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Lipoprotein-associated phospholipase A_2 is related to risk of subclinical atherosclerosis but is not supported by Mendelian randomization analysis in a general Japanese population

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ABSTRACT

Objective: Lipoprotein-associated phospholipase A_2 (Lp-PLA_2) is an enzyme predominantly bound to low-density lipoprotein (LDL). Lp-PLA_2 is recognized as playing a key role in inflammatory processes and the development of atherosclerosis. This study aimed to investigate whether Lp-PLA_2 is related to subclinical atherosclerosis, independently from traditional risk factors, in a general Japanese population by analyses of both the observational study and Mendelian randomization using V279F polymorphism.
Methods and results: We cross-sectionally examined community-based sample of 929 Japanese men aged 40–79 years, without statin treatment, who were randomly selected from the resident registration. Multiple regression analyses of Lp-PLA_2 activity and concentration were undertaken separately for men aged 40–49 years and 50–79 years, to clarify interactions of age and Lp-PLA_2. Lp-PLA_2 activity for men aged 50–79 years was significantly and positively related to intima-media thickness (IMT) ($P = 0.013$) and plaque index ($P = 0.008$) independent of traditional risk factors including small LDL particles, but not to coronary artery calcification (CAC) score. Associations with Lp-PLA_2 concentration were qualitatively similar to those of activity. Corresponding relationships were not observed in men aged 40–49 years. Mendelian randomization analyses based on V279F genotype did not show any significant associations with subclinical atherosclerosis, although the homozygote and heterozygote of V279F showed low Lp-PLA_2 activity and concentration.
Conclusions: Lp-PLA_2 activity in Japanese men aged 50–79 years was associated significantly and positively with IMT and plaque in the carotid artery but Mendelian randomization did not support that Lp-PLA_2 is a causative factor for subclinical atherosclerosis.

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1. Introduction

Lipoprotein-associated phospholipase A_2 (Lp-PLA_2) is an enzyme that is predominantly bound to low-density lipoprotein (LDL), particularly small LDL particles [1]. Lp-PLA_2 is recognized as playing a key role in the inflammatory processes leading to plaque formation and the development of atherosclerosis [2,3]. A variant-type Lp-PLA_2 gene, V279F, cannot produce normal amounts of Lp-PLA_2 [4–6]. Some Japanese studies have reported that the proportion of homozygous variant V279F is higher among Japanese (3–4%) than in Western populations, with a heterozygote frequency of around 30% in Japanese, compared to Caucasians almost all showing the null variant type [7–9]. Therefore, based on the uniquely high proportion of V279F polymorphism in this population, analysis of samples via Mendelian randomization [10] is possible whereby they may be included in a randomized clinical trial of Lp-PLA_2 without the need to consider unknown confounding factors.

The largest meta-analysis covered 32 prospective studies [11], mainly based on Caucasian populations, reported that Lp-PLA_2 activity and concentration were positively related to risk of coronary heart disease (CHD), stroke and all vascular death after adjustment for traditional risk factors including non-high-density lipoprotein-cholesterol. However, adjustment for small LDL particles was not conducted in that study, leaving unanswered the crucial question of whether increased Lp-PLA_2 levels are associated with CHD and/or other vascular diseases independent of LDL particles [1,12]. Additionally, the recent large cohort study, the Atherosclerosis Risk in Communities (ARIC) study, reported that small dense LDL-cholesterol (LDL-c) was associated with incident CHD [13]. The present epidemiological cross-sectional study therefore investigated whether Lp-PLA_2 is related to subclinical atherosclerosis independent of small LDL particles or LDL-c and other traditional risk factors in Japanese men randomly selected from a general population. Furthermore, whether V279F polymorphism is related to subclinical atherosclerosis was examined using Mendelian randomization.

2. Participants and methods

2.1. Study population

All the recruitment and examination was conducted from 2006 through 2008. Participants were population-based samples randomly selected from among Japanese nationals living in Kusatsu City, Shiga, between 40 and 79 years old. They were originally selected for a case–control study to examine the relationship between Lp-PLA_2 and coronary stenosis and/or myocardial infarction. Of the 2994 eligible individuals selected, 1238 (1094 men) agreed to participate. Details of the study were described elsewhere [14–16]. The present study was approved by the Institutional Review Board of the Shiga University of Medical Science (No. 17–19, 17–83) and written informed consent was obtained from all participants.

A total of 165 men were excluded from this analysis for the following reasons: use of statin (n = 118), missing information (n = 30), and triglyceride level at or above 400 mg/dl (n = 17). The exclusion of statin users was introduced in order to avoid distortion of any true associations between Lp-PLA_2 and subclinical atherosclerosis. The last criterion was also used to adequately estimate LDL-c levels following Friedwald's formula. Therefore, 929 participants were finally included in the present analyses (mean [SD] age, 63.8 [10.1] years).

2.2. Carotid atherosclerosis

The protocol was previously described [14,16–18]. Using a 7.5 MHz probe (Xario-660A, Toshiba Medical Systems, Japan), detailed B-mode images of the right and left common carotid artery (CCA), common carotid bifurcation, and the internal carotid artery were obtained with a standardized method established by the Ultrasound Research Laboratory of University of Pittsburgh [19]. Images from the following segments were digitized: near and far walls of the distal CCA (1 cm proximal to the carotid bulb), far wall of the bulb, and first centimeter of the far wall of the internal carotid artery (a total of 4 locations per side). Carotid intima-media thickness (IMT) was traced with the automatic image reading program of AMS (Chalmers University of Technology, Gotenburg, Sweden). The mean of all average readings across the 8 locations comprised the average IMT. In this study, mean IMT was estimated using IMT values from the CCA, bulb and ICA. Plaque grade for each segment was defined using the sum of plaque percentages compared to the lumen as follows: 0; 1, <30%; 2, ≥30% but <50%; or 3, ≥50%. Total plaque index was then determined as the total of all plaque grades [19,20].

2.3. Coronary artery calcification (CAC)

The scanning protocol was previously described [15,21]. We assessed CAC using either electron-beam computed tomography (EBCT, n = 681) using a C-150 scanner (Imatron, South San Francisco, CA, USA) or multi-detector row computed tomography (MDCT, n = 297) using an Aquilion scanner (Toshiba, Tokyo, Japan). Images were obtained from the level of the root of the aorta through the heart at a slice thickness of 3 mm with a scan time of 100 ms (EBCT) or 320 ms (MDCT). We acquired images at 70% of the cardiac cycle, using electrocardiogram triggering, during a single breath-hold. Quantification of CAC was performed using a DICOM workstation and AccuImage software (AccuImage Diagnostics, South San Francisco, CA, USA). The presence of CAC was defined as a minimum of three contiguous pixels (area = 1 mm^2) with density >130 Hounsfield units. We calculated CAC scores according to the Agatston method [22]. All computed tomography (CT) images were read by a single physician who was trained in CT reading at the Cardiovascular Institute, University of Pittsburgh, and who was blinded to clinical information of participants. The protocol described above was adopted from a separate cohort study of ours [23], in which the reproducibility of the scans showed an intraclass correlation of 0.98 [18]. In a stratified analysis by CT-type, we found similar results between participants assessed by EBCT and those by 16-channel MDCT (data not shown). In addition, CAC assessment by EBCT and MDCT has been reported to be comparable [24].

2.4. Biochemical examinations

Venipuncture was performed early in the clinic visit after a 12-h fast. We separated serum by centrifugation (3000 revolutions per min, for 15 min) at 4 °C within 90 min of sample collection. Samples were sent for routine laboratory tests that included lipid and glucose levels. Otherwise, they were stored at −80 °C, then shipped on dry ice later for specific tests. Standard lipids, including total cholesterol and triglycerides (TG), were measured using enzymatic techniques. High-density lipoprotein (HDL) cholesterol (HDL-c) was measured after heparin-calcium precipitation. Measurements were standardized according to guidelines from the Center for Disease Control and Prevention/Cholesterol Reference Method Laboratory Network (CDC/CRMLN) [25]. Friedewald's formula was used to estimate LDL-c levels.

Lp-PLA_2 activity was measured using a colorimetric activity