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The gene for the Lp(a)-specific glycoprotein is closely linked to the gene for plasminogen on chromosome 6

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Summary. We have studied the segregation of the Lp(a) glycoprotein phenotypes and of the plasminogen (PLG) polymorphism in three two-generation families. The inheritance of the Lp(a) gene was followed using the Lp(a) glycoprotein size polymorphism and that of the plasminogen gene, using protein and DNA polymorphisms. In the three families studied, no recombination was observed in 18 meioses. The lod score for linkage between the Lp(a) glycoprotein locus and the plasminogen locus in these families is greater than 5.0 at a recombination fraction of $\theta = 0$. Our results show that the structural gene for the Lp(a) glycoprotein is closely linked to the gene for plasminogen on chromosome 6.

Introduction

The lipoprotein Lp(a) was first described in 1963 by Berg as a new inherited antigen system in human plasma, with the trait being transmitted in an autosomal dominant manner. More recent studies have demonstrated that plasma Lp(a) levels are controlled by a major gene and represent a quantitative trait (Hassted et al. 1983; Sing et al. 1974). In many studies high levels of Lp(a) have been shown to be an independent risk factor for the development of ischemic heart disease (Kostner et al. 1981; Dahlen et al. 1986; Armstrong et al. 1986; Rhoads et al. 1986).

The lipid and protein composition of Lp(a) is very similar to that of low density lipoprotein (LDL), but in addition to apo B-100, Lp(a) has a unique glycoprotein attached to apo B through a cysteine residue (Ehnholm et al. 1972; Utermann and Weber 1983; Gaubatz et al. 1987; Armstrong et al. 1985; Fless et al. 1986). This gives the particle a density similar to that of the high density lipoproteins. The apparent molecular weight of the Lp(a) glycoprotein varies from individual to individual and ranges between 400 and 700 kd. These differences are not explained by differences in glycosylation, but are due to polypeptides of different sizes (Fless et al. 1984; Utermann et al. 1987). Six different Lp(a) protein species have been identified using SDS-PAGE and immunoblotting (Utermann et al. 1987, 1988a), and family studies demonstrate a codominant genetic transmission of these Lp(a) glycoprotein isoforms (Utermann et al. 1987, 1988b). Recent studies have shown that there is a strong association between the Lp(a) phenotypes and the levels of Lp(a) lipoprotein in plasma (Utermann et al. 1987, 1988a).

Amino acid and nucleotide sequence information have revealed a very high degree of homology between the Lp(a) glycoprotein and plasminogen (Eaton et al. 1987; McLean et al. 1987; Kratzin et al. 1987). This raises the possibility that the genes for plasminogen and the Lp(a) glycoprotein may be closely linked in the genome because of their evolutionary relationship. By means of somatic cell hybrids and in situ hybridization techniques, the plasminogen gene has been localized to the long arm of chromosome 6 (Murray et al. 1987). We have used the Lp(a) isoform phenotypes and protein and DNA polymorphisms for plasminogen (Murray et al. 1987) to study the segregation of these loci in three two-generation families. Our data show that the structural gene for the Lp(a) glycoprotein [apo(a)] is closely linked to the plasminogen locus on chromosome 6.

Materials and methods

Subjects

Three families with a total of 18 offspring were selected primarily on the basis of expected informativeness [different apo (a) types of parents] and sibship size (e.g., the D family) from a large family material (Utermann et al. 1988b).

Gene probe for plasminogen

The plasminogen DNA probe we have used (a kind gift from Dr. E. Davie, Seattle) is the JES-VI-56-5 800-bp genomic clone, which consists of a 342-bp sequence of the first exon of kringle IV (Malinowsky et al. 1984). This exon is equivalent to the 5′ half of the kringle IV repeates in apo (a) with a homology of at least 80% to apo (a) cDNA sequence (McLean et al. 1987). The clone is inserted into a pUC8 vector using the *Bam*HI and *Hin*dIII sites. Plasmid DNA was prepared using standard techniques (Maniatis et al. 1982). DNA was labeled to a specific activity of $(10–20 \times 10^6$ cpm/50 ng) by oligolabeling (Feinberg and Vogelstein 1983) using random hexanucleotide primers, Klenow Enzyme (Anglian Biotechnology, UK), and $\alpha[^{32}P]$dCTP at Ci/mmol (Radiochemical Centre, Amersham).

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DNA analysis

Blood samples for DNA analysis were taken in EDTA tubes and frozen at −70°C. Total genomic DNA was prepared from the leukocytes of 10 ml of blood by a Triton X100 lysis method (Kunkel et al. 1977). One to three micrograms of DNA were digested with *Sac*I (Anglian Biotechnology) under conditions recommended by the manufacturer, and the fragments were separated using an 0.8% agarose gel (BRL, US). Southern blots (Southern 1975) were performed using Hybond-N membranes (Amersham). Hybridizations were carried out for 1–2 days at 65°C in 4 × SSC (4 × 0.15 *M* NaCl, 0.015 *M* Na citrate); 1 × Denharts solution; 100 μg/ml salmon sperm DNA; 1 m*M* EDTA, 50 m*M* sodium phosphate pH 7.5; 0.1% sodium dodecyl sulfate (SDS); 6% polyethylene glycol. Filters were washed in 0.1–1 × SSC, 0.1% SDS at 65°C and exposed at −70°C to preflashed Fuji X-ray film for 2–5 days using an Ilford tungstate intensifying screen.

Lp(a) phenotype determination

Lp(a) phenotypes were determined from total plasma by Western blotting essentially as described by Utermann et al. (1987). Apo(a) phenotypes in the D family have been reported by Utermann et al. (1988b).

Plasminogen phenotype determination

Plasminogen phenotypes (Hobart 1979) were determined by isoelectric focusing on agarose followed by immunofixation essentially as described by Leifheit et al. (1987).

Lod score analysis of family data

Lod scores were calculated using the LINKAGE program (Lathrop et al. 1984).

Results

We have studied the inheritance of the structural gene for the Lp(a) glycoprotein in three independent families. Family pedigrees indicating the members' Lp(a) phenotypes and plasminogen phenotypes and genotypes are shown in Fig. 1. As shown in previous studies, the inheritance of the Lp(a) glycoprotein phenotypes in these families is compatible with the codominant inheritance of a series of autosomal alleles including one null allele at a single locus. Where a parent showed only one Lp(a) band, information from the children was used to determine if this individual is homozygous for that Lp(a) glycoprotein species (e.g., S4/S4) or heterozygous with one null allele (e.g., S4/0). For example, in the K family the children with the single-band phenotypes S4 and B make it possible to deduce that both the parents must carry a null allele.

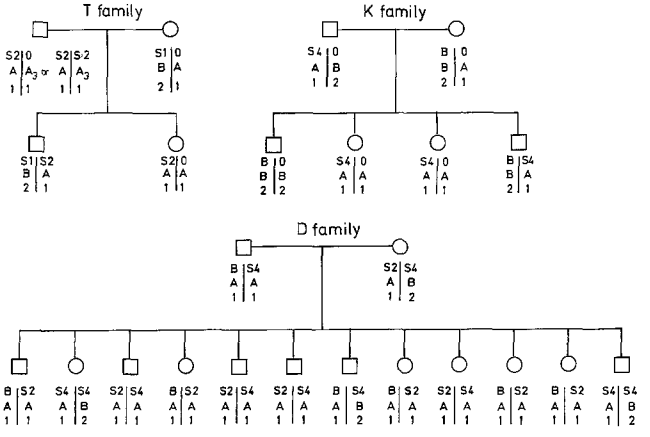

Fig. 1. Pedigree charts showing Lp(a) phenotypes and plasminogen phenotypes and genotypes. X X, Lp(a) phenotype; Y Y, plasminogen phenotype; Z Z, *Sac*I polymorphism

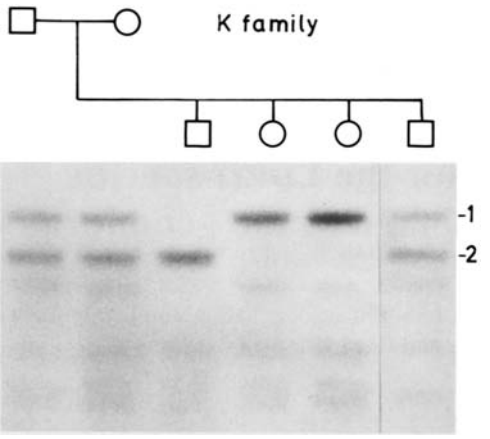

Fig. 2. Pedigree of the K family. DNA digested with the enzyme *Sac*I and hybridized with the genomic probe JES-IV-56-5

Table 1. Lod scores between the gene for the Lp(a) glycoprotein and the gene for plasminogen

Family	No. of offspring scored	Lod score at $\theta = 0.0$
D	12	3.31
K	4	1.51
T	2	0.30
Total	18	5.12

The DNA polymorphism of plasminogen used in this study is shown in Fig. 2. We designated the larger DNA fragment (15 kb) as allele 1 and the smaller fragment (10 kb) as allele 2. In addition to the polymorphic bands a number of weaker bands were detected by hybridization with the kringle IV probe when filters were washed at low stringency (1 × SSC, 65°C). Washing the filters at higher stringency (0.1 × SSC, 65°C) reduces the intensity of these bands. For the protein polymorphism, two bands are distinguished, A (fast) and B (slow). One individual in the T family was heterozygous for the A3 variant. From the pedigrees it can be seen that the DNA and protein polymorphisms of plasminogen were completely correlated, with all individuals with the phenotype PLG-A having the *Sac*I 1-allele and all those with PLG-B, the *Sac*I 2-allele. This linkage disequilibrium between the protein and DNA polymorphisms has been noted previously (Murray et al. 1987).

In the three families studied, no recombination was observed in 18 meioses. The lod score for linkage between the Lp(a) glycoprotein locus and the plasminogen locus in these families is greater than 5.0 at a recombination fraction of $\theta = 0$ (Table 1).

Discussion

Recently the gene for Lp(a) lipoprotein plasma levels was demonstrated to be closely linked to the plasminogen gene (Weitkamp et al. 1988). This finding is indicative, but does not necessarily prove, that the apo(a) structural gene is linked to the plasminogen gene. Only 40% of the variability in Lp(a) lipoprotein plasma levels is explained by the measured size variation determined by the apo(a) locus (Boerwinkle et al. 1989). Hence other genes might be involved in determining Lp(a) levels, and theoretically the observed linkage could be with such genes, and not with apo(a). Therefore previous claims of the linkage of apo(a) with plasminogen are not unequivocal.

Our results clearly show that the structural gene for the Lp(a) glycoprotein (apo a) is closely linked to the locus for plasminogen (PLG) on chromosome 6. This is in agreement with recent studies that have shown a high degree of sequence homology between plasminogen and the Lp(a) glycoprotein, suggesting that the genes are closely related and probably arose from a gene duplication event (Eaton et al. 1987; McLean et al. 1987; Kratzin et al. 1987). Subsequent deletions and homologous recombination events could have led to the loss of sequences for Kringles I–III and to the gain of a large number of repeats of the plasminogen kringle IV sequence found in the Lp(a) glycoprotein cDNA sequence (McLean et al. 1987). Individual variation in the number of kringle-IV-like repeats is a likely cause of the different size isoforms of the Lp(a) glycoprotein. Our results together with a recently published report on linkage between Lp(a) lipoprotein *levels* and the plasminogen gene loci (Weitkamp et al. 1988) are further evidence for previous suggestions that the same gene locus is involved in determining the Lp(a) glycoprotein size isoforms and Lp(a) lipoprotein concentration in plasma (Utermann et al. 1988a).

The probe we have used to detect the plasminogen polymorphism contains the coding sequence for the 5′ half of kringle IV. This sequence has at least an 80% homology to the kringle-IV-like sequence in the Lp(a) glycoprotein cDNA (McLean et al. 1987), which is repeated many times in the apo(a) gene. This raises the possibility that DNA polymorphisms detected with this probe by Southern blot hybridization might be due to polymorphism at the Lp(a), and not the PLG, locus. Although the RFLP used in this study, which is detected using the enzyme *Sac*I, is in strong linkage disequilibrium with the plasminogen protein polymorphism, this possibility cannot be ruled out. In addition, because of cross hybridization between the apo(a) and plasminogen sequences, studies using the kringle IV plasminogen probe for chromosomal localization of the gene by in situ hybridization and hybridoma cell techniques may have assigned apo (a), rather than plasminogen, to the long arm of chromosome 6. Hence our studies on the *protein* polymorphism of plasminogen and apo (a) firmly establish the linkage of both loci and, together with previous studies, the localization of both genes on chromosome 6. This localization has recently been confirmed using somatic cell hybrids and apo(a)- and plasminogen-specific probes (Frank et al. 1988).

Further studies of the gene organization of the Lp(a)/PLG gene cluster on chromosome 6 will lead to a better understanding of the spatial and evolutionary relationship of these genes. The finding of two related genes in close linkage, one of which is involved in lipoprotein metabolism and the other, in the clotting system, is intriguing and opens new perspectives to research in the genetic aspect of atherosclerosis.

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