[Frontiers in Bioscience S2, 866-875, June 1, 2010]

The physiopathology of lipoprotein (a)

Fabio Galvano[1], Michele Malaguarnera[1], Marco Vacante[2], Massimo Motta[2], Cristina Russo[2], Giulia Malaguarnera[3] Nicolantonio D'Orazio[4], Lucia Malaguarnera[3]

[1]Department of Biological Chemistry, Medical Chemistry and Molecular Biology, University of Catania, [2]Department of Senescence, Urological and Neurological Sciences, University of Catania, Italy, [3]Department of Biomedical Sciences, University of Catania, Italy, [4]Department of Biomedical Sciences, University of Chieti, Italy

TABLE OF CONTENTS

1. ABSTRACT

Lipoprotein(a) (also called Lp(a)) is a lipoprotein subclass. Different studies have identified Lp(a) as a putative risk factor for atherosclerotic diseases such as coronary artery disease and stroke. The physiological role of Lp(a) in humans is still unclear, but it seems that individuals with low concentrations of plasma Lp(a) manifest no deficiency syndrome or disease. Because of the high homology between plasminogen and apo(a) it is conceivable that Lp(a) plays a role in the coagulation system, especially in to thrombosis and impaired fibrinolysis processes. It can also contribute to coronary disease and can accumulate in the arterial walls and cerebral vessels. Lp(a) seems to play an active role in acute inflammation promoting the enhancement of intercellular adhesion molecules; that way it can contribute to develop atherosclerosis. Finally, we underline the relationship among Lp(a) levels and others inflammations molecules such as fibrinogen, fibronectin and TGF- β.

2. INTRODUCTION

Lipoprotein(a) (Lp(a)) was described for the first time about forty years ago by Berg (1) but still now little is known about it. The physiological role of Lp(a) is still unclear; nevertheless numerous epidemiological studies have identified elevated plasma Lp(a) concentrations as a risk factor for the development of atherosclerotic disorders, including coronary heart disease. Lp(a) is composed of a low-density lipoprotein (LDL)-like particle linked, by a single disulfide bridge, to a large, highly glycosylated apolipoprotein(a) (apo(a)); besides Lp(a) particles contain apo(a) and apo B in a 1:1 molar ratio. Apo B-100 is the major apolipoprotein component of the atherogenic lipoproteins (VLDL, LDL, IDL). Although epidemiological data relating apo B concentrations to CHD are limited, some case-control studies in patients with coronary heart disease (CHD) have found plasma apo B levels to be more discriminating than other plasma lipids and

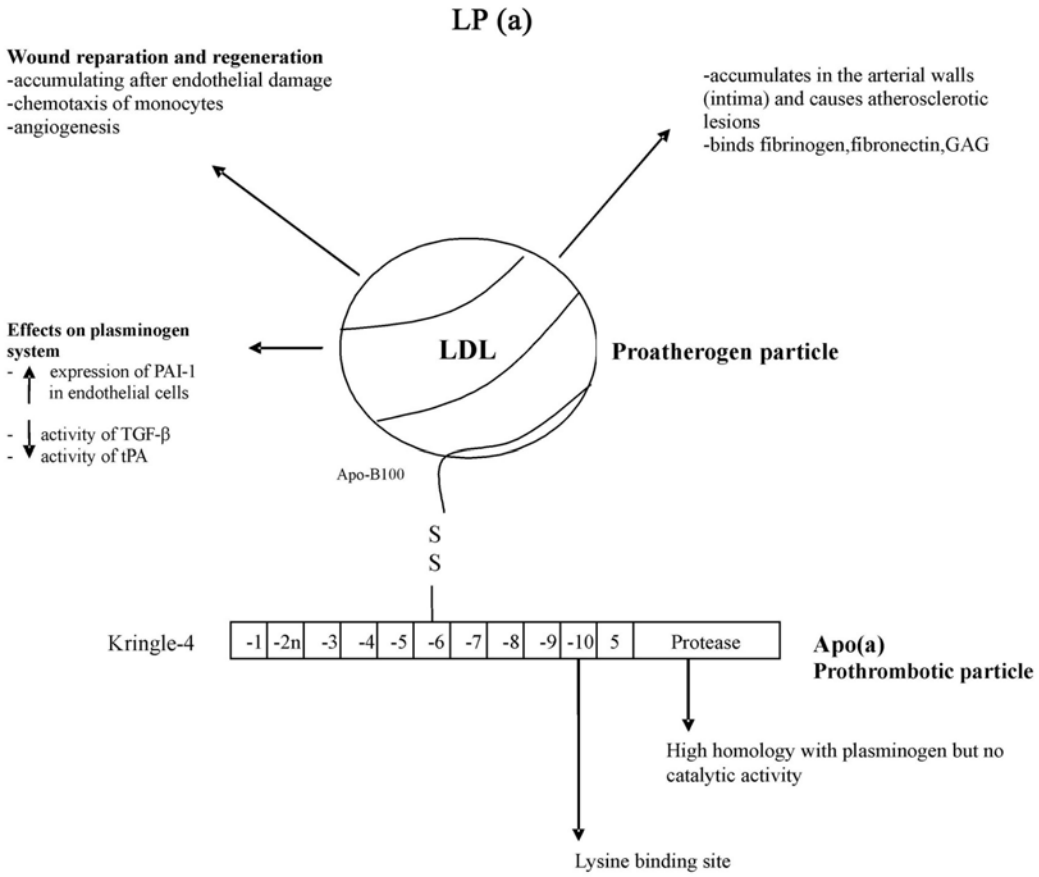

Figure 1. Lipoprotein(a). PAI-1= plasminogen activator inhibitor-1; TGF-β = transforming growth factor; tPA= tissuetype plasminogen activator.

lipoproteins (2). The special structure of Lp(a) makes difficult to understand the role of the single component in the development of cardiovascular diseases (CVD) and the right treatment to use to normalize the molecular levels. However, Lp(a) is a recognized independent risk factor for atherosclerotic CVD, although the mechanisms by which it contributes to IMA, stroke and CVD remain to be established. Early studies shown that Lp(a) plays a potential role in several steps of the fibrinolytic pathway leading to thrombogenesis. Over the last years, new biological functions have been increasingly assigned to Lp(a), including activation of various cell types that play important roles in atherogenesis (2). On the contrary few studies assessed the physiology and physiopathology of this molecule. For this reason the aim of this work is to focus on the physiological and pathophysiological role of Lp(a).

3. STRUCTURE, ISOFORMS, SERUM LEVELS AND GENETIC OF Lp(a)

Lipoprotein(a) was first described in 1963 by Berg in an electrophoretic band of pre-beta lipoprotein (1). Apoprotein(a) [Apo(a)] is linked to apolipoprotein B100 (ApoB100) by a single disulphide bridge in both humans and human-apo(a)/apoB transgenic mice (3-4-5-6-7) (Figure 1).

Biophysical studies of Lp(a) revealed several aspects of its structure. Lp(a) particle examined by electron microscopy appeared to be roughly circular, with a diameter of 25-30 nanometres and molecular weight ranging between 280,000 and 700,000 daltons.

In 1986 Fless and coll. made a chemical reduction of human plasma lipoprotein (a) by zonal ultracentrifugation (3). Apolipoprotein(a) was completely recovered from the bottom of the gradient, whereas lipoprotein(a-) (Lp(a-)), which contained all of the lipids and apo-B100 of Lp(a), floated. Further investigation by the techniques of circular dichroism and viscometry revealed that Lp(a-) was identical to low density lipoprotein (LDL). Lp(a-) was slightly larger in mass than autologous LDL and contained proportionally more triglycerides. The different mass between Lp(a) and Lp(a-) was accounted for by the loss of 2 molecules of apo(a) from the Lp(a) particle. By circular dichroism the structure of apo(a) was mostly random (71%) with the remainder representing 8% alpha-helix and 21% beta-sheet.

The amino acid composition was characterized by an unusually high content of proline (11.4 mol %) as well as tryptophan, tyrosine, arginine, threonine, and a low amount of lysine, phenylalanine, and isoleucine. Apo(a)

contained 28.1% carbohydrate by weight represented by mannose, galactose, galactosamine, glucosamine, and sialic acid. Overall, the structure of Lp(a) appears to be consistent with a rigid spherical LDL-like core particle which, as a consequence of its association with a flexible glycoprotein such as apo(a). Furthermore, the Lp(a-) remnant generated by the removal of apo(a) from Lp(a) was similar in structure but not identical to autologous LDL (3-8-9).

The LDL moiety of Lp(a) is similar to the plasma-derived LDL with reference to the lipid composition and the presence of apolipoprotein B-100 (apoB-100).

As such, the apo(a) component likely confers the unique structural and functional properties attributed to Lp(a). Apo(a) contains tandem repeats of a sequence that closely resembles plasminogen kringle IV ..similarity), followed by sequences that are highly homologous to the kringle V and protease domains of plasminogen.

Apo(a) contains 10 distinct subclasses of plasminogen kringle IV-like domains (KIV1−KIV10). Apo(a) KIV types 1 and 3−10 are present as single copies, whereas the kringle IV type 2 domain (KIV2) is present in a variable number of identically repeated copies and is the molecular basis for the observed isoform size heterogeneity of Lp(a) (10).

The apo(a) gene is a member of the plasminogen superfamily of evolved genes and is located on chromosome 6. It has been described in primates and the hedge-hog (11).

The discovery that the LPA gene has evolved from the plasminogen gene lead to hypothesize that LPA genes could influence components or processes related to thrombogenesis or thrombolysis in different ways.

Berg showed the interrelationship between fibrinogen levels and Lp(a) levels (12); LPA genes appear to affect levels as well as variability of fibrinogen; there could be “level gene” as well as “variability gene” effects on fibrinogen (12).

The effect of LPA genes on absolute fibrinogen level may be more pronounced at higher age than at young age. The same may be true respect to the “variability gene” effect of LPA genes and this effect may also be stronger in women than in men (12).

Apo(a) proteins vary in size due to a polymorphism caused by a variable number of so called kringle IV repeats in the LPA gene(each of the variable kringle IV consists of 114 amino acids). These variable apo(a) sizes are known as "apo(a) isoforms".

Originally were discovered six different isoforms (F, B, S1, S2, S3 and S4 according to different electrophoretic motilities) that vary in size from 300 to 800 kDa (13-14). More recently, Guerra *et al* described a seventh isoform category, S5 (15). Therefore improved techniques at higher resolution revealed >20 protein isoforms. These different isoforms can be grouped into low (LMW) and high molecular weight (HMW) isoforms according to the number of kringle IV repeats in the apo (a) molecules. In healthy subjects Lp(a) levels are mainly dependent on the isoform type, with those with LMW isoforms having high levels and those with HMW isoforms having lower levels (15).

There is a general inverse correlation between the size of the apo(a)isoform and the Lp(a) plasma concentration which is caused by a variable rate of degradation before the apo(a) protein has matured for Lp(a) assembly. Apo(a) is expressed by the liver cells (hepatocytes) and the assembly of apo(a) and LDL particles seems to take place at the outer hepatocyte surface. The half-life of Lp(a) in the circulation is about 3 to 4 days.

The values for Lp(a) serum levels are between 0.1 and 300 mg/dl (16). Lp (a) levels do not seem to be significantly affected by age or sex, diabetes, dietary cholesterol or HMG-CoA-reductase inhibitors, but studies are controversial (25).

The mechanism by which the apo(a) polymorphism determines Lp(a) concentrations in plasma is unknown.

Lp(a) particles can suffer oxidative modification and scavenger receptor uptake, with cholesterol accumulation and foam cell formation, thus leading to atherogenesis. (Box 2)

Oxidation of LDL and Lp(a) affects the catabolism of the lipoproteins, including changes in receptor recognition, catabolic rate, retention in the vessel wall, and propensity to accelerate atherosclerosis; oxidative modification of apo(a) may have an influence on Lprecognition by scavenger receptors of macrophages. Some studies show that Lp(a) particles are prone to oxidation and that the increased risk of coronary artery disease associated with elevated Lp(a) levels may be related in part to their oxidative modification and uptake by macrophages, resulting in the formation of macrophage-derived foam cells. Additionally, non enzymatic glycation of lipoprotein may contribute to the premature atherogenesis.

It has been observed that the proportion of plasma lipoprotein(a) Lp(a) in glycated form is significantly higher in diabetic patients rather than in non-diabetic controls.

Glycation does not appear significantly related to the atherogenic potential respect of unmodified Lp(a) (17-18).

Previous studies have demonstrated that oxidized phospholipids (OxPL) can be directly bound to apo(a) forming covalent bonds with the active lysine of its kringle-V domain, although it is possible that there may be additional or alternative binding sites for OxPL on other kringles of apo(a). Furthermore, others data suggest that additional OxPLs are present in the lipid phase of Lp(a) . These results may be influenced by both the heterogeneity of Lp(a).

The biological effects of Lp(a) have been attributed either to apo(a) or to constituents of its LDL-like particle. Among the components of the LDL portion of Lp(a) is the enzyme platelet-activating factor acetylhydrolase. Platelet-activating factor acetylhydrolase primarily exhibits a Ca2+-independent phospholipase A2 activity and is complexed to lipoproteins in plasma; thus, it is also referred to as lipoprotein-associated phospholipase A2 (Lp-PLA2). Substrates for Lp-PLA2 are the proinflammatory phospholipids platelet activating factor, as well as phospholipids containing oxidative fragmented residues (oxidized phospholipids; OxPLs). Such phospholipids are thought to play key roles in inflammatory reactions and particularly in vascular inflammation and atherosclerosis (19-20).

4. METABOLISM OF Lp(a)

Many evidences suggest that Lp(a) assembly occurs in circulation after secretion of apo(a) from the liver and metabolism of VLDL to LDL into circulation. Apo(a) is biosynthesized in liver cells and the size of the isoform determines its rate of synthesis and excretion. It is generally accepted that the assembly of LDL and apo(a) into the Lp(a) particle is a two-step process in which a noncovalent interaction between apo(a) and apoB-100 precedes disulfide bond formation between a free cysteine residue (Cys4507) within the KIV9 module of apo(a) and a cysteine residue of apoB-100, whose identity remains to be definitively identified. Biochemical and biophysical studies have implicated Cys3734 of apoB-100 in the covalent step, while more recent site-directed mutagenesis studies suggested that Cys4326 in apoB-100 was required for Lp(a) formation (8).

Specific kringle-4 domains in apo(a), mainly T-6 and T-7, bind in a first step to circulating LDL, followed by the stabilization of the newly formed Lp(a) complex. Circulating Lp(a) interacts specifically with kidney cells, or possibly other tissues, causing cleavage of 2/3-3/4 of the N-terminal part of apo(a) by a collagenase-type protease. Part of the apo(a) fragments is found in the urine, but there are indications that they represent the biologically active form of apo(a). The core portion of Lp(a) is, in turn, cleared by the LDL-receptor or by another liver's specific binding system. Strategies for reducing plasma Lp(a) levels with medication should aim, on one hand, at interfering with the assembly of Lp(a) and, on the other hand, to stimulate apo(a) fragmentation.

The mechanism and sites of Lp(a) catabolism are largely unknown. Uptake via the LDL receptor is not a major pathway of Lp(a) metabolism like several studies demonstrated (21-22) .

5. Lp(a) IN POPULATION

Lp(a) concentrations vary over one thousand fold between individuals, from < 0.2 to > 200 mg/dL. Such a wide range of concentrations is observed in all populations studied so far. The mean and median concentrations between different world populations show distinct particularities, the main being the two- to threefold higher Lp(a) plasma concentration of populations of African descent compared to Asian, Oceanic, or European populations (22).The general inverse correlation between apo(a) isoform size and Lp(a)plasma concentration is generally observed in all populations, whereas mean Lp(a) associated with certain apo(a) isoforms varies between populations. In Caucasians more than 90% of the variation in Lp(a) levels is explained by variation in the number of K-IV repeats and by a largely unknown sequence variation at the apo(a) gene locus. Several studies have shown that Lp(a) levels may be partly determined by other variables, such as age, sex, waist-hip ratio, glucose tolerance, alcohol consumption and smoking, but the results of such studies are untypical. Akita *et al.* concluded that ageing elevates plasma Lp(a) concentrations, thus having a putative role in the prevalence of coronary heart disease in the elderly (23-24).

6. PHYSYOLOGY OF Lp(a)

The physiology and function of Lp(a) are still poorly understood.

It is well known that a high plasma concentration of lipoprotein Lp(a) is considered to be a major and independent risk factor for cerebro- and cardiovascular atherothrombosis. We are going to shortly analyze the relationship between Lpa and several factors involved in cardiovascular risks.

6.1. Lp(a), plasminogen and fibrinolysis

Apolipoprotein(a) homology (75-90%) with plasminogen suggests that Lp(a) might contribute to the thrombotic and to the atherogenic aspects of ischemic heart disease (25).

Lp(a) decreases t-PA (tissue-type plasminogen activator) secretion from human umbilical vein endothelial cells; Lp(a) was also shown to inhibit competitively the binding of plasminogen to its endothelial receptor as well as to fibrinogen or fibrin, which might reduce the surface-dependent activation of plasminogen (26). (Box 1)

Particularly, one of the plasminogen-like kringle 4 copies present in apo(a), kringle IV type 10, contains a lysine binding site (LBS) that is similar to that of plasminogen. This structure allows binding of these proteins to fibrin and cell membranes. Bound plasminogen is cleaved at Arg561-Val562 by plasminogen activators and transformed into plasmin. This mechanism ensures fibrinolysis and pericellular proteolysis. Because of this structural/functional homology between Lp(a) and plasminogen and enzymatic difference, Lp(a) may compete with plasminogen for binding to lysine residues and impair, thereby, fibrinolysis and pericellular proteolysis. (Figure 2)

High concentrations of Lp(a) in plasma may, therefore, represent a potential source of antifibrinolytic activity. Indeed, a recent study has recently shown that during the course of the nephrotic syndrome the amount of plasminogen bound and plasmin formed at the surface of

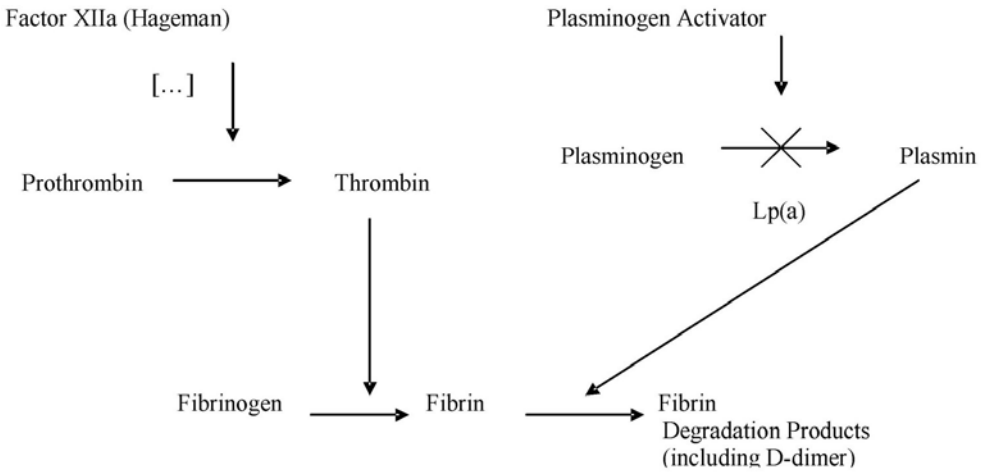

Figure 2. Lp(a) and coagulative cascade: inhibition of the activation of plasminogen.

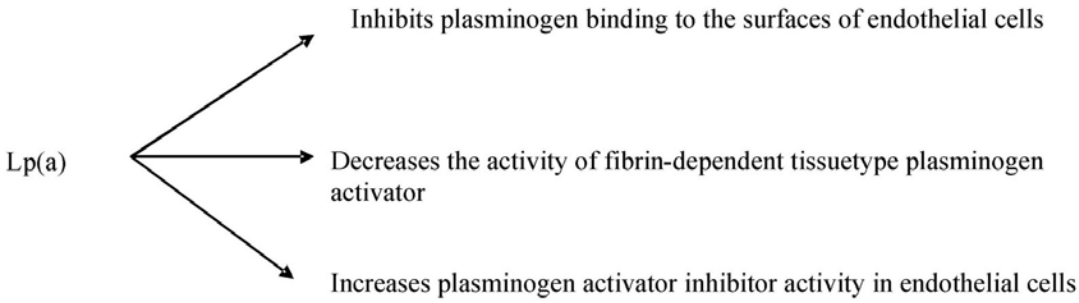

Figure 3. Effects of lipoprotein(a) on fibrinolytic system.

fibrin are directly related to *in vivo* variations in the circulating concentration of Lp(a) (27-28). This antifibrinolytic effect is primarily defined by the size of the apo(a) polymorphs, which show heterogeneity in their fibrin-binding activity, as only small size isoforms display high affinity binding to fibrin (29). The effect of the different isoforms was directly proportional to the amount of Lp(a) bound to the carboxy-terminal lysine residues of degraded fibrin. The relative affinity of the binding (kd range, 16 to 180 nmol/L) reflected the ability of individual Lp(a) isoforms to inhibit the binding of plasminogen (kd, 600 nmol/L). These data suggest that apo(a) isoform types with high affinity for fibrin may influence the ability of Lp(a) to interfere with fibrinolysis, thereby contributing to the association of elevated levels of Lp(a) with atherosclerotic and thrombotic risks. (Figure 3)

Thus, the real risk factor is the Lp(a) subpopulation with high affinity for fibrin. According to this concept, some Lp(a) phenotypes may not be related to atherothrombosis and, therefore, high Lp(a) in some individuals might not represent a risk factor for CVD. In agreement with these data, it has been recently reported that Lp(a) particles containing low molecular mass apo(a) emerged as one of the leading risk conditions in advanced stenotic atherosclerosis (30). The predictive value of high Lp(a) as a risk factor, therefore, depends on the relative concentration of Lp(a) particles containing small apo(a) isoforms with the highest affinity for fibrin.

Moreover, lipoproteins receptors that belong to the LDL receptor superfamily bind fibrinolytic enzymes and participate in regulating the proteolytic surface activity of the cell (29).

6.2. Lp(a) and fibrinogen

Some studies demonstrated that serum Lp(a) concentrations correlate significantly with plasma fibrinogen levels. Plasma fibrinogen has been recognized as an independent risk factor for atherosclerosis and its thrombotic complications in adults too. Fibrinogen enhances platelet activity, and elevated plasma fibrinogen concentrations are predictors of vascular events. Fibrinogen promotes the binding of apo(a) to the vessel walls *in vivo*, thus being crucial in the pathogenesis of atherosclerosis. Experiments on transgenic mice demonstrated that in vessel wall of apo(a) transgenic mice, fibrin(ogen) deposition was found to be essentially co-localized with focal apo(a) deposition and fatty-streak type atherosclerotic lesions. Fibrinogen deficiency in apo(a) transgenic mice decreased the average accumulation of apo(a) in vessel walls by 78% and the average lesion (fatty streak type) development by 81%. Fibrinogen deficiency in wild-type mice did not significantly reduce lesion development. Study results suggested that fibrin(ogen) provides one of the major sites

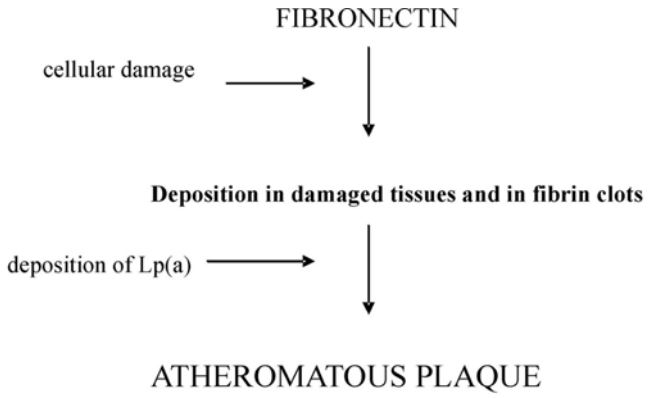

Figure 4. Role of Lipoprotein(a) and fibronectin in the develop of atherosclerotic plaque.

to which apo(a) binds to the vessel wall and participates in the generation of atherosclerosis (31).

Moreover, fibrinogen is an independent risk factor for ischemic atherothrombotic stroke (32). Fibrinogen levels remain elevated after stroke, with an increased risk of recurrent vascular events. Fibrinogen is also associated with a decrease in white blood cell elasticity and red blood cell deformability, with increased plasma erythrocyte viscosity.

However, most *in vitro* studies have shown that human Lp (a) decreases agonist-stimulated platelet aggregation whereas *in vivo* it appears to decrease aggregation as implied by increased bleeding times with higher blood serum concentrations of Lp(a). Lp (a) binding to platelets mediated by apo (a) increases platelet intracellular c-AMP levels in resting platelets, and decreases platelet production of thromboxane A2 and fibrinogen binding to platelets all of which reduce platelet aggregation. One, though not the only, explanation of these conflicting data may be that Lp(a) self-regulates its interference with fibrinolysis by reducing platelet aggregation and platelet binding of fibrinogen and hence the degree of requirement for fibrinolysis. However, further *in vivo* studies are needed to fully understand whether, if at all, Lp(a), at various concentrations and isoforms, favors reduced platelet aggregation or fibrinolysis (33).

6.3. Lp(a) and fibronectin

It has been demonstrated that increased levels of fibronectin together with Lp(a) are present in early atherosclerotic lesions and in atherosclerotic plaques. Fibronectin is a high molecular weight glycoprotein present in plasma in a soluble form and in connective tissue matrices in an insoluble form (34).

The apo(a) portion of Lp(a) binds to the carboxyterminal heparin binding domain of fibronectin. Lp(a) bound to fibronectin is internalized through the fibronectin receptor pathway and thereby causes increased accumulation of lipid and foam cell formation (Figure 4).

A peculiarity of fibronectin is its multiple interaction with other proteins including fibrin, collagen and others components of the coagulation system, such as plasminogen and its activators (35).

Fibronectin has been shown to be an early marker for atherosclerotic lesions.

Thus, following cellular damage, fibronectin is deposited in damaged tissues and in fibrin clots before tissue repair commences. This could be followed by deposition of Lp(a) and the formation of an early atheromatous plaque. This hypothesis would explain the atherogenecity of Lp(a) and provide a link between thrombus formation and atheroma (36-37).

It is well known that inflammation plays a central role in the pathogenesis of atherosclerosis and its complications (38), and that atherogenic lipoproteins, such as oxidized low density lipoprotein (LDL), remnant lipoprotein (beta-VLDL) and lipoprotein(a), take part in the pro-inflammatory reaction. On the contrary high density lipoproteins (HDL), anti-atherogenic lipoproteins, exert anti-inflammatory functions. All these studies demonstrate that Lp(a) is a pleiotropic molecule involved in the activation of acute inflammation (30). Lp(a) can increases the production of cytokines by vascular cells and through the autocrine and paracrine mechanisms (39-40).

6.4. Lp(a) and TGF-β

TGF-β is now recognized to be a multifunctional cytokine involved in growth promoting and growth inhibiting activities. TGF- β inhibits growth of epithelial cells, lymphocytes, endothelial cells, and smooth muscle cells (41-42-43-44-45-46).

Lp(a) inhibits the activation of transforming growth factor β (TGF-β) and contributes to the growth of arterial atherosclerotic lesions by promoting the proliferation of vascular smooth muscle cells and the migration of smooth muscle cells to endothelial cells.

The inverse relationship between Lp(a) levels and activated TGF-β is related to the inhibition of the

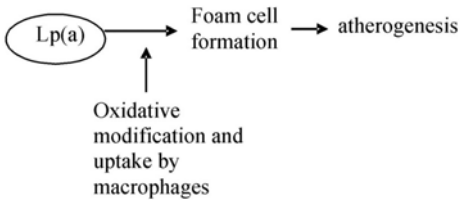

Figure 5. Lp(a) and oxidation.

conversion of plasminogen to plasmin, which is indispensable for the activation of TGF- β (45).

Lp(a) and TGF- β1 are thought to influence the expression of cellular adhesion molecules (CAMs), also involved in the process of atherogenesis (48).

Lp(a) can inhibit the activation of TGF- β by competing with the binding of plasminogen to cell or matrix surfaces.

It should be noted that the amount of Lp(a) required to block LTGF-β (latent molecule of TGF-β requiring activation for function) is relatively high and exceeds the circulating levels found in normal and pathological conditions. The circulating level of plasminogen is ~1 μM, while the Lp(a) plasma concentration is 1 nM-1μM. However, recent reports indicate that the tissue concentrations of Lp(a) in the intima of the arterial wall may be much higher than the circulating levels (49-50-51).

Recently it has been proposed a molecular mechanism for the Lp(a)- induced inhibition of LTGF-β activation and suggested a relationship between the activation of LTGF- β and atherosclerosis.

Kojiima *et al.* suggest that under normal conditions, LTGF- β may be activated at sites where endothelial cells and smooth muscle cells are in contact and here its role is to maintain the tissue architecture. Thus, excess tissue levels of Lp(a) may decrease TGF- β production thereby reducing the block on smooth muscle cells migration. This may account for the migration of smooth muscle cells from the media to the intima and thereby contribute to the generation of atheroma (52).

6.5. Lp(a) and adhesion molecules

Lp(a) may lead to an inflammatory process by inducing the expression of adhesion molecules on endothelial cells, the chemotaxis of monocytes, and the proliferation of smooth muscle cells.

Among these molecules, intercellular adhesion molecule (ICAM-1) was shown to be expressed in human atherosclerotic plaques by an immunohistochemical method and may be a candidate that plays an important role in mediating the localization of monocytes in the intima of arteries. Adhesion molecules were shown to contribute to the development not only of atherosclerotic but also of inflammatory vascular disorders by regulating cell adhesion between leukocytes and endothelial cells; given that Lp(a) might play an essential role in both the earliest stage of atherosclerosis and inflammatory CVDs by the enhancement of intercellular adhesion molecule (ICAM-1) (53-54).

Finally, it is of interest the demonstration that Lp(a) stimulates the production of vascular cell adhesion molecule (VCAM-1) in cultured human coronary artery endothelium(55-56).

P-selectin is one of the major adhesion molecules able to mediate the interaction between monocytes, platelets and endothelial cells. Increased expression of P-selectin has been frequently found in atherosclerotic lesions. Zhao *et al.* assessed the effects of native and oxidized LDL (n-LDL and ox-LDL) and Lp(a) (n-Lp(a) and ox-Lp(a)) on the expression of P-selectin in cultured human umbilical vein endothelial cells (HUVECs). Results showed that P-selectin protein expression was not influenced by n-LDL, but was moderately increased by ox-LDL and n-Lp(a). Ox-Lp(a) was the most potent stimulus for P-selectin expression. In addition, authors showed a dose dependent induced monocyte adherence to endothelial cells after incubation of HUVECs with ox-Lp(a). These results demonstrate that ox-Lp(a) can induce P-selectin expression in HUVECs, which may thereby influence the pathogenesis of atherosclerosis (55-57). (Figure 5)

7. SUMMARY AND PERSPECTIVE

Lp(a) is involved in the development of atherothrombosis and activation of acute inflammation exerting a pro-atherogenic and hypofibrinolytic effect. This molecule can be considered as a common linkage among different metabolic systems. Moreover, lipoprotein(a) plays a critical role in the pro-inflammatory reaction.

This pleiotropic effect is primarily regulated by the size of the apo(a) polymorphs: for example, only small size isoforms display high affinity binding to fibrin (58-60). According to this concept, some Lp(a) phenotypes may not be related to atherothrombosis and, therefore, high Lp(a) in some individuals might not represent a risk factor for CVD (61-62). Future studies need to clarify better its own function. We suppose that Lp(a) lipoprotein evolution during centuries could be due to the need to provide protection against several oxidative stressors. For example, Lp(a) lipoprotein has been demonstrated to be involved in wound healing (63). Others activities have been demonstrated *in vitro* and *in vivo* tumor models where Lp(a) exhibited an anti-angiogenic action.

Moreover Thillet et al. showed elevated Lp(a) levels in human centenarians, where probably Lp(a) correlated with human longevity (64-68).

Recently Tregouet et al. (69) identify the SLC22A3-LPAL2-LPA gene cluster as a strong susceptibility locus for coronary artery disease through a genome-wide haplotype association study. This locus was not identified from previous genome-wide association

studies focused on univariate analyses of SNPs (69). Further studies will be required to assess this relationship.

8. REFERENCES

1. K Berg. A new serum type system in man – the Lp system. *Acta Pathol Microbiol Scand.* 59, 369–82 (1963)

2. PO Kwiterovich, J Coresh, HH Smith, *et al.* Comparison of the plasma levels of apolipoproteins B and A-1, and other risk factors in men and women with premature coronary artery disease. *Am J Cardiol*. 69, 1015–21 (1992)

3. GM Fless, ME ZumMallen, and AM Scanu. Physicochemical properties of apolipoprotein(a) and lipoprotein(a) derived from the dissociation of human plasma lipoprotein (a). *J Biol Chem*. 261, 8712−18 (1986)

4. SM Marcovina, ML Koschinsky. Lipoprotein(a) as a risk factor for coronary artery disease. *Am J Cardiol*. 82, 57U-66U (1998)

5. S Chitayat, V Kanelis, ML Koschinsky, SP Smith. Nuclear magnetic resonance (NMR) solution structure, dynamics, and binding properties of the kringle IV type 8 module of apolipoprotein(a). *Biochemistry*. 20, 1732-42 (2007)

6. MJ Callow, EM Rubin. Site-specific mutagenesis demonstrates that cysteine 4326 of apolipoprotein B is required for covalent linkage with apolipoprotein (a) in vivo. *J Biol Chem*. 270, 23914−17 (1995).

7. SP McCormick, JK Ng, S Taylor, LM Flynn, RE Hammer, SG Young. Mutagenesis of the human apolipoprotein B gene in a yeast artificial chromosome reveals the site of attachment for apolipoprotein(a). *Proc Natl Acad Sci*. 92, 10147−51. (1995)

8. J Guevara Jr, NV Valentinova, O Garcia, AM Gotto, CY Yang, S Legal, J Gaubatz, JT Sparrow. Interaction of apolipoprotein[a] with apolipoproteinB-100 Cys3734 region in lipoprotein[a] is confirmed immunochemically. *J Protein Chem*. 15, 17−25 (1996)

9. MJ McQueen, S Hawken, X Wang Ounpuu S, Sniderman A, Probstfield J, Steyn K, Sanderson JE, Hasani M, Volkova E, Kazmi K, S Yusuf. Lipids, lipoproteins, and apolipoproteins as risk markers of myocardial infarction in 52 countries (the INTERHEART study): a case-control study. *Lancet.* 372, 224–33 (2008)

10. C Lackner, JC Cohen, HH Hobbs. Molecular definition of the extreme size polymorphism in apolipoprotein(a). *Hum Mol Genet*. 2, 933−40 (1993)

11. G Lippi , G Guidi. Lipoprotein(a): an emerging cardiovascular risk factor. *Crit Rev Clin Lab Sci.* 40:1-42 (2003)

12. K Berg. Risk factor variability and coronary heart disease. *Acta Genet Med.* 39:15-24 (1990)

13. A Edjème-Aké, R Garnotel, S Vallée-Polneau, E Anglés-Cano, D Monnet, P Gillery. Relationship between apo(a) length polymorphism and lipoprotein(a) concentration in healthy Ivorian subjects with single or double apo(a) isoforms. *Clin Biochem*. 41:1039-43. (2008)

14. H Dieplinger, C Lackner, FKronenberg, C Sandholzer, Lhotta K, Hoppichler F, Graf H, Konig P. Elevated plasma concentrations of lipoprotein(a) in patients with end-stage renal disease are not related to the size polymorphism of apolipoprotein(a). *J Clin Invest.* 91:397-401 (1993)

15. R Guerra, Z Yu, S Marcovina, R Peshock, JC Cohen, HH Hobbs. Lipoprotein(a) and apolipoprotein(a) isoforms: no association with coronary artery calcification in the Dallas Heart Study. *Circulation*. 111:1471-9 (2005)

16. C Sandholzer, E Boerwinkle, N Saha, MC Tong, G Utermann. Apolipoprotein(a) phenotypes, Lp(a) concentration and plasma lipid levels in relation to coronary heart disease in a Chinese population: evidence for the role of the apo(a) gene in coronary heart disease. *J Clin Invest.* 3:1040-6 (1992)

17. GX Shen. Impact and mechanism for oxidized and glycated lipoproteins on generation of fibrinolytic regulators from vascular endothelial cells. *Mol Cell Biochem*. 246:69-74 (2003)

18. J Zhang, S Ren, GX Shen. Glycation amplifies lipoprotein(a)-induced alterations in the generation of fibrinolytic regulators from human vascular endothelial cells. *Atherosclerosis*. 2:299-308 (2000)

19. C Edelstein, D Pfaffinger, J Hinman, E Miller, G Lipkind, S Tsimikas, C Bergmark, GS Getz, JL Witztum, AM Scanu. Lysine-phosphatidylcholine adducts in kringle V impart unique immunological and potential proinflammatory properties to human apolipoprotein (a). *J Biol Chem.* 278: 52841–47 (2003)

20. M Malaguarnera, Vacante M, Motta M, Malaguarnera M, Li Volti G, Galvano F. Effect of L-carnitine on the size of low-density lipoprotein particles in type 2 diabetes mellitus patients treated with simvastatin. *Metabolism*. 58, 1618-23. 2009

21. DJ Rader, W Cain, LA Zech, D Usher, HB Brewer. "Variation in lipoprotein(a) concentrations among individuals with the same apolipoprotein (a) isoform is determined by the rate of lipoprotein(a) production". *J. Clin. Invest.* 2: 443–7 (1993)

22. BL Knight, YF Perombelon, AK Soutar, DP Wade, M Seed. Catabolism of lipoprotein(a) in familial hypercholesterolaemic subjects. *Atherosclerosis.* 87: 227–37 (1991)

23. CL Scholtz, A Lingenhel, R Hillermann, IA Stander, JA Kriek, MP Marais, HJ Odendaal, HG Kraft, G Utermann, MJ Kotze. Lipoprotein(a) determination and risk of cardiovascular

disease in South African patients with familial hypercholesterolaemia. *S Afr Med J.* 4:374-8 (2000)

24. SS Anand, EA Enas, J Pogue, S Haffner, T Pearson, S Yusuf. Elevated lipoprotein(a) levels in South Asians in North America. *Metabolism.* 2:182-4 (1998)

25. H Akita, M Matsubara, H Shibuya, H Fuda, H Chiba. Effect of ageing on plasma lipoprotein(a) levels. *Ann Clin Biochem.* 3:237-40 (2002)

26. J Hoover-Plow, E Hart, Y Gong, A Shchurin, TA Schneeman. Physiological Function for Apolipoprotein(a): A natural regulator of the inflammatory response. *Exp Biol Med.* 234:28-34 (2009)

27. BF Asztalos, PS Roheim, RL Milani, M Lefevre, JR McNamara, KV Horvath, EJ Schaefer. Distribution of ApoA-I-containing HDL subpopulations in patients with coronary heart disease. *Arterioscler Thromb Vasc Biol*. 12:2670-6 (2000)

28. T Soulat, S Loyau, V Baudouin, V Durlach, P Gillery, R Garnotel, C Loirat, Anglès-Cano E. Evidence that modifications of Lp(a) *in vivo* inhibit plasmin formation on fibrin-a study with individual plasmas presenting natural variations of Lp(a). *J Thromb Haemost.* 1:121-7 (1999)

29. L Hervio, MJ Chapman, J Thillet, S Loyau, Anglès-Cano E. Does apolipoprotein(a) heterogeneity influence lipoprotein(a) effects on fibrinolysis? *Blood.* 2:392-7 (1993)

30. F Kronenberg, MF Kronenberg, S Kiechl, E Trenkwalder, P Santer, F Oberhollenzer, G Egger, G Utermann, J Willeit. Role of lipoprotein(a) and apolipoprotein(a) phenotype in atherogenesis: prospective results from the Bruneck study. *Circulation*. 100:1154-60 (1999)

31. XJ Lou, NW Boonmark, FT Horrigan, JL Degen, RM Lawn. Fibrinogen deficiency reduces vascular accumulation of apolipoprotein(a) and development of atherosclerosis in apolipoprotein(a) transgenic mice. *Proc Natl Acad Sci U S A*. 21:12591-5 (1998)

32. J Pineda, F Marín, P Marco, V Roldán, J Valencia, JM Ruiz-Nodar, F Sogorb, GY Lip. Premature coronary artery disease in young (age <45) subjects: Interactions of lipid profile, thrombophilic and haemostatic markers. *Int J Cardiol*. 136:222-5. (2008)

33. DE Barre. The molecular nature and consequences of lipoprotein (a)'s association with platelets. *Protein Pept Lett*. 14:839-42 (2007)

34. MW Mosesson. Fibrinogen and fibrin structure and functions. *J Thromb Haemost*. 8:1894-904 (2005)

35. R Testa, AR Bonfigli, C Sirolla, G De Grazia, P Compagnucci, S Manfrini, D Fumelli, I Testa. Fibronectin and lipoprotein(a) are inversely related to plasminogen activator inhibitor type-1 levels in Type 2 diabetic patients without complications. *Diabetes Nutr Metab*. 5:269-75. (2000)

36. EM Salonen, O Saksela, T Vartio, A Vaheri, LS Nielsen, J Zeuthen. Plasminogen and tissue-type plasminogen activator bind to immobilized fibronectin. *J Biol Chem.* 260:12302-7. (1985)

37. S Stenman, K von Smitten, A Vaheri. Fibronectin and atherosclerosis. *Acta Med Scand.* 642:165-70 (1980)

38. M Woodward, A Rumley, P Welsh, S MacMahon, GA Lowe. comparison of the associations between seven hemostatic or inflammatory variables and coronary heart disease. *J Thromb Haemost*. 9:1795-800 (2007)

39. A Imhof, D Rothenbacher, N Khuseyinova, A Hoffmeister, W Maerz, M Nauck, H Scharnagl, W Koenig, H Brenner. Plasma lipoprotein Lp(a), markers of haemostasis and inflammation, and risk and severity of coronary heart disease. *Eur J Cardiovasc Prev Rehabil.* 5:362-70 (2003)

40. J Fan, T Watanabe. Inflammatory reactions in the pathogenesis of atherosclerosis. *J Atheroscler Thromb.* 102:63-71 (2003)

41. JA Barnard, RM Lyons, HL Moses. The cell biology of transforming growth factor beta. *Biochim Biophys Acta.* 1032:79-87 (1990).

42. MB Sporn, AB Roberts. TGF-beta: problems and prospects. *Cell Regul.* 12:875-82 (1990)

43. R Derynck, JA Jarrett, EY Chen, DH Eaton, JR Bell, RK Assoian, AB Roberts, MB Sporn, DV Goeddel. Human transforming growth factor-beta complementary DNA sequence and expression in normal and transformed cells. *Nature.* 316:701-5 (1985)

44. JH Kehrl, AB Roberts, LM Wakefield, S Jakowlew, MB Sporn, AS Fauci. Transforming growth factor beta is an important immunomodulatory protein for human B lymphocytes. *J Immunol.* 137:3855-60 (1986)

45. RL Heimark, DR Twardzik, SM Schwartz. Inhibition of endothelial regeneration by type-beta transforming growth factor from platelets. *Science.* 233:1078-80 (1986)

46. GK Owens, AA Geisterfer, YW Yang, A Komoriya. Transforming growth factor-beta-induced growth inhibition and cellular hypertrophy in cultured vascular smooth muscle cells. *J Cell Biol.* 2:771-80 (1988)

47. S Stefoni, G Cianciolo, G Donati, A Dormi, MG Silvestri, L Colì, A De Pascalis, S Iannelli. Low TGF-beta1 serum levels are a risk factor for atherosclerosis disease in ESRD patients. *Kidney Int.* 61:324-35 (2002)

48. N Bogavac-Stanojevic, S Djurovic, Z Jelic-Ivanovic, V Spasojevic-Kalimanovska, D Kalimanovska-Ostric. Circulating transforming growth factor-beta1,

lipoprotein(a) and cellular adhesion molecules in angiographically assessed coronary artery disease. *Clin Chem Lab Med*. 7:893-8 (2003)

49. M Rath, A Niendorf, T Reblin, M Dietel, HJ Krebber, U Beisiegel. Detection and quantification of lipoprotein(a) in the arterial wall of 107 coronary bypass patients. *Arteriosclerosis*. 5:579-92 (1989)

50. A Niendorf, M Rath, K Wolf, S Peters, H Arps, U Beisiegel, M Dietel. Morphological detection and quantification of lipoprotein(a) deposition in atheromatous lesions of human aorta and coronary arteries. *Virchows Arch A Pathol Anat Histopathol.* 105-11. (1990)

51. Y Sato, R Tsuboi, R Lyons, H Moses, DB Rifkin. Characterization of the activation of latent TGF-beta by co-cultures of endothelial cells and pericytes or smooth muscle cells: a self-regulating system. *J Cell Biol.* 757-63. (1990)

52. S Kojima, PC Harpel, DB Rifkin. Lipoprotein (a) inhibits the generation of transforming growth factor beta: an endogenous inhibitor of smooth muscle cell migration. *J Cell Biol.* 6:1439-45 (1991)

53. S Allen,S Khan, S Tam, M Koschinsky, P Taylor, M Yacoub. Expression of adhesion molecules by lp(a): a potential novel mechanism for its atherogenicity. *FASEB J.* 15:1765-76. (1998)

54. T Ichikawa, H Unoki, H Sun, H Shimoyamada,, S Amrcovina, H Shikama, T Watanabe, J Fan. Lipoprotein(a) promotes smooth muscle cell proliferation and dedifferentiation in atherosclerotic lesions of human apo(a) transgenic rabbits. *Am J Pathol.* 227-36. (2002).

55. S Allen, S Khan, S Tam, M Koschinsky, P Taylor, M Yacoub. Expression of adhesion molecules by lp(a): a potential novel mechanism for its atherogenicity. *FASEB J.* 15:1765-76. (1998)

56. S Takami, S Yamashita, S Kihara, M Ishigami, K Takemura, N Kume, T Kita, Y Matsuzawa. Lipoprotein(a) enhances the expression of intercellular adhesion molecule-1 in cultured human umbilical vein endothelial cells. *Circulation.* 8:721-8 (1998)

57. SP Zhao, DY Xu. Oxidized lipoprotein(a) enhanced the expression of P-selectin in cultured human umbilical vein endothelial cells. *Thromb Res*. 6:501-10. (2000)

58. F Galvano, Li Volti G, Malaguarnera M, Avitabile T, Antic T, Vacante M, M Malaguarnera. Effects of simvastatin and carnitine versus simvastatin on lipoprotein(a) and apoprotein(a) in type 2 diabetes mellitus. *Expert Opin Pharmacother.* 10**:**1875-82. (2009)

59. W Patsch, AM Jr Gotto. Apolipoproteins: pathophysiology and clinical implications. *Methods Enzymol.* 263:3-32 (1996)

60. M. Malaguarnera, I. Giugno, BA Trovato, MP Panebianco, N Restuccia, P Ruello. Lipoprotein(a) in cirrhosis. A new index of liver functions? *Curr Med Res Opin.* 13:479-85. (1996)

61. JH Stein, RS Rosenson. Lipoprotein Lp(a) excess and coronary heart disease. *Arch Intern Med.* 11:1170-6 (1997)

62. M Motta, Giugno I, Bosco S, Pistone G, Ruello P, Maugeri D, M. Malaguarnera. Serum lipoprotein(a) changes in acute myocardial infarction. *Panminerva Medica.* 43:77-80**.** (2001)

63. Y Yano, K Shimokawa, Y Okada, A Noma. Immunolocalization of lipoprotein(a) in wounded tissues. *J Histochem Cytochem*. 4:559-68 (1997)

64. J Thillet, C Doucet, J Chapman, E Herbeth, D Cohen, L Faure-Delanef. Elevated lipoprotein(a) levels and small apo(a) isoforms are compatible with longevity: evidence from a large population of French centenarians. *Atherosclerosis.* 2:389-94 (1998)

65. G Pistone, AD Marino, C Leotta, S Dell'Arte, G Finocchiaro, M Malaguarnera. Levocarnitine administration in elderly subjects with rapid muscle fatigue - Effect on body composition, lipid profile and fatigue. *Drugs Aging.* 20:761-7. (2003)

66. M. Malaguarnera, Receputo G, Giugno I, Ruello P, Di Fazio I, M. Motta. Lipid profile in centenarians. *Clin Drug Investig.* 11:240-244. (1996)

67. M. Malaguarnera, Ruello P, Rizzo M, Receputo G, Rapisarda R, Restuccia N, G Pistone. Lipoprotein(a) levels in centenarians. *Arch Gerontol Geriatr.* Suppl. 5 385-8. (1996)

68. M Malaguarnera, I Giugno, P Ruello, M Rizzo, MP Panebianco, G Pistone, FB Tomasello. Lipid profile variations in a group of healthy elderly and centenarians. *Eur Rev Med Pharmacol Sci*. 2:75-9 (1998)

69. DA Trégouët, IR König, J Erdmann, A Munteanu, PS Braund, AS Hall, A Grosshennig, P Linsel-Nitschke, C Perret, M DeSuremain, T Meitinger, BJ Wright, M Preuss, AJ Balmforth, SG Ball, C Meisinger, C Germain, A Evans, D Arveiler, G Luc, JB Ruidavets, C Morrison, P van der Harst, S Schreiber, K Neureuther, A Schäfer, P Bugert, NE El Mokhtari, J Schrezenmeir, K Stark, D Rubin, HE Wichmann, C Hengstenberg, W Ouwehand; Wellcome Trust Case Control Consortium; Cardiogenics Consortium, A Ziegler, L Tiret, JR Thompson, F Cambien, H Schunkert, NJ Samani. Genome-wide haplotype association study identifies the SLC22A3-LPAL2-LPA gene cluster as a risk locus for coronary artery disease. *Nat Genet.* 41:283-5. (2009)

Key Words: Lipoprotein(a), Atherothrombosis, Fibrinolysis, Adhesion molecules, Review

Send correspondence to: Mariano Malaguarnera, A.P. Via Messina, 829, 95100 Catania , Italy, Tel: 39-95-7262008, Fax: 39-95-7262011 E-mail: malaguar@unict.it

http://www.bioscience.org/current/vol2S.htm