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Lipoprotein-associated phospholipase A_2 and risk of incident peripheral arterial disease: Findings from The Atherosclerosis Risk in Communities study (ARIC)

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ABSTRACT

Background and aims: Results from prospective studies evaluating the relationship between elevated lipoprotein-associated phospholipase A_2 (Lp-PLA_2) activity and incident peripheral arterial disease (PAD) have been mixed. We investigated whether higher Lp-PLA_2 levels are associated with increased risk of incident PAD and whether *PLA2G7* gene variants, which result in lower Lp-PLA_2 levels, are associated with reduced risk of incident PAD.

Methods: Our analysis included 9922 participants (56% female; 21% African-American; mean age 63 years) without baseline PAD at ARIC Visit 4 (1996–1998), who had Lp-PLA_2 activity measured and were subsequently followed for the development of PAD, defined by occurrence of a PAD-related hospitalization, through 2012. Cox proportional hazard models were performed to determine the association of Lp-PLA_2 levels and *PLA2G7* gene variants with incident PAD.

Results: During a median follow-up of 14.9 years, we identified 756 incident cases of PAD. In analyses adjusting for age, race, and sex, each standard deviation increment in Lp-PLA_2 activity (62 nmol/ml/min) was associated with a higher risk of developing PAD (hazard ratio (HR) 1.17; 95% confidence interval (CI) 1.09, 1.26). This association remained significant after additional adjustment for risk factors, other cardiovascular disease, and medication use, but was strongly attenuated (HR: 1.09; 95% CI 1.00, 1.20). *PLA2G7* variants were not associated with a lower risk of PAD in both white carriers (HR: 1.21; 95% CI: 0.17–8.56) and African-American carriers (HR: 0.83; 95% CI: 0.41–1.67), although statistical power was quite limited for this analysis, particularly in whites.

Conclusions: While higher Lp-PLA_2 activity was associated with an increased risk for incident PAD, it is likely a risk marker largely represented by traditional risk factors.

1. Introduction

Lipoprotein-associated phospholipase A_2 (Lp-PLA_2) is an enzyme highly expressed by macrophages in atherosclerotic lesions and is responsible for the hydrolysis of oxidized phospholipids on LDL particles [1,2]. Although the relationship of both

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higher Lp-PLA_2 mass and activity with greater risk of incident cardiovascular disease (CVD) is well-established [3–8], only two prospective studies have evaluated its association with incident peripheral arterial disease (PAD) [9,10]. These two studies obtained conflicting results, leaving uncertainty regarding this association.

Individuals with PAD have high mortality rates [11]. Eleven percent of US adults older than 40 years and over 200 million people worldwide are estimated to have PAD [12,13]. Prevalence of PAD is nearly twice as high in African-Americans compared to non-Hispanic whites, and established risk factors alone do not explain ethnic-specific variations in PAD prevalence [14–16]. Building upon the existing literature to better define whether a relationship between Lp-PLA_2 levels and incident PAD exists is important. If an association is observed, future studies could investigate whether individuals with high baseline Lp-PLA_2 levels would benefit from more intensive cardiovascular risk modification to reduce their risk of incident PAD.

We prospectively examined the relationship between Lp-PLA_2 activity and the development of PAD in The Atherosclerosis Risk in Communities (ARIC) study, a large population-based cohort with long-term follow-up. To better understand possible mechanisms of associations, we also examined the relationship between *PLA2G7* gene variants, which results in lower Lp-PLA_2 levels, and risk for incident PAD.

2. Materials and methods

The ARIC study included 15,792 men and women aged 45–64 years sampled from four U.S. communities in 1987–1989 [17]. Participants were re-examined in 1990–1992 (93% response), 1993–1995 (86%), 1996–1998 (80%), and 2011–2013 (65%) and followed for cardiovascular events. Due to the availability of Lp-PLA_2 activity data, participants in ARIC Visit 4 (1996–98) served as the eligible cohort and baseline Visit for the present analysis. Institutional review boards at each participating institution (University of Minnesota, Johns Hopkins University, University of North Carolina, and University of Mississippi Medical Center) approved the study, and all participants gave written informed consent at each study Visit.

2.1. Lp-PLA_2 activity measurement

Lp-PLA_2 activity was assessed from stored plasma at Visit 4 by an automated Colorimetric Activity Method (CAM) assay (diaDexus Inc., South San Francisco, CA) using a Beckman Coulter (Olympus) AU400e autoanalyzer. The Lp-PLA_2 activity assay had an inter-assay variation coefficient of 4.4% and a reliability coefficient (R) of 0.92, based on 419 blinded replicate samples.

Determination of *PLA2G7* gene mutations associated with a loss of Lp-PLA_2 activity function among ARIC participants has been described previously [18]. DNA sequencing was previously performed on Illumina HiSeq 2000 after exome capture with NimbleGen's VCRome2.1 on ARIC participants consenting to genomic use [19]. Whole exome sequencing was performed in 6325 ARIC participants to determine genetic variants that lower Lp-PLA_2 activity. There were 4 *PLA2G7* gene loss-of-function (LOF) variants observed on whole exome sequencing in white participants, of which one variant was already present on Illumina's HumanExome BeadChip array, rs140020965 Q287X. LOF variants were defined as mutations resulting in premature stop codons, splice sites, and small indels, which were predicted to disrupt protein production. Joint genotype calling on the exome chip with improved calling of rare variation was previously described [20]. There was 1 rare nonsynonymous *PLA2G7* mutation noted in African-American participants, which also was present on the exome chip, rs34159425 L389S. These two exome chip variants (one LOF stop codon and one nonsynonymous) along with sequenced LOF variants were used for the analysis.

2.2. Peripheral artery disease

The diagnosis of PAD was determined by ankle-brachial index (ABI) measurement, administration of the Rose Questionnaire, or confirmation of PAD-related hospitalizations.

The ABI was measured by trained technicians on nearly all participants at Visit 1 (96%) and on a selected random number of participants at Visits 3 (n = 4197) and 4 (n = 5882), using the Dinamap Model 1846 SX, an oscillometric device that obtains repeated blood pressure measurements automatically [21]. Trained technicians measured the ankle blood pressure at the posterior tibial artery in a randomly selected leg, and the brachial artery systolic blood pressure was measured in the right arm. Both were measured while the patient was in the supine position. The ABI was defined as the ratio of a single ankle SBP in one leg to a single brachial BP. According to a previous study, the reliability of the ABI based on single ankle and arm systolic blood pressure was 0.61 (95% confidence interval (CI) 0.50, 0.70) [22].

The Rose Questionnaire was used to evaluate whether participants had developed intermittent claudication, which was defined as exertional leg pain relieved within 10 min by resting [23]. Interviewers contacted participants by telephone annually through 1998 to directly identify intermittent claudication symptoms.

PAD-related hospitalizations were determined through use of ICD-9 codes. A trained abstractor obtained and recorded all ICD-9 hospital discharge diagnoses when a hospitalization occurred. All records with an ICD-9 code of 443.9 (peripheral vascular disease, unspecified), 440.2 (atherosclerosis of native arteries and extremities), 440.3 (atherosclerosis of bypass graft of the extremities), 440.4 (chronic total occlusion artery extremities), 84.11 (toe amputation), 84.12 (foot amputation), 84.15 (below-knee amputation), 84.17 (above-knee amputation), 38.18 (leg endarterectomy), 39.25 (aorto-iliac-femoral bypass), 39.29 (leg bypass surgery), and 39.50 (percutaneous transluminal angioplasty of non-coronary vessels) qualified as hospitalized PAD. Surveillance for all hospitalizations including revascularization procedures and amputations occurred through the year 2012.

We defined prevalent PAD as an ABI $\leq$0.9 at Visit 1, 3 or 4, a positive Rose Questionnaire through Visit 4, or a diagnosis of hospitalized PAD through Visit 4. These individuals were excluded from the study (n = 1381). Given the caveats of ABI measurements (nearly all participants but only single leg at Visit 1, only on a subsample and single leg [sometimes different leg than Visit 1] at Visits 3 and 4) and that the Rose Questionnaire was not administered beyond the time Visit 4 ended, we defined incident PAD as a hospital discharge diagnosis consistent with PAD.

Because *PLA2G7* LOF variants are rare exposures that are present throughout the entire life span, Visit 1 served as the baseline (instead of Visit 4) for the Lp-PLA_2 LOF analysis and exclusion criteria were applied to this visit only. As a result, new diagnoses of hospitalized PAD occurring between Visit 1 and Visit 4 were counted as incident PAD in this analysis (as opposed to prevalent PAD for the Lp-PLA_2 activity analysis). For this reason, more cases of incident PAD were observed in the LOF analysis.

2.3. Baseline covariates

All covariates were assessed at Visit 4 (1996–98) and included age (years), sex, race/ARIC clinic site, body mass index (BMI), education level (<12 years or $\geq$12 years), smoking status (current, former, or never), alcohol consumption (drinks/week), physical