

Localization of the structural gene for human apolipoprotein A-I on the long arm of human chromosome 11

(atherosclerosis/high density lipoprotein/apolipoprotein C-III/cell hybrid/Southern blot analysis)

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ABSTRACT Apolipoprotein A-I (apo A-I), the major apolipoprotein in human high density lipoproteins, is involved in the disease atherosclerosis. Cloned apo A-I cDNA (pA1-3) was used as a probe in chromosome mapping studies to detect the human apo A-I structural gene sequence in human–Chinese hamster cell hybrids. Southern blot analysis of 13 hybrids localized the gene to human chromosome 11. Confirmation of the chromosomal assignment was obtained by analysis of a hybrid (J1) containing a single human chromosome, no. 11. Regional mapping was achieved by using deletion subclones of J1 that localized the human apo A-I structural gene to the region 11q13→qter. Since the human apolipoprotein C-III (apo C-III) structural gene is closely linked to apo A-I, it can be assigned to the same region on the long arm of chromosome 11. By extension of methods previously described, it now appears possible to carry out fine-structure analysis of this and related gene regions on chromosome 11 and to study the biochemical concomitants of these genes and of genes on other chromosomes for analysis of their role in atherosclerosis.

High density lipoproteins (HDL) are a heterogeneous group of lipoproteins important in atherosclerosis and distinguished from other lipoproteins by their flotation properties. Plasma HDL are inversely correlated with the atherogenic process (1–4), although the mechanism by which they confer protection against atherosclerosis is unclear. *In vitro* experiments involving isolated erythrocytes and cultured cells suggest that HDL may be involved in delivery of cholesterol from the peripheral tissues to the liver for disposal (5–8). Metabolic studies in man have suggested an inverse correlation between the size of the total body cholesterol pool and the plasma HDL level (9). It is also possible that HDL may simply be a marker for a certain pattern of lipid transport and metabolism that confers protection against the development of atherosclerosis (10).

Apolipoprotein A-I (apo A-I), the major apoprotein in HDL, is a polypeptide of 243 amino acids (11, 12) and has a M_r of $\approx 28,000$. It is a co-factor for lecithin-cholesterol acyltransferase, a plasma enzyme that catalyzes the conversion of cholesterol and phosphatidylcholine to cholesteryl esters and lysophosphatidylcholine (13). This protein was found to be a necessary component in mixtures with phospholipid that can remove cholesterol from ascites cell membranes (14, 15).

Apo A-I is synthesized mainly in the liver and small intestine in mammals (16–20), with the synthesis in the latter presumably regulated by dietary lipid ingestion (20, 21). Recently, Cheung and Chan (12), as well as Shoulders and Barelle (22) and Breslow *et al.* (23), have cloned the cDNA for human apo A-I. Cheung and Chan also determined the nucleo-

tide sequence of a nearly complete apo A-I cDNA clone (12). The genomic DNA for apo A-I has been isolated and its sequence has been determined (24). There is evidence that the apo A-I gene is located close to another apolipoprotein gene, apo C-III (25), the latter being located approximately 2.6 kilobases (kb) below the 3' end of apo A-I on the same cloned genomic fragment. The chromosomal identifications and mapping positions of both genes have not been described previously.

We have used cloned apo A-I cDNA as a hybridization probe to map the human apo A-I gene by Southern blot analysis of human–Chinese hamster somatic cell hybrids. By this technique, the human apo A-I structural gene has been localized on chromosome 11. Regional mapping was carried out, indicating that apo A-I is on the long arm of chromosome 11, in the region encompassed by 11q13→qter. Thus, the apo C-III structural gene can also be assigned to this region on chromosome 11. The further steps now possible that permit genetic-biochemical analysis of the role of this and other genes important in atherosclerosis are indicated.

MATERIALS AND METHODS

Cell Culture. The Chinese hamster cell line CHO-K1 and the human cell line HT-1080 (26, 27) were cultured in F12 medium/8% fetal calf serum. The various human–CHO-K1 cell hybrids used were prepared from fusions between several different auxotrophic mutants of CHO-K1 cells and various human cells as described (26–28). The following cell hybrids were analyzed for the presence or absence of the human apo A-I gene by the Southern blotting procedure (numbers in parentheses indicate human chromosomes carried in each hybrid): CP3 (2, 3, 4, 5, 7, 8, 11, 12, 14, 16, 18, 19, 20, 21, X), CP5 (1, 3, 4, 5, 6, 8, 9, 10, 12, 14, 15, 17, 18, 21, 22, X), CP6 (3, 4, 6, 7, 9, 10, 12, 14, 17, 18, 19, 20, 21, 22), CP12 (2, 4, 8, 9, 11, 12, 13, 14, 16, 18, 22, X), CP14 (2, 4, 5, 9, 12, 14, 17, 18, 19, 20, 21, 22, X), CP15 (2, 3, 4, 5, 9, 11, 12, 14, 17, 19), CP16 (3, 5, 7, 14, 16, 17, 19, 20, 21, X), CP17 (1, 4, 5, 9, 12, 14, 15, 18, 19, 21, 22), CP18 (1, 3, 8, 11, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22), CP20 (2, 3, 4, 5, 8, 9, 14, 17, 19, 21), CP26 (1, 4, 5, 6, 9, 10, 11, 12, 13, 14, 15, 17, 18, 19, 21), CP27 (2, 3, 4, 5, 6, 7, 12, 13, 14, 16, 18, 21, X), and CP28 (1, 3, 4, 5, 6, 9, 16, 18, 19, 20, 21, 22). The preparation of each hybrid and its human chromosome content have been described (27, 28). In addition to the isozyme analysis conducted previously for identification of the human chromosomal content of each hybrid, we have also analyzed the hybrids by cytogenetic techniques using trypsin banding (29) and the Giemsa 11 differential staining (30) in sequential steps (31).

The cell hybrid clone J1, which contains a complete CHO-K1 genome together with a single human chromosome 11 (32), and three of its deletion mutant subclones, J1-7, J1-11,

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Abbreviations: apo, apolipoprotein; HDL, high density lipoproteins; CHO, Chinese hamster ovary; kb, kilobase(s).

and J1-23 (33, 34), were cultured in F12 medium. In clone J1-7, part of the short arm including 11pter→p11 has been deleted, in J1-11 most of the long arm including 11q13→qter has been deleted, and J1-23 has a deletion in the short arm including 11pter→p13, as described (33, 34).

Preparation of DNA from Cultured Cells and Cell Hybrids. Cells were grown in 150-mm dishes to confluency and harvested by trypsinization. The cells were treated with proteinase K after washing and DNA was isolated using described procedures (26, 35).

Digestion and Gel Electrophoresis of DNA. Aliquots (10 μ g) of DNA were digested with restriction enzymes in 30- μ l (final) volumes under conditions recommended by the suppliers (*HincII*, Amersham; *BamHI*, *HindIII*, *Kpn I*, *EcoRI*, *Sst I*, New England BioLabs). Digestions were carried out for 6 hr in the presence of excess enzyme (3–5 units/ μ g of DNA). The digested DNA samples were electrophoresed at 60 V for 16 hr on 1% agarose gels in a horizontal gel apparatus (Bethesda Research Laboratories). The gel was stained with ethidium bromide and the DNA was visualized with ultraviolet light. The DNA was transferred to nitrocellulose paper (Schleicher & Schuell) by the method of Southern (36).

Hybridization of DNA. Cloned apo A-I cDNA (pA1-3) was isolated from the recombinant plasmid by *Pst I* digestion. The cDNA consists of the complete coding and 3'-noncoding sequence together with part of the 5'-noncoding region of apo A-I mRNA (12). The DNA was labeled with [α -³²P]-dCTP and [α -³²P]dTTP by nick-translation to a specific activity of approximately 5×10^8 cpm/ μ g as described (12, 37).

Filters were prehybridized in a sealed plastic bag at 60°C for 8 hr in 10 \times Denhardt's solution (38)/5 \times SET (SET = 0.15 M NaCl/0.01 M Tris-HCl, pH 7.8/1 mM EDTA)/0.1 M Na phosphate, pH 7.0/10 mM Na pyrophosphate/0.1% NaDodSO₄. For hybridization, the solution was replaced by fresh prehybridization solution containing the nick-translated pA1-3 probe (2×10^6 cpm/ml) in a volume of 50 μ l per 1-cm² filter. Hybridization was carried out at 65°C for 16 hr. The filters were then washed with 15 mM NaCl/1.5 mM Na citrate, pH 7/1% NaDodSO₄ at 65°C for 5 min and again for 60 min with shaking. The filters were blotted dry and exposed to Kodak XAR-5 film with Cronex Lighting Plus intensifying screens (Dupont) at -70°C for 24–72 hr.

EXPERIMENTAL RESULTS

Hybridization of pA1-3 Probe with Genomic DNAs. Pilot experiments indicated that pA1-3 cross-hybridized to CHO DNA. Because all of the cell hybrids contained CHO DNA, it was important to select for restriction enzymes that produced different restriction patterns in human as opposed to CHO DNA. DNAs extracted from CHO and human cell lines, respectively, were digested with various restriction enzymes. The resulting fragments were separated by electrophoresis on 1% agarose and blotted onto nitrocellulose paper (36). Hybridization to the nick-translated human apo A-I cDNA clone, pA1-3, indicated that a number of enzymes, including *BamHI*, *HincII*, *HindIII*, *Kpn I*, and *Sst I* yielded clearly different patterns of restriction fragments that hybridized to the probe (data not shown). We selected *HincII* as the major enzyme for chromosomal mapping because of the simple patterns generated (an \approx 15-kb fragment from human DNA and an \approx 7-kb fragment from CHO DNA). In some studies, *BamHI* was used as a second enzyme because it produced an 11-kb fragment from human DNA and a 6-kb fragment from CHO DNA.

Hybridization of pA1-3 Probe with Cell Hybrid DNAs. The cell hybrid DNAs were hybridized to ³²P-labeled human apo A-I cDNA probe, pA1-3. The positive (+) hybrids are those containing both the 7-kb CHO fragment and the 15-kb human fragment, while the negative (-) hybrids are those containing only the 7.0-kb CHO fragment (Fig. 1). Initial synteny

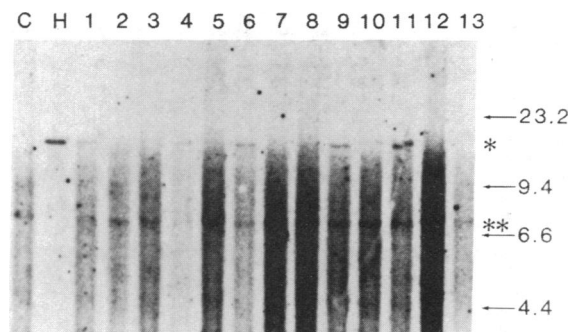


FIG. 1. Hybridization of pA1-3 to *HincII*-digested DNA from human–Chinese hamster somatic cell hybrids and their parental cells. Lanes: C, Chinese hamster cell CHO-K1; H, human cell HT-1080 (a similar pattern was obtained from DNA from human leukocytes); 1–13, to *HincII*-digested DNA from somatic cell hybrids CP3, CP5, CP6, CP12, CP14, CP15, CP16, CP17, CP18, CP20, CP26, CP27, and CP28, respectively. Molecular weight markers consisted of *HindIII*-digested λ phage DNA. *, Position of human-specific band hybridizing to pA1-3; **, position of CHO-specific band cross-hybridizing to pA1-3. Lanes 1, 4, 6, 9, and 11 represent positive hybrids showing the presence of the human band as defined in lane H.

analysis in the 13 hybrids revealed high concordance between the apo A-I gene and human chromosome 11. Further karyotype analysis of the hybrids indicated the presence of intact human chromosome 11 at high percentages in hybrids CP3 (90%), CP12 (100%), CP15 (100%), CP18 (75%), and CP26 (50%) and the absence of intact chromosome 11 in hybrids CP5, CP6, CP14, CP16, CP17, CP20, CP27, and CP28. The concordance frequencies for the 13 hybrids analyzed are given in Table 1. Concordant hybrids are those that have the particular human chromosome and the apo A-I gene either present or absent together. Conversely, those hybrids having either the chromosome or the apo A-I gene present singly are discordant hybrids. Concordant segregation frequency is expressed by dividing the number of concordant hybrids by

Table 1. Synteny analysis of the human apo A-I gene in 13 human–CHO-K1 cell hybrids by molecular hybridization using a cDNA probe pA1-3

Human chromosome	Concordant hybrids, no.	Concordant frequency, %
1	8	61.5
2	8	61.5
3	5	38.5
4	5	38.5
5	4	30.8
6	5	38.5
7	6	46.2
8	8	61.5
9	6	46.2
10	7	53.8
11	13	100.0
12	7	53.8
13	10	76.9
14	5	38.5
15	8	61.5
16	8	61.5
17	5	38.5
18	6	46.2
19	6	46.2
20	6	46.2
21	3	23.1
22	4	30.8
X	6	46.2

Only chromosome 11 exhibits a concordance frequency consistent with gene assignment to that chromosome.

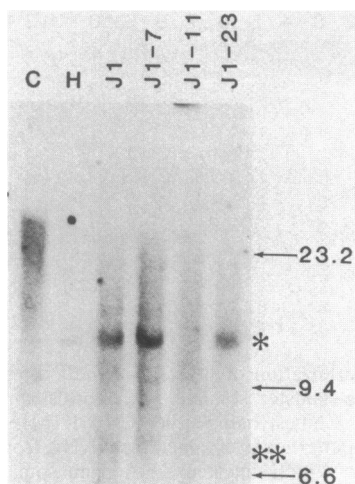


FIG. 2. Hybridization of pA1-3 to *HincII*-digested DNA from cell hybrids containing intact human chromosome 11 (J1) or clones containing chromosome 11 with deletions (J1-7, J1-11, and J1-23) (32-34). The specific deletions are indicated in Table 2. Lanes: C, CHO-K1; H, human cell HT-1080. This autoradiograph represents a 24-hr exposure of the x-ray film to the filter. *, Position of human-specific band hybridizing to pA1-3. **, Position of CHO-specific band cross-hybridizing to pA1-3; the hamster band showed up on all the lanes except H on more prolonged exposure of the x-ray film (96 hr) to the filter.

the total number of hybrids analyzed (13 hybrids in this study). Thus, the presence or absence of the 15-kb band specific to human apo A-I probe correlates well with human chromosome 11 (Table 1 and Fig. 1). These results allow assignment of the structural gene for human apo A-I to chromosome 11 and to no other chromosomes.

Confirmation of the Apo A-I Gene Assignment by Using the Cell Hybrid Containing a Single Human Chromosome 11. Molecular hybridization between the apo A-I gene probe and the J1 DNA revealed the 15-kb *HincII* band characteristic of the human apo A-I gene sequence (Fig. 2). Because no other human chromosomes are present in this hybrid clone, this result confirms assignment of the apo A-I gene to human chromosome 11.

Regional Mapping of the Apo A-I Gene Using Deletion Hybrids of Human Chromosome 11. For a regional assignment of the apo A-I gene in human chromosome 11, we used three subclones derived from J1 after mutation that introduced deletions in different parts of the chromosome. Southern blot hybridization between the gene probe and these deletion hybrid DNAs localized the apo A-I gene to the long arm of chromosome 11, in the region 11q13→qter (Table 2 and Fig. 2).

DISCUSSION

The cell hybrids used in the present study involved primary hybrid clones formed between different CHO-K1 auxotroph-

ic mutants and various human cells including lymphocytes and fibroblasts (27). Because some hybrids may still segregate certain human chromosomes at a slow rate over long periods in culture, periodic examination of the hybrids by cytogenetic analysis is essential in determining the percentage of a particular human chromosome present in a particular hybrid at any given time. Karyotypic analysis can elucidate the nature of discordant clones resulting from chromosomal segregation or breakage. It is especially important to analyze the human chromosome content at about the same time that the chromosomal analyses are carried out. Confirmation of the gene assignment obtained from synteny analysis by use of a hybrid containing only one or two relevant chromosomes as was done here is highly desirable because it minimizes the probability of error due to chromosome loss or translocation.

Atherosclerosis is one of the most important diseases of industrialized countries. This paper describes the mapping of a human gene important in this disease by means of a battery of human-CHO-K1 hybrid cell strains and selected deletion mutants. These developments make possible further genetic analysis of this disease, and the principles appear applicable to other genetic pathologies.

In a previous publication (41), it was shown that regional mapping can be followed up by more detailed fine-structure mapping through the use of deletion analysis in a series of hybrids mutagenized so as to produce a collection of deletions selected for their loss of particular markers. The DNA from each such deletion mutant is extracted, treated with an appropriate restriction nuclease, subjected to gel electrophoresis in which the fragments are separated according to their size, and then banded by exposure in the Southern blot procedure to a labeled repetitive human DNA sequence that we have shown is highly specific in recognizing only fragments of human DNA. Each of these human DNA fragments constitutes a new marker for the given chromosome whose relative mapping positions can be ascertained by the minimal marker loss principle (41). Thus, the probe for apo A-I can now be assigned to a more accurately defined position with a resolving power well beyond cytogenetic capability by associating it with the appropriate marker fragment. The further fine-structure analysis over the extended chromosomal region of the long arm of chromosome 11 that is now possible may well illuminate a number of significant relationships. In particular, it appears possible to elucidate details of both common and unique regulatory features of the closely linked apo A-I and apo C-III genes and to test for regulatory relationships with other markers on human chromosome 11.

Other apolipoprotein structural genes presumably are either weakly linked to or asyntenic with apo A-I. Thus, study of two kin with mutant apo A-I genes has shown that these genes segregate independently from the apo E phenotype (42). Moreover, the mouse genes for apo A-I and apo A-II, another major apolipoprotein in HDL, are located on different chromosomes (43). Finally, the mouse apo A-I gene has

Table 2. Molecular hybridization assay for the presence (+) or absence (-) of the human apo A-I structural gene in cell hybrids containing partial deletions of human chromosome 11

Cell hybrid	Possession of human chromosome 11 marker*							Portion of human chromosome 11 retained†	Human apo A-I gene
	Short arm				Long arm				
	a ₁	a ₃	LDHA	ACP2	a ₂	PBGD	ESA4		
J1	+	+	+	+	+	+	+	Intact	+
J1-7	-	-	-	-	+	+	+	p11→qter	+
J1-23	-	-	+	+	+	+	+	p13→qter	+
J1-11	+	+	+	+	-	-	-	pter→q13	-

*a₁, a₂, a₃, cell surface antigen markers (32-34); LDHA, lactate dehydrogenase A (33, 34); ACP2, acid phosphatase-2 (34); PBGD, uroporphyrinogen I synthase (39, 40); ESA4, esterase-A₄ (40).

†Cytogenetically detectable.

been assigned to mouse chromosome 9 (43), on which the mouse gene *Ups* (uroporphyrinogen I synthetase) is located. We have previously mapped the human gene for uroporphyrinogen I synthetase (PBGD), which is equivalent to the mouse *Ups*, to the long arm of human chromosome 11, in the same region as the apo A-I gene (39). Wang *et al.* (40) further localized PBGD to 11q23-qter. Thus, the linkage between the apo A-I gene and PBGD/*Ups* has been preserved in the two species through evolution and it can be expected that other genes on these homologous segments may find counterparts in the two species.

In addition to the fine-structure mapping of the apo A-I gene region and associated DNA regions, it is now possible to study the biosynthesis of the gene product in hybrids containing human chromosome 11, including effects of hormones, drugs, and other agents on this gene expression (44, 45); to study the effects of human chromosomes other than 11 on this expression; to identify and map specific loci other than that of the structural gene itself affecting this expression; and to study the effects of a variety of mutations in various loci on gene expression and its regulation.

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