Human Apolipoprotein A-I–C-III Gene Complex is Located on Chromosome 11

Gail A. P. Bruns, Sotirios K. Karathanasis, and Jan L. Breslow

The genes for two of the apolipoproteins, apo A-I and apo C-III, previously shown to be within 3kb in the genome, were localized to human chromosome 11 by Southern blot analysis of DNA from human-rodent somatic cell hybrids. These two genes were shown to exhibit polymorphisms associated with dyslipoproteinemia and premature atherosclerosis, and it will now be possible to examine the relationship of these genes to the many others that have been assigned to this chromosome. (Arteriosclerosis 4:97–102, March/April 1984)

Apolipoproteins are macromolecular complexes of noncovalently bound lipid and apolipoproteins. There are eight well-characterized apolipoproteins: apo A-I, apo A-II, apo A-IV, apo B, apo C-I, apo C-II, apo C-III, and apo E. Regulatory or structural mutations in the genes specifying these apolipoproteins may predispose individuals to dyslipoproteinemas, some of which have been associated with premature atherosclerosis. For example, variant apo E isoproteins due to amino acid substitutions in a receptor binding region have been described, and are presumably due to apo E structural gene mutations. These are common in the population and appear to underlie susceptibility to Type III hyperlipoproteinemia, a disorder associated with defective chylomicron remnant removal, hypercholesterolemia, hypertriglyceridemia, xanthomatosi, and premature atherosclerosis. Variant apo A-I isoproteins due to amino acid substitutions have also been described, but are relatively rare. Recently, two DNA polymorphisms in or near the apo A-I gene locus have been described. In one situation apparent homozygosity for the polymorphism was associated with deficient plasma levels of apo A-I and apo C-III, high density lipoproteins (HDL), and severe premature atherosclerosis. In the other situation a group of severely hypertriglyceridemic patients with Types IV and V hyperlipoproteinemia were shown to have an increased incidence of a restriction fragment length polymorphism (RFLP) associated with the apo A-I gene.

The DNA and genomic clones for human apo A-I and apo C-III have been isolated and characterized, and the genes specifying these two apolipoproteins have been shown to be within 3kb in the genome and convergently transcribed. Furthermore, the gene lesion associated with apo A-I–apo C-III deficiency has been shown to be due to a large DNA insertion in the coding region of the apo A-I gene, whereas the RFLP associated with hypertriglyceridemia is explained by a single base substitution in the DNA corresponding to the 3' noncoding region of apo C-III mRNA. The chromosomal localization of these lesions will facilitate linkage studies to the RFLP map being compiled for the human genome. This will aid in family studies of atherosclerosis susceptibility; also polymorphisms in the apolipoprotein genes may prove to be valuable genetic markers in the study of other linked genes.

Methods

In the current study we investigated the chromosomal localization of the apo A-I–apo C-III gene complex. For this purpose we used a panel of DNA from human-rodent somatic cell hybrids that have been extensively characterized for human chromosome complements. The hybrids were derived from fusions of HPRT deficient Chinese hamster E36...
The inserts of clones pAI-113, pAI-101, and pCIII-606 were digested with the restriction endonuclease EcoRI, electrophoresed on 0.8% agarose gels, and transferred to nitrocellulose filters. Prehybridization and hybridization were carried out as previously described. The hybridization probes were the inserts of clones pAI-113, pAI-101, and pCIII-606 excised with PstI, and labelled with ^3P by nick-translation. After hybridization for 15 to 20 hours at 65°C, the excess probe was washed off by incubation in 0.1xSSC and 0.1% SDS for 1 hour at 65°C. X-ray film was sandwiched between the filters and an intensification screen and exposed at -70°C for 3 days.

**Table 1. Hybridization Pattern of Apo A-I Probe with DNA Mapping Panels**

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Discordant fraction
Informative clones (no.)

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**Results**

In our initial studies, the pAI-113 probe was used as the hybridization probe with parental and hybrid DNA. This DNA is approximately 500bp in length, corresponds to the mRNA coding for apo A-I amino acids 94 to 243 as well as to a portion of the apo A-I mRNA 3' untranslated region, and in the genome is uninterrupted by intronic DNA. After EcoRI digestion, under the hybridization conditions, the single 13kb component in human DNA was easily distinguishable from the single 2.2kb component in Chinese hamster DNA, and from the two less intense mouse components (Figure 1). Comparable hybridization patterns were observed with a probe made from cDNA clone pAI-101 that contained sequences corresponding to the mRNA coding for apo A-I amino acids -4 to 143 (Figure 1). The 13kb component characteristic of human DNA exhibited concordant segregation with human chromosome 11 in all 30 primary human-Chinese hamster and human-mouse hybrids in the DNA mapping panels. Chromosome 11 segregation in the hybrids was monitored by expression of the LDH-A and lysosomal acid phosphatase ACP-2 isozymes, hybridization of the DNA with a cloned probe for beta-globin sequences, and cytogenetic identification of the chromosome. The hybridization pattern of the pAI-113 probe was discordant with the segregation of the other 21 autosomes and the X and the Y chromosomes in the somatic cell hybrids examined. The discordancy indices varied from 0.23 to 0.57 (Table 1). In comparable studies, the apo C-III probe, pCIII-606, was also examined and showed similar segregation characteristics. Twenty-one independent human-mouse and human-hamster hybrid clones were examined for hybridization with the apo C-III probe, pCIII-606, and for their human chromosome complements by both isozyme and cytogenetic techniques. In all 21 clones, the segregation of the apo C-III hybridization signal was concordant with that of the apo A-I probes and of chromosome 11. The segregation of chromosome 11 in these hybrids was determined by analysis of the acid phosphatase ACP-2 isozymes, LDH-A isozymes and coding sequence, beta globin sequences and by cytogenetic techniques. The discordancy fractions for apo C-III and chromosomes 1-10, 12-22, the X and the Y were 0.25-0.63 (Table 2).

**Table 2. Segregation of Apo C-III with Chromosome 11 in Human-Rodent Somatic Cell Hybrids**

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Figure 1. A. Schematic representation of the region of the apo A-I–apo C-III gene complex that corresponds to the apo A-I and apo C-III cDNA probes used in this study. Probe pAI-113 corresponds to apo A-I codons 94 to 243 (C. terminus) of apo A-I mRNA and also includes a portion of the 3'-noncoding region. Probe pAI-101 corresponds to apo A-I codons -4 to 143. Probe pCIII-606 corresponds to apo C-III codons 58 to 79 (C. terminus) and also includes the entire 3'-noncoding region of apo C-III mRNA and a part of the poly (A) tail. Restriction sites for EcoRI (R), BamHI (B), HindIII (H3), PstI (P), and Hpal1 (H) are indicated.

B. Hybridization patterns of probe pAI-113 and DNAs from human, rodent, and human-rodent somatic cell hybrids. The DNAs are: Lane 1, Mouse RAG cells. Lane 2, Chinese hamster E36 cells. Lane 3, HeLa cells. Lanes 4–8, Lane 15, Lane 16, Human-Chinese hamster hybrid clones. Lanes 9–14, Lane 17–20, Human-mouse hybrid clones. The cross-reactivity of pAI-113 with rodent sequences is well visualized in Lanes 6–8 and 15, 16 (the hamster sequence) and Lanes 9 and 17 (the mouse sequence). The difference in intensity of the hamster component in Lanes 15, 16 from that in Lanes 4–6 reflects a difference in hybridization efficiency of the two blots resulting from differences in the DNA density per lane and in the transfer conditions. Although not well visualized in Lane 2, the hamster component was observed in all E36 DNAs. In these two DNA panels, the 13kb human apo A-I sequence corresponding to pAI-113 segregated with chromosome 11 as indicated at the top of the panel. The intensity of this component in the several lanes correlated with the intensity of the LDH-A isozymes in the hybrid lines and with the fraction of metaphases with human chromosome 11. A HindIII digest of lambda DNA was used for the size markers. The black marks below the chromosome segregation data indicate the gel lanes.
Discussion

Apolipoproteins have in common the requirement that they bind and transport lipid in the blood stream. Structurally the apolipoproteins that have been sequenced have all possessed amphipathic alpha-helical regions capable of interacting with aqueous and nonpolar environments.1 In 1977 Barker and Dayhoff², using amino-acid sequence data available only for apo A-I, apo A-II, apo C-I, and apo C-III, proposed that the apolipoproteins were all derived from a common evolutionary precursor. The common function, structure, and perhaps even evolutionary background of the apolipoproteins suggests that their genes comprise a multigene family. Families of related DNA sequences have been described for the globins, immunoglobulins, histocompatibility antigens, ribosomal proteins, myosin heavy chain, inter- 

trons, have remained clustered.31 The assignment of the apo A-I—apo C-III gene complex to chromosome 11 raises the interesting question as to whether the other apolipoprotein genes are also located in this region of the genome. Although mapping with DNA probes has not been done for the other apolipoprotein genes, there is some evidence from linkage analysis, based on apolipoprotein electrophoretic variants, that apo A-II and apo E do not cosegregate with apo A-I and may, in fact, be on different chromosomes. In the mouse, apo A-I has been linked to other genes on chromosome 9, whereas apo A-II is linked to genes on chromosome 1.32 In humans it was observed that an electrophoretic variant of apo A-I, apo A-I Marburg, did not cosegregate with apo E electrophoretic variants in one informative family studied.33 Another group has studied two families in which polymorphic forms of apo E demonstrated linkage with electrophoretic variants of the third component of complement in males.34 Since the latter has been mapped to chromosome 19,35 apo E may reside on this chromosome and therefore would not be linked to the apo A-I—apo C-III gene complex. Thus, although two of the apolipoprotein genes, apo A-I and apo C-III, are closely linked in the genome, it appears that at least some of the apolipoprotein genes, apo A-II and apo E, are dispersed. There is currently no data on the chromosomal localization of the other apolipoprotein genes.

In addition to the apo A-I—apo C-III gene complex, many other human genes have been mapped to chromosome 11, including the cellular homologue of the Harvey murine sarcoma-transforming gene, the Harvey murine sarcoma transforming gene, the non-alpha-globin gene complex, a locus for catalase, several cell surface antigen loci, insulin, collagenase, uroporphyrinogen I synthase (the enzyme deficient in acute intermittent porphyria), and the gene complex that predisposes to the aniridia-Wilm's tumor syndrome.36—59 (Figure 2). Commonly occurring RFLPs have been described for several of these loci.⁰⁻⁶⁰ It may be possible, using RFLPs in the apo A-I—apo C-III gene complex, through linkage studies in large kindreds, to establish the relative order and recombination distances between a number of these genes and the apo A-I—apo C-III gene complex.

Acknowledgments

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