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REVIEW ARTICLE

Lipoprotein-associated phospholipase A2 (Lp-PLA2): a review of its role and significance as a cardiovascular biomarker

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Abstract

Objective: To conduct a comprehensive, systematic review of studies assessing the significance of lipoprotein-associated phospholipase A2 in cardiovascular diseases (CVDs).

Material and methods: A review of the literature was performed using the search term "Lipoprotein-associated phospholipase A2 (Lp-PLA2)" and each of the following terms: "cardiovascular risk," "cardiovascular death," "atherosclerotic disease," "coronary events," "transient ischemic attack (TIA)," "stroke," and "heart failure." The searches were performed on Medline, Google Scholar and ClinicalTrails.gov.

Results: The majority of published studies showed a significant association between Lp-PLA2 levels and cardiovascular events after multivariate adjustment. The association was consistent across a wide variety of subjects of both sexes and different ethnic backgrounds.

Conclusions: The role of Lp-PLA2 as a significant biomarker of vascular inflammation was confirmed, and Lp-PLA2 seems to be closely correlated to cardiovascular events. It may be an important therapeutic target and may have an important role in prevention, risk stratification and personalised medicine.

Keywords: Cardiovascular disease, growth factors/cytokines/inflammatory mediators, oxidative stress

Introduction

According to the World Health Organization (WHO), cardiovascular diseases (CVDs) are the number one cause of deaths worldwide and are responsible for over 30% of all global deaths annually, a fact that underscores the importance of primary and secondary prevention. The traditional cardiovascular risk factors as diabetes mellitus, hypertension, hyperlipidemia and smoking are helping clinicians to identify subjects at risk to develop CVDs. Despite of this, about 10–20% of individuals who develop coronary heart disease (CHD) have none or only one of the identifiable risk factors (Greenland et al., 2003; Khot et al., 2003).

Individuals with none or very few clinical risk factors are the least likely to be targeted and included in preventive therapy programs, but as a group, they experience a large number of cardiovascular events, being estimated that 35% of cardiovascular deaths occur in patients who have total serum cholesterol levels below 200 mg/dL (Castelli, 1996).

In 2001, the National Institutes of Health (NIH) Definition Working Group generated the following working definitions:

- biomarker—a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention
- clinical end point—a characteristic or variable that reflects how a patient feels, functions, or survives
- surrogate end point—a biomarker intended to substitute for a clinical end point.

A surrogate end point should predict clinical benefit (or harm or lack of benefit or harm) on the basis

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Abbreviations

ACS, Acute Coronary Syndrome;
apoB, apolipoprotein B;
ARIC, Atherosclerosis Risk in Communities;
CAD, cardiac allograph vasculopathy;
CABG, Coronary artery bypass surgery;
CHD, coronary heart disease;
CHF, Congestive Heart Failure;
CHS, Cardiovascular Health Study;
cPLA2, cytosolicphospholipase A2;
CV, Cardiovascular;
CVD, Cardiovascular disease;
HDL-C, High-density lipoprotein cholesterol;
HF, Heart Failure;
HFpEF, Heart failure with preserved ejection fraction;
HFrEF, Heart failure with reduced ejection fraction;
hs-CRP, high sensitive C-reactive protein;
ICAM-1, intercellular adhesion molecule 1;
IFN-γ, Interferon-γ;
IL-1β, Interleukin-1β;
IL-6, Interleukin-6;
IMHS, Intermountain Health Study;
IMT, Intima-media thickness;
iPLA2, Ca2+-independent phospholipase A2;
LDL-C, low-density-lipoprotein cholesterol;
Lp-PLA2, lipoprotein-associated phospholipase A2;
Lp(a), Lipoprotein a;
LURIC, The Ludwigshafen Risk and Cardiovascular Health Study;
LVEF, Left ventricular ejection fraction;
LysoPL, lysophospholipid;
LysoPC, lysophosphatidylcholine;
MCP-1, Monocytechemotactic protein-1;
MDCS, Malmo Diet & Cancer study;
MI, Myocardial Infarction;
MONICA, Monitoring of Trends & Determinants in Cardiovascular Disease;
NHS, Nurses' Health Study;
NOMASS, Northern Manhattan Stroke Study;
oxFFA, oxidised free fatty acids;
oxLDL, oxidised low-density lipoprotein;
oxPC, oxidized phosphatidylcholine;
PAF, platelet-activating factor;
PAF-AH, platelet-activating factor acetylhydrolases;
PDFG, platelet-derived growth factor;
PEACE, Prevention of Events with Angiotensin-Converting Enzyme Inhibitor;
PLA2, Phospholipase A2;
PROVE IT-TIMI22, Pravastatin or Atorvastatin Evaluation & Infection Therapy-Thrombolysis in Myocardial Infarction;
PTCA, Percutaneous transluminal coronary angioplast;
sPLA2, secreted phospholipase A2;
TIA, Transient Ischemic Attack;
TNF-α, tumour necrosis factor-α;
VCAM-1, vascular cell adhesion molecule-1;
VLDL, very-low-density lipoproteins;
UA, Unstable Angina;
WOSCOPS, West of Scotland Coronary Prevention Study

of epidemiological, therapeutic, pathophysiological, or other scientific evidence (Biomarkers Definitions Working Group, 2001).

Unfortunately, identifying such markers has proven to be difficult in the cardiovascular field. Recently, there has been an increased focus on the true role in CVD of Lp-PLA2, a lipid-associated biomarker, and it has been proposed as a valuable predictor of CVD. Lp-LPA2 is the link between lipid metabolism and the low-grade inflammation that is characteristic of cardiovascular or metabolic diseases. The degree of inflammation defines the vulnerability of the atherosclerotic plaque to rupture, and many studies during the last decade have attempted to identify high-risk, vulnerable plaques by identifying correlations between different biomarkers and cardiovascular complications. Lp-PLA2 level and activity may be directly associated with negative CVD outcome; therefore it may be useful as a potential biomarker of vascular inflammatory activity (Braun and Davidson, 2010) and as a prognostic biomarker for future cardiovascular events.

Recent epidemiologic studies demonstrated that increased Lp-PLA2 levels have a prognostic value and that they are associated with a high risk of future coronary and cerebrovascular events. Additionally, Lp-PLA2 may have predictive value for CHD or cardiovascular complications in healthy individuals and for recurrent events in patients with known atherosclerosis. In recent years, the value of Lp-PLA2 for risk determination in patients with heart failure has been established, making Lp-PLA2 one of the most promising biomarkers.

This review summarises the PLA2 superfamily. It provides a brief presentation of each enzyme type, with a focus on the structure/function, mechanism and properties of Lp-PLA2. This review also discusses Lp-PLA2 connection to cardiovascular events and summarises the available epidemiological and clinical data regarding Lp-PLA2 as a risk factor, prognostic biomarker and therapeutic target, grouped in terms of different CVDs (coronary disease, stroke and transient ischemic attack (TIA) and heart failure). Additionally, this review also contains a brief description of Lp-PLA2 modulation by different commonly used medications for the primary or secondary prevention or treatment of CVD.

Methods

A review of the literature was performed using the search term "*Lipoprotein-associated phospholipase A2 (Lp-PLA2)"* and each of the following terms*: "cardiovascular risk," "cardiovascular death," "atherosclerotic disease," "coronary events," "TIA," "stroke,"* and *"heart failure"* on Medline, Google Scholar and ClinicalTrails.gov. Publication dates ranged from Jan 1998 through December 2011. We aim to assess the role of Lp-PLA2 in the prediction of cardiovascular events in the primary

and secondary prevention settings and as a therapeutic target. The criteria used for our review included population-based epidemiological and clinical studies and the presence of clinical outcomes of interest, including cardiovascular death, atherosclerotic disease, coronary events, TIA, stroke and heart failure. We exclude studies without clinical outcomes.

PLA2 superfamily and Lp-PLA2's structure and properties

The superfamily of phospholipase A2 (PLA2) enzymes currently consists of 15 groups and numerous subgroups and includes five distinct types of enzymes: the secreted PLA2s (sPLA2), the cytosolic PLA2s (cPLA2), the Ca^{2+}-independent PLA2s (iPLA2), the platelet-activating factor acetylhydrolases (PAF-AH), and the lysosomal PLA2s (Schaloske and Dennis, 2006). In the past two decades, there has been a tremendous increase in our knowledge of the PLA2 enzyme superfamily, while Lp-PLA2 has generated particularly great interest.

The common enzymatic activity of PLA2 enzymes is to hydrolyse the sn-2 ester bond in phospholipids. The enzymes reaction products are lysophospholipids (lysoPL), especially lysophosphatidylcholine (lysoPC), and different fatty acids (Suckling, 2010).

The secreted PLA2s are characterised by low molecular weight (14–18 kDa), a requirement for histidine in the active site, a requirement for $Ca2^{+}$ for catalysis and the presence of six disulphide bonds (Schaloske and Dennis, 2006).

Cytosolic PLA2s have larger molecular weights (61–114 kDa), do not have bisulfide bonds and are dependent on a catalytic serine (Schaloske and Dennis, 2006). These enzymes are also dependent on $Ca2^{+}$, which (unlike the sPLA2 group) is required for translocation of the enzyme to the intracellular membrane and not for catalysis (Evans and Leslie, 2004; Shirai et al., 2005).

The $Ca2^{+}$-independent PLA2 (iPLA2) group comprises enzymes that do not require $Ca2^{+}$ for activity and utilise serine for catalysis (Schaloske and Dennis, 2006).

Lysosomal PLA2 is the most recent identified type. It has a molecular weight of 45 kDa and contains a conserved Ser-His-Asp triad and four cysteine residues for catalytic activity (Hiraoka et al., 2005; Schaloske and Dennis, 2006).

Lipoprotein associated phospholipase A2 (Lp-PLA2) belongs to the secretory phospholipase A2 group VII (sPLA2-VII) and was discovered because of its ability to catalyse the hydrolysis of PAF. It was therefore initially known as PAF-AH (Stafforini et al., 1987). Lp-PLA2 is a $Ca2^{+}$-independent 45-kDA protein that is composed of 441 amino acids. It is a serine-lipase that hydrolyses oxidised phospholipids at the *sn*-2 position with remarkable specificity, yielding oxidised fatty acids (oxFFA) and lysoPC (Six and Dennis, 2000; Winstead et al., 2000; Schaloske and Dennis, 2006). This enzyme is not interfacially activated (Min et al., 1999). Recently, regions in the catalytic domain that are important for binding low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol were identified; however, the mechanisms underlying the preferential binding of one molecule or the other is currently poorly understood (Gardner et al., 2008). Lp-PLA2 is secreted in active form by T lymphocytes, monocyte-derived macrophages and mast cells, and these cells seem to be the major source of Lp-PLA2 in plasma (McIntyre et al., 2009).

The gene for Lp-PLA2 is formed by 12 exons located on chromosome 6p21.2 to 12 (Tew et al., 1996). Variants of the Lp-PLA2 gene have been linked to different diseases. Lp-PLA2 gene polymorphisms may be proinflammatory or anti-inflammatory; these polymorphisms may confer increased or decreased cardiovascular risk or have no clinical significance at all. Further studies are needed in order to define the clinical significance of Lp-PLA2 gene polymorphisms.

Enzymatic function of Lp-PLA2

Lp-PLA2 is mostly produced in cells involved in atherosclerosis, such as macrophages, T-cells, lymphocytes, and mast cells (Stafforini et al., 1999). Through a protein–protein interaction between the N-terminus of Lp-PLA2 and the C-terminus of apolipoprotein B (apoB), Lp-PLA2 is mainly bound to LDL in the circulation, while the remaining Lp-PLA2 is distributed among HDL, very-low-density lipoproteins (VLDL) and lipoprotein a (Lp(a)) (Asano et al., 1999).

It has been determined that over 80% of plasma Lp-PLA2 may be linked to LDL cholesterol, while 10% may be associated with HDL cholesterol (Tsimihodimos et al., 2002).

Present in both plasma and atherosclerotic lesions oxLDL exhibits a wide array of proatherogenic properties and many of these are due to the presence of oxidized phospholipids (oxPL) within oxLDL (Steinberg, 2009). OxLDL is produced under oxidative stress generated by damaging free radicals (reactive oxygen and nitrogen species) both, in plasma and in the vascular wall cells.

Oxidation of LDL within the arterial wall take place following retention of LDL upon binding to proteoglicans and other extracellular matrix components and provides the substrate for the hydrolytic action of Lp-PLA2 (Figure 1).

By cleaving the oxidized phosphatidylcholine (oxPC) component of oxLDL particles, Lp-PLA2 generates two potent proinflammatory and proatherogenic mediators oxFFAs and lysoPC, which activate redox-sensitive inflammatory pathways in the vascular wall (Zalewski and Macphee, 2005).

Both the substrate for Lp-PLA2, oxLDL, and the products of its activity, lysoPC and oxFFA are associated with pro-apoptotic effects on macrophages (Carpenter et al., 2001).

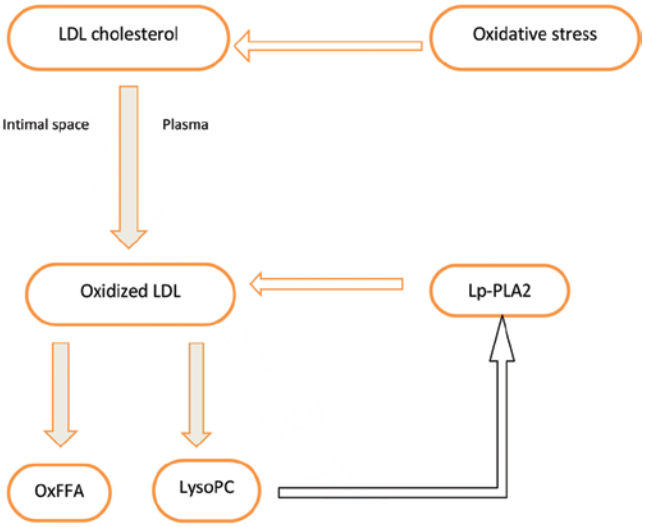


Figure 1. Lp-PLA2 pathway.

Atherogenic role of Lp-PLA2

Lp-PLA2 hydrolyses potentially dangerous oxidised phospholipids, reducing their ability to promote monocyte chemotaxis and adhesion, and it decreases the bioavailability of the prothrombotic PAF. Thus, in a physiological state, Lp-PLA2 may have atheroprotective and anti-inflammatory functions (Stafforini, 2009). Those roles have been supported by a few human epidemiological studies (Yamada et al., 1998) in populations (especially Asian) where a genetic deficiency of PLA2s has been associated with an increased incidence and severity of CVD, in particular stroke and myocardial infarction. These studies have resulted in some early controversy regarding the role of Lp-PLA2 in atherogenesis. Additionally, Lp-PLA2 activity is important for reducing the immunogenicity of oxLDL, a phenomenon that can be attributed to a decrease in oxidised phospholipids in patients with coronary artery disease (CAD) and healthy individuals (Lourida et al., 2006).

Recent evidence appears to show that Lp-PLA2 has a definite proatherogenic role. The bioproducts of the Lp-PLA2-mediated hydrolysis of oxidised phospholipids, such as lysoPC and oxFFA, are proinflammatory and have an important role in inflammation and accordingly in atherogenesis. LysoPC in particular is involved in inflammatory cytokine production and in the induction of the expression of adhesion molecules and cytokines. It has a chemoattractant property for macrophages, and it induces vascular smooth muscle migration (Zalewski and Macphee, 2005). LysoPC also up-regulates Lp-PLA2 activity, resulting in a vicious cycle in which proinflammatory mediators are increasingly up-regulated, contributing to plaque progression and destabilisation (Macphee et al., 2005). Furthermore, oxFFAs promote atherosclerosis by directly and indirectly increasing oxidative stress and the presence of oxidised LDL and other lipoproteins in the plasma and arterial walls, thereby initiating fatty streak formation.

Inflammatory cells in atherosclerotic plaques produce more Lp-PLA2, resulting in a self-enhancing cycle of upregulation of Lp-PLA2 and progression of atheromas.

It is worth mentioning the association between Lp-PLA2 and apoptosis; this association should be further investigated because lysoPC is a potent chemoattractant for monocytes and T-cells, which promotes endothelial dysfunction, stimulates macrophage proliferation and induces apoptosis in smooth muscle cells, with potential implications for atherogenesis (Takahashi et al., 2002).

There has been some controversy regarding the biological role of Lp-PLA2 in atherosclerosis, and Lp-PLA2 may have a dual action (Figure 2) that is dependent on its association either with LDL (proatherogenic) or HDL (antiatherogenic) (Silva et al., 2011). However, the mechanism for preferential binding of one molecule over the other is currently poorly understood (Gardner et al., 2008).

Lp-PLA2 as cardiovascular biomarker

CHD and cardiovascular death

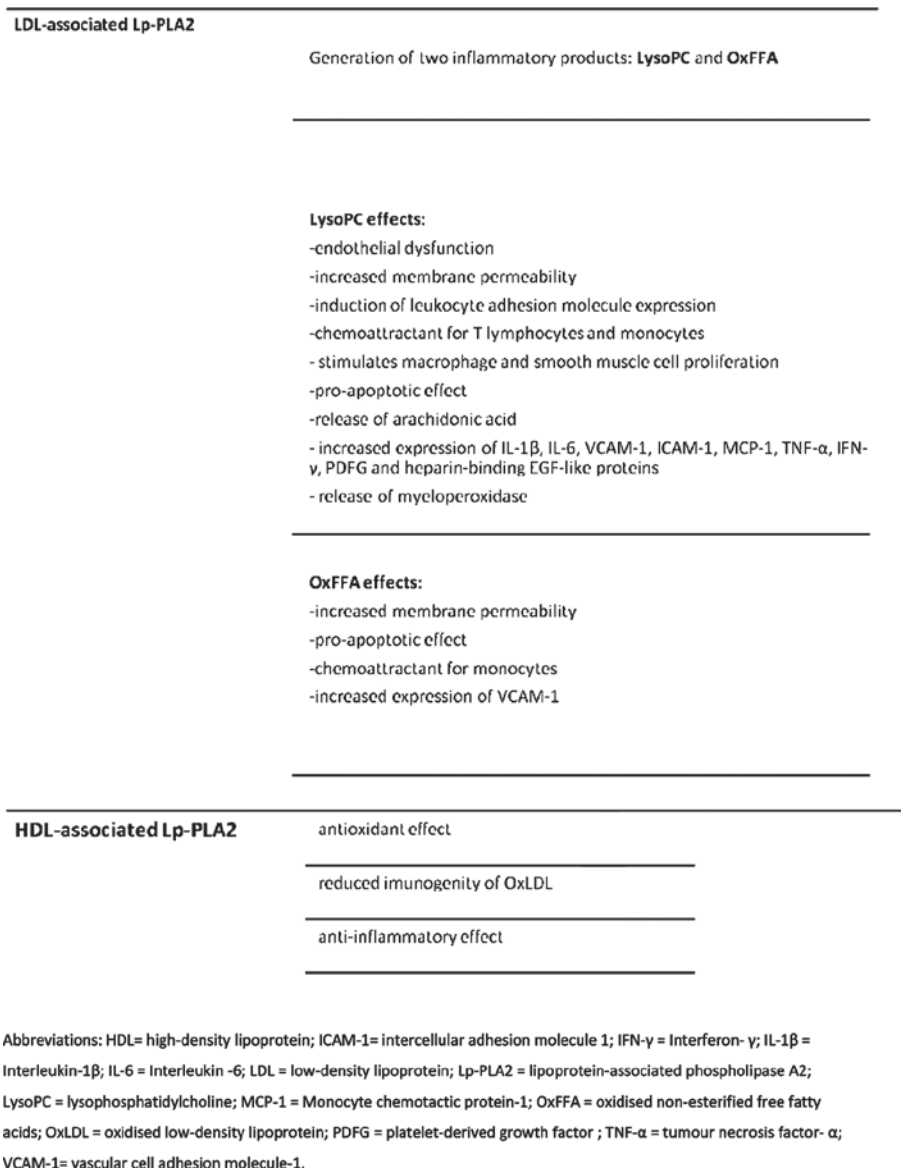
Over the past several years, established and novel biomarkers have been tested as correlates of CVD risk. C-reactive protein was the soluble marker most reproducibly associated with cardiovascular risk (Ridker et al., 2005).

In 2000, for the West of Scotland Coronary Prevention Study (WOSCOPS) a twofold greater risk of CHD was observed for patients in the highest quintile of Lp-PLA2 levels compared with those in the lowest quintile. This relationship remained valid after taking traditional CV risk factors and other inflammatory markers (including hs-CRP) into account (Packard et al., 2000; Table 1).

In the Atherosclerosis Risk in Communities (ARIC) study individuals with increased levels of Lp-PLA2 and hs-CRP were three times more likely to have a coronary event compared with individuals with low Lp-PLA2 and hs-CRP levels (Ballantyne et al., 2004).

The expression of Lp-PLA2 is higher in macrophages associated with vulnerable and ruptured plaques, and within the necrotic core, compared with less advanced plaques. Moreover, elevated plasma Lp-PLA2 is indicative of rupture-prone plaques and is a strong independent predictor of CHD and stroke (Ballantyne et al., 2005). All those findings could make Lp-PLA2 a novel biomarker, representing a non-invasive tool for assessing plaque stability (Ballantyne et al., 2005).

To date, there is a vast body of evidence derived from prospective studies (Brilakis et al., 2005; Elkind et al., 2006; Sabatine et al., 2007) and meta-analyses (Garza et al., 2007) underlining the association between Lp-PLA2 and cardiovascular (CV) events (Table 2). A strong, statistically significant correlation exists between increased Lp-PLA2 activity and future CV risk (Corson et al., 2008; Ali and Madjid, 2009) because high levels of Lp-PLA2 are found in rupture-prone plaques, and Lp-PLA2 may



Abbreviations: HDL= high-density lipoprotein; ICAM-1= intercellular adhesion molecule 1; IFN-γ = Interferon- γ; IL-1β = Interleukin-1β; IL-6 = Interleukin -6; LDL = low-density lipoprotein; Lp-PLA2 = lipoprotein-associated phospholipase A2; LysoPC = lysophosphatidylcholine; MCP-1 = Monocyte chemotactic protein-1; OxFFA = oxidised non-esterified free fatty acids; OxLDL = oxidised low-density lipoprotein; PDFG = platelet-derived growth factor ; TNF-α = tumour necrosis factor- α; VCAM-1= vascular cell adhesion molecule-1.

Figure 2. Possible HDL- and LDL-associated Lp-PLA2 effects and potential dual role in atherosclerosis.

actually be released from these plaques into the circulation. Staining of coronary tissue demonstrates the presence of Lp-PLA2 in the thin fibrous cap of rupture-prone plaques (Kolodgie et al., 2006).

Lp-PLA2 has been confirmed to predict the presence of CAD, even among patients undergoing coronary angiography. Uniquely, Lp-PLA2 predicted the risk of CAD death, but not all cause death (May et al., 2006).

Lp-PLA2 has recently gained much attention because of its potential to be used as an adjunct to traditional risk factors. Several epidemiologic studies have shown a correlation between Lp-PLA2 levels and traditional cardiovascular risk factors, including age, LDL, HDL, LDL/HDL ratio, total cholesterol, triglycerides, obesity and metabolic syndrome (Koenig et al., 2004; Ballantyne et al., 2005; Gazi et al., 2005; Persson et al., 2007; Daniels et al., 2008; Tsimikas et al., 2009). Interestingly, even after adjusting for these traditional risk factors, increased Lp-PLA2 levels are still strongly associated with future cardiovascular events (Koenig et al., 2006).

There are some advantages noticed for Lp-PLA2 as a marker; one of those is the fact that the Lp-PLA2 level is independent of insulin resistance (Anderson, 2008). Another one is the fact that Lp-PLA2 has high specificity for vascular inflammation and has minimal bio-variation, facts that could actually make Lp-PLA2 superior to other inflammatory markers, such as high sensitivity C-reactive protein (hs-CRP) (Ali and Madjid, 2009). This finding was also supported by other recent data from the Cardiovascular Health Study. In this study, both mass and activity were associated with risk of MI independent of CVD risk factors and CRP. Lp-PLA2 activity, but not mass, was associated with CVD death. In addition, there was some synergism when Lp-PLA2 mass or activity was

Table 1. Lp-PLA2 and first or secondary coronary events studies.*

Author, Year	Study name	Demographics	# Cases	CV endpoint	Lp-PLA2 assay	Relative risk (95% CI)**	Hazard ration/odds ratio (95% CI)**
Packard et al., 2000	WOSCOPS	Men with hypercholesterolemia	580/1160	CHD death, nonfatal MI, need of revascularization	Mass	1.18 (1.05–1.33)	1.80 (1.3–2.6)
Koenig et al., 2004	MONICA	Men with moderate hypercholesterolemia	97/837	CHD death, nonfatal MI	Mass	—	1.23 (1.02–1.47)
Ballentyne et al., 2005	ARIC	Healthy subjects	608/740	CHD death, nonfatal MI, surgical revascularization	Mass	—	LDL-C<130mg/dl(tertiles)*** 1.83 (1.10–3.05) 2.08 (1.20–3.62) LDL-C>130 mg/dl (tertiles) 1.83 (1.11–3.00) 1.99 (1.17–3.38)
Oei et al., 2005	Rotterdam	Healthy subjects age > 55years	308/1820	CHD death, nonfatal MI	Activity	—	CHD quartiles 1.39 (0.92–2.10) 1.99 (1.32–3.00) 1.97 (1.28–3.02)
Brilakis et al., 2005	Mayo	Consecutive angiography cases, age 26–76 years	61/405	CHD death, nonfatal MI, revascularization, stroke	Mass		For 1 SD increase in Lp-PLA2 1.3 (1.06–1.59)
Koenig et al., 2006	—	CHD patients entering rehabilitation program (age 30–70 years)	95/956	CVD death, MI, stroke,	Mass activity		Mass: Lp-PLA2 tertiles 2.16 (1.20–3.90) 2.09 (1.10–3.96) Activity: Lp-PLA2 tertiles 1.79 (0.99–3.22) 1.81 (0.94–3.49)
May et al., 2006	IMHS	Consecutive patients undergoing coronary angiography	1493	All cause of death, CAD death, non CAD CV death, MI and CVA	Mass		CV events quartiles OR 1.15 (0.78–1.71) 1.53 (1.02–2.31) 2.44 (1.58–3.79) CAD death HR 1.73 (0.84–3.61) Q4 vs.. Q1 2.18 (1.04–4.57) Q3 vs. Q1 1.27 (0.58–2.78) Q2 vs. Q1
Elkind et al., 2006	NOMASS	1st stroke patients	467 (80 recurrent stroke)	Recurrent stroke, MI, vascular death, all cause of death	Mass		Combined endpoints (recurrent stroke, MI, death) 1.86 (1.01–3.42)
O'Donoghue et al., 2006	PROVE-IT TIMI22	ACS patients randomized on 2 statins doses	995/2653	Death, MI, UA requiring revascularization, Stroke	Mass activity		1.33 (1.01–1.74)
Sabatine et al., 2007	PEACE	Stable CAD	3766	CV death, MI, Coronary revascularization, UA, stroke	Mass		Lp-PLA2 quartiles 1.13 (0.94–1.36) 1.23 (1.02–1.48) 1.41 (1.17–1.70)

(*Continued*)

Table 1. *(Continued).*

Author, Year	Study name	Demographics	# Cases	CV endpoint	Lp-PLA2 assay	Relative risk (95%CI)**	Hazard ration/odds ratio (95%CI)**
Persson et al., 2007	MDCS	Male and female residents of Malmo city, nondiabetic, no CDV	4480	MI and stroke	Mass activity		Activity (Lp-PLA2 increased, with MetS) MI 1.97 (1.34–2.90) Activity (Lp-PLA2 increased, without MetS) MI 1.40 (1.03–1.92)
Winkler et al., 2007	LURIC	CAD	3232	Cardiac death, total death	Activity		Lp-PLA2 tertiles 1.45 (1.08–1.92) 1.59 (1.14–2.22)
Daniels et al., 2008	Rancho Bernardo	Man and women, median age 72 years, no CVD	1077	MI, angina and coronary revascularization	Mass		Lp-PLA2 Quartiles 1.43 (0.89–2.31) 1.96 (1.23–3.10) 1.75 (1.10–2.78)
Tsimikas et al., 2009	Bruneck	Sex and age stratified random sample, age 45–84 years	765	CV death, MI, stroke, TIA and non-CVD death	Activity		MI Tertiles 2.4 (0.8–7.1) 3.6 (1.2–10.3)
Jenny et al., 2010	CHS	White and black men and women, age > 65 years, no CVD	4318	Ischemic stroke and MI	Mass activity		Mass: MI 1.49 (1.19–1.85) Death 1.11 (0.92–1.33) Activity: MI 1.36 (1.09–1.70) CVD death 1.23 (1.02–1.50)
Hatoum et al., 2011	NHS	Healthy women	421/842	Incidence of fatal and nonfatal MI	Activity	1.75 (1.09–2.84)	—

ACS, acute coronary syndrome; ARIC, Atherosclerosis Risk in Communities; CHD, coronary heart disease; CHS, cardiovascular health study; CVD, cardiovascular disease; IMHS, Intermountain Health Study; LDL-C, low-density lipoprotein cholesterol; Lp-PLA2, lipoprotein-associated phospholipase A2; LURIC, The Ludwigshafen Risk and Cardiovascular Health Study; MDCS, Malmo Diet & Cancer study; MI, myocardial infarction; MONICA, Monitoring of Trends & Determinants in Cardiovascular Disease; NHS, Nurses' Health Study; NOMASS, Northern Manhattan Stroke Study; PEACE, Prevention of Events with Angiotensin-Converting Enzyme Inhibitor; PROVE IT-TIMI22, Pravastatin or Atorvastatin Evaluation & Infection Therapy-Thrombolysis in Myocardial Infarction; TIA, transient ischemic attack; UA, unstable angina; WOSCOPS, West of Scotland Coronary Prevention Study.
*All results were multivariate adjusted to traditional risk factors and CRP, lipids, race, education, medication used, alcohol intake and other inflammatory markers.
**Analysis by tertiles, quartiles, dichotomous.
***Without CRP adjustment.

Table 2. Lp-PLA2 and stroke, TIA or subclinical extracoronary atherosclerosis studies.*

Author, year	Study name	Demographics	# subjects	CV endpoint (other than CHD death or nonfatal MI)	Lp-PLA2 assay	Relative risk (95% CI)**	Hazard ratio/odds ratio (95% CI)**
Oei et al., 2005	Rotterdam	Healthy subjects age > 55 years	308(CHD)/1820	Ischemic stroke	Activity	—	Ischemic Stroke quartiles 1.08 (0.55–2.11) 1.58 (0.82–3.04) 1.97 (1.03–3.79)
Kardys et al., 2006	Rotterdam	Healthy subjects age > 55 years	1609	Extracoronary atherosclerosis	Activity	—	Carotid IMT: OR 0.86 (0.53–1.4)
Elkind et al., 2006	NOMASS	1st stroke patients	467 (80 recurrent stroke)	Recurrent stroke, MI, vascular death, all cause of death	Mass		Recurrent stroke: 2.08 (1.04–4.18)
Persson et al., 2007	MDCS	Male and female residents of Malmo city, nondiabetic, no CDV	4480/130 strokes	Stroke	Mass activity	1.46 (1.01–2.13)	
Persson et al., 2007	MDCS	Male and female	5402	Carotid IMT	Mass activity		No association
Tsimikas et al., 2009	Bruneck	Sex and age stratified random sample, age 45–84 years	765	stroke, TIA and non-CVD death	Activity		Stroke/TIA tertiles 1.2 (0.5–2.9) 2.0 (0.8–4.8)
Jenny et al., 2010	CHS	White and black men and women, age > 65 years, no CVD	4318	Ischemic stroke	Mass activity		Mass: stroke1.21 (0.98–1.49)

CHD, coronary heart disease; CHS, cardiovascular health study; CV, cardiovascular; CVD, cardiovascular disease; IMT, intima-media thickness; Lp-PLA2, lipoprotein-associated phospholipase A2; MDCS, Malmo Diet & Cancer Study; MI, myocardial infarction; NOMASS, Northern Manhattan Stroke Study; TIA, transient ischemic attack.
*All results were multivariate adjusted to traditional risk factors and CRP, lipids, race, education, medication used, alcohol intake and other inflammatory markers.
**Analysis by tertiles, quartiles, dichotomous.

combined with CRP. For MI risk, both Lp-PLA2 mass and activity added excess risk to elevated CRP alone (~20% excess risk), while for CVD death, Lp-PLA2 activity combined with CRP added excess risk (~12% excess risk; Jenny et al., 2010; Table 1).

These data were strengthened by a recent meta-analysis confirming the log-linear-like correlation between the mass and activity of Lp-PLA2 with the risk of CHD and cardiovascular death (Thompson et al., 2010). The same meta-analysis, which examined records from 79,036 individuals participating in 32 prospective studies (yielding 17,722 incident fatal or non-fatal outcomes) revealed an interesting finding. It showed that the correlations of Lp-LPA2 mass and activity are not exclusive to vascular events and that vascular outcome associations depend, at least partially, on plasma lipids. According to this recent meta-analysis, the relative risk for CHD (adjusted for conventional risk factors) is 1.11 (95% CI 1.07–1.16) for increase Lp-PLA2 mass and 1.10 (95% CI 1.05–1.16) for Lp-PLA2 activity. For vascular mortality, the adjusted relative risk is 1.13 (1.05–1.22) for Lp-PLA2 mass and 1.16 (1.09–1.24) for Lp-PLA2 activity; for ischaemic stroke, the relative risk is 1.14 (1.02–1.27) (Lp-PLA2 mass) and 1.08 (0.97–1.20) (Lp-PLA 2 activity); and for non-vascular mortality, the relative risk is 1.10 (1.03–1.18) (Lp-PLA2 mass) and 1.10 (1.04–1.17) (Lp-PLA 2 activity; Thompson et al., 2010).

Different studies have shown that Lp-PLA2 activity and mass levels are higher in men than in women. In Rotterdam study, Lp-PLA2 activity values were 46.8 nmol/min/mL for men compared with 43.0 nmol/min/mL for women (Oei et al., 2005).

The Dallas Heart Study, performed in a multi-ethnic population, confirmed those findings. This finding may be due to oestrogen's ability to down-regulate Lp-PLA2 expression (Miyaura et al., 1991). Oestrogen replacement therapy can significantly reduce Lp-PLA2 activity in healthy post-menopausal women (Yoshimura et al., 1999), while administration of steroids with progesterone-like activity increases Lp-PLA2 activity (Ohshige et al., 1994).

A recent study found that Lp-PLA2 activity was associated with a 7.9% net increase in the prediction of CHD for women, with a global 3.5% gain in predictive ability. Lp-PLA2 activity adds value to the traditional risk factors, thus improving the risk classification (Hatoum et al., 2010).

Recent data revealed that Lp-PLA2 values are independently associated with good coronary collateral development and Gensini score in patients with isolated

left CAD (Orem et al., 2011). On the other hand, the Rotterdam Coronary Calcification Study demonstrated a moderate association between Lp-PLA2 activity and coronary calcifications after age adjustment, suggesting at the same time that the effect of Lp-PLA2 on coronary calcifications may be exerted through its effects on LDL cholesterol (Kardys et al., 2007).

The relationship between Lp-PLA2 levels and coronary artery ectasia (CAE) was recently investigated, and the results of the research showed increased levels of Lp-PLA2 in patients with isolated CAE, suggesting that Lp-PLA2 may be implicated in the pathogenesis of this disease (Korkmaz et al., 2011).

It should be noted that the studies presented above used different cut-off points for Lp-PLA2 values. Starting with 2008, the Consensus Panel has recommended that the Lp-PLA2 mass cut-off point should be 200 ng/mL together with lipid lowering goals based on risk stratification, while the earlier recommendation was 235 ng/mL.

According to current recommendations, values of Lp-PLA2 mass higher than 200 ng/mL place the individual in a high-risk category for CAD (Davidson et al., 2008).

Stroke and TIA

It was suggested that Lp-PLA2 is an independent risk predictor of stroke and TIAs (Oei et al., 2005; Persson et al., 2008; Table 2). Through recent research it has been noticed that atherosclerotic plaques from symptomatic patients with a history of prior ipsilateral stroke or TIA demonstrated greater levels of Lp-PLA2 and its product, lysoPC, than plaques obtained from asymptomatic patients (Mannheim et al., 2008), indicating the clinical significance of Lp-PLA2 in cerebrovascular disease.

In the Rotterdam study, the risk of stroke increased with each increase in quartile of Lp-PLA2 (Oei et al., 2005), nevertheless, the association between Lp-PLA2 activity and subclinical extracoronary atherosclerosis was strongly attenuated or even disappeared after adjusting for cholesterol levels (Kardys et al., 2006). This finding was confirmed by another study that failed to identify an association between Lp-PLA2 and carotid intima-media thickness (IMT) (Persson et al., 2007). On the other hand, a recent study showed that in patients with beta-thalassemia, Lp-PLA2 is correlated with the IMT, suggesting that this enzyme may be implicated in premature carotid atherosclerosis (Tselepis et al., 2010).

In the ARIC study, individuals with increased levels of both Lp-PLA2 and hs-CRP had an 11-fold higher incidence of stroke than individuals with low Lp-PLA2 and hs-CRP levels (Ballantyne et al., 2005). This association between Lp-PLA2 levels and first ischaemic stroke was confirmed in the Malmo Diet and Cancer Study (Persson et al., 2005), in the Bruneck study (Tsimikas et al., 2009) and in the Cardiovascular Health Study (Jenny et al., 2010).

Further strengthening these data, some studies demonstrated that circulating Lp-PLA2 is increased in asymptomatic patients with high-grade carotid stenosis and unstable plaques, suggesting that Lp-PLA2 may be a relevant biomarker to guide decisions regarding invasive therapy in asymptomatic patients with carotid artery disease (Sarlon-Bartoli et al., 2011).

Heart failure and heart transplant

In addition to data regarding CHD or stroke, Lp-PLA2 levels could provide data regarding the risk of developing heart failure or other heart diseases. Baseline Lp-PLA2 levels are associated with a high risk of developing heart failure in older adults, independent of coronary risk factors (Suzuki et al., 2009; Table 3). In patients with established heart failure, Lp-PLA2 levels are associated with mortality rates, with an age-sensitive cut-off value. Therefore, for patients older than 80 years, elevated Lp-PLA2 ceases to be a risk marker. Notably, no gender differences were shown in the Lp-PLA2-mortality association (Gerber et al., 2009).

Moreover, Lp-PLA2 activity levels are significantly associated with renal disease, left ventricular dysfunction and congestive heart failure in elderly persons (Furberg et al., 2008).

Lp-PLA2 may be consistently correlated with HF, regardless of its aetiology and it has been identified that elevated plasma values of Lp-PLA2 in heart failure with preserved ejection fraction (HFpEF) are consistent with the exacerbated inflammatory status (Moldoveanu et al., 2011).

For heart transplant patients, Lp-PLA2 levels are independently correlated with the progression of cardiac allograft vasculopathy (CAV) and increased rates of cardiovascular events and cardiovascular death. These findings make Lp-PLA2 a useful marker for assessing the risk of developing CAV and suggest that it is a possible therapeutic target in heart transplant patients (Raichlin et al., 2008).

Modulation of Lp-PLA2

Previous data from clinical trials have shown that many drugs commonly used in clinical practice for the primary or secondary prevention or treatment of CVD can modify Lp-PLA2 mass and activities.

There are contradictory data regarding the effect of some drugs used in the treatment of diabetes. A study in diabetic patients taking pioglitazone and glipizide showed no effect on Lp-PLA2 plasma values (Basu et al., 2007), while another study showed that pioglitazone administration enhances Lp-PLA2 activity (Sumita et al., 2004).

Statins and fibrates are classes of lipid-lowering medications that are commonly used for cardiovascular primary and secondary prevention. Administration of atorvastatin (20 mg/day) significantly reduced Lp-PLA2 activity in patients with primary dyslipidaemia types IIA and IIB by 28.6% and 42.4%, respectively (Tsimihodimos

Table 3. Lp-PLA2 and heart failure studies.*

Author, Year	Study name	Demographics	#subjects	CV endpoint	Lp-PLA2 assay	Relative risk (95% CI)**	Hazard ration/odds ratio (95% CI)**
Raichlin et al., 2008	—	Heart transplant recipients	112	CV death, reduction in LVEF ≤ 45%,secondary to CAV; PTCI, CABG	Mass	—	Lp-PLA2 > 236 ng/ml (tertiles) 2.4 (1.16–5.19)
Furberg et al., 2008	CHS	Men and women age > 65 years	5531	CVD	Activity	—	27% greater risk of prevalent CHF per standard deviation increment of Lp-PLA2
Gerber et al., 2009	Olmstead	HF patients	646	Mortality	Mass	—	3.83 (1.93–7.61) in the <80 years old group 0.82 (0.44–1.51) in the >80 years old group
Suzuki et al., 2009	CHS	Men and women age > 65 years, no CHF, no CVD	3991	CHF	Antigen activity		Lp-PLA2 antigen (quartiles) 1.44 (1.16–1.79) Q4 vs. Q1 Lp-PLA2 activity (quartiles) 1.06 (0.84–1.32)
Moldoveanu et al., 2011	—	HF (HFrEF and HFpEF)	228 HF/20 controls	—	Activity		HFpEF Lp-PLA2 levels > HFrEF lp-PLA2 levels > Controls Lp-PLA2 levels

CAD, cardiac allograph vasculopathy; CABG, coronary artery bypass surgery; CHF, congestive heart failure; CHS, cardiovascular health study; CV, cardiovascular; CVD, cardiovascular disease; HF, heart failure; HFpEF, heart failure with preserved ejection fraction; HFrEF, heart failure with reduced ejection fraction; Lp-PLA2, lipoprotein-associated phospholipase A2; LVEF, left ventricular ejection fraction; MI, myocardial infarction; PTCA, percutaneous transluminal coronary angioplast; TIA, transient ischemic attack.
*All results were multivariate adjusted to traditional risk factors and CRP, lipids, race, education, medication used, alcohol intake and other inflammatory markers.
**Analysis by tertiles, quartiles, dichotomous.

et al., 2002). Rosuvastatin administration in type IIA dyslipidaemia decreased both Lp-PLA2 mass and activity (Saougos et al., 2007).

In the PROVE IT-TIMI 22 trial, designed to determine whether intensive lowering of blood cholesterol levels beyond the recommended level of 100 mg/dL would further increase the clinical benefits of statins, patients were randomised either to pravastatin (40 mg/day) or atorvastatin (80 mg/day). Interestingly, Lp-PLA2 was not useful for risk stratification when measured early after acute coronary syndrome (ACS). Importantly, however, a clinical benefit emerged as early as 30 days after ACS; Lp-PLA2 activity was significantly lowered with high-dose statin therapy and 30-day Lp-PLA2 activity in the highest quintile was associated with a statistically significant 33% increase in the relative risk of CV events, independent of C-reactive protein and LDL cholesterol levels (O'Donoghue et al., 2006). The effects of simvastatin, pravastatin, and lovastatin on Lp-PLA2 activity are controversial (Schaefer et al., 2005; O'Donoghue et al., 2006; Kom et al., 2007; Ky et al., 2008).

Fenofibrate, however, has confirmed efficacy in reducing Lp-PLA2 mass (Rosenson, 2008). Moreover, in a study investigating the combination of a low-lipid diet and orlistat, fenofibrate or both drugs over six months, a significant reduction of Lp-PLA2 activity was observed in all groups (14%, 22% and 35%, respectively) when compared with baseline (Filippatos et al., 2007).

Ezetimibe, a selective cholesterol absorption inhibitor, significantly decreases both Lp-PLA2 activity and mass. This reduction in mass and activity is associated with all apolipoprotein-B subtypes (Saougos et al., 2007).

In the LURIC study, Lp-PLA2 activity was lower in women than in men and was affected by the intake of lipid-lowering drugs (–12%; $P < 0.001$), aspirin (–6%; $P < 0.001$), β-blockers (–6%; $P < 0.001$), and digitalis (+7%; $P < 0.001$). Lp-PLA2 activity was not elevated in unstable angina, non-ST-elevation myocardial infarction, or ST-elevation myocardial infarction. When non-users of lipid-lowering drugs were examined, Lp-PLA2 activity was associated with the severity of CAD and the number of coronary vessels with significant stenosis (Winkler et al., 2005).

The PEACE Trial investigated the prognostic utility of Lp-PLA2 for cardiovascular outcomes in patients with stable CAD who were treated with angiotensin converting enzyme (ACE) inhibitors. The study concluded that for patients with stable CAD, an elevated level of Lp-PLA2 was a significant predictor of nonfatal adverse cardiovascular outcomes independent of hs-CRP and traditional clinical risk factors and that further investigation would be needed to establish whether therapies that lower Lp-PLA2 reduce cardiovascular risk (Sabatine et al., 2007).

An interesting observation comes from the Nurses' Health Study, a cross-sectional study that showed that lower Lp-PLA2 activity was related to several dietary factors. These factors included a higher percentage of energy consumed as protein instead of carbohydrate, a higher intake of mono-unsaturated fat instead of carbohydrate, mild to moderate alcohol consumption and

not being overweight or obese. It is unknown whether dietary Lp-PLA2 changes affect the cardiovascular risk (Hatoum et al., 2010).

Lp-PLA2 as a therapeutic target

Lp-PLA2 may be both a specific marker and a causal mediator of plaque progression and instability. Moreover, numerous epidemiologic studies clearly demonstrated an association between Lp-PLA2 and increased cardiovascular risk. There is also histological evidence of increased vascular Lp-PLA2 in rupture-prone coronary and carotid lesions. For all these reasons, there has been an increasing interest in pharmacologically targeting Lp-PLA2 to reduce cardiovascular risk.

Darapladib is a member of a new class of drugs, the selective Lp-PLA2 inhibitors. These agents block the active serine residue and effectively reduce Lp-PLA2 activity. Darapladib reduces lysoPC content and expression of genes associated with macrophage and T-lymphocyte functioning, thereby decreasing plaques and necrotic core areas. Because Lp-PLA2 is also involved in lipid modifications within the atheroma, it may be a complementary therapeutic target for the reduction of LDL-C in patients with high-risk plaques. Recent data support the hypothesis that darapladib is an anti-atherosclerotic therapy whose efficacy can be gauged through Lp-PLA2 activity levels (McCullough, 2009).

In preclinical studies, darapladib reduced coronary atherosclerosis by reducing the necrotic core area of the coronary plaque and medial destruction, confirming the crucial role of vascular inflammation (independent of hypercholesterolaemia) in the development of lesions implicated in the pathogenesis of myocardial infarction and stroke (Wilensky et al., 2008).

Phase I and II studies have shown a dose-dependent reduction in Lp-PLA2 activity with darapladib, with no effect on levels of plasma lipids, while levels of hs-CRP and interleukin-6 (IL-6) were slightly lowered.

In the IBIS-2 study, after 12 months of treatment with darapladib, necrotic core growth was substantially reduced, while plaque volume as measured by intravascular ultrasound (IVUS), was not affected (Serruys et al., 2008).

Because of encouraging preclinical data, imaging results and clinical biomarkers, currently darapladib is being tested in two large-scale studies, the Stabilisation of Atherosclerotic Plaque by Initiation of Darapladib Therapy (STABILITY) trial, with 15,828 randomised patients, and the Stabilisation of Plaques using Darapladib-Thrombolysis In Myocardial Infarction 52 Trial (SOLID-TIMI 52), with an estimated recruitment of 13,000 patients. Reports of those studies are expected in 2013 and 2014, respectively. It is expected that these on-going trials will determine the safety of darapladib and evaluate its ability to lower the risk of cardiovascular events in CHD patients (ClinicalTrails.gov, 2011). If it proves to reduce the rate of cardiovascular events, the role of darapladib in reducing the progression toward high-risk plaques will be further elucidated. At the same time, Lp-PLA2 will be solidified as a marker along with LDL-C and as a key treatment target for decreasing cardiovascular risk and prevention of stroke, myocardial infarction and cardiovascular death (Colley et al., 2011).

On the basis of the body of evidence from preclinical, clinical and imaging data might be predicted that PLA2 inhibitors will show some benefit in reducing cardiovascular events, particularly darapladib, which is currently being investigated in longer-term trials. However, it is still too early to predict whether this effect will be robust (>25% reduction of cardiovascular events; Charo and Taub, 2011).

Conclusions

Lp-PLA2 plays a central role in the pathophysiology of atherosclerosis, from its initiation to the progression of cardiovascular complications. The association of Lp-PLA2 with HDL or LDL determines whether its effects are anti-atherosclerotic or pro-atherosclerotic, respectively, however the mechanism determining preferential binding to LDL or HDL is poorly understood. Recently, increased evidence has proven the role of Lp-PLA2 in atherosclerosis.

The majority of published studies showed a significant relationship between Lp-PLA2 levels and CV events, with consistent results across a wide variety of subjects of both sexes and different ethnic backgrounds. This association was observed in both primary and secondary prevention studies. The available evidence supports Lp-PLA2 as a reliable marker of cardiovascular risk and a potential biomarker of inflammatory activity, with an advantage over C-reactive protein in terms of specificity for CVD.

Lp-PLA2 represents a promising target for the treatment of atherosclerotic CVD. On the basis of the weight of existing evidence, darapladib, a selective Lp-PLA2 inhibitor, is expected to reduce cardiovascular events.

In conclusion, Lp-PLA2 is potentially a marker of vascular inflammation, a risk factor, a prognostic biomarker, and most recently, a therapeutic target. Increasing evidence supports the role and utility of Lp-PLA2 as a marker or as a therapeutic target in preventive and personalised medicine. Future studies need to focus on exploring the potential of this biomarker and evaluating the effects of Lp-PLA2 inhibition on human populations.

Declaration of interest

The authors declared no conflict of interest.

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