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Lipoprotein-Associated Phospholipase A₂ (Lp-PLA₂) and Risk of Cardiovascular Disease in Older Adults: Results from the Cardiovascular Health Study

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Abstract

Objective—To examine associations between lipoprotein-associated phospholipase A₂ (Lp-PLA₂) antigen level (mass) and enzymatic activity (activity) and cardiovascular disease (CVD) in older adults.

Methods—We examined associations of Lp-PLA₂ mass and activity with incident myocardial infarction (MI; n=508), stroke (n= 565) and CVD death (n=665) using Cox regressions adjusted for age, sex, ethnicity and CVD risk factors in 3,949 older adults, aged ≥ 65 years at baseline, from the Cardiovascular Health Study (CHS).

Results—Lp-PLA₂ was associated with incident CVD events in these older adults. Hazard ratios (95% confidence intervals) for highest versus lowest tertiles of Lp-PLA₂ mass were 1.49 (1.19–1.85) for MI, 1.21 (0.98–1.49) for stroke and 1.11 (0.92–1.33) for CVD death. The highest tertile of Lp-PLA₂ activity was associated with MI (1.36; 1.09–1.70) and CVD death (1.23; 1.02–1.50). Combined Lp-PLA₂ tertile 3 and CRP >3mg/l, compared to Lp-PLA₂ tertile 1 and CRP <1 mg/l, was associated with MI (2.29; 1.49–3.52) for Lp-PLA₂ mass and MI (1.66; 1.10–2.51) and CVD death (1.57; 1.08–2.26) for activity. For MI, both mass and activity added excess risk to elevated CRP alone (~20% excess risk) and activity added excess risk for CVD death (~12%).

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Conclusion—Lp-PLA₂ mass and activity were associated with incident CVD events in older adults in CHS. Lp-PLA₂ and CRP were independent and additive in prediction of events. While associations were modest, these results support further exploration of Lp-PLA₂ to identify older individuals at risk for CVD.

Keywords

Epidemiology; Inflammation; Cardiovascular diseases; Lipoprotein-associated phospholipase A₂

Introduction

Lipoprotein-associated phospholipase A₂ (Lp-PLA₂), is an enzyme produced by inflammatory cells that acts to cleave oxidized phospholipids. Lp-PLA₂ generates pro-inflammatory moieties lysophosphatidylcholine and oxidized fatty acids which act as monocyte chemoattractants and activate macrophages[1]. The localization of Lp-PLA₂ in atherosclerotic lesions and its association with plaque instability support a potential causal role for Lp-PLA₂ in cardiovascular disease (CVD)[2].

Associations of Lp-PLA₂ mass (circulating antigen level) and/or activity (circulating enzymatic activity) with CVD risk have been examined in several prospective studies. Most [3–7], but not all[8], reported associations of Lp-PLA₂ with risk of future CVD events. One study suggested that activity may be more strongly associated with CVD risk factors than mass [9]. These studies were primarily in middle-aged adults where vulnerable and ruptured plaques are commonly associated with sudden cardiac death[10]. Lp-PLA₂ is reported to be strongly expressed in these types of lesions[2]. There are limited data on associations between Lp-PLA₂ and CVD in older adults who are more likely to have stable plaque[10]. As plaque morphology may change with age[10], associations of Lp-PLA₂ with CVD may likewise vary with age. We therefore examined associations of both Lp-PLA₂ mass and activity with CVD events in older adults from the Cardiovascular Health Study (CHS). Since C-reactive protein (CRP) and Lp-PLA₂ have been reported to be complementary in risk prediction[6,7], we also assessed potential interactions between these two inflammatory biomarkers.

Materials and Methods

Cardiovascular Health Study

The CHS comprises 5,888 men and women ≥ 65 years of age at baseline[11]. The original cohort (n = 5,201) was enrolled in 1989–1990. An additional primarily African-American cohort (minority cohort; n = 687) was recruited in 1992–1993. Baseline examinations for both cohorts included anthropometry, medical and lifestyle histories, blood collection, resting 12-lead electrocardiography (ECG), carotid ultrasonography and ankle-brachial blood pressure index (ABI). Prevalence and extent of clinical CVD (confirmed angina, myocardial infarction (MI), transient ischemic attack, stroke, carotid endarterectomy and peripheral arterial disease surgery) were confirmed by physical examination or review of medical records. We excluded 1,565 participants with prevalent CVD. All subjects gave informed consent for participation in the study and all procedures were conducted under institutionally approved protocols.

Ascertainment of Events

Potential events were identified through contact with participants or proxies. Deaths were ascertained by review of local obituaries and contact with proxies for those who died. Medical documentation was reviewed by a committee using standardized, published criteria to classify events and cause of death. CVD death included death due to atherosclerotic coronary heart disease, cerebrovascular disease, atherosclerotic disease or other CVD. Follow-up was 100%

complete for death status. Event ascertainment has been completed through June 30, 2006. Mean (standard deviation) follow-up times for original and minority cohorts were 12.3 (4.2) and 10.1 (3.3) years, respectively.

Nonexclusive case groups were: incident MI (n = 508), ischemic stroke (n = 565) and CVD death (n = 665). There were 1,265 CVD events (MI, stroke and/or CVD death cases). 2,684 participants remained free of any event of interest through June 30, 2002.

Clinical Definitions

Glycemic status was classified by American Diabetic Association guidelines as normal (fasting glucose <110 mg/dl), impaired (fasting glucose 110–125 mg/dl) and diabetes (fasting glucose $\geq$ 126 mg/dl and/or taking insulin or oral hypoglycemics). Body mass index (BMI, kg/m²) was calculated. Smoking was defined as never, former (more than 30 days since last cigarette) or current. Hypertension was defined as systolic blood pressure >140 mm Hg, diastolic blood pressure >90 mm Hg, or history of hypertension and use of antihypertensive medication. Dyslipidemia was defined as total/HDL cholesterol ratio > 5 or taking lipid-lowering medication.

Laboratory Methods

Blood collection and laboratory procedures have been described[12]. C-reactive protein (CRP) was measured by high sensitivity assay (analytical coefficient of variation (CV) 8.9%)[13]. Lp-PLA₂ mass was measured at the Laboratory for Clinical Biochemistry Research (University of Vermont, Burlington, VT) using the PLACTM Test (Diadexus, San Francisco, CA); analytical CV 6.3%. Lp-PLA₂ activity was measured at GlaxoSmithKline (Research Triangle Park, NC) by radiometric assay using a tritium-labeled platelet activating factor (H³-PAF) substrate [14]; analytical CV 7.5%. Lp-PLA₂ activity was expressed as nanomoles PAF hydrolyzed per minute per milliliter of plasma sample (nmol/min/ml). All measurements were made on baseline blood samples not previously thawed. Technicians were blinded to case status.

3,949 CHS participants were included in the current analyses; 3,948 with Lp-PLA₂ mass measurements and 3,943 with activity measurements. 3,929 participants also had CRP measurements.

Statistical Analyses

Data were analyzed using STATA (version 8.0). Differences in baseline characteristics between participants with and without incident CVD events were assessed by *t* tests or χ^2 tests. Because Lp-PLA₂ mass and activity levels differed significantly by sex and ethnicity, we used sex- and ethnic-specific cutpoints to define tertiles. Cox proportional hazards models were used to determine hazard ratios (HRs) and 95% confidence intervals (95% CIs) for mass and activity tertiles. Those with an event were compared to all remaining participants. Analyses were minimally adjusted for age, sex and ethnicity. Fully adjusted models included CVD risk factors (BMI, diabetes, smoking, hypertension and dyslipidemia).

Potential complementary associations of CRP and Lp-PLA₂ were assessed. CRP was divided into three categories, low (<1 mg/l), intermediate (1–3 mg/l) and high ($\geq$ 3 mg/l). Using an additive scale[15], the relative risk of interaction (RERI) is the risk in excess of that expected if Lp-PLA₂ and CRP interacted in a purely additive manner. In terms of the proportional hazards model, RERI is equivalent to HR(high Lp-PLA₂ and high CRP) – HR(high Lp-PLA₂) – HR(high CRP) + 1 for each outcome. P-values and 95% CIs were computed using the delta method[16]. The proportion of risk due to high Lp-PLA₂ and high CRP attributable to

their interaction was calculated as RERI% ($(\text{RERI}/[\text{HR}(\text{high Lp-PLA}_2 \text{ and high CRP}) - 1] \times 100)$ [15].

Results

Baseline Characteristics

Baseline characteristics are shown in Table 1. Cases were older than those free of events ($p < 0.001$ for all comparisons). MI and CVD-related death cases were more likely to be male ($p < 0.001$ and $p = 0.002$, respectively), hypertensive ($p < 0.001$ for both) and diabetic ($p < 0.001$ for both) than the event free group. Stroke cases were more likely to be hypertensive ($p < 0.001$) and diabetic ($p < 0.001$). Smoking, ethnicity, BMI and dyslipidemia did not differ between cases and event-free participants (all comparisons $p > 0.05$).

Mean Lp-PLA₂ activity levels were higher in MI ($p < 0.001$) and CVD death ($p = 0.004$) cases than the event-free group (Figure 1B). Lp-PLA₂ mass was higher in MI cases ($p = 0.001$; Figure 1A). CRP levels were higher in MI ($p = 0.001$), stroke ($p = 0.047$) and CVD death cases ($p = 0.001$) compared to event-free participants (Figure 1C).

Associations of Lp-PLA₂ Mass and Activity with Incident CVD Events

In Cox proportional hazards models adjusted for age, sex and ethnicity, the highest tertile of Lp-PLA₂ mass, compared to the lowest, was associated with risk of MI and stroke (Model 1, Table 2). Results were attenuated with additional adjustments for CVD risk factors, BMI, diabetes, smoking, hypertension and dyslipidemia, (Model 2, Table 2). Higher Lp-PLA₂ mass remained significantly associated with risk of MI in these models; associations of mass with stroke and CVD death were no longer significant. We also stratified on LDL cholesterol levels. Associations were similar in those with LDL cholesterol levels < 130 mg/dl and those with levels ≥ 130 mg/dl.

The highest tertile of Lp-PLA₂ activity, compared to the lowest, was associated with increased risk of MI, stroke and CVD death in minimally adjusted models (Model 1, Table 2). In fully adjusted models, higher Lp-PLA₂ activity remained significantly associated with MI and CVD death but not stroke (Model 2, Table 2). Prediction of MI and stroke was similar in those with LDL cholesterol levels < 130 mg/dl and those with levels ≥ 130 mg/dl; however, prediction of CVD death appeared to be limited to those with lower LDL cholesterol (HR 1.52; 95% CI 1.18–1.96 for LDL < 130 mg/dl versus 1.08; 0.76–1.53 for those with higher LDL cholesterol).

For comparison, associations of CRP with events were also determined. High CRP (≥ 3 mg/l), compared to low (< 1 mg/l), was associated with increased risk of MI, stroke and CVD death in models adjusted for age, sex and ethnicity (Model 1, Table 2). In fully adjusted models, high CRP remained associated with risk of MI and CVD death (Model 2, Table 2).

Associations of Combined Lp-PLA₂ and CRP with CVD Events

We examined the influence of CRP on Lp-PLA₂ associations with events. In all models tested, there were no statistically significant multiplicative interactions of CRP with Lp-PLA₂ mass or activity. Addition of CRP (by cutpoints, < 1 mg/l, 1–3 mg/l, ≥ 3 mg/l) to the multivariable models for Lp-PLA₂ mass or activity did not appreciably alter associations of these factors with CVD events (Model 3, Table 2).

We determined the RERI to evaluate the complementary associations of CRP and Lp-PLA₂ with events. In fully adjusted models, those with high Lp-PLA₂ mass (tertile 3) and high CRP (≥ 3 mg/l) had an increased risk of MI and stroke compared to those with low Lp-PLA₂ mass (tertile 1) and low CRP (< 1 mg/l; Table 3). The RERI percent was significant for MI. The

presence of both high Lp-PLA₂ activity and high CRP was associated with risk of MI and CVD death (Table 3), with a significant RERI percent for both events.

Discussion

In this study of both Lp-PLA₂ mass and activity associations with CVD in older adults, both mass and activity were associated with risk of MI independent of CVD risk factors and CRP. Lp-PLA₂ activity, but not mass, was associated with CVD death. HRs for the top tertiles of Lp-PLA₂ mass and activity and risk of MI were similar to that for CRP >3 mg/l. In addition, there was some synergism when Lp-PLA₂ mass or activity was combined with CRP. For MI, there was approximately 20% excess risk for mass or activity combined with CRP and 12% excess risk for activity combined with CRP for CVD death.

Our findings are similar to those reported for coronary heart disease in middle-aged individuals [5–7] and similar to results for Lp-PLA₂ mass in older adults in the Rancho Bernardo Study [17]. In contrast to our null findings, Lp-PLA₂ activity was associated with risk of stroke in older adults in the Rotterdam Study[3] and mass was associated with risk of stroke in middle-aged adults in the Atherosclerosis Risk in Communities (ARIC) study[18]. Differences in associations of Lp-PLA₂ with stroke between our study and the Rotterdam and ARIC studies may be due to chance or may be due to dissimilarities, such as age range and selection criteria, between study participants. Differences in risk prediction between older and middle-aged adults have been reported[19]. CRP and fibrinogen predict CVD events in both older and middle-aged individuals; however, the relative risks in older adults appear lower than in younger adults[19]. Similarly, lipid measures are strongly associated with CVD risk in middle-aged adults[20] and weakly associated with risk in older adults in the CHS[21]. Lp-PLA₂ is bound to LDL and HDL moieties and the weaker association of Lp-PLA₂ with CVD in older adults may reflect the overall weaker association of lipids with CVD in this age range. These lower relative risks should not diminish interest in evaluation of novel biomarkers in the elderly. While relative risks for biomarkers tend to be lower in older populations, attributable risks are usually higher because of the higher absolute risk with aging. In addition, the association of Lp-PLA₂ activity with CVD death in older adults with low LDL may be useful clinically in identifying individuals who would benefit from more aggressive treatment.

As seen in middle-aged populations[6,18], we observed enhanced prediction of CVD events when CRP was combined with either Lp-PLA₂ mass or activity. In ARIC, the additive effect for coronary heart disease was only seen in participants with LDL cholesterol values <130 mg/dl[7]. We did note in CHS that associations of Lp-PLA₂ activity with CVD death were likewise limited to those with LDL cholesterol < 130 mg/dl. Circulating Lp-PLA₂ is bound primarily to LDL, with the highest concentrations in small, dense LDL[1]. In those with high Lp-PLA₂ levels, increased hydrolysis of atherogenic small dense LDL particles likely leads to increased vascular inflammation. Similarly, higher levels of CRP are associated with vascular inflammation. Individuals with low LDL but high levels of Lp-PLA₂ and CRP may therefore be at increased risk of CVD[7].

Strengths of this study include the large number of CVD events in a population-based sample of apparently healthy older adults from a prospective epidemiologic study. Limitations include measuring levels of biomarkers only once; intra-individual variation cannot be accounted for. However, assay variability would be expected to bias findings towards the null so observed associations are potentially underestimations. CHS participants are older adults and predominantly Caucasian and results of this study may not generalize to other populations. In addition, it is not clear from our study or previous studies whether Lp-PLA₂ mass or activity would be the best marker for potential clinical use. This question may be addressed by the ongoing Lp-PLA₂ Studies Collaboration[22].

In summary, we report that Lp-PLA₂ mass and activity were associated with risk of incident CVD events in older adults in a population-based prospective study. CRP and Lp-PLA₂ were independent predictors of events and there was a significant additive effect when the biomarkers were combined. Given recent evidence suggesting that Lp-PLA₂ may be a specific marker of plaque progression and stability[2], may be a specific marker for vascular inflammation and has minimal biovariability[23], our results support further study of this novel biomarker as a tool for CVD risk stratification. Ongoing studies of Lp-PLA₂ inhibitors will further elucidate the clinical utility of this interesting molecule.

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A

B

C

Figure 1.

Biomarker levels by case status. Means (points) and standard error of the means (bars) for Lp-PLA₂ mass (A), Lp-PLA₂ activity (B) and CRP (C) for event-free and case groups. CRP values derived from ln-transformed values. *** $p \leq 0.001$, ** $p \leq 0.01$, * $p < 0.05$ compared to event-free.

Table 1

Baseline characteristics by case status

Variable	Event-free (n = 2684)	MI (n = 508)	Stroke (n = 565)	CVD Death (n = 665)
Age, years	71.7 (5.2)	72.8 (5.2)	73.4 (5.4)	74.5 (5.6)
Male, n (%)	946 (35.2)	249 (49.0)	195 (34.5)	277 (41.7)
White, n (%)	2251 (83.9)	438 (86.2)	471 (83.4)	566 (85.1)
Hypertension, n (%)	948 (35.3)	236 (46.5)	278 (49.3)	325 (48.9)
Ever smoked, n (%)	1382 (51.6)	283 (55.7)	285 (50.5)	350 (52.6)
Diabetes, n (%)	280 (10.5)	108 (21.3)	105 (18.7)	147 (22.2)
BMI, kg/m ²	26.6 (4.7)	27.1 (4.6)	26.6 (4.6)	26.6 (4.8)
Dyslipidemia, n (%)	594 (22.1)	132 (26.0)	137 (24.2)	167 (25.1)

Values are mean (standard deviation) unless otherwise noted.

Table 2

Risk of CVD events by biomarker levels

Model 1		Hazard Ratio (95% Confidence Interval) for Event		
		MI	Stroke	CVD Death
Lp-PLA ₂ mass	Tertile 2	1.27 (1.02 – 1.59)	1.28 (1.05 – 1.58)	1.11 (0.92 – 1.35)
	Tertile 3	1.51 (1.21 – 1.87)	1.26 (1.03 – 1.56)	1.17 (0.97 – 1.41)
	Tertile 2	1.19 (0.95 – 1.48)	1.05 (0.85 – 1.29)	1.14 (0.94 – 1.38)
Lp-PLA ₂ activity	Tertile 3	1.42 (1.14 – 1.76)	1.14 (0.93 – 1.39)	1.29 (1.07 – 1.56)
	1–3 mg/l	1.01 (0.81 – 1.25)	1.06 (0.86 – 1.29)	1.07 (0.89 – 1.30)
	≥ 3mg/l	1.69 (1.34 – 2.13)	1.31 (1.05 – 1.63)	1.61 (1.32 – 1.98)
Model 2				
	Lp-PLA ₂ mass			
	Tertile 2	1.26 (1.01 – 1.57)	1.27 (1.03 – 1.56)	1.10 (0.91 – 1.33)
Lp-PLA ₂ activity	Tertile 3	1.49 (1.19 – 1.85)	1.21 (0.98 – 1.49)	1.11 (0.92 – 1.35)
	Tertile 2	1.16 (0.93 – 1.45)	1.03 (0.84 – 1.27)	1.15 (0.95 – 1.40)
	Tertile 3	1.36 (1.09 – 1.70)	1.06 (0.86 – 1.31)	1.23 (1.02 – 1.50)
CRP	1–3 mg/l	0.94 (0.75 – 1.18)	1.01 (0.82 – 1.24)	0.99 (0.82 – 1.21)
	≥ 3mg/l	1.44 (1.13 – 1.85)	1.17 (0.92 – 1.49)	1.33 (1.07 – 1.66)
Model 3				
	Lp-PLA ₂ mass			
	Tertile 2	1.30 (1.04 – 1.63)	1.26 (1.03 – 1.55)	1.11 (0.92 – 1.34)
Lp-PLA ₂ activity	Tertile 3	1.54 (1.24 – 1.93)	1.19 (0.97 – 1.47)	1.10 (0.91 – 1.33)
	Tertile 2	1.12 (0.93 – 1.46)	1.04 (0.84 – 1.28)	1.17 (0.96 – 1.41)
	Tertile 3	1.40 (1.12 – 1.75)	1.05 (0.85 – 1.30)	1.22 (1.00 – 1.49)

Model 1 adjustments: age, sex, ethnicity. Model 2: Model 1 plus BMI, diabetes, smoking, hypertension, dyslipidemia.

Model 3: Model 2 plus CRP (by cupoints). Lp-PLA₂ mass and activity tertile 1 and CRP <1 mg/l are referents (HR=1.00). Bold values significant at p<0.05.

Table 3Associations of combined high Lp-PLA₂ and high CRP with CVD events

Lp-PLA ₂ Mass and CRP			
Event	HR (95% CI) [#]	RERI* (%)	p-value
MI	2.29 (1.49 – 3.52)	19.5	<0.001
Stroke	1.29 (0.87 – 1.91)	8.3	0.037
CVD Death	1.47 (1.04 – 2.09)	6.4	0.28
Lp-PLA ₂ Activity and CRP			
MI	1.66 (1.10 – 2.51)	17.6	<0.001
Stroke	0.94 (0.63 – 1.39)	4.0	0.62
CVD Death	1.57 (1.08 – 2.26)	11.9	0.003

[#] Hazard ratio (HR) and 95% confidence interval (95% CI) adjusted for age, sex, ethnicity, BMI, diabetes, smoking, hypertension, dyslipidemia. Combined high Lp-PLA₂ (tertile 3) and high CRP (≥ 3 mg/l) compared to low Lp-PLA₂ (tertile 1) and low CRP (<1 mg/l).

* RERI is the percentage of relative excess risk from high Lp-PLA₂ and high CRP due to interaction. Bold values significant at $p < 0.05$.