Published in final edited form as:
Am J Hematol. 2008 July ; 83(7): 524–527. doi:10.1002/ajh.21182.

Lipoprotein-Associated Phospholipase A_2 and Risk of Venous Thrombosis in Older Adults

Nels Olson, B.S.[1], **Ellen S. O'Meara, Ph.D**[2], **Nancy S. Jenny, Ph.D**[1], **Aaron R. Folsom, M.D.**[3], **Edwin G. Bovill, M.D.**[1], **Curt D. Furberg, M.D.**[4], **Susan R. Heckbert, M.D.,Ph.D.**[5], **Bruce M. Psaty, M.D., Ph.D**[6], and **Mary Cushman, M.D., M.Sc.**[7]

1 *Department of Pathology, University of Vermont College of Medicine, Burlington, VT.*

2 *Department of Biostatistics, University of Washington, Seattle, WA.*

3 *Division of Epidemiology and Community Health, University of Minnesota, Minneapolis, MN.*

4 *Division of Public Health Sciences, Wake Forest University School of Medicine, Winston-Salem, NC.*

5 *Department of Epidemiology, University of Washington, Seattle, WA.*

6 *Departments of Medicine, Epidemiology and Health Services, University of Washington, Seattle, WA.*

7 *Departments of Medicine and Pathology, University of Vermont College of Medicine, Burlington, VT.*

Abstract

Lipoprotein-associated phospholipase A_2 (Lp-PLA_2) is an enzyme involved in inflammation and platelet function. Inherited deficiency and elevated levels are associated with atherosclerosis. Given potential common etiologies of atherosclerosis and venous thrombosis (VT), we hypothesized that low and high Lp-PLA_2 would be associated with VT risk. Lp-PLA_2 mass and activity were measured in baseline samples of Cardiovascular Health Study participants (5,888 men and women age ≥65), excluding 354 reporting pre-baseline VT. The study endpoint was VT unrelated to cancer after 11.6 years follow-up. Hazard ratios were estimated using Cox proportional hazard models, adjusting for age, race, sex and body-mass index. With 129 cases of VT, there was no association of Lp-PLA_2 activity with risk. Adjusted hazard ratios were 1.19 (CI 0.62, 2.29) and 0.87 (CI 0.43, 1.76) for the lowest and highest decile, respectively, compared to the 10-25th percentile. Corresponding hazard ratios for Lp-PLA_2 mass were 1.63 (CI 0.79, 3.34) and 1.33 (CI 0.61, 2.87). Results were robust to several definitions of low or high Lp-PLA_2. While the association of Lp-PLA_2 levels with arterial disease events implies a role for this enzyme in atherogenesis, our findings suggest that it is not prothrombotic.

Keywords

Lipoprotein-associated phospholipase A_2; risk factor; venous thrombosis; deep vein thrombosis; pulmonary embolus

Platelet activating factor acetylhydrolase (PAFAH) is an enzyme in the phospholipase A_2 family. PAFAH inactivates platelet activating factor (PAF) by hydrolyzing the acetyl group at the sn-2 position [1] and is synthesized by a variety of cell types [2]. Three isoforms of PAFAH have been identified: intracellular types Ib and II and a plasma form [3]. Plasma PAFAH, which

Correspondence: Mary Cushman, MD, MSc., University of Vermont, 208 South Park Drive, Suite 2, Colchester, VT 05446. Email: Mary.Cushman@uvm.edu, Fax: 802-656-8965, Phone: 802-656-8968.

is also called lipoprotein-associated phospholipase A_2 (Lp-PLA_2), is a 45 kDa monomeric enzyme associated primarily with high and low density lipoproteins (HDL and LDL) [4]. PAF is a phospholipid messenger involved in the mediation of a variety of biological activities [5]. Lp-PLA_2 regulates the inflammatory effects of PAF in the vasculature by terminating PAF signaling [6]. A loss of function mutation in the Lp-PLA_2 gene was reported in 4% of a Japanese population and this deficiency state has been associated with thrombotic inflammatory diseases including myocardial infarction, stroke and non-familial dilated cardiomyopathy [7-11]. Conversely, several studies of middle aged men and women have reported that elevated circulating levels of Lp-PLA_2 was associated with increased risk of coronary heart disease and stroke [12-15].

Although Lp-PLA_2 is related to thrombosis and inflammation status, there is no information on the association of Lp-PLA_2 levels and venous thrombosis (VT), a disease that millions of patients annually are at risk for [16]. Given its biological roles in coagulation and inflammation and the recently described links between atherosclerosis and VT [17], we examined the association of Lp-PLA_2 concentration with subsequent VT. Because both low and high levels of Lp-PLA_2 levels are associated with atherosclerotic disease, we hypothesized that both low and high Lp-PLA_2 activity and mass levels would be associated with increased rates of VT.

Results

Of the 5,287 participants eligible for analysis, Lp-PLA_2 mass was available in 5,154 (mean 344.7 ng/ml; SD 118.2 ng/ml). Of these participants 4,343 were white and 2,989 were women. Lp-PLA_2 activity was available in 5,142 participants (mean 39.5 nmoles/min/ml; SD 12.9 nmoles/min/ml); 4,333 of these participants were white and 2,984 were women. The Spearman correlation coefficient between Lp-PLA_2 mass and activity among the 5142 participants who had measurements for both values was 0.53. There were 129 incident VT cases with Lp-PLA_2 mass data (64 were idiopathic) and 128 incident VT cases with activity data (63 were idiopathic). The median follow up was 11.6 years.

The mean (SD) Lp-PLA_2 mass and activity concentrations were greater in men than women (mass 357 (121) vs. 336 (115) ng/ml; activity 42.6 (13.1) vs. 37.3 (12.3) nmoles/min/ml). Caucasians had higher levels than African Americans (mass 354 (119) vs. 298 (103) ng/ml; activity 40.8 (13.0) vs. 33.2 (10.6) nmoles/min/ml) (all p-values <0.0001). Because of these differences, percentile categories were defined using sex and race specific values.

Table I shows the distribution of VT risk factors in this cohort by Lp-PLA_2 categories. Participants with higher Lp-PLA_2 mass, but not activity, were older. BMI was positively associated with Lp-PLA_2 activity, but not mass. Factor VIII levels were higher with increasing levels of Lp-PLA_2 mass and activity.

Table II shows the incidence rates of overall and idiopathic VT by baseline Lp-PLA_2 mass and activity categories. Compared to those in the 10-25th percentile, participants with lower or higher baseline Lp-PLA_2 mass, except for those in the 75-90th percentile, had higher incidence rates of VT. The unadjusted hazard ratios followed the same pattern, but were generally not statistically significant. For example, the HR of VT for Lp-PLA_2 mass in the 0-10th percentile compared to the 10-25th percentile was 1.66 (95% CI 0.81, 3.41). Above the 90th percentile the HR was 1.40 (95% CI 0.65, 3.03) compared to those in the 10-25th percentile. Adjustment for age, sex, race, and BMI did not alter the interpretation of the results. In analyses limited to idiopathic cases of VT, hazard ratios were higher but remained non-significant. For Lp-PLA_2 activity, differences in VT incidence among categories were less apparent than for Lp-PLA_2 mass. No hazard ratios for lower or higher Lp-PLA_2 activity approached statistical

significance. Further adjustment for factor VIII in the original cohort did not alter the interpretation of the results for either Lp-PLA_2 activity or mass (data not shown).

Sensitivity analyses assessing other forms of the data were considered. Comparing those with Lp-PLA_2 levels below the 10^{th} percentile to the rest of the cohort, the hazard ratio for overall VT was 1.24 (95% CI 0.74-2.10) for mass and 1.34 (CI 0.80-2.27) for activity, adjusted for age, sex, race and BMI. Expressing Lp-PLA_2 mass or activity in tertiles of the distribution, compared to the first tertile, the adjusted hazard ratio of overall VT for Lp-PLA_2 mass was 1.06 (95% CI 0.69-1.62) for the middle tertile and 0.69 (95% CI 0.42-1.12) for the top tertile. For Lp-PLA_2 activity these hazard ratios were 0.77 (95% CI 0.48-1.22) and 0.92 (95% CI 0.59-1.44), respectively. Results were similar for idiopathic VT (data not shown). Considering Lp-PLA_2 mass and activity as continuous variables, using linear and quadratic functions, none were significant (data not shown).

In a separate analysis we included cancer related VT cases in order to increase event numbers. For Lp-PLA_2 mass, results were only slightly altered in comparison to the non cancer related VT analysis and followed the same trends described above. For Lp-PLA_2 activity, participants in every category had lower incidence rates of VT compared to those in the $10\text{-}25^{th}$ percentile, although this finding was not statistically significant. For example, compared to the $10\text{-}25^{th}$ percentile, the HR of VT for Lp-PLA_2 activity in the $0\text{-}10^{th}$ and $>90^{th}$ percentiles were 0.84 (95% CI 0.46, 1.51) and 0.63 (95% CI 0.33, 1.20), respectively. Adjustment for age, sex, race, BMI and/or factor VIII did not alter the hazard ratios for either Lp-PLA_2 mass or activity in this analysis.

Discussion

In this population of 5,154 older adults, we observed no significant association of either high or low baseline Lp-PLA_2 mass or activity concentrations with risk of future VT. Separate analyses of idiopathic cases or using various definitions of low or high Lp-PLA_2 levels did not alter the results.

Several studies have implicated Lp-PLA_2 as a risk factor for cardiovascular disease. While recent studies have suggested etiologic links between venous and arterial thrombosis [17], our results suggest Lp-PLA_2 is not an etiologic factor for both. Lp-PLA_2 was a risk factor for first coronary heart disease in men with hypercholesterolemia who were in the West of Scotland Coronary Prevention Study [12], in healthy middle aged men in the MONICA-Augsburg cohort [13], and in the general populations of the Rotterdam study and the Cardiovascular Health Study [18], Jenny et. al., manuscript submitted]. The Atherosclerosis Risk in Communities study (ARIC) of middle-aged individuals concluded that higher Lp-PLA_2 predicted myocardial infarction in those with lower LDL cholesterol and was a risk marker for stroke [14]. Conversely, a loss of function mutation resulting in Lp-PLA_2 deficiency, found in 4 percent of the Japanese population [19], has been linked to myocardial infarction [8] and stroke [7]. To date this mutation has not been detected in a North American population [20]. We could not demonstrate that either low or high Lp-PLA_2 concentrations were related to VT risk.

Although neither approached statistical significance, Lp-PLA_2 mass and activity hazard ratios differed with respect to the null. For Lp-PLA_2 mass, many of the hazard ratios were increased, in contrast to reduced hazard ratios for Lp-PLA_2 activity. Lp-PLA_2 activity and mass were not highly correlated and were correlated differently with some risk factors, suggesting that these assays represent different biological aspects of inflammation.

The strengths of this study include the prospective design in a large cohort of participants with baseline Lp-PLA_2 mass and activity measurements. The cohort included people age 65 and older residing in four communities in the United States. As such, our results are not

generalizable to individuals below this age or from other regions. The main limitation of this study is the relatively small number of incident VT events and restricted power to detect small hazard ratios. While the effects of long term-storage of blood samples on Lp-PLA_2 determination are unknown, they are likely small given our storage temperature.

We conclude that Lp-PLA_2 is not a risk factor for VT. We are unaware of other studies examining this question. While the association of Lp-PLA_2 levels with myocardial infarction and stroke imply a role for this enzyme in atherogenesis, our findings suggest that it is not related to thrombotic potential.

Methods

Study population and baseline assessment

The Cardiovascular Health Study (CHS) is a prospective, observational study of risk factors for cardiovascular disease. It includes 5,888 men and women who were ≥65 years of age at baseline [21]. Participants were randomly sampled from Medicare eligibility lists. The original cohort (n = 5,201) was recruited from 1989 -1990 from four U.S. communities (Forsyth County, North Carolina; Sacramento County, California; Washington County, Maryland; and Allegheny County, Pennsylvania). An additional cohort of 687 primarily African American men and women were recruited in 1992 – 1993. All participants provided written informed consent in accordance with local human subjects committees.

At the baseline visit demographic information and medical history were ascertained, and physical measurements performed. Body mass index (BMI) was calculated using height and weight (kg/m^2). Following an 8-12 hour fast, blood samples were collected and centrifuged for 10 minutes at 3,000g at 4° C [22]. Samples were frozen, shipped to a central laboratory and stored at − 70°C. In 2005, baseline plasma samples that had not been previously thawed were used to measure Lp-PLA_2. Lp-PLA_2 activity was measured by GlaxoSmithKline using a tritium-labeled form of platelet activating factor [3H PAF] as substrate in a 96-well microplate, as described previously [18]. Samples were tested in duplicate and were retested if the replicate coefficient of variation was >25%. The analytic CV was 7.5%. Plasma Lp-PLA_2 mass was measured at the University of Vermont using an enzyme-linked immunosorbent assay kit (second generation PLAC™ Test; diaDexus Inc., South San Francisco, CA, USA). All samples were analyzed in duplicate. The analytic CV was 6.3%. Factor VIII coagulant activity was measured in the original cohort only (n = 5,201) on the Coag-A-Mate X2 (Organon-Teknika, Durham, NC) using factor VIlI-deficient plasma and partial thromboplastin (Organon - Teknika, Durham, NC).

We excluded participants who reported pre-baseline VT (n=354), were taking warfarin at baseline (n=98), did not have a blood sample for analysis or a previously unthawed blood sample available (n=330), or more than one of these. Exclusions resulted in 5,287 participants for analysis.

Case Ascertainment

Follow-up involved alternating telephone calls and clinic visits every 6 months. Hospitalizations were identified by self-report of the participant or proxy, and by search of Health Care Financing Administration records. For every hospitalization, hospital discharge summaries and *ICD-9-CM* discharge codes were obtained. Cases of possible VT (deep vein thrombosis and or pulmonary embolism) between enrollment and Dec. 31, 2001 were identified and validated using standardized criteria requiring positive imaging studies as previously described [23]. Thrombosis events were classified as idiopathic or secondary (within 90 days of acute medical conditions such as major trauma, associated surgery, marked immobility, or

cancer). Cancer related VT was not included as an endpoint; participants with cancer-related VT (n=36) were censored as non-events from the analysis at the time of their VT.

Statistical Analysis

Means or frequencies of VT risk factors were examined by Lp-PLA_2 categories. Categories were based on sex and race (African American or other) specific percentiles of the distribution: 0-10%, 10-25%, 25-50%, 50-75%, 75-90%, and 90+ %. These percentiles were chosen to test the hypothesis of increased risk at low and high levels. Characteristics were compared across categories using the chi-square test of independence or the Wald test of inequality of means. Follow-up time was calculated as time from baseline to incident non-cancer related VT, death or December 31, 2001, whichever occurred first. Rates were calculated by dividing the number of VT events by the total number of person-years of follow-up. Hazard ratios and 95% confidence intervals of incident VT by Lp-PLA_2 activity and mass categories were estimated using Cox proportional hazard regression. The hypothesized J-curve response relationship was evaluated by testing the significance of a quadratic term in the model for each assay. Following this hypothesis, on an a priori basis participants in the 10-25th percentile group for each assay were used as the reference group for categorical analyses. Other forms of the data were assessed in secondary analyses. Regression models were adjusted for the VT risk factors in this cohort; age, race, sex and higher BMI [24]. Models that also adjusted for factor VIII were restricted to the original cohort. Models were fit to estimate associations with overall non-cancer related VT and idiopathic VT separately. Data were analyzed using Stata statistical software (StataCorp. 2003. Stata Statistical Software: Release 8.0. College Station, TX: StataCorp LP). All statistical tests were two-sided.

Acknowledgements

The authors thank the participants and staff of the CHS for their contributions over many years. A full list of participating CHS investigators and institutions can be found at http://www.chs-nhlbi.org. CHS was funded by contracts N01-HC-85079 to N01-HC-85086 and N01 HC-55222 from the National Heart, Lung, and Blood Institute. The LITE study was funded by R01 HL59367 from the National Heart, Lung, and Blood Institute. National Heart, Lung, and Blood Institute investigators were involved in the design and acquisition of data for the CHS, but not the LITE study. Funding for Lp-PLA_2 assays was provided by GlaxoSmithKline, who had the opportunity to review the manuscript but was not involved in the analysis and interpretation of the findings or in the decision to publish.

References

1. Blank ML, Lee T, Fitzgerald V, et al. A specific acetylhydrolase for 1-alkyl-2-acetyl-sn-glycero-3-phosphocholine (a hypotensive and platelet-activating lipid). J Biol Chem 1981;256:175–178. [PubMed: 7451433]
2. Asano K, Okamoto S, Fukunaga K, et al. Cellular source(s) of platelet-activating-factor acetylhydrolase activity in plasma. Biochem Biophys Res Commun 1999;261:511–514. [PubMed: 10425216]
3. Castro Faria Neto HC, Stafforini DM, Prescott SM, et al. Regulating inflammation through the anti-inflammatory enzyme platelet-activating factor-acetylhydrolase. Mem Inst Oswaldo Cruz 2005;100 (Suppl 1):83–91. [PubMed: 15962103]
4. Stafforini DM, McIntyre TM, Carter ME, et al. Human plasma platelet-activating factor acetylhydrolase. Association with lipoprotein particles and role in the degradation of platelet-activating factor. J Biol Chem 1987;262:4215–4222. [PubMed: 3549727]
5. Stafforini DM, McIntyre TM, Zimmerman GA, et al. Platelet-activating factor, a pleiotrophic mediator of physiological and pathological processes. Crit Rev Clin Lab Sci 2003;40:643–672. [PubMed: 14708958]
6. Evangelou AM. Platelet-activating factor (PAF): implications for coronary heart and vascular diseases. Prostaglandins Leukot Essent Fatty Acids 1994;50:1–28. [PubMed: 8146205]

7. Hiramoto M, Yoshida H, Imaizumi T, et al. A mutation in plasma platelet-activating factor acetylhydrolase (Val279-->Phe) is a genetic risk factor for stroke. Stroke 1997;28:2417–2420. [PubMed: 9412624]
8. Yamada Y, Ichihara S, Fujimura T, et al. Identification of the G994--> T missense in exon 9 of the plasma platelet-activating factor acetylhydrolase gene as an independent risk factor for coronary artery disease in Japanese men. Metabolism 1998;47:177–181. [PubMed: 9472966]
9. Ichihara S, Yamada Y, Yokota M. Association of a G994-->T missense mutation in the plasma platelet-activating factor acetylhydrolase gene with genetic susceptibility to nonfamilial dilated cardiomyopathy in Japanese. Circulation 1998;98:1881–1885. [PubMed: 9799208]
10. Unno N, Nakamura T, Kaneko H, et al. Plasma platelet-activating factor acetylhydrolase deficiency is associated with atherosclerotic occlusive disease in japan. J Vasc Surg 2000;32:263–267. [PubMed: 10917985]
11. Karasawa K, Harada A, Satoh N, et al. Plasma platelet activating factor-acetylhydrolase (PAF-AH). Prog Lipid Res 2003;42:93–114. [PubMed: 12547653]
12. Packard CJ, O'Reilly DS, Caslake MJ, et al. Lipoprotein-associated phospholipase A2 as an independent predictor of coronary heart disease. West of Scotland Coronary Prevention Study Group. N Engl J Med 2000;343:1148–1155. [PubMed: 11036120]
13. Koenig W, Khuseyinova N, Lowel H, et al. Lipoprotein-associated phospholipase A2 adds to risk prediction of incident coronary events by C-reactive protein in apparently healthy middle-aged men from the general population: results from the 14-year follow-up of a large cohort from southern Germany. Circulation 2004;110:1903–1908. [PubMed: 15451783]
14. Ballantyne CM, Hoogeveen RC, Bang H, et al. Lipoprotein-associated phospholipase A2, high-sensitivity C-reactive protein, and risk for incident ischemic stroke in middle-aged men and women in the Atherosclerosis Risk in Communities (ARIC) study. Arch Intern Med 2005;165:2479–2484. [PubMed: 16314544]
15. Ballantyne CM, Hoogeveen RC, Bang H, et al. Lipoprotein-associated phospholipase A2, high-sensitivity C-reactive protein, and risk for incident coronary heart disease in middle-aged men and women in the Atherosclerosis Risk in Communities (ARIC) study. Circulation 2004;109:837–842. [PubMed: 14757686]
16. Anderson FA Jr. Zayaruzny M, Heit JA, et al. Estimated annual numbers of US acute-care hospital patients at risk for venous thromboembolism. Am J Hematol 2007;82:777–782. [PubMed: 17626254]
17. Prandoni P. Venous thromboembolism and atherosclerosis: is there a link? J Thromb Haemost 2007;5 (Suppl 1):270–275. [PubMed: 17635736]
18. Oei HH, van der Meer IM, Hofman A, et al. Lipoprotein-associated phospholipase A2 activity is associated with risk of coronary heart disease and ischemic stroke: the Rotterdam Study. Circulation 2005;111:570–575. [PubMed: 15699277]
19. Miwa M, Miyake T, Yamanaka T, et al. Characterization of serum platelet-activating factor (PAF) acetylhydrolase. Correlation between deficiency of serum PAF acetylhydrolase and respiratory symptoms in asthmatic children. J Clin Invest 1988;82:1983–1991. [PubMed: 3198761]
20. Stafforini DM, Satoh K, Atkinson DL, et al. Platelet-activating factor acetylhydrolase deficiency. A missense mutation near the active site of an anti-inflammatory phospholipase. J Clin Invest 1996;97:2784–2791. [PubMed: 8675689]
21. Fried LP, Borhani NO, Enright P, et al. The Cardiovascular Health Study: design and rationale. Ann Epidemiol 1991;1:263–276. [PubMed: 1669507]
22. Cushman M, Cornell ES, Howard PR, et al. Laboratory methods and quality assurance in the Cardiovascular Health Study. Clin Chem 1995;42:264–270. [PubMed: 7874780]
23. Cushman M, Tsai AW, White RH, et al. Deep vein thrombosis and pulmonary embolism in two cohorts: the Longitudinal Investigation of Thromboembolism Etiology. Am J Med 2004;117:19–25. [PubMed: 15210384]
24. Tsai AW, Cushman M, Rosamond WD, et al. Cardiovascular risk factors and venous thromboembolism incidence: the Longitudinal Investigation of Thromboembolism Etiology. Arch Intern Med 2002;162:1182–1189. [PubMed: 12020191]