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Lipoprotein-associated phospholipase A2 Mass and Activity and PLA2G7 gene polymorphisms as Markers for Cardiovascular Risk Stratification In High Risk Coronary Artery Disease Patients.

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Abbreviations

ACE: angiotensin-converting enzyme
ACS acute coronary syndrome
ADMA: asymmetric dimethylarginine
ALP: alkaline phosphatase
AngII: angiotensin II
AMI acute miocardial infarction
ApoE-/-: apoliproteinE deficient mice
AT1: angiotensin receptor-1
BMI: body mass index
CAD: coronary artery disease
CHD coronary heart disease
CRP C-reactive protein
DDAH: dimethylarginine dimethylaminohydrolase
EC: endothelial cells
ECM: extracellular matrix
EMT: endothelial mesenchymal transition
ESRD: end-stage renale disease
FGF-23: fibroblast growth factor-23
Hsp: heat shock protein
ICAM-1: intracellular adhesion molecule-1
IL: interleukin
LDL: low density lipoprotein
LDLr-/-: LDL receptor deficient mice
NEFA non esterified fatty acids
MACE major adverse cardiovascular events
MGP: matrix-gla protein
MMP: matrix metalloproteinase
NO: nitric oxide
oxLDL: oxidized LDL
UA unstable angina

1. ABSTRACT

Objective. We investigated if Lp-PLA2 titer predicts cardiovascular (CV) events in a prospective cohort study and we sought to investigate if LpPLA2 mass and activity bear any prognostic information in high-risk coronary artery disease patients

We also analyzed the association of three polymorphisms (Arg92His, Ile198Thr, Ala379Val) and related haplotypes at the PLA2G7 locus with angiographic coronary artery disease (CAD), plasma LpPLA2 mass, LpPLA2 activity, and long-term survival.

Background Lipoprotein-associated phospholipase A2 (Lp-PLA2), which is secreted by different cell types (e.g. monocytes, macrophages and T lymphocytes) and in plasma is associated with low-density lipoprotein (LDL) place a controversial function in the atherosclerotic process : the degradation of pro-inflammatory phospholipids in LDL would suggest anti-atherogenic properties, while the production of pro-inflammatory species (lysoPC and NEFA) would support a pro-atherogenic role

Methods. The titer of Lp-PLA2 was measured in 749 randomly selected Caucasian patients of the GENICA Study, who underwent coronary angiography and were followed-up for incident CV events. Patients were classified including the last and the first three quartiles, respectively by Lp-pLA2 and after we determined the best cut-off value in predicting CV deaths and MACE with ROC curves and into a high and a low titer group. Finally we analyzed the association of three polymorphisms (Arg92His, Ile198Thr, and Ala379Val) and related haplotypes at the PLA2G7 locus with angiographic coronary artery disease (CAD), plasma LpPLA2 activity and mass and long-term survival in the same cohort of high risk patients

Results. Complete follow-up data were obtained in 78% of the patients with a median follow-up of 7.2 years. Patients in the high Lp-PLA2 activity group again showed a worse CV death-free survival includes AMI, ACS, stroke, death from other cardiovascular causes (17.64% vs. 8.4%, respectively, p=0.1) and a significantly worse CV events-free survival (33.3% vs. 20.5%, respectively, p=0.023) than those in the low Lp-PLA2 activity group at the Kaplan-Meyer plot. The 92His and 198Thr polymorphisms those are associated with a modest change in plasma LpPLA2 activity. However, these alleles are neither associated with CAD nor with long-term survival.

Conclusions The analysis performed to correct for the imbalance of variables distribution between the patients with low and high Lp-PLA2 with the propensity score matching has somewhat reduced the role of Lp-PLA2 mass for the prediction of CV events and confirm the role of Lp-PLA2 activity in CV events-free survival and a borderline significance for AMI and ACS events. The genetic approach has allowed us in an important way of there are at least two coding variants 92His and 198Thr that are associated with a modest change in plasma LpPLA2 activity. However, these alleles are neither associated with CAD nor with long-term survival.

Key words: coronary artery disease, Lp-PLA2,cardiovascular prognosis, atherosclerosis.

2. Riassunto

Premesse . La lipoproteina associata alla fosfolipasi A2 (Lp-PLA2), che è secreta da diversi tipi di cellule (monociti, i macrofagi e linfociti T) e che nel plasma è associato con le lipoproteine a bassa densità (LDL) mantiene ancora una funzione controversa nel processo eziopatogenico dell'aterosclerosi. Se da un lato l'induzione da lei mediata della degradazione di fosfolipidi infiammatori in LDL suggerisce proprietà anti-aterogeniche, la produzione di specie pro-infiammatorie (lysoPC e NEFA) sosterrebbe un ruolo pro-aterogenico

Obiettivo. Ho cercato di studiare se l'enzima Lp-PLA2 è capace di predire gli eventi cardiovascolari (CV) in uno studio prospettico di coorte andando a indagare se i valori di massa e di attività correlavono con le stime prognostiche nei pazienti ad alto rischio coronarico
Abbiamo anche analizzato l'associazione di tre polimorfismi (Arg92His, Ile198Thr, Ala379Val) e degli aplotipi correlati al locus PLA2G7 negli stessi pazienti, e rapportato i valori plasmatici di massa e attività enzimatica di LpPLA2 per valutare se la presenza o meno di queste variazioni genotipiche possano essere a loro volta predittive nella sopravvivenza a lungo termine.

Metodi. Valori di massa e attività di Lp-PLA2 sono stato misurati in 749 pazienti dello studio GENICA (Genetic and ENvironmental factors InCoronary Atherosclerosis) sottoposti ad angiografia coronarica e seguiti in lungo follow-up monitorandone l'incidenza degli eventi cardiovascolari. I pazienti sono stati classificati in base al titolo di Lp-PLA2, ed è stato determinato il miglior cut-off utile nel predire le mortalità CV e gli eventi (MACE).
Infine abbiamo analizzato l'associazione di tre polimorfismi (Arg92His, Ile198Thr e Ala379Val) e gli aplotipi correlati al locus PLA2G7 con gli stessi livelli di attività e di massa di LpPLA2 ed è stata valutata la sopravvivenza a lungo termine nella stessa coorte di pazienti ad alto rischio.

Risultati. I dati completi di follow-up sono stati ottenuti nel 78% dei pazienti con un follow-up mediano di 7,2 anni. I pazienti con alta Lp-PLA2 dimostravano una peggior sopravvivenza per end-point morte.(17,64% vs 8,4%, rispettivamente, p = 0,1) e inoltre una maggiore incidenza di MACE (33,3% vs 20,5%, rispettivamente, p = 0,023) rispetto a quelli con bassa attività di Lp-PLA2.
I polimorfismi 92His e 198Thr si sono dimostrati associati ad un modesto cambiamento nell'attività plasmatica di LpPLA2. Tuttavia, questi alleli non sono associati a significative differenze nella sopravvivenza a lungo termine.

Conclusioni. L'analisi effettuata per correggere lo squilibrio della distribuzione delle variabili tra i pazienti con bassa e alta Lp-PLA2 tramite il Propensity Score ha in qualche modo ridotto il ruolo di Lp-PLA2 massa per la previsione di eventi cardiovascolari e ma confermando il ruolo dell'attività enzimatica di Lp-PLA2 come variabile indipendente predittiva per MACE. L'approccio genetico invece ci ha permesso di considerare che almeno due varianti polimorfiche come 92His e 198Thr sono associate con un cambiamento modesto nell' attività plasmatica di LpPLA2. Tuttavia, questi alleli non sono associati con alla prognosi di malattia coronarica ne con le sopravvivenze a lungo termine

3. Introduction

Atherosclerosis, largest killer of men and women in the westernized countries is the principal cause of coronary artery disease (CAD). It is known that CAD is a progressive disease that generally begins in childhood and manifests clinically during the mean and the elderly ages. [1,2].

The term atherosclerosis derives from the Greek and refers to the thickening of the arteries (sclerosis, indurations) and the accumulation of lipids (ather, jelly, fatty material) that characterize the lesions. The sites most affected by disease are the aorta and its main branches, the vessels of the cerebral circulation, lower limb and the coronary arteries, where the disease affects epicardial arteries causing reduced blood flow reserve in the vessels. Despite this, so-called “hardening of the arteries” was first described only in the 1700s, and it was not until the 1900s that a good description of myocardial infarction (MI) was forthcoming [3]

In the past, CAD was considered to be a simple, process of fat deposition in the arteries which leads a progressive shrinkage resulting in an in complete vessel obstruction. However, nowadays atherosclerosis is not considered a "passive disease” characterized by a simple accumulation of lipids in the arterial wall, a diverse and active process, characterized by a number of cellular interactions in response to defined molecular signals. The blood vessels are no longer considered mere inert conductors, but are a viable structures, and interaction between different components of blood and normal constituents of the arterial wall, as the endothelium and smooth muscle cells, contribute to the pathogenesis of the disease

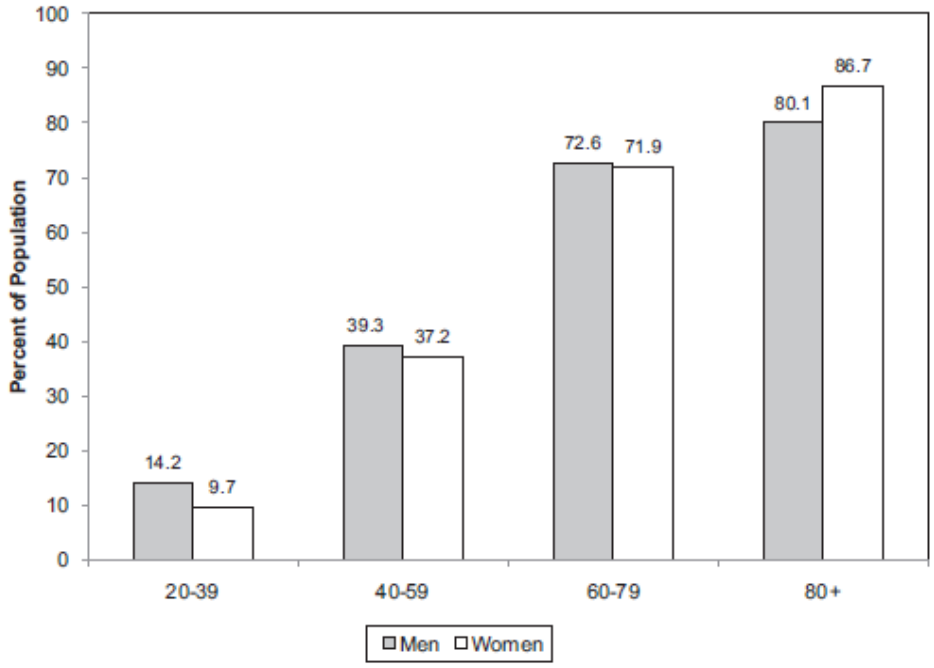


Figure 1Prevalence of cardiovascular disease in adults _20 years of age by age and sex (National Health and Nutrition Examination

3.1 Coronary Heart Disease: epidemiology.

Coronary heart disease [CHD] mortality rates have declined over the last fourty years but, it remains responsible for about one-third of all deaths in people over age 35 [1,2]. Atherosclerosis is the leading cause of death and disability in developed societies. In the U.S. estimated that 16300000 Americans ≥20 years of age are suffering from ischemic heart disease. In addition, current estimates suggest that by the year 2020, cardiovascular diseases, will be the most common cause of death worldwide. In the U.S. and Europe the incidence of cardiovascular disease, in particular of ischemic heart disease, is rising, in low-income groups above all, but not in high-income groups (who adopt healthier lifestyles). Looking more specifically to European countries, it turns out be a marked variability in the data of morbidity and mortality from cardiovascular disease (CVD) between nation to nation, largely explained by differences in risk factors, lifestyle, and to a lesser extent by a different susceptibility genetically determined

The 2011 Heart Disease and Stroke Statistics update of the American Heart Association reported that 82600000 American adults have 1 or more types of CVD. Of these, 40400000 are estimated to be ≥60 years of age. Total CVD includes hypertension, CHD including 7.9 million with myocardial infarction (MI) and 9 million with angina pectoris, heart failure, stroke and congenital cardiovascular defects. Is also indicated that the prevalence of these diseases increases with age for both women and men. Some data from National Health and Nutrition Examination Survey report pointed that MI prevalence was similar by sex in middle-aged individuals (35 to 54 years) during the 1988 to 1994 and 1999 to 2004 time periods. Although MI prevalence was significantly greater in men than women in both time periods, there were trends toward a decrease in men and an increase in women [2].

The drawback of these databases is also that patient with few symptoms or without clinical manifestations like silent ischemia which is thought to account for 75 percent of all ischemic episodes [4] are underestimated. Moreover is known that prevalence of anatomic CHD from post-mortem studies of Roger et al. have documented a reduced since 1979. In a report of 2562 autopsies performed between 1979 and 1994, the prevalence of significant anatomic coronary disease in subjects aged 20 to 59 years fell from 42 to 32 percent in men and from 29 to 16 percent in women when the periods 1979 to 1983 and 1990 to 1994 were compared, and there was no significant change in prevalence in those ≥60 years of age [5].

The original Framingham Study cohort with its 44 years of follow-up and 20 years of surveillance has allowed assessment of the incidence of initial coronary events such MI, angina pectoris, unstable angina, and sudden and non-sudden coronary deaths [6-8]. The principal observations were: for persons aged 40 years, the lifetime risk of developing CHD is 49 percent in men and 32 percent in women. For those reaching age 70 years, the lifetime risk is 35 percent in men and 24 percent in women. For total coronary events, the incidence rises steeply with age, with women lagging behind men by 10 years. The fatal manifestations of coronary disease, such as MI and sudden death, women lag behind men in incidence by 20 years, but the sex ratio for incidence shrinks progressively with advancing age and the incidence at ages

65 to 94 compared to ages 35 to 64 more than doubles in men and triples in women [2] then taking into account that in premenopausal women, serious manifestations of coronary disease, such as MI and sudden death, are relatively rare an beyond the menopause, the incidence and severity of coronary disease increases abruptly, with rates three times those of women the same age who remain premenopausal [6].

Additionally to sex and gender, other factors may influence w the initial presentation of CHD and two reports illustrate the incidence trend of CHD decrease in developed countries. First, in an analysis from the NHANES follow-up study comparing two cohorts of subjects, from 1971 to 1982 (10,869 patients) and from 1982 to 1992 (9774 patients), [9] the incidence of CHD decreased from 133 to 114 cases per 10,000 persons per year of follow-up. An even larger decline was seen in cardiovascular disease overall (from 294 to 225 cases per 10,000 persons per year).

Second, a report from the Mayo Clinic tests the incidence of CHD over time in Olmsted County, Minnesota . During the interval from 1988 to 1998, there was a declining trend in the age-adjusted incidence of any new coronary disease (MI, sudden death, unstable angina, or angiographically diagnosed CHD) from 57 to 50 cases per 10,000 persons.

Heart disease mortality has been declining in the United States and in regions where economies and health care systems are relatively advanced, but the experience is often quite different around the world [9].

Cardiovascular diseases are still the leading cause of death in Italy, being responsible for 44% of all deaths. In particular, ischemic heart disease is the leading cause of death, accounting for 28% of all deaths, and cerebrovascular accidents are third place with 13%, after tumors. Those who survive a heart attack become chronic patients. The disease changes the quality of life and results in significant economic costs to society. In Italy the prevalence of people suffering from cardiovascular disability accounted for 4.4 per thousand (Istat -National Institute for Statistics). 23.5 percent of the Italian pharmaceutical expenditure (equal to 1.34 in PIL), is intended to drugs for the cardiovascular system (Report on the health status of the country, 2000). The

data from the Italian National Register of coronary and cerebrovascular events show a substantially uniform incidence throughout Italy

The comparison between the rates of coronary and cerebrovascular events shows that men predominate in coronary events and women in those cerebrovascular. As for cases of stroke is confirmed that the mortality is greater in the southern regions than the north, as expected for the higher prevalence of hypertension and smoking.

Is expected that coronary heart disease mortality in the world will increase approximately 29 percent in women and 48 percent in men in developed countries between 1990 and 2020 and the corresponding estimated increases in developing countries were 120 percent in women and 137 percent in men [10] with the most dramatic increments in ischemic heart disease events for the Middle East and Latin America. The experience in Asia is especially important because of the large populations involved.

In India for example, ischemic heart disease may not be largely explained by traditional risk factors [11], in China, risk factor trends complement tracking of event rates such, the dramatic increase in CHD mortality in Beijing area is attributable to greater cholesterol levels. The mean cholesterol level was 166 mg/dL in 1984 and 206 mg/dL only 15 years later [12]. In Latin America, declines in vascular disease rates have been less favourable than in the United States; unfavourable trends in physical activity, obesity, and smoking contribute to these differences [13,14].

Figure 2 Gini index (a measure of statistical dispersion is a measure of the inequality of a distribution) and CVD mortality by World Bank Income group in men and women, (age standardized,per 100 000)

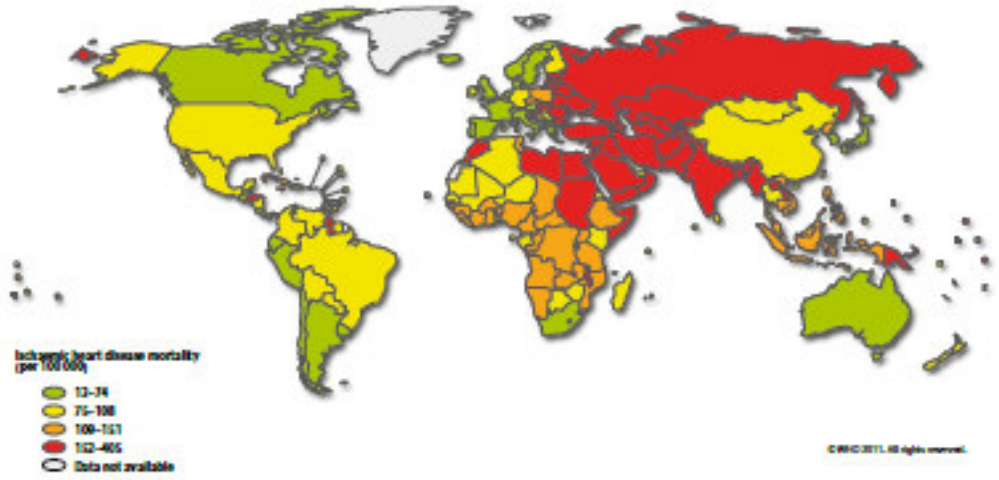


Figure 3 World map showing ischemic heart disease mortality rates (age standardized, per 100 000)

4.Pathogenesis of Atherosclerosis

In the far 1933 the term "atherosclerosis" describing the association of fatty degeneration and vessel stiffening was introduced.[15] This process affects medium and large-sized arteries and is characterized by patchy intramural thickening of the subintima that encroaches on the arterial lumen.

The earliest visible lesion of atherosclerosis is the fatty streak, secondary to an accumulation of lipid-laden foam cells under the intimal layer of the artery. With time, the fatty streak evolves into a fibrous plaque, the hallmark of established atherosclerosis and finally the lesion may evolve to contain large amounts of lipids. If it becomes unstable, because of denudation of the overlying endothelium or plaque rupture, a thrombotic occlusion of the overlying artery may result.

Atherosclerotic lesions are composed of three major features. The first is the cellular component comprised predominately of macrophages and smooth muscle cells. The second component is the connective tissue matrix and extracellular lipids. The third component are the intracellular lipids that accumulates within macrophages, thereby converting them into foam cells.

Atherosclerotic lesion initiation

Contemporary views on atherosclerosis postulate perturbations of the endothelial function as an early stage in this process. Endothelial cells undergo qualitative changes when subjected to irritative stimuli, i.e. hyperlipidemia, hypertension, and pro-inflammatory cytokines, expressing adhesion molecules. The crucial role of these molecules, i.e. vascular cell adhesion molecule-1 (VCAM-1), intracellular adhesion molecule 1 (ICAM-1), P- and E-selectin, was demonstrated by knock-out studies in mice models[16-19].

Despite the systemic action of the stimuli activating endothelial cells the atherosclerotic disease has a localized distribution, preferably at branching points of the arterial tree. This probably reflects different patterns of blood flow in specific segments of the arterial tree, because it was demonstrated that laminar shear stress elicits an atheroprotective pattern of genes in endothelial cells[20]

Monocyte diapedesis

Once activated, endothelial cells secrete chemokines, e.g., monocyte chemoattractant protein 1 (MCP-1), which, together with adhesion molecules expression, attract monocytes, dendritic cells, and T-cells in the subendothelial space. This process is characterized by monocytes tethering and rolling on endothelial cells through the interaction of monocyte P-selectin glycoprotein ligand-1 (PSGL-1) with endothelial selectins. After firm adhesion to the dysfunctional endothelium, a process where is probably implicated monocyte type 1 interferon signaling pathway monocytes entry the subendothelial space (diapedesis)[21]. T-cells gain access to the intima with similar mechanisms involving chemokines and adhesion molecules[22].

Importantly, it was demonstrated that blocking of chemokines or their receptors, i.e. CC chemokine receptor 2 (CCR2), CX chemokine receptor 2 (CXCR2), to inhibit monocyte intima entry, retards or prevents lesion development in mouse models of atherosclerosis[23].

Monocyte differentiation into macrophages

Once in the intima monocytes are stimulated by macrophage colony-stimulating factor (M-CSF) produced by activated endothelial cells to differentiate into macrophage and/or dendritic cell-like features[24,25]. These cells upregulate the scavenger receptors and acquire a pro-inflammatory pattern of functions characteristic of M1 macrophages, producing high levels of cytokines like interleukin-(IL)1ß and tumor necrosis factor (TNF)[26].

Foam cells formation

Low-density lipoproteins (LDLs) have a shell of phospholipids, free cholesterol and apolipoprotein B100 (ApoB100) with a core comprising esterified cholesterol and triglycerides. These particles can accumulate in the subendothelial space, where the ApoB100 binds to proteoglycans of the extracellular matrix, trapping the LDLs[27].

Once LDLs accumulate in the sub-endothelial extracellular space they promote recruitment of monocytes and their differentiation into macrophages Under the influence of pro-inflammatory cytokines, these cells produce reactive nitrogen and oxygen species (ROS) that oxidize LDLs, which are deemed to play a major role in the initiation and progression of the atherosclerotic process. This is proved by the

very low uptake of LDLs by resident mouse peritoneal macrophages, with small increase in cellular cholesterol content and no formation of foam cells even when exposed to very high LDL concentrations[28]. Whereas, oxidative modified LDLs bind with high affinity to macrophages, are actively internalized, and lead to intracellular cholesterol accumulation[28]. Oxidation of LDL causes a loss of their affinity for the LDL receptor, which is downregulated by cholesterol intracellular overload, and a gain of affinity for the scavenger receptors (SR)A and CD36, which are up-regulated by cholesterol excess in macrophages. Thus, upon binding of oxidized LDL (oxLDL) these receptors are internalized with ensuing foam cells formation.

Presumably the rate of production of oxLDL in the arterial intima is a function of the concentration of native LDL present and is proportional to the plasma LDL. Oxidation of LDL was originally thought to be involved in pathogenesis because this could account for the loading of macrophages with cholesterol, but it quickly became apparent that oxLDL had many other properties that were potentially pro-atherogenic. For example, oxLDL is itself directly chemotactic for monocytes and T cells. Among other biological effects, oxLDL (and its various oxidized lipid components) are cytotoxic for endothelial cells, for macrophages and smooth muscle cells (SMC) and stimulate the release of MCP-1 and of M-CSF from endothelial cells[29,30].

However, additional mechanisms of foam cells production are presumably active in atherosclerosis, like phagocytosis of matrix-retained and aggregated lipoproteins and pinocytosis of nonretained native LDLs, as suggested by *in vitro* experiments[31,32].

Once internalized by macrophages cholesteryl esters of lipoproteins are hydrolyzed to free cholesterol and fatty acids in late endosomes[33]. Here free cholesterol is transferred to other cell sites, like endoplasmic reticulum, downregulating LDL receptors and cholesterol synthesis through inhibition of the sterol-regulatory element binding pathway (SREBP)[34]. Free cholesterol could also migrate, probably through a Golgi vescicular transport[33,35],to the plasma membrane where it is removed from the cells by the ATP-binding cassette transporter (ABC)A1- and ABCG1-mediated transport to apolipoprotein A1 and HDL, respectively, or by ‘‘passive diffusion’’ to cholesterol-poor HDL[36,37].

Other atheroma cell components

Atheroma formation includes migration of smooth muscle cells (SMC) from the tunica media into the intima. Here they proliferate under the stimulus of platelet derived growth factor (PDGF) and produce extracellular matrix molecules, like collagen and elastin, forming the fibrous cap that overlies the atherosclerotic plaque[38].

T-cells, which are recruited simultaneously with monocytes, are another crucial component in human atheroma, with a macrophage/T-cells ratio from 4:1 to 10:1. These cells are activated in the intima, producing proatherogenic mediators and contributing to lesion growth and plaque progression[22,39].

B-cells and mast-cells are occasionally present in the atheroma, but they are abundant in the adventitial side of the atherosclerotic artery[40,41].

Atherosclerotic plaque evolution and destabilization

Evolution of the atherosclerotic plaque is characterized by enlargement over time due to the accumulation of foam cells. Macrophages are crucial in plaque morphology modification, which could lend to vulnerable plaques through necrotic core formation and thinning of the fibrous cap. In fact, most culprit lesions responsible of acute events recognized at pathology are ruptured sites at plaque shoulders, in close proximity to areas of plaque necrosis, with a thinned overlying fibrous cap[42].

The fibrous cap thinning is secondary to processes that either decrease the collagen deposition by SMCs or enhances its degradation. Macrophages are crucial in this mechanism, reducing their local release of transforming growth factor ß (TGF ß)[43] which decreases collagen deposition by SMCs, and triggering SMCs apoptosis through the Fas apoptotic pathway and by secreting proapoptotic TNFa and nitric oxide[44]. Moreover, macrophages might be involved in collagen degradation through the production of matrix metalloproteinases and serine proteases[45,46].

The plaque necrosis is secondary to advanced lesion macrophages apoptosis and insufficient phagocytic clearance (efferocytosis) in advanced atherosclerotic plaques[47]. These mechanisms are important because they lead towards necrotic core formation, which is proinflammatory, prothrombotic, contributes to proteolytic plaque breakdown and physical stress[42,48].

Macrophage apoptosis may be triggered by lack of growth factors, oxidative stress,

death receptor activation, prolonged activation of endoplasmic reticulum stress pathways. One of the main inducer of the last mechanism is insulin resistance. When endoplasmic reticulum is subjected to an abnormally prolonged stress the unfolded protein response (UPR), which in normal conditions correct the disequilibria in the endoplasmic reticulum functions, shows a persistent expression of its effector C/EBP-homologous protein (CHOP), which triggers macrophages apoptosis[48],[49]and was demonstrated having an increased expression in atherosclerotic plaques with vulnerable features[49]. Among the downstream mediators of the CHOP pathway it is worth citing the Ca2+/calmodulin-dependent protein kinase II (CaMKII) and the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase[50]. However, these mechanisms are balanced by compensatory cell survival pathways, including those involving NF-kB, Akt, p38a, and autophagy[47]

As stated above plaque necrosis requires also insufficient apoptotic cells removal, which is not thought to be secondary to overwhelming macrophage apoptosis, because this process is highly efficient[47],but is likely due to defective efferocytosis. Possible mechanisms implicated in this defect are oxidative stress-induced efferocyte death[51],LP-associated hydrolysis of oxidized phosphatidylserine on the surface of apoptotic cells by phospholipase A2 (PLA2), which is a ligand for efferocytes[52], and protease-mediated cleavage of the efferocytosis receptor MerTK[53]. The protective effects of an efficient clearance of apoptotic macrophages results are mediated by the removal of the cells before membrane damage leads to extracellular leakage of toxic intracellular material, the stimulation of an anti-inflammatory response in the efferocytes mediated by IL-10 and TGFß, the increase of the efferocytes survival. This cell survival effect involves robust esterification and efflux of cholesterol, efflux of proapoptotic oxidized lipids, and activation of Akt and NF-**k**B cell survival pathways.

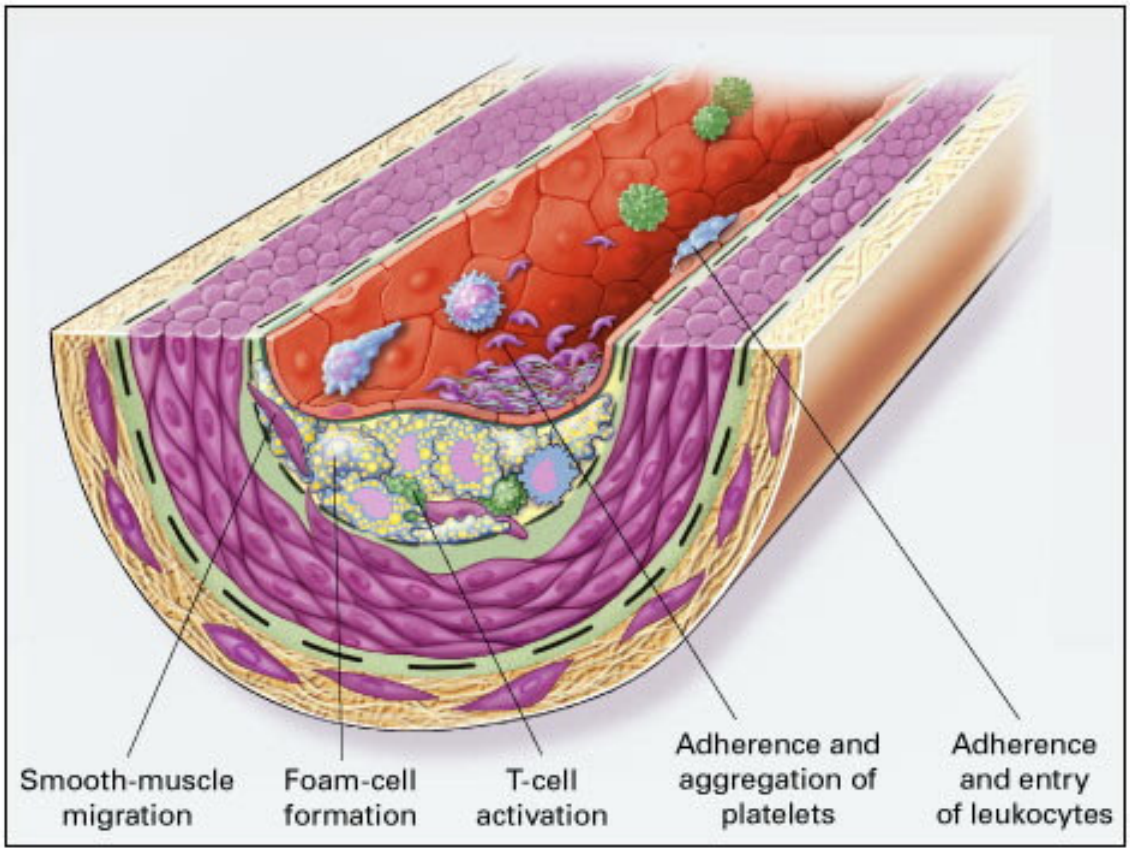


Figure 4 Fatty streaks initially consist of lipid-laden monocytes and macrophages (foam cells) together with T lymphocytes. modified from Ross R. N Engl J Med 1999;340:115-126.

5. Risk factors for Atherosclerotic Vascular Disease

A variety of factors, often acting in concert, are associated with an increased risk for atherosclerotic plaques in coronary arteries and other arterial beds

The major reason for the limited success in the field of CAD and myocardial infarction (MI) genetics is that CAD is a complex disease and MI is very difficult to be predicted. Both are believed to be caused by many genetic factors and environmental and by an interactions among these

The importance of identifying risk factors for atherosclerosis emerged from a large amount of experimental studies, as well as epidemiological studies. The Framingham Heart Study was the first to provide a rigorous support for the concept that hypercholesterolemia, hypertension, smoking, diabetes, age and sex predicts cardiovascular risk, allowing you to define the concept of "risk factors" for cardiovascular disease and in practice, the cardiovascular risk factors fall into two categories:

a) Non-modifiable risk factors such as age, sex and familiar for early cardiovascular disease;

b) Modifiable risk factors by lifestyle and / or drug treatment

More recently it has been shown that nine easily measured risk factors and potentially modifiable (smoking, lipid profile, hypertension, diabetes, obesity, poor diet of fruits and vegetables, physical inactivity, no consumption of alcohol and psychosocial factors) together predict about 90% of the risk of myocardial infarction.

The simultaneous presence of the nine risk factors mentioned above increases the risk of myocardial infarction is about 300 times (see in contrast, regular exercise activity, a diet rich in fruits and vegetables and moderate consumption of wine are associated with a significant reduction in risk cardiovascular system.

5.1 Established Risk Factors for CHD

Sex and age

Observational studies carried out over dozens of years have been an excess of cardiovascular risk in men than in women before menopause. The relative protection against cardiovascular disease in women before menopause comes from the protective

effects of so-called "umbrella estrogens" that determines, above all, a more favourable lipid profile Therefore, before 50 years of age, the incidence of cardiovascular disease is markedly higher in men than in women, and subsequently increases progressively in women, approaching that seen in men around the eighth decade of life The incidence of cardiovascular disease increases with age, especially above 45 years in men and above 55 years in women. Some of the risk factors, such as smoking, an abnormal lipid profile, hypertension and diabetes, have a greater negative effect on cardiovascular risk in individuals younger than in older individuals.

Family history

It considers the presence of familiar early-onset cardiovascular disease in the father of the first 55 years of age and mother before age 65, although these age limits are arbitrary.

The risk of cerebral-vascular events was higher in subjects with positive family history. In most cases, this familiarity is attributable to the transmission of other risk factors such as hyperlipidemia, diabetes, and hyperhomocysteinemia. However, we know families with high incidence of premature cardiovascular death in which there are no best-known risk factors.

Everything passes then to the information given by the genetic endowment. Genes can pass on the risk of cardiovascular disease, and they can also be responsible for passing on other risk conditions Lifestyle habits, such as smoking or poor diet passed on from one generation to the next can increase the risk of cardiovascular disease. There's no single gene that increases risk of getting heart disease. It's likely that several genes are responsible, and see all this in detail shortly in the section on the role of genetic determinants

Lipids and lipoproteins

Hypercholesterolemia is consistently associated with cardiovascular disease through endothelial dysfunction. Oxidized low-density lipoprotein (LDL) how we have seen is an important mediator of endothelial damage, but individuals with smaller LDL particles can also have endothelial dysfunction, independently of LDL plasma concentration[54] .

As previously described, several epidemiological studies have shown a relationship between the continuous and increasing LDL cholesterol and 'incidence of cardiovascular events, but for HDL-C has been reported an inverse correlation. As for triglycerides, it is unclear whether hypertriglyceridemia is an independent risk factor for atherosclerosis, or

whether it is correlated with metabolic changes associated how the reduced concentration of HDL in the presence of small dense LDL or insulin resistance.

Hypertension

Hypertension is a well-established risk factor for adverse cardiovascular outcomes, including CHD mortality and stroke[55,56]. In the worldwide INTERHEART study of patients from 52 countries, hypertension accounted for 18 percent of the population-attributable risk of a first MI.

Hypertension contributes of atherogenesis producing hemodynamic stress on the arterial wall that promotes oxidative processes. A number of epidemiological studies have established a direct relationship in geographically and ethnically diverse populations between the elevation of blood pressure and the incidence of cardiovascular and cerebrovascular diseases.

The concentrations of Angiotensin II, the main product of the renin-angiotensin system are often increased in patients with hypertension. Angiotensin II is a potent vasoconstrictor, which helps stimulate the atherogenesis proliferation of smooth muscle cells. In addition, angiotensin II NTA also increases the activity of lipoxygenase of smooth muscle cells promoting the oxidation of LDL and inflammatory processes.

Endothelial dysfunction is the hallmark of hypertension[57] both primary (essential) and secondary. This has been documented in different vascular beds with receptor-operated (acetylcholine, radykinin, substance P), mechanical (increase in shear stress) or mixed (dynamic exercise and cold pressure test) stimuli. Endothelial dysfunction associated with essential (primary) hypertension is characterized by impaired nitric oxide bioactivity determined by ROS, which scavenge nitric oxide. ROS can be generated by non-enzymatic and enzymatic sources, including NAD(P)H oxidases or xanthine oxidase, COX and NOS-induced superoxide production caused by depletion of the cofactor, tetrahydrobiopterin.

In the presence of reduced availability of nitric oxide, alternative pathways, including hyper polarization, account for endothelium-dependent vasodilatation. Furthermore, part of endothelial dysfunction can be explained by variations in the *eNOS* gene. An interaction of the NO and ET-1 systems may participate in the pathogenesis of endothelial dysfunction. Although inconclusive evidence exists of increased plasma concentrations of ET-1 in essential hypertension, the vasoconstrictor activity of the peptide is increased along with diminished availability of NO. Mounting evidence for an association of

endothelial dysfunction with markers of vascular damage and cardiovascular events in patients with essential hypertension supports this contention. In patients with essential hypertension, an impaired forearm response to acetylcholine correlates with intima-media thickening of the carotid arteries. Moreover, in epicardial coronary arteries of normotensive individuals, the response to acetylcholine showed an inverse correlation with intramural plaque. The presence of coronary endothelial dysfunction has also been associated with cardiovascular events in longitudinal studies

Diabetes mellitus

Cardiovascular disease is the major complication of diabetes (particularly type 2).

Insulin resistance is a key component in the etiopathogenetic Type 2 diabetes and is frequently associated with the development of atherosclerotic injury making possible to formulate hypotheses about the insulinergic interaction pathogenesis between the system and the formation of plaque atherosclerotic.

Insulin normally lowers the free fatty acids plasma concentration and its lack of effect favors the increase, in diabetic patients. In the presence of high concentrations of glucose, lipoproteins may be modified by glycation (non-enzymatic glycosides), which alters the recognition and binding by the receptors. For example, the glycation of LDL and thus causes the prolongation of the increase their plasma concentration. The continuous interaction of aldoses with proteins can lead to the formation of Advanced glycation end product defined highly reactive AGEs (Advanced Glycosilation End products). The continuous accumulation of AGEs in proteins plays an important role in the development of complications and was observed that the interaction of AGEs with their receptors generates oxygen radicals thereby modulating the physiology of endothelial[58-60]

Obesity

Obesity, or excessive body weight associated with an increased tissue adipose tissue, plays an important role in the development of atherosclerotic disease, influencing all diseases associated with it, such as diabetes type II, dyslipidemia and hypertension[61,62]and low levels of adiponectin[63]. In 1998 the World Health Organization (WHO) has recognized the phrase "metabolic syndrome" to indicate the simultaneous presence of these diseases. In a study of young subjects, from 15 to 34 years, deaths from external causes, there was a strong association between body mass index (BMI) and the presence of atherosclerotic lesions[64].

Chronic kidney disease

The increased coronary risk in patients with end-stage renal disease has been well described, but there is now clear evidence that mild to moderate renal dysfunction is also associated with a substantial increase in CHD risk. Practice guidelines from the National Kidney Foundation in 2008 and the American College of Cardiology task force in 2010 recommended that chronic kidney disease be considered a CHD risk equivalent.

Cigarette smoking

The association between smoking and cardiovascular mortality has been recognized for decades and an independent relationship between smoking and an increased cardiovascular risk has been widely documented. Smoking is directly responsible both for the deaths of approximately 310000 men and 84000 women yearly and for premature cardiovascular events.

The recent World Health Organization report identified smoking as the fourth greatest global threat to health.

Smoking places a significant physiological stress on the vasculature by acutely decreasing coronary blood flow and myocardial oxygen delivery and by inducing profound, predominantly silent, regional disturbances in myocardial perfusion. Numerous mechanisms contribute to the increased cardiovascular risk in smokers, including increased activation of platelets and leucocytes, and adverse effects on lipids, blood pressure and insulin resistance. However, more recently, the harmful effects of tobacco smoke on the endothelium are of critical importance, and assume that tobacco smoke have an irregular appearance with disturbances in membrane architecture, and decreased activity of endothelial (e)NOS.

Infection

The theorem that acute infectious illnesses may be associated with a transient increase in the risk of cardiovascular events has been supported since the first hours[65,66] and it has been proposed that certain types of infections may play a role in the pathogenesis of atherosclerosis by establishing a low-grade of persistent inflammatory process or that acute/chronic inflammation may result in endothelial dysfunction and may be responsible for a cardiac event. The major organisms that have been studied with respect to chronic inflammation are Chlamydophila (formerly Chlamydia) pneumoniae, cytomegalovirus,

and Helicobacter pylori; however, enterovirus (coxsackie viral infection), hepatitis A virus and herpes simplex virus type 1 and type 2 have also been implicated. Large randomized trials do not support benefit from antibiotic therapy against C. pneumoniae to reduce coronary events.

5.2 Novel Risk Factors for Atherosclerotic Vascular Disease

According to a recent study 88% of heart attack victims would have been considered low to moderate risk if they were tested with current national guidelines [AHA, ACC, ESC] before their heart attack. Moreover, most myocardial infarction occurs in less severe coronary artery stenosis.

It is apparent, however, that a substantial proportion of cardiovascular events occur in individuals without these established risk factors, despite the fact that most cardiovascular events can be explained by conventional risk factors, the search for additional etiologic agents must continue. and additional tests to assist in the prediction of risk in these individuals may be warranted[10,67,68]. Below we frame a series of emerging risk factors that bind well to the risk construction and on which several studies have been made

C-reactive protein

C-reactive protein is part of the acute-phase reactors. During inflammation C-reactive protein levels were increased significantly due to high concentration of IL-6 in plasma, produced by adipocytes and macrophages. This protein is associated with fosfocolin in microbes, perhaps complementary to assist the union for foreign or defective cells and enhances phagocytosis by macrophages. Also plays an important role as a first innate immunity defense system against infection The normal levels of C-reactive protein increase in 6 hours and come to assimilate them in 48 hours. Its average life is constant and its level is mainly determined by the rate of production and therefore the severity of the case. Serum amyloid A is an acute phase indicator that responds more rapidly in similar circumstances. C-reactive protein was discovered by Tillett and Francis in 1930 as a substance in the serum of patients with acute inflammation that reacted to the C polysaccharide pneumococco. Initially it was thought that this protein was a pathogenic segregation, since it was present in high amounts in people with diseases such as cancer.

The baseline level of inflammation, as assessed by the plasma concentration of C-reactive protein (CRP), predicts the long-term risk of a first MI ischemic stroke, or peripheral

artery disease[69-71]. Measurement of CRP levels improves risk stratification, a statement from the Centres for Disease Control and Prevention and the American Heart Association published in 2003 concluded that, in patients at intermediate risk for CHD, serum hs-CRP may, at the discretion of the physician, help direct further evaluation and therapy for primary prevention [70]. Cardiovascular risk has also been associated with a variety of other markers of inflammation, further supporting the role of inflammation in atherosclerosis.

Microalbuminuria

Microalbuminuria reflects vascular damage and appears to be a marker of early arterial disease. A number of studies have shown that microalbuminuria is an important risk factor for cardiovascular disease and early cardiovascular mortality

Endothelial dysfunction

Dysfunction of the vascular endothelium is a hallmark of most conditions that are associated with atherosclerosis and is therefore held to be an early feature in atherogenesis. However, the mechanisms by which endothelial dysfunction occurs in smoking, dyslipidaemia, hyperhomocysteinaemia, diabetes mellitus, arterial hypertension, cerebrovascular diseases, coronary artery disease and heart failure are complex and heterogeneous

Dysfunction of the vascular endothelium has been documented in most conditions that are associated with atherosclerosis and therefore is an early feature in atherogenesis. Besides inducing vasodilatation, nitric oxide has numerous effects that can be regarded as protective from atherosclerosis. It prevents the adhesion of leucocytes and their migration into the arterial wall, proliferation of VSMCs, platelet adhesion and aggregation. Maintaining the balance of blood flow and thrombus formation is also a major task of the vascular endothelium. It has been shown that both nitric oxide and prostacyclin (PGI2), another endothelium derived relaxing factor, inhibit the activation of platelets. The endothelium dynamically releases tissue plasminogen activator (tPA), which promotes breakdown of fibrin clot and, therefore, maintains endogenous fibrinolysis Given the profound involvement of endothelial dysfunction in the pathogenesis of cardiovascular disease, it is not surprising that endothelial dysfunction has been consistently associated with cardiovascular risk factors and also demonstrated in most disease conditions that predict high risk of cardiovascular events. Thus the purpose of this paper will be to

provide a concise overview of the impact of the different cardiovascular risk factors and disease conditions on the mechanisms causing endothelial
dysfunction

Endothelial progenitor cells

Endothelial progenitor cells (EPCs) are derived from the bone marrow and are thought to support the vascular endothelium and prevent the development of atherosclerosis.

Decreased numbers of circulation EPCs have been associated with endothelial dysfunction and a higher Framingham risk factor score in patients without established cardiovascular disease[72].

The mechanisms by which EPCs might be reduced in patients with or at risk for CHD are not well understood. One possible contributing factor is selective functional exhaustion of hematopoietic progenitor cells in bone marrow and peripheral blood in ischemic cardiomyopathy[73].

Asymmetrical dimethylarginine

Asymmetrical dimethylarginine (ADMA) is an endogenous nitric oxide synthase inhibitor that may be an independent risk factor for endothelial dysfunction and CHD[74]. Patients with and without established coronary artery disease and elevated levels of serum ADMA have an increased risk of acute coronary events compared to individuals with lower levels[75].

Arterial intima-media thickness

The analysis of the arterial ultrasound allows obtaining precise information about the morphology of the vessel wall and the possible presence of carotid atherosclerotic stenosing lesions. The normal arterial wall shows the presence of two parallel hyper echoic lines separated by a hypo echoic area, which corresponds to the lumen-intima and media-adventitia of the vessel, which together constitute more than the average intima thickness (IMT) An increase of this thickening is one of the earliest changes in the structure of the atherosclerotic process that can be analyzed at the level of the carotid arteries. Several prospective studies have shown an association between increased carotid IMT and the occurrence of cerebrovascular and cardiovascular events has been observed an exponential increase in risk with increasing extent of coronary atherosclerotic lesions in the common carotid and bulb the increase in IMT is, therefore, a risk factor for

myocardial infarction and / or cerebrovascular disease and is a non-invasive marker of atherosclerotic disease. For several years, moreover, several studies have documented that many cardiovascular risk factors correlate independently with increasing values of IMT[76-78]. The study of IMT is also used as an intermediate endpoint for evaluating the effectiveness of different drug treatments, especially anti-hypertensive and cholesterol lowering drugs, inducing a slowing of the progression of atherosclerotic disease and a recovery of endothelial function.

Arterial stiffness

Arterial stiffness, measured as the aortic pulse wave velocity (PWV) between the carotid and femoral arteries, is a predictor of cardiovascular events. This was demonstrated in a meta-analysis of 17 studies that included over 15,000 patients in whom aortic PWV had been correlated to clinical outcome[79]. The pooled relative risks for total cardiovascular events, cardiovascular mortality, and all-cause mortality were significantly increased comparing high versus low aortic PWV groups.

Serum phosphate

The first evidence for an association between serum phosphate levels and cardiovascular events in individuals without either CHD or CKD comes from analysis of the Framingham Offspring cohort[80] A significant association between serum phosphorus levels and coronary artery calcium levels obtained at the time of computed tomography scanning has also been found in healthy, younger adults (mean age 25 years)[81]. In multivariate models higher baseline phosphorus levels, including those within the normal range, were significantly associated with higher coronary artery calcium scores.

Coagulation factors

The haemostatic system is responsible for the arrest of bleeding after vascular damage, it is also implicated in the risk of thrombosis associated with atherosclerosis. Alterations of platelet function and blood coagulation and fibrinolytic systems, in part, genetically determined, may influence the risk of thrombosis. Of all the factors of the waterfall coagulation, fibrinogen, factor VII, factor V, the tissue activator of plasminogen activator (t-PA) and plasminogen activator inhibitor (PAI-1) are appeared the most affected. It is known as fibrinogen, contributing to increase plasma viscosity and platelet aggregation, facilitates blood clotting. The inherited defect of the coagulation system best known is

associated precisely at a certain resistance to degradation of the factor V by activated protein C. If the correlation between this polymorphism and the occurrence of venous thrombosis is revealed quite strong, the majority of studies more or less extensive and recent showed no association between myocardial infarction and factor V Leiden[82]

The increase in circle of t-PA, the main endogenous profibrinolytic enzyme, as well as PAI- 1, has been linked to the onset of acute thrombotic events. Respect the effect of genetic variants of PAI-1, and in particular the variant investigated for a better 4G guanine in the promoter region, the susceptibility to CHD observed results rather controversial and not exhaustive[8283].

Fibrinogen also is a circulating glycoprotein that acts at the final step in the coagulation response to vascular and tissue injury. Epidemiological data support an independent association between elevated levels of fibrinogen and cardiovascular morbidity and mortality. Several factors other than inflammation have been shown to modulate fibrinogen levels. Smoking and smoking cessation are associated with an increase or decrease, respectively, in plasma fibrinogen. Furthermore, there is a dose response relationship between number of cigarettes smoked and fibrinogen level. Fibrinogen levels tend to be higher in patients with diabetes, hypertension, obesity, and those with sedentary lifestyles. Fibrates and niacin lower fibrinogen levels (in addition to lipid parameters), whereas statins and aspirin do not. Further clinical trials are necessary before it can be determined whether fibrinogen has a causal role in atherothrombosis or is merely a marker of the degree of vascular damage taking place

Air pollution

Multiple observational studies have demonstrated an association between fine particulate air pollution and cardiovascular and cardiopulmonary mortality as well as an increased risk for the development of acute coronary syndromes

Iron overload

Heart disease is a manifestation of iron overload in hereditary hemochromatosis (HH). Whether minimal degrees of iron overload in HH heterozygotes increases cardiovascular risk remains controversial.

Obstructive sleep apnoea

Obstructive sleep apnoea is associated with and increased risk for coronary artery disease, cardiac arrhythmias, systemic hypertension, and pulmonary hypertension. These relationships are discussed in scientific community, but it is confirmed especially in optical common of pathogenic mechanisms and associated detection of an increase of inflammatory and / or vascular stress substances in patients with confirmed diagnosis

5.2.1 New Biomarkers of Atherosclerotic

Cytokines IL-6

IL-6 is a 26-kDa single chain glycoprotein, produced by many cell types including activated monocytes/macrophages and endothelial cells, as well as by adipose tissue[84].

The IL-6 is one of the most important pro-inflammatory cytokines, represents an interesting target for a variety of diseases, and its involvement in atherogenic process has been extensively studied. As described previously, the IL-6 is to be generated locally by the cells of the atherosclerotic lesion, is released into the blood, for example, in adipose tissue, where it is able to exert numerous adverse effects that increase the atherogenesis. In addition, it has been previously demonstrated that IL-6 affects the expression of receptors at the local level scavenger SR-A and CD36, involved on uptake of modified LDL, favoring the formation of macrophage foam cells, a sign distinguishing early and advanced formation of atherosclerotic lesions, Many studies in vitro and in vivo clearly indicate a proatherogenic role of IL-6, the lack of mediated signaling by IL-6 in fact, seems to reduce the development of atherosclerosis and its over-expression seems to promote it. Nevertheless, other experimental results on the function of IL-6 atherosclerosis are still quite controversial

IL-18

The IL-18 and IL-12 are involved in the generation of Th1 effectors cells. Unexpectedly, the administration of IL-18 antibody accelerates the development atherosclerosis in mice ApoE - / -, but the over-expression of IL-18 binding protein reduces instead of IL-18 anti-inflammatory effect, ceding stab the plate. The simultaneous deletion of the gene of IL-18 and E determines a reduction of the expression of apoprotein IFN-d, a smaller development of the lesion and an increase in plasma levels of cholesterol and triglycerides, compared to the control mice[85]

Type II Secretory Phospholipase A2

Type II secretory phospholipase A2 (sPLA2-II) is another member of the phospholipase 2 family and is widely expressed in hepatocytes, macrophages, EDs, platelets and vascular SMCs. SPLA2 type II is a Ca2_-dependent, 14-kDa enzyme which belongs to the group of acute phase reactants. SPLA2-II production is up regulated in response to proinflammatory compounds such as IL-1_, IL-6, tumor necrosis factor (TNF)-, INF-, and oxLDL. Possible atherogenic mechanisms of sPLA2-II include its effects on lipoproteins which results in the release of various lipid mediators at the site of lipoprotein retention in the arterial wall that in turn may trigger local inflammatory cellular responses

BNP and N-terminal pro-BNP

Brain natriuretic peptide (BNP) is a natriuretic hormone released from myocardial cells in response to volume expansion and possibly increased wall stress. The N-terminal fragment, N-pro-BNP, is also released into the circulation. Serum BNP and N-pro-BNP are increased in patients with HF and are predictors of death and cardiovascular events in asymptomatic patients without HF[86].

Lipoprotein(a)

Lipoprotein(a) is an LDL-like particle in which an apolipoprotein(a) [Apo(a)] moiety is linked via a disulfide bond to apoB-100. Concentrations of Lp(a) are largely under genetic control and vary substantially between individuals depending on the size of the Apo(a) isoform present; conversely, Lp(a) levels vary little with diet or exercise, unlike other lipoproteins such as LDL and high-density lipoprotein (HDL). The wide range of Lp(a) in plasma within a population is due in large part to a variable number of plasminogen-like kringle IV repeats, and an inverse correlation between the number of kringle IV type 2 repeats in the Apo(a) gene and Lp(a) plasma concentration exists. The biological function of Lp(a) is still unclear, but there is strong evidence that its phylogenetic role may have been to respond to tissue injury and vascular lesions, prevent infectious pathogens from invading cells, and promote wound healing . The use of Lp(a) as a screening tool has some limitations. No universally accepted, standardized method of determination for Lp(a) exists, although recently, a working group of the International Federation of Clinical Chemistry demonstrated the inaccuracy of Lp(a) values determined by methods sensitive to Apo(a) size and recommended the widespread implementation of

a proposed reference material for those Lp(a) assays that are validated to be unaffected by Apo(a) size heterogeneity[87]. The incremental predictive value of Lp(a) measurement additive to that of traditional screening methods for global risk assessment has not been formally studied..

Homocysteine

Homocysteine is a highly reactive, sulphur-containing amino acid formed as a by-product of the metabolism of the essential amino acid methionine[88]. Cells re-metabolize homocysteine by a number of possible pathways involving several different enzymes; these enzymes variously use B vitamins as substrates or cofactors, namely folate, cobalamin (vitamin B12), and pyridoxine (vitamin B6). It has been postulated that mild to moderate elevations of homocysteine in the general population predispose to atherosclerosis in a manner akin to the classic risk factors. This is important because of the availability of an inexpensive, safe, and effective therapy for lowering homocysteine (B vitamins) and folic acid supplementation. Mechanistic studies have demonstrated that homocysteine may induce vascular damage by promoting platelet activation, oxidative stress, endothelial dysfunction, hypercoagulability, vascular smooth muscle cell proliferation, and endoplasmic reticulum stress.

Myeloperoxidase

Myeloperoxidase (MPO), a member of the heme-peroxidises super family, is a leukocyte-derived enzyme, and is secreted on leukocyte activation and degranulation. There are several pathways through which MPO could exert its deleterious effects but above. MPO could be also involved in the development of endothelial dysfunction, because MPO uses the athero-protective endothelial-derived NO as a substrate.

Matrix Metalloproteinases

MMPs might be implicated in vascular and cardiac remodelling as a result of deregulated activation of these enzymes. Recently, several lines of evidences have demonstrated that MMPs play an important role in atherogenesis

Cross-sectional studies are being conducted on other emergent biomarkers as *Monocyte Chemo attractant Protein-1*, *Placental Growth Factor*, *Pregnancy-Associated Plasma Protein* and on *Soluble CD40 Ligand.*

Finally, the aim of this work will then evaluate the role of *Lp-PLA2* and *oxLDLab* in the pathogenesis of atherosclerosis and their weight as a marker of risk and/or disease progression and subsequent chapters will be devoted specifically

5.2.2 Genetic Determinants of CAD

I mentioned earlier in the section on non-modifiable risk factors that, family history is one of the most significant independent risk factor for CAD/MI. Twin studies also suggest the hypothesis that genetic factors contribute to the development of CAD and MI. Several polymorphisms of candidate genes that code for coagulation proteins as well as inflammation, as well as lipid metabolism, have been linked to risk of myocardial infarction, especially juvenile onset, but they explain only a minority of cases. The application of advanced molecular biology techniques that allow the comparison of many genes (and not just a candidate gene at a time) should help to identify novel polymorphisms associated with cardiovascular risk and could possibly shed light on new pathophysiological mechanisms.

The most frequently used method for identifying the susceptibility genes for CAD and MI has been candidate gene case-control association studies. Methodologically, this is the easiest approach by which a candidate gene is selected based on its potential involvement in CAD/MI.

Several gene loci that affect the risk for the development of coronary heart disease (CHD) have been identified, with locus 9p21 single nucleotide polymorphisms (SNPs) showing the strongest association. However, locus 9p21 SNPs have not been shown to significantly improve on the discrimination or classification of predicted risk compared to the use of traditional risk factors [89-91]. A retrospective analysis evaluated the dose effect of abnormal 9p21 SNPs on the burden of CHD and among 950 non-diabetic patients with early-onset CHD (mean age 56 years) displaying at least one angiographic epicardial stenosis >50 percent seen by coronary angiography, the 9p21 genotype was associated with a risk of:

A. Left main CHD (OR 2.38 per copy of risk allele, 95% CI 1.48-3.85)
B. 3-vessel CHD (OR 1.45 per copy of risk allele, 95% CI 1.18-1.79)
C. Need for bypass surgery (OR 1.37 per copy of risk allele, 95% CI 1.04-1.79) [91].

The results from the candidate gene association studies should be interpreted with caution as many of these studies are confounded by the selection bias of cases and controls, population admixture, imperfect matching of cases and controls, phenotyping errors, and small sample size. But significant progress has been made in mapping and identifying disease-causing genes and susceptibility genes for CAD and MI using genome-wide linkage analysis with large families of hundreds of small nuclear families and genome-wide association studies.

Disease-causing genes have been identified for familial hypercholesterolemia increase risk of premature arteriosclerosis and CAD[92]. Hypercholesterolemia is characterized by elevation in levels of plasma cholesterol bound to LDL, tendon xanthomas, and atheroma and can be caused by mutations in the LDL receptor (*LDLR*) gene, the ApoB-100 gene, the pro-protein convertase subtilisin/kexin-type 9 gene (*PCSK9*), the cholesterol 7-[-hydroxylase gene (*CYP7A1*)], and the *ARH* gene.

Two different types of linkage analyses have been used to map the chromosomal locations of genes for CAD and MI. In *linkage analysis*, the goal is to identify at least one polymorphic marker at a specific chromosomal location that co-inherits with the disease, which then suggests that the marker and disease gene are located close to each other, and the location of the marker is taken as the location of the disease gene. The first type of linkage analysis is model-bases linkage analysis using large families in which the inheritance pattern of the disease gene in the families is clearly defined.

The second type of linkage analysis is model-free analysis using hundreds of small nuclear families with at least two affected siblings in each family. Similar to model-based linkage analysis, genome-wide genotyping is usually performed with about 400 markers providing genome coverage.

When a genetic locus is mapped, candidates genes that are located in the region and potentially relevant to the disease physiology are selected for identification of apthogentic mutations that cause the disease (*disease-causing gene*, monogenic trait) or for identification of single nucleotide polymorphisms (SNPs) and SNP haplotypes that are associated with the disease (*susceptibility gene*, complex trait). *Disease-linked genes* are the genes that are connected to the disease by molecular biology, microarray, or proteomic analyses, but their relation to the disease as a cause or a consequence is not established. Some disease-linked gene may serve as biomarkers for the disease.

Genetic variants or SNPs in many candidate genes with physiologic relevance to CAD and MI have been found to be associated with increased or decreased risks for CAD and

MI. Numerous possible susceptibility genes have been identified for CAD and MI. Many of these studies need to be further validated or replicated as false positive results can be generated easily in a case-control association study due to selection bias of cases and controls and population admixture. This article reviews only susceptibility genes identified by genome-wide association studies and by positional cloning based on genetic linkage analysis with small nuclear families. Transcriptional factor gene *USF1* and familial combined hyperlipidemia .Familial combined hyperlipidemia (FCHL) is present in about 20% of patients with CAD and is characterized by elevated serum total cholesterol or triglycerides. A major susceptibility locus for FCHL was mapped to chromosome 1q21–23 in a Finnish population. Recently, two synonymous SNPs in the *USF1* gene were found to be significantly associated with FCHL (P =0.00002)[93]. *USF1* encodes a transcriptional factor belonging to the basic helix-loop-helix leucine zipper family and regulates genes involved in glucose and lipid metabolism, including *ABCA1* and *apolipoproteins CIII*, *AII*, and *E*. The characteristics of the downstream genes regulated by *USF1* make it an attractive gene for the pathogenesis of FCHL; however, it remains to be determined whether the two SNPs associated with FCHL are the true causative variants, a common problem with association studies.

The first genome-wide case-control association study for CAD and MI was carried out using 92 788 gene-based SNPs with 94 Japanese patients with MI. Positive SNPs with a nominal significance P value of 0.01 were then genotyped in 1133 MI cases and 1006 controls[94]. Three SNPs in the *LTA* gene (exon 1 10G/A, intron 1 252A/G, exon 3 p.Thr26Asn) were found to be significantly associated with a high risk of MI when they were homozygous (odds ratio = 1.69–1.78; $P = 2.2 \times 10\_5$ to $3.3 \times 10\_6$). Lymphotoxin-[is a cytokine that mediates immune responses and inflammation.

Two other genetic loci have been identified for MI on chromosomes 1p and 14q, and four significant linkages were reported for CAD on chromosomes 2q, 3q, 16p, and Xq but the specific genes at these loci remain to be identified.

	Chromosomal location	Gene (function)	Phenotype	
Disease-causing genes				
adCAD1 (autosomal dominant CAD locus 1)	15q26	*MEF2A* (transcription factor in endothelium of coronary arteries)	CAD, MI	
adCAD2	*?*	*?*	*?*	*?*
Susceptibility genes				
1	1p34–36	?	MI	
2	2q21.1–22	?	CAD	
3	2q36–37	?	Acute coronary syndrome	
4	3q13	?	CAD	
5	5q12	*PDE4D* (phosphodiesterase 4D, cAMP signaling, inflammation)	Ischemic stroke	
6	6p21	*LTA* (lymphotoxin-α, cytokine, inflammation, immune response)	MI	
7	13q12–13	*ALOX5AP* (FLAP, generating leukotrienes, inflammatory mediators)	MI, stroke	
8	14q	?	MI	
9	16p13-ter	?	CAD	
10	22q13.1	*LGALS2* (galectin-2, regulates secretion of LTA; inflammation)	MI	
11	Xq23–26	?	CAD	

CAD, coronary artery disease; LTA, lymphotoxin-α; MI, myocardial infarction; PDE4D, phosphodiesterase 4D.

New genomic and proteomic approaches have begun to identify genes whose expression is linked to CAD and MI. Microarray analysis allows simultaneous analysis of expression of thousands of genes in CAD tissues *vs* non-CAD tissues. As an example, expression of 49 genes was newly linked to CAD, and these genes include intercellular adhesion molecule-2, PIM2, ECGF1, fusin, B cell activator (BL34, GOS8), Rho GTPase activating protein-4, retinoic acid receptor responder, Y2-arrestin, and many others. Many other genes have been linked to CAD by microarray analysis and have been extensively reviewed[95] .

The first proteomic study of CAD was reported recently[96]. Proteins from CAD and non-CAD coronary arteries were separated by two-dimensional gel electrophoresis. Protein spots that showed different expression levels in two tissues were excised from the gels and identified by mass spectrometric analysis. The results from the two-dimensional gel analysis were confirmed by Western blot analysis. Expression of the ferritin light chain was found to be significantly increased in the diseased coronary arteries by about twofold. This result links the ferritin light chain gene to CAD and supports the 'iron hypothesis' that proposes an association between excessive iron storage and a high risk of CAD. It remains to be determined whether an elevated ferritin level is a contributor or causative

factor for atherosclerotic CAD or is merely associated with the disease process. Nevertheless, increased ferritin expression in coronary arteries may become a significant biomarker for atherosclerotic CAD and may be developed as a diagnostic marker for the disease with more studies in the future. Only one disease-causing gene, *myocyte enhancer factor-2 (MEF2A),* encoding a member of the MEF2 family of transcription factors, has been identified for primary CAD and MI without other accompanying clinical feature like hypercholesterolemia and Tangier disease [93,97-101].

6. Lipoprotein-associated phospholipase A2 and atherosclerosis

The phospholipases A2 superfamily comprises enzymes catalyzing the hydrolysis of glycerophospholipids at the sn-2 ester bond, generating free fatty acids and lysophospholipids[102,103]. This reaction produces a wide variety of signaling molecules, e.g., prostaglandins, leukotrienes, lysophospholipids, platelet activating factor (PAF), and oxidized lipids, which induce a multitude of biological actions in several tissues, including the cardiovascular system[104-106]. The PLA2 are divided into five main categories: the secreted small molecular weight sPLA2s, the larger cytosolic Ca2+-dependent cPLA2s, the Ca2+ independentiPLA2s, the PAF acetylhydrolases or lipoprotein-associated phospholipase A2 (Lp-PLA2), and the lysosomal PLA2s[107].

6.1 Lipoprotein-associated phospholipase A2: Biochemistry

Lipoprotein-associated phospholipase A2 is a Ca2+-independent protein of 45 kDa, composed by 441 amino acid residues[108] and exclusively produced by the hematopoietic cell lineage[109]. The tertiary sructure of the protein is characterized by ß sheets with helical connections[110-112] with the catalytic site, which comprises the triad Ser-273 , Asp-296, and His-351[113], lying within the hydrophobic pocket, just above the interface-binding surface accessing the lipoprotein particle, facing the acqueous phase and oriented toward its substrate[114]. This allows the catalytic site to exert its activity on substrates entering from the lipoproteins and from the acqueous phase. Two alpha-helices contributes to the enzyme lipoproteins binding, one (residues 114-126) is thought to access the LDLs, and the other (residues 362-369) influences the binding to HDLs[114].

Lipoprotein associated phospholipase A2 is specific for short sn-2 acyl chain of the phospholipid substrate, up to 9 methylene groups. However, the enzyme can also hydrolyze oxidized phospholipids produced by radical mediated oxidation of phosphatidylcholines containing an sn-2 polyunsaturated fatty acyl residue with chains longer than 9 carbon atom residues[115,116] This is secondary to the relaxation of sn-2 chain length selectivity when its ω-end contains oxidized aldehydic or carboxylic moieties[116], which undergo chain fragmentation in their peroxidized acyl chains and become truncated and therefore can be hydrolyzed more efficiently by Lp-PLA2 than oxidized phospholipids containing nonfragmented-peroxidized chains[117] [116,118]. Hydrolysis of

oxidized phospholipids leads to the generation of lysophosphatidylcholine (lysoPC) and oxidized non-esterified fatty acids (NEFA) that have pro-inflammatory properties[119].

Moreover, LpPLA2 is susceptible to oxidative irreversible inactivation, which may facilitate the accumulation of enzyme substrates formed under oxidative stress[120-122].

The cellular sources of LpPLA2 are monocytes/macrophages, T lymphocytes, platelets, and mast cells, therefore is restricted to cells of the hematopoietic lineage[109,123-126] This was elegantly demonstrated by Asano et al. who showed that patients with normal plasma LpPLA2 activity before bone marrow transplant from a donor who was homozygous for the Lp-PLA2 mutation V279F, resulting in complete Lp-PLA2 inactivation, were lacking Lp-PLA2 activity in their serum after the transplant[109]. Thus, the release of Lp-PLA2 occurs independently of lipoprotein secretion and it associates with them afterwards (see Imaizumis, in: R. Cross (Ed.), Advances in Lipobiology, JAI Press Inc., Greenwich, CT., 1996).

Secretion of LpPLA2 is regulated by several mediators like the pro-inflammatory IFN-γ, IL-1, IL-4, IL-6, TNF-α, GM-CSF, and M-CSF[127] which reduce secretion of the enzyme by human macrophages. However, the regulation by pro- and anti-inflammatory agents is dependent on the cellular state of differentiation, as demonstrated by an increase in LpPLA2 secretion when LPS, IL-1β, G-CSF, and TNF-α can act on less differentiated cells such as monocytes[128].

The gene for Lp-PLA2 (PLA2G7) has 12 exons and is located on chromosome 6p21. [138-145]. Its promoter region contains a set of GC-rich motifs surrounding the transcription start site[127,129] and a number of transcription factors are involved in the regulation of its expression[127]. Among others, the transcription factors Sp1 and Sp3 are worth to mention, because they participate in the constitutive expression of human plasma Lp-PLA2 gene in macrophages[129]. Moreover, the levels of Sp1 are high in developing hematopoietic cells and may explain the elevated expression of Lp-PLA2 associated with differentiation of monocytes into macrophages[129]. In normolipidemic subjects with no detectable Lp (a) levels LpPLA2 circulates in plasma mostly associated to LDL, preferably with the densest and more electronegative LDL fractions[130-132], and to a much lesser extent to HDL[133,134]. However, most LDL particles do not contain LpPLA2[135] as demonstrated by Gaubatz et al., who showed that the molar ratio between LpPLA2 and apolipoprotein B-100, the main protein component of LDL, ranged between 1:100 and 1:10,000[131]. A major role in the association of Lp-PLA2 with LDL is played by the apolipoprotein B-100 and the enzyme residues Tyr-205, Trp-115, and Leu-116 of Lp-

PLA2 and to lesser extent Met-117, which are crucial for the enzyme association with LDL[136].

The string of C-terminal residues His-367, Met-368, Leu-369, and Lys-370 is necessary, instead, for binding to HDL[137]. A prominent role seems to play Met-368 and Leu-369 residues, which are necessary but not sufficient for binding to HDL and either directly participate in Lp-PLA2 association with HDL or contribute to the formation of a binding pocket that optimizes interaction of Met-368 and Leu-369 with the lipoprotein[137]. However, additional regions of human Lp-PLA2 are possibly required for interaction with HDL, as demonstrated by the removal of the carbohydrate content of the macrophage derived Lp-PLA2 that enhances the enzyme association with HDL[138]. These results provide evidence that a factor contributing to the preferential association of Lp-PLA2 with LDL versus HDL in human plasma is the enzyme glycosylation.

Furthermore, in plasma with normal LDL-cholesterol levels, the Lp-PLA2 activity associated with total HDL represents the 4.9% of the total plasma enzyme activity, whereas its mass represents the 28.5 of total plasma enzyme mass. Thus, the Lp-PLA2 activity associated with HDL does not significantly contribute to the total plasma enzyme activity, whereas its mass significantly influences the total plasma enzyme mass[139]

However, it is worth underscoring that the LpPLA2 distribution among its carriers varies in patients with dyslipidemia, owing to the presence of lipoprotein(a), an LDL-like particle in which apo-B100 is linked to a unique glycoprotein, apolipoprotein(a) [Am J Cardiol. 2008;101:44B]). In subjects with a lipoprotein(a) level >0.1 mg/mL, LpPLA2 preferentially associates with it[140], and this is relevant because some studies showed that this lipoprotein is the preferential carrier of oxidized phospholipids in human plasma[141-143]. The current view is that binding and transport of oxidized phospholipids by lipoprotein(a) may prevent their pro-inflammatory properties and perhaps enhance their degradation by LpPLA2 and supports the possibility that low lipoprotein(a) levels may have beneficial anti-inflammatory functions and attenuate cardiovascular risk[143].

6.2 Lipoprotein-associated phospholipase A2: Genetics

The PLA2G7 harbors several synonymous and nonsynonymous polymorphisms, which have been characterized in a comprehensive analysis by Sutton et al. in patients with coronary artery disease[144]. The study comprised two independent datasets including Caucasian and African-American patients and 19 single nucleotide polymorphisms

(SNPs) were investigated. Three of the SNPs were non synonymous amino acid substitutions in the coding region of the PLA2G7 whereas 16 SNPs were identified in regions located outside the putative transcription binding sites, enhancer elements, splicing junctions, intron/ exon boundaries, triplex repeats or additional known[144]

Patients with undetectable levels of plasma LpPLA2 activity were initially described in 4% of the Japanese populations[145] and the molecular basis for the deficiency was recognized in a point mutation (V279F) near the active site of the enzyme[146]. The mutation has been subsequently reported in other populations of Asian[147,148] and Caucasian ethnicity[149], however there have been no reports of this defect in the North American population. Much less common mutations (Q281R)[136,150,151] I317N, and an insertion in exon 3 (Ins191)[150] were demonstrated to impair LpPLA2 function. It is relevant to point out that LpPLA2 deficiency has no major physiological effect by itself, but becomes evident when combined with other genetic defects or environmental factors. Deficiency of LpPLA2 has been associated with atherosclerosis[152-154] coronary artery disease in Japanese men[155], stroke[156], dilated cardiomyopathy in Japanese[157], atherosclerotic occlusive disease[158], abdominal aortic aneurysm[159]. However, opposite results were reported in a Korean population, where deficiency of LpPLA2 was demonstrated to reduce the risk of cardiovascular disease[147,160] [.

Three nonsynonymous SNPs in the PLA2G7, R92H, I198T, and A379V are not limited to defined populations, and were found in subjects of diverse ethnicity instead. However, studies investigating the contribution of these variants to cardiovascular disease led to conflicting results. For example, the A379V variant has been associated with both increased[161-164] and decreased[148,165] activity levels. The picture is even more complicated in the R92H SNP, which was associated with a reduced LpPLA2 activity level[163,164,166], but with an increased LpPLA2 mass[166] More conclusive data were recently published in a meta analysis including more than twelve thousands subjects and investigating the PLA2G7 gene SNPs, which demonstrated an association of the Arg92His mutant allele with increased levels of LpPLA2 mass and of Ala379Val and rs7756935 SNPs with LpPLA2 activity [grallert Eur Heart J 2012v33p238]. Moreover, studies investigating the protective or disease promoting effect of these SNPs reported conflicting results. Sutton and co-workers reported that the R92H and A379V variants are associated with coronary artery disease significantly more than the I198T polymorphism[144] and a Taiwanese population carrying the A379V SNP had increased severity of coronary atherosclerosis[148]. Moreover, in a cross-sectional study which enrolled a Chinese population with coronary

artery disease the A379V SNP was associated to myocardial infarction[167]. However, opposite results were reported in Caucasian patients harboring the V379 allele, which was associated with a decreased atherosclerosis risk[162,165], and no association in a third study[144,161]. In a prospective study which enrolled 3234 Caucasian patients undergoing coronary angiography the Arg92His and Ala379Val variants were not associated with coronary artery disease, whereas the Ile198Thr was associated with a protective effect, with an OR of patients carrying the mutant allele of 0.69 (95%CI: 0.49–0.96), however none of the tested SNPs was significantly associated with death- or cardiovascular death-free surviuval[163]. Moreover, two recently published meta-analysis both including more than ten thousands subjects of European ancestry there was no association of any of the PLA2G7 SNPs with risk of coronary artery disease[164,168]. Thus, the role of these SNPs is still under debate, and larger and better designed controlled studies are required to clarify this issue.

6.3 Lipoprotein-associated phospholipase A2: Pathology

The biological role of LpPLA2 is still under debate, because anti- and pro-atherogenic properties of this enzyme have been proposed (for a review see[169,170]).

Some studies suggest that Lp-PLA2 may be anti-atherogenic based on the reduced oxidative stress[171,172] and lower risk of myocardial infarction, stroke, and peripheral arterial disease[155]. Moreover, increasing the Lp-PLA2 activity by transferring human genes into apolipoprotein E-deficient mice caused a reduction in atherosclerosis extent and macrophages homing to the endothelium[173,174]. However, the relevance of data gathered from animal models is undermined by the fact that Lp-PLA2 is mostly carried on HDL, opposite to human beings[130-132].

However, pathological data in white populations suggest that Lp-PLA2 may be proatherogenic, because it is present within atherosclerotic plaques and co-localizes with intima-based macrophages and foam cells, which are the primary source of Lp-PLA2 production[175]. Although in vitro studies have shown that peripheral mononuclear cells and platelets may produce Lp-PLA2, in vivo, it is almost exclusively produced by macrophages and foam cells based in the intima[138] as confirmed by an in vivo study showing the net production of this enzyme in blood flowing across an atherosclerotic versus non-atherosclerotic coronary vascular bed[176].

In advanced lesions, LpPLA2 staining is intense in regions abundant in lipids and oxidation products, and it is also present in thin-cap fibroatheromas, in necrotic cores of human ruptured plaques, and in apoptotic macrophages, whereas in early plaques with fatty streaks or thick fibrous caps the staining minimal[177]. This suggests that it is at least associated with plaque progression and vulnerability, although its exact role is not fully defined.

Moreover, consistent with the proatherogenic role of Lp-PLA2 are the results from large white population studies, demonstrating an independent association between plasma Lp-PLA2 mass and cardiovascular disease risk and a recent meta-analysis showing an association with cardiovascular disease[178].

The mechanism leading to the pro-atherogenic effect of LpPLA2 is still under debate, but some light was shed by Macphee and colleagues in 1995 who demonstrated that oxidized phospholipids on surface of LDL are hydrolyzed by the Lp-PLA2, forming two important triggers of the inflammation cascade, oxidized fatty acids and lysophosphatidylcholine, which elicit several potentially pro-atherogenic effects[179-188,188-190]. These molecules stimulate the expression of adhesion molecules such as VCAM-1 and ICAM-1 and cytokines by endothelial cells, macrophages, and other leukocytes, downregulate the endothelial nitric oxide, enhances the production of reactive oxygen species and oxidative stress, induces endothelial cell apoptosis[191-194]. Moreover, selective inhibition of LpPLA2 prevents lysophosphatidylcholine and oxidized fatty acids generation in oxidized LDL, resulting in inhibition of monocyte chemotaxis and protection of macrophages against apoptotic death[188,190].

Further supporting the pro-atherogenic role of Lp-PLA2 are studies carried out with its inhibitors like azetidinones. Studying human leukocytes in presence of oxidized LDLs in vitro Shi et al demonstrated that inhibition of the LpPLA2 abolished the hydrolysis of oxidized phospholipids, inhibiting interleukin-6, interleukin-1, and tumor necrosis factor–alpha production[195].

6.4 Lipoprotein-associated phospholipase A2: Clinical studies

The first study highlighting the Lp-PLA2 role as an independent risk predictor for cardiovascular disease was the West of Scotland Coronary Prevention Study (WOSCOPS), which showed an association between increased baseline levels of Lp-PLA2 and risk of cardiovascular events in dyslipidemic patients (OR 1.18, CI 1.05–1.33)

independently from traditional risk factors and other inflammatory markers[196].

Despite the vast majority of epidemiologic studies designed to investigate the relationship between Lp-PLA2 and cardiovascular events in patients with and without prior history of cardiovascular disease have demonstrated an association among the two (reviewed in[197]), some of the studies carried out in apparently healthy populations have not been able to confirm this relationship. For example in one of the first studies completed after the WOSCOPS trial, which enrolled apparently healthy middle-aged women, the predictive value of LpPLA2 mass for cardiovascular events, was present only at univariate analysis, whereas was lost after multivariate correction for cardiovascular risk factors[198], results confirmed by the ARIC and the EPIC studies in larger cohorts including apparently healthy middle-aged men and women[199,200]. However, WOSCOPS trial confirmatory findings came from other studies enrolling apparently healthy patients and demonstrating the value of LpPLA2 levels in predicting cardiovascular events, like the MONICA[201], the Rotterdam[202], the Malmo[203], and the Bruneck[169] studies. Moreover, confirmatory results were also obtained in selected populations like in elderly as in the Rancho Bernardo[204], the PROSPER[205], and in the Cardiovascular Health[206] studies, in diabetics[207] , in high risk patients[208].

A wealth of data confirms also the prognostic role Lp-PLA2 either mass or activity on patients already known to have cardiovascular disease. In patients with peripheral artery disease neither Lp-PLA2 mass nor activity were able to predict cardiovascular death[209]. Whereas, in subjects with stroke[210], heart transplant[211], congestive heart failure[212] Lp-PLA2 mass was able to predict either CV events or CV death.

Focusing on patients with coronary artery disease Brilakis et al. enrolled 466 patients who underwent coronary angiography and after a median follow up of 4 years found a strong and independent association between Lp-PLA2 mass and CV events (OR 1.3, 95% CI 1.06-1.59)[213]. However, although Lp-PLA2 mass correlated with the extent of angiographic coronary artery disease at univariate analysis, the association was no longer significant after adjustment for common risks factors. Therefore, Lp-PLA2 could be a good predictor of CV events, secondary to plaque destabilization, but poorly related to plaque burden. Consistent with this postulate are the results of the study completed by Serruys et al. in 330 subjects with angiographically documented coronary disease randomized to oral darapladib, a selective Lp-PLA2 inhibitor[214], and placebo , as part of the IBIS-2 trial. Using virtual histology intravascular ultrasound they demonstrated that there was no difference in atheroma volume, whereas darapladib significantly inhibited

the increase of necrotic core volume[215]. Other studies on patients undergoing coronary angiography extended in larger cohorts the results reported by Brilakis et al. demonstrating that Lp-PLA2 mass[216] and activity[217] predict also CV death.

Finally, in patients with coronary artery disease[218-220], previous myocardial infarction[208,221,222], acute coronary syndrome[223] either Lp-PLA2 mass or activity were able to predict cardiovascular events.

Lipoprotein associated phospholipase A2 is also useful in predicting an increased risk of future stroke. In the Rotterdam Study, a prospective case cohort study enrolling healthy subjects, 110 cases of ischaemic stroke were identified. After adjustment for traditional cardiovascular risk factors, the hazard ratios for the 2nd, 3rd and 4th quartiles of Lp-PLA2 were 1.08, 1.58 and 1.97 (P = 0.03), respectively, when compared to the first quartile[202]. The association between Lp-PLA2 levels and first ischaemic stroke has been confirmed in the NOMAS[210], and the Malmo Diet and Cancer[224] studies.

To consolidate the data published in numerous epidemiologic studies, the Lp-PLA2 Studies Collaboration group performed a meta-analysis on all the prospective studies of Lp-PLA2 published and cardiovascular events [Lp-PLA(2) Studies Collaboration[225],]. The investigators analyzed the data of 79 036 subjects from 32 prospective studies. As the relative risks for Lp-PLA2 activity and mass did not differ significantly in healthy and vascular disease patients, the meta-analysis combined data on primary and secondary cardiovascular events. F or each standard deviation Lp-PLA2 increase, the relative risks for the primary end-point of coronary heart disease were 1.10 (1.05–1.16) and 1.11 (1.07–1.16) for Lp-PLA2 activity and mass, respectively. The relative adjusted risks for ischaemic stroke were 1.08 (0.97–1.20) and 1.14 (1.02–1.27); vascular mortality 1.16 (1.09–1.24) and 1.13 (1.05–1.22); and nonvascular mortality 1.10 (1.04–1.17) and 1.10 (1.03–1.18).

The wealth of data available in the literature suggests that Lp-PLA2 is involved in determining cardiovascular events and basic science data provide insights on the pathological mechanisms that are possibly involved. However, the only definitive proof of Lp-PLA2 involvement in enlargement and/or destabilization of the atherosclerotic plaque would come only from studies involving the clinical use of its inhibitors. Two trials are currently underway on this issue. The STABILITY (Stabilization of Atherosclerotic plaque By Initiation of Therapy darapLadIb) trial was started in 2008 with more than 27 000 patients enrolled and aims to evaluate the efficacy of prolonged treatment with darapladib in reducing the risk of cardiovascular events in patients with

chronic coronary artery disease[226]. A second trial, the SOLID-TIMI 52 (stabilization of plaques using Darapladib-Thrombolysis in Myocardial Infarction 52 trial), enrolled 11 500 patients with acute coronary syndrome[227].

7. Aim of the study and Methods

We investigated if Lp-PLA2 titer predicts cardiovascular (CV) events in a prospective cohort study and we sought to investigate if LpPLA2 mass and activity bear any prognostic information in high-risk coronary artery disease patients considering that the GENICA study patients were a high-risk population with over 97% in the highest class of risk according to the NCEP criteria, (NCEP).

We also analyzed the association of three polymorphisms (Arg92His, Ile198Thr, Ala379Val) and related haplotypes at the PLA2G7 locus with angiographic coronary artery disease (CAD), plasma LpPLA2 mass, LpPLA2 activity, and long-term survival.

8. Methods

8.1 Patient selection

Consecutive caucasian patients of both genders consecutively referred to the Division of Cardiology of the Cittadella General Hospital for coronary angiography for investigation of chest pain and/or suspected CAD were enrolled between 1999 and 2001 in THE GENICA STUDY[228,229]. The Medical Ethics Committee of our university approved the study protocol, and a written consent after explanation of the aims and details of the study was obtained from each participant. The refusal to participate in this study was the only exclusion criterion. Two groups served as controls: group 1 entailed patients in whom significant (e.g., stenosis d 50%) CAD was eventually ruled out by coronary angiography; group 2 comprised 119 consecutive healthy normotensive blood donors enrolled at the local blood bank during the same period . In these latter subjects, it was unethical to perform coronary angiography to rule out the presence of asymptomatic CAD. Therefore, the following inclusion criteria were used: negative family history of CAD, MI, and stroke; nonsmoking status; absence of hypercholesterolemia, hypertriglyceridemia, diabetes mellitus, all defined as specified in the following text.

Based on available data from epidemiologic and family studies, a cohort fulfilling these criteria is expected to have a very low prevalence of asymptomatic CAD. Information on the long-term outcome was gathered with a predefined form: through review of medical charts, for the patients regularly seen at referring hospitals and through telephone interviews of family doctors and/or patients and first-degree relatives for those not attending regular follow-up visits.

Predetermined primary endpoint were CV events, including acute coronary syndromes, stroke, and CV deaths. The latter comprised sudden death or due to congestive heart failure, acute coronary syndromes, or stroke according to the Syst-Eur Trial criteria[230]

Demographic and clinical measurements

A standard questionnaire was used to carefully ascertain medical history in all participants (transient ischemic attack, stroke, angina, MI, coronary artery bypass, percutaneous transluminal coronary angioplasty, renal failure, peripheral artery disease, and history of 67 vascular surgical interventions), smoking habits, presence/absence of

hypertension, diabetes, hypercholesterolemia, hypertriglyceridemia, and current medications. Body mass index was calculated as weight/height2 (kg/m2). Patients were classified into three groups: current smokers, nonsmokers, and ex-smokers (who had stopped smoking for at least one year). Diabetes mellitus (type I or II) was defined as a previous diagnosis of the disease, history of antidiabetic medications, or plasma fasting levels of glucose 126 mg/dl (7.0 mmol/l) on at least two occasions. Impaired glucose tolerance was defined as plasma fasting levels of glucose ranging between 110 to 126 mg/dl (6.1 to 6.9 mmol/l)[231].

Hypercholesterolemia was defined as a low-density lipoprotein (LDL) cholesterol 100 mg/dl according to the National Cholesterol Education Program guidelines for patients with CAD; hypertriglyceridemia was defined as plasma fasting levels 134 mg/dl, that is, higher than the 95th percentile value of our group 1 control subjects. Blood pressure was measured by mercury sphygmomanometer using Korotkoff phase V for diastolic, according to the World Health Organization guidelines. Hypertension was defined as systolic pressure 140 mm Hg, and/or diastolic pressure 90 mm Hg , or use of any antihypertensive agents.

Coronary angiography

Angiography was carried out and evaluated by experienced cardiologists who were blinded to patients' genotype. Angiography and measurement of left ventricular ejection fraction (LVEF) and the grading of the CAD burden with the modified Duke Prognostic Index score was carried out as described[232]. This score considers only major epicardial coronary arteries with ≥ 50% diameter stenosis and goes from 0 (all major coronary arteries with lesions < 50% diameter stenosis) to 100 (≥ 95% left main stenosis). It was reported to accurately predict five-year mortality of medically treated patients[233].

Of the 1,271 patients originally recruited in the GENICA study who had complete coronary angiography data, 17% (n = 217) were found to have normal coronary arteries, and 14% (n =178) stenosis <50%, 23% (n = 290), 24% (n = 305), and 22% (n = 281) had significant (50%) stenosis in one, two, or three major epicardial vessels, respectively.

Laboratory measurements

Each patient was studied between 8:30 and 12:00. Before coronary angiography, blood samples were taken from the femoral artery and were immediately put on ice and centrifuged at 3,000× *g* (at 4°C for 10 min). Total cholesterol, high-density lipoprotein

(HDL) cholesterol, triglycerides, glycemia, sodium, potassium, blood urea nitrogen, and creatinine levels were measured with conventional methods.

8.2 Blood sampling and plasma LpPLA2

Blood was drawn at about 9 a.m. after an overnight fast and 15- min. supine rest from an antecubital vein without stasis. Five ml of whole blood with 100 µl 6% Na2EDTA was immediately put on ice. After centrifugation at 3000×*g* (at 4 ◦C for 10 min), aliquots of plasma and buffy coat were stored at −20 ◦C.

Lp-PLA2 concentration (mass) was measured at diaDexus by an ELISAmethod (PLAC® test, diaDexus South San Francisco, CA), which is a sandwich enzyme immunoassay that uses two highly specific monoclonal antibodies for the direct measurement of Lp-PLA2 concentration. A set of Lp-PLA2 calibrators is used to plot a standard curve of absorbance versus Lp-PLA2 concentration from which the Lp-PLA2 concentration in the test sample can be determined.

The ELISA diaDexus PLAC kit for analysis of the company is a sandwich enzyme immunoassay using two monoclonal antibodies specific for the direct measurement of the concentration of Lp-PLA2 in human plasma and blood.

The analysis system uses a monoclonal anti-Lp-PLA2 (2C10) against Lp-PLA2 for immobilization on the solid phase microtiter plate. The sample is added to the plate and incubated for 10 minutes at 20-26° C. A second antibody Lp-PLA2 monoclonal antibody (4B4) labeled with the enzyme horseradish peroxidase (HRP) is then added and reacts with the antigen immobilized to 20- 6° C for 180 minutes, resulting in the capture of Lp-PLA2 molecules between the solid phase and enzyme-labeled antibodies. The tetramethylbenzidine (TMB) substrate, is then added and incubated at 20 to 26 ° C for 20 minutes, developing a blue color. The color development is stopped with the addition of stop solution that changes the color yellow. Measure the absorbance of the reaction at 450 nm and is directly proportional to the concentration of Lp-PLA2 present. It is used a set of Lp-PLA2 calibrators to plot the standard curve of absorbance compared to the concentration of Lp-PLA2, which is used to determine the concentration of Lp-PLA2 in the patient sample. Provides two levels of control to monitor the performance within the clinical tests.

Lp-PLA2 activity was determined at diaDexus using a Colorimetric Activity Method (CAM assay, diaDexus). The assay is performed in a 96-well microplate with a colorimetric substrate that is converted, via hydrolysis, by the phospholipase enzyme. Briefly, 25 lof sample, standard or control are added per well, followed by addition of assay buffer plus substrate. The change in absorbance is immediately measured at 405 nm. The level of Lp-PLA2 activity in nmol/(min ml) was calculated from the slope of the signal generated over time, based on a standard conversion factor from a *p*-nitrophenol calibration curve.

For the Lp-PLA2 mass assay the coefficient of variation was within 3% in control samples and between 7.5% and 10% in 89% of the twins samples. For the Lp-PLA2 activity assay the coefficient of variation was within 2% in control samples and between 2.5% and 5% in 79–97% of the samples.

8.3 Extraction of deoxyribonucleic acid (DNA) and SNPs genotyping.

The blood was collected in ethylenediamine-tetracetic (EDTA) acid and stored at -20°C until DNA was extracted according to standard procedures, and quantified by spectrophotometer; 200 µl of whole blood mixed with 100 µl lysis buffer (3 M guanidine thiocyanate, 20 mM EDTA, 10 mM tris HCL, ph 6.8; 40mg/ml Triton x-100, 10 mg/ml DTT) were added to 100 µl binding solution (40 mg/ml silica [Sigma] directly suspended in the lysis buffer), mixed and incubated for 3 minutes at room temperature and centrifuge for 15 seconds at 700 rpm and discard supernatant repeat once.

We added after 100 µl lysis buffer and mix, centrifugated for 15 sec. and repeated once also with succeeding addition of absolute ethanol. After alcohol mix the supernatant was discard and vacuum-dry the pellet. Finally 50 µl of eluition buffer, resuspend the pellet and it was incubated for 3 min. at 65 °C and centrifugate for 1 min for transfer supernatant in a clean tube.

SNP genotyping was performed in *Roche Light-cycler 480* by real-Time Polymorphism Chain Reaction (PCR) using *Endpoint Genotyping Analysis* (EGA)

EGA uses 2 sequence-specific probes that are designed for Wild Type (WT) and Mutant target DNAand these probes are labeled with different fluorescent dyes in our application. The specific probe for WT and mutant was 6-carboxyfluorescein, acronym *FAM* and high-affinity F-actin probe conjugated photostable, red fluorescent *Texas Red-X* .

Each probe contains two labels (a fluorescent reporter and a quencher) wich are in close proximity to each other. When the probe is intact, the quencher suppresses the report signal. During the elongation step the probe is cleaved and reporter fluorescence measured.

The EGA sets the genotype of the sample by measuring the intensity distribution of used probe after PCR.

Amplification primers and hydrolysis for each polymorphism are shown under:

OLIGO	SEQUENCE
FORHIS92	5′-TAA CAG GGC ACC TTC TTG C-3′
REVHIS92	5′-CCC ATA AGC CAG TGT GTT C-3′
FORALA379	5′-ATG AGG AAG GGA AGG AG- 3′
REVALA379	5′-TCA GGG TTC TAA GGT AGA G-3
REVTHR198	5′-AGA TTC ATC TGG TTT AGG TCA TGA-3′
FORTHR198	5′-GCA ACT GGC AAA ATA ATT GGA C-3′

The PCR reaction mixture consisted of Lightcycler DNA Master Hybridization Probes 10uml_ (Roche Diagnostics, Milan, Italy) to which primers 200nM) and probes 100nM) for the Arg92His or Ala379Val or Ile198Thr polymorphisms, respectively, were added. Below they show the sequences of hydrolysis probe.

HYDROLYSIS PROBE	SEQUENCE	MODIFICATION
PB1HIS92	5′-AGA TAA TGA TCG CCT TGA CAC-3	3′ Blackhole Quencer 5′FAM (6-FAM)
PB2HIS92	5′-AGA TAA TGA TCA CCT TGA CAC C-3′	3′ Blackhole Quencer 5′ Texas Red
PB1ALA379	5′-CAG AAA CAG GGG ACA AGT-3′	3′ Blackhole Quencer 5′FAM (6-FAM)
PB2ALA379	5′-CAG AAA TAG GGG ACA AGT C-3′	3′ Blackhole Quencer 5′ Texas Red
PB1THR198	5′-CTT AGC AAC AAA GCT TCA TTA GCA T-3′	3′ Blackhole Quencer 5′FAM (6-FAM)
PB2THR198	TAG CAA CAA AGC TCC ATT AGC A-3′	3′ Blackhole Quencer 5′ Texas Red

The cycling condition were 95°C for 10 min followed by 45 repeats of 95°C for 10 s ; 54°C, 30 s; 72°C, 15 s for each polymorphism, respectively

Hence, distinction of wild type, mutant, and eterozygous genotype can be easily accomplished by differences in their respective fluorescence emission .

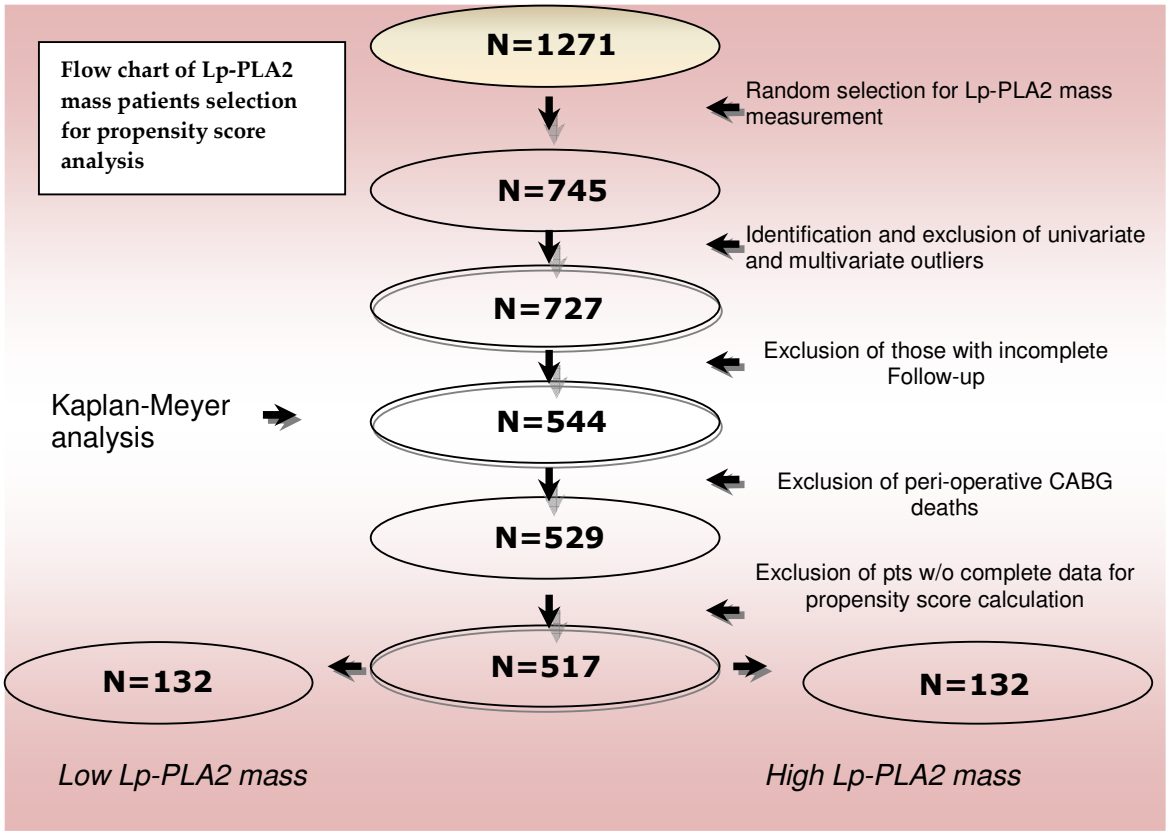
8.4 Statistical analysis

Serum triglycerides, HDL- and LDL-cholesterol, age, creatinine, CAD Duke index score, LVEF, and oxLDLAbs were examined after log or square root transformation to achieve a Gaussian distribution. A random sample comprising 748 of the CAD patients originally recruited in the GENICA study was obtained with single random number generation through SPSS. Patients in this sample were selected for the Lp-PLA2 mass titer measurement, which was performed blindly with respect to all clinical and anthropometric data.

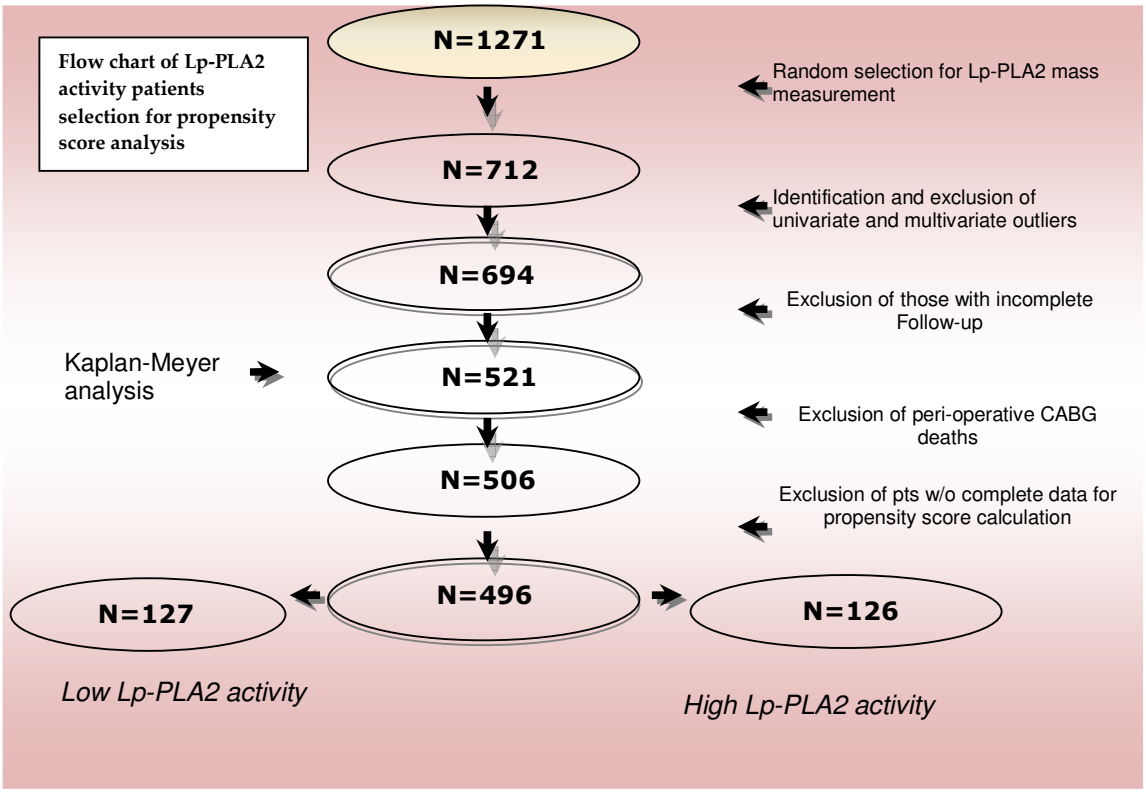
Standardized z scores were calculated to identify univariate outliers and exclusion of cases with z scores exceeding $|3.29|$ that corresponded to a $p<0.001$, was decided *a priori*. Mahalanobis distance was assessed by regression analysis to identify multivariate outliers; cases with χ^2 in excess of 32.909 (12 df at $\alpha=0.001$) were considered outliers and removed from further analysis (14 patients) according to the technique of Tabachnick and Fidell [29].

Comparison of quantitative variables across groups was done by ANOVA followed by Bonferroni's *post hoc* test. Chi-square analysis was used to compare the frequencies of categorical CAD risk factors.

Standard multiple regression analysis was used beforehand to verify the assumption that cases lost at follow up did not differ significantly from those available for survival analysis. Propensity score was calculated with logistic regression analysis including all available variables that are known to potentially affect the outcomes, including gender, age, BMI, LDL- and HDL-cholesterol, triglycerides, serum creatinine, homocysteine, glycaemia, serum sodium concentration, heart rate, arterial hypertension, smoking habit, LVEF, the Duke Prognostic Index of coronary atherosclerotic burden, length of follow up, history, and treatment variables. To correct for the imbalance of variables distribution between the patients with low and high Lp-PLA2 mass we did a greedy matching without replacement using a caliper of 0.2 standard deviations of the logit of the propensity score[234].



In the same way and condition patients in this sample were selected for the Lp-PLA2 activity titer measurement, which was performed blindly with respect to all clinical and anthropometric data and similarly was calculated correction for the imbalance of variables distribution between the patients with low and high Lp-PLA2 activity



To identify variables independently associated with Lp-PLA2 mass and activity we performed a regression analysis using inclusion and exclusion criteria of 0.05 and 0.10, respectively. The backward variable elimination was preferred to the forward inclusion because it carries a lower risk of missing relevant predictor variables. Collinearity tests were used to avoid including highly correlated variables in the model.

The cut-off points were determined by analyzing the best Youden index (sensitivity - specificity – 1) and the maximized area under the ROC curve (AUC)

The distribution of measured baseline covariates between low and high Lp-PLA2 groups in the matched samples was then compared, assessing the balance in measured variables with standardized differences. We plotted the event occurrence with time using the Kaplan-Meier method and compared the survival curves for the matched set with the test proposed by Klein and Moeschberger. Statistical significance was defined as $P< 0.05$. SPSS 18 for Windows (SPSS Italy Inc., Bologna, Italy) was used for all analyses.

9. Results

The main clinical and demographic features of these patients are summarized in table 1

The demographic characteristics of the GENICA Study	
Variable	CAD (n=749)
Age (yrs)	64.3±9.4
Gender (M/F)	611(82%)/138 (18%)
Non-Smokers/Smokers/Ex (%)	35.0/14.2/50.8
BMI (Kg/m2)	26.9±3.8
Heart Rate (b/min)	66±10
Systolic BP, (mmHg)	135±18
Diastolic BP, (mmHg)	78±10
Mean BP, (mmHg)	97±11
Total Cholesterol, (mg/dL)	206±44
HDL-Cholesterol, (mg/dL)	45±11
LDL Cholesterol, (mg/dL)	131±36
Triglycerides, (mg/dL)	147±98
Serum Glycemia, (mg/dL)	115±40
Left Ventricular EF (%)	60±14

Tab. 1. Demographic and clinical characteristics of theGENICA subjects. *Results are expressed as mean ± SD. BMI, body mass index; K^+, potassium; Na^+, sodium; BP, Blood Pressure; HDL, high density lipoprotein; LDL, low density lipoprotein; EF, ejection fraction*

The clinical characteristics of the GENICA Study	
Variable	CAD (n=749)
Diabetes mellitus (%)	19.0
Hypertensives (%)	61.4
Hypercholesterolemia (%)	59.4
Hypertriglyceridemia (%)	22.7
Cholesterol-lowering drugs (%)	38.6
History of	
Transient ischemic attack (%)	3.5
Ischemic Stroke (%)	1.7
Acute myocardial infarction (%)	45.6
Coronary artery bypass surgery (%)	12.1
Percutaneous angioplasty (%)	9.3
Peripheral arterial disease (%)	17.5
Vascular surgery (%)	5.4
Chronic renal failure (%)	6.7

Tab.2 Clinical characteristics of the of theGENICA subjects

After exclusion of patients with no or incomplete follow-up data and of univariate and multivariate outliers we could obtain full follow-up data in 544 patients (73.1%) randomly selected for measurement of theLp-PLA2 mass titer. Fifteen further cases of perioperative death at coronary artery by-pass surgery were also excluded from the analysis as death was judged to be related to surgery or its complications in these patients. Therefore, the final analysis was carried out in 529 patients.

The anthropometric and clinical features of these patients divided according to the Lp-PLA2 mass quartiles are shown in Table 3 Overall there were no significant differences across quartiles with the exception of the Lp-PLA2 mass titer, heart rate, total cholesterol,

LDL cholesterol, mean triglycerides and left ventricular ejection fraction.

The coronary angiography findings in the cohort of patients selected for this study did not differ from those of the whole population of the GENICA study [228,229,235] and moreover as shown in table 3 we could fully confirm in this larger cohort the lack of association between the CAD burden and Lp-PLA2 mass and activity quartiles at baseline

Quartile of Lp-PLA2 mass					
Variable	**1st (n=133)**	**2nd (n=132)**	**3rd (n=132)**	**4th (n=132)**	**P=**
Age (yrs)	63.14± 9.01	62.64±9.34	64.47±9.76	62.39±10.40	NS
Gender (M/F)	100(75.2%)/33(24.8%)	109(82.6%)/23(17.4%)	104(78.8%)/28(21.2%)	108(81.8%)/24(18.2%	NS
Non-Smokers/Smokers/Ex (%)	41/16/43	35/14/51	38/8/54	32/21/47	NS
BMI (Kg/m2)	26.3±3.5	26.4±3.0	27.4±3.9	26.6±3.9	NS
Serum Creatinine (micromol/L)	90±26	89±21	95±26	96±28	NS
Serum K+ (mmol/L)	4.2±0.3	4.1±0.3	4.0±0.3	4.2±0.3	NS
Serum Na+ (mmol/L)	140.3±2.4	139.6±2.5	139.6±3.01	139.5±2.4	NS
Heart Rate (b/min)	64±9	66±10	66±10	67±9	0.037
Systolic BP, (mmHg)	134±18	135±17	135±17	132±17	NS
Diastolic BP, (mmHg)	78±9	78±10	78±8	78±9	NS
Serum Glycemia, (mg/dL)	113.2±34.8	115.0±38.1	112.4±36.7	109.0±29.3	NS
Total Cholesterol, (mg/dL)	191.4±36.6	206.5±43.0	211.27±38.2	223.8±46.7	<0.001
Mean HDL-Chol. (mg/dL)	46.9±12.0	46.6±11.4	45.2±9.9	45.5±11.2	NS
LDL Cholesterol, (mg/dL)	120.5±28.7	130.95±30.52	135.78±31.48	145.49±37.35	<0.001
Triglycerides, (mg/dL)	126.8±57.7	140.5±88.3	142.6±59.8	164.1±131.4	0.008
Homocysteine, (μmol/L)	11.3±5.2	12.5±6.9	13..2±5.8	13.3±9.1	NS
Left Ventricular EF (%)	65±12	62±12	60±14	61±15	0.049
Lp-PLA2 mass (ng/ml)	253.46±1.8	337.82±0.21	393.23±0.12	490.17±2.8	<0.001
Duke CAD score	35±21	38±18	37±19	36±21	NS
Follow-up (years)	7.1±1.9	7.1±1.9	7.1±2.3	6.6±2.5	NS

Tab 3. Demographic and clinical characteristics of the subjects classified by Lp-PLA2 mass quartiles.
Results are expressed as mean ± SD. BMI, body mass index; K^+, potassium; Na^+, sodium; BP, Blood Pressure; HDL, high density lipoprotein; LDL, low density lipoprotein; EF, ejection fraction; CAD, coronary artery disease.

The anthropometric and clinical features of these patients divided according to the Lp-PLA2 activity quartiles are shown in Table 4. Overall there were no significant differences across quartiles with the obvious exception of the total Cholesterol, (mg/dL), HDL, LDL, homocysteine Lp-PLA2 activity, triglycerides and left ventricular ejection fraction

Quartile of Lp-PLA2 activity					
Variable	**1st (n=127)**	**2nd (n=126)**	**3rd (n=126)**	**4th (n=127)**	**P=**
Age (yrs)	61.7±9.2	62.5±8.7	64.7±9.9	63.3±10.6	NS
Gender (M/F)	92(72.4%)/35(27.6%	100(79.4%)/26(20.6%)	102(81%)/24(19%)	110(79.8%)/17(13.4.2'	NS
Non-Smokers/Smokers/Ex (%)	16/37/47	14/38/48	11/35/54	18/38/44	NS
BMI (Kg/m2)	26.4±3.3	26.7±3.9	26.8±3.6	27.3±3.9	NS
Serum Creatinine (micromol/L)	89±26	92±22	92±26	95±27	NS
Serum K+ (mmol/L)	4.1±0.3	4.2±0.3	4.2±0.3	4.2±0.3	NS
Serum Na+ (mmol/L)	139.9±2.5	139.2±2.6	139.7±2.5	139.9±2.6	NS
Heart Rate (b/min)	64±9	66±10	66±9	67±9	NS
Systolic BP, (mmHg)	134±17	135±17	134±19	133±17	NS
Diastolic BP, (mmHg)	78±9	78±9	78±9	78±9	NS
Serum Glycemia, (mg/dL)	115±33	113±38	109±32	111±34	NS
Total Cholesterol, (mg/dL)	194.2±42.4	207.5±36.9	213.3±41.2	218.0±44.1	<0.001
Mean HDL-Chol. (mg/dL)	48.2±12.7	46.6±9.6	46.3±10.6	43.4±9.8	<0.001
LDL Cholesterol, (mg/dL)	121.4±30.9	133.86±30.5	136.4±33.5	141.9±35.4	<0.001
Triglycerides, (mg/dL)	124.7±69.7	135.7±61.3	145.2±78.8	157.5±78.9	<0.001
Homocysteine, (μmol/L)	11.5±7.0	11.4±4.3	12.4±5.5	14.6±9.3	<0.001
Left Ventricular EF (%)	65±12	64±12	60±13	61±15	0.037
Lp-PLA2 activity nmol/ml/min	80.65±11.62	104.12±4.86	121.01±5.51	148.73±16.0	<0.001
Duke CAD score	34±20	37±19	37±20	37±20	NS
Follow-up (years)	7.1±1.7	7.0±2.3	6.9±2.3	6.7±2.4	NS

Tab. 4 Demographic and clinical characteristics of the subjects classified by Lp-PLA2 activity quartiles. *Results are expressed as mean ± SD. BMI, body mass index; K^+, potassium; Na^+, sodium; BP, Blood Pressure; HDL, high density lipoprotein; LDL, low density lipoprotein; EF, ejection fraction;; CAD, coronary artery disease.*

Significant predictors of the Lp-PLA2 mass were HDL, LDL e triglycerides (adjusted R^2= 0.0062, p=<0.0001) (Table 5). In the models using Lp-PLA2 activity as the dependent variable, significant predictors were similarly gender , LDL, HDL, triglycerides and homocysteinemia adjusted R^2= 0.114, p=<0.0001 (Table 6).

Variable in the model	B	β	pValue
HDLc	-1.245	-0.119	0,042
LDLC	2.190	0.230	0,000
Triglycerides	0.004	0.104	0,072

Tab.5 Stepwise linear regression analysis of determinants of Lp-PLA2 mass

Variable in the model	B	β	pValue
Gender	-9,497	-0.144	0,014
HDLc	-15,171	-0.140	0,018
LDLc	23,523	0.238	0,000
Triglycerides	0,044	0.111	0,054
Homocysteinemia	6,170	0.117	0,040

Tab.6 Stepwise linear regression analysis of determinants of Lp-PLA2 activity

We determined the best cut-off value in predicting CV deaths and MACE with ROC curves and we found that the Lp-PLA2 activity Youden Index is 136.146 (Area under the

curve AUC 0.707, 95% Confidence interval 0,663 to 0,749; p <0,0001) and 130.221(AUC 0.565, 95% CI 0,520 to 0,610; p 0,029) respectively, whereas at ROC curves Lp-LPA2 mass was significant for CV death (AUC 0.668, 95% CI 0,623 to 0,710; p <0,0001) but not for MACE. (Fig. 5 and 6)

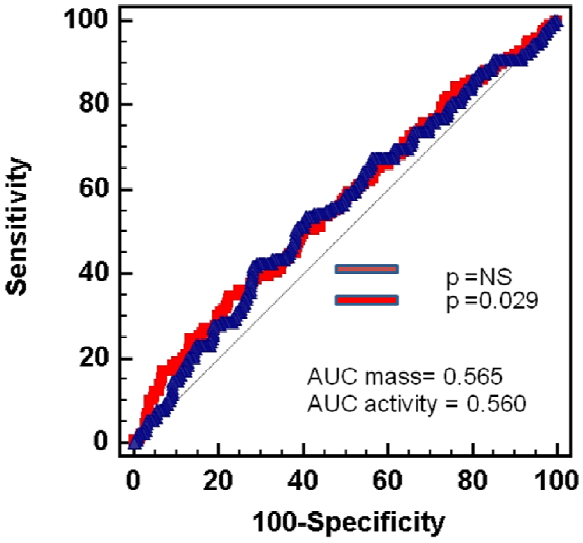


Figure 5 Roc curve comparison between LPPLA2 mass and activity for prediction of cardiovascular death

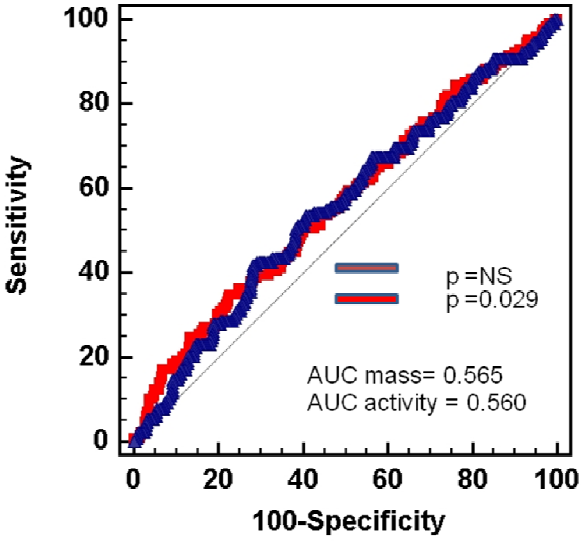
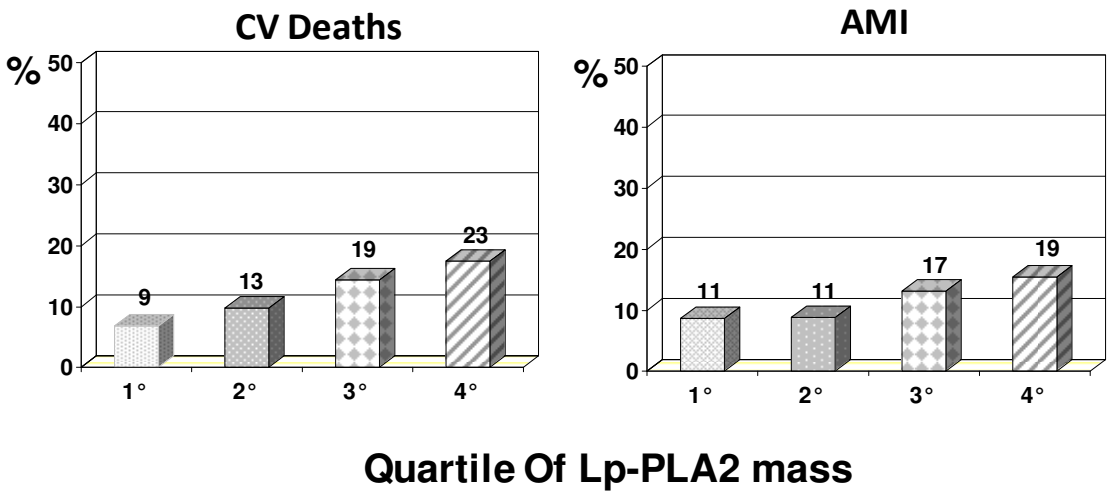


Figure 6 Roc curve comparison between LPPLA2 mass and activity for prediction of of cardiovascular events

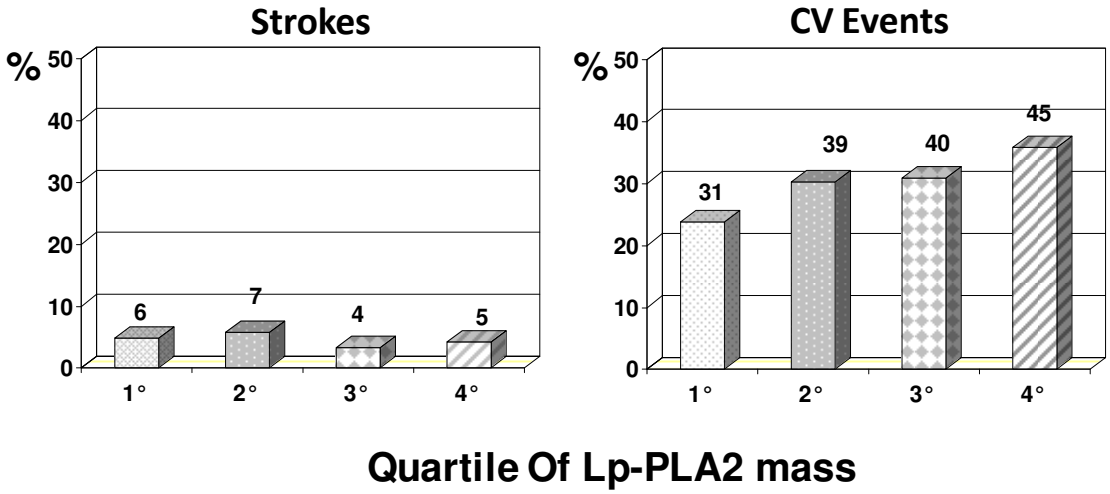
The CV death and events according to quartiles of **Lp-PLA2 mass** titer is shown under in Fig.

The median length of follow-up was 7.2 years (range 1-12.7 years). Of the 529 patients 64 (12%) had a CV death and 155 (29.3%) a CV event. (Fig. 7A-B)

7A

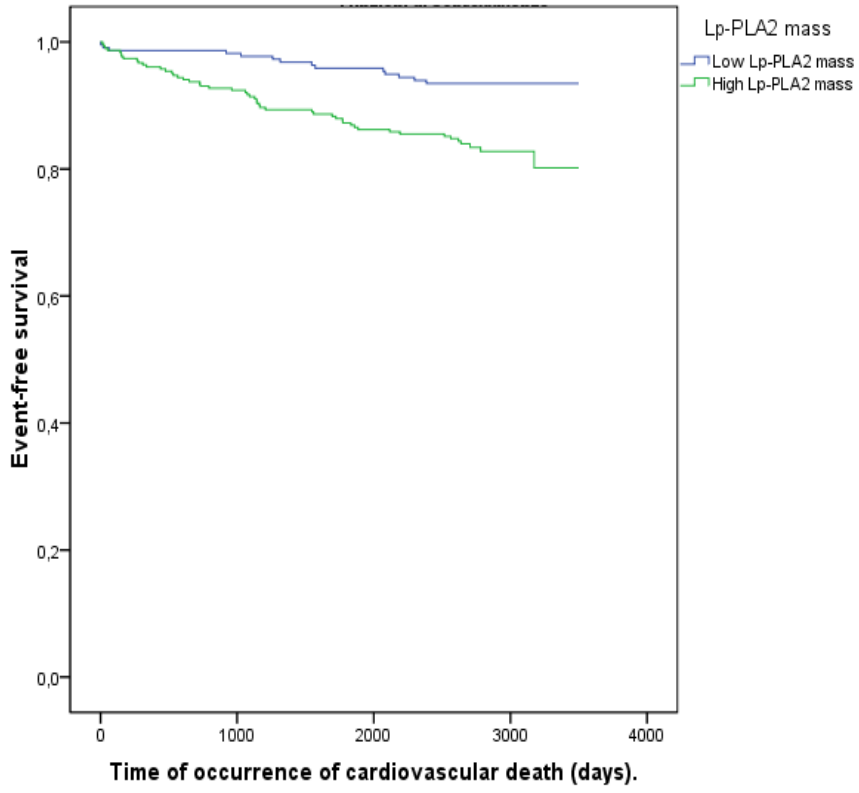


7B

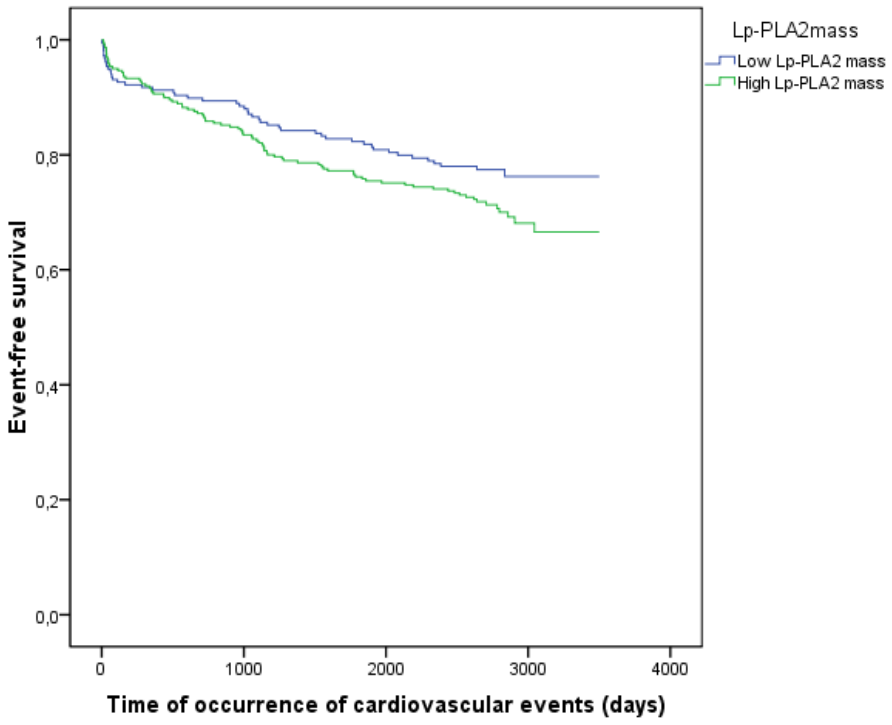


In the group with the highest **Lp-PLA2 mass** titer had a worse CV-death free survival (16.3% vs.6.2%, respectively, p = <0.0001 at Kaplan-Meyer analysis) and a lower CV event-free survival includes AMI, ACS, stroke, death from other cardiovascular causes (29.4% vs. 22.4%, respectively, p =0.099 at Kaplan-Meyer analysis) than those in the low **Lp-PLA2 mass** group comprising the first three quartiles.(Fig 8A & B)

Fig 8A & B



p =<.0001

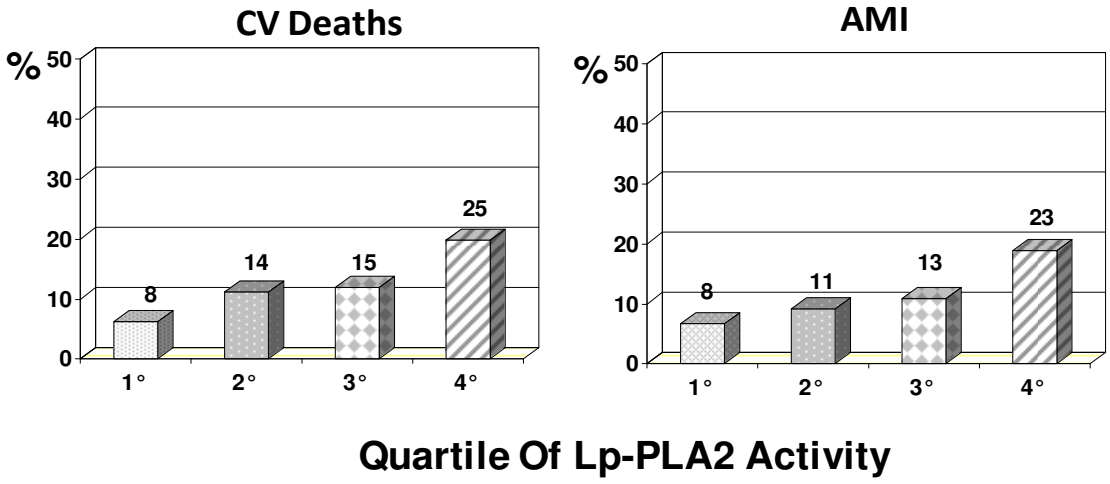


p =0.099

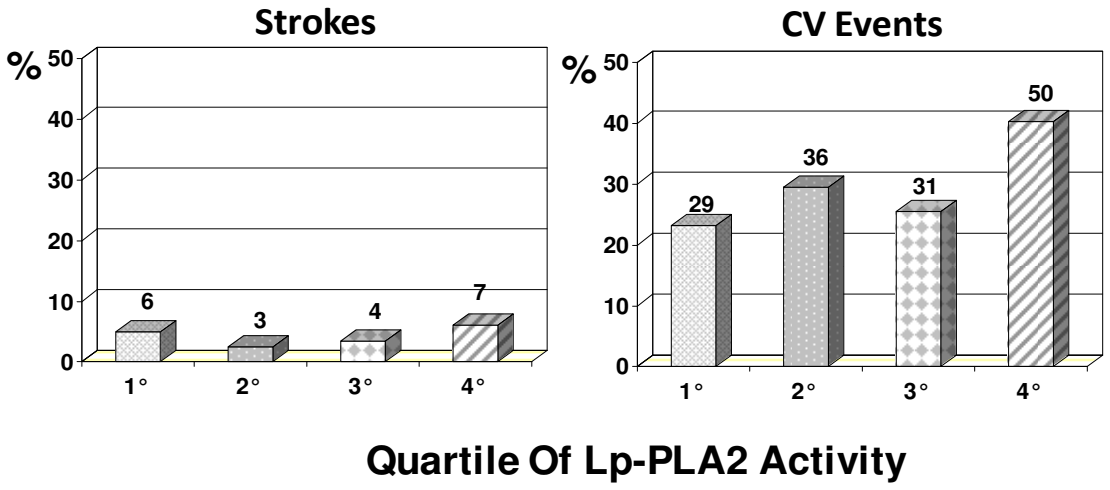
In the same way the CV death and events according to quartiles of **Lp-PLA2 activity** titer is shown under in Fig.9A-B Cardiovascular death and events rate by quartiles of **Lp-PLA2 activity** titer (the absolute number of events is shown above each column)

The median length of follow-up was 7.2 years (range 1-12.7 years). Of the 506 patients 62 (12.2%) had a CV death and 146 (28.8%) a CV event.

9A



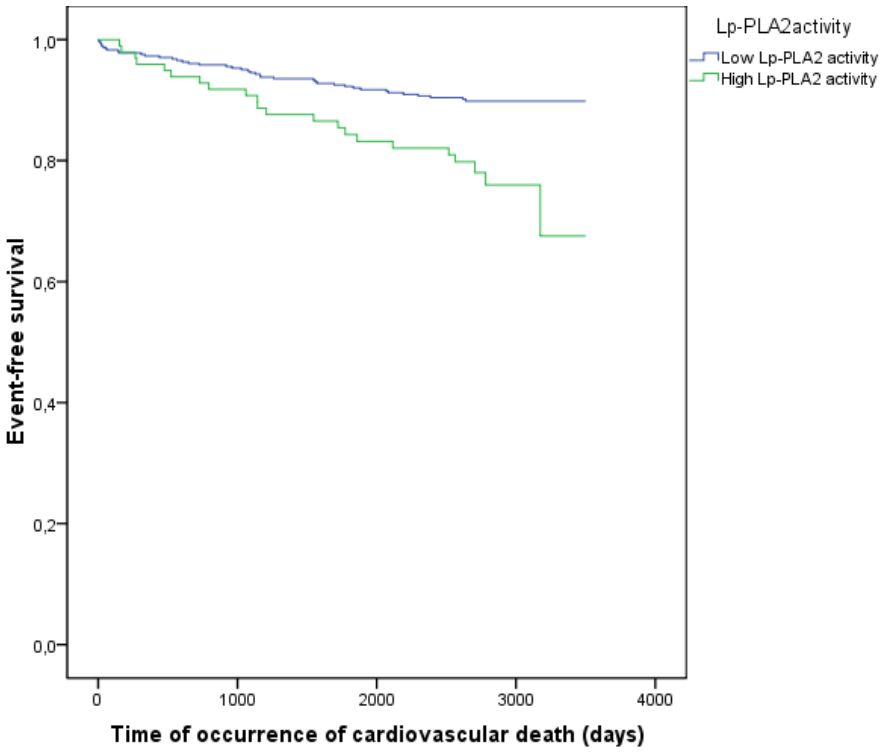
9B

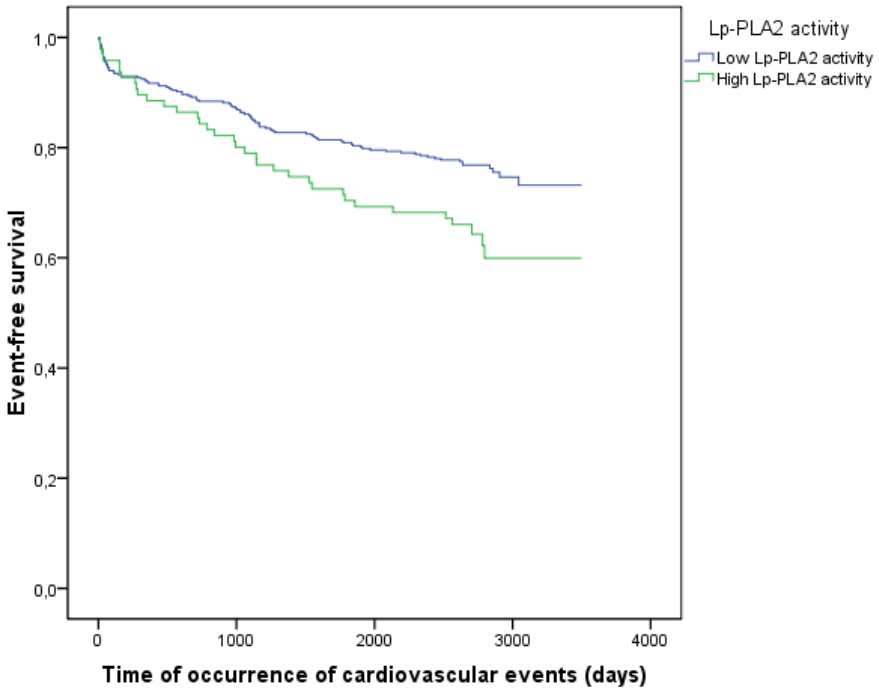


The patients in the highest group of **Lp-PLA2 activity** titer had a worse CV-death free survival (22.4% vs.9.7%, respectively, p = <0.001 at Kaplan-Meyer analysis) and a lower CV event-free survival includes AMI,ACS, stroke, death from other cardiovascular

causes (36.4% vs.23.5%, respectively, p =0.011 at Kaplan-Meyer analysis) than those in the low Lp-PLA2 activity group comprising the first three quartiles. (Fig 10A &-B)

Fig 10 A&B





Further analysis was carried out in 517 patients who had complete data for all the variables necessary to compute the propensity score. Among these patients 132 of the patients in the group with highest Lp-PLA2 mass could be matched with 132 patients of the low Lp-PLA2 mass group by propensity score. After this matching overall 64 deaths were observed, corresponding to a death rate of 12 %. Patients in the high **Lp-PLA2 mass** group again showed a worse CV death-free survival includes AMI,ACS, stroke, death from other cardiovascular causes (11.4 % vs. 6.2 %, respectively, p=<0.1) and a worse AMI events-free survival (13.3% vs.7.6%, respectively, p=0.07) but not significant than those in the low **Lp-PLA2 mass** group at the Kaplan-Meyer plot (Figures 11 A-B)

Fig.11A

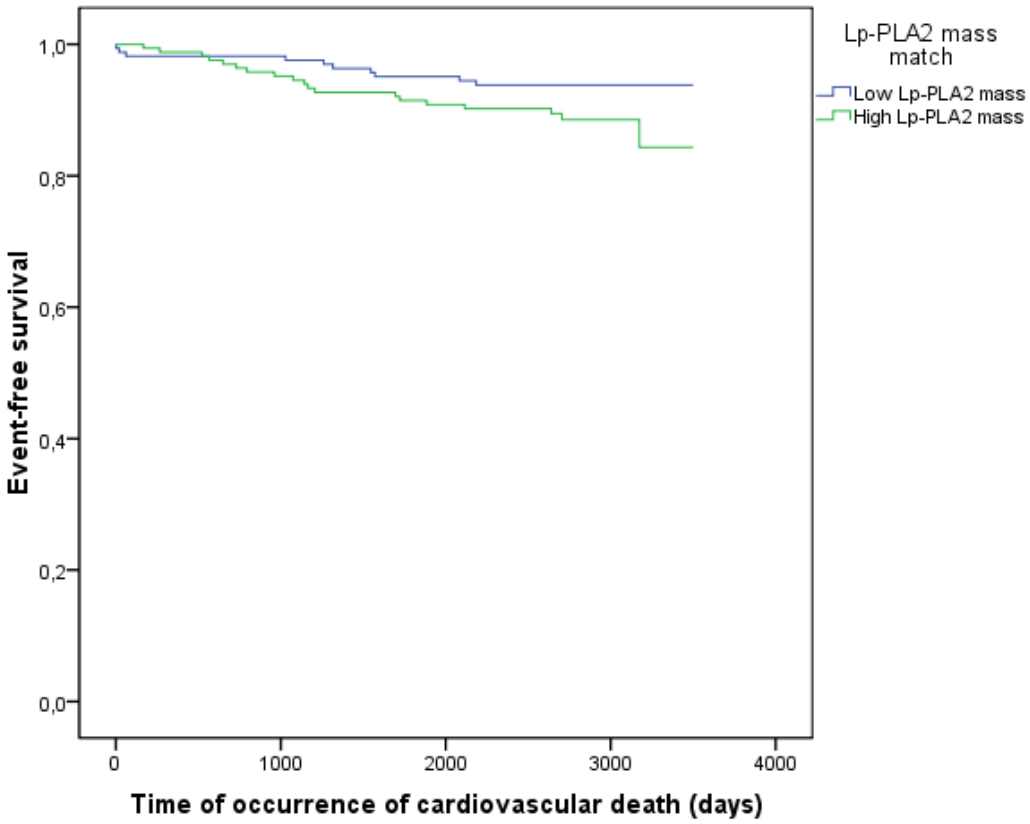
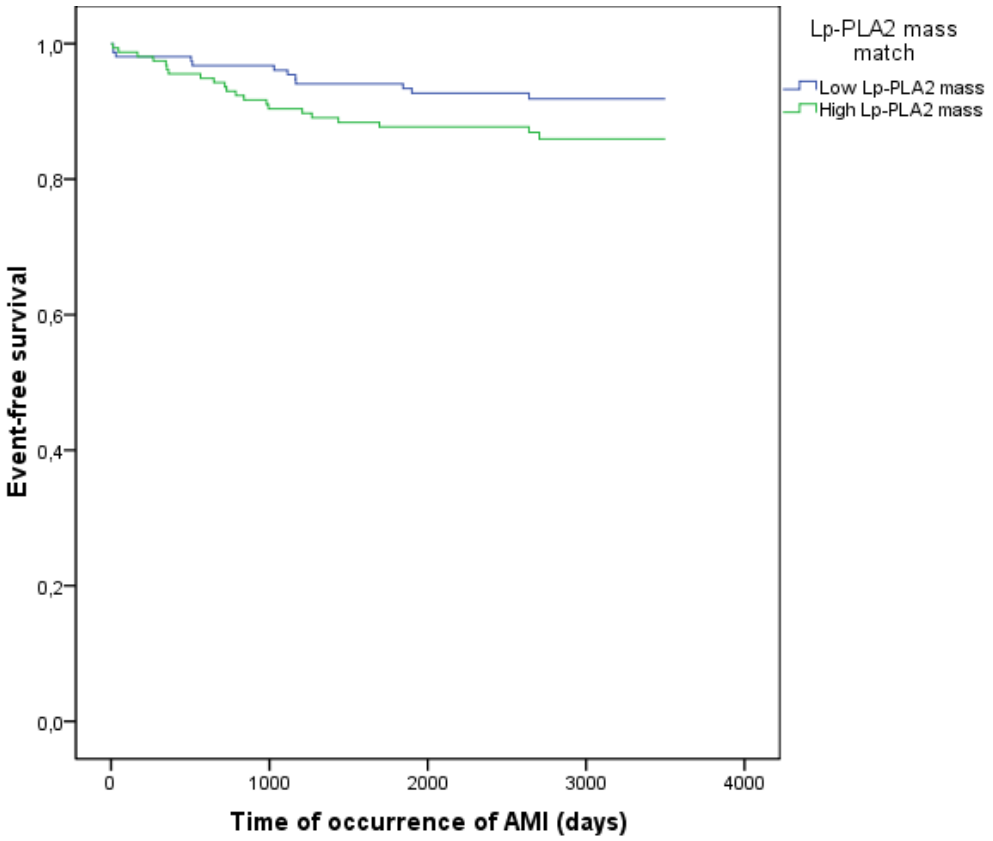


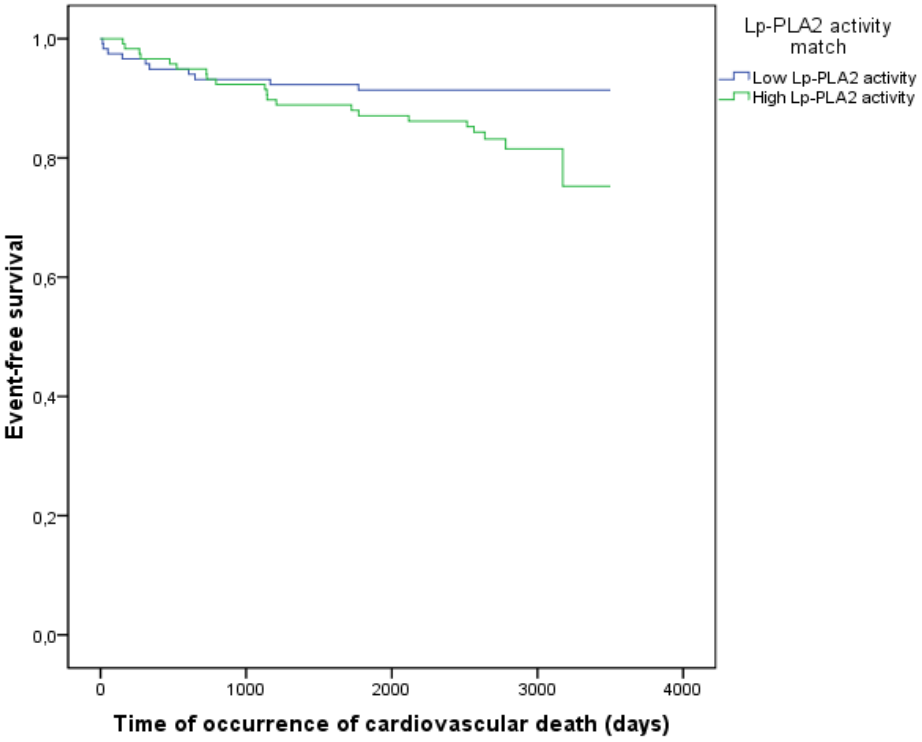
Fig 11B

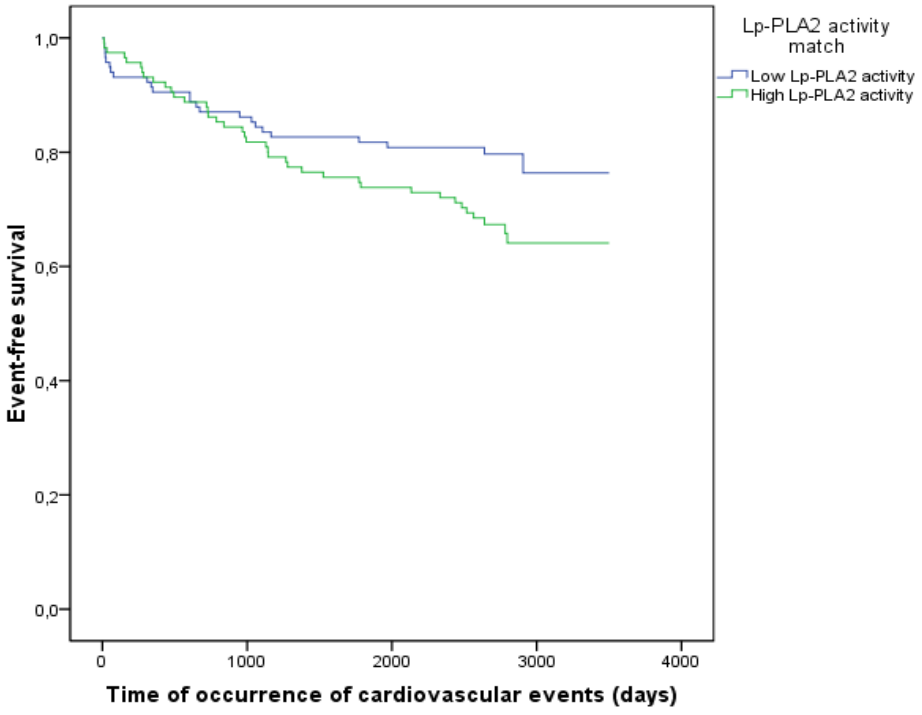


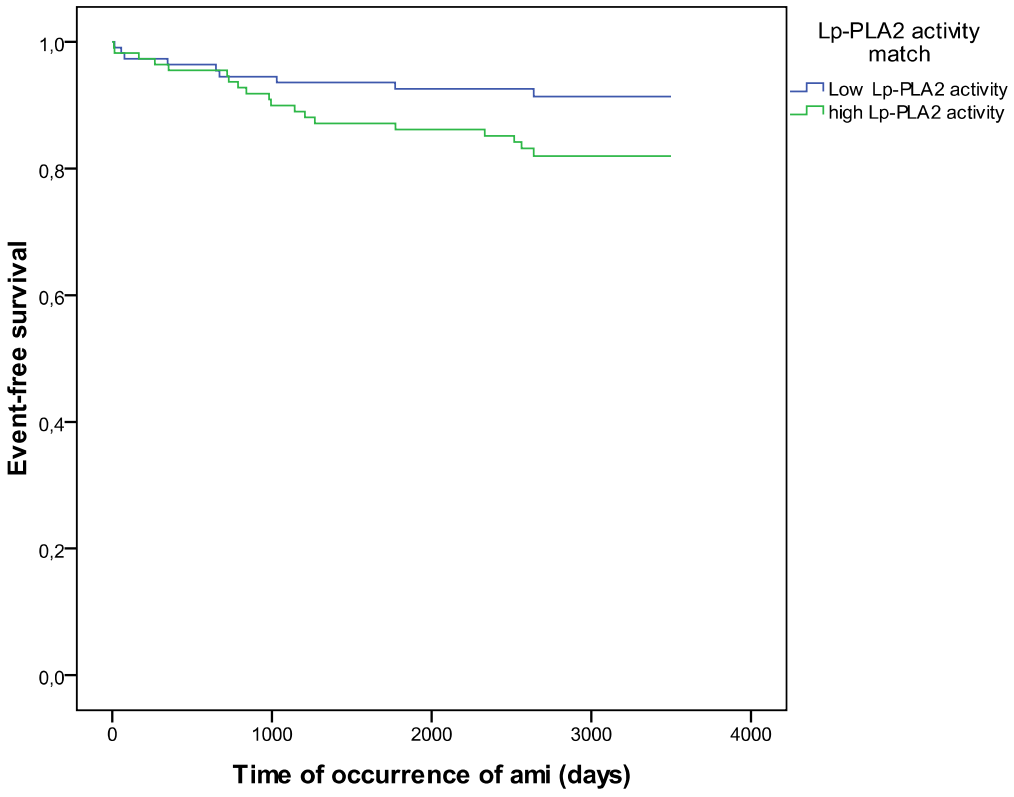
Analysis was carried out also in 496 patients who had complete data for all the variables necessary to compute the propensity score. Among these patients 126 of the patients in the group with highest Lp-PLA2 activity could be matched with 126 patients of the low Lp-PLA2 activity group by propensity score.

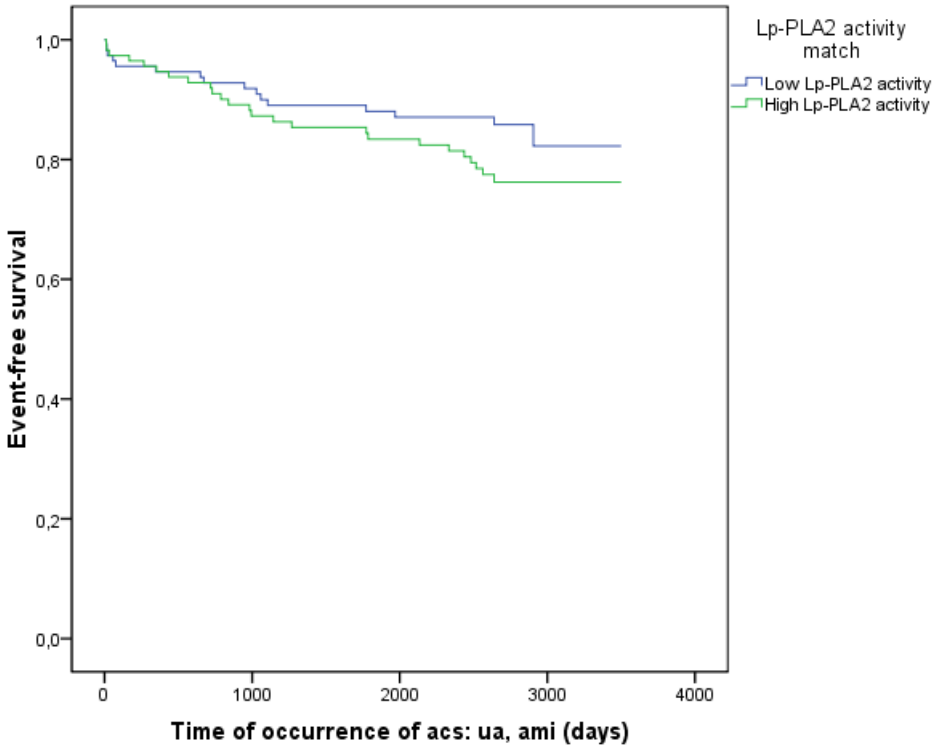
Patients in the high **Lp-PLA2 activity** group again showed a worse CV death-free survival includes AMI, ACS, stroke, death from other cardiovascular causes (17.64% vs. 8.4%, respectively, p=0.1) and a significantly worse CV events-free survival (33.3% vs. 20.5%, respectively, p=0.023) than those in the low **Lp-PLA2 activity** group at the Kaplan-Meyer plot. We show also the plot for AMI events-free survival (p=0.06) and ACS events-free survival (p=0.05) (Figures12 A-B-C-D)

Figures 12 A-B-C-D






Lp-PLA2 activity match
Low Lp-PLA2 activity
high Lp-PLA2 activity
Event-free survival
1,0
0,8
0,6
0,4
0,2
0,0
0
1000
2000
3000
4000
Time of occurrence of ami (days)


Lp-PLA2 activity match
Low Lp-PLA2 activity
High Lp-PLA2 activity
Event-free survival
1,0
0,8
0,6
0,4
0,2
0,0
0
1000
2000
3000
4000
Time of occurrence of acs: ua, ami (days)

He took into consideration the analysis of genetic polymorphisms and their role in the activity and Lp-PLA2 mass. They were first described the values as a function of various genotypes on enzyme functions. The figure 13 shows the variations of mass and activities titer in the various haplotypes His92 has shown to act by increasing the mass but surprisingly decreased enzyme activity were decreased, whereas 198Thr increased activity and mass

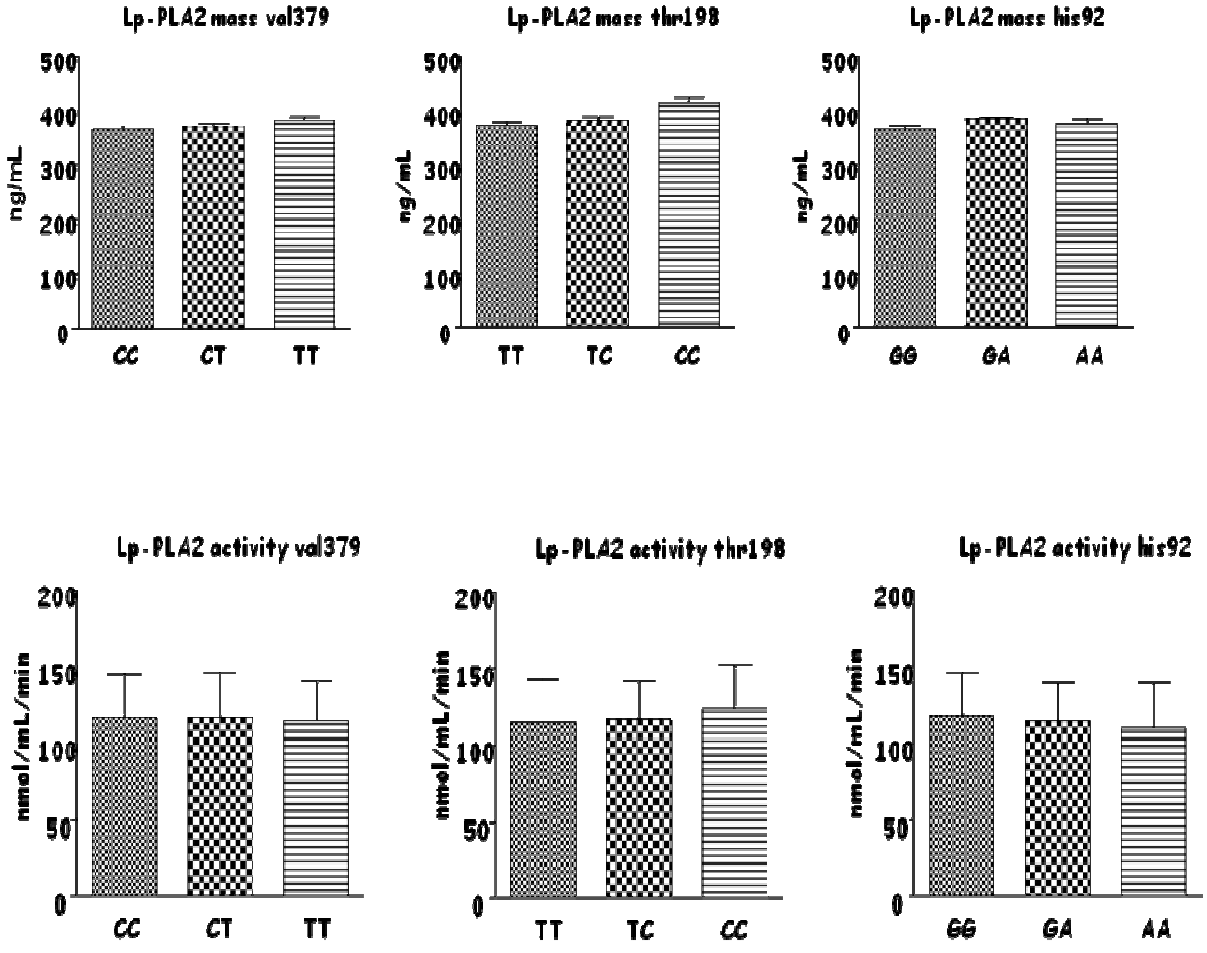


Figure 13 variations of mass and activities titer in the various haplotypes

Even creating a variable that would allow us to evaluate the cumulative genotypes in which there was a difference in mass and activities to verify that the different genotypes have functional relevance has not led to significant changes. This data are show in Fig 14

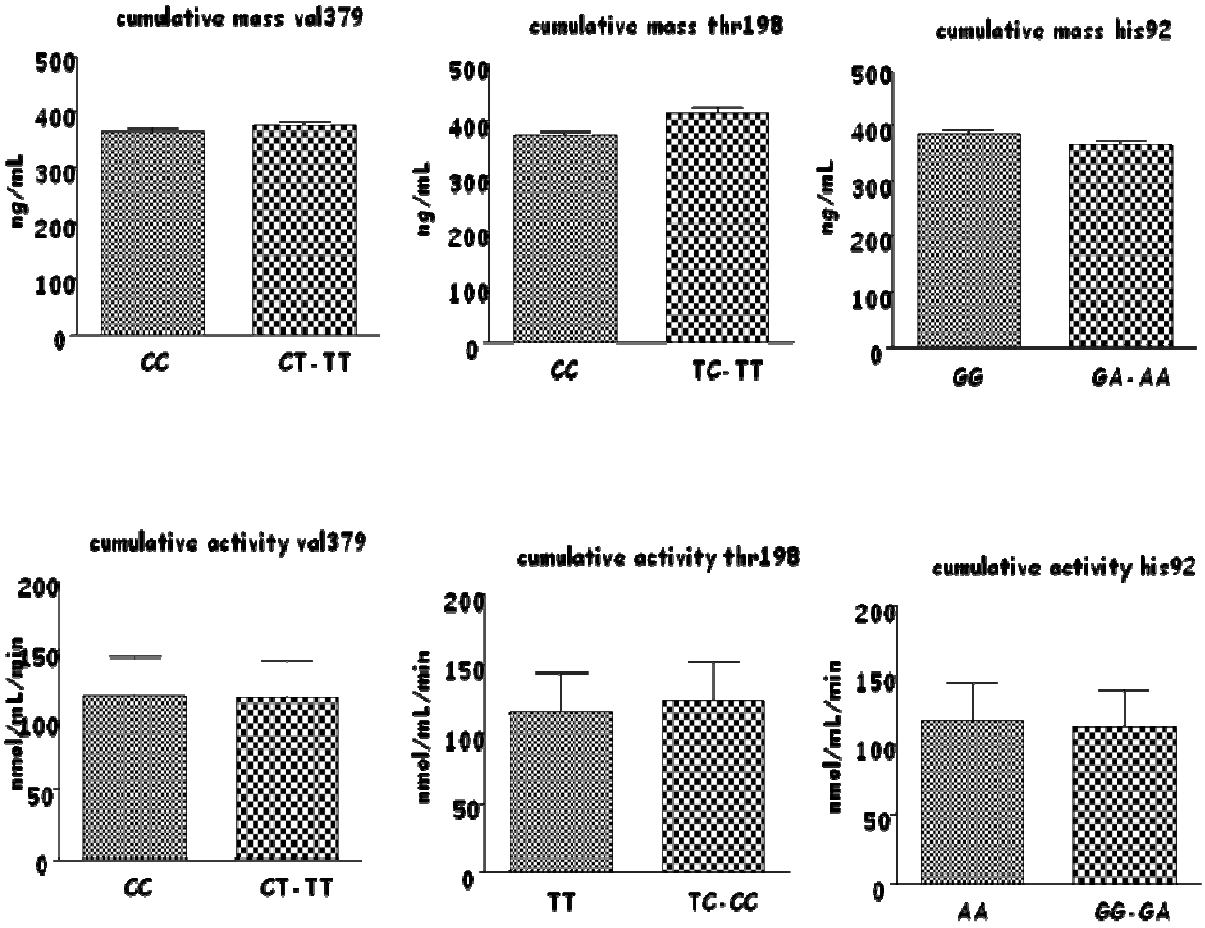
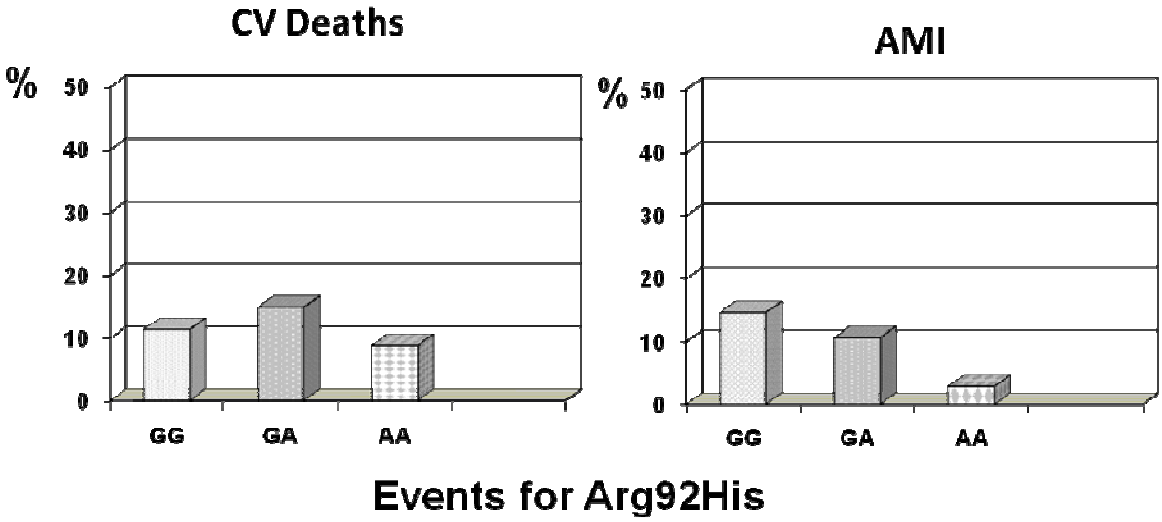


Figure 14

The CV death and events according to percentage of episodes occurrence by different genotype titer is shown under in Fig. Only THR198 genotype for CV death and stroke show significant link (Fig 15A-B-C-)



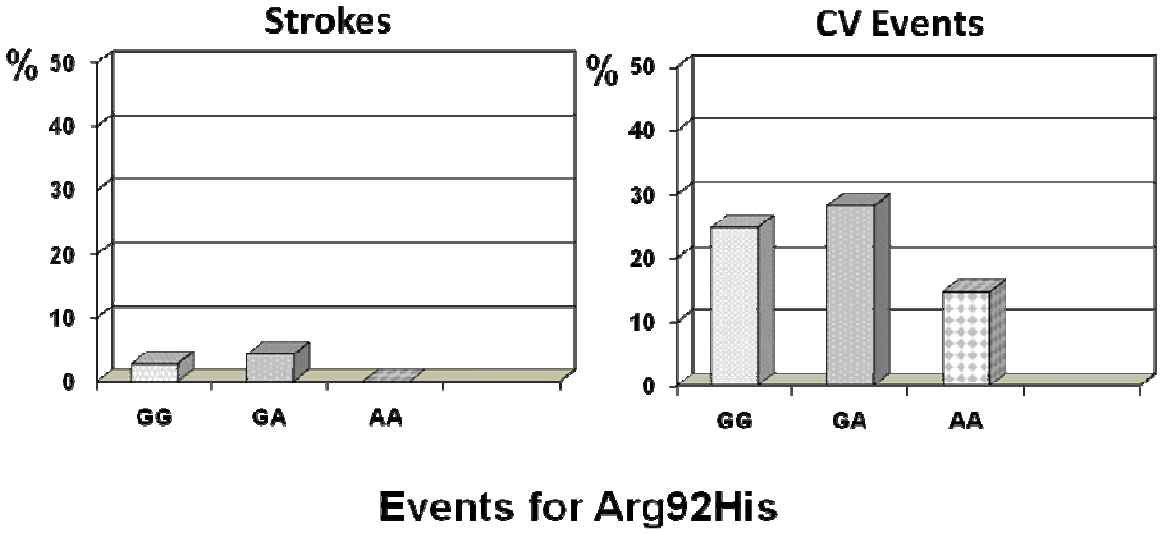
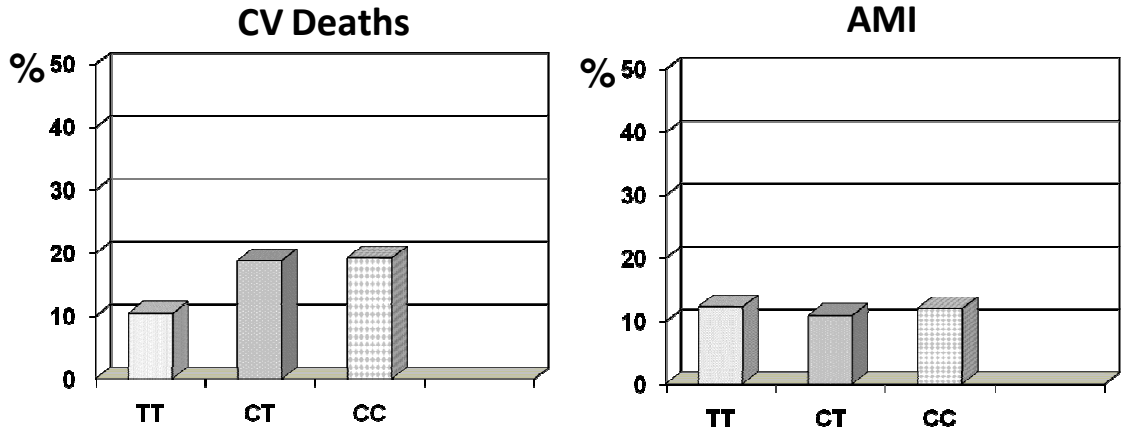


Fig 15 A


CV Deaths
AMI
%
50
40
30
20
10
0
TT
CT
CC
Events for Ile198Thr

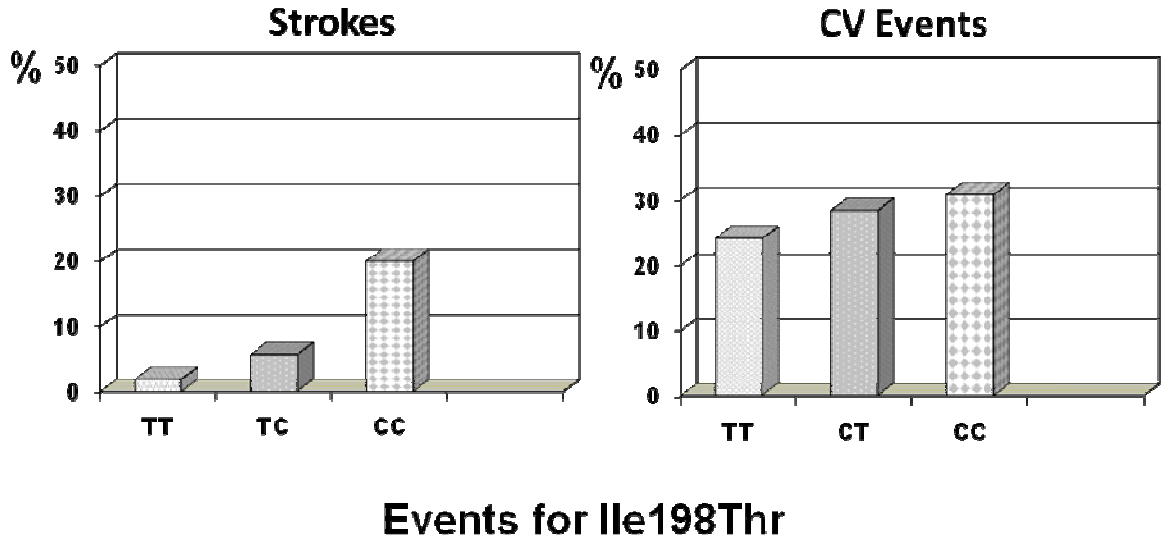
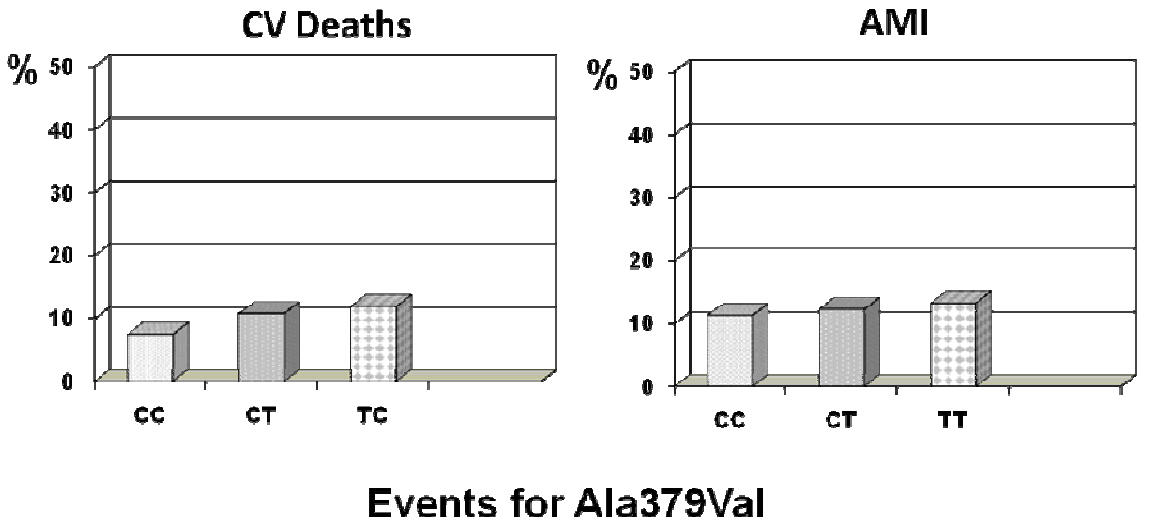

Strokes
CV Events
%
50
40
30
20
10
0
TT
TC
CC
CT
Events for Ile198Thr

Fig 15 B


CV Deaths
%
50
40
30
20
10
0
CC
CT
TC
AMI
%
50
40
30
20
10
0
CC
CT
TT
Events for Ala379Val

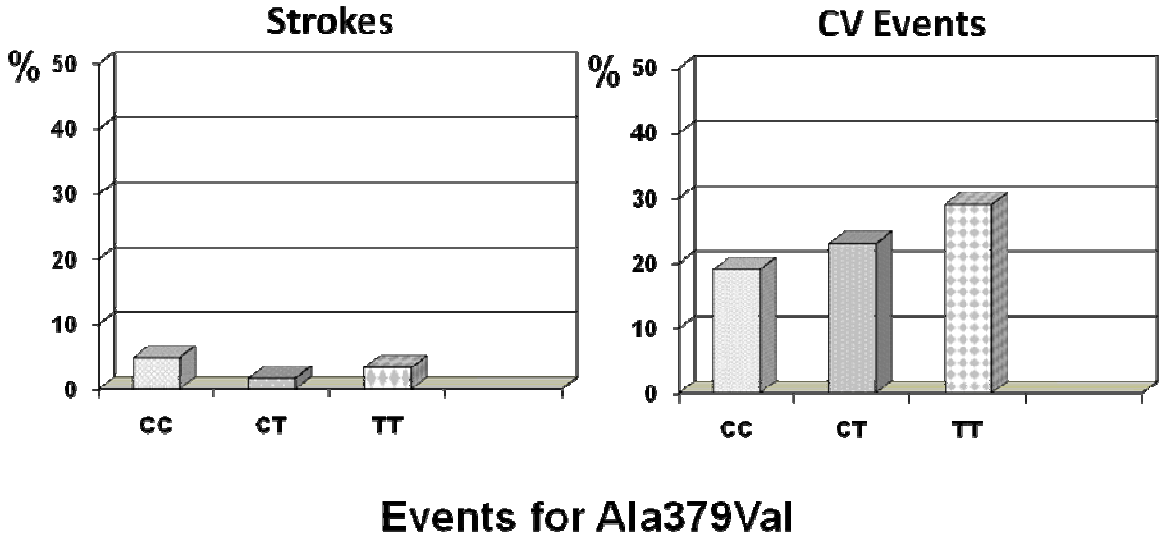
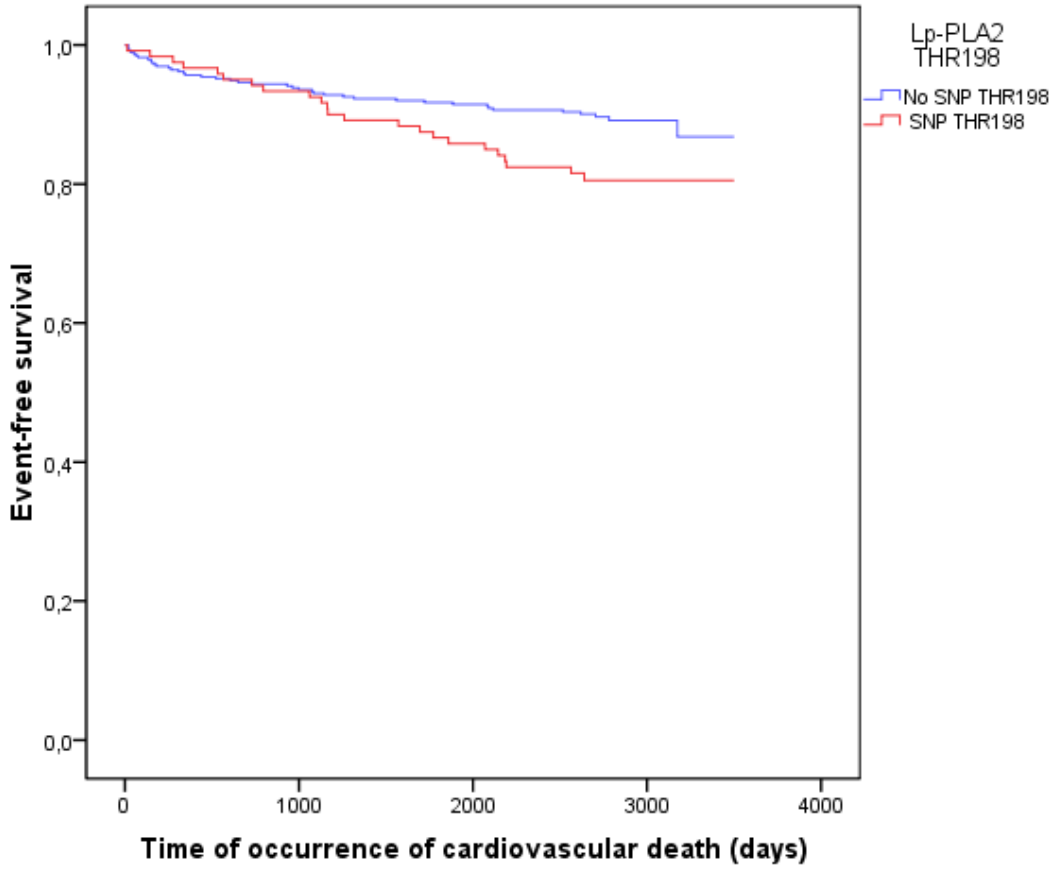
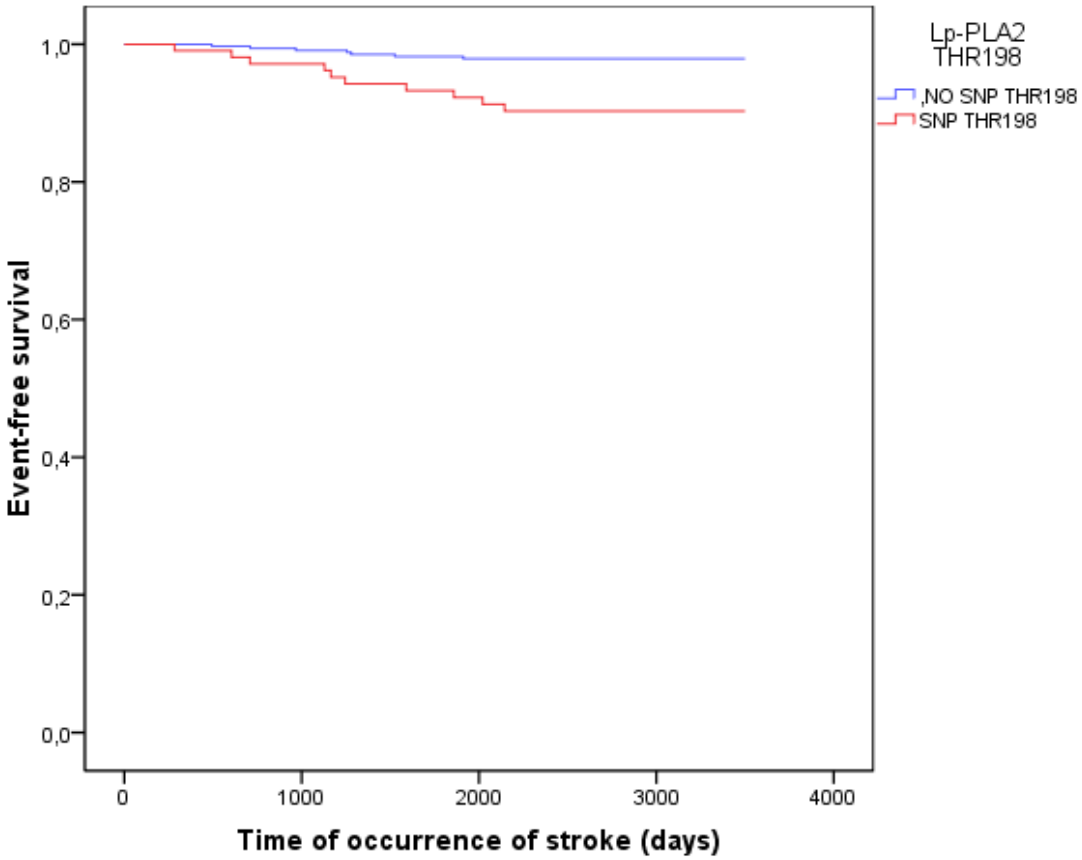

Strokes
%
50
40
30
20
10
0
CC
CT
TT
CV Events
%
50
40
30
20
10
0
CC
CT
TT
Events for Ala379Val

Fig 15 c

The patients with the influence of genotype 198THR (show in red in Fig 16-17) had a worse CV-death free survival (p = 0.018 at Kaplan-Meyer analysis) and for stroke free survival (p =<0.0001) than those patients without the cumulative power of genotype.





Further analysis was carried out in patients who had complete data for all the variables necessary to compute the propensity score for cumulative presence of 198THR genotype influence. Among these patients 115 could be matched with other 115 patients for evaluation of CV-death free survival and for stroke free survival. Kaplan-Meier analysis were not significant (data not shown)

10. Discussion

Lipoprotein-associated phospholipase A2 (Lp-PLA2), is a calcium-independent lipase belonging to the phospholipase A2 super family, which is secreted by different cell types (e.g. monocytes, macrophages and T lymphocytes) and in plasma it is associated with low-density lipoprotein (LDL) and lipoprotein (a) (Lp(a)[136,143,189] . The Lp-PLA2 fraction bound to Lp(a) has a greater activity than that associated with LDL[140]. Initially identified as the platelet-activating factor acetylhydrolase (PAF-AH) [134,135], Lp-PLA2 plays a key role in degradation of pro-inflammatory phospholipids and in the production of lysophosphatidylcholine (lysoPC) and oxidized non-esterified fatty acids (NEFA) that have pro-inflammatory properties. Hence, the function of Lp-PLA2 in the atherosclerotic process is controversial: in the degradation of pro-inflammatory phospholipids in LDL would suggest anti-atherogenic properties, while the production of pro-inflammatory species (lysoPC and NEFA) would support a pro-atherogenic role [170]. The latter is also supported by two lines of evidence Studies of Caucasian populations demonstrated a higher risk of developing cardiovascular events in the subjects with higher levels of Lp-PLA2 (mass or activity) after adjustment for several risk factors [196,198,199,201,202,217,220]. In our study we have confirmed a significant incidence of cardiovascular death in individuals with higher levels of both the mass and enzyme activity in line with the available data. Persons in the second and third and fourth quartiles for Lp-PLA2 were at higher cardiovascular risk than individuals in the lowest quartile It is important also to remember that the population was undoubtedly considered a population at high risk but the coronary angiography findings in the cohort of patients selected did not differ from those of the whole population of the GENICA study [228,229,235]. Lp-PLA2 activity and mass are associated with each other, proatherogenic lipids, and vascular risk. Lp-PLA2 activity was more strongly associated with various lipid markers than Lp-PLA2 mass was. This fact could indicate their varying distributions across lipoprotein classes, differences in measurement precision, or both.

Significant predictors of the Lp-PLA2 mass were HDL, LDL e triglycerides and in the models using Lp-PLA2 activity as the dependent variable, significant predictors were similarly gender, LDL, HDL, triglycerides and homocysteinemia likely to confirm that a central role in the development of the disease is the individual lipid profile.

It is very interesting to evaluate the association of Lp-PLA2 enzyme activity with homocysteinemia, as mentioned by Atik et. al [236] forty-two patients underwent elective carotid endarterectomy and where plaque Lp-PLA2 correlated with serum homocysteine levels, plaque macrophages, and plaque C. pneumoniae. This might suggest that also Lp-PLA2 could co-participate to induce vascular damage by promoting platelet activation, oxidative stress, endothelial dysfunction, hypercoagulability, vascular smooth muscle cell proliferation, and endoplasmic reticulum stress

On the other hand it is true that the analysis performed with the propensity score matching has somewhat reduced the role of Lp-PLA2 mass for the prediction of CV events and confirms the role of Lp-PLA2 activity in CV events-free survival and a borderline significance for AMI and ACS events.

The propensity score matching attempts to reduce the confounding effects of covariates, and so it allows differences of responses to be attributed to differences of treatments or exposures. In effect it employs a predicted probability of group membership based on observed predictors, usually obtained from logistic regression to create a counterfactual group and may be used for matching or as covariates alone or with other matching variables or covariates.

A similar population was investigated by May et. al[216] where Lipoprotein-associated phospholipase A2 was 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, myocardial infarction, or cerebrovascular accident. Winkler et. al[217] demonstrated instead how LpPLA2 activity predicts risk for 5-years cardiac mortality independently from the established risk factors and indicates the risk for cardiac death in patients with low and medium-high hsCRP concentrations. Therefore, LpPLA2 activity may provide information for the identification and management of patients at risk beyond established risk stratification strategies

The shape of relations of circulating Lp-PLA2 with ischemic stroke is less clear than it is that with coronary heart disease however, confirmation of the higher incidence of patients with high mass or high activity does not find consolation for what concerns the occurrence of cerebrovascular events in contrast with those expressed in such Atherosclerosis Risk in Communities study. ARIC[237] it was an NIH-sponsored case cohort study in four U.S. communities on 12,773 African-American and Caucasian men and women with an approximate six-year follow-up period. In these people elevated Lp-PLA2 levels conferred approximately a twofold increase in stroke risk, independent of

other risk factors bbut considering that the population remained without any doubt the essential role of hypertension and, since hypertension is a strong risk factor for stroke, therefore the high prevalence of hypertension in the US population reduces its predictive power of the study.

These differences among Lp-PLA2 and their correlation with clinical manifestations have not been clarified thus far therefore there are no robust answers to the question on whether different titers have different effects on CV events. It could also be that the available conflicting data are attributable to the study design, insufficient power, and the lack of taking into proper consideration the potentially confounding effect of an uneven distribution of most CV risk factors between patients with different levels of Lp-PLA2 titer.

In the GENICA study exhaustive information on all potentially relevant confounders and also on the coronary atherosclerotic burden have been collected using a modified Duke Index score to take into account also the left main lesions. Furthermore, these patients were clearly at high-risk as confirmed by the observation of a high rate of CV events and an 11.6% death rate at follow-up. With the power conferred by these features as well as by a long-term follow-up and a careful matching for potential confounders this study demonstrates that in a cohort of high-risk Caucasian patients referred for coronary angiography a titer of oxLDLAbs exceeding the 75^{th} percentile of the value distribution is associated with a worse CV prognosis, as compared to the patients with a lower titer. This negative prognostic effect was independent of major CV risk factors, as shown by adjustment for potential confounders in our analysis.

The strength of this conclusion is supported by the prospective design of our study and the application of a statistical analysis based on the propensity score matching, which allows to balance the groups based on the known baseline determinants of CV events at follow up.

The receiver operating characteristic (ROC) curve can be used to evaluate the effectiveness of a certain biomarker in the determination of a diseased on the Youden Index (J), another main summary statistic of the ROC curve used in the interpretation and evaluation of a biomarker, which defines the maximum potential effectiveness of a biomarker The cut-point that achieves this maximum is referred to as the optimal one because it is the cut-point that optimizes the biomarker's differentiating ability when equal weight is given to sensitivity and specificity An ideal risk marker, studied in an

ideal population might yield a consistent cut point associated with a sudden increase in cardiovascular risk.

The Lp-PLA2 levels cut point is not proposed as a treatment target, but rather as a level above which clinicians should consider a patient to be at higher risk for cardiovascular events, independently from established risk factors, high- and low-density lipoprotein cholesterol, and high-sensitivity C-reactive protein. We determined the best cut-off value in predicting CV deaths and MACE with ROC curves and we found that the Lp-PLA2 activity Youden Index was 136.146 nmol/ml/min and 130.221 nmol/ml/min respectively

By using an identical approach we therefore investigated the question on whether the plasma levels (mass) and the activity of Lp-PLA2 are under tight genetic control. We have finally completed the study simultaneously investigating the relationship between PLA2G7 genotypes and LpPLA2, angiographic CAD status and survival. We analyzed the association of three polymorphisms (Arg92His, Ile198Thr, and Ala379Val) and related haplotypes at the PLA2G7 locus with angiographic coronary artery disease (CAD), plasma LpPLA2 activity and mass and long-term survival in the same cohort of high risk patients

Only SNPs Arg92His, Ile198Thr were associated with a significant change in LpPLA2 plasma levels. The promoter variant His92 has shown to act by increasing the mass but surprisingly enzyme activity decreased, whereas 198Thr increased activity and mass mimicking a gene-dose effect involving all haplotypes. These results partially confirmed the study of Hoffmann et. al[163] where thess three SNPs were associated with a significant change in LpPLA2 plasma levels and the promoter variant 403C and His92 were decreased, whereas Val379 increased in activity, both coding variants showing a clear gene-dose effect. Partially corresponding results were obtained when they analyzed the haplotypes. Haplotype 2 (Val379) was highly significantly associated with an increase in plasma LpPLA2 activity, whereas the decrease in activity for the haplotypes 3 and 5, carrying the variants 403C and His92, was not consistently significant in both the subgroups.

As regards the determinants of Lp-PLA2 a study of nuclear families attributed 62% variances of platelet-activating factor acetylhydrolase (PAF-AH), e.g. Lp-PLA2, activity to heritability [238]. The Lp-PLA2 gene (*PLA2G7*) on chromosome 6 entails 12 exons and some functional polymorphisms (SNPs): the Val279Phe (exon 9) occurs in 31% of Japaneses and implies reduced plasma Lp-PLA2 levels[146].

Studies have associated this variant with a higher prevalence of cardiovascular disease, thus suggesting an anti-atherogenic role of Lp-PLA2 [155,156,159]; however, this mutation was never found in Caucasians[162] . By contrast, the Ala379Val variant in exon 11 that implies in a reduced affinity of the enzyme for its substrate[239], has been associated with a reduced risk of myocardial infarction in homozygous in Caucasians subjects [162,165]. For the other gene variants, the Arg92His (R92H, exon 4) and the Ile198Thr (exon 7) described in Whites [162,239], data in association with altered Lp-PLA2 activity or coronary artery disease are weak or not significant[162] Studies of monozygotic (MZ) and dizygotic (DZ) twins offers a powerful method of partitioning genetic and environmental sources of covariance of quantitative traits that are potentially relevant for cardiovascular disease [240,241].

In our cohort the patients with the influence of genotype 198THR had a worse CV-death free survival and a lower stroke free survival than those patients without the cumulative power of genotype.

Further analysis was carried out in patients who had complete data for all the variables necessary to compute the propensity score for cumulative presence of 198THR genotype influence. Among these patients 115 could be matched with other 115 patients for evaluation of CV-death free survival and for stroke free survivalNone of the analyzed PLA2G7 polymorphisms or haplotypes was significantly associated with the survival outcome, neither the neutral nor the CAD

Conclusion

Significant predictors of the Lp-PLA2 mass were HDL, LDL e triglycerides and for Lp-PLA2 activity as the dependent variable, significant predictors were similarly gender, LDL, HDL, triglycerides and homocysteinemia likely to confirm that a central role in the development of the disease is taken from the lipid profile.

Remains very interesting to evaluate the association of Lp-PLA2 enzyme activity with homocysteinemia that at present it is unclear and deserves in-depth studies

The analysis performed to correct for the imbalance of variables distribution between the patients with low and high Lp-PLA2 with the propensity score matching has somewhat reduced the role of Lp-PLA2 mass for the prediction of CV events and confirm the role of Lp-PLA2 activity in CV events-free survival and a borderline significance for AMI and ACS events.

The genetic approach has allowed us in an important way of there are at least two coding variants 92His and 198Thr that are associated with a modest change in plasma LpPLA2 activity. However, these alleles are neither associated with CAD nor with long-term survival. On the other hand, our results raise the possibility that the rare variant 92His, which is not associated with a change in activity, might be protective.

11. References

1. Rosamond W, Flegal K, Furie K, et al. Heart disease and stroke statistics--2008 update: a report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Circulation 2008;117:e25-146.

2. Lloyd-Jones D, Adams RJ, Brown TM, et al. Executive summary: heart disease and stroke statistics--2010 update: a report from the American Heart Association. Circulation 2010;121:948-54.

3. Euan A. Ashley, Josef Niebauer. Cardiology Explained. Remedica Publishing, Remedica Publishing 2003.

4. Deedwania PC, Carbajal EV. Silent myocardial ischemia. A clinical perspective. Arch Intern Med 1991;151:2373-82.

5. Roger VL, Weston SA, Killian JM, et al. Time trends in the prevalence of atherosclerosis: a population-based autopsy study. Am J Med 2001;110:267-73.

6. Gordon T, Kannel WB, Hjortland MC, McNamara PM. Menopause and coronary heart disease. The Framingham Study. Ann Intern Med 1978;89:157-61.

7. Lerner DJ, Kannel WB. Patterns of coronary heart disease morbidity and mortality in the sexes: a 26-year follow-up of the Framingham population. Am Heart J 1986;111:383-90.

8. Kannel WB. Prevalence and clinical aspects of unrecognized myocardial infarction and sudden unexpected death. Circulation 1987;75:II4-5.

9. Yusuf S, Reddy S, Ounpuu S, Anand S. Global burden of cardiovascular diseases: Part II: variations in cardiovascular disease by specific ethnic groups and geographic regions and prevention strategies. Circulation 2001;104:2855-64.

10. Yusuf S, Reddy S, Ounpuu S, Anand S. Global burden of cardiovascular diseases: part I: general considerations, the epidemiologic transition, risk factors, and impact of urbanization. Circulation 2001;104:2746-53.

11. Goyal A, Yusuf S. The burden of cardiovascular disease in the Indian subcontinent. Indian J Med Res 2006;124:235-44.

12. Critchley J, Liu J, Zhao D, Wei W, Capewell S. Explaining the increase in coronary heart disease mortality in Beijing between 1984 and 1999. Circulation 2004;110:1236-44.

13. Rodriguez T, Malvezzi M, Chatenoud L, et al. Trends in mortality from coronary heart and cerebrovascular diseases in the Americas: 1970-2000. Heart 2006;92:453-60.

14. Beaglehole R, Reddy S, Leeder SR. Poverty and human development: the global implications of cardiovascular disease. Circulation 2007;116:1871-3.

15. Aschoff L. Observations CONCERNING THE RELATIONSHIP BETWEEN CHOLESTEROL METABOLISM AND VASCULAR DISEASE. Br Med J 1932;2:1131-4.

16. Li H, Cybulsky MI, Gimbrone MA,Jr, Libby P. An atherogenic diet rapidly induces VCAM-1, a cytokine-regulatable mononuclear leukocyte adhesion molecule, in rabbit aortic endothelium. Arterioscler Thromb 1993;13:197-204.

17. Cybulsky MI, Iiyama K, Li H, et al. A major role for VCAM-1, but not ICAM-1, in early atherosclerosis. J Clin Invest 2001;107:1255-62.

18. Dong ZM, Chapman SM, Brown AA, Frenette PS, Hynes RO, Wagner DD. The combined role of P- and E-selectins in atherosclerosis. J Clin Invest 1998;102:145-52.

19. Bourdillon MC, Poston RN, Covacho C, Chignier E, Bricca G, McGregor JL. ICAM-1 deficiency reduces atherosclerotic lesions in double-knockout mice (ApoE(-/-)/ICAM-1(-/-)) fed a fat or a chow diet. Arterioscler Thromb Vasc Biol 2000;20:2630-5.

20. Gimbrone MA,Jr, Topper JN, Nagel T, Anderson KR, Garcia-Cardena G. Endothelial dysfunction, hemodynamic forces, and atherogenesis. Ann N Y Acad Sci 2000;902:230,9; discussion 239-40.

21. Kamei M, Carman CV. New observations on the trafficking and diapedesis of monocytes. Curr Opin Hematol 2010;17:43-52.

22. Hansson GK, Robertson AK, Soderberg-Naucler C. Inflammation and atherosclerosis. Annu Rev Pathol 2006;1:297-329.

23. Mestas J, Ley K. Monocyte-endothelial cell interactions in the development of atherosclerosis. Trends Cardiovasc Med 2008;18:228-32.

24. Smith JD, Trogan E, Ginsberg M, Grigaux C, Tian J, Miyata M. Decreased atherosclerosis in mice deficient in both macrophage colony-stimulating factor (op) and apolipoprotein E. Proc Natl Acad Sci U S A 1995;92:8264-8.

25. Johnson JL, Newby AC. Macrophage heterogeneity in atherosclerotic plaques. Curr Opin Lipidol 2009;20:370-8.

26. Bouhlel MA, Derudas B, Rigamonti E, et al. PPARgamma activation primes human monocytes into alternative M2 macrophages with anti-inflammatory properties. Cell Metab 2007;6:137-43.

27. Tabas I, Williams KJ, Boren J. Subendothelial lipoprotein retention as the initiating process in atherosclerosis: update and therapeutic implications. Circulation 2007;116:1832-44.

28. Steinberg D. The LDL modification hypothesis of atherogenesis: an update. J Lipid Res 2009;50 Suppl:S376-81.

29. Yui S, Sasaki T, Miyazaki A, Horiuchi S, Yamazaki M. Induction of murine macrophage growth by modified LDLs. Arterioscler Thromb 1993;13:331-7.

30. Chatterjee S, Ghosh N. Oxidized low density lipoprotein stimulates aortic smooth muscle cell proliferation. Glycobiology 1996;6:303-11.

31. Kruth HS, Jones NL, Huang W, et al. Macropinocytosis is the endocytic pathway that mediates macrophage foam cell formation with native low density lipoprotein. J Biol Chem 2005;280:2352-60.

32. Tabas I, Li Y, Brocia RW, Xu SW, Swenson TL, Williams KJ. Lipoprotein lipase and sphingomyelinase synergistically enhance the association of atherogenic lipoproteins with smooth muscle cells and extracellular matrix. A possible mechanism for low density lipoprotein and lipoprotein(a) retention and macrophage foam cell formation. J Biol Chem 1993;268:20419-32.

33. Maxfield FR, Tabas I. Role of cholesterol and lipid organization in disease. Nature 2005;438:612-21.

34. Brown MS, Goldstein JL. The SREBP pathway: regulation of cholesterol metabolism by proteolysis of a membrane-bound transcription factor. Cell 1997;89:331-40.

35. Chang TY, Chang CC, Ohgami N, Yamauchi Y. Cholesterol sensing, trafficking, and esterification. Annu Rev Cell Dev Biol 2006;22:129-57.

36. Tall AR, Yvan-Charvet L, Terasaka N, Pagler T, Wang N. HDL, ABC transporters, and cholesterol efflux: implications for the treatment of atherosclerosis. Cell Metab 2008;7:365-75.

37. Rothblat GH, Phillips MC. High-density lipoprotein heterogeneity and function in reverse cholesterol transport. Curr Opin Lipidol 2010;21:229-38.

38. Libby P, Ridker PM, Hansson GK. Progress and challenges in translating the biology of atherosclerosis. Nature 2011;473:317-25.

39. Tedgui A, Mallat Z. Cytokines in atherosclerosis: pathogenic and regulatory pathways. Physiol Rev 2006;86:515-81.

40. Kovanen PT. Mast cells: multipotent local effector cells in atherothrombosis. Immunol Rev 2007;217:105-22.

41. Grabner R, Lotzer K, Dopping S, et al. Lymphotoxin beta receptor signaling promotes tertiary lymphoid organogenesis in the aorta adventitia of aged ApoE-/- mice. J Exp Med 2009;206:233-48.

42. Virmani R, Burke AP, Kolodgie FD, Farb A. Vulnerable plaque: the pathology of unstable coronary lesions. J Interv Cardiol 2002;15:439-46.

43. Fadok VA, Bratton DL, Konowal A, Freed PW, Westcott JY, Henson PM. Macrophages that have ingested apoptotic cells in vitro inhibit proinflammatory cytokine production through autocrine/paracrine mechanisms involving TGF-beta, PGE2, and PAF. J Clin Invest 1998;101:890-8.

44. Boyle JJ, Weissberg PL, Bennett MR. Tumor necrosis factor-alpha promotes macrophage-induced vascular smooth muscle cell apoptosis by direct and autocrine mechanisms. Arterioscler Thromb Vasc Biol 2003;23:1553-8.

45. Galis ZS, Sukhova GK, Lark MW, Libby P. Increased expression of matrix metalloproteinases and matrix degrading activity in vulnerable regions of human atherosclerotic plaques. J Clin Invest 1994;94:2493-503.

46. Liu J, Sukhova GK, Sun JS, Xu WH, Libby P, Shi GP. Lysosomal cysteine proteases in atherosclerosis. Arterioscler Thromb Vasc Biol 2004;24:1359-66.

47. Tabas I. Macrophage death and defective inflammation resolution in atherosclerosis. Nat Rev Immunol 2010;10:36-46.

48. Tabas I. The role of endoplasmic reticulum stress in the progression of atherosclerosis. Circ Res 2010;107:839-50.

49. Myoishi M, Hao H, Minamino T, et al. Increased endoplasmic reticulum stress in atherosclerotic plaques associated with acute coronary syndrome. Circulation 2007;116:1226-33.

50. Timmins JM, Ozcan L, Seimon TA, et al. Calcium/calmodulin-dependent protein kinase II links ER stress with Fas and mitochondrial apoptosis pathways. J Clin Invest 2009;119:2925-41.

51. Yvan-Charvet L, Pagler TA, Seimon TA, et al. ABCA1 and ABCG1 protect against oxidative stress-induced macrophage apoptosis during efferocytosis. Circ Res 2010;106:1861-9.

52. Wilensky RL, Macphee CH. Lipoprotein-associated phospholipase A(2) and atherosclerosis. Curr Opin Lipidol 2009;20:415-20.

53. Sather S, Kenyon KD, Lefkowitz JB, et al. A soluble form of the Mer receptor tyrosine kinase inhibits macrophage clearance of apoptotic cells and platelet aggregation. Blood 2007;109:1026-33.

54. Quyyumi AA, Mulcahy D, Andrews NP, Husain S, Panza JA, Cannon RO,3rd. Coronary vascular nitric oxide activity in hypertension and hypercholesterolemia. Comparison of acetylcholine and substance P. Circulation 1997;95:104-10.

55. Miura K, Daviglus ML, Dyer AR, et al. Relationship of blood pressure to 25-year mortality due to coronary heart disease, cardiovascular diseases, and all causes in young

adult men: the Chicago Heart Association Detection Project in Industry. Arch Intern Med 2001;161:1501-8.

56. Lewington S, Clarke R, Qizilbash N, Peto R, Collins R, Prospective Studies Collaboration. Age-specific relevance of usual blood pressure to vascular mortality: a meta-analysis of individual data for one million adults in 61 prospective studies. Lancet 2002;360:1903-13.

57. Brunner H, Cockcroft JR, Deanfield J, et al. Endothelial function and dysfunction. Part II: Association with cardiovascular risk factors and diseases. A statement by the Working Group on Endothelins and Endothelial Factors of the European Society of Hypertension. J Hypertens 2005;23:233-46.

58. Schmidt AM, Yan SD, Wautier JL, Stern D. Activation of receptor for advanced glycation end products: a mechanism for chronic vascular dysfunction in diabetic vasculopathy and atherosclerosis. Circ Res 1999;84:489-97.

59. Wei M, Gibbons LW, Mitchell TL, Kampert JB, Stern MP, Blair SN. Low fasting plasma glucose level as a predictor of cardiovascular disease and all-cause mortality. Circulation 2000;101:2047-52.

60. Selvin E, Steffes MW, Zhu H, et al. Glycated hemoglobin, diabetes, and cardiovascular risk in nondiabetic adults. N Engl J Med 2010;362:800-11.

61. Poirier P, Giles TD, Bray GA, et al. Obesity and cardiovascular disease: pathophysiology, evaluation, and effect of weight loss. Arterioscler Thromb Vasc Biol 2006;26:968-76.

62. Klein S, Burke LE, Bray GA, et al. Clinical implications of obesity with specific focus on cardiovascular disease: a statement for professionals from the American Heart Association Council on Nutrition, Physical Activity, and Metabolism: endorsed by the American College of Cardiology Foundation. Circulation 2004;110:2952-67.

63. Maiolino G, Cesari M, Sticchi D, et al. Plasma adiponectin for prediction of cardiovascular events and mortality in high-risk patients. J Clin Endocrinol Metab 2008;93:3333-40.

64. McGill HC,Jr, McMahan CA, Herderick EE, et al. Obesity accelerates the progression of coronary atherosclerosis in young men. Circulation 2002;105:2712-8.

65. Smeeth L, Thomas SL, Hall AJ, Hubbard R, Farrington P, Vallance P. Risk of myocardial infarction and stroke after acute infection or vaccination. N Engl J Med 2004;351:2611-8.

66. Madjid M, Miller CC, Zarubaev VV, et al. Influenza epidemics and acute respiratory disease activity are associated with a surge in autopsy-confirmed coronary heart disease death: results from 8 years of autopsies in 34,892 subjects. Eur Heart J 2007;28:1205-10.

67. Fye WB. Introduction: The origins and implications of a growing shortage of cardiologists. J Am Coll Cardiol 2004;44:221-32.

68. Hackam DG, Anand SS. Emerging risk factors for atherosclerotic vascular disease: a critical review of the evidence. JAMA 2003;290:932-40.

69. Ridker PM, Glynn RJ, Hennekens CH. C-reactive protein adds to the predictive value of total and HDL cholesterol in determining risk of first myocardial infarction. Circulation 1998;97:2007-11.

70. Koenig W, Sund M, Frohlich M, et al. C-Reactive protein, a sensitive marker of inflammation, predicts future risk of coronary heart disease in initially healthy middle-aged men: results from the MONICA (Monitoring Trends and Determinants in Cardiovascular Disease) Augsburg Cohort Study, 1984 to 1992. Circulation 1999;99:237-42.

71. Ridker PM, Buring JE, Shih J, Matias M, Hennekens CH. Prospective study of C-reactive protein and the risk of future cardiovascular events among apparently healthy women. Circulation 1998;98:731-3.

72. Hill JM, Zalos G, Halcox JP, et al. Circulating endothelial progenitor cells, vascular function, and cardiovascular risk. N Engl J Med 2003;348:593-600.

73. Kissel CK, Lehmann R, Assmus B, et al. Selective functional exhaustion of hematopoietic progenitor cells in the bone marrow of patients with postinfarction heart failure. J Am Coll Cardiol 2007;49:2341-9.

74. Cooke JP. Asymmetrical dimethylarginine: the Uber marker? Circulation 2004;109:1813-8.

75. Thum T, Tsikas D, Stein S, et al. Suppression of endothelial progenitor cells in human coronary artery disease by the endogenous nitric oxide synthase inhibitor asymmetric dimethylarginine. J Am Coll Cardiol 2005;46:1693-701.

76. Salonen JT, Salonen R. Ultrasonographically assessed carotid morphology and the risk of coronary heart disease. Arterioscler Thromb 1991;11:1245-9.

77. Salonen R, Salonen JT. Determinants of carotid intima-media thickness: a population-based ultrasonography study in eastern Finnish men. J Intern Med 1991;229:225-31.

78. Puato M, Palatini P, Zanardo M, et al. Increase in carotid intima-media thickness in grade I hypertensive subjects: white-coat versus sustained hypertension. Hypertension 2008;51:1300-5.

79. Vlachopoulos C, Aznaouridis K, Stefanadis C. Prediction of cardiovascular events and all-cause mortality with arterial stiffness: a systematic review and meta-analysis. J Am Coll Cardiol 2010;55:1318-27.

80. Dhingra R, Sullivan LM, Fox CS, et al. Relations of serum phosphorus and calcium levels to the incidence of cardiovascular disease in the community. Arch Intern Med 2007;167:879-85.

81. Foley RN, Collins AJ, Herzog CA, Ishani A, Kalra PA. Serum phosphorus levels associate with coronary atherosclerosis in young adults. J Am Soc Nephrol 2009;20:397-404.

82. Mizuno T, Sugimoto M, Matsui H, Hamada M, Shida Y, Yoshioka A. Visual evaluation of blood coagulation during mural thrombogenesis under high shear blood flow. Thromb Res 2008;121:855-64.

83. Croce K, Libby P. Intertwining of thrombosis and inflammation in atherosclerosis. Curr Opin Hematol 2007;14:55-61.

84. Rattazzi M, Puato M, Faggin E, Bertipaglia B, Zambon A, Pauletto P. C-reactive protein and interleukin-6 in vascular disease: culprits or passive bystanders? J Hypertens 2003;21:1787-803.

85. Elhage R, Jawien J, Rudling M, et al. Reduced atherosclerosis in interleukin-18 deficient apolipoprotein E-knockout mice. Cardiovasc Res 2003;59:234-40.

86. Wang TJ, Larson MG, Levy D, et al. Plasma natriuretic peptide levels and the risk of cardiovascular events and death. N Engl J Med 2004;350:655-63.

87. Marcovina SM, Albers JJ, Scanu AM, et al. Use of a reference material proposed by the International Federation of Clinical Chemistry and Laboratory Medicine to evaluate analytical methods for the determination of plasma lipoprotein(a). Clin Chem 2000;46:1956-67.

88. Mangoni AA, Jackson SH. Homocysteine and cardiovascular disease: current evidence and future prospects. Am J Med 2002;112:556-65.

89. Samani NJ, Erdmann J, Hall AS, et al. Genomewide association analysis of coronary artery disease. N Engl J Med 2007;357:443-53.

90. Paynter NP, Chasman DI, Buring JE, Shiffman D, Cook NR, Ridker PM. Cardiovascular disease risk prediction with and without knowledge of genetic variation at chromosome 9p21.3. Ann Intern Med 2009;150:65-72.

91. Palomaki GE, Melillo S, Bradley LA. Association between 9p21 genomic markers and heart disease: a meta-analysis. JAMA 2010;303:648-56.

92. Wang Q. Molecular genetics of coronary artery disease. Curr Opin Cardiol 2005;20:182-8.

93. Pajukanta P, Cargill M, Viitanen L, et al. Two loci on chromosomes 2 and X for premature coronary heart disease identified in early- and late-settlement populations of Finland. Am J Hum Genet 2000;67:1481-93.

94. Ozaki K, Ohnishi Y, Iida A, et al. Functional SNPs in the lymphotoxin-alpha gene that are associated with susceptibility to myocardial infarction. Nat Genet 2002;32:650-4.

95. Archacki S, Wang Q. Expression profiling of cardiovascular disease. Hum Genomics 2004;1:355-70.

96. You SA, Archacki SR, Angheloiu G, et al. Proteomic approach to coronary atherosclerosis shows ferritin light chain as a significant marker: evidence consistent with iron hypothesis in atherosclerosis. Physiol Genomics 2003;13:25-30.

97. Gretarsdottir S, Sveinbjornsdottir S, Jonsson HH, et al. Localization of a susceptibility gene for common forms of stroke to 5q12. Am J Hum Genet 2002;70:593-603.

98. Bhagavatula MR, Fan C, Shen GQ, et al. Transcription factor MEF2A mutations in patients with coronary artery disease. Hum Mol Genet 2004;13:3181-8.

99. Hauser ER, Crossman DC, Granger CB, et al. A genomewide scan for early-onset coronary artery disease in 438 families: the GENECARD Study. Am J Hum Genet 2004;75:436-47.

100. Wang L, Fan C, Topol SE, Topol EJ, Wang Q. Mutation of MEF2A in an inherited disorder with features of coronary artery disease. Science 2003;302:1578-81.

101. Wang Q, Rao S, Shen GQ, et al. Premature myocardial infarction novel susceptibility locus on chromosome 1P34-36 identified by genomewide linkage analysis. Am J Hum Genet 2004;74:262-71.

102. Kudo I, Murakami M. Phospholipase A2 enzymes. Prostaglandins Other Lipid Mediat 2002;68-69:3-58.

103. Schaloske RH, Dennis EA. The phospholipase A2 superfamily and its group numbering system. Biochim Biophys Acta 2006;1761:1246-59.

104. Pratico D. Prostanoid and isoprostanoid pathways in atherogenesis. Atherosclerosis 2008;201:8-16.

105. Das UN. Can endogenous lipid molecules serve as predictors and prognostic markers of coronary heart disease? Lipids Health Dis 2008;7:19.

106. Cedars A, Jenkins CM, Mancuso DJ, Gross RW. Calcium-independent phospholipases in the heart: mediators of cellular signaling, bioenergetics, and ischemia-induced electrophysiologic dysfunction. J Cardiovasc Pharmacol 2009;53:277-89.

107. Burke JE, Dennis EA. Phospholipase A2 biochemistry. Cardiovasc Drugs Ther 2009;23:49-59.

108. Tjoelker LW, Wilder C, Eberhardt C, et al. Anti-inflammatory properties of a platelet-activating factor acetylhydrolase. Nature 1995;374:549-53.

109. Asano K, Okamoto S, Fukunaga K, et al. Cellular source(s) of platelet-activating-factor acetylhydrolase activity in plasma. Biochem Biophys Res Commun 1999;261:511-4.

110. Wei Y, Swenson L, Castro C, et al. Structure of a microbial homologue of mammalian platelet-activating factor acetylhydrolases: Streptomyces exfoliatus lipase at 1.9 A resolution. Structure 1998;6:511-9.

111. Derewenda ZS, Ho YS. PAF-acetylhydrolases. Biochim Biophys Acta 1999;1441:229-36.

112. Stafforini DM. Biology of platelet-activating factor acetylhydrolase (PAF-AH, lipoprotein associated phospholipase A2). Cardiovasc Drugs Ther 2009;23:73-83.

113. Tjoelker LW, Eberhardt C, Unger J, et al. Plasma platelet-activating factor acetylhydrolase is a secreted phospholipase A2 with a catalytic triad. J Biol Chem 1995;270:25481-7.

114. Samanta U, Bahnson BJ. Crystal structure of human plasma platelet-activating factor acetylhydrolase: structural implication to lipoprotein binding and catalysis. J Biol Chem 2008;283:31617-24.

115. Steinbrecher UP, Pritchard PH. Hydrolysis of phosphatidylcholine during LDL oxidation is mediated by platelet-activating factor acetylhydrolase. J Lipid Res 1989;30:305-15.

116. Stremler KE, Stafforini DM, Prescott SM, McIntyre TM. Human plasma platelet-activating factor acetylhydrolase. Oxidatively fragmented phospholipids as substrates. J Biol Chem 1991;266:11095-103.

117. Davis B, Koster G, Douet LJ, et al. Electrospray ionization mass spectrometry identifies substrates and products of lipoprotein-associated phospholipase A2 in oxidized human low density lipoprotein. J Biol Chem 2008;283:6428-37.

118. Stremler KE, Stafforini DM, Prescott SM, Zimmerman GA, McIntyre TM. An oxidized derivative of phosphatidylcholine is a substrate for the platelet-activating factor acetylhydrolase from human plasma. J Biol Chem 1989;264:5331-4.

119. Bui QT, Prempeh M, Wilensky RL. Atherosclerotic plaque development. Int J Biochem Cell Biol 2009;41:2109-13.

120. Liapikos TA, Antonopoulou S, Karabina SP, Tsoukatos DC, Demopoulos CA, Tselepis AD. Platelet-activating factor formation during oxidative modification of low-density lipoprotein when PAF-acetylhydrolase has been inactivated. Biochim Biophys Acta 1994;1212:353-60.

121. Dentan C, Lesnik P, Chapman MJ, Ninio E. PAF-acether-degrading acetylhydrolase in plasma LDL is inactivated by copper- and cell-mediated oxidation. Arterioscler Thromb 1994;14:353-60.

122. Ambrosio G, Oriente A, Napoli C, et al. Oxygen radicals inhibit human plasma acetylhydrolase, the enzyme that catabolizes platelet-activating factor. J Clin Invest 1994;93:2408-16.

123. Mitsios JV, Vini MP, Stengel D, Ninio E, Tselepis AD. Human platelets secrete the plasma type of platelet-activating factor acetylhydrolase primarily associated with microparticles. Arterioscler Thromb Vasc Biol 2006;26:1907-13.

124. Narahara H, Frenkel RA, Johnston JM. Secretion of platelet-activating factor acetylhydrolase following phorbol ester-stimulated differentiation of HL-60 cells. Arch Biochem Biophys 1993;301:275-81.

125. Nakajima K, Murakami M, Yanoshita R, et al. Activated mast cells release extracellular type platelet-activating factor acetylhydrolase that contributes to autocrine inactivation of platelet-activating factor. J Biol Chem 1997;272:19708-13.

126. Goudevenos J, Tselepis AD, Vini MP, et al. Platelet-associated and secreted PAF-acetylhydrolase activity in patients with stable angina: sequential changes of the enzyme activity after angioplasty. Eur J Clin Invest 2001;31:15-23.

127. Cao Y, Stafforini DM, Zimmerman GA, McIntyre TM, Prescott SM. Expression of plasma platelet-activating factor acetylhydrolase is transcriptionally regulated by mediators of inflammation. J Biol Chem 1998;273:4012-20.

128. Wu X, Zimmerman GA, Prescott SM, Stafforini DM. The p38 MAPK pathway mediates transcriptional activation of the plasma platelet-activating factor acetylhydrolase gene in macrophages stimulated with lipopolysaccharide. J Biol Chem 2004;279:36158-65.

129. 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;375:351-63.

130. Sanchez-Quesada JL, Benitez S, Perez A, et al. The inflammatory properties of electronegative low-density lipoprotein from type 1 diabetic patients are related to increased platelet-activating factor acetylhydrolase activity. Diabetologia 2005;48:2162-9.

131. Gaubatz JW, Gillard BK, Massey JB, et al. Dynamics of dense electronegative low density lipoproteins and their preferential association with lipoprotein phospholipase A(2). J Lipid Res 2007;48:348-57.

132. Bancells C, Benitez S, Villegas S, Jorba O, Ordonez-Llanos J, Sanchez-Quesada JL. Novel phospholipolytic activities associated with electronegative low-density lipoprotein are involved in increased self-aggregation. Biochemistry 2008;47:8186-94.

133. McCall MR, La Belle M, Forte TM, Krauss RM, Takanami Y, Tribble DL. Dissociable and nondissociable forms of platelet-activating factor acetylhydrolase in

human plasma LDL: implications for LDL oxidative susceptibility. Biochim Biophys Acta 1999;1437:23-36.

134. Stafforini DM, Prescott SM, McIntyre TM. Human plasma platelet-activating factor acetylhydrolase. Purification and properties. J Biol Chem 1987;262:4223-30.

135. Stafforini DM, McIntyre TM, Carter ME, Prescott SM. Human plasma platelet-activating factor acetylhydrolase. Association with lipoprotein particles and role in the degradation of platelet-activating factor. J Biol Chem 1987;262:4215-22.

136. Stafforini DM, Tjoelker LW, McCormick SP, et al. Molecular basis of the interaction between plasma platelet-activating factor acetylhydrolase and low density lipoprotein. J Biol Chem 1999;274:7018-24.

137. Gardner AA, Reichert EC, Topham MK, Stafforini DM. Identification of a domain that mediates association of platelet-activating factor acetylhydrolase with high density lipoprotein. J Biol Chem 2008;283:17099-106.

138. Tselepis AD, John Chapman M. Inflammation, bioactive lipids and atherosclerosis: potential roles of a lipoprotein-associated phospholipase A2, platelet activating factor-acetylhydrolase. Atheroscler Suppl 2002;3:57-68.

139. Gazi I, Lourida ES, Filippatos T, Tsimihodimos V, Elisaf M, Tselepis AD. Lipoprotein-associated phospholipase A2 activity is a marker of small, dense LDL particles in human plasma. Clin Chem 2005;51:2264-73.

140. Blencowe C, Hermetter A, Kostner GM, Deigner HP. Enhanced association of platelet-activating factor acetylhydrolase with lipoprotein (a) in comparison with low density lipoprotein. J Biol Chem 1995;270:31151-7.

141. Tsimikas S, Brilakis ES, Miller ER, et al. Oxidized phospholipids, Lp(a) lipoprotein, and coronary artery disease. N Engl J Med 2005;353:46-57.

142. Edelstein C, Pfaffinger D, Hinman J, et al. Lysine-phosphatidylcholine adducts in kringle V impart unique immunological and potential pro-inflammatory properties to human apolipoprotein(a). J Biol Chem 2003;278:52841-7.

143. Kiechl S, Willeit J, Mayr M, et al. Oxidized phospholipids, lipoprotein(a), lipoprotein-associated phospholipase A2 activity, and 10-year cardiovascular outcomes: prospective results from the Bruneck study. Arterioscler Thromb Vasc Biol 2007;27:1788-95.

144. Sutton BS, Crosslin DR, Shah SH, et al. Comprehensive genetic analysis of the platelet activating factor acetylhydrolase (PLA2G7) gene and cardiovascular disease in case-control and family datasets. Hum Mol Genet 2008;17:1318-28.

145. Miwa M, Miyake T, Yamanaka T, et al. Characterization of serum platelet-activating factor (PAF) acetylhydrolase. Correlation between deficiency of serum PAF acetylhydrolase and respiratory symptoms in asthmatic children. J Clin Invest 1988;82:1983-91.

146. Stafforini DM, Satoh K, Atkinson DL, et al. Platelet-activating factor acetylhydrolase deficiency. A missense mutation near the active site of an anti-inflammatory phospholipase. J Clin Invest 1996;97:2784-91.

147. 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;91:3521-7.

148. 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;4:1023-8.

149. Balta G, Gurgey A, Kudayarov DK, Tunc B, Altay C. Evidence for the existence of the PAF acetylhydrolase mutation (Val279Phe) in non-Japanese populations: a preliminary study in Turkey, Azerbaijan, and Kyrgyzstan. Thromb Res 2001;101:231-4.

150. Ishihara M, Iwasaki T, Nagano M, et al. Functional impairment of two novel mutations detected in lipoprotein-associated phospholipase A2 (Lp-PLA2) deficiency patients. J Hum Genet 2004;49:302-7.

151. Yamada Y, Yokota M. Loss of activity of plasma platelet-activating factor acetylhydrolase due to a novel Gln281-->Arg mutation. Biochem Biophys Res Commun 1997;236:772-5.

152. Yamada Y, Yoshida H, Ichihara S, Imaizumi T, Satoh K, Yokota M. Correlations between plasma platelet-activating factor acetylhydrolase (PAF-AH) activity and PAF-AH genotype, age, and atherosclerosis in a Japanese population. Atherosclerosis 2000;150:209-16.

153. Unno N, Sakaguchi T, Nakamura T, et al. A single nucleotide polymorphism in the plasma PAF acetylhydrolase gene and risk of atherosclerosis in Japanese patients with peripheral artery occlusive disease. J Surg Res 2006;134:36-43.

154. Yamamoto I, Fujitsu J, Nohnen S, et al. Association of plasma PAF acetylhydrolase gene polymorphism with IMT of carotid arteries in Japanese type 2 diabetic patients. Diabetes Res Clin Pract 2003;59:219-24.

155. 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;47:177-81.

156. Hiramoto M, Yoshida H, Imaizumi T, Yoshimizu N, Satoh K. A mutation in plasma platelet-activating factor acetylhydrolase (Val279-->Phe) is a genetic risk factor for stroke. Stroke 1997;28:2417-20.

157. Ichihara S, Yamada Y, Yokota M. Association of a G994-->T missense mutation in the plasma platelet-activating factor acetylhydrolase gene with genetic susceptibility to nonfamilial dilated cardiomyopathy in Japanese. Circulation 1998;98:1881-5.

158. Unno N, Nakamura T, Kaneko H, et al. Plasma platelet-activating factor acetylhydrolase deficiency is associated with atherosclerotic occlusive disease in japan. J Vasc Surg 2000;32:263-7.

159. Unno N, Nakamura T, Mitsuoka H, et al. Association of a G994 -->T missense mutation in the plasma platelet-activating factor acetylhydrolase gene with risk of abdominal aortic aneurysm in Japanese. Ann Surg 2002;235:297-302.

160. Jang Y, Waterworth D, Lee JE, et al. Carriage of the V279F null allele within the gene encoding Lp-PLA is protective from coronary artery disease in South Korean males. PLoS One 2011;6:e18208.

161. Wootton PT, Stephens JW, Hurel SJ, et al. Lp-PLA2 activity and PLA2G7 A379V genotype in patients with diabetes mellitus. Atherosclerosis 2006;189:149-56.

162. 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;13:1341-51.

163. 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;7:41-8.

164. 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;121:2284-93.

165. Abuzeid AM, Hawe E, Humphries SE, Talmud PJ, HIFMECH Study Group. 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;168:283-8.

166. Suchindran S, Rivedal D, Guyton JR, et al. Genome-wide association study of Lp-PLA(2) activity and mass in the Framingham Heart Study. PLoS Genet 2010;6:e1000928.

167. 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;75:605-11.

168. Grallert H, Dupuis J, Bis JC, et al. Eight genetic loci associated with variation in lipoprotein-associated phospholipase A2 mass and activity and coronary heart disease:

meta-analysis of genome-wide association studies from five community-based studies. Eur Heart J 2012;33:238-51.

169. 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;30:107-15.

170. Zalewski A, Macphee C. Role of lipoprotein-associated phospholipase A2 in atherosclerosis: biology, epidemiology, and possible therapeutic target. Arterioscler Thromb Vasc Biol 2005;25:923-31.

171. 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;23:829-35.

172. Quarck R, De Geest B, Stengel D, et al. Adenovirus-mediated gene transfer of human platelet-activating factor-acetylhydrolase prevents injury-induced neointima formation and reduces spontaneous atherosclerosis in apolipoprotein E-deficient mice. Circulation 2001;103:2495-500.

173. Hase M, Tanaka M, Yokota M, Yamada Y. Reduction in the extent of atherosclerosis in apolipoprotein E-deficient mice induced by electroporation-mediated transfer of the human plasma platelet-activating factor acetylhydrolase gene into skeletal muscle. Prostaglandins Other Lipid Mediat 2002;70:107-18.

174. Theilmeier G, De Geest B, Van Veldhoven PP, et al. HDL-associated PAF-AH reduces endothelial adhesiveness in apoE-/- mice. FASEB J 2000;14:2032-9.

175. 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;19:2909-17.

176. 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;115:2715-21.

177. 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;26:2523-9.

178. 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;82:159-65.

179. Kume N, Cybulsky MI, Gimbrone MA,Jr. Lysophosphatidylcholine, a component of atherogenic lipoproteins, induces mononuclear leukocyte adhesion molecules in cultured human and rabbit arterial endothelial cells. J Clin Invest 1992;90:1138-44.

180. Wong JT, Tran K, Pierce GN, Chan AC, O K, Choy PC. Lysophosphatidylcholine stimulates the release of arachidonic acid in human endothelial cells. J Biol Chem 1998;273:6830-6.

181. Rikitake Y, Kawashima S, Yamashita T, et al. Lysophosphatidylcholine inhibits endothelial cell migration and proliferation via inhibition of the extracellular signal-regulated kinase pathway. Arterioscler Thromb Vasc Biol 2000;20:1006-12.

182. Rong JX, Berman JW, Taubman MB, Fisher EA. Lysophosphatidylcholine stimulates monocyte chemoattractant protein-1 gene expression in rat aortic smooth muscle cells. Arterioscler Thromb Vasc Biol 2002;22:1617-23.

183. Yamakawa T, Tanaka S, Yamakawa Y, et al. Lysophosphatidylcholine activates extracellular signal-regulated kinases 1/2 through reactive oxygen species in rat vascular smooth muscle cells. Arterioscler Thromb Vasc Biol 2002;22:752-8.

184. Chai YC, Howe PH, DiCorleto PE, Chisolm GM. Oxidized low density lipoprotein and lysophosphatidylcholine stimulate cell cycle entry in vascular smooth muscle cells. Evidence for release of fibroblast growth factor-2. J Biol Chem 1996;271:17791-7.

185. Kohno M, Yokokawa K, Yasunari K, et al. Induction by lysophosphatidylcholine, a major phospholipid component of atherogenic lipoproteins, of human coronary artery smooth muscle cell migration. Circulation 1998;98:353-9.

186. Chang MY, Tsoi C, Wight TN, Chait A. Lysophosphatidylcholine regulates synthesis of biglycan and the proteoglycan form of macrophage colony stimulating factor. Arterioscler Thromb Vasc Biol 2003;23:809-15.

187. Oestvang J, Anthonsen MW, Johansen B. Role of secretory and cytosolic phospholipase A(2) enzymes in lysophosphatidylcholine-stimulated monocyte arachidonic acid release. FEBS Lett 2003;555:257-62.

188. Carpenter KL, Dennis IF, Challis IR, et al. Inhibition of lipoprotein-associated phospholipase A2 diminishes the death-inducing effects of oxidised LDL on human monocyte-macrophages. FEBS Lett 2001;505:357-63.

189. Macphee CH, Nelson J, Zalewski A. Role of lipoprotein-associated phospholipase A2 in atherosclerosis and its potential as a therapeutic target. Curr Opin Pharmacol 2006;6:154-61.

190. 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.

191. Glass CK, Witztum JL. Atherosclerosis. the road ahead. Cell 2001;104:503-16.

192. Safaya R, Chai H, Kougias P, et al. Effect of lysophosphatidylcholine on vasomotor functions of porcine coronary arteries. J Surg Res 2005;126:182-8.

193. Inoue N, Takeshita S, Gao D, et al. Lysophosphatidylcholine increases the secretion of matrix metalloproteinase 2 through the activation of NADH/NADPH oxidase in cultured aortic endothelial cells. Atherosclerosis 2001;155:45-52.

194. Takahashi M, Okazaki H, Ogata Y, Takeuchi K, Ikeda U, Shimada K. Lysophosphatidylcholine induces apoptosis in human endothelial cells through a p38-mitogen-activated protein kinase-dependent mechanism. Atherosclerosis 2002;161:387-94.

195. Shi Y, Zhang P, Zhang L, et al. Role of lipoprotein-associated phospholipase A2 in leukocyte activation and inflammatory responses. Atherosclerosis 2007;191:54-62.

196. 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;343:1148-55.

197. Anderson JL. Lipoprotein-associated phospholipase A2: an independent predictor of coronary artery disease events in primary and secondary prevention. Am J Cardiol 2008;101:23F-33F.

198. 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;38:1302-6.

199. 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;109:837-42.

200. Rana JS, Arsenault BJ, Despres JP, et al. Inflammatory biomarkers, physical activity, waist circumference, and risk of future coronary heart disease in healthy men and women. Eur Heart J 2011;32:336-44.

201. 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;110:1903-8.

202. Oei HH, van der Meer IM, 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;111:570-5.

203. 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;27:1411-6.

204. 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;51:913-9.

205. Caslake MJ, Packard CJ, Robertson M, et al. Lipoprotein-associated phospholipase A(2), inflammatory biomarkers, and risk of cardiovascular disease in the Prospective Study of Pravastatin in the Elderly at Risk (PROSPER). Atherosclerosis 2010;210:28-34.

206. 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;209:528-32.

207. Hatoum IJ, Hu FB, Nelson JJ, Rimm EB. Lipoprotein-associated phospholipase A2 activity and incident coronary heart disease among men and women with type 2 diabetes. Diabetes 2010;59:1239-43.

208. Hatoum IJ, Cook NR, Nelson JJ, Rexrode KM, Rimm EB. Lipoprotein-associated phospholipase A2 activity improves risk discrimination of incident coronary heart disease among women. Am Heart J 2011;161:516-22.

209. Allison MA, Denenberg JO, Nelson JJ, Natarajan L, Criqui MH. The association between lipoprotein-associated phospholipase A2 and cardiovascular disease and total mortality in vascular medicine patients. J Vasc Surg 2007;46:500-6.

210. 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;166:2073-80.

211. Raichlin E, McConnell JP, Bae JH, Kremers WK, Lerman A, Frantz RP. Lipoprotein-associated phospholipase A2 predicts progression of cardiac allograft vasculopathy and increased risk of cardiovascular events in heart transplant patients. Transplantation 2008;85:963-8.

212. Gerber Y, Dunlay SM, Jaffe AS, et al. Plasma lipoprotein-associated phospholipase A2 levels in heart failure: association with mortality in the community. Atherosclerosis 2009;203:593-8.

213. 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;26:137-44.

214. Blackie JA, Bloomer JC, Brown MJ, et al. The identification of clinical candidate SB-480848: a potent inhibitor of lipoprotein-associated phospholipase A2. Bioorg Med Chem Lett 2003;13:1067-70.

215. 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;118:1172-82.

216. 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;152:997-1003.

217. 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;53:1440-7.

218. Koenig W, Twardella D, Brenner H, Rothenbacher D. Lipoprotein-associated phospholipase A2 predicts future cardiovascular events in patients with coronary heart disease independently of traditional risk factors, markers of inflammation, renal function, and hemodynamic stress. Arterioscler Thromb Vasc Biol 2006;26:1586-93.

219. 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;28:1172-8.

220. 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;27:2463-9.

221. Corsetti JP, Rainwater DL, Moss AJ, Zareba W, Sparks CE. High lipoprotein-associated phospholipase A2 is a risk factor for recurrent coronary events in postinfarction patients. Clin Chem 2006;52:1331-8.

222. Gerber Y, McConnell JP, Jaffe AS, Weston SA, Killian JM, Roger VL. Lipoprotein-associated phospholipase A2 and prognosis after myocardial infarction in the community. Arterioscler Thromb Vasc Biol 2006;26:2517-22.

223. O'Donoghue M, Morrow DA, Sabatine MS, et al. Lipoprotein-associated phospholipase A2 and its association with cardiovascular outcomes in patients with acute coronary syndromes in the PROVE IT-TIMI 22 (PRavastatin Or atorVastatin Evaluation and Infection Therapy-Thrombolysis In Myocardial Infarction) trial. Circulation 2006;113:1745-52.

224. Persson M, Berglund G, Nelson JJ, Hedblad B. Lp-PLA2 activity and mass are associated with increased incidence of ischemic stroke: a population-based cohort study from Malmo, Sweden. Atherosclerosis 2008;200:191-8.

225. Lp-PLA(2) Studies Collaboration, Thompson A, Gao P, et al. Lipoprotein-associated phospholipase A(2) and risk of coronary disease, stroke, and mortality: collaborative analysis of 32 prospective studies. Lancet 2010;375:1536-44.

226. 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;160:655-61.

227. 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;162:613,619.e1.

228. Cesari M, Maiolino G, Colonna S, et al. Under treatment with lipid-lowering drugs of high-risk coronary heart disease patients of the GENICA study. J Cardiovasc Pharmacol 2003;42:484-90.

229. Rossi GP, Cesari M, Zanchetta M, et al. The T-786C endothelial nitric oxide synthase genotype is a novel risk factor for coronary artery disease in Caucasian patients of the GENICA study. J Am Coll Cardiol 2003;41:930-7.

230. Staessen JA, Birkenhager WH, Fagard R. Implications of the Systolic Hypertension in Europe (Syst-Eur) Trial for clinical practice. Nephrol Dial Transplant 1997;12:2220-2.

231. Puavilai G, Chanprasertyotin S, Sriphrapradaeng A. Diagnostic criteria for diabetes mellitus and other categories of glucose intolerance: 1997 criteria by the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus (ADA), 1998 WHO consultation criteria, and 1985 WHO criteria. World Health Organization. Diabetes Res Clin Pract 1999;44:21-6.

232. Cesari M, Zanchetta M, Burlina A, et al. Hyperhomocysteinemia is inversely related with left ventricular ejection fraction and predicts cardiovascular mortality in high-risk coronary artery disease hypertensives. Arterioscler Thromb Vasc Biol 2005;25:115-21.

233. Califf RM, Armstrong PW, Carver JR, D'Agostino RB, Strauss WE. 27th Bethesda Conference: matching the intensity of risk factor management with the hazard for

coronary disease events. Task Force 5. Stratification of patients into high, medium and low risk subgroups for purposes of risk factor management. J Am Coll Cardiol 1996;27:1007-19.

234. Austin PC. Some methods of propensity-score matching had superior performance to others: results of an empirical investigation and Monte Carlo simulations. Biom J 2009;51:171-84.

235. Rossi GP, Maiolino G, Zanchetta M, et al. The T(-786)C endothelial nitric oxide synthase genotype predicts cardiovascular mortality in high-risk patients. J Am Coll Cardiol 2006;48:1166-74.

236. Atik B, Johnston SC, Dean D. Association of carotid plaque Lp-PLA(2) with macrophages and Chlamydia pneumoniae infection among patients at risk for stroke. PLoS One 2010;5:e11026.

237. Ballantyne CM, Hoogeveen RC, Bang H, et al. Lipoprotein-associated phospholipase A2, high-sensitivity C-reactive protein, and risk for incident ischemic stroke in middle-aged men and women in the Atherosclerosis Risk in Communities (ARIC) study. Arch Intern Med 2005;165:2479-84.

238. Guerra R, Zhao B, Mooser V, Stafforini D, Johnston JM, Cohen JC. Determinants of plasma platelet-activating factor acetylhydrolase: heritability and relationship to plasma lipoproteins. J Lipid Res 1997;38:2281-8.

239. Kruse S, Mao XQ, Heinzmann A, et al. The Ile198Thr and Ala379Val variants of plasmatic PAF-acetylhydrolase impair catalytical activities and are associated with atopy and asthma. Am J Hum Genet 2000;66:1522-30.

240. Cesari M, Narkiewicz K, De Toni R, Aldighieri E, Williams CJ, Rossi GP. Heritability of plasma adiponectin levels and body mass index in twins. J Clin Endocrinol Metab 2007;92:3082-8.

241. Lenzini L, Antezza K, Caroccia B, et al. A twin study of heritability of plasma lipoprotein-associated phospholipase A2 (Lp-PLA2) mass and activity. Atherosclerosis 2009;205:181-5.