Structural and Functional Polymorphism of Lipoprotein(a): Biological and Clinical Implications

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Lipoprotein(a) [Lp(a)], a variant of low-density lipoprotein, is heterogeneous in density because of variability in the content and composition of its core lipids and size polymorphism of its specific glycoprotein component, apolipoprotein(a) [apo(a)]. In some individuals, density polymorphism may also derive from the fact that Lp(a) contains 2 mol of apo(a) per mole of apoB100, contrary to the more common 1:1 molar stoichiometry. Moreover, the size of apo(a) is polymorphic because of variations in the number of kringle 4 type 2 repeats. Another type of apo(a) polymorphism is related to sequence mutations at the kringle level. Two mutations can occur in kringle 4 type 10: one, Trp to Arg, is affiliated with an Lp(a) that is lysine-binding defective; the other, Met to Thr, with a normal lysine-binding function. Thus, Lp(a) is structurally and functionally polymorphic, a notion that must be considered in assessing the cardiovascular pathogenicity of this lipoprotein variant and in immunquantification assays.

Lipoprotein(a) [Lp(a)] is a lipoprotein particle similar to low-density lipoprotein (LDL) in terms of lipid content and composition; however, the protein moiety consists of apolipoprotein (apo)B100 linked by a disulfide bridge to apo(a), a glycoprotein containing about 30% carbohydrates by weight (1, 2). Apo(a) is synthesized in the liver but the site(s) where this protein links with apoB100 has not been clearly established (1, 2). Evidence indicates that this linkage can take place either intracellularly (3) or extracellularly (4, 5), with both events being possible depending upon the metabolic state of the individual (1, 2). The plasma concentrations of Lp(a) are determined mainly by synthesis and may vary as much as 1000-fold among subjects, although they remain relatively constant within the same individual. Lp(a), discovered >25 years ago (6), has been receiving increasing attention since the work of McLean et al. (7) showed that apo(a) has a striking structural similarity to plasminogen and that, by potentially interfering with the function of this zymogen in the fibrinolytic system, apo(a) can be a risk factor for atherosclerotic cardiovascular disease (ASCVD) (7, 8). This postulate has been supported by several epidemiological studies, mostly retrospective, and by in vitro and ex vivo observations (1, 9, 10). However, discrepant views that have appeared in the literature (11, 12) suggest a need for more studies to clarify the reasons for these apparent discrepancies and also to explore the potential role of the structural/functional polymorphism of Lp(a). Some of the recent progress in this research area is outlined below.

Factors Accounting for the Structural Polymorphism of Lp(a)

The following factors contribute to the polymorphism of Lp(a):

Lipid content and composition of Lp(a). Lp(a) is an LDL-like particle that, like LDL, varies in the content and composition of its core lipids, i.e., triglycerides and cholesteryl esters. At one end of a density-gradient profile are light Lp(a) species enriched in triglycerides, whereas at the other end are denser particles with a core mainly consisting of cholesteryl esters; in between are the intermediate-density particles (1). Thus, from the technical standpoint, it is difficult to a priori assign precise density limits to Lp(a). Moreover, Lp(a) density is also affected by the size polymorphism of apo(a) (see next section).

Size polymorphism of apo(a). Apo(a) is highly polymorphic, varying in mass between 300 and 800 kDa (7). This size/mass variability is related to the number of kringle 4 type 2 repeats (3 to 42 kringles) of apo(a). Lp(a) has only 1 mol of apoB100 per particle and, commonly, 1 mol of apo(a) linked to it. Consequently, variations in the size of apo(a) affect the total mass of the Lp(a) protein and, thus, the density of the whole particle. In principle, subjects homozygous for a given apo(a) isoform should have an Lp(a) exhibiting a single symmetrical peak in an ultracentrifugal density-gradient profile. However, because of variations in lipid content and composition in each lipoprotein class, a broad band is usually seen. In heterozygous subjects, the two apo(a) size isoforms segregate into distinct particles that may be technically either easy or difficult to separate, depending on the size differential between the two apo(a)s. Under optimal conditions, Lp(a) particles isolated from the plasma of homozygous subjects should have one only type of apo(a) isoform.

In some subjects, Lp(a) contains 2 mol of apo(a) per mole of apoB100 (1). In this case, the Lp(a) density will depend not only on apo(a) size but also on the number of apo(a)s. At present, apo(a) appears to be the major determinant of Lp(a) size, although variations in LDL components may also play a role in Lp(a) size heterogeneity.

The size polymorphism of Lp(a) is under the control of the apo(a) gene localized in the long arm of chromosome.

References

1. Nonstandard abbreviations: Lp(a), lipoprotein(a); apo, apolipoprotein; LDL, low-density lipoprotein; ASCVD, atherosclerotic cardiovascular disease.

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6 (13). This polymorphism is related to the number of kringle 4 type 2 repeats of apo(a). The other classes of kringle 4, i.e., kringle 1 and kringles 30 to 37, are usually present in apo(a) as a single copy (14). This is also the case of kringle 5 (7). The understanding of the apo(a) size polymorphism is still incomplete except that, as already mentioned, it has a major role in determining Lp(a) density and plasma concentrations of Lp(a) (1, 15). Moreover, this polymorphism may have an effect on Lp(a) function (discussed later).

Apo(a) sequence polymorphism. Recent studies have shown possible interindividual differences in the amino acid sequence of apo(a) kringle 4 type 10 (also referred as kringle 4-37). These studies were prompted by the observation that rhesus monkey apo(a), contrary to its human counterpart, has a defective lysine-binding function, probably related to a substitution for Trp 72 of apo(a) kringle 4 type 10 by Arg, as suggested by both amino acid sequence data (16) and structural information from crystallographic analyses of plasminogen kringle 4 (17). Recently, several coworkers and I found human subjects in whom apo(a) kringle 4 type 10 has the same Trp 72 → Arg mutation as in rhesus apo(a), albeit significantly less frequently than in rhesus apo(a) (18). Another mutation, Met 66 → Thr, has also been found in human apo(a) kringle 4 type 10, but at a relatively higher frequency (in ~40% of the population studied) (19, 20). These observations were made possible by the development of techniques able to amplify specifically the DNA isolated from the leukocytes in peripheral blood, in the region coding for kringle 4 type 10 (21). These techniques can be readily applied to the analysis of the other apo(a) kringles, opening up additional possibilities for encountering new mutations. However, it is already established that apo(a) is polymorphic not only in size but also in sequence; study of this type of polymorphism can be expected to shed important new insights into the biology of apo(a) and Lp(a).

Degree of apo(a) glycosylation. As already mentioned, apo(a) contains about 30% carbohydrates by weight (1). In view of the difference in kringle number among apo(a) size isoforms, it is theoretically possible, though not yet documented, that the degree of glycosylation varies among Lp(a) species. Of interest is the kringle 4 type 10 Met 66 → Thr mutation because having Thr instead of Met in position 66 introduces an additional potential glycosylation site.

Relationship of Apo(a) Structure to Functional Polymorphism of Lp(a) in Terms of Lysine Binding

Both plasminogen and apo(a) have lysine-binding capacity (1, 7). In plasminogen, this capacity is related to kringle 1 and 4 and is due to a special domain referred to as the "lysine-binding pocket" (16, 17). This domain comprises seven amino acids: two anionic, Asp 55 and Asp 57; two cationic, lys 58 and Arg 71; and three nonpolar, Trp 66, Trp 72, and Phe 84. The lysine-binding pocket is also present in human and rhesus apo(a) kringle 4 type 10, except that Lys 58 is replaced by Arg in the wild-type form. The naturally occurring Trp 72 → Arg mutation observed both in human and simian apo(a) affects one of the amino acids of the lysine-binding pocket of kringle 4 type 10, predicted from the crystallographic studies on plasminogen kringle 4 to be an important determinant in lysine binding. The fact that the Lp(a) species containing the Trp 72 → Arg mutation are defective in lysine binding supports the structural prediction that Trp 72 of kringle 4 type 10 plays a dominant role in this binding (16, 17). Additional support for this role comes from recent studies showing that kringle 4 type 10, when expressed as a glycosylated product in mammalian cells, has the capacity to bind lysine (14). Because this binding is also present in the kringle 4 type 10 expressed in Escherichia coli (22), a bacterial system that does not promote glycosylation, apparently carbohydrates are not required for lysine binding (at least in the case of kringle 4 type 10). Additional support, although indirect, for the importance of the lysine-binding pocket in lysine binding comes from the observation that the Met 66 → Thr mutation, located outside this pocket, is not associated with a binding defect (20). Overall, the results of both clinical and laboratory studies favor the conclusion that kringle 4 type 10 is the kringle that plays a major role in the binding of Lp(a) to lysine. These results, however, do not exclude the possibility that other kringles may have an effect on this binding, although their contribution is likely to be less.

Lysine binding can be also influenced by apo(a) size polymorphism and the attending heterogeneity in Lp(a) density. The literature is limited on the subject, but the binding to lysine and to fibrin may differ between light and dense Lp(a) (23).

Clinical Significance of Apo(a) Polymorphism

High plasma concentrations of Lp(a) have been associated with an increased incidence of ASCVD and inversely to small-size apo(a) phenotypes (1, 9, 10, 15). However, not all published data agree with the notion of concentration dependency of the cardiovascular pathogenicity of Lp(a) (11, 12); moreover, plasma concentrations of Lp(a) may also depend on the functional state of the 5' region of apo(a), now under intense investigation (24–27). We have also to consider the sequence polymorphism of apo(a) and species of Lp(a) that, by virtue of a single point mutation, no longer bind to lysine and may potentially be less atherothrombogenic than their wild-type counterpart. Thus, to critically establish the pathological role of Lp(a), we need to know in every individual under study their apo(a) genotype and phenotype as well as the lysine-binding function of their Lp(a). If lysine binding relates to the cardiovascular pathogenicity of Lp(a), as suggested, then lysine-binding-defective species of this lipoprotein should be considered "good" or "benign" from that viewpoint. Our personal experience on this topic is rather limited. At present we know only that the few subjects homozygous for the Trp 72 → Arg mutation have neither a personal nor a familial history of ASCVD (18). A rhesus monkey we followed that had the same mutation and a hypercholesterolemia second-
ary to a genetically determined deficiency of the LDL receptor died at an advanced age due to an intervening pulmonary infection; autopsy revealed little evidence of thrombotic disease (28).

The structural polymorphism of Lp(a) may be also relevant to the issue of immunoquantification. Because the size polymorphism of apo(a) depends on the number of kringle 4 type 2 repeats, it is important to establish that polyclonal antibodies reacting against this kringle will exhibit the same affinity for the various apo(a) size isoforms. Ideally, one should use an antibody raised against one of the nonrepeating kringle 4 units or against kringle 5, which in apo(a) is present as a single copy. Particular attention should also be paid to point mutations that may change antibody affinity. From the information available on the mutability of kringle 4 type 10, we conclude that monoclonal antibodies raised against this kringle must be used with caution in quantifying Lp(a).

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