Disease Markers 34 (2013) 323–331
DOI 10.3233/DMA-130976
IOS Press

Lipoprotein-associated phospholipase A2 (Lp-PLA$_2$): A novel and promising biomarker for cardiovascular risks assessment

Anping Cai[a,1], Dongdan Zheng[b,1], Ruofeng Qiu[b], Weiyi Mai[b] and Yingling Zhou[a,*]
[a]*Department of Cardiology, Guangdong Cardiovascular Institute, Guangdong General Hospital, Guangdong Academy of Medical Sciences, Guangzhou, Guangdong, China*
[b]*Department of Cardiology, The First Affiliated Hospital of Sun Yat-sen University, Guangzhou, Guangdong, China*

Abstract. Atherosclerosis and its manifestations namely cardiovascular diseases (CVD) are still the leading cause of morbidity and mortality worldwide. Although intensified interventions have been applied, the residual cardiovascular (CV) risks are still very high. Lipoprotein-associated phospholipase A2 (Lp-PLA$_2$) is a novel and unique biomarker highly specific for vascular inflammation and atherosclerosis. Both pro-atherogenic property of Lp-PLA$_2$ and positive correlation with CV events have already been demonstrated by a large number of scientific and clinical studies. Currently, in the Adult Treatment Panel III (ATP III) guideline, Lp-PLA$_2$ has been recommended as an adjunct to traditional risk factors in assessing future CV risks. Encouragingly, darapladib, an orally Lp-PLA$_2$ specific inhibitor, has been tested in basic research and preclinical trials and the outcomes are quite striking. Additionally, there are two phase III ongoing clinical trials in evaluating the efficacy and safety of darapladib on cardiovascular outcomes. With regard to the potential values of Lp-PLA$_2$ in risk stratification, therapeutic regimen establishment and prognosis evaluation in patients with moderate or high risk, our present review is going to summarize the relevant data about the bio-chemical characteristics of Lp-PLA$_2$, the actions of Lp-PLA$_2$ on atherosclerosis and the results of Lp-PLA$_2$ in scientific research and clinical studies.

Keywords: Lipoprotein-associated phospholipase A2, cardiovascular diseases, inflammatory biomarker

1. Introduction

Despite great advances in terms of screening, diagnosis, treatment and prevention have been achieved in the past decades, cardiovascular diseases (CVD) are still the leading cause of morbidity and mortality worldwide [1]. It has been well documented that the initiation and progress of CVD is largely associated with the severity of atherosclerosis. Literally, atherosclerosis is recognized as a chronic and dynamic status of vascular inflammation, and usually has already existed for years or decades before cardiovascular (CV) events occurring [2–4]. Therefore, quantitatively and accurately evaluating the severity of atherosclerosis will be helpful and beneficial to identify an individual at high risk for CV events. A variety of approaches (e.g., Framingham risk criteria and Reynolds risk scoring) have been used to effectively evaluate CV risk in population with risk factors. Furthermore, promotion of risk stratification would not only raise concerns for potential CV risk but also directly improve prognosis. However, there are still some deficiencies, including complexity and inaccuracy, especially when applied these risk estimating systems to individuals with moderate risk, which is defined as equal or more than 2 risk factors or the 10 years Framingham CV risk is 10–20% [5]. Consequently,

[1]These authors contributed equally.

*Corresponding author: Yingling Zhou, Department of Cardiology, Guangdong Cardiovascular Institute, Guangdong General Hospital, Guangdong Academy of Medical Sciences, Guangzhou, Guangdong, China. Tel.: +86 20 83827812; Fax: +86 20 83827812; E-mail: drcacocolacai@gmail.com.

ISSN 0278-0240/13/$27.50

AHA/CDC incorporates high sensitivity C-reactive protein (Hs-CRP) into traditional risk factors for further enhancing the sensitivity and accuracy of risk estimating systems [6]. Nonetheless, Hs-CRP is an indicator of general inflammation, rather than exclusive to vascular inflammation [7]. Hence, the analysis of Hs-CRP elevation sometimes can be confounded by other conditions such as infection, adiposity, or rheumatic disease [8]. Lipoprotein-associated phospholipase A2 (Lp-PLA$_2$) is a novel and unique biomarker, highly specific for vascular inflammation and atherosclerosis [7,8], and pro-atherogenesis and positive correlation with CV events have been sufficiently demonstrated by a large number of scientific and clinical studies [9–13]. Currently, in the Adult Treatment Panel III (ATP III) guideline, Lp-PLA$_2$ has been recommended as an adjunct to traditional risk factors in the process of CV risk assessment [14]. Moreover, results from clinical studies indicate that the combination of Lp-PLA$_2$ and Hs-CRP would be much better than Hs-CRP alone for reclassifying CV risk [15,16]. Encouragingly, darapladib, an oral Lp-PLA$_2$ specific inhibitor, has been tested in basic research and preclinical trials and the outcomes are quite striking [17–20]. With regard to the potential values of Lp-PLA$_2$ in risk stratification, therapeutic regimen establishment and prognosis evaluation in patients with moderate or high CV risk, our present review is going to summarize the relevant data about the bio-chemical characteristics of Lp-PLA$_2$, the actions of Lp-PLA$_2$ on atherosclerosis and the results of Lp-PLA$_2$ in scientific research and clinical studies.

2. Atherosclerosis initiation and development

It's well known that vascular inflammation, hallmark of the beginning of atherosclerosis, is primarily incurred by endothelial dysfunction. Accordingly [21, 22], after exposed to traditional risk factors for a certain period, such as obesity, smoking, hypertension, dyslipidemia and diabetes mellitus, endothelial cells gradually become dysfunction and the natural barrier built among endothelia is impaired. Subsequently, lipids begin accumulating in sub-endothelial spaces [23]. Macrophages infiltrate and engulf lipids and turn into foam cells, and concomitantly produce inflammatory cytokines, reactive oxygen species and chemotactic factors, which are largely derived from oxidized low density lipoprotein (oxLDL) hydrolysis. Afterward, more leukocytes infiltrate and accumulate, lipid oxidation aggravates, endothelial cells deteriorate, and consequently vascular inflammation propagates. With numerous vicious cycles, atherosclerotic plaque, featured by necrotic lipid core, inflammatory cells and fibrous cap, is gradually established, which directly lead to the risk of CV events significantly increase. Basically, the occurrence of CV events largely depends on the stability of atherosclerotic plaque, and many characteristics have been identified to evaluate the plaque stability. For example, compared with other phenotypes, plaque with thinner fibrous cap and larger volume of necrotic lipid core is more vulnerable and easier to rupture. Therefore, identifying a prone-rupture plaque would be beneficial and helpful to prevent CV events. Currently, however, the risk assessment algorithms could not provide reliable evidences to identify a potential prone-rupture plaque [24]. Intravascular ultrasound (IVUS) and carotid magnetic resonance imaging have been used to successfully assess the component of atherosclerotic plaques in few studies. Nevertheless, these approaches are neither invasive nor inexpensive and simple, which hampers its broad application. Intriguingly, many studies reveal that comparing to stable plaque with small lipid core and thick fibrous cap, the activity of Lp-PLA$_2$ in the prone-rupture plaque is much higher [25,26]. Moreover, the distribution of Lp-PLA$_2$ is found predominantly adjacent to areas with massive macrophages aggregation and oxLDL accumulation [9,27], indicating that Lp-PLA$_2$ activity or mass is significantly related to the plaque stability and Lp-PLA$_2$ may be useful and reliable to identify vulnerable plaques and to evaluate future CV risks.

3. Bio-chemical characteristics of Lp-PLA$_2$

Lp-PLA$_2$, also known as platelet-activating factor acetylhydrolase or type VIIA PLA$_2$, is encoded by PLA2G7 gene and composes of 441 amino acids. Lp-PLA$_2$ is a Ca^{2+}-independent phospholipase which belongs to phospholipase A2 superfamily [28]. There are two kinds of Lp-PLA$_2$, namely secreted Lp-PLA$_2$ in circulating system and Lp-PLA$_2$ within atherosclerotic plaque. After mainly produced by macrophages in atherosclerotic plaque, Lp-PLA$_2$ enters into circulating system and turns to be secreted Lp-PLA$_2$ [8, 29]. Approximately 70% of secreted Lp-PLA$_2$ binds to LDL-C and the residual 30% binds to HDL-C and other lipoproteins [30]. Biologically, secreted Lp-PLA$_2$ mainly binds to ApoB portion of LDL and hydrolyzes LDL into lysophosphotidylcholine (Lyso-

PC) and arachidonic acid. While in the atherosclerotic plaque, Lp-PLA$_2$ hydrolyzes oxLDL into Lyso-PC and oxidized non-esterified fatty acids (oxNEFAs), both of which play important and multiple roles on atherogenesis [31–33]. Since secreted Lp-PLA$_2$ is derived from atherosclerotic plaque and with regard to its properties of high specificity and low bio-variability [34], measurement of secreted Lp-PLA$_2$ can accurately and quantitatively reflect the degree of inflammatory reaction within atherosclerotic plaque. Nowadays, secreted Lp-PLA$_2$ measurement can be classified into enzyme activity and enzyme mass [35]. Although activity and mass is significantly correlative [36,37], only Lp-PLA$_2$ mass measurement has already been approved by US Food and Drug Administration for clinical use [38]. Additionally, some studies recently report that the correlation of mass and activity may not be as high as previously expected, which necessitates further investigation. At the very beginning, based on the 50^{th} percentile serum level in healthy population, $>$ 235 ng/mL of Lp-PLA$_2$ is defined as the cut-point for risk stratification [39]. Afterward, many studies reveal that comparing to those with Lp-PLA$_2$ level less than 200 ng/mL, patients with Lp-PLA$_2$ level more than 200 ng/mL has higher CV risk [10,12,40–42]. With respect to the definition and function of biomarker [43], in terms of enhancing risk assessment and increasing diagnostic and prognostic values, to our knowledge, it may be more appropriate to set Lp-PLA$_2$ $>$ 200 ng/mL as the cut-point, so as to find out truly high risk patients and carry out more specific therapy. In addition, one issue needed to be addressed here is that currently setting this cut-point is for better discriminating moderate or high risk patients rather than as a therapeutic target for treatment [5]. Recently, one meta-analysis shows that by contrast with the cut-point, roughly linear correlation between Lp-PLA$_2$ and CV risk and mortality is observed [37]. However, to our knowledge, appropriately specify Lp-PLA$_2$ therapeutic target for each patient with different risk stratification would be essential in the future when convincing and solid evidences, regarding lowering Lp-PLA$_2$ serum level with specific inhibitor could safely and significantly reduce CV events, can be achieved from ongoing phase III clinical trials [44,45].

4. Effects of Lp-PLA$_2$ on atherosclerosis

Currently, Lp-PLA$_2$ is recognized as a pro-atherogenic enzyme responsible for regulating lipid metabolism and inflammatory respond. However, at the very beginning, there are some controversies regarding the dual anti- and pro-atherogenic effects of Lp-PLA$_2$ on atherosclerosis. Lp-PLA$_2$ is primarily recognized as anti-atherogenic enzyme because of its capability of hydrolyzing platelet activating factor (PAF) and LDL-C, both of which are considered detrimental to vessel wall [9,46]. Some scientific research in different animal models also shows that increase serum level of Lp-PLA$_2$ could mitigate vascular inflammation and attenuate atherosclerosis. On the contrary, reduction of Lp-PLA$_2$ due to missense mutation could lead to CV risk profoundly increase [9,47,48]. Intriguingly, a substantial amount of studies gradually report that the activity or mass of Lp-PLA$_2$ positively associates with the severity of atherosclerosis and CV risk [49–51]. The two detrimental substrates, namely Lyso-PC and oxNEFAs, degraded by Lp-PLA$_2$ play crucial roles on the development and progress of atherosclerosis [52, 53]. Both Lyso-PC and oxNEFAs are capable of recruiting leukocytes, up-regulating inflammatory cytokine, amplifying oxidation, enhancing matrix metalloproteinase expression, and finally expanding necrotic lipid core and thinning fibrous cap [53–55]. Emerging evidences have also consistently shown that comparing to healthy individuals, activity or mass of Lp-PLA$_2$ is significantly increased in patients with CVD. Histologically, in prone-rupture plaques, the activity or mass of Lp-PLA$_2$ is also much higher than that in the relatively stable plaques. Furthermore, after the activity or mass of Lp-PLA$_2$ is decreased by darapladib or statins, the volume of necrotic lipid core and the number of macrophages and foam cells are profoundly reduced when compared with control group. Encouragingly, many published studies and meta-analyses have also consistently demonstrated that after fully adjusted for traditional risk factors, elevated Lp-PLA$_2$ is associated with increased risk of CVD. Recently, one meta-analysis shows that Lp-PLA$_2$ activity is positively correlated with non-HDL-C (r = 0.49, 95% IC 0.45–0.52), LDL-C (r = 0.48, 0.41–0.55), apo-lipoprotein B (r = 0.45, 0.38–0.51), and $\log_e$ triglycerides (r = 0.22, 0.19–0.26), while inversely correlated with HDL-C (r = -0.24, -0.29 to -0.19) and apo-lipoprotein AI (r = -0.15, -0.23 to -0.05) [37], indicating that the atherogenic effect of Lp-PLA$_2$ is largely associated with conventional atherogenic-lipids. Collectively, the adverse effects, regarding pro-atherogenesis, Lp-PLA$_2$ imposes on cardiovascular system is outweighed the so-call anti-atherogenesis as evidenced by a large number of scientific and clinical research.

Table 1
Epidemiological studies in evaluating the associations of Lp-PLA$_2$ and primary outcomes

Study	Year	Study population	Cardiovascular end-point	Study major findings
WOSCOPS [7]	2000	580 CVD cases 1160 controls	CHD	The highest quintile of Lp-PLA$_2$was a doubling risk for CHD as compared with the lowest quintile
WHI [75]	2001	123 cases 123 controls	CHD, non-fatal MI and stroke	Lp-PLA$_2$was not a strong predictor of future cardiovascular risk among unselected women
ARIC [15]	2004	608 cases 740 controls	CHD	Lp-PLA$_2$ and CRP might be complementary in identifying individuals at high CHD risk in whom LDL-C <130 mg/dL
The Rotterdam Study [76]	2005	308 CHD cases 110 strokes and1820 controls	CHD and ischemic stroke	Lp-PLA$_2$ activity was an independent predictor of CHD and ischemic stroke in the general population
Malmo [77]	2007	131 strokes 131 MIs	CVD	Higher plasma level of Lp-PLA$_2$ increased incident CVD risk
The Rancho Bernardo Study [78]	2008	1077 community men and women	CHD	Elevated Lp-PLA$_2$ levels predict CHD events in apparently healthy older adults
The Bruneck Study [79]	2009	765 subjects	CVD	Increased Lp-PLA$_2$ activity was associated with incident fatal and non-fatal CVD
The Cardiovascular Health Study [80]	2010	508 MIs 565 Strokes 665 CVD death	CVD	Lp-PLA$_2$ mass and activity were associated with incident CVD events in older adults

Table 2
Epidemiological studies in evaluating the associations of Lp-PLA2 and secondary outcomes

Study	Year	Study population	Cardiovascular end-point	Study major findings
Association of Lp-PLA$_2$ with CAD and the major adverse events [81]	2005	504 consecutive CAD patients	CAD and major adverse events	Higher Lp-PLA$_2$ levels were associated with a higher incidence of major adverse events
The HELICOR study [82]	2005	312 patients with CAD and 479 controls	CAD	Elevated Lp-PLA$_2$ concentrations were associated with the presence of stable CAD
The PROVE IT-TIMI [22]	2006	3648 ACS patients	CV events	Lp-PLA$_2$ activity was associated with an increased risk of CV events
The KAROLA study [83]	2006	1051 patients with CHD	CHD	Increased concentrations of Lp-PLA$_2$ predict future cardiovascular events in patients with manifest CHD
The PEACE trial [13]	2007	3766 stable CAD patients	CV events	In stable CAD, an elevated level of Lp-PLA2 was a significant predictor of nonfatal adverse CV outcomes
The Veterans Affairs HDL Intervention Trial [84]	2008	1451 men	CV events	High Lp-PLA$_2$ independently predicted CV events
Expression of Lp-PLA$_2$ in carotid artery plaques predicts cardiac outcome [26]	2009	162 consecutive patients	CV events	Lp-PLA$_2$ expression in in carotid artery plaques is a predictor of long-term cardiac outcome

5. Basic and clinical research of Lp-PLA$_2$

As aforementioned, Lp-PLA$_2$ is firstly recognized as an anti-atherogenic enzyme. The efficacies of Lp-PLA$_2$ over-expression have been tested in different animal models. The first study to document the anti-inflammatory effect of Lp-PLA$_2$ is performed by Tjoelker and co-workers in 1995 [56]. Thereafter, Morgan and colleagues report that in rabbit with myocardial ischemia-reperfusion injury, infusion of recombinant Lp-PLA$_2$ decreases leukocyte infiltration and reduces myocardial necrosis when compares to control group [57]. In the mice model with apo-lipoprotein E deficiency, adenoviral gene transfer of human Lp-PLA$_2$ diminishes macrophages infiltration and reactive oxygen species production [58]. In another study carried out by Noto H and colleagues [59], they find that Lp-PLA$_2$ has potential to ameliorate lipid oxidation and concomitantly reduce serum level of pro-atherogenic lipoproteins. However, in addition to anti-atherogenic evidences, a substantial amount of studies from scientific to clinical ranges also consistently show that Lp-PLA$_2$ not only involves in the initiation and progress of athesclerosis, but also relates to plaque rupture and CV events occurring. For instance, in the swine model with diabetes mellitus and hyper-

lipidemia, inhibiting Lp-PLA$_2$ activity effectively prevents coronary artery lesions progress [20]. In the mice model with apo-lipoprotein E deficiency, attenuating Lp-PLA$_2$ profoundly ameliorates inflammatory reaction and deters plaque formation [19]. Since the first report of WOSCOPS in 2000 [7], many epidemiological studies and meta-analyses have also been conducted to further investigate and clarify the associations between Lp-PLA$_2$ and the prognosis of patients with CVD. In the clinical trial of WOSCOPS [7], results indicate that Lp-PLA$_2$ elevation appears to be a risk factor for coronary heart disease (CHD), which strongly implicates the effects of Lp-PLA$_2$ on atherogenesis and CV risk assessment. Garza CA and colleagues report that after adjusted for traditional risk factors, Lp-PLA$_2$ is still significantly associated with CV risk and Lp-PLA$_2$ measurement is helpful and beneficial for risk stratification [38]. In a cross-section study conducted by Blankenberg and co-workers [60], they observe that participants with the highest quartile of Lp-PLA$_2$ activity have a 1.8-fold increase risk of CHD when compared to those in the first quartile after fully adjusted for other clinical and metabolic factors. In 2008, Marshall A. Corson and colleagues conducted a meta-analysis and they included 25 clinical trials in evaluating the relationship between Lp-PLA$_2$ and CV risk [8]. Of these, ten of 11 primary studies and 12 of 13 recurrent CV events studies consistently demonstrate the positive correlation between Lp-PLA$_2$ and future CV risk. Another 6 studies also observe the positive correlation between Lp-PLA$_2$ and ischemic stroke. Recently, a meta-analysis includes 32 clinical studies in evaluating the relationship of circulating Lp-PLA$_2$ mass or activity with future risk of CHD, ischemic stroke, and mortality [37]. Notably, after adjusted for conventional risk factors, relative risks with Lp-PLA$_2$ elevation significantly increase for CHD [1.11 (95% CI 1.07–1.16) and 1.10 (1.05–1.16)], ischemic stroke [1.14 (1.02–1.27) and 1.08 (0.97–1.20)], vascular mortality [1.13 (1.05–1.22) and 1.16 (1.09–1.24)] and non-vascular mortality [1.10 (1.03–1.18) and 1.10 (1.04–1.17)], strongly supporting the notion that Lp-PLA$_2$ is a reliable indicator for the evaluation of future CV risk. Furthermore, this meta-analysis also shows that the risk of Lp-PLA$_2$ for CVD is comparable in magnitude to that with non-HDL cholesterol and systolic blood pressure, further indicating that the novel biomarker Lp-PLA$_2$ may be as valuable and significant as conventional risk factors. Finally, other epidemiological studies in investigating the correlation between Lp-PLA$_2$ and the primary or secondary outcomes of CVD are also summarized in Tables 1 and 2 respectively.

6. Lp-PLA$_2$ gene polymorphism and CVD

It is worth to be noted that the activity or mass of Lp-PLA$_2$ is variable among different ethnic groups, and the variants of Lp-PLA$_2$ encoding gene (PLA2G7), which locates on chromosome 6p21-p12, predominantly contributes to this phenomenon. Furthermore, many studies on the single nucleotide polymorphism (SNP) of PLA2G7 reveal that the biological functions of similar variant are quite contrary in different ethnic groups [61–64]. For example, in the Chinese Han population, Li and co-workers find that there is significant association between V279F variant (PLA2G7, rs16874954) and CVD, indicating that carrier of rare allele F increases the risk of CV events [65], which is consistent to the Japanese population as reported by Yamada [66] and Shimokata [67]. Nevertheless, in the South Korean population, V279F variant results in an unexpectedly opposite outcome [68]. A379V variant (PLA2G7, rs1051931), in which alanine is substituted by valine, leads to the modification of Lp-PLA$_2$ function and consequently enhance the anti-atherogenic effects as reported by Ninio E and co-workers [69]. Intriguingly, in the study conducted by Liu and colleagues [70], the outcome is quite contradictory. They find 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 [71]. To our knowledge, these disparities among different studies may be at least partially ascribed to the following mechanisms. First of all, the prime differences of ethnicity. Secondly, the clinical characteristics of subjects between each study are not always comparable, therefore, the outcome relates to the same PLA2G7 variant may be quite contrary. Thirdly, the different frequencies of PLA2G7 variant among studied subjects may also contribute to the discrepancy. Last but not the least, genetic variants other than PLA2G7 per se may also influence Lp-PLA$_2$ activity or mass. Although it is still uncertain about the relationship of PLA2G7 variants with Lp-PLA$_2$ activity or mass and prognosis among different ethnic groups, we consider that the two ongoing clinical trials (STABILITY and SOLID-TIMI 52) which involve different ethnic groups will finally demonstrate the effects of Lp-PLA$_2$ reduction on the outcomes of patients with CVD irrespective of PLA2G7 variants.

7. Further perspective

Nowadays, although intensified interventions have been applied, a significant residual CV risk is still observed when takes Lp-PLA$_2$ into account for risk assessment [51,72] indicating that incorporation of Lp-PLA$_2$ would be more accurate and reliable to identify patients with different degree of CV risk. Currently, Lp-PLA$_2$ measurement has only been reserved to patients with moderate or high CV risk, rather than unselectively applied to apparently healthy population or low risk patients, since the values of Lp-PLA$_2$ in these population groups are insignificant. For example, in the ARIC study [15], the investigators recruit an apparently healthy middle-aged population, and the result suggests that c-statistic improvement is obtained when incorporating Lp-PLA$_2$ to traditional risk factors, however, this effect is quite modest. In another Women's Health Study [73], the authors enroll 28,263 apparently healthy middle-aged women for assessing the relationship of baseline Lp-PLA$_2$ level and the mortality risk of CVD over a mean follow-up of three years. They conclude that in the healthy women, Lp-PLA$_2$ is not a strong predictor for future CV risk. In summary, both of the studies indicate that on the basis of current evidences, Lp-PLA$_2$ should not be routinely used in low-risk or apparently healthy populations.

As mentioned before, traditional risk estimating models are neither reliable nor simple. Incorporation of a highly specific and sensitive biomarker endorsed by consensus panel would absolutely facilitate clinicians to easily and accurately recognize patients at high risk for CV events [5]. Moreover, since previous risk assessment models could not identify a prone-rupture plaque [74], Lp-PLA$_2$ incorporation not only would be helpful to identify patients who are at high risk for CV events, but also could raise awareness so as to perform more intensified interventions. Due to lack of large, prospective and randomized clinical trials to support darapladib application in clinical practices at present time, it is recommended that patients with higher level or activity of Lp-PLA$_2$, serum LDL-C level should be lowered by additional 30 mg/dL.

8. Conclusion

In summary, based on present scientific and clinical evidences, Lp-PLA$_2$ appears to be a valuable biomarker for better discriminating patients with moderate or high CV risks. In spite of intensified interventions, a majority of patients are still with high residual CV risk, Lp-PLA$_2$ incorporation could provide additive values to traditional risk factors in identifying a prone-rupture plaque and assessing future CV risks. Last but not least, to our knowledge, if the two ongoing phase III clinical trials finally are able to demonstrate the efficacy and safety of Lp-PLA$_2$ specific inhibitor darapladib, the future strategies will be significantly shifted and the outcomes will definitely be overwhelmingly improved.

Funding

This work was supported by the grants from the Technology Project Foundation of Guangdong Province, China (2009A030301004, 2011B031800021 and 2011B031800263). The research grant of cardiovascular medication of Guangdong Province (2011 X25). Medical Scientific Research Grant of the Health Ministry of Guangdong province, China (B2011310, A2012663).

Conflict of interest

All authors declare that there is no conflict interest.

References

[1] Pearson TA, Bazzarre TL, Daniels SR, et al. American Heart Association guide for improving cardiovascular health at the community level: A statement for public health practitioners, healthcare providers, and health policy makers from the American Heart Association Expert Panel on Population and Prevention Science. Circulation. 2003. 107(4): 645-51.

[2] Ross R. Atherosclerosis – an inflammatory disease. N Engl J Med. 1999. 340(2): 115-26.

[3] Libby P. What have we learned about the biology of atherosclerosis? The role of inflammation. Am J Cardiol. 2001. 88(7B): 3J-6J.

[4] Libby P. Act local, act global: Inflammation and the multiplicity of "vulnerable" coronary plaques. J Am Coll Cardiol. 2005. 45(10): 1600-2.

[5] Davidson MH, Corson MA, Alberts MJ, et al. Consensus panel recommendation for incorporating lipoprotein-associated phospholipase A2 testing into cardiovascular disease risk assessment guidelines. Am J Cardiol. 2008. 101(12A): 51F-57F.

[6] Pearson TA, Mensah GA, Alexander RW, et al. Markers of inflammation and cardiovascular disease: Application to clinical and public health practice: A statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. Circulation. 2003. 107(3): 499-511.

[7] Packard CJ, O'Reilly DS, Caslake MJ, et al. Lipoprotein-associated phospholipase A2 as an independent predictor of coronary heart disease. West of Scotland Coronary Prevention Study Group. N Engl J Med. 2000. 343(16): 1148-55.
[8] 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(12A): 41F-50F.
[9] Hakkinen T, Luoma JS, Hiltunen MO, et al. Lipoprotein-associated phospholipase A(2), platelet-activating factor acetylhydrolase, is expressed by macrophages in human and rabbit atherosclerotic lesions. Arterioscler Thromb Vasc Biol. 1999. 19(12): 2909-17.
[10] 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(7): 1586-93.
[11] Koenig W, Khuseyinova N, Lowel 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(14): 1903-8.
[12] Gerber Y, McConnell JP, Jaffe AS, Weston SA, Killian JM, Roger VL. Lipoprotein-associated phospholipase A2 and prognosis after myocardial infarction in the community. downloaded. Arterioscler Thromb Vasc Biol. 2006. 26(11): 2517-22.
[13] Sabatine MS, Morrow DA, O'Donoghue M, et al. Prognostic utility of lipoprotein-associated phospholipase A2 for cardiovascular outcomes in patients with stable coronary artery disease. Arterioscler Thromb Vasc Biol. 2007. 27(11): 2463-9.
[14] Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. Circulation. 2002. 106(25): 3143-421.
[15] Ballantyne CM, Hoogeveen RC, Bang H, et al. Lipoprotein-associated phospholipase A2, high-sensitivity C-reactive protein, and risk for incident coronary heart disease in middle-aged men and women in the Atherosclerosis Risk in Communities (ARIC) study. Circulation. 2004. 109(7): 837-42.
[16] Nambi V, Hoogeveen RC, Chambless L, et al. Lipoprotein-associated phospholipase A2 and high-sensitivity C-reactive protein improve the stratification of ischemic stroke risk in the Atherosclerosis Risk in Communities (ARIC) study. Stroke. 2009. 40(2): 376-81.
[17] Mohler ER 3rd, Ballantyne CM, Davidson MH, et al. The effect of darapladib on plasma lipoprotein-associated phospholipase A2 activity and cardiovascular biomarkers in patients with stable coronary heart disease or coronary heart disease risk equivalent: the results of a multicenter, randomized, double-blind, placebo-controlled study. J Am Coll Cardiol. 2008. 51(17): 1632-41.
[18] Serruys PW, Garcia-Garcia HM, Buszman P, et al. Effects of the direct lipoprotein-associated phospholipase A(2) inhibitor darapladib on human coronary atherosclerotic plaque. Circulation. 2008. 118(11): 1172-82.
[19] Wang WY, Zhang J, Wu WY, et al. Inhibition of lipoprotein-associated phospholipase A2 ameliorates inflammation and decreases atherosclerotic plaque formation in ApoE-deficient mice. PLOS ONE. 2011. 6(8): e23425.
[20] Wilensky RL, Shi Y, Mohler ER 3rd, et al. Inhibition of lipoprotein-associated phospholipase A2 reduces complex coronary atherosclerotic plaque development. Nat Med. 2008. 14(10): 1059-66.
[21] Hansson GK, Libby P, Schonbeck U, Yan ZQ. Innate and adaptive immunity in the pathogenesis of atherosclerosis. Circ Res. 2002. 91(4): 281-91.
[22] Wilensky RL, Hamamdzic D. The molecular basis of vulnerable plaque: Potential therapeutic role for immunomodulation. Curr Opin Cardiol. 2007. 22(6): 545-51.
[23] Tabas I, Williams KJ, Boren J. Subendothelial lipoprotein retention as the initiating process in atherosclerosis: Update and therapeutic implications. Circulation. 2007. 116(16): 1832-44.
[24] Tuzcu EM, Kapadia SR, Tutar E, et al. High prevalence of coronary atherosclerosis in asymptomatic teenagers and young adults: Evidence from intravascular ultrasound. Circulation. 2001. 103(22): 2705-10.
[25] Kolodgie FD, Burke AP, Skorija KS, et al. Lipoprotein-associated phospholipase A2 protein expression in the natural progression of human coronary atherosclerosis. Arterioscler Thromb Vasc Biol. 2006. 26(11): 2523-9.
[26] Herrmann J, Mannheim D, Wohlert C, et al. Expression of lipoprotein-associated phospholipase A(2) in carotid artery plaques predicts long-term cardiac outcome. Eur Heart J. 2009. 30(23): 2930-8.
[27] Mannheim D, Herrmann J, Versari D, et al. Enhanced expression of Lp-PLA2 and lysophosphatidylcholine in symptomatic carotid atherosclerotic plaques. Stroke. 2008. 39(5): 1448-55.
[28] Dada N, Kim NW, Wolfert RL. Lp-PLA2: An emerging biomarker of coronary heart disease. Expert Rev Mol Diagn. 2002. 2(1): 17-22.
[29] Lavi S, McConnell JP, Rihal CS, 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(21): 2715-21.
[30] Wu X, McIntyre TM, Zimmerman GA, Prescott SM, Stafforini DM. Molecular characterization of the constitutive expression of the plasma platelet-activating factor acetylhydrolase gene in macrophages. Biochem J. 2003. 375(Pt 2): 351-63.
[31] Ridker PM, Cushman M, Stampfer MJ, Tracy RP, Hennekens CH. Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. N Engl J Med. 1997. 336(14): 973-9.
[32] Dada N, Kim NW, Wolfert RL. Lp-PLA2: An emerging biomarker of coronary heart disease. Expert Rev Mol Diagn. 2002. 2(1): 17-22.
[33] Quinn MT, Parthasarathy S, Steinberg D. Lysophosphatidylcholine: a chemotactic factor for human monocytes and its potential role in atherogenesis. Proc Natl Acad Sci U S A. 1988. 85(8): 2805-9.
[34] Khuseyinova N, Greven S, Ruckerl R, et al. Variability of serial lipoprotein-associated phospholipase A2 measurements in post myocardial infarction patients: Results from the AIRGENE Study Center Augsburg. Clin Chem. 2008. 54(1): 124-30.
[35] Brilakis ES, Khera A, McGuire DK, et al. Influence of race and sex on lipoprotein-associated phospholipase A2 levels: Observations from the Dallas Heart Study. Atherosclerosis. 2008. 199(1): 110-5.
[36] Caslake MJ, Packard CJ, Suckling KE, Holmes SD, Chamberlain P, Macphee CH. Lipoprotein-associated phospholipase

A(2), platelet-activating factor acetylhydrolase: A potential new risk factor for coronary artery disease. Atherosclerosis. 2000. 150(2): 413-9.

[37] Thompson A, Gao P, Orfei L, et al. Lipoprotein-associated phospholipase A(2) and risk of coronary disease, stroke, and mortality: Collaborative analysis of 32 prospective studies. Lancet. 2010. 375(9725): 1536-44.

[38] Garza CA, Montori VM, McConnell JP, Somers VK, Kullo IJ, Lopez-Jimenez F. Association between lipoprotein-associated phospholipase A2 and cardiovascular disease: A systematic review. Mayo Clin Proc. 2007. 82(2): 159-65.

[39] Lanman RB, Wolfert RL, Fleming JK, et al. Lipoprotein-associated phospholipase A2: Review and recommendation of a clinical cut point for adults. Prev Cardiol. 2006. 9(3): 138-43.

[40] Brilakis ES, McConnell JP, Lennon RJ, Elesber AA, Meyer JG, Berger PB. Association of lipoprotein-associated phospholipase A2 levels with coronary artery disease risk factors, angiographic coronary artery disease, and major adverse events at follow-up. Eur Heart J. 2005. 26(2): 137-44.

[41] Winkler K, Hoffmann MM, Winkelmann BR, et al. Lipoprotein-associated phospholipase A2 predicts 5-year cardiac mortality independently of established risk factors and adds prognostic information in patients with low and medium high-sensitivity C-reactive protein (the Ludwigshafen risk and cardiovascular health study). Clin Chem. 2007. 53(8): 1440-7.

[42] Elkind MS, Tai W, Coates K, Paik MC, Sacco RL. High-sensitivity C-reactive protein, lipoprotein-associated phospholipase A2, and outcome after ischemic stroke. Arch Intern Med. 2006. 166(19): 2073-80.

[43] Biomarkers and surrogate endpoints: Preferred definitions and conceptual framework. Clin Pharmacol Ther. 2001. 69(3): 89-95.

[44] White H, Held C, Stewart R, et al. Study design and rationale for the clinical outcomes of the STABILITY Trial (STabilization of Atherosclerotic plaque By Initiation of darapLadIb TherapY) comparing darapladib versus placebo in patients with coronary heart disease. Am Heart J. 2010. 160(4): 655-61.

[45] O'Donoghue ML, Braunwald E, White HD, et al. Study design and rationale for the Stabilization of pLaques usIng Darapladib-Thrombolysis in Myocardial Infarction (SOLID-TIMI 52) trial in patients after an acute coronary syndrome. Am Heart J. 2011. 162(4): 613-619.e1.

[46] Macphee CH. Lipoprotein-associated phospholipase A2: A potential new risk factor for coronary artery disease and a therapeutic target. Curr Opin Pharmacol. 2001. 1(2): 121-5.

[47] Turunen P, Jalkanen J, Heikura T, et al. Adenovirus-mediated gene transfer of Lp-PLA2 reduces LDL degradation and foam cell formation in vitro. J Lipid Res. 2004. 45(9): 1633-9.

[48] Jang Y, Waterworth D, Lee JE, et al. Carriage of the V279F null allele within the gene encoding Lp-PLA(2) is protective from coronary artery disease in South Korean males. PLOS ONE. 2011. 6(4): e18208.

[49] MacPhee CH, Moores KE, Boyd HF, et al. Lipoprotein-associated phospholipase A2, platelet-activating factor acetylhydrolase, generates two bioactive products during the oxidation of low-density lipoprotein: Use of a novel inhibitor. Biochem J. 1999. 338 (Pt 2): 479-87.

[50] May HT, Horne BD, Anderson JL, et al. Lipoprotein-associated phospholipase A2 independently predicts the angiographic diagnosis of coronary artery disease and coronary death. Am Heart J. 2006. 152(5): 997-1003.

[51] Anderson JL. Lipoprotein-associated phospholipase A2: An independent predictor of coronary artery disease events in primary and secondary prevention. Am J Cardiol. 2008. 101(12A): 23F-33F.

[52] Quinn MT, Parthasarathy S, Steinberg D. Lysophosphatidylcholine: A chemotactic factor for human monocytes and its potential role in atherogenesis. Proc Natl Acad Sci U S A. 1988. 85(8): 2805-9.

[53] Colley KJ, Wolfert RL, Cobble ME. Lipoprotein associated phospholipase A(2): Role in atherosclerosis and utility as a biomarker for cardiovascular risk. EPMA J. 2011. 2(1): 27-38.

[54] Benitez S, Camacho M, Arcelus R, et al. Increased lysophosphatidylcholine and non-esterified fatty acid content in LDL induces chemokine release in endothelial cells. Relationship with electronegative LDL. Atherosclerosis. 2004. 177(2): 299-305.

[55] Shi Y, Zhang P, Zhang L, et al. Role of lipoprotein-associated phospholipase A2 in leukocyte activation and inflammatory responses. Atherosclerosis. 2007. 191(1): 54-62.

[56] Tjoelker LW, Wilder C, Eberhardt C, et al. Anti-inflammatory properties of a platelet-activating factor acetylhydrolase. Nature. 1995. 374(6522): 549-53.

[57] Morgan EN, Boyle EM Jr, Yun W, et al. Platelet-activating factor acetylhydrolase prevents myocardial ischemia-reperfusion injury. Circulation. 1999. 100(19 Suppl): II365-8.

[58] Theilmeier G, De Geest B, Van Veldhoven PP, et al. HDL-associated PAF-AH reduces endothelial adhesiveness in apoE-/- mice. FASEB J. 2000. 14(13): 2032-9.

[59] Noto H, Hara M, Karasawa K, et al. Human plasma platelet-activating factor acetylhydrolase binds to all the murine lipoproteins, conferring protection against oxidative stress. Arterioscler Thromb Vasc Biol. 2003. 23(5): 829-35.

[60] Blankenberg S, Stengel D, Rupprecht HJ, et al. Plasma PAF-acetylhydrolase in patients with coronary artery disease: Results of a cross-sectional analysis. J Lipid Res. 2003. 44(7): 1381-6.

[61] Hoffmann MM, Winkler K, Renner W, et al. Genetic variants and haplotypes of lipoprotein associated phospholipase A2 and their influence on cardiovascular disease (The Ludwigshafen Risk and Cardiovascular Health Study). J Thromb Haemost. 2009. 7(1): 41-8.

[62] Hou L, Chen S, Yu H, et al. Associations of PLA2G7 gene polymorphisms with plasma lipoprotein-associated phospholipase A2 activity and coronary heart disease in a Chinese Han population: The Beijing atherosclerosis study. Hum Genet. 2009. 125(1): 11-20.

[63] Abuzeid AM, Hawe E, Humphries SE, Talmud PJ. Association between the Ala379Val variant of the lipoprotein associated phospholipase A2 and risk of myocardial infarction in the north and south of Europe. Atherosclerosis. 2003. 168(2): 283-8.

[64] Wang T, Karino K, Yamasaki M, et al. Effects of G994T in the Lp-PLA2 gene on the plasma oxidized LDL level and carotid intima-media thickness in Japanese: the Shimane study. Am J Hypertens. 2009. 22(7): 742-7.

[65] Li L, Qi L, Lv N, et al. Association between lipoprotein-associated phospholipase A2 gene polymorphism and coronary artery disease in the Chinese Han population. Ann Hum Genet. 2011. 75(5): 605-11.

[66] Yamada Y, Ichihara S, Fujimura T, Yokota M. Identification of the G994–> T missense in exon 9 of the plasma platelet-activating factor acetylhydrolase gene as an independent risk factor for coronary artery disease in Japanese men. Metabolism. 1998. 47(2): 177-81.

[67] Shimokata K, Yamada Y, Kondo T, et al. Association of gene polymorphisms with coronary artery disease in individuals with or without nonfamilial hypercholesterolemia. Atherosclerosis. 2004. 172(1): 167-73.

[68] Jang Y, Kim OY, Koh SJ, 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.

[69] Ninio E, Tregouet D, Carrier JL, et al. Platelet-activating factor-acetylhydrolase and PAF-receptor gene haplotypes in relation to future cardiovascular event in patients with coronary artery disease. Hum Mol Genet. 2004. 13(13): 1341-51.

[70] Liu PY, Li YH, Wu HL, et al. Platelet-activating factor-acetylhydrolase A379V (exon 11) gene polymorphism is an independent and functional risk factor for premature myocardial infarction. J Thromb Haemost. 2006. 4(5): 1023-8.

[71] Casas JP, Ninio E, Panayiotou A, et al. PLA2G7 genotype, lipoprotein-associated phospholipase A2 activity, and coronary heart disease risk in 10 494 cases and 15 624 controls of European Ancestry. Circulation. 2010. 121(21): 2284-93.

[72] Cannon CP, Braunwald E, McCabe CH, et al. Intensive versus moderate lipid lowering with statins after acute coronary syndromes. N Engl J Med. 2004. 350(15): 1495-504.

[73] Blake GJ, Dada N, Fox JC, Manson JE, Ridker PM. A prospective evaluation of lipoprotein-associated phospholipase A(2) levels and the risk of future cardiovascular events in women. J Am Coll Cardiol. 2001. 38(5): 1302-6.

[74] Tuzcu EM, Kapadia SR, Tutar E, et al. High prevalence of coronary atherosclerosis in asymptomatic teenagers and young adults: Evidence from intravascular ultrasound. Circulation. 2001. 103(22): 2705-10.

[75] Blake GJ, Dada N, Fox JC, Manson JE, Ridker PM. A prospective evaluation of lipoprotein-associated phospholipase A(2) levels and the risk of future cardiovascular events in women. J Am Coll Cardiol. 2001. 38(5): 1302-6.

[76] Oei HH, der Meer IM v, Hofman A, et al. Lipoprotein-associated phospholipase A2 activity is associated with risk of coronary heart disease and ischemic stroke: The Rotterdam Study. Circulation. 2005. 111(5): 570-5.

[77] Persson M, Hedblad B, Nelson JJ, Berglund G. Elevated Lp-PLA2 levels add prognostic information to the metabolic syndrome on incidence of cardiovascular events among middle-aged nondiabetic subjects. Arterioscler Thromb Vasc Biol. 2007. 27(6): 1411-6.

[78] Daniels LB, Laughlin GA, Sarno MJ, Bettencourt R, Wolfert RL, Barrett-Connor E. Lipoprotein-associated phospholipase A2 is an independent predictor of incident coronary heart disease in an apparently healthy older population: The Rancho Bernardo Study. J Am Coll Cardiol. 2008. 51(9): 913-9.

[79] Tsimikas S, Willeit J, Knoflach M, et al. Lipoprotein-associated phospholipase A2 activity, ferritin levels, metabolic syndrome, and 10-year cardiovascular and non-cardiovascular mortality: Results from the Bruneck study. Eur Heart J. 2009. 30(1): 107-15.

[80] Jenny NS, Solomon C, Cushman M, et al. Lipoprotein-associated phospholipase A(2) (Lp-PLA(2)) and risk of cardiovascular disease in older adults: Results from the Cardiovascular Health Study. Atherosclerosis. 2010. 209(2): 528-32.

[81] Brilakis ES, McConnell JP, Lennon RJ, Elesber AA, Meyer JG, Berger PB. Association of lipoprotein-associated phospholipase A2 levels with coronary artery disease risk factors, angiographic coronary artery disease, and major adverse events at follow-up. Eur Heart J. 2005. 26(2): 137-44.

[82] Khuseyinova N, Imhof A, Rothenbacher D, et al. Association between Lp-PLA2 and coronary artery disease: focus on its relationship with lipoproteins and markers of inflammation and hemostasis. Atherosclerosis. 2005. 182(1): 181-8.

[83] 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(7): 1586-93.

[84] Robins SJ, Collins D, Nelson JJ, Bloomfield HE, Asztalos BF. Cardiovascular events with increased lipoprotein-associated phospholipase A(2) and low high-density lipoprotein-cholesterol: The Veterans Affairs HDL Intervention Trial. Arterioscler Thromb Vasc Biol. 2008. 28(6): 1172-8.