Chapter 5

Lipoprotein-Associated Phospholipase A_2 – Pathophysiological Role and Clinical Significance as a Cardiovascular Biomarker

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Additional information is available at the end of the chapter

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Abstract

Within the last decade, a broad range of biomarkers associated with an increased risk for death and cardiovascular/cerebrovascular endpoints have been identified. Epidemiological studies clearly indicate that lipoprotein-associated phospholipase A_2 (Lp-PLA_2) has the potential to become clinically useful emerging biomarker in the true sense, linking plaque biology with cardiovascular/cerebrovascular event rate. Lipoprotein-associated phospholipase A_2 is a specific vascular inflammatory marker, a risk factor, a prognostic biomarker, and also a therapeutic target. This chapter will summarize our current knowledge on Lp-PLA_2 with emphasis on its potential pathophysiological mechanisms of action and on clinical relevance as cardiovascular/cerebrovascular biomarker. This chapter gives comprehensive, systematic review of studies assessing the significance of Lp-PLA_2 in cardiovascular/cerebrovascular diseases with emphasis on clinical benefit of pharmacologic inhibition of Lp-PLA_2.

Keywords: biomarker, cardiovascular disease, lipoprotein-associated phospholipase A_2

1. Introduction

Although an atherogenic lipoprotein phenotype was reported as a predictor of cardiovascular/cerebrovascular disease, numerous recent studies have recognized additional lipid-related markers as emerging biomarkers to identify patients with cardiovascular/cerebrovascular disease risk. Among them the most promising biomarkers for cardiovascular/cerebrovascular risk assessment is lipoprotein-associated phospholipase A_2 (Lp-PLA_2).

2. Lipoprotein-associated phospholipase A_2: structure and biology

Lipoprotein-associated phospholipase A_2 (Lp-PLA_2), also known as platelet-activating factor acetylhydrolase (PAF-AH), belongs to the phospholipase A_2 superfamily [1,2]. This Ca^{2+}-independent phospholipase is encoded by *PLA2G7* gene that consists of 12 exons and 11 introns located on chromosome 6p21.2 to 12 [3,4]. Lp-PLA_2 is protein of 45,4 kDa that consists of 441 amino acid residues [5]. The major sources of Lp-PLA_2 in plasma are T lymphocytes, monocytes/macrophages, activated bone marrow-derived mast cells, and liver cells [6-8]. The secreted Lp-PLA_2, circulates in plasma in active form. It predominantly binds to LDLs, and in a much smaller extent to HDLs, Lp(a), lipoprotein remnants, and platelet-borne microparticles [6,9-12]. Indeed, Lp-PLA_2 is highly associated with the smallest LDL and HDL subclasses [13] and with electronegative LDL, which overlaps with small dense LDL [14]. Lp-PLA_2 bound to HDL has a much lower specific activity compared to when bound to LDL [10]. Different distribution of Lp-PLA_2 in various plasma lipoproteins affects its functions.

Lp-PLA_2 catalyzes hydrolysis of the acetyl group at sn-2 position of PAF to generate lyso-PAF and acetate [15]. On the other side, Lp-PLA_2 cleaves oxidatively modified lipoproteins from the sn-2 position of the apoB100-containing lipoproteins into oxidized nonesterified fatty acids (oxFFAs) and lysophosphatidylcholine.

3. Actions of lipoprotein-associated phospholipase A_2

We can think about Lp-PLA_2 as a friend and foe at the same time. On the one hand, it functions as PAF-AH, which hydrolyzes inflammatory mediator PAF, inhibits foam cell formation, enhances cholesterol efflux in macrophages, and exerts its atheroprotective role [16].

On the other hand, Lp-PLA_2 has a proatherogenic role. Lp-PLA_2 relates to a number of different proatherogenic biological processes. Lp-PLA_2 hydrolyzes oxidized phospholipids on modified LDL particles within the arterial intima, and thus contributes to the initiation and progression of atheroma. Lp-PLA_2 is produced by macrophages and foam cells within atherosclerotic plaque. Its expression is mainly confined to plaque areas with massive lipid accumulation and leukocyte infiltration, cellular necrosis, and calcification, suggesting that Lp-PLA_2 is a marker for rupture-prone plaque [16]. The amount of Lp-PLA_2 and its by-product, lysophosphatidylcholine in the coronary circulation is proportional to the extent of the atheroma and indirectly affects local endothelial function [17]. Proatherogenic activities of lysophosphatidylcholine are: expression of adhesion molecules; upregulation of cytokines and CD40 ligand by T-cells, cytotoxic at concentrations higher than 30–50 μM; stimulation of macrophage proliferation; release of arachidonic acid from endothelial cells; induction of MCP-1 and genes for growth factors; release of myeloperoxidase; migration of vascular smooth muscle cells; chemoattractant for monocytes, macrophages, and T-cells; induction of apoptosis in smooth muscle cells and macrophages; involvement in the antigenicity of oxidized LDL; inhibition of endothelium-derived nitric oxide [18-20]. Furthermore, oxFFAs promote atherosclerosis by direct and

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indirect increasing of oxidative stress and the presence of oxidized LDL and other lipoproteins in the plasma and arterial walls, thereby initiating fatty streak formation [21].

4. Assays for Lp-PLA_2 mass and activity determination

It is well known that circulating Lp-PLA_2 can be measured by different assays ascertaining either its mass or activity. There is no definite decision about the potential superiority of the tests.

According to the literature data, Lp-PLA_2 levels were determined preferentially using PLAC® test. Lp-PLA_2 mass was determined by diaDexus PLAC Test ELISA, sandwich enzyme immunoassay, followed by diaDexus PLAC® test based for Lp-PLA_2 mass measurement on turbidimetric immunoassay technology. More recently, enzyme assay PLAC® Test for measurement of Lp-PLA_2 activity has been developed and commercially available. The preferred sample type is EDTA plasma, and serum is also acceptable. It should be noted that methodological issues associated with Lp-PLA_2 measurement make comparisons between studies difficult.

Recently published meta-analysis of 32 prospective studies in persons with stable vascular disease or recent acute ischemic event revealed a moderate correlation between mass and activity of Lp-PLA_2 [24]. The PROVEIT-TIMI 22 study [25] has also found a moderate correlation between Lp-PLA_2 activity and mass measured at baseline and at 30-day after ACS. There is still controversy about the method of estimating Lp-PLA_2 level. While Koenig et al. [26] reported that Lp-PLA_2 mass was the better risk predictor of future cardiovascular events than Lp-PLA_2 activity, Persson et al. [27] has reached the opposite conclusion. Jenny et al. [28] showed no difference in Lp-PLA_2 activity and mass with respect to risk prediction.

Currently, there is no consensus on the best method to estimate Lp-PLA_2 level. A consensus panel recommendation for incorporating Lp-PLA_2 testing into the cardiovascular disease risk assessment guidelines used Lp-PLA_2 mass for stratifying patients [23].

Blood samples should be refrigerated after processing and should be kept frozen for long-term storage. There are no restrictions to the time of day that the sample should be drawn and no dietary restrictions. In contrast to other emerging risk markers, a very minimal biological variation in Lp-PLA_2 concentrations has been demonstrated among individuals monitored serially over several weeks [29].

In addition, Lp-PLA_2 levels are typically unaffected by conditions of systemic inflammation, such as osteoarthritis and chronic obstructive pulmonary disease, whereas markers of inflammatory response are often elevated by these conditions. The normal population medians for men and women are in the range of 230–250 ng/mL, and a value of >300 ng/mL may be considered elevated [30].

Gender differences in Lp-PLA_2 levels were found; men had significantly higher levels than women. Also, a significant association between Lp-PLA_2 levels and smoking was noticed.

These finding has been observed in previous studies [31,32,33,34]. Lower Lp-PLA_2 levels in women could be explained by estrogen-mediated down-regulation of Lp-PLA_2 expression, due to lower concentrations of LDL cholesterol in women or estrogen-related decrease in platelet activating factor acetyl hydrolase activity [35,36]. Estrogen-replacement therapy can significantly reduce Lp-PLA_2 activity in healthy postmenopausal women [37], while administration of steroids with progesterone-like activity increases Lp-PLA_2 activity [38]. Smoking may increase the carrier (LDL) and the substrate (oxidized LDL) for Lp-PLA_2 [39].

5. Association of Lp-PLA_2 with cardiovascular disease risk

Lp-PLA_2 is an emerging inflammatory biomarker, characterized by high vascular specificity and low biovariability. High Lp-PLA_2 levels are indicative of rupture-prone plaque [22]. Numerous epidemiological and clinical studies examined association between Lp-PLA_2 concentration/enzyme activity and cardiovascular disease risk in apparently healthy individuals, in subjects with stable cardiovascular disease, acute coronary syndrome (ACS), heart failure (HF), stroke, transient ischemic attack (TIA) support, trying to answer in which patients would determination of Lp-PLA_2 be the most valuable. There are novel data about the prognostic significance of Lp-PLA_2 as a predictor of short-term or long-term outcome in patients with cardiovascular disease. To evaluate this, systematic literature review concerning the association of Lp-PLA_2 with cardiovascular disease risk and prognostic implications was done. The studies on this issue were extracted from relevant electronic databases (Medline (http://www.ncbi.nlm.nih. gov/pubmed/), Embase (http://www. embase.com/), Google Scholar (http://scholar. google.com/), Yahoo (http:// www. yahoo. com/), Kobson (http:// www.kobson.nb.rs/), ClinicalTrails.gov.) and the obtained results were included in the text.

In 2008, the panel's recommendation [23] incorporated Lp-PLA_2 testing as an adjunct to traditional risk factors in assessing future cardiovascular risks. It endorses Lp-PLA_2 testing in moderate-risk persons determined as having simply two risk factors. An Lp-PLA_2 >200 ng/mL warrants reclassification of the moderate-risk patient as high cardiovascular risk and should prompt reduction of the LDL-C target from 130 mg/dL to 100 mg/dL. The panel also recommends Lp-PLA_2 testing for patients with coronary artery disease (CAD) or CAD risk equivalents (diabetes, ischemic stroke, etc.) be considered at very high risk when Lp-PLA_2 is elevated, warranting reduction in the LDL-C target from 100 mg/dL to 70 mg/dL. Today, Lp-PLA_2 measurement for cardiovascular disease risk stratification of patients is included in four international clinical guidelines: 2012 European Guidelines on CVD Prevention in Clinical Practice European Society of Cardiology – Lp-PLA_2 may be measured as a part of a refined risk assessment in patients at high risk of a recurrent acute atherothrombotic event. Class IIb; 2010 ACCF/AHA Guideline for Assessment of Cardiovascular Risk in Asymptomatic Adults – Lp-PLA_2 testing may be considered in intermediate-risk asymptomatic adults; 2011 AHA/ASA Guidelines for the Primary Prevention of Stroke Measurement of inflammatory markers such as high sensitive C-reactive protein (hs-CRP) or Lp-PLA_2 in patients without CVD may be considered to identify patients who may be at increased risk of stroke; 2012 AACE Guidelines for Management of Dyslipidemia and Prevention of Atherosclerosis Test for Lp-

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PLA_2, which in some studies has demonstrated more specificity than highly sensitive CRP, when it is necessary to further stratify a patient's CVD risk [40-43].

Some studies suggested that Lp-PLA_2 predicts risk complementary to hs-CRP, although when compared with hs-CRP, Lp-PLA_2 in some studies is suggested to be a more promising marker of risk prediction. In multiple previous studies, correlation analysis revealed no association of Lp-PLA_2 with CRP [28,31,44-47]. It can be expected because CRP is an acute-phase reactant and its elevation can be caused by a wide range of inflammatory conditions. CRP shows intraindividual variability of ~40%. On the other hand, Lp-PLA_2 is not affected by systemic inflammation; it is a specific marker of vascular inflammation. Also, it shows significantly lower biologic variability than CRP, and higher stability in states of myocardial ischemia [48].

Lp-PLA_2 is suggested to be a more promising marker of risk prediction than CRP [49]. Winkler et al. [50] showed that increased Lp-PLA_2 levels in moderate-risk patients with hs-CRP<3 mg/L doubled the risk for cardiac death. The WOSCOPS study [44] found that Lp-PLA_2 was significantly associated with cardiovascular risk, compared with hs-CRP. Stankovic et al.'s [32] results confirmed that Lp-PLA_2 have better risk prediction than CRP.

Although, the majority of published studies showed a significant relationship between Lp-PLA_2 levels and cardiovascular events, there are several important differences across ethnic groups; for example, African-Americans and Caucasians with respect to Lp-PLA_2. Activity of Lp-PLA_2 was higher among African-Americans with CAD. The difference in Lp-PLA_2 activity levels between CAD and non-CAD patients was higher among African-Americans. Also, the Lp-PLA_2 index was independently associated with the extent of CAD among African-Americans [51-53].

From the WOSCOPS publication (West of Scotland Coronary Prevention Study, WOSCOPS) [44], which revealed a positive association between elevated circulating concentrations of Lp-PLA_2 and the risk of coronary heart disease (CHD), the interest in Lp-PLA_2 as a biomarker for cardiovascular disease rapidly increased. The vast body of evidence derived from prospective epidemiologic studies, two meta-analyses (79,036 participants in 32 prospective studies, and 52,995 subjects participated in 33 studies) and review revealed the positive association of elevated Lp-PLA_2 with cardiovascular risk [24,54-56]. Furthermore, Lp-PLA_2 has been confirmed to predict the presence of CAD, even among patients undergoing coronary angiography. Uniquely, Lp-PLA_2 predicted the risk of CAD death, but not all cause death [57].

LpPLA_2 has been considered as a prognostic marker in patients with CAD. Li et al. [58] identified prognostic value of Lp-PLA_2 baseline measurement for major adverse cardiac event (MACE) (cardiovascular death, nonfatal myocardial infarction, and target vessel revascularization) in ACS patients during follow-up for a median of 6 months. Mockel et al. [59] demonstrated that Lp-PLA_2 levels in the first 7 hours after onset of symptoms may be an independent predictor of MACE within 42 days in ACS patients. PROVEIT-TIMI 22 study [25] has shown that Lp-PLA_2 in patients randomized to atorvastatin or pravastatin measured 30 days after ACS are associated with an increased risk of cardiovascular events (death, myocardial infarction, unstable angina, revascularization, or stroke) over 24 months of follow-up. Gerber et al. MACE [48] found that Lp-PLA_2 level measured early after myocardial infarction is

strongly and independently associated with 1-year mortality. Stankovic et al. [32] demonstrated that the Lp-PLA_2 may have short-term predictive value in pure STEMI patients treated by primary percutaneous coronary intervention (PCI). They concluded that pre-interventional plasma Lp-PLA_2 level is an independent predictor of 30-day MACE in patients with first anterior STEMI treated by primary PCI, and suggested that Lp-PLA_2 level could help in very early risk stratification of STEMI patients treated by PCI.

Previously published studies examined and suggested the association between Lp-PLA_2 and heart failure (HF) incidence in a population-based cohort of healthy individuals, and in people older than 65 years [60]. Baseline Lp-PLA_2 levels are associated with a high risk of developing heart failure in 3,991 adults older than 65 years, independent of coronary risk factors [61]. Lp-PLA_2 activity is significantly associated with congestive heart failure in 5,531 persons older than 65 years [62]. Gerber et al. [63] evaluated the association of Lp-PLA_2 with mortality in subjects with diagnosed HF. Lp-PLA_2 was strongly and independently associated with mortality in patients under 80 years of age. Moldoveanu et al.'s study [64] in 208 patients with HF found significantly increased Lp-PLA_2 activity in HF patients with preserved ejection fraction (EF) than in HF with reduced EF. The literature data about the association of Lp-PLA_2 and heart failure on admission in patients with acute myocardial infarction are missing. Stankovic et al. [32] suggested that patients with the first anterior STEMI who had higher levels of Lp-PLA_2 had a worse prognosis, but not with a greater probability of developing HF. In Raichlin et al.'s [65] study in heart transplant patients, Lp-PLA_2 correlated with the progression of cardiac allograft vasculopathy and increased risk of cardiovascular events/death suggesting that it could be therapeutic target in heart transplant patients.

In 2005, the US Food and Drug Administration (FDA) approved Lp-PLA_2 blood test for assessing patients at risk for ischemic stroke. The Rotterdam Study was the first population-based study that determined the impact of elevated Lp-PLA_2 on stroke. It identified that Lp-PLA_2 activity was an independent predictor of ischemic stroke in the middle-aged healthy men and women population [31]**.** In the ARIC study, healthy middle-aged adults with increased levels of both Lp-PLA_2 and hs-CRP had an 11-fold higher incidence of stroke than individuals with low Lp-PLA_2 and hs-CRP levels [45]. This association between Lp-PLA_2 mass/activity and first ischemic stroke was confirmed in the Malmo Diet and Cancer Study [66], Bruneck Study [67], and Cardiovascular Health Study [28]. The Tsekepis et al. study [68] showed that Lp-PLA_2 correlated with the intima-media thickness in patients with beta-thalassemia, suggesting that Lp-PLA_2 may be implicated in premature carotid atherosclerosis.

Although some reports inconclusively found this positive association of Lp-PLA_2 and first-ever and recurrent stroke, little is known about its influence on stroke outcome. Elkind et al. [69] measured Lp-PLA_2 mass and activity in relation to outcome in first ischemic stroke patients, determined as recurrent stroke, recurrence of vascular events, and mortality. Lp-PLA_2 was a good predictor of recurrent stroke risk. Delgado et al. [70] investigated the temporal profile of Lp-PLA_2 mass and activity within the first 24 hours after stroke and found significant changes in Lp-PLA_2 concentrations early after stroke onset. Patients with higher Lp-PLA_2 mass were more likely to be resistant to intravenous t-PA administration with very low early recanalization rates.

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Lp-PLA_2, an inflammatory biomarker, has been described as able to predict risk of first-ever or recurrent stroke and myocardial infarction [71]. Moreover, Lp-PLA_2 may also have a role in the pathophysiology of cerebrovascular disease, particularly in strokes of atherosclerotic etiology, since its expression is enhanced in atherosclerotic carotid lesions together with markers of oxidative damage, inflammation, and instability [72].

Sarlon-Bartoli et al. [73] reported that Lp-PLA_2 mass is increased in patients with high-grade carotid stenosis and unstable plaque and suggest that Lp-PLA_2 could be an important biomarker for classifying carotid plaque as vulnerable and predict neurological risk of a carotid stenosis in asymptomatic subjects. Although these findings have the potential to improve cerebrovascular disease stratification, correlation with ultrasonic or MRI markers of plaque instability or the presence of infarction on brain imaging must be performed [74].

Shoamanesh et al. [75] investigated the association between circulating biomarkers of inflammation including Lp-PLA_2 and MRI markers of cerebral small vessel disease in 1,763 stroke-free Framingham offspring. They observed higher levels of lipoprotein-associated phospholipase A_2 mass in patients with greater white matter hyperintensity volumes and silent cerebral infarcts. These results could improve stroke risk prognosis.

The accumulating results of numerous studies demonstrate a significant positive association between Lp-PLA_2 levels and incident cardiovascular disease and heart failure. A dissociation could be noted between mass and activity in terms of risk prediction. Several of the epidemiology studies have measured only Lp-PLA_2 mass (26,30,33,44,47,57,63,65,69), whereas others measured only enzyme activity (31,50,62,64,67) and a few have measured both in the same study population (25,27,28, 76). Recently published meta-analysis assessed the 32 prospective studies of Lp-PLA_2 and cardiovascular outcomes. For each standard deviation Lp-PLA_2 increase, the relative risks for the primary endpoint of coronary heart disease were 1.10 (1.05–1.16) and 1.11 (1.07–1.16) for Lp-PLA_2 activity and mass, respectively. The relative adjusted risks for ischemic stroke were 1.08 (0.97–1.20) and 1.14 (1.02–1.27); vascular mortality 1.16 (1.09–1.24) and 1.13 (1.05–1.22); and nonvascular mortality 1.10 (1.04–1.17); and 1.10 (1.03–1.18) for Lp-PLA_2 activity and mass, respectively. The final decision whether to measure the Lp-PLA_2 mass or its enzyme activity may help transition of this biomarker from research to routine clinical practice.

6. Lp-PLA_2 and genetic influences

Several Lp-PLA_2 gene polymorphisms and their role in affecting the regulation or production of LpPLA_2 assessed as activity and mass were described; many in small studies and some in recently published genome-wide association studies. It is known that genetic factors account for 62% of the variation in Lp-PLA_2 activity [76,77].

Although familial factors explain about one-half and one-quarter of the variance in Lp-PLA_2 activity and mass, respectively [78,79], few genetic determinants of Lp-PLA_2 have been identified. The first genome-wide association study using data from 6,668 Caucasian subjects

in population-based Framingham Heart Study identified one locus associated with Lp-PLA_2 mass, and four loci associated with Lp-PLA_2 activity [80]. Twelve SNPs in the region of chromosome 6p12.3 near the gene for PLA2G7 were associated with Lp-PLA_2 mass at a genome-wide level of significance. The top hit SNP rs1805017, is a nonsynonymous change (H92R) within the PLA2G7 gene. It was found that T allele that corresponds to the amino acid histidine was associated with higher Lp-PLA_2 mass. On the other side, four loci achieving genome-wide significance for association with Lp-PLA_2 activity was identified: a) first within the APOE/APOC1 gene cluster on chromosome 19q13.32 (rs41377151); b) second locus on chromosome 1p13.3, which includes the genes PSRC1 (rs599839), CELSR2 (rs4970834); c) third locus within an intron of SCARB1 on chromosome 12q24.31 (rs10846744); d) fourth locus in ZNF259 gene and BUD13 gene on chromosome 11q23.3 near the apolipoprotein gene cluster APOA5/APOA4/APOC3/APOA1 (rs12286037, rs11820589). Investigation from Suchindran's study was extended by Grallert et al. who made meta-analysis with additional four cohorts [81]. They performed genome-wide association study as part of the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium in five population-based studies with the aim to identify genetic loci related to Lp-PLA_2 mass/activity [82]. This study, in the popoulation of 13,664 subjects, revealed the association of PLA2G7 loci variants with Lp-PLA_2 mass/activity, and genetic variants (APOC1, CELSR2, LDL, ZNF259, SCARB1) related to low-density lipoprotein cholesterol levels with Lp-PLA_2 activity.

Also, Lp-PLA_2 mass was associated with SNP rs247616 on chromosome 16 within the cholesteryl ester transfer protein gene. The T allele of rs247616 was associated with higher HDL-C concentration and higher mean Lp-PLA_2 mass [83]. The additional value of this study was the revealed significant association of four polymorphisms (in APOC1, CELSR2, SCARB1, ZNF259), but not PLA2G7 with coronary heart disease. Large-scale analysis focusing on variants in PLA2G7 did not identify any SNP that achieved experiment-wide statistical significance [79].

The most frequently studied SNPs within PLA2G7 are: variants in exon 9 (Val279Phe; rs45619133), exon 11 (Val379Ala; rs1051931), exon 7 (Ile198Thr, Iso195Thr; rs1805018), and exon 4 (Arg92His; rs1805017) [84-86].

The V279F SNP was found in subjects of Asian ancestry. The Val279Phe substitution is located within the catalytical domain of Lp-PLA_2 and leads to reduction of enzyme activity in V/F heterozygous individuals or complete loss of enzymatic activity in homozygous F/F individuals. The 279F null allele is relatively frequent in Japan, with approximately 25% and 2% of the population carrying one or two copies. Its prevalence declines toward the West, has intermediate frequencies in China and Korea, is rare in the Middle East, and almost completely absent in European populations. The results from the association studies on this V279F variant and coronary artery disease have thus far been inconclusive. Li et al. [87] in Chinese Han population, found significant association of V279F and coronary artery disease, indicating that carrier of F allele increases the risk of coronary artery disease. This result was consistent with the Yamada et al. [88] study (850 cases/1,684 controls), which found that subjects carrying the mutant allele are at higher risk of arterial events (MI or stroke), and the Shimokata study (3,085 subjects with coronary artery disease/2,163 controls) [89]. The Jang et al. [90] study in two

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different patient sets (2,890 men diagnosed with CAD before age 60/3,128 male controls and 877 CAD cases/1,230 controls) confirmed that deficiency in Lp-PLA_2 activity due to carriage of PLA2G7 279F allele protects from CAD in Korean men. Nevertheless, in the South Korean population (532 cases/670 controls) and Chinese study (827 cases/947 controls), V279F variant results in an unexpectedly opposite outcome [91,92]. Meta-analysis of 14 association studies focusing on R92H polymorphism in PLA2G7 gene and risk of CHD in 8,280 cases/5,656 controls indicate 92H allele had probably increased the risk of CHD [92]. The missense polymorphisms I198T and A379V are identified mainly in Caucasians. A379V variant in which alanine is substituted by valine is functional. Some studies explored the contribution of A379V to Lp-PLA_2 activity with contradictory results; some found the association of A379V variant with increased [86,93] and some with decreased Lp-PLA_2 activity [94].

There are also different reports about the association between the A379V variant and cardiovascular disease [94-97]. A recent Taiwanese population study reported that subjects carrying the 379 V allele had increased severity of coronary atherosclerosis [94]; two studies in Caucasian subjects reported that the 379 V allele was associated with decreased atherosclerosis risk [93,96]; a large meta-analysis showed that the 379 V allele was associated with Lp-PLA_2 activity, but not with cardiovascular risk markers [97]; and another study showed no association [95].

Intriguingly, in the study conducted by Liu and colleagues [94], the outcome was quite contradictory. They found that in the Chinese Taiwan Han population, A379V variant is significantly associated with Lp-PLA_2 activity and the severity of coronary atherosclerosis. Recently, a meta-analysis including a total of 12 studies shows that in the populations from European ancestry, among the 7 SNPs, A379V variant shows the strongest association with Lp-PLA_2 activity; however, no significant correlation is found between PLA2G7 variants and cardiovascular risk markers, coronary atheroma, or CHD [97].

7. Lp-PLA_2 as a therapeutic target

It is well known that lipid-altering medications, including statins, fenofibrate, prescription of omega-3 fatty acids, for weight loss, have been shown to reduce Lp-PLA_2 levels. The degree of its reduction correlates with the extent of lipid lowering.

Lp-PLA_2 was identified as a potential novel target of therapy. The most used therapy targeting Lp-PLA_2 in plasma in advanced stages of clinical investigation is darapladib. Darapladib is a selective, potent, reversibile, oral inhibitor of lipoprotein-associated phospholipase A_2. The basic idea of applying darapladib is to improve patient outcomes in addition to evidence-based treatments and potentially reduce cardiovascular and cerebrovascular events by decreasing cytokines concentrations, stabilizing atherosclerotic plaque, inhibiting macrophage infiltration, and thickening of the connective tissue cap.

The first study that showed as a secondary endpoint a change in coronary artery plaque necrotic core with darapladib after 12 months of treatment was Integrated Biomarkers and

Imaging Study (IBIS)-2 [98]. It was a multicenter, randomized, double-blind placebo-controlled study that included 330 patients with angiographically confirmed coronary artery disease. Inhibition of Lp-PLA_2 with darapladib also prevented necrotic core expansion of coronary plaque as measured on intravascular ultrasound.

One multicenter, randomized, double-blind placebo-controlled study examined the effects of darapladib on biomarkers of cardiovascular risk in 959 CAD and CAD-risk equivalent patients who were previously randomized to atorvastatin 20 mg or 80 mg and then randomized to oral darapladib 40, 80, 160 mg, or placebo for 12 weeks. Overall dose-dependent inhibition of Lp-PLA_2 activity was sustained over the study period and was present in both atorvastatin dose groups, at different baseline LDL cholesterol < or ≥70 mg/dl, and high-density lipoprotein cholesterol HDL-C < or ≥40 mg/dl [99].

The first study that examined the effects of darapladib on Lp-PLA_2 activity in Japanese dyslipidemic patients with/without the Val279Phe single-nucleotide polymorphism (SNP) of the *PLA2G7* gene showed that darapladib produced sustained inhibition of Lp-PLA_2 activity [100].

Two large-scale studies with hard clinical endpoints were completed: the Stabilization of Atherosclerotic Plaque by Initiation of Darapladib Therapy (STABILITY) trial, with 15,828 randomized patients, and the Stabilization of Plaques using Darapladib-Thrombolysis In Myocardial Infarction 52 Trial (SOLID-TIMI 52), with an estimated recruitment of 13,026 patients [101-103].

The SOLID-TIMI 52 was a randomized, double-blind, placebo-controlled, multicenter, and event-driven trial that determined the clinical benefit of direct inhibition of Lp-PLA_2 activity with darapladib in patients after an acute coronary syndrome (non-ST-elevation or ST-elevation myocardial infarction). Subjects were randomized to darapladib (160 mg enteric-coated tablet daily) or matching placebo within 30 days after acute coronary syndrome. The primary endpoint was the composite of coronary heart disease death, nonfatal myocardial infarction, or nonfatal stroke, and secondary endpoints were major and total coronary events, individual components of the primary endpoint, and all-cause mortality. Patients were followed up for a median of 2.5 years. In patients who experienced an ACS event, direct inhibition of Lp-PLA_2 with darapladib added to optimal medical therapy and initiated within 30 days of hospitalization did not reduce the risk of major coronary events.

The STabilization of Atherosclerotic plaque By Initiation of darapLadIb TherapY (STABILITY) trial was a double-blind trial in patients randomized more than one month after a myocardial infarction to double-blind darapladib (160 mg daily) or placebo (daily). The primary endpoint was a composite of cardiovascular death, myocardial infarction, or stroke. Secondary endpoints included the components of the primary endpoint as well as major coronary events (death from coronary heart disease, myocardial infarction, or urgent coronary revascularization for myocardial ischemia) and total coronary events (death from coronary heart disease, myocardial infarction, hospitalization for unstable angina, or any coronary revascularization). Patients were followed up for a median of 3.7 years. In patients with stable coronary heart disease, darapladib did not significantly reduce the risk of the primary composite endpoint of cardiovascular death, myocardial infarction, or stroke.

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Darapladib is a selective Lp-PLA_2 inhibitor that is under investigation for its potential to stabilize high-risk atherosclerotic plaques and potentially reduce cardiovascular events [104,105]. A phase 2 clinical trial in 959 patients with CHD or CHD-risk equivalents demonstrated that darapladib was effective at producing sustained inhibition of plasma Lp-PLA_2 activation in patients on atorvastatin therapy. This study was a post hoc analysis of this phase 2 trial studying high-risk patients with a diagnosis of peripheral arterial disease (PAD). Despite a more aggressive baseline risk factor profile, darapladib was equally effective at reducing Lp-PLA_2 in patients with and without PAD [106]. Johnson et al. [107] assessed the effects of darapladib on both plasma and plaque Lp-PLA_2 activity in patients undergoing elective carotid endarterectomy randomized to darapladib 40 mg (n = 34), 80 mg (n = 34), or placebo (n = 34) for 14 days. Patients were followed by carotid endarterectomy 24 hours after the last dose of study medication. Darapladib reduced plasma and plaque Lp-PLA_2 activity compared with placebo.

8. Conclusion

Within the last decade, a broad range of biomarkers associated with an increased risk for death and cardiovascular/cerebrovascular endpoints have been identified. Epidemiological studies clearly indicated that Lp-PLA_2 has the potential to become a clinically useful biomarker because it promotes independent information in the diagnosis, and especially cardiovascular/cerebrovascular risk stratification. In the future, we can expect new drugs (new Lp-PLA_2 inhibitors) that will affect patient's management, and assessing the effect of Lp-PLA_2 inhibition on cardiovascular endpoints can provide definitive answers.

However, further clinical validation in well-designed observational and interventional studies is needed before these recommendations can be properly evaluated in order to include them in the clinical diagnostic algorithms.

Currently, Lp-PLA_2 measurement has only been reserved to patients with moderate and high cardiovascular risk, rather than healthy population or low-risk patients, since the values of Lp-PLA_2 in these population groups are insignificant. Also, the evaluation of Lp-PLA_2 in combination with noninvasive imaging could be expected. The formation of best-case model from Lp-PLA_2 and other biomarkers can yield the best patient stratification algorithm. The next step could be cost-effectiveness analysis of more accurate risk stratification with biomarker testing. Future studies need to focus on exploring the potential of this biomarker and evaluating the effects of Lp-PLA_2 inhibition on human populations.

The final decision on which test to use for Lp-PLA_2 determination, test based on mass or activity of Lp-PLA_2, together with the development of commercially available automated, robust, valid, high throughput, cost-effective test capable of increasing agility and reducing the analytical imprecision can be adopted routinely in clinical practice for better risk stratification and therapeutic choice in patients with cardiovascular/cerebrovascular disease.

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