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Relationship between carotid artery intima-media thickness and small dense low-density lipoprotein cholesterol concentrations measured by homogenous assay in Japanese subjects

Hideki Yagi [a], Hiroyuki Sumino [a], Tomoyuki Aoki [a], Katsuhiko Tsunekawa [a], Osamu Araki [a], Takao Kimura [a], Makoto Nara [a], Takayuki Ogiwara [a], Katsuyuki Nakajima [b], Masami Murakami [a,*]

[a] *Department of Clinical Laboratory Medicine, Gunma University Graduate School of Medicine, 3-39-15 Showa-machi, Maebashi, Gunma 371-8511, Japan*
[b] *Department of Laboratory Sciences, Gunma University Graduate School of Health Sciences, Maebashi, 3-39-22 Showa-machi, Maebashi, Gunma 371-8511, Japan*

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ABSTRACT

Background: Small dense low-density lipoprotein cholesterol (sdlDL-C) concentrations correlate more strongly with coronary heart disease than other LDL-C and large LDL particle concentrations. We investigated the association between carotid artery intima-media thickness (IMT) and sdlDL-C concentrations in Japanese subjects.
Methods: Carotid artery IMT, blood pressure (BP), fasting plasma sdlDL-C, glucose metabolism, lipid, and C-reactive protein concentrations were measured in 97 native Japanese subjects. Carotid artery IMT was assessed by ultrasonography, and sdlDL-C concentrations were measured by a homogenous assay. Pearson's correlation coefficient analyses and multiple regression analyses were used to examine the relationships between carotid artery IMT values, sdlDL-C values, and other clinical variables.
Results: After multiple regression analysis, including age, sex, body mass index, systolic BP, diastolic BP, fasting plasma glucose, HbA1c, estimated glomerular filtration rate (eGFR), total-C, high-density lipoprotein (HDL)-C, triglyceride, LDL-C, non-HDL-C, large buoyant LDL-C, and sdlDL-C, carotid artery IMT remained significantly associated with age, systolic BP, diastolic BP, and sdlDL-C, whereas sdlDL-C remained significantly associated with age, total-C, HDL-C, triglycerides, and carotid artery IMT.
Conclusions: When measured by a homogenous assay, carotid artery IMT may have a closer relationship with sdlDL-C concentrations than other lipid parameters in Japanese subjects. sdlDL-C may be a potentially useful risk marker when assessing carotid artery IMT in Japanese subjects.

1. Introduction

Low-density lipoprotein (LDL) particles are heterogenous with respect to their size, density, and lipid composition. Compared with large buoyant LDL, small dense LDL cholesterol (sdlDL-C) is thought to be more atherogenic [1]. Indeed, sdlDL-C concentrations have shown to correlate more strongly with coronary heart disease (CHD) than either LDL or large LDL cholesterol concentrations [2–6]. Thus, the selective measurement of sdlDL-C concentrations is useful for evaluating the actual atherogenic risk of individuals.

The traditional methods to assess LDL size and density and obtain sdlDL-C concentrations are ultracentrifugation [7] and gradient gel electrophoresis (GGE) [8], but these are unsuitable for routine analysis because they require expensive equipment, complicated techniques, and long assay times. Additionally, nuclear magnetic resonance (NMR) imaging can simultaneously determine the size and number of LDL particles [9], whereas high performance liquid chromatography (HPLC) enables the determination of lipid concentrations in various lipoprotein subfractions [10]. However, HPLC is too laborious, time-consuming, and expensive for routine clinical use, and the instrumentation required for NMR is also too expensive for general clinical laboratories to acquire feasibly. Although a simple precipitation method was developed to enable the quantification of sdlDL-C using heparin-magnesium precipitation assays [11,12], it still required offline sample pretreatment that hindered its smooth integration into general clinical use.

Recently, a novel, fully automated homogenous assay for measuring sdlDL-C has been developed [13]. Using this new assay, sdlDL-C values showed excellent agreement with those obtained by isolation of the d = 1.044–1.063 g/ml plasma fraction by sequential ultracentrifugation [14]. This homogenous method for measuring plasma sdlDL-C concentrations is also rapid, taking just 10 min. This precise and rapid method allows the routine examination of large number of samples.

An increased carotid artery intima-media thickness (IMT) measured noninvasively by B-mode ultrasonography is considered a good

Abbreviations: BP, blood pressure; GGE, gradient gel electrophoresis; IMT, intima-media thickness

* Corresponding author. Tel.: +81 27 220 8550; fax: +81 27 220 8583.
E-mail address: mmurakam@gunma-u.ac.jp (M. Murakami).

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surrogate marker for early atherosclerosis. It is reportedly associated with cardiovascular risk [15] and the severity of coronary atherosclerosis [16], and can predict cardiovascular events, such as myocardial infarction and stroke, in several populations [17]. Several studies have reported an association between carotid artery IMT and LDL particle size distribution determined by GGE [18–20], LDL particle distribution measured by NMR [21], and sdlDL-C quantification measured by simple precipitation methods [22]. However, only one study using the homogenous assay in Japanese Americans has demonstrated that sdlDL-C concentrations were positively correlated with carotid artery IMT [23]. Japanese Americans, who share an identical genetic history with their Japanese progenitors, have typically lived with westernized lifestyle for decades, consuming diets high in fat and simple carbohydrates [24].

Carotid artery IMT is greater in Japanese Americans than in native Japanese [25], which means that the association between carotid artery IMT and sdlDL-C measured by homogenous assay remains unknown for native Japanese populations. Therefore, we investigated the association between carotid artery IMT and sdlDL-C concentrations in native Japanese subjects using the homogenous assay to measure carotid artery IMT and plasma sdlDL-C concentrations.

2. Materials and methods

2.1. Subjects

One hundred ninety-five consecutive native Japanese subjects who visited our department for assessment of cardiovascular disease or risk participated in this study. All were required to have lived in Japan since birth. A history was taken from all subjects. We excluded subjects with the following conditions that could have serious influence on carotid artery IMT, blood pressure (BP), circulating lipid, and glucose metabolism: those with a history of cardiovascular disease, including stroke, CHD, thromboembolic disease, or congestive heart failure; peripheral arterial disease; malignancy; infectious disease; liver or renal disease; overt endocrine disease; current smokers; and those currently on medication. However, subjects with hypertension, dyslipidemia, and diabetes mellitus, as defined by the diagnostic criteria [26–28], were included. Written informed consent was obtained from each subject, and the study was approved by the Institutional Review Board of Gunma University Hospital.

We performed physical examinations and carotid artery IMT examinations in the morning (after a 12 h fast) and obtained blood samples from the antecubital vein for serum and plasma analyses at the same time.

2.2. Physical examination

The height and weight of subjects were measured and the body mass index (BMI) was calculated (weight in kilograms divided by height in meters squared). BP was measured in the morning (after a 12 h overnight fast) by the same investigator with a sphygmomanometer on the right arm of the subject after a 10-min rest in the supine position.

2.3. Measurement of the carotid artery IMT

The wall thickness of the carotid arteries was evaluated bilaterally by ultrasonography (LOGIQ 9, GE Healthcare Japan Corp.) using a 7.5-MHz linear type B-mode probe [29,30]. After the subject had rested for at least 10 min in the supine position with the neck in slight hyperextension, we evaluated the optimal visualization of the common carotid arteries, carotid bulb, and extracranial internal and external carotid arteries bilaterally. IMT was assessed as the greatest IMT at any location in the far walls of these carotid arteries, including atheromatous plaques on both sides. The max IMT was defined as the greater of the 2 unilateral IMT values. A physician who was blinded to the clinical characteristics of subjects evaluated all the scans. The variability of the ultrasonographic measurements was assessed by performing five measurements over a 1-month period in 12 volunteers. The intra-observer coefficient of variation for the IMT measurement was 5.5% ± 0.8%.

2.4. Laboratory analyses

Serum total cholesterol, triglyceride, high-density lipoprotein cholesterol (HDL-C), and LDL-C concentrations were measured by enzymatic methods and C-reactive protein (CRP) concentrations were measured by latex immunoassay, respectively, using an automatic analyzer (Labospect 008; Hitachi). Plasma sdlDL-C concentrations were measured using a newly developed homogenous assay [13,31] in an automatic analyzer (Hitachi-7700; Hitachi, Tokyo, Japan). Serum insulin concentrations were measured by chemiluminescence immunoassay using an automatic analyzer (AIA-2000 LA; Tosoh, Tokyo, Japan). Plasma glucose concentrations were measured by hexokinase method and hemoglobin A1c (HbA1c) concentrations were measured by HPLC, using automatic analyzers (ADAMS Glucose GA-1170 and ADAMS A1c HA-8180, respectively; Arkray).

2.5. Statistical analysis

Data were expressed as mean ± SD. Pearson's correlation coefficient analyses were used to examine the relationships between carotid artery IMT values and sdlDL-C values and other clinical variables, and between sdlDL-C values and carotid artery IMT values and other clinical variables. Furthermore, multiple regression analysis was performed using carotid artery IMT values, sdlDL-C values, and those of the other clinical variables. All the probability values were 2-tailed. A $P < 0.05$ was considered statistically significant. All statistical analyses were performed using the IBM SPSS software, ver 21.0.

3. Results

3.1. Descriptive statistics

Of the 195 subjects available during the study period, 97 met the inclusion criteria. The average age was 49.3 ± 14.0 y (range, 23–77 y), and they comprised of 41 men (age: 52.3 ± 14.8 y; range, 24–77 y) and 56 women (age: 47.2 ± 13.1 y; range, 23–68 y). We included 22 subjects with essential hypertension, 45 with dyslipidemia, and 8 with type 2 diabetes mellitus. The characteristics of subjects are summarized in Table 1.

3.2. Univariate and multivariate analyses for correlations with carotid artery IMT

The results of the univariate regression analysis revealed that carotid artery IMT was positively correlated with age ($r = 0.640$, $P < 0.001$), systolic BP ($r = 0.411$, $P < 0.001$), diastolic BP ($r = 0.352$, $P < 0.001$), fasting plasma glucose ($r = 0.273$, $P = 0.007$), HbA1c ($r = 0.278$, $P = 0.006$), total cholesterol ($r = 0.428$, $P < 0.001$), triglycerides ($r = 0.245$, $P = 0.016$), LDL cholesterol ($r = 0.446$, $P < 0.001$), non-HDL cholesterol ($r = 0.474$, $P < 0.001$), large buoyant LDL cholesterol ($r = 0.343$, $P = 0.001$), and sdlDL-C ($r = 0.474$, $P < 0.001$; Fig. 1), but had no significant association with other clinical variables (Table 2). In addition, the results of the multiple regression analysis, including age, sex, systolic BP, diastolic BP, fasting plasma glucose, HbA1c, estimated glomerular filtration rate (eGFR), total cholesterol, HDL cholesterol, triglycerides, LDL cholesterol, non-HDL cholesterol, large buoyant LDL cholesterol, and sdlDL-C revealed that dependent carotid artery IMT determinants remained significantly associated with age, systolic BP, diastolic BP, and sdlDL-C (Table 3).

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