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HUMAN APOLIPOPROTEIN A-I AND C-III GENES RESIDE IN THE p11→q13 REGION OF CHROMOSOME 11

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SUMMARY: Apolipoprotein (apo) A-I is a major protein of high density lipoproteins (HDL). The gene for apoA-I has been localized to the p11→q13 region of chromosome 11 by filter hybridization analysis of mouse-human hybrid cell cDNAs containing chromosome 11 translocations utilizing a cloned human apoA-I cDNA probe. The known linkage of apoA-I and apoC-III also permitted the simultaneous assignment of the apoC-III gene to the same region on chromosome 11. Comparison with previously established gene linkages on the mouse and human genome suggests that apoA-I + apoC-III may be linked to the esterase A4 and uroporphyrinogen synthase genes which are present on the long arm of human chromosome 11. The localization of the apoA-I + apoC-III genes in the p11→q13 region of chromosome 11 represents a definitive chromosomal assignment of a human apolipoprotein gene, and will now enable more detailed analysis of the geneomic organization and linkages of the apolipoprotein genes.

Plasma lipoproteins, the major vehicle for lipid transport in man, can be separated by hydrated density into five major classes designated chylomicrons, VLDL , IDL, LDL, and HDL (1). These plasma lipoproteins are composed of varying proportions of triglycerides, cholesterol, cholesteryl esters, phospholipids, and proteins (apolipoproteins). Thirteen apolipoproteins have been isolated and characterized from human plasma lipoproteins and several have been sequenced (for reviews, see 2-5). Apolipoproteins function as cofactors for enzymes,

†Abbreviations: VLDL, very low density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins; apo, apolipoproteins; Kb, kilobase; TBE, Tris borate EDTA, pH 8.3; SSC, standard salin citrate, pH 7.1.

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ligands for interaction with specific high affinity cellular receptors, exchange proteins for the transfer of lipid constituents between lipoprotein particles, and structural components of lipoprotein particles (for reviews, see 5,6).

Plasma HDL is of major clinical importance since HDL-cholesterol as well as apoA-I, the major apolipoprotein of HDL, have been inversely associated with the development of premature cardiovascular disease (7-9). Human apoA-I has been shown to be synthesized as a preproapolipoprotein (10-12) and the complete nucleic acid sequence of preproapoA-I has been reported (13-15). In man, preproapoA-I undergoes co-translational cleavage to proapoA-I. In vivo studies in man have shown that following secretion from the cell plasma proapoA-I undergoes post-translational cleavage to mature apoA-I by an apparent specific peptidase (16). Variants of apoA-I including apoA-$I_{Tangier}$ (17-19), apoA-I_{Milano} (20,21), and apoA-$I_{Marburg}$ (22), are associated with mild hypertriglyceridemia, and reduced levels of plasma apoA-I as well as HDL. The factors which modulate the biosynthesis and processing of apoA-I may be of major importance in determining the plasma levels of apoA-I and HDL, thereby influencing the susceptibility of subjects to the development of premature vascular disease.

In order to improve our understanding of the biosynthesis and metabolism of apoA-I we have initiated a systematic analysis of the structure as well as the factors which modulate the expression of the apoA-I gene. We have previously reported the cloning and characterization of human liver apoA-I mRNA (10). Cloned apoA-I cDNAs have been utilized as hybridization probes for analysis of the genomic organization of the apoA-I gene in normal subjects and patients with variants of the A-I apolipoprotein (15). Recently, Rees et al reported DNA polymorphism in the 3'-flanking region of the apoA-I gene (23). This polymorphism was found in 44% of the patients with type IV and type V hyperlipoproteinemia, and 5% in the control population. DNA polymorphism has been shown to be extremely useful in the evaluation of monogenic diseases (e.g., hemoglobinpathies), however, its potential usefulness for evaluation

of genetic markers for hyperlipoproteinemia and atherosclerosis remains to be established.

A detailed knowledge of the genomic organization, chromosomal location, and expression of apolipoprotein genes will be required to establish the structural and functional linkages in the apolipoprotein gene complexes. Direct linkage data, to our knowledge, has not as yet been reported for any of the thirteen apolipoproteins. Recently, Olaisen et al reported a family study which indirectly localized apoE to chromosome 19 by establishing a linkage of apoE to complement C3 which resides on chromosome 19 (24).

Advances in recombinant DNA technology have provided methods to obtain specific DNA sequences which can be used as probes for direct chromosomal assignment of specific genes. In this present report, we assign the human apoA-I gene to chromosome 11 and a regional localization of apoA-I to p11→q13 using filter hybridization analysis of human-mouse cell hybrid DNAs. Recently, a kindred with apoA-I + apoC-III absence has been reported and the genomic DNA of both genes have been identified in a single recombined phage clone (25,26). The distance between the apoA-I and apoC-III genes was less than 3 Kb apart. Assuming that the apoC-III gene occupies a constant chromosomal position in the human population, our assignment of apoA-I to chromosome 11 p11→q13 simultaneously assigns the apoC-III gene to the same region on chromosome 11.

MATERIALS AND METHODS

Apolipoprotein A-I cDNA Probe: The cDNA probe used in this study was obtained from clone pMDB 1408 as described earlier (10, 15). This clone contains the nucleic acid sequence which encodes for the entire mature apoA-I protein and 10 amino acid residues of the prepropeptide. A 866 base pair long cDNA insert was obtained by cleavage with Msp I [1 ug DNA/2 units of enzyme for 1 hour at 37°C] and purified by agarose gel electrophoresis (1.1%). 0.25 ug of purified DNA was radiolabeled to high specific activity (2.5 x 10^8 cpm/ug) by nick translation (27).

Human-Mouse Somatic Cell Hybrids: Human-mouse somatic cell hybrids were constructed by fusing mouse LM/TK (thymidine kinase deficient) or RAG (hypoxanthine phosphoribosyl transferase deficient) fibroblasts with human fibroblasts or leukocytes. Proliferating cell hybrids were selected in hypoxanthine-aminopterin-thymidine medium (28). Cell hybrids were characterized for human chromosome content by trypsin-Giemsa banding (29).

Filter Hybridization: High molecular weight DNAs were isolated from human-mouse cell hybrids as previously reported (30), at the same passage as for karyotype analysis (31). Isolated DNAs (10 ug) were digested with 30 units of EcoRI

(Bethesda Research Laboratory) for 3 hrs at 37°C. The restriction fragments were separated by 0.7% agarose gel electrophoresis (0.8 cm x 11 cm x 14 cm) at 35 volts for 16 hours. DNA was transferred to nitrocellulose filter paper (Schleicher and Schull) for 16 hrs in TBE buffer. Hybridization was performed for 24 hrs at 42°C, and washed briefly at room temperature with 2 x SSC, 0.1% SDS, then for 30 mins at 50°C with 0.1 x SSC, 0.1% SDS as previously described (32,33).

RESULTS

As previously reported (15) the apoA-I cDNA probe hybridized to a single 12.5 Kb EcoRI fragment of human DNA by Southern blotting analysis (34) (Fig. 1, lane 1). Under the same hybridization condition, no similar 12.5 Kb fragment was observed with mouse DNA (Fig. 1, lane 2). Therefore, high molecular weight DNAs were extracted from a panel of 19 human-mouse cell hybrids and analyzed by the Southern blotting technique. Filter hybridization and karotype analyses were simultaneously performed on cells at the same passage.

Figure 1 illustrates representative results obtained with the hybridization techniques for hybrid cell lines which contained the apoA-I gene (lane 3, Xer-9;

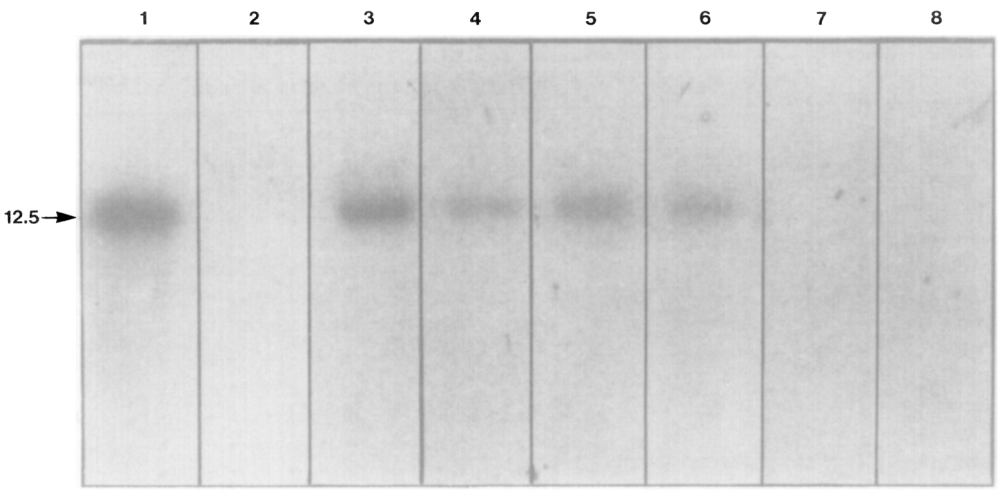

FIGURE 1. DNA from human diploid fibroblasts (lane 1), and mouse RAG cells (lane 2) and human-mouse cell hybrids (lanes 3-8) were hybridized with a ^{32}P-labelled apoA-I specific DNA fragment. The probe hybridizes to a single 12.5 kb EcoRI human DNA fragment (indicated by the arrow), but not to mouse DNA. Cell hybrid DNAs in lanes 3-6 contain the human apoA-I gene, whereas in lanes 7 and 8 the apoA-I gene was absent. The cell hybrid DNAs in lanes 3-8 are respectively: XER-9, XER-11, XER-7; REW-11; WSL-15; EXR-5 CSAZ (See Table I for the human chromosome content of each of the hybrids).

lane 4, XER-11; lane 5, XER-7; lane 6, REW 11) and when the apoA-I gene was absent (lane 7, NSL-15; lane 8, EXR-5 CsAZ).

The results of the complete analysis of the 19 human-mouse cell lines are shown in Table I. The apoA-I gene segregated with chromosome 11. To localize the apoA-I gene on chromosome 11 analyses were performed with previously well characterized cell hybrids, EXR and XER, which contain chromosome 11 translocations (35,36). Hybridization analysis with the apoA-I cDNA probe localized the apoA-I gene to the p11 → q13 region of chromosome 11 (Table I). A schematic diagram illustrating the regional assignment of the apoA-I gene on chromosome 11 is shown in Figure 2. This study permitted a direct assignment of an apolipoprotein gene to a specific chromosome.

DISCUSSION

The application of recombinant DNA techniques to human genetics has permitted the development of major new concepts of gene structure and organi-

Table 1

Segregation of the Apolipoprotein A-I Gene with Human Chromosomes in Human-Mouse Somatic Cell Hybrids

Hybrid	ApoA-I	Chromosomes																						Translocation Chromosomes
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	
TSL-2	−	−	+	−	−	+	+	−	−	−	+	−	+	−	−	−	−	−	+	−	+	+	−	+	17/3
ATR-13	−	+	+	+	+	−	+	+	+	−	+	−	+	−	+	+	−	+	+	−	−	−	−	−	5/X
NSL-9	−	−	−	−	−	+	−	−	+	−	+	−	+	+	+	+	+	+	−	−	+	+	+	−	17/9
NSL-15	−	−	+	−	+	+	−	+	+	−	−	−	+	+	+	+	−	+	+	+	−	+	+	+	
JSR-17S	+[W]	+	−	+	−	+	−	−	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	−	7q⁻
WIL-6	+	−	+	−	+	+	+	+	+	−	+	+	−	−	+	−	−	+	−	+	+	+	−	+	
WIL-2	−	−	−	−	−	−	−	−	+	−	−	−	+	−	−	−	−	+	−	−	−	+	−	+	
WIL-14	−	−	−	+	−	−	−	−	+	−	+	−	−	−	+	−	−	+	−	−	−	−	−	+	
WIL-13	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	+	+	−	−	+	−	−	
REW-11	+	−	−	−	+	−	−	−	−	−	−	+	−	+	−	−	+	−	−	−	+	+	−	+	
XTR-22	+	−	+	−	+	+	+	−	+	−	+	+	−	−	−	−	−	−	+	+	+	+	+	−	X/3
WIL-7	+	−	+	+	−	+	+	−	+	−	+	+	−	+	+	−	−	+	+	−	−	+	−	+	
WIL-8X	+	−	−	+	+	+	+	+	−	−	+	+	+	−	+	−	+	+	+	+	+	+	−	+	
JWR-26C	+	−	+	+	+	+	+	+	−	+	+	+	+	−	+	+	+	+	+	−	+	+	−	+	1p⁻
JWR-22H	+	−	−	−	+	−	+	−	−	−	+	+	−	−	+	−	+	+	+	−	+	+	−	−	2/1
XER-7	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	−	+	−	−	−	−	−	+	11/X
XER-9	+	−	+	−	+	−	−	−	+	−	+	−	+	−	−	+	+	+	+	−	−	+	−	−	11/X
XER-11	+	+	−	+	+	−	+	+	+	−	+	−	+	−	−	+	+	+	+	−	+	−	+	−	X/11, 11/X
EXR-5CSAz	−	+	−	+	+	+	+	+	+	−	+	−	+	+	+	+	+	+	+	+	+	+	+	+	X/11

A "+" in a chromosome column indicates its presence in 10% of the metaphases examined. The presence or absence of human apoA-I in a given cell hybrid is indicated by a "+" or "−", respectively, in the column designated apoA-I. The translocation chromosomes are present in the designated hybrids and originate from the human parental cells used for cell fusions (30). XER hybrids were constructed with human GM 2859 fibroblasts containing an X;11 translocation: 46, X, del (11) (p11q13), t (X;11) (q11; p11). EXR hybrids were constructed from human GM 3322 fibroblasts containing a different X;11 translocation: 46, X, t (X;11) (q22; q13).

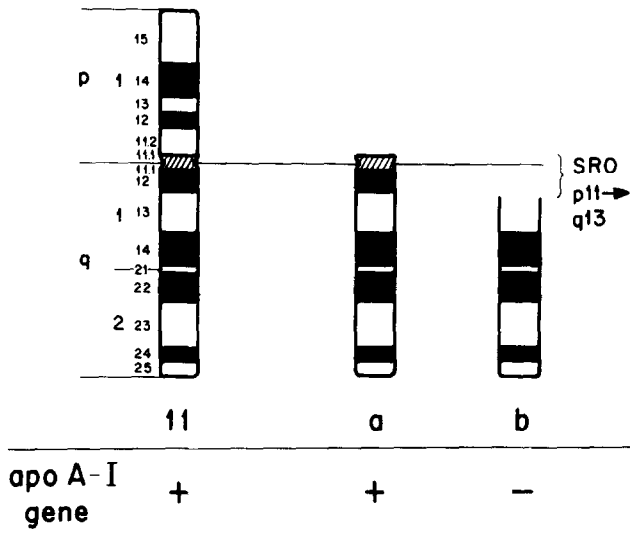

FIGURE 2. The region within the human chromosome 11 to which the apoA-I gene has been localized is shown in this schematic diagram. The normal human chromosome 11 is shown for reference on the left. In the center (a) is the region 11p11→qter involved in the 11/X translocation segregating in the XER hybrids. On the right (b) is the region 11q13→qter involved in the X/11 translocation segregating in EXR hybrids. Below each chromosome is indicated the presence ("+") or absence ("-") of the apoA-I gene. The smallest region of overlap (SRO) is p11→q13.

zation in normal subjects and patients with specific genetic diseases. In the field of lipoprotein research, there is a large body of information on the chemistry and physiological function of the plasma apolipoproteins, however, virtually nothing is known about the structure and organization of the apolipoprotein genes. Using inbred mouse strains, Lusis et al (37) have localized apoA-I and apoA-II to mouse chromosome 9 and 1, respectively. The human population, however, is genetically heterogeneous and genetic studies have been usually restricted to kindred analysis as was utilized in establishing the linkage of apoE to the C3 component of complement locus on chromosome 19 (24).

The availability of purified cloned DNA sequences as probes will now permit direct as well as unequivocal assignment of a gene to a specific chromosome. In the present study the identification of the presence of apoA-I sequences in 19 mouse-human somatic cell hybrids permitted the direct assignment of the apoA-I gene to human chromosome 11. Further analysis of cell hybrids containing chromosome 11 translocations localized apoA-I to the region p11→q13. Since the apoC-III gene has been identified in combination with apoA-I in a

single recombinant phage clone (26), the apoC-III gene can also be localized to the p11→q13 region of chromosome 11.

Current information available on the mouse and human gene map suggests that apoA-I + apoC-III may reside within a conserved group of linked genes based on the known location of other genes on the short- and long-arms of human chromosome 11 and the homologous mouse genes (Table 2). Several genes on the short-arm of human chromosome 11 including insulin, β-globin, LDHA, and c-Ha-ras1 have homologs on mouse chromosome 7 (38,39). Acid phosphatase-2 and catalase are proximal to this group of genes on the short-arm with respect to the centromere; the homologous mouse genes are on mouse chromosome 2. In the mouse, the gene Sep-1, recently identified as apoA-I, was located on chromosome 9 (37) and linked to esterase 17 and uroporphyrinogen synthase (39). The human homolog of esterase 17 is esterase A4. Both esterase A4 and uroporphyrinogen synthase are located on the long-arm of human chromosome 11 (39) which would suggest that apoA-I and apoC-III are located near esterase A4 and uroporphyrinogen synthase on the long arm of chromosome 11. Thus, the apoA-I-esterase 17-uroporhyrinogen synthase gene complex on mouse chromosome 9 may have a homologous linkage group on the long-arm of human chromosome 11. Examination of additional hybrids containing informative translocations of this region of human chromosome 11, and the use of high resolution *in situ* hybridization techniques (40) should

Table 2
Human Chromosome 11 Genes and the Locations of Homologous Genes in the Mouse

	Human Gene Symbol	Mouse Gene Symbol	Mouse Chromosome
Insulin	INS	Ins-1	7
β-Globin	HBB	Hbb	7
Lactate Dehydrogenase A	LDHA	Ldh-1	7
c-Ha-*ras*1	HRAS1	Hras	7
Acid Phosphatase	ACP2	Acp-2	2
Catalase	CAT	Cs-1	2
Apolipoprotein A-I	APOA-I	Sep-1	9
Uroporphyrinogen Synthetase	UPS	Ups	9
Esterase	ESA4	ES-17	9

Symbols and chromosome assignments for the human and mouse genes have been taken from reference (39).

add additional insights into the structural organization and linkages of the apoA-I and apoC-III genes.

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