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Clinical and genetic factors associated with lipoprotein-associated phospholipase A_2 in the Framingham Heart Study☆

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ABSTRACT

Objective: To conduct an investigation of clinical and genetic correlates of lipoprotein-associated phospholipase (Lp-PLA_2) activity and mass in a large community-based cohort. Higher circulating Lp-PLA_2 predicts cardiovascular disease risk, but sources of inter-individual variability are incompletely understood.
Methods: We conducted stepwise regression of clinical correlates of Lp-PLA_2 in four Framingham Heart Study cohorts ($n = 8185$; mean age 50 ± 14 years, 53.8% women, 9.8% ethnic/racial minority cohort). We also conducted heritability and linkage analyses in Offspring and Generation 3 cohorts ($n = 6945$). In Offspring cohort participants we performed association analyses ($n = 1535$ unrelated) with 1943 common tagging SNPs in 233 inflammatory candidate genes.
Results: Sixteen clinical variables explained 57% of the variability in Lp-PLA_2 activity; covariates associated with Lp-PLA_2 mass were similar but only explained 27% of the variability. Multivariable-adjusted heritability estimates for Lp-PLA_2 activity and mass were 41% and 25%, respectively. A linkage peak was observed for Lp-PLA_2 activity (chromosome 6, LOD score 2.4). None of the SNPs achieved experiment-wide statistical significance, though 12 had q values <0.50, and hence we expect at least 50% of these associations to be true positives. The strongest multivariable-association with Lp-PLA_2 activity was found for *MEF2A* (rs2033547; nominal $p = 3.20 \times 10^{-4}$); SNP rs1051931 in *PLA2G7* was nominally associated ($p = 1.26 \times 10^{-3}$). The most significant association to Lp-PLA_2 mass was in *VEGFC* (rs10520358, $p = 9.14 \times 10^{-4}$).
Conclusions: Cardiovascular risk factors and genetic variation contribute to variability in Lp-PLA_2 activity and mass. Our genetic association analyses need replication, which will be facilitated by web posting of our genetic association results.

Abbreviations: CVD, cardiovascular disease; HDL, high-density lipoprotein; LD, linkage disequilibrium; LDL, low-density lipoprotein; Lp-PLA_2, lipoprotein-associated phospholipase A_2; LOD, logarithm of the odds; SE, standard error; SNP, single nucleotide polymorphism.

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1. Introduction

Inflammation and oxidative stress contribute to atherogenesis. Circulating lipoprotein-associated phospholipase A2 (Lp-PLA_2) has been scrutinized intensively as a marker of cardiovascular disease (CVD) risk because the enzyme exhibits pro-inflammatory and oxidative activities. A key feature is the transformation of oxidized low-density lipoprotein (LDL) in the arterial wall into highly proatherogenic reactants like lysophosphatidylcholine and oxidized fatty acids.

Research implicates Lp-PLA_2 in multiple phases in the development of CVD. Circulating Lp-PLA_2 concentrations predict the presence of coronary artery disease and correlate with endothelial dysfunction and early atherosclerosis in the coronary circulation [1]. Clinical and epidemiological studies consistently demonstrate associations with incident and recurrent coronary artery disease events [2–4]. Accounting for traditional CVD risk factors, higher blood Lp-PLA_2 is associated with adverse long-term CVD outcomes [2,5,6].

Prior reports have suggested that circulating Lp-PLA_2 concentrations are related to both clinical and genetic factors [7,8], but the determinants in the community are incompletely reported. We hypothesized that Lp-PLA_2 activity and mass would be associated with CVD risk factors and genetic variation in the *PLA2G7* gene coding for Lp-PLA_2 and in other inflammatory SNPs. We report the association of Lp-PLA_2 activity and mass with clinical factors. In addition, we describe heritability, genetic linkage, and the relation of variation in LpPLA_2 activity and mass with variation in 13 common single nucleotide polymorphisms (SNPs) representing the *PLA2G7* gene, and SNPs in inflammatory candidate genes in the community-based Framingham Heart Study.

2. Materials and methods

2.1. Study sample

Plasma Lp-PLA_2 measurements were available from four Framingham Study cohort examinations, including: the Framingham Offspring cohort seventh follow-up examination (1998–2001; $n = 5124$); the Third Generation cohort first examination enrolled from 2002 to 2005 ($n = 4085$), and 804 Omni Study ethnic/racial minority participants (see Supplementary data). The study protocol was approved by the Boston University Medical Center Institutional Review Board and participants signed informed consent.

2.2. Lp-PLA_2 determination

Lp-PLA_2 activity and mass were measured from overnight fasting plasma specimens that were stored at −80 °C. Lp-PLA_2 activity was measured using a colorimetric activity method (diaDexus CAM Kit, Inc., San Francisco, CA) [3]. Lp-PLA_2 mass was measured using a commercially available sandwich enzyme immunoassays (diaDexus PLAC® test, Inc., San Francisco, CA). Details of laboratory analysis are provided in the Supplementary data.

2.3. Genotyping

Genotyping was conducted by Perlegen Sciences, Inc., Mountain View, CA and the Broad Institute of Harvard and Massachusetts Institute of Technology in members of Offspring and Generation 3 cohorts (not Omni) cohorts. A total of 1943 SNPs in 233 inflammatory candidate genes passed quality control and entered analyses, more details on the methods are available in the Supplementary data. Linkage analyses were conducted using 640 polymorphic markers covering 22 autosomal chromosomes.

2.4. Statistical analysis

2.4.1. Clinical correlates

Skewed distributions led us to employ natural logarithmic transformation of both markers. Lp-PLA_2 mass and activity stepwise linear regression models were performed with forwards selection (inclusion $p < 0.05$). Age, sex and cohort were forced into the model. The model was selected from the following clinical variables: current smoking, alcohol consumption, body mass index, waist circumference, systolic and diastolic blood pressures, fasting biomarkers (calculated low density lipoprotein [LDL]- and high density lipoprotein [HDL]-cholesterol, triglycerides, glucose), diabetes, medications (hypertension, lipid therapy, hormone replacement in women, aspirin [≥3 per week]), prevalent CVD, and season. R^2 for the overall model and partial R^2 for individual variables were assessed. In secondary analyses, we tested the interactions among age, sex, cohort, LDL- and HDL-cholesterol with respect to association with Lp-PLA_2. A two-sided $p < 0.05$ was considered statistically significant for the clinical correlates analysis. SAS version 8.1 (http://www.sas.com/presscenter/guidelines.html, Cary, NC) was used for clinical analyses and creation of phenotype residuals for genetic analyses adjusting for age, sex, cohort, smoking, alcohol consumption, body mass index, waist, systolic and diastolic blood pressure, total/HDL-cholesterol, triglycerides, glucose, diabetes, the four medication classes listed above, prevalent CVD, and season.

2.4.2. Heritability, linkage and association

Heritability analyses were restricted to Offspring and Generation 3 individuals in families with ≥2 phenotyped individuals ($n = 6945$ individuals, 782 families). Multivariable-adjusted Lp-PLA_2 residuals were examined in association with inflammatory SNPs. For each association, multiple testing was accounted for by computing the q value. See Supplementary data for details on genetic analyses.

3. Results

3.1. Participant characteristics

The clinical and laboratory characteristics of the study participants available for phenotype, linkage, and candidate gene analyses are presented in Table 1; Omni participants were unavailable for genetic analyses. The clinical characteristics by study cohort are displayed in Supplementary Table 1. The SNP study sample had an older mean age (62 vs. 49 years in the phenotype and linkage samples, respectively). Pearson's correlation coefficient between Lp-PLA_2 activity and mass was 0.46 (95% confidence interval 0.45, 0.48).

3.2. Multivariable clinical correlates of Lp-PLA_2

As displayed in Table 2, in stepwise multivariable linear regression models with age, sex and cohort forced in, Lp-PLA_2 activity and mass concentrations were positively associated with higher mean age, smoking, and LDL-cholesterol, and inversely associated with being a woman or a minority, alcohol consumption category, medications (hypertension, lipid-lowering, and hormone replacement therapy). Lp-PLA_2 activity was strongly inversely associated with HDL-cholesterol; however, mass was only weakly associated and the direction was positive. Both mass and activity were associated with season, though not with a consistent pattern. In addition, Lp-PLA_2 activity was positively associated with CVD, and inversely associated with body mass index, whereas Lp-PLA_2 mass was positively associated with diastolic blood pressure. Triglycerides