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MINIREVIEW

Apolipoproteins and Atherosclerosis. Apolipoprotein E and Apolipoprotein(a) as Candidate Genes of Premature Development of Atherosclerosis

B. HOŘEJŠÍ, R. ČEŠKA

Third Medical Department, First Faculty of Medicine, Charles University and General Faculty Hospital, Prague, Czech Republic

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Summary

Apolipoprotein E (apoE) is a plasma lipoprotein which plays a basic role in the degradation of particles rich in cholesterol and triglycerides. It is able to bind to LDL receptors, but also to receptors for chylomicron remnants. There are three major apoE isoforms, E2, E3, and E4. Their role in lipoprotein metabolism is related to their affinity for receptors. Allele E3 is predominant and apoE3 affects metabolism of lipoproteins in a standard way. When compared to allele E3, allele E2 is associated with lower LDL levels, whereas allele E4 with higher LDL levels. This has an impact on the progression of atherosclerosis. Allele E2 exhibits a protective role, whereas allele E4 is associated with a high risk factor. Lipoprotein(a) [Lp(a)] is a plasma lipoprotein, consisting of apolipoprotein(a), linked by a covalent bond with the LDL particle. Increased Lp(a) levels are associated with an increased incidence of diseases based on atherosclerosis, namely the ischemic heart disease. Another effect of Lp(a) is its competition with plasminogen, resulting in a decrease of fibrinolysis and thrombogenic activity. ApoE and Lp(a) are independent risk factors for premature development of atherosclerosis and therefore can be considered as candidate genes of premature atherosclerosis.

Key words

Atherosclerosis • Apolipoprotein E • Lipoprotein(a)

Atherosclerosis, risk factors and pathogenesis

Atherosclerosis is one of the major health problems in developed countries. It is a degenerative process of the vascular wall, resulting in the following cardiovascular diseases: ischemic heart disease, atherosclerotic occlusive disease of the lower extremities, ischemic cerebrovascular attacks and other organ damage according to the localization of vascular atherosclerotic changes. Atherosclerosis is defined as heterogeneous combined changes of the intima, the inner vascular layer. During this process, lipid accumulation and rebuilding of intercellular matrix occur with a predominance of fibrous tissue in the medial layer of the vascular wall. This

Fax +420 2 249 20 590
E-mail: physres@biomed.cas.cz
http://www.lf2.cuni.cz/physiolres/physiolres.htm

is caused by metabolic abnormalities, altered structural properties of the vascular wall, hemodynamic factors and the changes in particular blood components.

We distinguish the following modifiable risk factors: hyperlipoproteinemias (HLP), arterial hypertension, diabetes mellitus, smoking and thrombogenic factors, whereas non-modifiable risk factors include age, sex, positive family history of cardiovascular diseases and genetic disposition of the individual.

The general mechanisms of atherosclerosis include the proliferation of smooth muscle cells and macrophages, production of connective tissue matrix by smooth muscle cells (elastic filaments, collagen, proteoglycans) and accumulation of lipid particles including free and esterified cholesterol, particularly in the foam cells.

The development of atherogenesis is a gradual process from endothelial dysfunction and thickening of the vascular intima to the formation of fatty streaks and atheromatous plaques, which may progress to complicated plaques (by spasms, thrombosis, ulceration or calcification).

Apolipoprotein E

Apolipoprotein E (apoE) was first described by Shore and Shore (1973) as a part of VLDL (very low density lipoproteins) particles. Later, its structure, polymorphism and association with hyperlipoproteinemias were described, as a part of beta-VLDL. The effect of apoE on lipoprotein metabolism, receptor function and, recently on physiological reparative processes in the central nervous system (CNS) and their association with Alzheimer´s disease have become a subject of investigation (see below).

ApoE structure

Apolipoprotein E is a polypeptide, composed of 229 amino acids which binds to various types of lipoproteins. It is mostly bound with VLDL particles and chylomicrons, less frequently with HDL (high density lipoprotein) cholesterol. Approximately one third of its molecule, called the N-terminal, enables binding to LDL (low density lipoprotein) receptor; the domain for this binding being located between amino acids 130 and 150. ApoE is a ligand of both an LDL receptor and receptor for chylomicron remnants. The domain of apoE for this receptor has not yet been identified (Mahley and Rall 1999, Herz *et al.* 1988).

Three apoE isoforms are distinguished, which differ in the substitution of one amino acid. Their determination is genetically coded by various alleles for apoE. Amino acids on positions 112 and 158 are essential for distinguishing the apoE type. An amino acid substitution in the apoE2 molecule results in a decrease of LDL receptor affinity up to 1% of the affinity of other apoE isoforms. Even with a change in position 158, i.e. not directly in the domain for LDL binding (containing amino acids 120 to 150), the alpha helix structure of apoE molecule is altered and, consequently, so is its binding ability.

The most frequent type is apoE3 (relative frequency about 0.78). ApoE4 has a relative frequency 0.14 and the frequency of apoE2 is 0.08. With regard to the relative frequencies of alleles, geographical or ethnic differences have to be taken into account.

ApoE gene

The apoE gene is localized on the long arm of chromosome 19. Unlike other apolipoproteins, apoE is synthesized not only in the liver, but also in the brain (astrocytes), spleen, lungs, kidneys, smooth muscle cells and ovaries, but not in the intestinal epithelium (Mahley 1988). Due to the genetic polymorphism, there are several apoE isoforms coded by alleles of the human genome. Three major isoforms (E2, E3, and E4) are encoded by three alleles (epsilon 2, epsilon 3 and epsilon 4) of the apolipoprotein E gene. Considering all combinations of alleles, there is a possibility of six genotypes: E2/2, E2/3, E3/3, E3/4, E4/4 and E2/4.

ApoE metabolism

ApoE has an effect on the degradation of lipoproteins and, consequently, on plasma lipid levels. ApoE is a ligand of the LDL receptor, also called apo B/E receptor. The LDL receptor has a key position in the degradation of lipoprotein particles – LDL. ApoE has an even higher affinity for the LDL receptor than apolipoprotein B (apoB) itself. Another specific receptor for apoE binding is the receptor for chylomicron remnants, also called the LDL receptor-related protein (LRP). ApoE binds chylomicron remnants rich in triglycerides with the help of this receptor; they enter the circulation mainly by the alimentary route. While the LDL receptor (apo B/E receptor) is able to bind apoE and apoB-100 binding terminals, the receptor for remnants (LPR) can bind apoE only.

After synthesis in the liver, apolipoprotein E is bound in VLDL particles. The nascent synthesized VLDL contain one molecule of apoB, large amounts of apolipoprotein C (apoC) and a small quantity of apoE. During degradation, mediated mainly by lipoprotein lipase, the lipoprotein content in the particle changes. The apoE

proportion in IDL particles rises and the proportion of apoC decreases. The final LDL particles contain apoB-100 only.

As far as the apoE participation in the metabolism of chylomicrons is concerned, the chylomicrons from the ingested food, absorbed in the intestine, are transported to the liver. During this transport in the circulation they are subjected to lipoprotein lipase which degrades them to chylomicron remnants. Before entering the liver, the chylomicron remnants are enriched with apolipoprotein E that mediates the binding of chylomicron remnants (Hui *et al.* 1984).

All three apoE isoforms have different effects on the metabolism of lipoproteins. The predominant isoform in the population is allele E3, the most common genotype E3/3. ApoE3 has a standard effect on lipids metabolism. On the other hand, apoE2 and E4 affect the receptors in a different way and for this reason they differ in their effect on plasma lipid levels.

Allele E2 is associated with lower LDL levels, whereas allele E4 with higher LDL levels, when compared with allele E3. Despite the higher affinity of apoE4 to the LDL receptor, early "receptor occupation" and subsequent accumulation of LDL particles occur, accompanied by increased absorption of chylomicron particles from the intestinal lumen. Due to the changed apoE structure, the receptor affinity of apoE2 is lower, but an up-regulation is present so that the final clearance of the particles is higher. It is known that apoE2 is associated with lower LDH cholesterol plasma levels.

ApoE and type III hyperlipoproteinemia

The occurrence of apoE2 is often associated with type III hyperlipoproteinemia according to Fredrickson or with dysbetalipoproteinemia. The latter is characterized by the dysbeta fraction in electrophoresis and high plasma triglyceride levels, occurring mainly in the VLDL fraction (Utermann *et al.* 1977).

The low binding apoE2 activity to receptors decelerates the catabolic change of chylomicrons, VLDL and of remnant particles thus increasing their content in the plasma. Moreover, the enhanced activity of hepatic LDL receptors lowers LDL-cholesterol concentrations and increases HDL-cholesterol concentrations in the plasma. The presence of allele E2, even in homozygous E2/2 form, does not necessary imply type III hyperlipoproteinemia, and *vice versa*. This type of hyperlipoproteinemia may occur in patients with allele E3 or E4, but such an association is substantially lower.

The development and especially the manifestation of this type of hyperlipoproteinemia also depend on genetic and hormonal factors as well as on alimentary influences or the nutritional state. The manifestation of type III hyperlipoproteinemia is also affected by thyroidal function. Hypothyroidism is relevant for type III hyperlipoproteinemia expression. This is explained by the effect of thyroidal hormones on lipoprotein uptake in the liver and, in case of their insufficiency, to the decreased expression of LDL receptors (Hazzard and Bierman 1972, Thompson *et al.* 1981). Similar observations were made in our department (Kršek *et al.* 1999).

Another factor influencing the manifestation of the disease, is the hormonal estrogen disposition of individual patients. It is related to the ability of estrogens to affect LDL receptors, i.e. to their up-regulatory activity. In premenopausal women, the type III hyperlipoproteinemia occurs very rarely; the manifestation of this genetic predisposition occurs during the postmenopausal period. When estrogen replacement is introduced in the postmenopausal period, laboratory parameters of this hyperlipoproteinemia improve dramatically (Falko *et al.* 1979).

Food intake pattern is another factor, which may affect manifestations of the disease. An excess intake of energy results in lipoprotein overproduction and their accumulation in the plasma. Therefore, the diet is an important part of the treatment of type III hyperlipoproteinemia.

ApoE and atherosclerosis

Due to the different effects of individual apoE isoforms on the metabolism of lipoproteins, there is a direct relation between the apoE isoform and manifestations of premature atherosclerosis (Srinivasan *et al.* 1999).

This topic has been the subject of a number of publications. The main issue concerns the association between apoE isoforms and the occurrence of ischemic heart disease. The presence of allele E2 (with the exception of the association with type III hyperlipoproteinemia) is considered to be a protective factor against premature atherosclerosis symptoms, compared with the presence of allele E3 and particularly E4. The risk of ischemic heart disease depends on the occurrence of allele E4 which is connected with an increased risk of ischemic heart disease, whereas the allele E2 has a contrary effect (Kogawa *et al.* 1997, Cattin *et al.* 1997).

ApoE and neurobiology: Alzheimer's disease

Particular apoE isoforms seem to be associated with the occurrence of Alzheimer's disease. It is known that the physiological role of apoE includes the reparative functions in the central and peripheral nervous system.

Recent studies have shown a higher incidence of Alzheimer's disease in patients with the apoE4 allele, in comparison with E3 or E2 alleles (Hofman *et al.* 1997).

ApoE4 does not have such effective reparation abilities as apoE2 or E3. Deteriorated remodeling and reparation of specific classes of neurons may lead to neuronal degeneration and possible development of Alzheimer´s disease.

Lipoprotein(a)

Lipoprotein(a) [Lp(a)] is a lipoprotein that is rich in cholesterol esters. Its protein component is apolipoprotein(a) [apo(a)]. This molecule was discovered in 1963, but it did not arouse attention until 25 years later, when the association between apo(a) and ischemic heart disease was observed. A number of studies have investigated this relationship and, more generally, the association between Lp(a) and the premature manifestation of atherosclerosis (Nachman 1997, Gaw *et al.* 1999).

Lp(a) structure

Lipoprotein(a) consists of two components: a modified form of LDL (low density lipoproteins) and a hydrophilic glycoprotein – apolipoprotein(a). The basis of the apo(a) structure is apolipoprotein B-100 (apoB-100), which is linked by a disulphidic covalent bond with the modified LDL particle. The lipid composition of Lp(a) is similar as in the LDL particle, but the Lp(a) contains more glycosylated remnants. In electrophoresis, Lp(a) moves in the agar as a slow pre-beta fraction.

Lp(a) genetics

The structure of Lp(a) is markedly similar to that of plasminogen, a key component of the fibrinolytic system, which participates in the homeostasis of coagulation processes. The plasma concentration of Lp(a) is determined genetically. The gene for apo(a), is localized at the end of chromosome 6 (Malgaretti *et al.* 1992), near the gene for plasminogen. The gene exists in several isoforms – so far 34 of them have been discovered (Lackner *et al.* 1993). Apolipoprotein(a) is the major genetic determinant of the plasma concentration of lipoprotein(a). This is because the specific isoforms determine the different sizes of apo(a) particles, ranging from 300 to 850 kD. There is a negative correlation between the molecular weight of the particles and the plasma concentration of Lp(a).

Lp(a) metabolism

Lp(a) arises by the following mechanism: initially, the non-covalent binding between the free cystein of the apo(a) molecule and the disulphidic-linked cystein of the apolipoprotein B-100 C-terminal occurs. A mutation of the C-terminal of apoB-100 can affect the affinity of the binding between apo(a) and apoB-100 (Durovic *et al.* 1994). As an example, a mutation leading to familial apoB-100 defect (absence of an amino acid in position 3500) represents one of the possible etiological mechanisms of hypercholesterolemia. Heterozygotes of the familial apoB-100 defect have a higher variability in the plasma Lp(a) levels than other individuals, caused by their higher sensitivity to changes in the arrangement of the LDL particles (Hoek *et al.* 1997). Very low Lp(a) levels are found in patients with a lecithin-cholesterol acetyltransferase (LCAT) deficiency. This is because of the abnormality of their LDL particles, explained by insufficient cholesterol conversion to cholesterol esters due to LCAT deficiency. Cholesterol esters are responsible for the hydrophobic properties of LDL particles, The covalent binding between apo(a) and particles with such an abnormality is difficult and that is why Lp(a) plasma levels are low.

As far as Lp(a) clearance is concerned, it was shown that plasma Lp(a) levels are much more dependent on Lp(a) synthesis than on its degradation. The clearance of plasma Lp(a) *via* LDL receptors depends on particle size, LDL binding and apo(a). *In vivo* studies have proved that the LDL receptor does not predominate in Lp(a) catabolism. Drugs that have an effect on LDL receptor activity, e.g. statins or bile acid-binding resins, do not influence the plasma Lp(a) concentration. Similarly, hypothyroidism, characterized by decreased LDL receptor activity and increased plasma LDL particle levels, does not cause higher Lp(a) levels. Furthermore, Lp(a) levels in familial hypercholesterolemia have been studied with respect to the present functional alteration of the LDL receptor. Some studies have indicated an increased Lp(a) concentration in patients with familial hypercholesterolemia (FH) (Utermann 1989), whereas this was not observed by Hegele *et al.* (1990). There was also no association between Lp(a) and leptin (Haluzík *et al.* 1999). The assumed physiological significance of Lp(a) is its role in healing processes and tissue regeneration.

Factors affecting the plasma Lp(a) concentration

A normal Lp(a) level ranges from undetectable values to 0.3 g/l. The individual levels increase with age and are similar in both women and men. In pre-menopausal women, the concentrations are lower, whereas in the post-menopausal period they increase by about 15-20 % (Jenner *et al.* 1993). The Lp(a) plasma concentration is relatively stable and is not influenced by the body mass or food patterns, although the consumption of monounsaturated fatty acids lead to an increase. On the contrary, decreased Lp(a) levels are

associated with excessive alcohol consumption, as soon as alcoholic hepatopathy develops. Their levels do not correlate with concentrations of other lipoproteins. Only highly increased Lp(a) levels may elevate the total or LDL-cholesterol values (cholesterol esters form only 1/30 of the Lp(a) structure).

Significantly higher Lp(a) values are present in chronic renal insufficiency. After kidney transplantation, the value decreases by about 50 %. Interestingly, a Lp(a) decrease can be seen after heart transplantation, probably due to immunosuppressive therapy needed in both types of transplantation.

As has already been mentioned, thyreopathy also affects the Lp(a) levels. Therapeutic hormonal substitution of hypothyroidism decreases plasma lipoprotein(a), whereas antithyroidal drugs elevate its plasma concentrations.

Lp(a) and atherosclerosis

The first papers about the relationship between plasma Lp(a) and atherosclerosis appeared soon after Lp(a) discovery in 1965. Kostner *et al.* (1981) compared the group of patients with ischemic heart disease (after a myocardial infarct without hyperlipoproteinemia as a risk factor) with control healthy subjects matched by age and sex. The plasma Lp(a) levels were significantly higher in patients after myocardial infarct so that the lipoprotein(a) concentration in the plasma has been recognized as an independent coronary risk factor, separately from associated hyperlipoproteinemia. Subsequent studies have shown that this association is particularly valid in younger patients.

Therefore, Lp(a) elevation is a separate risk factor, independent of the concentration of other lipoproteins. If these factors predisposing to atherosclerosis development are present simultaneously, the risk rises significantly.

According to the above information, it might be supposed that a high Lp(a) level is associated with increased risk of the coronary disease. Nevertheless, the results of prospective studies are controversial. For example, the Helsinki Heart Study did cast doubts upon this conclusion. There were no differences in plasma Lp(a) concentration in patients who sustained myocardial infarct during the follow-up, and those who were without a coronary event (Jauhiainen *et al.* 1991).

The relationship between plasma lipoprotein(a) levels and atherosclerosis has been studied in different vascular beds including the coronary one. Schreiner *et al.* (1996) described a close association between the elevated Lp(a) and the atherosclerosis of cerebral vasculature or peripheral arteries.

Using the ultrasonographic method, the width of the intima in carotid arteries is monitored in relation to plasma Lp(a) levels. The results obtained by some authors are controversial. Several studies have suggested a significant association between plasma Lp(a) and intima width in arteria carotis communis (Schreiner *et al.* 1996, Baldassarre *et al.* 1996). In contrast, our study indicates a negative correlation between these values (Hořejší *et al.* 1999). To same results were also presented by Bonithon-Kopp *et al.* (1996).

The *in vitro* experiments dealt with the mechanism of endothelial alterations. Lp(a) contributes to the transformation of foam cells in sclerotic lesions. The competition between Lp(a) and plasminogen can also be demonstrated *in vitro* after plasminogen activation by tissue plasminogen or streptokinase. Lp(a) is bound to fibrinogen or fibrin. The interference between Lp(a) and plasminogen results in proliferation of smooth muscle cells in the vascular wall. The explanation is based upon the inhibitory effect of activated plasminogen on the generation of transforming growth factor β, which is an antimitogen for smooth muscle cells (Kojima *et al.* 1991).

Conclusions

ApoE isoforms have different effects on the metabolism of lipoproteins and there is a direct relation between these isoforms and premature atherosclerosis manifestations. High plasma concentrations of Lp(a), which are genetically determined, represent an independent risk factor for coronary heart disease, independent of plasma lipid concentrations.

These genes for apolipoprotein E isoforms and the gene encoding plasma lipoprotein(a) are considered as the candidate genes for premature development of atherosclerosis. Their determination can play a role in the estimation of coronary risk.

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Reprint requests

Dr. B. Hořejší, Third Internal Clinic, First Faculty of Medicine, U nemocnice 1, 128 21 Prague 2, Czech Republic.
e-mail: brohe@post.cz