The investigation of lipoprotein-related phospholipase A2 (LP-PLA2) V279F mutation in coronary artery disease

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Abstract

Aim: Despite advances in the treatment of cardiovascular diseases, coronary artery disease (CAD) is the leading cause of death worldwide. The media layer of normal and diseased arteries contains lipoprotein-associated phospholipase A2 (Lp-PLA2), which is mostly made by mast cells, T lymphocytes, and monocytes. Our research aimed to define how the Lp-PLA2 and V279F gene polymorphisms are related to CAD.

Materials and Methods: This study included 109 CAD patients and 71 controls. Fasting blood glucose (FBG) and lipid profiles were measured via the enzymatic colorimetric technique. Lp-PLA2 levels were detected with ELISA. The genomic DNAs were isolated from whole blood. Real-time polymerase chain reaction (RT-PCR) with the V279F mutation detection kit was used to identify the Lp-PLA2 V279F mutation.

Results: In the CAD group compared to controls, serum Lp-PLA2 levels were higher (p=0.001). While FF (mutant) genotypes and VF (heterozygous) genotypes were absent in both groups, the Lp-PLA2 V279F mutation was discovered as the VV (wild) genotype in both the CAD group and controls.

Conclusion: As a result, we determined that the Lp-PLA2 V279F mutation cannot be considered a genetic risk factor for CAD, but that Lp-PLA2 elevation may be an important parameter for CAD. Keywords: Lp-PLA2, Coronary artery disease, V279F.

Introduction

Coronary Artery Disease (CAD) is still the leading cause of death in humans in many countries today. Among the risk factors for CAD, smoking, dyslipoproteinemia, diabetes mellitus, high blood pressure, and family history all play significant roles. Atherosclerosis, which is known to be a chronic inflammatory disease, progresses in response to genetic, metabolic, and environmental damage [1, 2].

The Framingham Heart Study defined the early biomarker that could be employed for the pathogenesis of atherosclerosis and identified cholesterol as an essential factor in the pathology of CAD [3]. In many clinical studies, it has been observed that low-density lipoprotein (LDL) and total cholesterol (TC) increase in people at cardiovascular risk and that reducing their levels by administering medication has beneficial effects. However, many people with normal LDL continue to develop atherosclerosis, indicating that additional variables may possibly be involved in the onset of this condition [4-6]. Recently, a relationship between lipoprotein-associated phospholipase A2 (Lp-PLA2) and endothelial dysfunction has been identified independent of other CAD risk factors, and clinical studies and cohort studies indicated a relationship between CAD and Lp-PLA2 [7-10]. The liver, mast cells, macrophages, T-lymphocytes, and monocytes are the predominant sources from which Lp-PLA2 is produced. These cells participate in the formation of atherogenesis and atherosclerosis. The Lp-PLA2 is mostly bound to LDL cholesterol in humans, with very small amounts bound to very low-density lipoprotein (VLDL) and high-density lipoprotein (HDL). Oxidized phospholipids in the LDL particle are substrates for Lp-PLA2. When the phospholipids on the LDL particle are oxidized, Lp-PLA2 cleaves the fatty acid at the sn-2 position on glycerol and produces two potent mediators, oxidized free fatty acid (oxFFA), and lysophatidylcholine (LPC) [8-10]. OxFFA and LPC play a role in inflamma-

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tion and both have pro-apoptotic effects on macrophages and also have high solubility, so they can easily diffuse into atheroma and act on various cell types, which have a role in atherosclerosis. LPC is a potent chemoattractant for monocytes and T cells, inducing apoptosis in smooth muscle cells promoting endothelial dysfunction, and stimulating macrophage proliferation. Foam cells and activated macrophages produce more Lp-PLA2 [9-13]. A higher oxidation degree of LDL indicates higher LPC concentration. Studies have displayed that oxidized LDL increases the risk of atherosclerosis. In addition, OxFFA and LPC stimulate the formation of adhesion particles and cytokines by plaque-based macrophages, endothelial cells, and leukocytes [14,15].

The Lp-PLA2 gene is based at the 6q21.2-p12 region of the chromosome and consists of 12 exons. The V279F mutation, first identified in Lp-PLA2, is a single point mutation in exon 9 at nucleotide 994, with Thymine (G994T) replaced by Guanine. This nucleotide change causes the formation of Valine 279 Phenylalanine (V279F) in the mature protein and a lack of activity in the Lp-PLA2 enzyme. It has been defined that the molecular basis of Lp-PLA2 enzyme deficiency is caused by a single point mutation of V279F close to the active site of the enzyme [13,16].

The exposure of patients without traditional cardiovascular risk factors to cardiovascular events has prompted researchers to seek new markers that can better predict cardiovascular risks. Therefore, we examined Lp-PLA2 levels and Lp-PLA2 V279F single point mutation in relation to CAD to find whether Lp-PLA2 is a new risk factor.

Materials and Methods

Study subjects

Our study group consisted of 180 individuals, 46 females and 134 males, presented to the Mersin University Hospital Cardiology outpatient clinic with chest pain and underwent coronary angiography between September 2010 and September 2011. According to the angiography results, 109 subjects with a minimum of one coronary artery stenosis over 70% formed the patient group, and 71 subjects without coronary artery lesions formed the control group. Patients with a history of coronary artery bypass graft surgery, inflammatory disease, infectious disease, hematological disease, autoimmune disease, kidney disease, liver disease, or malignancy, were excluded from the study. Power analysis was performed using G Power 3.0.10 for Windows. In order for the difference between the patient and control groups in terms of the Lp-PLA2 parameter to be found statistically significant, at least 100 cases in each group are sufficient with a Type I error of 0.05 and 80% power. Fifteen subjects who did not meet the inclusion criteria in the control group were excluded from the study, while 13 subjects in the control group refused to participate in the study.

The Mersin University University Clinical Research Ethics Committee approved the study (05.15.2009, 2009/105) and informed consent was acquired from all individuals enrolled in the research.

Blood sampling

During the angiography procedure, 10 mL of blood was taken from the femoral artery, and it was divided into tubes containing ethylenediaminetetraacetic acid (EDTA) for DNA extraction and biochemistry tubes for separating the serums. Whole blood taken to detect the Lp-PLA2 V279F mutation in the patient and control groups was stored at +4 ºC for DNA isolation until analysis. Peripheral venous blood taken into biochemistry tubes for lipid profile and fasting blood glucose (FBG) levels were centrifuged at 4000 rpm for 10 minutes and serum was allocated and studied in a Cobas 501 autoanalyzer (Roche Diagnostics, Manheim GmbH, Germany). Serum Lp-PLA2 levels were measured by the enzyme-linked immunosorbent assay (ELISA) method using the ELISA (Cat. No. E90867Hu, USCN Life Science Inc., China) kit.

DNA extraction and genotyping

To perform genetic analysis, peripheral blood was drawn into tubes containing EDTA. DNA extraction was performed from circulating leukocytes by utilizing a high pure polymerase chain reaction (PCR) template preparation kit (Roche Diagnostics, GmbH, Mannheim, Germany, catalog no: 1 796 828). DNA was extracted according to the methodology laid out by Roche Diagnostics GmbH. For the DNA extraction step of this procedure, we employed proteinase K, binding buffer, inhibitor removal buffer, isopropanol, wash buffer, and elution buffer. Lp-PLA2 V279F gene polymorphism was analyzed by utilizing LightCycler mutation detection kits (TIB MOLBIOL) in real-time-PCR with a LightCycler instrument (Roche Diagnostics, Manheim, Germany). The primer sequences were:

- forward primer: 5'_ CAAATA ggACTC-TATTgATAgg g_3';

- reverse primer: 5'_ gCTTTACTATCTTATTTTCT-TACCTg_3'

- and the probes sequences were detection probe: 5'_AgTCTgAATAACCgTTgCTCC--FL_3'

- and anchor probe: 5'_LC-CCAAAA gAATgTCCAATTACTgCTATTTTT--PH_3'.

Statistical analysis

The obtained data were analyzed using IBM Statistical Package for the Social Sciences Demo Version 20.0 package (IBM SPSS Inc. Free Download, Chicago, Illinois, USA) program. The Kolomogorov-Smirnov and Shapiro-Wilk tests were used to evaluate the normal distribution, and the Levene test was used to examine the homogeneity of variances. Quantitative data conforming to the normal distribution were presented as mean ± standard deviation and compared with independent samples t-test or One-Way ANOVA depending on the number of groups compared. Qualitative data were summarized with frequency and percentage, and the chi-square test was used to analyze qualitative data. The logistic regression analysis was performed to determine the relationship between categorical variables. A p-value of <0.05 was considered a statistical significance level.

Results

The study included 109 healthy individuals with a mean age of 62.6 ± 9.4 years (17 females and 92 males) and 71 individuals with CAD with a mean age of 55.8 ± 11.6 years (29 females and 42 males). The distribution and descriptive information of risk factors in CAD and control groups are given in Table 1. The age difference between groups was statistically significant ($p=0.001$). Males were 3.7 times more likely to have CAD than females and represented a higher proportion of CAD patients compared to controls (OR: 3.73, 95% CI: 1.85-7.53; $p=0.001$). Smokers had a 2-fo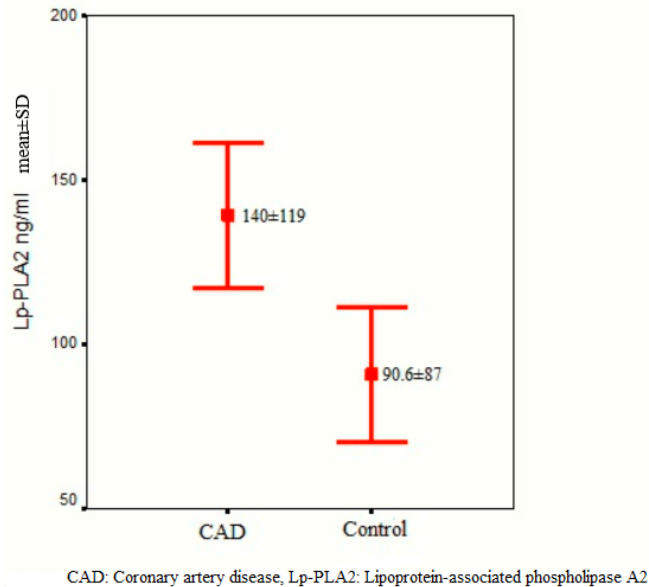ld ($p=0.023$) risk of CAD and those with a family history of CAD had a 2.89-fold ($p=0.002$) risk of CAD. The presence of hypertension ($p=0.092$) and diabetes ($p=0.16$), which are considered among the risk factors for CAD, was not a risk factor for CAD.

Compared to controls, serum FBG levels were higher in the CAD group ($p=0.01$) and due to the usage of statin group medications with cholesterol-lowering effects in the CAD group, there was no significant difference between the two groups' cholesterol levels, which is the main risk factor for CAD ($p=0.27$) (Table 2). Lp-PLA2 levels of both groups are presented in Figure 1. The Lp-PLA2 level was 90.6 ± 87 ng/mL in the control group, while it was 140 ± 119 ng/mL in the CAD group. Serum Lp-PLA2 levels were higher in the CAD group than in controls ($p=0.0029$). Lp-PLA2 levels were 102.31 ± 94.74, 142.44 ± 123.9, and 173.17 ± 122.3 ng/mL in vessel 1, vessel 2, and vessel 3, respectively (Figure 2). Levels of Lp-PLA2 increased according to the number of occluded coronary arteries, and patients with 3 involved vessels and above had the highest Lp-PLA2 levels. A statistically significant difference was detected only between those with 3 involved vessels and above and those with 1 involved vessel ($p=0.016$). In both groups, only the Lp-PLA2 V279V (wild) genotype was detected as 100%. Lp-PLA2 V279F heterozygous and homozygous mutant genotypes were not detected.

CAD: Coronary artery disease, Lp-PLA2: Lipoprotein-associated phospholipase A2

Figure 1. Lp-PLA2 levels in coronary artery patients and control groups.

Lp-PLA2: Lipoprotein-associated phospholipase A2
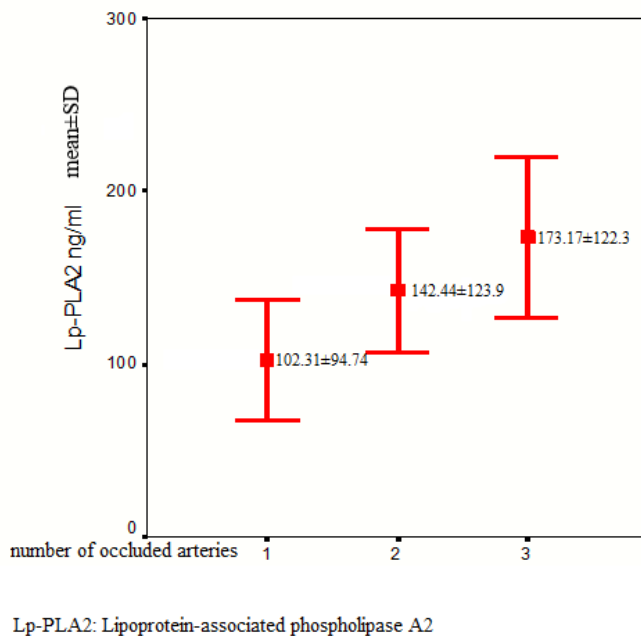
Figure 2. Lp-PLA2 levels according to the number of occluded coronary arteries in coronary artery patients.

Discussion

Platelet-activating factor acetylhydrolase (PAFAH), also known as LP-PLA2, has a major role in the pathogenesis of atherosclerosis. In our findings, the Lp-PLA2 level was significantly higher in the CAD ($p=0.002$). When the Lp-PLA2 levels were compared according to the number of occluded coronary arteries, we found that the number of CAD patients with 3 or more occluded coronary arteries was higher than those with one occluded coronary artery ($p=0.016$). In the literature, we found no study comparing the number of occluded coronary arteries and Lp-PLA2 levels. Many researchers have suggested that Lp-PLA2 may be a new inflammatory marker as an independent risk factor associated with CAD [17,18]. Koenig et al. [19] found a high level of Lp-PLA2 in CAD, independent of other cardiac risk markers. Kicchl et al. [20] found levels of Lp-PLA2 one standard deviation (SD) higher in the CAD group compared to controls.

In a prospective study evaluating atherosclerosis in more than 12 000 healthy middle-aged individuals over 6 years, Ballantyne et al. [21] reported that CRP and levels of Lp-PLA2 were significantly related to CAD. Caslake et al. [22] found higher serum Lp-PLA2 in 94 CAD patients compared to 54 controls. Wang et al. [23] reported that patients with severe stenosis in intracranial atherosclerosis had higher Lp-PLA2 levels. Xi et al. [24] detected elevated levels of Lp-PLA2 related to the risk of cardiovascular events and death in a middle-aged population. Lp-PLA2 levels can be affected by various factors related to the severity, phase (acute vs. chronic), and process of the disease [17, 19-25]. In order to identify whether the change in Lp-PLA2 activity is the result of an underlying cause of atherosclerotic disease or potentially a reaction to atherosclerotic changes, it is also critical to identify the patient's Lp-PLA2 genotype. The Lp-PLA2 V279F genotype

Table 1. Distribution of risk factors and descriptive information in control and CAD groups.

		Control Group n=71	CAD Group n=109	Odd's Ratio	Confidence Interval (%95)	p-value
Age		55.89±11.68	62.69±9.44			0.001
Gender	Female	29 (40.8)	17 (15.6)	-------		0.001
	Male	42 (59.2)	92 (84.4)	3.73	1.85-7.53	
Diabetes (Type 2)	-	55 (77.5)	74 (68.0)	-------		0.16
	+	16 (22.5)	35 (32.0)	-------		
Hypertension	-	33 (46.5)	37 (32.1)	-------		0.092
	+	38 (53.5)	72 (67.9)	-------		
Smoking	-	54 (76.1)	67 (61.5)	-------		0.04
	+	17 (23.9)	42 (38,5)	2.00	1.02-3.88	
Family History	-	54 (72.6)	57 (52.3)	-------		0.002
	+	17 (27.4)	52 (47.7)	2.89	0.473-1.86	
Number of occluded coronary arteries	0	71	----			
	1	----	31 (30.0)			0.106
	2	----	49 (31.0)			0.291
	3 and above	----	29 (39.0)			0.016

Continuous variables are given as mean±standard deviation. n: Number of samples, p: Significance between group. CAD: Coronary artery disease.

Table 2. Fasting Blood Sugar and lipid profile levels of control and CAD groups.

	Control Group	CAD Group	p-value
Fasting Blood Sugar*	99.87±19.99	112.89±45.44	0.01
Total Cholesterol*	183.07 ± 50.88	175.52 ± 39.87	0.27
HDL-Cholesterol*	42.05 ± 12.02	40.64 ± 10.07	0.41
LDL-Cholesterol*	99.11 ± 35.44	111.6 ± 41.59	0.56
VLDL-Cholesterol*	31.18 ± 18.91	31.51 ± 19.45	0.91
Triglyceride*	155.91±94.59	170.00±88.88	0.15

* Continuous variables are given as mean±standard deviation, and concentration units are mg/dl. p: Significance between groups. CAD:Coronary artery disease, FBG: Fasting blood glucose, TC: Total cholesterol, TG: Triglyceride, HDL: high-density lipoprotein, LDL: low-density lipoprotein.

distribution differs between races. In the study in which more than 2000 individuals participated in South America and Europe, no heterozygous or homozygous genotype was detected [26]. The V279F mutation has primarily been found in the Japanese population. Although it was later detected in ethnic groups such as Turks and Kyrgyz, it was not detected in Caucasians. The V279F mutation is associated with decreased Lp-PLA2 levels in 27% of heterozygous and 4% of homozygous Japanese individuals due to a defect in enzyme secretion. Researchers have discovered that the V279F mutation is linked to cerebral hemorrhage, non-familial dilated hypertrophic cardiomyopathy, abdominal aortic aneurysm, atherosclerotic occlusive diseases, and stroke in men in the Japanese population. [24-28].

According to Yamada et al. [29] there was a larger prevalence of the V279F mutant allele in male Japanese patients with MI (homozygous, 2.1%; heterozygous, 33.0%) than in controls (homozygous, 2.2%; heterozygous, 21.0%). No significant correlation was found between the mutant genotype and CAD. Hiramoto et al. [30] found the V279F mutation in Japanese stroke patients as the heterozygous genotype as 39.2% and the homozygous genotype as 4.2%, and in the control group, the heterozygous genotype was 22.4% and the homozygous genotype was 3%. They found higher homozygous genotypes in the patient group than in controls and suggested that the V279F mutation may be a genetic risk factor in atherosclerotic diseases [30]. Balta et al. [27] verified the presence of the V279F mutation in non-Japanese populations. In their study, healthy 358 Turks, 100 Azeris, and 143 Kyrgyz were examined and heterozygous mutations were defined to be 3 (0.84%), 0, and 12 (8.4%), respectively, and no mutant genotype was detected among these individuals [27].

Contrary to other studies in Korea, Jang et al. [31] displayed that the homozygous F279F genotype reduced the risk of CAD in a study conducted with 532 men with CAD and 670 healthy individuals. In the study displayed by Şekuri et al [28] with 164 patients and 142 controls, they found the heterozygous mutation rate as 2.60% in the patient group, while all individuals in the controls were VV genotypes. They found no significant relationship between V279F heterozygous genotype and CAD. According to Ma et al. [32], there was no discernible difference in the frequency of the V279F genotype in controls and CAD patients.

Although there was no Lp-PLA2 V279F mutation in Caucasians, epidemiological studies have displayed that the risk of CAD increases with an increase in Lp-PLA2 levels [20-27,30,33]. However, in studies conducted in Asians, it was suggested that the V279F mutation, which reduces enzyme activity, increases the risk of stroke, CAD, and MI, and therefore LP-PA2 has an anti-atherogenic role in these diseases [29,30,34-36]. This difference between studies is due to genetic variation between the Caucasian and Asian

populations and also due to both the proatherogenic and anti-atherogenic effects of the Lp-PLA2 enzyme.

In our study, all individuals in both CAD and control groups had the VV genotype. Balta et al. [27] found 3 heterozygous genotypes in 358 healthy Turkish individuals. Şekuri et al. [28] determined that there were only 3 heterozygous genotypes in 164 individuals with CAD. The mutant genotype was not detected in either study. In these studies, which included Turkish participants, the V279F heterozygous genotype rates were very low and the mutant genotype was not observed. In our findings, we could not detect the V279F heterozygous and mutant genotype. Therefore, studies in larger populations are required to confirm these findings in the Turkish population. All these studies have shown that Lp-PLA2 has been researched extensively. When the research data are evaluated in general, although it is generally believed that there may be an association between high levels of Lp-PLA2 and CAD, the opposite findings are also noteworthy.

Some restrictions must be noted regarding the current study. Our study's primary limitation is its small participant pool. More extensive research is needed to address this. A significant correlation between Lp-PLA2 V279F mutation and CAD may have been seen if the size of our study group had been large enough.

Conclusion

In conclusion, the Lp-PLA2 level was significantly higher in the CAD group than in controls, while the Lp-PLA2 V279F mutation was not defined in either group. We think that this is because other mutations in the Lp-PLA2 gene such as Arg92His, Ile198Thr, Ile317N, and A379V affect Lp-PLA2 activity, and sequencing analysis with Lp-PLA2 protein expression is required to confirm these findings.

Ethical approval

The Mersin University Ethics Committee approved the study. (05.15.2009, 2009/105).

References

1. Libby P, Theroux P. Pathophysiology of coronary artery disease. Circulation. 2005; 111:3481-3488.
2. Ross R. Atherosclerosis is an inflammatory disease. Am Heart J. 1999; 138 (52): 19-20.
3. Koenig W, Löwel H, Baumert J, Meisinger C. C-reactive protein modulates risk prediction based on the Framingham score: implications for future risk assessment: results from a large cohort study in Southern Germany. Circulation. 2004; 109:1349-1353.
4. Vaina S, Stefanadis C. Detection of the vulnerable coronary atheromatous plaque. Where are we now? Int J Cardiovasc Intervent. 2005; 7:75-87.
5. Atkinson AJ, Colburn WA, DeGruttola VG, DeMets DL, Downing GJ, Hoth DF, et al. Biomarkers Definitions Working Group. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. Clin Pharmacol Ther. 2001; 69:89-95.
6. Ferri N, Paoletti R, Corsini A. Lipid-modified proteins as biomarkers for cardiovascular disease: a review. Biomarkers. 2005; 10:219-237.
7. Lavi S, McConnell JP, Rihal CS, Prasad A, Mathew V, Lerman LO, et al. Local production of lipoprotein-associated phospholipase A2 and lysophosphatidylcholine in the coronary circulation: association with early coronary atherosclerosis and endothelial dysfunction in humans. Circulation. 2007; 115:2715–2721.
8. Kolodgie FD, Burke AP, Skorija KS, Ladich E, Kutys R, Makuria AT, et al. Lipoprotein-associated phospholipase A2 protein expression in the natural progression of human coronary atherosclerosis. Arterioscler Thromb Vasc Biol. 2006; 26:2523–2529.
9. Stafforini DM. Biology of platelet-activating factor acetylhydrolase (PAF-AH, lipoprotein-associated phospholipase A2). Cardiovasc Drugs Ther. 2009; 23:73–83.
10. Corson MA, Jones PH, Davidson MH. Review of the evidence for the clinical utility of lipoprotein-associated phospholipase A2 as a cardiovascular risk marker. Am J Cardiol. 2008; 101:51-50.
11. Lerman A, McConnell JP. Lipoprotein-associated phospholipase A2: A risk marker or a risk factor? Am J Cardiol. 2008; 101:11–22.
12. Anderson JL. Lipoprotein-associated phospholipase A2: An independent predictor of coronary artery disease events in primary and secondary prevention. Am J Cardiol. 2008; 101:23–33.
13. Jang Y, Kim OY, Koh SJ, Chae JS, Ko YG, Kim JY, et al. The Val279Phe variant of the lipoprotein-associated phospholipase A2 gene is associated with catalytic activities and cardiovascular disease in Korean men. J Clin Endocrinol Metab. 2006; 91(9):3521-7.
14. Safaya R, Chai H, Kougias P, Lin P, Lumsden A, Yao Q, et al. Effect of lysophosphatidylcholine on vasomotor functions of porcine coronary arteries. J Surg Res. 2005; 126:182-8.
15. Tselepis AD, John Chapman M. Inflammation, bioactive lipids, and atherosclerosis: potential roles of a lipoprotein-associated phospholipase A2, platelet-activating factor- acetylhydrolase. Atheroscler Suppl. 2002; 3:57-68.
16. Prescott SM, Zimmerman GA, Stafforini DM, McIntyre TM. Platelet-activating factor and related lipid mediators. Annu Rev Biochem. 2000; 69:419–45.
17. Packard CJ, Denis DS. Lipoprotein-associated phospholipase A2 as an independent predictor of coronary heart disease. N Engl J Med. 2000; 343:1148–1155.
18. Rader DJ. Inflammatory markers of coronary risk. N Engl J Med, 2000; 343:1179–11825.
19. Koenig W, Twardella D, Brenner H, Rothenbacher D. Lipoprotein-associated phospholipase A2 predicts future cardiovascular events in patients with coronary heart disease independently of traditional risk factors, markers of inflammation, renal function, and hemodynamic stress. Arterioscler Thromb Vasc Biol. 2006; 26: 1586-93.
20. Kiechl S, Willeit J, Mayr M, Viehweider B, Oberhollenzer M, Kronenberg F, et al. Oxidized phospholipids, lipoprotein(a), lipoprotein-associated phospholipase A2 activity, and 10-year cardiovascular outcomes: prospective results from the Bruneck study. Arterioscler Thromb Vasc Biol. 2007;27(8):1788-95
21. Ballantyne CM, Hoogeveen RC, Bang H, Coresh J, Folson AR, Heiss G, et al. Lipoprotein-associated phospholipase A2, high sensitivity C reactive protein and risk for incident coronary heart disease in middle-aged men and woman in the Atherosclerosis Risk in Communities study (ARIC). Circulation. 2004; 109:837-42.
22. Caslake MJ, Packard CJ, Suckling KE, Holmes SD, Chamberlain P, Macphee CH. Lipoprotein-associated phospholipase A2, platelet-activating factor acetylhydrolase: a potential new risk factor for coronary heart disease. Atherosclerosis. 2000; 150:413–419.
23. Wang Y, Liu G, Song H, Cao C, Ji X, Cao G. Elevated Lipoprotein-Associated Phospholipase A2 Is Associated With Intracranial Atherosclerosis. Front Neurol. 2022;10; 13:858302.
24. Xi H, Cheng GL, Hu FF, Li SN, Deng X, Zhou Y. The Use of Lipoprotein-Associated Phospholipase A2 in a Chinese Population to Predict Cardiovascular Events. Biomed Environ Sci. 2022, 20;35(3):206-214.
25. Khakpour H, Frishman WH. Lipoprotein-associated phospholipase A2: an independent predictor of cardiovascular risk and a novel target for immunomodulation therapy. Cardiol Rev. 2009;17(5):222-229.
26. Karasawa K, Harada A, Satoh N, Inoue K, Setaka M. Plasma platelet-activating factor- acetylhydrolase (PAF-AH). Progress in Lipid Research. 2003, 42:93–114.

27. Balta, G, Gurney, A, Kudayarov, DK, Tunc, B, Altay, C. Evidence for the existence of the PAF acetylhydrolase mutation (Val279Phe) in non-Japanese populations: a preliminary study in Turkey, Azerbaijan, and Kyrgyzstan. Thromb Res. 2001; 101:231-234.

28. Şekuri C, Çam S, Tengiz İ, Ercan E, BayturanM Ö, Berdeli A. Association of platelet-activating factor acetylhydrolase gene polymorphism with premature coronary artery disease in Turkish patients. Anadolu Kardiyol Derg. 2006;6(2):132-4.

29. Yamada Y, Ichihara S, Fujimura T, Yokota M. Identification of the G994T missense mutation 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: 177-81.

30. Hiramoto M, Yoshida H, Imaizumi T, Yoshizumi N, Satoh K. A mutation in plasma platelet-activating factor acetylhydrolase (Val279Phe) is a genetic risk factor for stroke. Stroke. 1997; 28: 2417-20.

31. Jang Y, Waterworth D, Lee JE, Song K, Kim S, Kim HS. Carriage of the V279F Null Allele within the Gene Encoding Lp-PLA2 Is Protective from Coronary Artery Disease in South Korean Males. Plos One. 2011;5;6(4): e18208.

32. Ma S, Ding L, Cai M, Chen L, Yan B, Yang J. Association Lp-PLA2 Gene Polymorphisms with Coronary Heart Disease. Dis Markers. 2022, 2;2022:9775699.

33. Koenig W, Khuseyinova N, Löwel H, Trischler G, Meisinger C. 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; 110:1903-8.

34. Yamada Y, Izawa H, Ichihara S, Takatsu F, Ishihara H, Hirayama H, et al. Predıctıon of the rısk of myocardial ınfarctıon from polymorphısms ın candıdate genes. N Engl J Med. 2002; 12;347(24):1916-23.

35. Paik JK, Chae JS, Jang Y, Kim JY, Kim OY, Jeong TS, et al. Effects of V279F in the Lp-PLA2 gene on markers of oxidative stress and inflammation in Koreans. Clim Chin Acta. 2010; 2:411(7-8):486-93.

36. Hou L, Chen S, Yu H, Lu X, Chen J, Wang L. Association between Lipoprotein-Associated Phospholipase A2 Gene Polymorphism and Coronary Artery Disease in the Chinese Han Population: the Beijing atherosclerosis study. Ann Hum Genet. 2011;75(5):605-11.