Published in final edited form as:

Diab Vasc Dis Res. 2010 January ; 7(1): 47–55. doi:10.1177/1479164109346358.

Lipoprotein-Associated Phospholipase A₂, C-Reactive Protein, and Coronary Artery Disease in Individuals with Type 1 Diabetes and Macroalbuminuria

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Abstract

Given the paucity of data in type 1 diabetes concerning lipoprotein-associated phospholipase A₂ (Lp-PLA₂), we examined its prospective relationship with coronary artery disease (CAD), as well as effect modification by C-reactive protein (CRP) and haptoglobin genotype, in individuals with type 1 diabetes who are at an increased risk for CAD due to also having macroalbuminuria (n=96).

Although Lp-PLA₂ activity was univariately predictive of CAD (HR=1.54 per sd, p=0.009), this relationship was not significant after covariate adjustment (p=0.59). There was a significant interaction between Lp-PLA₂ and CRP (p=0.02), ie. those with both markers greater than median level were more likely to have a CAD event than those persons with low levels of both (HR=2.89, p=0.06). When stratified by haptoglobin genotype, Lp-PLA₂ was predictive of CAD in persons with the 2/1 (HR=2.40, p=0.05), but not 2/2 (HR=0.66, p=0.27), genotype.

The association between Lp-PLA₂ activity and CAD differs by CRP and haptoglobin genotype in this group of persons with type 1 diabetes and macroalbuminuria.

Keywords

Lp-PLA₂; Type 1 Diabetes; Macroalbuminuria; Coronary Artery Disease; C-Reactive Protein; Haptoglobin Genotype

INTRODUCTION

As atherosclerosis has been increasingly considered an inflammatory process¹, researchers have turned their focus to evaluating the prognostic value of biomarkers of inflammation. Lipoprotein-associated phospholipase A₂ (Lp-PLA₂) has recently been added to this biomarker list. Lp-PLA₂ is an enzyme produced by macrophages in advanced, rupture prone, atherosclerotic plaques. It circulates bound primarily to lipoproteins in the plasma and hydrolyses oxidized low-density lipoprotein (LDL) generating two proinflammatory mediators, oxidized free fatty acids and lysophosphatidylcholine. Through this action on LDL particles, it was therefore thought that Lp-PLA₂ may be directly involved in the formation of atherosclerotic lesions². However, it is now recognized that Lp-PLA₂ induces a broad range of biological responses, is involved in a wide variety of biological actions³ and

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CONFLICT OF INTEREST DISCLOSURE:

Nothing to disclose.

can thus be attributed a variety of properties, including both pro-^{4,5} and anti-inflammatory.^{6–8} Nevertheless, increasing evidence from epidemiological studies in humans suggests that Lp-PLA₂ is independently associated with coronary artery disease risk (CAD).^{9–17} Thus far, the presence of such an association has not been evaluated among individuals with type 1 diabetes (T1D), despite persons with T1D being at increased risk of developing cardiovascular disease.^{18–25}

High sensitivity C-reactive protein (CRP), a marker of systemic inflammation produced in the liver, has also been associated with increased vascular disease risk in both individuals with type 2 diabetes and the general population.^{26–29} Interestingly, in the Atherosclerotic Risk in Communities (ARIC) study, the relationship between Lp-PLA₂ and coronary heart disease was found to be modified by the level of C-reactive protein (CRP) in persons with lower levels of LDL, so that persons with high levels of both markers were at an increased risk for coronary heart disease compared to persons with high levels of either marker alone.¹² While CRP has been found to be associated with vascular disease in nondiabetic^{27,28,30–34} and type 2 diabetic^{29, 35, 36} subjects, the relationship between CRP and CAD in type 1 diabetes is not clear.^{37–39}

Recently, several longitudinal studies have provided evidence that a polymorphism of the Haptoglobin gene more than doubles cardiovascular disease risk among persons with type 2 diabetes.^{40–42} We have also shown that the haptoglobin genotype is associated with CAD in the Pittsburgh Epidemiology of Diabetes Complications study of type 1 diabetes, with the 2/2 genotype conferring the greatest risk.⁴³ We assessed the prospective association between Lp-PLA₂ with CAD risk, and examined whether this relationship is modified by CRP and haptoglobin genotype, in a group of individuals with T1D, who are at particularly high risk for CAD due to also having diabetic renal disease.^{24, 44–49} We also examined whether HDL or LDL cholesterol concentration modified the prediction of CAD by Lp-PLA₂.

METHODS

Study Population

The participants were identified from the Pittsburgh Epidemiology of Diabetes Complications (EDC) study, a prospective study of childhood-onset (<18 years old at diagnosis) T1D. All participants were diagnosed with T1D or seen within one year of diagnosis at Children's Hospital of Pittsburgh between 1950 and 1980. A total of 658 individuals met the eligibility criteria and participated in the EDC baseline examination, conducted between 1986 and 1988, and participants were assessed biennially thereafter. Lp-PLA₂ activity was measured on a subgroup of the EDC study cohort with macroalbuminuria (albumin excretion rate ≥ 200 $\mu\text{g}/\text{min}$) at study baseline; the current analyses were performed on these 96 individuals.

Measurement of Biomarkers

Lp-PLA₂ activity was assayed using a colorimetric assay (diaDexus, South San Francisco, CA, USA). CRP was measured using a high sensitivity turbidimetric method (reagents developed by Carolina Liquid Chemicals, Brea, CA, USA). A PureGene kit (Gentra Systems, Minneapolis, MN) was used to isolate high-molecular weight genomic DNA and haptoglobin was genotyped by the amplification method of Koch et al.⁵⁰ Genotypes were assigned visually by comparison to controls of known genotype.

Ascertainment of Coronary Artery Disease Outcome

CAD was defined as CAD death, MI and/or Q-waves with Minnesota Codes 1.1 or 1.2, stenosis $\geq 50\%$ or revascularization, ischemic ECG (Minnesota Code 1.3, 4.1–4.3, 5.1–5.3,

7.1), or EDC physician diagnosed angina. Events were confirmed by medical records and verified by an EDC study physician masked to Lp-PLA₂ measurements.

Clinical Measurements

Participants completed questionnaires concerning demographic and medical history information. Weight was measured using a balanced-beam scale with clothing during the clinical examination. Height was measured using the clinic stadiometer, with the Frankfort plane held horizontal. BMI was calculated as weight in kilograms divided by height in meters squared. Waist circumference was measured twice at the midpoint between the highest point of the iliac crest and the lowest part of the costal margin in the midaxillary line. If the two measurements differed by >0.5 cm, a third measurement was performed. The mean of the waist measurements was recorded as waist circumference. Hip girth measurement was performed at the widest point of the glutei, usually at the level of the greater femoral trochanter, and the mean of two measures was recorded as the hip circumference, in the same manner as the waist measurements. The ratio of the waist circumference to the hip circumference (WHR) was used in analyses. For the first 18 months of the study, fasting blood samples were analyzed for HbA1c (microcolumn cation exchange; Isolab, Akron, OH, USA). For the remainder of the baseline examinations, automated HPLC (Diamat; BioRad, Hercules, CA, USA) was performed. The two assays were highly correlated ($r=0.95$; Diamat HbA1c = $-0.18 + 1.00$ [Isolab HbA1c]). For analysis purposes, the HbA1c values were converted to a DCCT-aligned value using a regression equation derived from duplicate assays ($DCCT\ HbA1c = 0.14 + 0.83[EDC\ HbA1c]$). Serum total cholesterol and triglycerides were determined enzymatically,^{51,52} HDL-cholesterol was determined using a precipitation technique with a modification⁵³ of the Lipid Research Clinics method⁵⁴ and LDL-cholesterol levels were calculated from the measurements of total cholesterol, triglycerides and HDL-cholesterol using the Friedewald equation.⁵⁵ All blood samples were taken after at least 8 hours of fasting. Three seated blood pressure readings were taken with a random-zero sphygmomanometer and the mean of the second and third readings was used in analyses, according to the Hypertension Detection and Follow-up Program Protocol.⁵⁶ Hypertension was defined as $\geq 140/90$ mmHg or use of antihypertensive medication. White blood cell count (WBC) was obtained using a counter S-plus IV.

Statistical Analyses

Baseline characteristics were compared between CAD cases and noncases using Student's t-test, Wilcoxon 2-sample test for non-normally distributed variables, and chi-square test for binary variables. T1D duration-adjusted correlations between Lp-PLA₂ and CAD risk factors were assessed using Pearson partial correlations or Spearman partial correlations, when variables were not normally distributed. Cox proportional hazard models were used to estimate the relative risk of CAD associated with a 1-standard deviation increase in Lp-PLA₂ activity. Follow-up time was defined as the time from the baseline examination to the date of the first CAD event or, for noncases, follow-up continued until death, last contact, or censoring during 18-year follow-up. Adjusted regression models were built using forward selection. Because age and duration of diabetes are highly correlated in this cohort ($r=0.85$), only duration was made available to multivariate models. The proportional hazards assumption was assessed visually by plotting the log cumulative hazard function of CAD by Lp-PLA₂ activity and verified by showing that time-dependent Lp-PLA₂ interaction variables were not statistically significant. An Lp-PLA₂-CRP interaction term was tested with respect to risk of developing CAD and additional proportional hazards models were fit to examine combined categories of the two biomarkers, using indicator variables, in order to further explore the nature of the interaction. The combined categories were created by stratifying baseline Lp-PLA₂ and CRP by their median values and combining as follows: Low Lp-PLA₂-Low CRP, Low Lp-PLA₂-High CRP, High Lp-PLA₂-Low CRP, High Lp-

PLA₂– High CRP. Analyses were repeated after stratifying by haptoglobin genotype in the participants with available genetic material (n=80). Persons with the 2/1 genotype were compared to those with the higher risk genotype (2/2). Due to the low frequency of the 1/1 genotype, those persons (n=10) were excluded from the genotype comparison analysis. All analyses were performed using SAS 9.1.3 (SAS Institute Inc., Cary, NC).

RESULTS

A comparison of baseline characteristics between CAD cases and noncases is shown in Table 1. Lp-PLA₂ activity was significantly higher in persons who went on to develop CAD compared to those who did not (p=0.02, Table 1). After adjusting for LDL-cholesterol, the difference in Lp-PLA₂ by CAD status was no longer statistically significant (p=0.12). Adjustment for HDL also attenuated the difference in Lp-PLA₂ by CAD cases and noncases, but it remained statistically significant (p=0.04). Lp-PLA₂ activity was significantly and positively correlated with total and LDL-cholesterol, diastolic blood pressure, white blood cell count, and waist-hip ratio, but not body mass index (Table 2). Lp-PLA₂ also had an inverse significant correlation with HDL-cholesterol and marginally significant positive correlations with triglycerides and systolic blood pressure (Table 2).

In an unadjusted Cox proportional hazards model, higher Lp-PLA₂ activity was significantly associated with an increased risk of developing CAD (hazard ratio (HR) =1.54 per one standard deviation increase in Lp-PLA₂ activity, p=0.009) (Table 3). However, after forward selection, in a model adjusting for T1D duration, sex, CRP, LDL-cholesterol, systolic blood pressure, blood pressure medication use, and white blood cell count, Lp-PLA₂ activity was no longer significantly associated with CAD risk (HR=1.15, p=0.59). Alternative models were fit including HDL-cholesterol both in place of, and in addition to, LDL-cholesterol, age in place of T1D duration, and waist circumference in place of waist-hip ratio, but the results showed little difference from those presented in Table 3 (data not shown). No interactions were found between Lp-PLA₂ activity and either of the lipoproteins. An Lp-PLA₂–CRP interaction term, added to the final model shown in Table 3, was found to be statistically significant (p=0.02)

In an unadjusted model assessing the relationship between combined categories of Lp-PLA₂ activity and CRP with CAD, those in the high Lp-PLA₂ activity – high CRP group had significantly elevated risk of developing CAD (HR=3.79, p=0.004) compared to the low-low group (Table 4). After covariate adjustment, this relationship remained, but a little attenuated, with the high Lp-PLA₂ activity – high CRP group being nearly 3 times more likely to develop CAD than the low Lp-PLA₂ – low CRP group (HR=2.89, p=0.06).

Lp-PLA₂ activity level did not differ by haptoglobin genotype, with a mean of 146.4 (SD 32.1) in the Haptoglobin 2/1 genotype and 145.1 (SD 32.6) in the Haptoglobin 2/2 genotype (p=0.89). Although an Lp-PLA₂ activity – haptoglobin interaction term was not significant with respect to CAD incidence (p=0.45), after stratifying by haptoglobin genotype, in the 2/1 genotype, the association between Lp-PLA₂ activity and CAD was statistically significant (HR 1.80, p=0.02) and remained so after adjustment for CRP, T1D duration, LDL-cholesterol, HbA1c, and triglycerides (HR 2.40, p=0.05) (Table 5). The increased HR was largely due to the addition of triglycerides into the model. In contrast, there was no apparent association between Lp-PLA₂ activity and CAD in the 2/2 genotype (unadjusted HR 1.18 p=0.47, fully adjusted HR 0.66 p=0.27) (Table 5). The results remained similar when both genotype models were forced to contain the same covariates for adjustment, namely, T1D duration, sex, LDL-cholesterol, ln(Triglycerides), and CRP, covariates that were significant predictors of CAD in an overall model of the participants with genetic data available, (Hp 2/1 HR=1.97 (0.98, 3.95), p=0.05, Hp 2/2 HR=0.56 (0.23, 1.34), p=0.19). The

relationship between CRP and CAD was slightly weaker in the 2/2 genotype (adjusted HR 1.14, $p=0.01$) compared to the 2/1 genotypes (adjusted HR 1.63, $p=0.001$). A comparison of the incidence of CAD per 100 person years by stratified groups suggested that having both high Lp-PLA₂ activity and high CRP may lead to an increased risk of CAD in persons with the 2/1 genotype (11.9 events per 100 person years compared to the low Lp-PLA₂ activity, low CRP group rate of 1.15 events per 100 person years, $p=0.01$). This was not seen in those with the 2/2 genotype (Figure 1), where rates were similar across groups ($p=0.73$). While no analyses were possible by subtype of CAD events due to small sample size, in both Hp 2/1 and 2/2, angina comprised approximately 50% of the CAD events in the high Lp-PLA₂ activity and high CRP category.

DISCUSSION

In this cohort of childhood-onset type 1 diabetic persons with proteinuria, elevated CRP levels are associated with an increased risk of CAD, while Lp-PLA₂ activity is only associated with an increased risk of CAD if CRP is also high. Intriguingly, however, Lp-PLA₂ activity further increases risk in the haptoglobin 2/1 subgroup (but not Hp 2/2), even after adjustment for CRP and other factors. These results suggest that Lp-PLA₂ activity may add to the prediction of CAD in T1D persons who are thought to have a lower genetic predisposition to cardiovascular disease. We are unaware of any previous reports of this relationship in type 1 diabetes.

The mean level of Lp-PLA₂ activity in the current report, of 145.4 nmol/min/mL (sd 32.9), is higher than the levels reported by most other studies. In reports by Furberg *et al.*,⁵⁷ Kim *et al.*,¹⁷ and Oei *et al.*,¹³ mean Lp-PLA₂ activity was <50 nmol/min/mL. In contrast, however, reports by Allison *et al.*⁵⁸ and Koenig *et al.*¹⁶ reported levels similar to those seen in the current study (mean Lp-PLA₂ activity 145.2 (33.5) and 122.4 (26.2) nmol/min/mL, respectively). One possible reason for these higher levels is that the participants in these studies had either prior coronary heart disease¹⁶ or had previously been referred to a vascular testing center⁵⁸, whereas most of the participants in the studies with lower levels of Lp-PLA₂ activity were healthy at the time the sample was collected, except for the report by Furberg, where approximately 10% had a history of myocardial infarction.⁵⁷ The participants in our study are thus more comparable to those in the Allison⁵⁸ and Koenig¹⁶ reports, as having both type 1 diabetes and proteinuria puts them at an increased risk of developing CAD.

Lp-PLA₂ activity was correlated with traditional cardiovascular disease risk factors, including total, LDL, and HDL-cholesterol, both of which, to different extents, carry Lp-PLA₂ in the blood. Lp-PLA₂ activity was also correlated with diastolic blood pressure, white blood cell count, and waist-hip ratio. It was not correlated with CRP or measures of proteinuria and kidney function. The lack of correlation with CRP is similar to the findings of the West of Scotland Coronary Prevention Study (WOSCOPS)¹⁰, ARIC¹², the Rotterdam study¹³, the report by Brilakis *et al.*¹⁴, the Ludwigshafen Risk and Cardiovascular Health Study¹⁵, but is in contrast to Koenig *et al.* who reported a positive correlation between the two markers.¹⁶

A few studies of the general population have examined the Lp-PLA₂ –CRP relationship with respect to cardiovascular disease incidence.^{11–13, 59} Most of these studies looked at Lp-PLA₂ mass. In ARIC, a significant three way interaction between Lp-PLA₂ mass, CRP, and LDL was found, so that in those with LDL <130 mg/dl, high Lp-PLA₂ was associated with coronary heart disease when CRP is also elevated.¹² In the current analysis, no interaction between Lp-PLA₂ activity and LDL cholesterol was observed (data not shown). Similarly, analysis of middle-age men in the Monitoring of Trends and Determinants in Cardiovascular

Disease Augsburg (MONICA) survey database also showed that persons with elevations in both Lp-PLA₂ mass and CRP were at an increased risk of coronary heart disease compared to those with an elevation in either marker alone, but did not report directly testing for an interaction.¹¹ In contrast, the Women's Health Study (WHS) did not find an association between Lp-PLA₂ mass and cardiovascular disease, though CRP was significantly associated with disease incidence.⁵⁹ As in our study, the Rotterdam Study examined Lp-PLA₂ activity and reported an independent association between Lp-PLA₂ activity and coronary heart disease, however there was no interaction between Lp-PLA₂ activity and CRP.¹³ We have also shown that while the level of Lp-PLA₂ did not differ between haptoglobin genotype, Lp-PLA₂ activity was associated with CAD in the 2/1 genotype but not in the 2/2 genotype. Similarly, the incidence of CAD is elevated in those with high Lp-PLA₂ and high CRP in persons with the 2/1 genotype but not the 2/2 genotype. We are unaware of any prior reports suggesting this relationship between haptoglobin and Lp-PLA₂ with respect to CAD.

It is interesting to speculate why Lp-PLA₂ would only predict CAD in the lower risk Hp 2/1 subgroup. It is clearly not simply due to absolute levels being increased only in Hp 2/1 CAD cases, as the mean values for CAD cases are similar in both Hp 2/1 and 2/2 (data not shown). The most likely explanation, beyond chance, is that of a complex interplay with other risk factors and time to CAD event. For example, given one's genetic background (Hp 2/2), one may be predisposed to CAD events, thus the additive effect of Lp-PLA₂ activity is difficult to demonstrate. However, in a lower risk group (Hp 2/1), this added risk (Lp-PLA₂ activity) becomes evident. While in Hp 2/1, those with Lp-PLA₂ activity below the median level seemed to be protected in terms of time to first CAD event (mean follow-up time to event approximately 11.5 years), those with Lp-PLA₂ activity above the median level had a mean time to event that was similar to that seen in Hp 2/2 (mean follow-up time to event approximately 7 years). This difference in time to CAD event demonstrates that Lp-PLA₂ activity yields a comparable risk in Hp 2/1 to that of Hp 2/2 overall. This result is also consistent with events being generally more inflammatory induced in Hp 2/1 and less so in Hp 2/2 where oxidative mechanisms may predominate. However, these results should be viewed with caution, owing to the small sample size (ie. a total of 70 individuals and 35 CAD events), although, the sample size is quite large for long term follow-up of the type of participant being studied, ie. persons with type 1 diabetes and renal disease.

Our study has many strengths, including a prospective design with long term follow-up (through 18 years) to examine the incidence of CAD events which were confirmed by medical records. The major limitation of the study is the small sample size, particularly the small number of study participants with the 1/1 haptoglobin genotype, which does not allow us to run multivariate analysis within that subgroup. Additionally, there is a potential for a survivor bias in the high CAD risk 2/2 genotype group, such that the most at-risk individuals are not represented due to death prior to commencement of the study.

In conclusion, this study among individuals with type 1 diabetes and renal disease has demonstrated an interaction between Lp-PLA₂ activity and CRP with respect to CAD risk. However, this relationship does not seem to exist in individuals with the higher-risk 2/2 haptoglobin genotype. Additionally, in persons with the 2/1 haptoglobin genotype, Lp-PLA₂ activity is an independent predictor of CAD incidence, suggesting that it may be useful as a marker of risk in persons expected to have a lower genetic susceptibility to developing CAD.

Acknowledgments

This research was supported by National Institutes of Health Grant DK34818.

We thank the EDC staff and all study participants for their contributions.

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Figure 1.
Coronary artery disease (CAD) incidence per 100 person years of follow-up by lipoprotein-associated phospholipase A2 (Lp-PLA2) activity and C-reactive protein combined category and Haptoglobin genotype

Table 1

Baseline Characteristics by Coronary Artery Disease Status

	CAD Cases (n=41)	Non-Cases (n=55)	P-value
Diabetes Duration (years)	25.5 (6.0)	19.0 (5.4)	<.0001
Age (years)	32.4 (5.7)	27.5 (5.4)	<.0001
Sex (% female, n)	39.0% (16)	58.2% (32)	0.06
Lp-PLA₂ activity (nmol/min/mL)	154.6 (32.3)	138.6 (31.8)	0.02
C-Reactive Protein (CRP) (mg/dL)	3.3 (3.5)	2.8 (5.7)	0.67
Haptoglobin 2/2 genotype ^{*,†}	56.4% (22)	51.2% (21)	0.64
White Blood Cell Count (× 10³/mm²)	7.8 (2.2)	6.7 (1.7)	0.006
HbA1c (%)	8.7 (1.2)	9.0 (1.8)	0.29
Body Mass Index (kg/m²)	24.6 (3.2)	23.2 (2.9)	0.03
Waist-Hip Ratio	0.85 (0.07)	0.82 (0.08)	0.03
Current Smoker [†]	36.6% (15)	27.3% (15)	0.38
Ever Smoker [†]	46.3% (19)	40.0% (22)	0.53
Systolic Blood Pressure (mmHg)	123.3 (15.7)	118.8 (15.6)	0.16
Diastolic Blood Pressure (mmHg)	77.4 (12.4)	72.2 (11.3)	0.01
Hypertension[†]	58.5% (24)	25.5% (14)	0.001
Blood Pressure Medication Use[†]	39.0% (16)	20.8% (11)	0.05
Total Cholesterol (mg/dl)	217.2 (39.9)	205.4 (47.2)	0.19
HDL Cholesterol (mg/dl)	51.4 (11.5)	56.3 (14.1)	0.07
LDL Cholesterol (mg/dl)	139.6 (31.5)	123.3 (35.9)	0.03
Triglycerides [‡] (mg/dl)	113 (88–169)	91 (68–135)	0.06
Albumin Excretion Rate [‡] (μg/min)	881.3 (436.2–1399.0)	514.6 (357.3–1297.9)	0.35

Data are means (sd) unless otherwise noted.

* n=80 participants with available genetic material

[†] % (n)

[‡] Median (Interquartile Range)

Table 2

Type 1 Diabetes Duration-Adjusted Partial Correlations between Lipoprotein-Associated Phospholipase A₂ (Lp-PLA₂) Activity and Coronary Artery Disease Risk Factors

Risk Factor	partial r	p-value
HbA _{1c}	−0.007	0.95
Total Cholesterol	0.48	<.0001
HDL-Cholesterol	−0.28	0.01
LDL-Cholesterol	0.58	<.0001
Triglycerides ^I	0.19	0.08
Systolic Blood Pressure	0.19	0.09
Diastolic Blood Pressure	0.23	0.04
Body Mass Index	−0.01	0.93
Waist-Hip Ratio	0.30	0.002
White Blood Cell Count	0.30	0.006
C-Reactive Protein	−0.02	0.88
Albumin Excretion Rate ^I	0.11	0.33

Pearson's partial correlation unless indicated

^I Spearman's rank partial correlation

Table 3

Hazard Ratio of Coronary Artery Disease (CAD) Event Associated with a 1 Standard Deviation Increase in Lipoprotein-Associated Phospholipase A₂ (Lp-PLA₂) Activity

Model 1 ^a	Model 2 ^b	Model 3 ^c	Model 4 ^d
Hazard Ratio (95% Confidence Interval), P-value			
1.54 (1.11, 2.12), 0.009	1.60 (1.15, 2.23), 0.005	1.31 (0.91, 1.87), 0.13	1.15 (0.70, 1.87), 0.59

^aModel 1: Lp-PLA₂ Activity (unadjusted)

^bModel 2: Lp-PLA₂ Activity and CRP

^cModel 3: Lp-PLA₂ Activity, CRP, T1D duration, sex

^dModel 4: Final model after forward selection - Lp-PLA₂ Activity, CRP, T1D duration, sex, LDL-cholesterol, systolic blood pressure, blood pressure medication use, white blood cells

In addition to the variables included in the final model, the following were also made available to forward selection models: HbA1c, waist-hip ratio, triglycerides, diastolic blood pressure, and smoking status.

Table 4

Hazard Ratios of Coronary Artery Disease (CAD) Event Associated with Combined Lipoprotein-Associated Phospholipase A₂ (Lp-PLA₂) Activity and C-Reactive Protein (CRP) Level

Model 1 ^a	Model 2 ^b	Model 3 ^c
Hazard Ratio (95% Confidence Interval), P-value		
Low Lp-PLA ₂ Activity – Low CRP	(Reference)	(Reference)
Low Lp-PLA ₂ Activity – High CRP	2.28 (0.85, 6.12), 0.10	3.67 (1.35, 9.93), 0.01
High Lp-PLA ₂ Activity – Low CRP	2.06 (0.78, 5.40), 0.14	1.43 (0.53, 3.85), 0.48
High Lp-PLA ₂ Activity – High CRP	3.79 (1.54, 9.34), 0.004	3.44 (1.37, 8.66), 0.009

Low Lp-PLA₂ Activity defined as Lp-PLA₂ activity < median value (147.20 (nmol/min/mL))

High Lp-PLA₂ Activity defined as Lp-PLA₂ activity ≥ median value (147.20 (nmol/min/mL))

Low CRP defined as CRP < median value (1.56 (mg/dl))

High CRP defined as CRP ≥ median value (1.56 (mg/dl))

^aModel 1: unadjusted

^bModel 2: Combined Lp-PLA₂ Activity-CRP level, T1D duration, sex

^cModel 3: Combined Lp-PLA₂ Activity-CRP level, T1D duration, sex, LDL-cholesterol, systolic and diastolic blood pressures, white blood cell count

In addition to the variables included in the final model, the following were also made available to forward selection models: HbA1c, waist-hip ratio, triglycerides, blood pressure medication use, and smoking status.

Table 5

Hazard Ratios of Coronary Artery Disease (CAD) Event Associated with a 1 Standard Deviation Increase in Lipoprotein-Associated Phospholipase A₂ (Lp-PLA₂) Activity Stratified by Haptoglobin (Hp) Genotype

	Hp 2/1 (n=34, 16 events)	Hp 2/2 (n=36, 19 events)
	Hazard Ratio (95% CI), P-value	Hazard Ratio (95% CI), P-value
Model 1^a	1.80 (1.10, 2.97), 0.02	1.18 (0.75, 1.85), 0.47
Model 2^{b,c}	2.40 (1.02, 5.64), 0.05	0.66 (0.31, 1.39), 0.27

^aModel 1: Lp-PLA₂ Activity (unadjusted)

^bModel 2, Hp 2/1: Final model after forward selection, Lp-PLA₂ Activity, C-reactive protein, type 1 diabetes duration, sex, HbA1c, LDL-cholesterol, ln(Triglycerides)

^cModel 2, Hp 2/2: Final model after forward selection, Lp-PLA₂ Activity, type 1 diabetes duration, sex, LDL-cholesterol

In addition to the variables included in the final model, the following were also made available to forward selection models: waist-hip ratio, blood pressure, blood pressure medication use, smoking status.