = 313)	59 (56.7%)	160 (49.5%)	94 (47.2%)	NS	
CVD group ( <i>n</i> = 313)	45 (43.3%)	163 (50.5%)	105 (52.8%)		
Total	104	323	199		
<b>(b)</b>					
LDL size	LDL size (nm)				<i>P</i> -value
	<25.5	25.5–26.4	26.5–26.9	≥27.0	
Control group ( <i>n</i> = 313)	13 (41.9%)	80 (50.5%)	127 (48.8%)	93 (51.1%)	NS
CVD group ( <i>n</i> = 313)	18 (58.1%)	73 (49.5%)	133 (51.2%)	89 (48.9%)	
Total	31	153	260	182	

Data are summarized as numbers with percentages. CVD, cardiovascular disease; LDL, low-density lipoprotein; MetS, metabolic syndrome; NS, not significant.

**Table 4** | Logistic regression analysis of baseline clinical and laboratory characteristics with cardiovascular disease events

<i>n</i> = 626	OR	95% CI	<i>P</i> -value
BMI	1.015	0.964–1.068	NS
SBP	1.014	0.998–1.030	NS
DBP	0.987	0.963–1.012	NS
LDL	1.000	0.994–1.005	NS
Diabetes mellitus	4.244	1.693–10.640	0.002
Current smoker	1.237	0.857–1.786	NS
Family history of premature CHD	1.525	0.956–2.433	NS
LDL size			
≥ 27.0 nm	1		
26.5–26.9 nm	1.083	0.734–1.599	NS
<26.5 nm	1.070	0.698–1.642	NS

BMI, body mass index; CHD, coronary heart disease; CI, confidence interval; DBP, diastolic blood pressure; LDL, low-density lipoprotein; NS, not significant; SBP, systolic blood pressure.

1.693–10.640,  $P = 0.002$ ) was the only independent predictive factor of CVD in the present case-control study. Subgroup analysis for patients without diabetes ( $n = 595$ ) or with CHD ( $n = 594$ ) are also shown in Tables S1 and S2, respectively. LDL size was not associated with the risk for future CVD in any of the analysis.

## DISCUSSION

The results suggest individuals with DM were at greater risk of CVD than those without. In the multivariate analysis, DM was the independent predictive factor of CVD. However, we could not verify an association between LDL particle size and CVD. We found a prevalence of sdLDL of 29.4%, which is similar to previously reported values<sup>13,14</sup>. The overall prevalence rate of CVD in KHGS during the 8-years follow-up period was 18.7%. This number is higher than the rate in the previous Korean studies<sup>15,16</sup>. This discrepancy might be as a result of older age, longer follow-up duration and the rural community of the present study. It is well-known that individuals with diabetes have a two- to fourfold increased risk of CHD compared with non-diabetic individuals<sup>17,18</sup>. Fasting plasma glucose and HbA<sub>1c</sub> were higher in the CVD group, and the differences in HbA<sub>1c</sub> were statistically significant. Furthermore, diabetes was the only independent predictive factor of CVD in the present study. There is evidence that patients with diabetes with no history of CHD had the same risk of myocardial infarction (MI) as that observed in non-diabetic subjects with a history of MI<sup>19</sup>. This similar level of risk of diabetes and previous CHD has led to the suggestion that individuals with diabetes should be treated as CHD-risk equivalents<sup>20</sup>. The present results fit well with these previous results.

Low-density lipoprotein size did not differ between the two groups. Our attempt to divide the participants into four groups according to mean LDL particle size levels in order to analyze the correlations with CVD did not yield any significant results (Table 3b). Contrary to previous studies<sup>6–8</sup>, LDL particle size was not an independent risk factor for CVD in the present study. Our attempt to analyze the correlations of CVD with mean LDL particle size, sdLDL (phenotype B) and percentage of sdLDL to total LDL did not show any significant risk factor (data not shown). Furthermore, multivariate analysis with very sdLDL also failed to show any significance (OR 1.285, 95% CI 0.604–2.734,  $P = 0.515$ ; data not shown). Subgroup analysis for patients without diabetes or with CHD also failed to show a significant correlation with sdLDL or LDL size (Tables S1 and S2, respectively). A recent review by Ip *et al.*<sup>10</sup> found that LDL particle size and small LDL particle fraction were not consistently associated with CVD incidence. Furthermore, none of the studies reported adequate analyses to determine the relative or incremental value of LDL subfraction measurement as a predictor of CVD compared with traditional risk factors. Ip *et al.*<sup>10</sup> also noted that the clinical value of treatment based on the results of LDL subfraction testing is lacking. Another review by Gazi *et al.*<sup>9</sup> also found no definite causal relationship between sdLDL and CVD,

probably because of the close association between sdLDL and triglyceride (TG) levels, and other risk factors. These disagreements could also be attributed to differences in age, ethnicity, sex and geographical distribution among the study populations. In addition, the method of measuring sdLDL was different in the previous studies<sup>6,8</sup>.

MetS is known to increase the risk of CVD<sup>21</sup>. The percentage of participants with MetS was not higher in the CVD group. In addition, tertile division of participants according to the number of components of MetS did not show a significant association with CVD (Table 3a). However, in the multivariate analysis, the number of MetS components was associated with the risk for CVD (OR 1.240, 95% CI 1.056–1.457,  $P = 0.009$ ; data not shown). Compared with those without MetS, participants with MetS had a significantly smaller mean LDL particle size (26.30 nm vs 26.77 nm), higher percentage of sdLDL to total LDL (21.14% vs 10.06%) and more phenotype B (50.3% vs 18.3%; data not shown). In the present study, LDL particle size was significantly correlated with each component of MetS, as shown by others<sup>22</sup> (data not shown). LDL particle size decreased, whereas the percentage of sdLDL to total LDL and proportion of phenotype B increased as the number of MetS components escalated (Table 2). This might offer proof for validity of our sdLDL assay, even if MetS failed as an independent risk factor for CVD. Yet, DM is one of the components, and its predictive power was more significant than MetS. The influence of other components of MetS (such as waist circumference, TG, HDL and blood pressure) might have been obscured by the significantly larger (4.24-fold) impact of DM on CVD in the multivariable analysis. Furthermore, DM itself is a complex metabolic disorder with a preponderance of sdLDL particles<sup>23</sup>.

Other known risk factors of CVD, such as smoking<sup>24,25</sup> and family history of premature CHD<sup>26–28</sup>, were not significant enough to predict CVD in the present study. However, we should not ignore the benefits of smoking cessation<sup>29</sup> and incorporating family history of premature CHD into the risk estimation process that guides treatment decisions<sup>1</sup>. A larger prospective study focusing on these associations is required.

The limitations of the present study should be considered when interpreting the results. First, analyses were carried out using samples that had been kept at  $-70^{\circ}\text{C}$  for several years; therefore, we cannot exclude some degree of protein and/or membrane degradation. However, we evaluated the LDL subfraction in all of the patients who developed CVD during follow up. Second, data on the use of lipid-lowering agents (statins, fibrate, nicotinic acid or ezetimibe) are lacking.

We conclude that sdLDLs are not significant predictors of CVD in the present Korean community-based prospective study. In this case-control study, DM was the only independent predictor of CVD.

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## SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

**Table S1** | Logistic regression analysis of patients without diabetes with cardiovascular disease events.

**Table S2** | Logistic regression analysis of baseline clinical and laboratory characteristics with coronary heart disease events.