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MINIREVIEW

Lipoprotein(a), Atherosclerosis, and Apolipoprotein(a) Gene Polymorphism

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High plasma lipoprotein(a) [Lp(a)] levels have been implicated as an independent risk factor for coronary artery disease in Caucasians, Chinese, Africans, and Indians. Apo(a) that evolved from a duplicated plasminogen gene during recent primate evolution is responsible for the concentration of Lp(a) in the artery wall leading to atherosclerosis, by virtue of its ability to bind to the extracellular matrix and its role in stimulating the proliferation and migration of human smooth muscle cells. Several types of polymorphisms, size as well as sequence changes both in the coding and regulatory sequences, have been reported to influence the variability of Lp(a) concentration. Apo(a) exhibits genetic size polymorphism varying between 300 and 800 kDa that could be attributed to the number of k-4 VNTR (variable number of transcribed kringle-4 repeats). An inverse relationship between Lp(a) level and apo(a) allele sizes is a general trend in all ethnic populations although apo(a) allele size distribution could be significantly variable in ethnic types. A negative correlation between the number of pentanucleotide TTTTA$_n$ repeat (PNR) sequences in the regulatory region of the apo(a) gene and Lp(a) level has also been observed in Caucasians and Indians, but not in African Americans. However, a significant linkage disequilibrium was noted between the PNR number and k-4 VNTR. In order to correlate the role of apo(a) gene polymorphisms to apo(a) gene regulation, we have proposed that liver-specific transcriptional activators and repressors might contribute to the differential expression of apo(a) gene, in an individual-specific manner.

The role of serum lipoprotein(a) [Lp(a)] level in the pathogenesis of coronary artery disease (CAD) was first described by Berg in 1963 (1). The plasma Lp(a), a low-density lipoprotein-like particle containing one molecule of apo(a) and apoB, varies in the human population, ranging from <0.1 to 100 mg/dl (2). Apo(a) is synthesized in the liver and the linking of this protein with apoB might take place either intracellularly or extracellularly, with both events being possible depending upon the metabolic state of the individual (3). The high plasma Lp(a) levels have been implicated as a major independent risk factor for premature coronary artery diseases (4–6), cerebrovascular disease (7), and restenosis of coronary lesions (8).

HERITABILITY

The Lp(a) levels are highly heritable (1) but remain remarkably constant in an individual over time (9). In Caucasians and Chinese, the distribution serum of Lp(a) level is highly skewed toward lower levels (1,2,10,11) whereas in Africans and African Americans the Lp(a) levels are two- to threefold higher and the distribution curve of Lp(a) levels has a more Gaussian type (11–17). The observed modes showing skewing of African American Lp(a) concentration to lower levels might be due to the genetic admixture as 25% of the genes in African Americans are Caucasian in origin (18). The Lp(a) levels were significantly higher ($P < 0.001$) in North Indian CAD patients than in controls (19). In all populations studied to date there has been no

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significant difference between Lp(a) levels in men and women. The one exception is in postmenopausal women where the Lp(a) level is increased by an average of 15% (20).

PATHOGENIC MECHANISMS

Lp(a) is concentrated in the artery wall by virtue of binding to fibrin, plasminogen receptors, matrix, and other targets (21–24). As an inactive homolog of plasminogen, apo(a) competes for the binding and activation of plasminogen and interferes with clot lysis (2,25–27). The inhibition of plasminogen activation and the prolonged presence of thrombus on the vessel wall may promote the growth and migration of smooth muscle cells and the development of atherosclerotic lesions through several intermediate pathways (28,29).

The close homology of apo(a) to plasminogen has raised the question of whether Lp(a) is associated with an increased tendency to thrombosis, because of competition with plasminogen for its binding sites on endothelial cells and fibrin. As the Lp(a) competes with plasminogen *in vitro,* it was suggested that the binding of apo(a) to proline and hydroxyproline could be responsible for the binding of apo(a) to the extracellular matrix such as collagen and elastin that are rich in proline and hydroxyproline (30).

Further, tissue transglutaminase binds to Lp(a) (31) whereas Lp(a) binds to macrophages (32). This suggests that apo(a) leads to an increased presence of oxidized lipids in the artery that could be atherogenic in nature.

It has also been shown that Lp(a) as well as recombinant apo(a) might stimulate the proliferation and migration of human smooth muscle cells (29). This activity is due to the inhibition of plasminogen activation by apo(a), and the subsequent plasmin-dependent activation of latent TGF-β.

EVOLUTION

The existence of the Lp(a) particle and the apo(a) protein are restricted to Old World monkeys, apes, and humans, although most mammals lack apo(a) (33–36). In humans, the apo(a) and plasminogen genes are separated by about 50,000 bp on chromosome 6, and are flanked by two other extremely similar genes or pseudogenes (37–41).

Interestingly, hedgehogs produce an apo(a)-like protein composed of highly repeated copies of plasminogen k-3 like domain, with complete absense of the protease domain sequence (34). DNA sequence comparison and phylogenetic analysis indicate that the human type apo(a) gene evolved from a duplicated plasminogen gene during recent primate evolution. In contrast, the k-3-based type of apo(a) evolved from an independent duplication of the plasminogen gene approximately 90 million years ago (the primitive extant mammal hedgehog whose ancestors diverged from other orders of placental mammals) (42–44). It was proposed that in a type of convergent evolution, the plasminogen gene has probably been independently remodeled twice during mammalian evolution to produce similar forms of apo(a) in two widely divergent groups of species (42). It appears that the primate and hedgehog of apo(a) genes evolved independently by duplication and modification of different domains of the plasminogen gene, providing a novel type of convergent molecular evolution. It has been observed that the primate and hedgehog apo(a) represent a remarkable case of independent, parallel gene assembly, utilizing a common genetic precursor, the plasminogen gene (34).

POLYMORPHISMS

It has been hypothesized that the polymorphisms of the apo(a) gene other than size could contribute to the increase of Lp(a) level in coronary heart disease (CHD) patients as 90% of the variance in Lp(a) levels has been suggested to be attributable to the apo(a) locus. Several types of polymorphisms, size as well as sequence changes in the apo(a) gene, both in the coding as well as in the regulatory regions, have been reported which influence the variability of plasma Lp(a) concentration (3,45). The apo(a) gene was implicated as a major determinant of Lp(a) levels in individuals when it was noted that the apo(a) glycoprotein varied in size over a wide range and that its size tended to be inversely related to plasma concentration of Lp(a) (13,46).

Kringle Repeats

Apo(a) exhibits genetic size polymorphism with over 30 alleles varying in mass between 300 and 800 kDa and the number of k-4 VNTR (variable number of transcribed kringle-4 repeats) in apo(a) alleles varies from 12 to 51 (47). This size/mass variability is related to the number of k-4 type 2 repeats (3 to 42 kringles) of apo(a) (48). The other classes of kringle-4, i.e., kringle-1 and kringle 30–37, are usually

present in apo(a) as a single copy (49). This is also the case with kringle-5 (48).

An inverse relationship between apo(a) allele sizes and Lp(a) level has been a general trend. Apo(a) alleles containing fewer k-4 repeats tend to be associated with high Lp(a) level but they are also associated with little or no detectable plasma apo(a).

In both Caucasian and Chinese, the apo(a) allele containing more than 28 k-4 repeats is associated with low Lp(a) (50). The apo(a) allele frequencies and phenotype distributions differed significantly between Blacks and Whites ($P < 0.0001$). Blacks had a higher relative frequency of the intermediate allele (k-$4_{22–28}$) whereas Whites had a higher relative frequency of the small alleles (k-$4_{17–24}$) and large alleles (k-$4_{29–33}$). However, the frequency of the null allele was low in Blacks (1.0%) in comparison to Whites (6.7%). For large polymorphs (>31 k-4 repeats) both Blacks and Whites exhibited uniformly low Lp(a) levels. However, for the small apo(a) size (<k-4_{20}) both Blacks and Whites exhibited high median Lp(a) levels and a wide variation of Lp(a) level (11). In American Blacks, some very large alleles associated with significant amounts of Lp(a) concentrations are inversely related to the number of k-4 repeats in the apo(a) alleles.

Although Caucasians and Blacks had very different distributions of Lp(a) concentrations, there was no significant difference in the overall frequency distribution of their apo(a) alleles. Caucasians and Chinese had similar Lp(a) concentration but significantly different apo(a) allele size distributions. In the North Indian population, an inverse relation was also observed between Lp(a) level and apo(a) isoform size ($k_{12–50}$) (19). It is striking that the relationship between apo(a) allele size and plasma Lp(a) level was similar in Caucasians, African Americans, and Chinese (51). The relationship between apo(a) allele size and Lp(a) level was almost identical in other reports (11,12,14,15). Ninety-four percent Caucasian, 95% Chinese, and 94% African Americans have two apo(a) alleles that contain different numbers of k-4 repeats (52).

It could be anticipated, given the inverse relationship between apo(a) allele size and Lp(a) level, that apo(a) alleles associated with no detectable circulating apo(a) would have been of larger size, but this was not the case. The apo(a) alleles which were associated with little to no apo(a) protein had a very similar size distribution to those alleles with detectable protein products. There are two possible explanations for the fact that apo(a) alleles associated with little or no plasma apo(a) protein are distributed throughout the entire size spectrum. The apo(a) allele associated with a very low concentration of plasma protein may be the result of several different mutations that interfere with apo(a) synthesis or secretion. Alternatively, there may be a few common mutations in the apo(a) gene that arose early in the gene's evolution and now are associated with apo(a) alleles of widely varying sizes due to subsequent recombination at the locus. The interracial differences in Lp(a) concentration are not due to differences in the frequency distributions of apo(a) alleles (51). A large variation in the Lp(a) concentration can be explained on the basis of the variable number of transcribed k-4 type-2 repeat polymorphisms (53). The other classes of K-4 type-1 and type-3 to type-10 are usually present as a single copy. Two other mutations can occur in k-4 type 10: W72R is associated with an Lp(a) that is lysine-binding defective, and M66T affects aa 66 with a normal lysine binding function (3). The notion that small size apo(a) phenotypes have high Lp(a) level and have an increased incidence of CAD cannot explain the pathogenecity of the disease as the Lp(a) level may also depend on the functional state of the 5′-region of apo(a).

Pentanucleotide Repeat Polymorphisms

The other kind of polymorphism of the apo(a) gene has been observed in the 1.4-kb regulatory region. The most striking one is the pentanucleotide repeat polymorphism (PNRP) at −1373 (54). The reports show that the number of pentanucleotide sequence $TTTTA_n$ (PNR) varies between numbers 8 and 11 in various populations. The high number repeats have been associated with low Lp(a) level whereas a low number of repeats was common among individuals with high Lp(a) level. A significant negative correlation between the number of repeats and the plasma Lp(a) had been observed in Caucasians that included Tyroleans, Danes (55), and North Indians (19), although no significant effect of the PNRP was present in Blacks. The PNR-9 was associated with low Lp(a) level (56) and PNR-8 allele was most frequent among Japanese (57).

However, a significant linkage disequilibrium was noted between the PNR number and K-4 repeats. Apo(a) alleles with 11 PNR contained fewer than 24 k-4 VNTR and were paradoxically associated with low Lp(a) (58). The effect of PNRP on Lp(a) concentrations was independent from the K4-VNTR. Thus,

in Caucasians but not in Blacks, concentrations of the Lp(a) are strongly associated with two repeat polymorphisms in apo(a) gene (55). Increased Lp(a) levels in 172 Japanese patients on chronic hemodialysis were mainly attributable to the combination of eight PNR and large sized apo(a) isoform (A16–A25) (59). Plasma levels in 184 Korean patients with CAD showed a significant difference between PNR 8/5 versus 8/8 genotype whereas Lp(a) levels varied significantly among the genotypes of a Met/Thr polymorphism (60). Other mutations such as +93C/T polymorphism in the 5′ untranslated region that introduces an upstream ATG and reduces *in vitro* translation showed a significant impact on Lp(a) concentration in Africans but not in Caucasians (61). Over eight PNR was most common among 289 Russian MI patients and therefore could be pathogenic whereas the prevalence of T (+93) allele in children without a family history of CHD may have a protective effect for the inherited predisposition to heart disease (62). A negative association of Lp(a) level with apo(a) size isoform and PNR 8/8 was considered a possible predictor for the degree of atherosclerosis in 94 Japanese CHD patients (56). Further, C/T-49, G/A-21 (63), and simultaneous double alterations (−418A > G; −384C > T) in individuals with low Lp(a) level, have been reported (63). However, an increase of Lp(a) levels observed in 594 Irish and French MI (myocardial infarction) cases (that was not directly attributable to apo(a) size variation) was not related to the five polymorphism of the apo(a) gene considered (PNR at −1373, −914G/A, −49C/T, −21G/A, and a Met/Thr affecting aa 4168 (63).

HYPOTHESIS

Although apo(a) gene polymorphism and its correlation to Lp(a) level and CAD have been well studied in different populations, no data have been available in correlating the role of these polymorphisms with apo(a) gene regulation. In a reporter gene analysis experiment, it was reported that a 1.5-kb apo(a) promoter fragment in different sequence variations from two different individuals with high or low Lp(a) levels had variable promoter strength (54). However, a gene reporter analysis with 10 other allelic 1.5-kb promoter fragments led to comparable promoter activities (65). In order to correlate the role of various mutations in the regulatory region with the differential apo(a) gene expression, hence the Lp(a) level, in different individuals, an explanation could lie at the transcription level. We propose that different transcriptional activators and repressors might contribute to the differential expression of apo(a) gene. If such would be the case, these hypothetical activators and repressors could be individual-specific. Specific liver-specific activators and repressors might recognize specific cis-acting elements in order to activate or repress apo(a) gene transcription. Any mutations in these DNA elements could then enhance or repress factor binding in order to regulate gene transcription. Further, probable interactions between these opposing factors to compete for a specific DNA site might contribute to a closely regulated apo(a) level. However, any repetitive, polymorphic sites, such as PNR sequences, in the apo(a) promoter, could also alter the DNA structure so that sequential binding of factors might contribute to the gene transcription. Otherwise, how could one explain an inverse correlation between the number of PNR and Lp(a) level? High numbers of PNR repeats have been observed in individuals with low Lp(a) levels. Assuming that only one or two extra PNR sequences could contribute to repression of apo(a) gene, two things might happen. The topology of this sequence alone might contribute to a sequential binding of factors to an adjacent DNA element; otherwise PNR sequence in association with other distal cis-elements (with or without mutations) might contribute to specific factor binding. Planned experiments utilizing the reported mutations would test our proposed model.

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