Role of homocysteine and lipoprotein (A) in atherosclerosis: An update.

Devika Tayal [#@]**, Binita Goswami**[*@]**, Bidhan Chandra Koner**[$] **and V. Mallika**[*]

\# Department of Biochemistry, Lady Hardinge Medical College and Associated Hospital, New Delhi, India
* Department of Biochemistry, G B Pant Hospital, New Delhi, India
\$ Department of Biochemistry, Maulana Azad Medical College, and Associated Hospitals, New Delhi, India
@ Both DT and BG have contributed equally for the paper.

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Abstract

**Lipoprotein (a) and homocysteine are two important independent risk factors for coronary atherosclerosis. Lipoprotein (a) is a low-density lipoprotein -like particle formed by the association of the highly polymorphic glycosylated apolipoprotein (a) with apolipoprotein ¯B100. Homocysteine is formed as a by-product of the metabolism of amino acid methionine. The present review attempts to elaborate the roles of homocysteine and lipoprotein (a) in the etiopathogenesis of CAD and the inter relationship between these two established risk factors. The literature was searched from the websites of the National Library of Medicine (http://www.ncbi.nlm.nih.gov/) and Pub Med Central, the U.S. National Library of Medicine's digital archive of life sciences journal literature. The role of homocysteine and lipoprotein (a) has been extensively investigated during the past few decades. The outcomes have been varied with both favourable and unfavourable inferences. Certain researchers have also pointed towards interplay between these two risk factors at the molecular level.
A better understanding of the interaction between the risk factors of CAD will help in better risk stratification and management of this disease.**

Introduction

Cardiovascular disease (CVD) is the leading cause of death and disability in developed nations and is increasing rapidly in the developing world [1]. In more than 90% of instances, the cause of myocardial ischemia is reduction of coronary blood flow owing to atherosclerotic coronary arterial obstruction [2]. By the year 2020, it is estimated that CVD will surpass infectious diseases as the world's leading cause of death and disability [3]. Indians are predisposed to CVD due to a multitude of factors and present with an aggressive form of the disease. It has been predicted that coronary artery disease might become the most prevalent disease in India by the year 2020 due to the rapid changes in demography and life styles consequent to economic development [4]. Much research has gone into understanding the pathogenesis of CAD and atherosclerosis; and the identification of the causative factors of this condition is imperative for tackling this pandemic.

Pathphysiology of atherosclerosis

The hallmark of atherosclerosis is the atherosclerotic plaque, which contains lipids (intracellular and extra cellular cholesterol and phospholipids), inflammatory cells (e.g., macrophages, T-cells), smooth muscle cells, connective tissue (e.g., collagen, glycosaminoglycans, elastic fibers), thrombi, and calcium deposits. The mechanism of atherosclerosis involves several highly interrelated processes, including lipid disturbances, platelet activation, thrombosis, endothelial dysfunction, inflammation, oxidative stress, vascular smooth cell activation, altered matrix metabolism, remodeling, and genetic factors [5].

Risk factors

Risk factors play an important role in initiating and accelerating the complex process of atherosclerosis. The major modifiable risk factors include elevated blood pressure, dyslipidemia, smoking, and diabetes mellitus. Unmodifi-

able risk factors include age, gender, and family history/genetics. A number of emerging risk factors for atherosclerosis have recently been proposed to help identify high-risk individuals. Newer risk factors include Lipoprotein (a) [Lp (a)], homocysteine, thrombotic risk factors and inflammatory risk factors [2]. In this present review we will discuss the role of Lp (a) and homocysteine and the inter-relation between these two risk factors and whether it leads to increased risk of atherothrombosis.

Lipoprotein (a) [Lp(a)]

Biochemistry

Lipoprotein (a) [Lp(a)] was identified by Berg in 1963[6]. It has been observed that individuals with low or null concentrations of plasma lipoprotein (a) manifest no deficiency syndrome or disease [7]. Lp (a) is a low-density lipoprotein (LDL)-like particle formed by the association of the highly polymorphic glycosylated apolipoprotein (a) (apo (a)) with apolipoprotein B100 (apo B100), the classic protein moiety of LDL [8]. Apo(a) is attached to Apo B100 through a single disulphide link between apo B100 Cys 3734 and apo(a) kringle IV type 9 Cys67[9]. The cysteine residue in apoB involved in the covalent bond between apoB and apo(a) is close to the postulated LDL receptor-binding region of apoB[10].

The human apo(a) gene is located in a gene cluster within 400 kb of genomic DNA on the telomeric region of chromosome 6 (6q26-27) [11]. The apo(a) gene belongs to a gene family that includes several similar sequences encoding prothrombin, tissue-type plasminogen activator (t-PA), urokinase A-chain, plasminogen, coagulation factor XII, macrophage stimulating factor, hepatocyte growth factor and other proteins and polypeptides of unclear function[12]. The apo (a) gene shares the highest homologies with the gene of the zymogen plasminogen. The plasminogen gene contains coding sequences for 5 different K domains (K1 to K5), and 2 of these are present in the apo (a) gene, K4 and K5. Due to size heterogeneity, apo (a) exhibits a genetic size polymorphism with apparent molecular weight of isoforms ranging from 300 to 800KDa [13, 14]. An inverse correlation between the number of kringle IV type 2 repeats in the apo (a) gene and Lp(a) plasma concentration exists[15]. The presence of apo (a) influences to a major extent metabolic and physicochemical properties of Lp(a) [16,17]. Although many studies have addressed differences between blacks and whites, population differences with regard to Lp(a) and apo(a) size are not limited to these groups. Differences in both Lp(a) levels and apo(a) size distribution have been noted among Asian populations. Thus, whereas East Asians have been reported to have a relatively narrow apo(a) size distribution and low Lp(a) levels, South Asians have higher mean Lp(a) levels. [18-23] Several polymorphisms in the apo(a) gene have been reported. Of these, a C/T variation in the promoter region of the apo(a) gene and a pentanucleotide repeat (TTTTA$_n$) ≈ 1 kb upstream of the apo(a) gene have been studied .[24-26]

Mechanism of atherogenicity (Fig. 1)

Raised levels of Lp(a) are associated with an increased incidence of a variety of cardiovascular diseases, including silent coronary artery disease (CAD), acute myocardial infarction (AMI), asymptomatic carotid atherosclerosis, stroke, and peripheral artery occlusive disease (PAOD) [27,28]. The mechanism implicated in the atherogenicity of Lp (a) include

a) tendency to self-aggregate and precipitate and a greater capacity to bind to glycosaminoglycans and other structures in the vascular wall. Lp(a) binds avidly to endothelial cells, macrophages, fibroblasts, and platelets, as well as to the subendothelial matrix; there, it promotes proliferation of vascular smooth muscle cells and chemotaxis of human monocytes [29-31]. Foam cell production is also enhanced by lp(a) [32].

b) Due to its unique structural homology to plasminogen, Lp(a) competes with plasminogen for binding to plasminogen receptors, fibrinogen, and fibrin[33].It also induces the production of plasminogen activator inhibitor 1 (the main inhibitor of the fibrinolytic system) and inhibits the secretion of tissue-plasminogen activator by endothelial cells [34,35]. It promotes generation of free radicals in monocytes [36].

c) In a series of studies, Witztum et al have demonstrated that a key oxidized phospholipid is preferentially associated with Lp(a) [37-39]. Proinflammatory, oxidized phospholipids are covalently bound to kringle V in apo(a) [37]. Another study suggests that oxidized phospholipids are physically associated with Lp(a) lipoprotein bound to lysine residues on isolated fragments of kringle V of apo(a) [40] and also in the lipid phase of Lp(a) lipoprotein. The presence of oxidized phospholipids in Lp (a), potentially being taken up by the vessel wall, accelerates development of atherosclerosis.

d) In an endothelial cell system, native Lp (a), via apo (a), stimulates the production of adhesion molecules such as intercellular adhesion molecule [41], vascular cell adhesion molecule-1[42], and E-selectin[42], as well as endothelin-1[43] and I-309, a potent chemo attractant for monocytes[44]. In a vascular smooth muscle cell system, native Lp(a), and particularly apo(a), inhibits the proteolytic activation of transforming factor β via a decrease in cell surface generation of plasmin, resulting in increased vascular smooth muscle cells proliferation[45].

Fig 1: Pathogenic mechanisms of Lipoprotein (a)
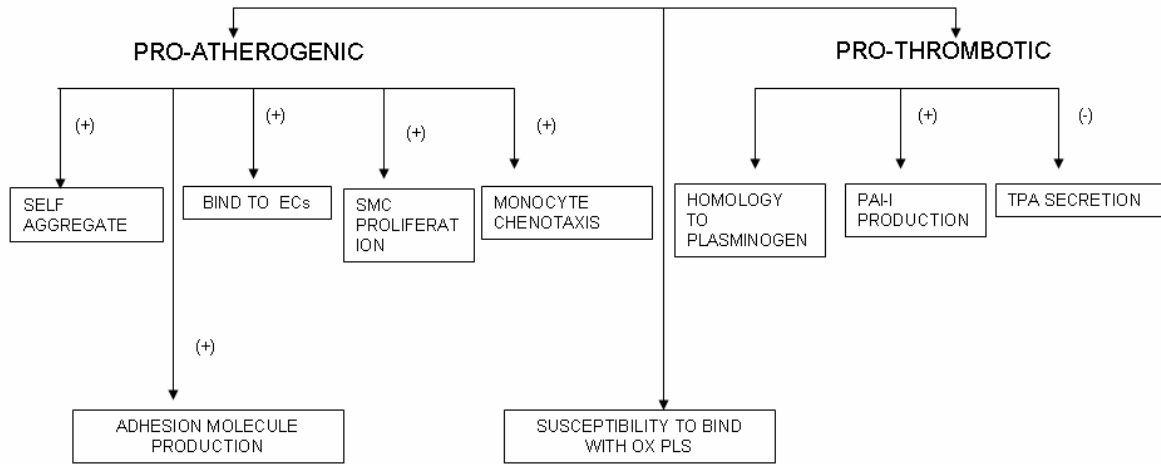
Figure 1.: Atherothrombotic mechanisms initiated by lipoprotein (a)

Retrospective case-control studies have found a strong association between elevated Lp(a) levels and CVD. On the other hand, the results of prospective studies have been mixed. That plasma Lp(a) concentration is an independent risk factor for CVD in both men and women was confirmed by the Prospective Epidemiological Study of Myocardial Infarction (PRIME), which included 9133 French and Northern Irish men, aged 50 to 59 years at entry, without any manifestations of cardiovascular disease. In these subjects, Lp (a) was significantly associated with CVD development and appeared to be a significant risk factor ($P<0.0006$) in the cohort as a whole. [46]

Studies have suggested an association between high levels of Lp (a) [>30 mg/dL]) and the presence and extent of coronary artery disease, premature coronary artery disease, myocardial infarction, restenosis after balloon angioplasty, cerebrovascular disease, saphenous vein bypass graft disease, and cardiac allograft vasculopathy. This association has been documented in men and women and in white persons, African Americans, and Asian Indians [47-69].

However some studies have shown no association between Lp (a) levels and vascular disease. In the Physicians' Health Study, which involved 14,916 predominantly white, middle-aged men, serum Lp (a) level did not correlate with future risk for myocardial infarction or stroke[70,71]. In the Helsinki Heart Study, baseline Lp (a) levels were similar in patients who developed coronary events over 5 years of follow-up and in those who did not suffer from any such event [72]. A 5-year prospective follow-up study of 2156 French Canadian men showed that elevated Lp(a) level was not an independent risk factor for coronary artery disease[73].In another study involving 140 African-American patients, plasma Lp(a) levels were found to be similar in those with and without coronary disease[74].

Indian perspective

Sharobeem et al conducted a study on 55 South Asian patients with ischaemic stroke (confirmed on computerised scan of the brain) and 85 controls and concluded that Lp(a) and the Apo B to AI ratio are associated with ischemic stroke in South Asians [75]. Rajappa et al assessed the role of lp(a) in the prediction of CAD in south Indians with Diabetes Mellitus [76]. They confirmed that high concentrations of Lp(a) along with high prevalence of non insulin dependent Diabetes Mellitus (NIDDM), predispose Indians to atherosclerosis at an earlier age. We have also demonstrated the role of lp(a) as a risk factor in patients of acute myocardial infarction [77]. Singh et al concluded from their study that Lp(a) alone can correctly discriminate a CVD individual from a control subjects by 95% [78]. In few studies conducted in India higher mean levels of Lp(a) were observed in cases than controls- 12 to 41mg/dl in patients and 8 to 24 mg/dl in healthy controls [79-83]. Hence, lp(a) is a strong risk factor in Indians.

Homocysteine

Biochemistry

Homocysteine "hypothesis of arteriosclerosis" was first proposed by McCully in 1969, when he observed premature atherothrombosis of the peripheral, coronary, and cerebral vasculature in children with homocystinuria, an inborn error in methionine metabolism [84]. Homocysteine, a sulphur-containing amino acid, is an intermediate formed during the catabolism of the essential dietary amino acid methionine. Approximately 80% of plasma

homocysteine is protein bound. Free and protein bound homocysteine and its disulfides are globally referred to as total homocysteine (tHcy) [85-87]. Homocysteine is metabolized by two pathways: vitamin B_6-dependent trans-sulfuration and vitamin B_{12}-dependent and folate-dependent remethylation [88]. In 1976 Wilcken and Wilcken first showed that patients with coronary heart disease have elevated concentrations of plasma cysteine – homocysteine disulphide after an oral methionine load [89].

Mechanisms involved in the athero-thrombotic effects of homocysteine (Figs. 2,3)

The mechanisms by which hyperhomocysteinemia might contribute to atherogenesis and thrombosis are incompletely understood and various theories have been propounded for the same. Some of them are highlighted under the following subheads.

a) *Effects on platelet function and thrombosis*: The vascular endothelium maintains a non-thrombogenic surface with the help of antithrombin III and thrombomodulin-protein C mediated anticoagulant pathways [90, 91]; synthesis of plasminogen Activators [92-94] and the inhibition of platelet activation by ecto-ADPase, prostacyclin and nitric oxide [92]. The endothelium also synthesises clotting factors and maintains the balance between procoagulant and anticoagulant mechanisms, which is vital to the maintenance of vascular haemostasis. Homocysteine may upset this balance and predispose to thrombogenesis via stimulation of procoagulant factors and promotion of coagulation in the absence of thrombin [95]. Homocysteine stimulates platelet generation of thromboxane A2 which is a vaso-constrictor and proaggregant. Hyper homocysteinemia causes: activation of factor V and interferes with protein C activation and thrombomodulin expression [96], interferes with the generation and bioavailability of prostacyclin and the expression of anticoagulant substance heparan sulphate on vessel wall [97]. It also inhibits the activity of ecto-ADPase[98]. Platelet activation due to hyperhomocystenemia results in thrombosis and smooth muscle proliferation and is therefore important in the pathogenesis of atherothrombosis [99, 100].

b) *Effects on endothelial and smooth muscle function*: Vascular intimal smooth muscle proliferation with subsequent formation of extracellular matrix collagen is an integral component of atherosclerosis [100]. This process may be directly stimulated by homocysteine or may be secondary to the mitogenic effect of endothelial and/or platelet-derived growth factors released by homocysteine-induced endothelial cell damage. The signal pathway for the stimulation of DNA synthesis in smooth muscle cells by homocysteine involves cyclins ,cyclin-dependent

kinases, neutrophil docking protein complex CD11b/ CD18 [101] and endothelial expression of monocyte chemoattractant protein-1[102]. Homocysteine causes endothelial cell desquamation, smooth muscle cell proliferation and intimal thickening [103]. High concentrations of homocysteine are also toxic to endothelial cells in vitro [104]. Homocysteine inhibits DNA synthesis in vascular endothelial cells and causes growth arrest at the G1 phase of cell cycle [105]. Pathological concentrations of homocysteine also increase the interaction between neutrophils and endothelial cells. This results in neutrophil migration across the endothelium with concurrent damage and detachment of endothelial cells [106]. Further, homocysteine induces mitogenesis in vascular smooth muscle cells by stimulating MAP kinase signal transduction pathway and by the induction of C-fos and C-myb genes [107].

c) *Effect on the oxidant antioxidant dynamics*:
Homocysteine can undergo auto-oxidation forming hydrogen peroxide (H_2O_2) as a by product. H_2O_2 can then lyse endothelial cells [108]. Homocysteine can oxidize LDL as well as combine with LDL to form thiolated LDL which is taken up by foam cells much faster [109]. Homocysteine has been shown to inhibit intracellular antioxidant enzymes, including glutathione peroxidase thus decreasing the cell's ability to neutralize oxidant radicals [110]. Peroxynitrite is also increased in peripheral blood vessels during hyperhomocysteinemia. Peroxynitrite is known to activate poly ADP-ribose polymerase (PARP), and activation of PARP may be an important mediator of vascular dysfunction. Homocysteine reduces bioavailability of nitric oxide (NO) [111] and causes deterioration of the elastic structure of the arterial wall through alteration in metalloproteinase activity. It may also increase vascular rigidity by augmenting breakdown of elastin in vascular cells [112]. Liao et al have demonstrated that plasma Hcy level has a significant, negative correlation with apoA-I concentration in human CAD [13]. Hyperhomocysteinemia may produce vascular dysfunction and promote oxidative stress by increasing levels of asymmetric dimethylarginine (ADMA), an endogenous inhibitor of nitric oxide synthase (NOS). ADMA also inhibits basal relaxation in cerebral blood vessels [114].

d) *Effects at the genetic level*:
DNA methylation is a critical component of epigenetic regulation of gene expression, particularly during development. DNA hypomethylation is induced by increase in homocysteine levels. Thus global or selective DNA methylation may contribute to alterations in gene expression and vascular changes during hyperhomocysteinemia [115]. Homocysteine at high concentration also increases the transcription and activity of tissue factor [116]. Homocysteine elicits a DNA damage response in neurons that promotes apoptosis and hypersensitivity to excitotoxins. DNA strand breaks and associated activation of PARP and NAD (nicotinamide adenine dinucleotide) de-

pletion occur rapidly after exposure to homocysteine and precede mitochondrial dysfunction, oxidative stress and caspase activation [117].

Studies evaluating the role of homocysteine in cad

Despite huge literature available, a uniform and widely acceptable view regarding the role of homocysteine has not been established. Medical fraternity is still witnessing differing opinions regarding need to treat hyperhomocysteinemia. The association between homocysteine levels and CAD has been observed more in retrospective studies as compared to prospective studies. Several studies have reported that CAD is associated with high homocysteine levels. In the Framingham Heart Study, and in women enrolled in the Atherosclerosis Risk in Communities (ARIC) study, homocysteine levels were higher in adults with CHD [118,119]. In the British Regional Heart Study, homocysteine levels were significantly (P=0.004) higher in patients with stroke [120]. The frequency of hospitalization for cardiovascular disease was correlated with baseline homocysteine levels, particularly in the oldest age group (hospitalization-rate ratio per 5 µmol/L increase in homocysteine: 1.29 versus 1.10; probability value for interaction, 0.02) [121].

A meta-analysis of cross-sectional epidemiological studies has suggested that a 5µM increment in plasma homocysteine is associated with 60% higher incidence of ischemic heart disease [122]. The mean tHcy concentration in plasma samples from 271 subjects who developed a myocardial infarction during the 5 years of follow up were significantly higher than those of 271 matched controls[123].Hyperhomocysteineimia is associated with more severe and diffuse coronary atherosclerosis[124]. Plasma homocysteine level is the strongest predictor of mortality in a prospective study of patients with angiographically confirmed coronary artery disease and previous myocardial infarction. The risk of death was increased 4.5 fold when homocysteine was> 20 µM as compared to < 9 µM [125].

The Homocysteine Studies Collaboration combined evidence from 12 prospective and 18 retrospective studies conducted during 1966 to 1999. A total of 5,073 CAD events and 1,113 stroke events were observed among 16,786 healthy individuals. The results showed that a 25% increase in the serum homocysteine concentration (an increase of approximately 3 µmol/l) is associated with a

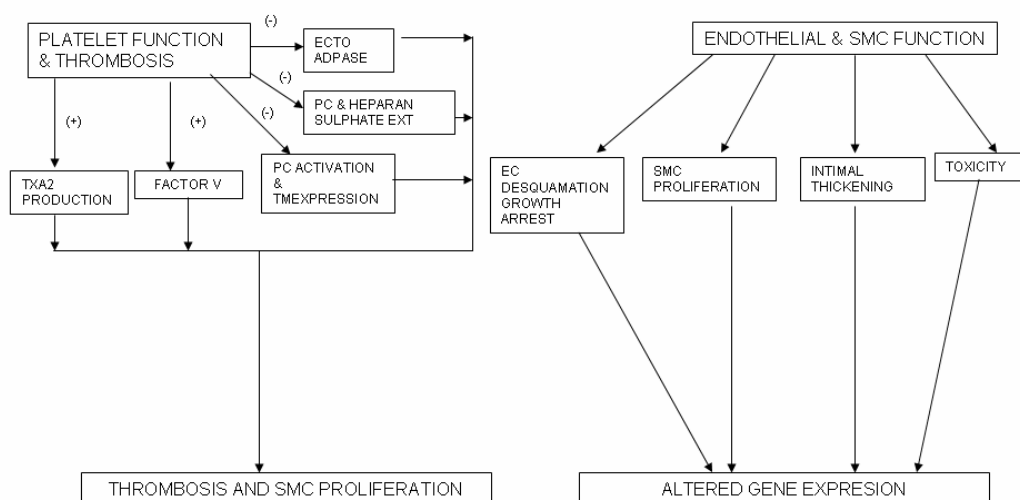49% higher risk of ischemic heart disease (IHD) in the retrospective studies [126].

Homocysteine lowering, achieved after the introduction of the folate food fortification programme in North America, was accompanied by a decline in cardiovascular risk especially of stroke. Although the initial clinical trials suggested that homocysteine-lowering treatment with folates and B vitamins induces coronary plaque regression, this finding was not confirmed by more recent clinical studies [127].

Several large-scale studies totaling nearly 50,000 subjects are currently under way in the U.S., Canada, and Europe-WENBIT (the Western Norway B-vitamin Intervention Trial), SEARCH (Study of Effectiveness of Additional Reduction in Cholesterol and Homocysteine) trial, PACIFIC (Prevention with a Combined Inhibitor and Folate in Coronary Heart Disease) trial, VITATOPS (Vitamins to Prevent Stroke) trial, and others. One will have to await the results from these trial data before finally confirming or refuting the homocysteine hypothesis in atherothrombotic vascular disease [128].

The results of the Physicians' Health Study, the Nurses' Health Study, the European Concerted Action Study, and the Hordaland Homocysteine Study all support the validity of the homocysteine theory of arteriosclerosis [129].Meta-analysis of published studies suggests that elevation of homocysteine is a causal factor in atherogenesis; such studies predicted that lowering homocysteine concentrations would be estimated to benefit ≈15–40% of the population by preventing vascular disease[130].

Humphrey et al concluded from their recent meta analysis in 2008 that homocysteine has a positive predictive value in CAD risk assessment. Meta-analysis yielded a combined risk ratio for coronary events of 1.18 (95% confidence interval, 1.10-1.26) for each increase of 5 micromol/L in homocysteine level. The association between homocysteine and CHD was similar when analyzed by sex, length of follow-up, outcome, study quality, and study design. They concluded that each increase of 5 micromol/L in homocysteine level increases the risk of CHD events by approximately 20%, independently of traditional CHD risk factors [131].

Fig 2: PATHOGENIC MECHANISMS OF HOMOCYTSTEINE

Figure 2.: Pro Thrombotic effects of homocysteine

Fig 3: PATHOGENIC MECHANISMS OF HOMOCYSTEINE
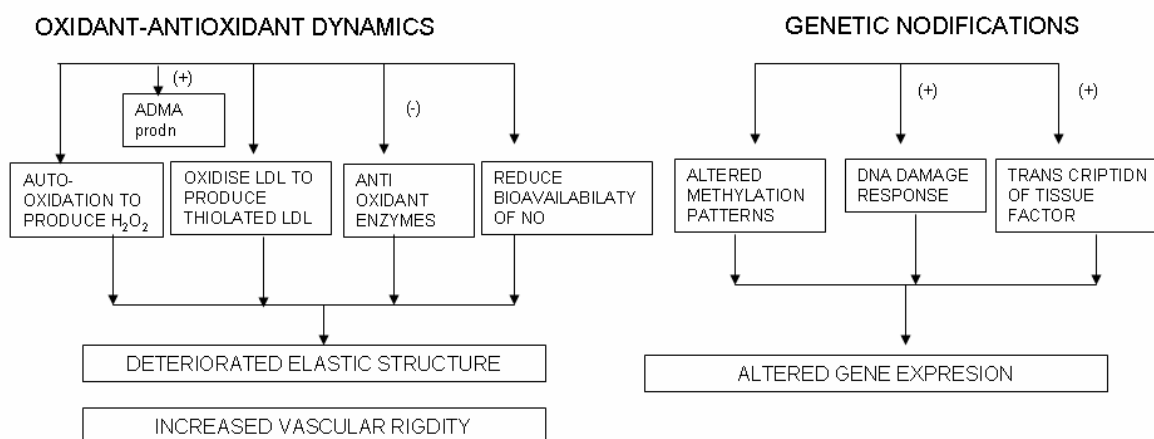
Figure.3. Pro oxidant effects and genetic modifications initiated by homocysteine

However, these positive findings need to be interpreted with caution as some studies have not confirmed the homocysteine theory of atherosclerosis. There is evidence to suggest that homocysteine is released from damaged vascular tissue after myocardial infarction and stroke. Hence, it is often considered that increased homocysteine levels may be an effect rather than cause of CAD It is proposed that during the repair of damaged tissue, there is an increased demand for DNA. These repair processes require the methylation of DNA, RNA and proteins - reactions that lead to the generation of homocysteine as the end point of methylation. Cleophas et al concluded from their meta-analysis that although the data analysed till date demonstrate a strong association between homocysteine and CAD, they do not support the hypothesis that the rela-

tionship is causative. Homocysteine may not be as harmful to the heart as it appears to be, but it may be an important indicator for an unhealthy lifestyle, making it an important variable in assessing patients for CAD risk [132].

Brilakis et al , concluded from their study on 500 patients, that multivariate analysis which included age, gender, smoking, LDL, HDL, Lp(a), apo A1, and apo B revealed no independent association between quartile of homocysteine and odds ratio (OR) for CAD[133].

Indian perspective
Due to a predominant vegetarian diet, Indians are deficient in vitamin B12 and consequently have higher serum levels of homocysteine [134]. There are studies which

support as well as refute the role of this elevated level of homocysteine in the pathogenesis of CAD among Indians. Puri et al evaluated the role of homocysteine in patients of premature CAD. They found that plasma homocysteine was a significant (OR 6.05) independent risk factor for young CAD patients in India [135]. Bhargav et al observed that Hcy is significantly higher in patients of vascular diseases as compared to normal controls [136]. Abraham et al and Rao et al also demonstrated significantly higher homocysteine levels in patients of CAD as compared to healthy controls [137, 138].

Pandey et al concluded from their study conducted on 137 consecutive women who attended a health care program that no positive correlation between elevated hcy levels and CAD was demonstrated in spite of a large percentage of persons showing elevated homocysteine levels [139]. Deepa et al conducted a study analysing the homocysteine levels in Indian diabetic males. They concluded that homocysteine did not play a role in CAD risk assessment in diabetic males [140]. There is a need for further population based studies to evaluate the atherogenecity of homocysteine in the Indian scenario.

Inter-relation between Lp (a) and homocysteine (Figs. 4, 5)

There is a growing body of evidence that atherosclerosis is the final outcome of the complex interplay of a multitude of risk factors interacting through various intertwined molecular mechanisms. Evidence for an interaction between Lp(a) and other established or emerging risk factors for cardiovascular disease, such as LDL cholesterol, high-density lipoprotein cholesterol, and homocysteine, have been found[141-143].

Harpel et al demonstrated an inter-relation between homocysteine and Lp(a) concentrations. He found that homocysteine alters the intact Lp (a) particle so as to increase the reactivity of the plasminogen-like apolipoprotein (a) portion of the molecule. Homocysteine, at concentrations as low as 8micromol/L, significantly increases the affinity of Lp (a) for fibrin. Homocysteine induces a 20-fold increase in the affinity between Lp (a) and plasmin-treated fibrin and a 4-fold increase with untreated fibrin. Thus, the largest effect of homocysteine was on the binding of Lp(a) to plasmin modified fibrin. The dissociation constant (Kd) for the interaction between Lp (a) and plasmin-treated fibrin was 0.18 nM in the presence of homocysteine and 3.67 nM in its absence. The effect of homocysteine on increasing Lp(a) binding to fibrin was proportional to the concentration of the sulfhydryl compound added[144]. This study illustrated the remarkable susceptibility of Lp(a) to sulfhydryl compounds of physiological significance, including homocysteine, glutathione, and cysteine. In the presence of these compounds, the binding of Lp (a) to fibrin is significantly enhanced. The binding of homocysteine-treated Lp (a) to plasmin-modified fibrin was found to be specific since homocysteine did not increase the binding of Lp (a) to other immobilized proteins.

Lp (a) may be altered by homocysteine in individuals with hyperhomocysteinemia, by glutathione in the micro-environment of the thrombus, or by other reductive mechanisms that remain to be established. Limited reduction may produce a reorientation of apo (a) kringle(s) with exposure of additional binding sites not previously accessible to the fibrin surface. Lp(a) particles with enhanced fibrin affinity may prove to be an important link between thrombosis, atherogenesis, and sulfhydryl compound metabolism[145].

Western blot analysis of Lp(a) documented that homocysteine caused a concentration dependent decrease in the amount of Lp(a) migrating at the position of the intact particle, and an increase in free apoB-100 and apo(a). This indicated that homocysteine had reduced the putative disulfide bond between apoB- 100 and apo(a) . In preliminary immunoblot ligand studies, it was found that fibrin degradation products bind to the free apo(a) produced by homocysteine, suggesting that reduced apo(a) loses functional activity[145].

Foody et al carried out a cross-sectional study [146] for baseline characteristics of all patients referred to the Cleveland Clinic Foundation (CCF) Preventive Cardiology and Rehabilitation Program (PCRP) from 1996 to 1998. It was found that when both high homocysteine and lipoprotein (a) were present as risk factors in women, the associated risk was greater than what would be expected if the risk due to these two factors were simply added up. This cross-sectional analysis demonstrates that Lp (a) and tHcy interact to increase the risk of CAD in women. Higher serum concentrations of both factors increase the CAD risk by nearly 5 times. This theory is consistent with the suggestion that apo (a) is the atherogenic moiety of Lp(a), as noted in transgenic mouse models[147].

Ghorbanihaghjo et al observed an association between homocysteine and Lp(a) in the pathogenesis of retinal arteriosclerosis[148]. Nardulli et al demonstrated by immunochemical, ligand-binding and plasminogen activation studies, that homocysteine modifies the structure and function of Lp (a) in human plasma by reducing the apo(a)/apoB disulfide bond causing the appearance of free apo(a) with high affinity for fibrin that inhibits plasminogen binding and plasmin formation . Lp (a) and homocysteine may therefore be considered to be acting in a consortium in order to increase the risk of atherothrombosis [149].

Fig 4: INTERACTION BETWEEN HOMOCYSTEINE AND LIPOPROTEIN (a)
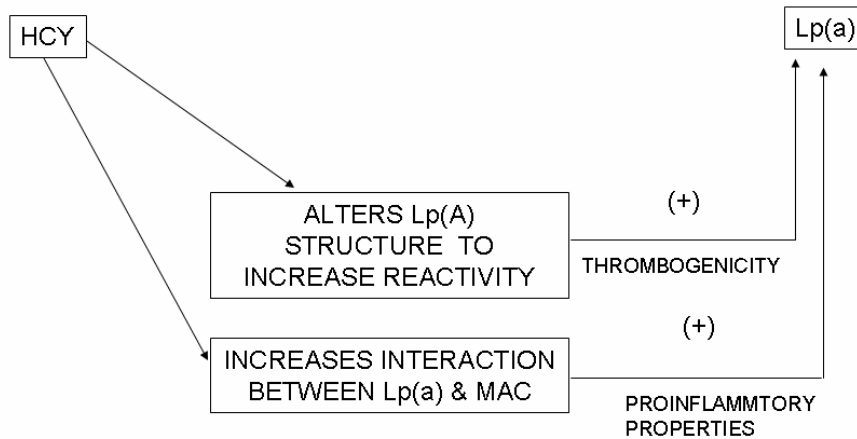
Figure 4. Interaction between lipoprotein (a) and homocysteine

Fig 5: INTERACTION BETWEEN HOMOCYSTEINE AND LIPOPROTEIN (a)
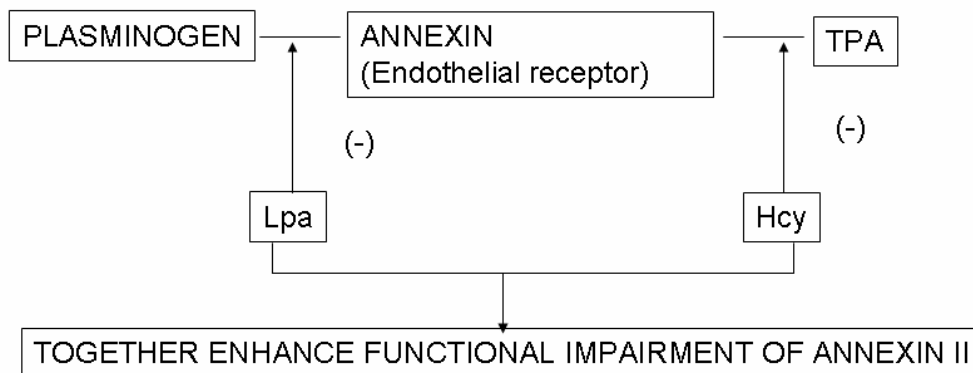
Figure 5. Interaction between lipoprotein (a) and homocysteine in altering annexin functions

Sotiriou et al also evaluated the interaction between homocysteine and Lp(a).They found that proatherogenic factor, homocysteine, can augment the interaction between Lp(a) and Mac-1 integrin, potentiating the proinflammatory action of Lp(a). Lp (a) and homocysteine synergize to increase the risk for coronary artery disease: when both risk factors are present, the associated risk is greater than what would be expected if these two risk factors were acting independently. Their data showing that homocysteine enhances the proinflammatory action of Lp(a) may provide a novel clue as to how the interaction of these two risk factors increases cardiovascular risk[150].

The interaction of Lp(a)/apo(a) with the ternary complex is attributed in part to lysine binding sites within the kringle 4-like domains of apo(a). Thus, the interaction of apo(a) with Mac-1 shares similarities with the antifibrinolytic activity of apo(a). The fact that smaller isoforms of Lp(a) have been recognized as a risk factor for coronary heart disease independent of plasma Lp(a) concentrations has been partially attributed to the fact that smaller apo(a) isoforms bind more avidly to fibrin and are more effective in the inhibition of plasmin formation. Sotiriou's work describes a novel proinflammatory function of Lp(a)/apo(a) that directly interacts with Mac-1 and mediates inflammatory cell recruitment as well as concomitant up-regulation of the prothrombotic tissue factor. The proinflammatory mechanism of Lp(a) potentiated in the presence of homocysteine improves our understanding of the proatherogenic potential of these emerging cardiovascular risk factors.

Recent evidence indicates a potential role for the plasmin/plasminogen activator system in the prevention of atherosclerotic vascular disease. Fibrin deposition is a common histologic feature of the tissues of mice that are genetically deficient in one or more key components of the fibrinolytic system. Cell surface receptors may support fibrinolytic surveillance in both intravascular and

extravascular locations by stimulating the efficiency of plasmin generation and by protecting plasmin from its inhibitors. In vitro studies suggest that the endothelial cell receptor, annexin II, which independently binds both plasminogen and t-PA, could play a key role in the process. Binding of plasminogen to annexin II is specifically inhibited in the presence of excess concentrations of the atherogenic LDL-like particle Lp(a). Similarly, t-PA binding to annexin II is blocked by homocysteine, a sulf-hydryl-containing amino acid that is associated with atherogenesis and that directly derivatizes the t-PA binding domain of annexin II. The presence of both the risk factors, thus, accentuates the functional impairment of annexin II [151]. Hopkins et al [152] analyzed plasma Lp(a), lipids, and other coronary risk factors in a case-control study of men and women with premature athero-sclerosis. The relative risk for CAD in patients with Lp(a) values >40 mg/dL was 2.9. An Lp(a) level >40 mg/dL and high tHcy resulted in an OR of 32, with a CI between 6.5 and 155.

These findings support the hypothesis that tHcy and Lp (a) interact to increase the risk of CAD. A high tHcy level may act in concert with a high Lp (a) level to promote atherosclerosis and or vascular disease. These data provide an interesting hypothesis-generating finding regarding the differential interactive effects of 2 emerging cardiovascular risk factors and may have important implications for the prevention and treatment of CAD in select high-risk populations.

In contrast, in a prospective analysis of young men with premature peripheral atherosclerosis (N=95), Valentine et al [153] reported no significant interaction between Lp(a) and tHcy in defining risk of CAD. Other studies have also reported non existence of any synergism between homocysteine and Lp (a) [154,155] In one study, high serum total homocysteine (tHcy) and lipoprotein (a) [Lp(a)] levels were found to be independent risk factors for cardiovascular disease [154]. An investigator in another study found a positive correlation between the levels of homocysteine and those of Lp(a) (Spermann's rho = 0.54, p < 0.001) but their effect on coronary heart disease was found to be independent [155].

The above mentioned studies highlight the need for further studies to validate or refute Harper's hypothesis that suggested a synergestic interaction between the two risk factors -homocysteine and Lp (a) in atherothrombosis. It may provide an important link between thrombosis, atherogenesis and sulphhydryl compound metabolism.

Perspective
Homocysteine and Lipoprotein (a) are two well known risk factors for atherothrombosis. To the best of our knowledge, ours is the first broad review that attempts to evaluate the inter relationship between these two risk factors. The association between these two can be further potentiated by extensive meta analysis of the studies that have attempted to evaluate this intricate relationship. A randomised control trial that evaluates the effect of medication on homocysteine and Lp (a) and their further effect in ameliorating or attenuating CAD risk , is the need of the hour.

Last but definitely, not the least, such elaborate research and studies should be helpful to the general population and this can be accomplished by the development of drugs that can manage the multiple aetiologies effectively in a comprehensive manner.

Conclusion

The current understanding of atherosclerosis is as a chronic inflammatory process, developing in response to some metabolic disorders (dyslipidemia, insulin resistance), infections and environmental processes that initiate and promote lesion development to the point of acute thrombotic complications. The growing knowledge about the interplay between homocysteine and lipoprotein (a) has many far reaching implications. Their synergism calls for more stringent treatment modalities in subjects having high homocysteine as well as lipoprotein (a) levels.

Understanding the complex interplay between the various risk factors will be very beneficial in devising lifestyle modifications and pharmacological interventions to deal with CAD, a disease that has assumed epidemic proportions.

Abbreviations:
ADMA – Dimethyl arginine
AMI- Acute Myocardial Infarction
CI- Confidence Interval
CAD- Coronary Artery Disease
CVD- Cardiovascular Disease
EC- Endothelial Cell
HDL- High Density Lipoprotein
LDL- Low Density Lipoprotein
NAD- Nicotinamide Adenine Dinucleotide
NO- Nitric Oxide
NOS- Nitric Oxide Synthase
OR- Odds Ratio
PAOD- Peripheral Artery Occlusive Disease
PARP- Poly ADP Ribose Polymerase
PC- Protein C
SMC- Smooth Muscle Cell
TM- Thrombomodulin
TPA- Tissue Plasminogen Activator
TXA2- Thromboxane A2

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Correspondence:

Binita Goswami
B 406 Panchsheel apartments
Plot 9, Sector 10, Dwarka
Delhi 110075
India