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The apolipoprotein(a) gene resides on human chromosome 6q26-27, in close proximity to the homologous gene for plasminogen

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Summary. Apolipoprotein(a) [apo(a)], the glycoprotein associated with the lipoprotein(a) [Lp(a)] subfraction of plasma lipoproteins, has been shown to exhibit heritable molecular weight isoforms ranging from 400–700 kDa. Increased serum concentrations of Lp(a) correlate positively with the risk of atherosclerosis. Variations in Lp(a) plasma levels among individuals are inherited as a codominant quantitative trait. As part of an effect to define the basis of these variations and further clarify the expression of the protein, we have determined the chromosomal location of the human apo(a) gene. Blot hybridization analysis of DNA from a panel of mouse-human somatic cell hybrids with an apo(a) cDNA probe revealed a complex pattern of bands, all of which segregated with chromosome 6. In situ hybridization yielded a single peak of grain density located on chromosome 6q26-27. Apo(a) cDNA sequences exhibit striking homology to those of the plasma protease plasminogen, and, therefore, we have reexamined the chromosome assignment of the plasminogen gene. We conclude that both the apo(a) and plasminogen genes reside on human chromosome 6q22-27, consistent with a gene duplication mechanism for their evolutionary origin. The results are of significance for the genetic control of apo(a) expression and genetic influences predisposing to atherosclerosis.

Introduction

High serum levels of lipoprotein(a) [Lp(a)] are an independent risk factor for coronary artery disease (Kostner et al. 1981; Dahlen et al. 1986; Armstrong et al. 1986; Rhoads et al. 1986). Although the role of Lp(a) in normal physiology and in atherogenesis is unclear, recent studies have clarified the structure of the lipoprotein. The composition of Lp(a) closely resembles that of low-density lipoprotein (LDL), with the exception that the particle contains an additional protein moiety. This moiety is a large, highly glycosylated protien, termed apo(a), that is linked by one or more disulfide bonds to apoB100 (Utermann and Weber 1983; Gaubatz et al. 1983; Fless et al. 1984).

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Lp(a) has been shown to exhibit density heterogeneity both within and among individuals (Gaubatz et al. 1983). Various studies (Fless et al. 1984; Gaubatz et al. 1987; Utermann et al. 1987) indicate that the density heterogeneity observed is a result of the molecular weight heterogeneity of apo(a); isoforms of apo(a) have been reported with molecular weights ranging from 400 to 700 kilodaltons (kDa). Utermann et al. (1987), have shown that these differences are heritable and result from variations of a single genetic locus. The size differences are not a consequence of the degree of sialyation.

Examination of partial protein sequence (Eaton et al. 1987) and the complete cDNA sequence (McLean et al. 1987) of apo(a) has revealed remarkable similarity to plasminogen. Plasminogen is a fibrinolytic protease that consists of five tandemly repeated, homologous domains, called kringles, and a single proteolytic domain (for review see Castellino 1981). Human apo(a) contains 37 tandem repeats of sequences similar to kringle 4 of plasminogen, followed by domains that resemble kringle 5 and the protease domain of plasminogen (McLean et al. 1987). The various untranslated and coding regions of the apo(a) gene show homologies ranging from 78%–100% when compared to corresponding regions of the human plasminogen gene. This suggests that the two genes arose from duplication of a common precursor during recent mammalian evolution. The degree of sequence similarity also results in considerable cross-hybridization of nucleic acid probes. However, sequences corresponding to the amino terminus and kringles 1–3 plasminogen are absent in apo(a).

We now report the use of the cDNA for apo(a) to map the gene to human chromosome 6q26-27. Previously, the gene for plasminogen has been mapped to the same region by in situ hybridization and somatic cell hybrid studies (Murray et al. 1987). We have ruled out the possibility that the localization of the two homologous genes to the same chromosomal region resulted from cross-hybridization.

Materials and methods

Probes

The human apo(a) cDNA probe corresponds to 5500 bases of the 3′ end of the mRNA (McLean et al. 1987). The plasmino-

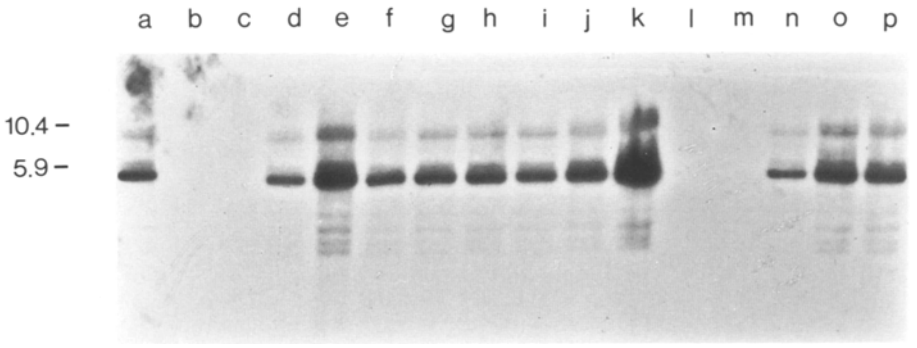

Fig. 1. Southern hybridization analysis of apo(a) sequences in *Eco*RI digested DNA isolated from a panel of mouse-human somatic cell hybrid clones. *Lane a* is human parental, *lane b* is mouse parental. *Lanes c–p* are hybrid clones 84-39, 84-38, 84-37, 84-35, 84-34, 84-30, 84-27, 84-26, 84-25, 84-21, 84-20, 84-13, 84-7, 84-5, respectively

gen probe was a cDNA fragment, 863 bases in length, encoding a portion of the amino terminal peptide, and kringles 1, 2, and 3, regions which are not present in apo(a) (Forsgren et al. 1987).

In situ hybridization to chromosomes

The apo(a) cDNA probe was labeled by oligonucleotide priming with ^{3}H-labeled deoxyribonucleotides to a specific activity of about 4×10^8 cpm/μg. The probe was then hybridized to chromosomes from normal human lymphocytes using a method developed by Harper and Saunders (1981) as modified by Cannizzaro and Emanuel (1984). The slides were exposed for 10 days, and all silver grains on or touching chromosomes were scored.

Somatic cell hybrid analysis

A panel of 17 mouse-human somatic cell hybrid clones was constructed and analyzed for chromosome content as described previously (Mohandas et al. 1986). DNA was isolated from nuclei of these clones as well as the parental mouse cell line (B82, GM 0347A) and the human male fibroblast parental cell line (IMR91). DNA was isolated using sodium dodecyl sulfate (SDS) and proteinase K followed by phenol-chloroform extraction. Following cleavage with restriction enzyme, 10 μg of DNA from each sample was subjected to electrophoresis through a 1% agarose gel and transferred by blotting to a nylon filter as described (Southern 1975). The filters were then probed with the cDNA probes radiolabeled with ^{32}P-dCTP by a random priming method (Feinberg and Vogelstein 1983) to a specific activity of about 1×10^9 cpm/μg. Filter hybridization was performed using 2×10^6 cpm probe per milliliter in 0.5 *M* sodium phosphate, pH 7.0, 7% SDS, 1% bovine serum albumin, 1 m*M* ethylenediaminetetraacetate (EDTA) and 100 μg/ml denatured salmon sperm DNA at 65°C for 24 h. Filters were washed twice for 20 min in 2 × SSC (1 × SSC = 150 m*M* NaCl, 14 m*M* sodium citrate, pH 7.0) containing 0.1% SDS at 65°C and twice in 0.1 × SSC. Autoradiograms were prepared by exposing the filters to Kodak XAR-5 film at −70°C.

Results

*Eco*RI digested DNA from a mouse-human somatic cell hybrid panel was probed with a cDNA probe for human apo(a). Figure 1 shows a Southern blot of human parental, mouse parental, and 14 representative hybrid clones. Human parental DNA yielded two intense bands corresponding to 10.4 and 5.9 kilobase pairs (kb), followed by several less intense bands ranging from 3.9 to 1.7 kb. No hybridizing bands were detected in the murine genome. Of the 17 mouse-human hybrids, 14 were positive for all bands detected in the human parent and 3 were negative (Table 1, Fig. 1). Examination of the chromosome content of these clones shows complete concordance of the apo(a) gene sequences with chromosome 6 (Table 1).

Regional localization of the gene for apo(a) was carried out by in situ hybridization of the apo(a) cDNA probe to human metaphase chromosomes. Figure 2a shows the distribution of silver grains over metaphase chromosomes obtained from analysis of 102 cells. The accumulation of grains over the long arm of chromosome 6 (Fig. 2a), with peak grain density occurring in the q26-27 region (Fig. 2b), is consistent with the somatic cell hybrid results and indicates that the apo(a) gene resides on human chromosome 6q26-27.

Since the plasminogen gene is highly homologous to the apo(a) gene and has previously been localized to the same region of human chromosome 6, we examined whether the assignments could have been affected by cross-hybridization of probe sequences to the two genes. Figure 3 shows the hybridization pattern of plasminogen to representative mouse-human somatic cell hybrid clones. The portion of the plasminogen gene represented in the probe encodes kringles 1–3 and is not present in the apo(a) gene. The mouse parental pattern shows a single band of 7.3 kb whereas the human parental pattern shows bands of 7.5, 5.7, 4.2, 3.7, 2.4, and <1 kb. Thirteen of the hybrid clones are positive for human parental bands 7.5, 5.7, 3.7, 2.4, and <1 klb (Table 1, Fig. 3). The chromosome content of these clones indicates that these bands originate from chromosome 6 (Table 1). Four of the hybrid clones are positive for the 4.2-kb band, a pattern consistent with localization to chromosome 2 (Table 1, Fig. 3). The failure to observe hybridization in hybrid clone 84-20 is presumably due to the low content of chromosome 2 in that clone. The identity of the cross-hybridizing sequence on chromosome 2 is unclear, but it could represent a plasminogen pseudogene. The plasminogen probe exhibits greater homology to the chromosome 6 locus than the chromosome 2 locus, since the 4.2-kb band can be selectively removed by high stringency washing (0.1 × SSC at 70°C) of the filters (data not shown). Previous in situ hybridization studies have indicated that the plasminogen gene resides on human chromosome 6q26-27, the same chromosomal region as the apo(a) gene. Since our Southern blotting results allow distinction between

Table 1. Segregation of apo(a) and plasminogen (plg) genes with human chromosomes in mouse-human somatic cell hybrids

Hybrid clone	apo(a)	plg	4.2-kb band[b]	Human chromosomes[a]																							
				1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	Y
84-2	+	+	+	+	+	−	+	+	+	+	+	−	−	+	+	−	+	+	+	+	+	+	−	+	−	−
84-7	+	+	−	−	−	+	+	−	+	+	+	−	−	+	+	−	+	−	+	+	−	−	+	−	−	−
84-26	+	+	−	+	−	+	+	+	+	+	−	−	+	−	+	+	+	+	+	+	−	+	+	−	+	−
84-30	+	+	−	−	−	+	+	(+)	+	−	−	−	+	−	(+)	+	+	−	−	+	+	−	(+)	+	+	−	−
84-3	+	+	+	+	+	+	+	−	+	+	+	−	+	−	−	+	+	+	−	+	+	+	+	−	−	−	−
84-4	+	+	+	+	+	+	+	+	+	+	+	−	+	−	−	+	+	+	−	+	+	+	−	−	−	−	−
84-5	+	+	+	+	+	+	−	+	+	−	+	−	−	−	−	(+)	+	+	−	+	−	+	−	+	+	+	+
84-13	+	+	−	−	−	−	−	−	+	−	−	−	+	−	+	+	+	+	−	+	+	−	+	−	−	−	−
84-20	−	−	−	−	(+)	+	+	+	−	−	+	−	−	(+)	(+)	+	+	−	−	+	+	−	+	+	(+)	−	−
84-21	−	−	−	−	−	−	−	−	−	+	−	−	+	−	+	−	+	−	−	−	−	−	+	+	−	−	(+)
84-25	+	+	−	−	−	−	+	+	+	−	+	−	+	+	(+)	−	−	+	−	+	+	−	−	+	−	−	−
84-27	+	ND[c]	ND	−	+	−	−	+	+	−	+	−	−	+	+	−	−	+	−	+	−	−	+	+	−	−	−
84-34	+	+	−	−	−	−	+	−	+	+	+	−	+	+	−	−	+	+	−	+	−	(+)	+	−	+	−	−
84-35	+	+	−	−	−	+	+	−	+	+	+	−	−	−	+	−	+	+	+	+	−	−	−	−	−	−	−
84-37	+	+	−	−	−	−	+	+	+	+	(+)	−	−	+	+	−	−	+	−	+	−	−	+	+	+	−	−
84-38	+	+	−	+	+	−	+	(+)	+	+	+	−	+	−	+	+	+	+	+	+	+	(+)	−	+	+	−	−
84-39	−	−	−	−	−	+	−	−	−	+	+	−	−	−	−	−	+	+	−	+	−	−	−	−	−	−	−
No. of discordant hybrids for apo(a)				8	10	7	4	7	0	8	4	14	7	9	6	8	4	6	11	3	6	7	5	9	8	14	13

[a] Clones that contain the chromosome in 10%–30% of cells surveyed are indicated by parenthesis
[b] kb, Kilobase pairs
[c] ND, Not determined

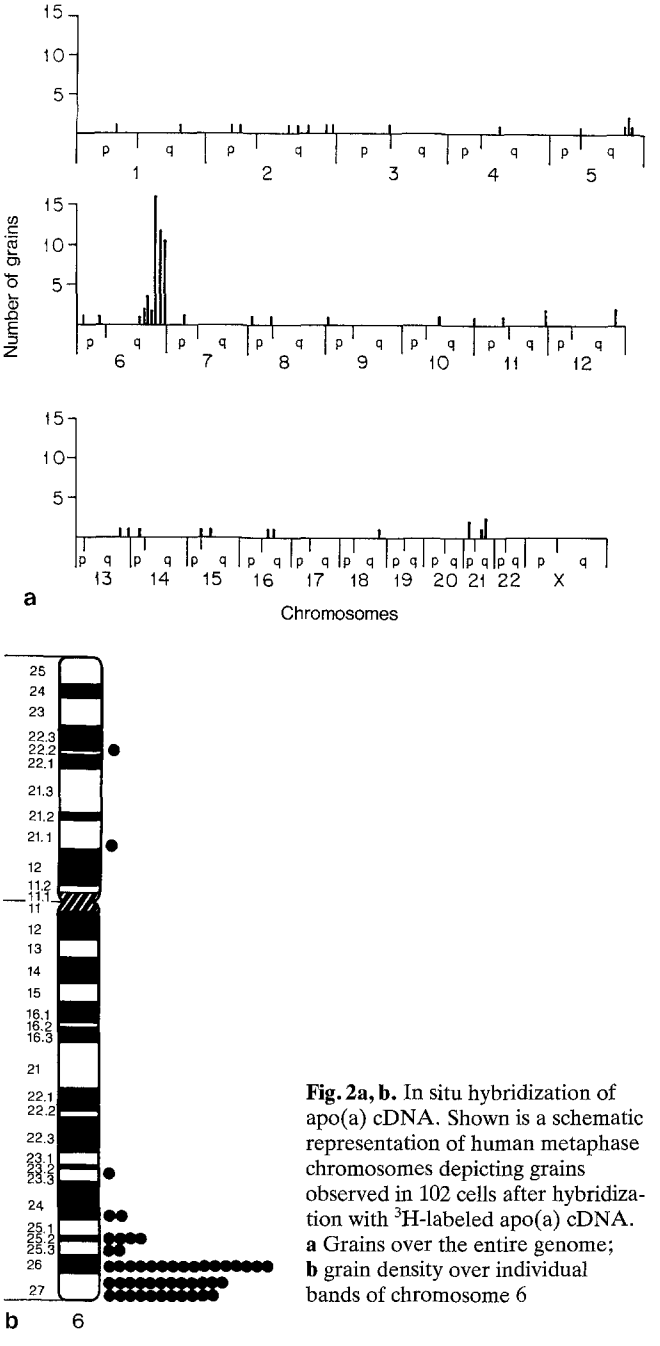

Fig. 2a, b. In situ hybridization of apo(a) cDNA. Shown is a schematic representation of human metaphase chromosomes depicting grains observed in 102 cells after hybridization with ^{3}H-labeled apo(a) cDNA. **a** Grains over the entire genome; **b** grain density over individual bands of chromosome 6

plasminogen and apo(a), and since only a single major peak was observed in the in situ hybridization studies with the apo(a) probe, we conclude that both genes are located on human chromosome 6q26-27.

Discussion

We have used a cDNA probe for apo(a) to determine the chromosomal location of the apo(a) gene. Our results indicate that the apo(a) gene resides on chromosome 6 at band q26-27, a site previously assigned to the plasminogen gene by Murray et al. (1987) using somatic cell hybrid studies and in situ hybridization. As the high degree of homology between the apo(a) and plasminogen genes raised the possibility of cross-hybridization, we felt it worthwhile to confirm the mapping of

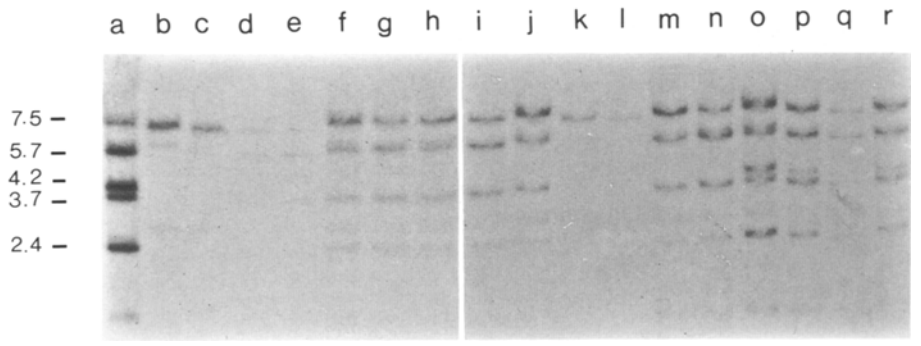

Fig. 3. Southern hybridization analysis of plasminogen sequences in *Eco*RI digested DNA. *Lane a* is human parental, *lane b* is mouse parental. *Lanes c–r* are hybrid clones 84-39, 84-38, 84-37, 84-35, 84-34, 84-30, 84-26, 84-25, 84-21, 84-20, 84-13, 84-7, 84-5, 84-4, 84-3, 84-2, respectively

plasminogen. Complex hybridization patterns on Southern blots have been reported for plasminogen (Murray et al. 1987) and this may have been due to cross-hybridization with apo(a). The blot hybridization patterns observed for apo(a) and plasminogen were distinct and demonstrated that both genes reside on chromosome 6. The striking homology between plasminogen and apo(a) indicates these two genes are members of a gene family. The proximity of these two genes is consistent with a gene duplication mechanism for their evolutionary origin, and with subsequent gene conversion to account for the nearly 100% identity of the 5′ ends.

Much of the current interest in Lp(a) stems from studies showing a positive correlation between Lp(a) serum levels and coronary artery disease. The mechanism by which Lp(a) contributes to atherosclerosis is unclear. Lp(a) typically carries less than 15% of total plasma cholesterol, indicating that the role Lp(a) plays in atherogenesis is not one affecting total cholesterol levels (Berg 1983). Amino acid differences between apo(a) and plasminogen probably preclude its having plasminlike proteolytic activity (Eaton et al. 1987; McLean et al. 1987). However, the presence of apo(a) is likely to affect the uptake of this lipid-laden particle. Armstrong et al. (1985) have shown that apo(a) inhibits binding of the LDL-like Lp(a) particle to the LDL receptor, presumably by masking key regions of apo B-100. Apo(a) possibly contributes to the uptake of the lipoprotein by endothelial cells or by macrophages in the artery wall, events which are also likely to contribute to atherogenesis.

High levels of Lp(a) are inherited as a dominant quantitative trait (Harvie and Schultz 1970; Sing et al. 1974; Hassted et al. 1983). Utermann et al. (1987) have recently shown that the interindividual variations in the molecular weight of apo(a) are also inherited, and that these phenotypes are determined by a single locus. Moreover, family studies have suggested a correlation between apo(a) molecular weight phenotypes and Lp(a) serum concentrations. By analysis of plasminogen phenotypes, Weitkamp et al. (1988) recently obtained evidence for the linkage of the plasminogen locus to a locus controlling quantitative variations of Lp(a). Together with our results, demonstrating that the structural locus for apo(a) is linked to the plasminogen gene, it now appears that quantitative variations of Lp(a) are due to polymorphisms of the apo(a) gene.

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