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# CHROMOSOMAL LOCALIZATION OF THE HUMAN APOPROTEIN CI GENE AND OF A POLYMORPHIC APOPROTEIN AII GENE

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SUMMARY Human apoprotein(apo) CI and apo AII cDNA probes have been used to analyze the segregation of the human genes in panels of human-mouse hybrids. The apo CI (APOCI) gene segregates with chromosome 19 and the apo AII (APOA2) gene with chromosome 1. Somatic cell hybrids containing chromosome translocations were used to map the apo AII gene to the 1p21-1qter region. Human APOA2 is polymorphic for the restriction endonuclease Msp I. Comparison of human and mouse chromosome 1 reveals a conserved group including apo AII, renin and peptidase genes and suggests that APOA2 will be found distal to this group on human chromosome 1. The mouse apo AII gene is closely linked with genes that regulate HDL structure. Similar HDL regulatory genes will probably be found near human APOA2. 

The human plasma lipoproteins are separated by hydrated density into five classes: chylomicrons, very low density lipoprotein (VLDL), intermediate density lipoprotein (IDL), low density lipoprotein (LDL) and high density lipoprotein (HDL) (1). The lipoproteins are noncovalently associated macromolecular complexes of triglyceride, cholesterol and apoprotein. Eight well-defined apoproteins have been isolated from the lipoproteins: apoprotein(apo) AI, apo AII, apo AIV, apo B, apo CI, apo CII, apo CIII and apo E (2). The apoproteins share functional and structural properties (2,3). Apo AI and apo AII are the main structural components of HDL. Apo AI is the physiological activator of lecithin cholesterol acyl transferase (LCAT), but no function is known for apo AII. Apo AIV and apo CI can also activate LCAT in vitro. Apo B and apo E are both ligands for the LDL receptor. The peptides apo CII and apo CIII regulate lipoprotein lipase and block the interaction between the apo E of VLDL and the hepatic LDL receptor (4).

All of the apoproteins that have been sequenced possess homologous amphipathic alpha-helical regions that are capable of interacting with both aqueous and lipid environments (2,3,5). In apo AI and apo E this generates prominent internal periodicity punctuated by proline every twenty-second

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amino acid residue. The homology is also reflected at the DNA level (6). Apo AII, apo AIV, apo CI and CIII have a more or less similar amino acid periodicity. It has, therefore, been suggested that apoproteins evolved from a common ancestral gene by duplication (3).

Lipoprotein metabolism is perturbed by various mutations of the apoprotein genes. Homozygous inactivation of the locus containing the two genes apo AI and apo CIII produces a deficiency of HDL and severe premature atherosclerosis (7). Rare variants of apo AI give rise to mildly reduced HDL concentrations (8). Moreover, a Sst I restriction fragment length polymorphism at the 3' end of the apo CIII gene is associated with hypertriglyceridaemia (9,10). Structural mutations of the apo E gene and apo CII deficiency cause dyslipoproteinaemia (11-12).

To understand more clearly the genetic and structural relationship between the apoprotein genes, their possible involvement in the atherosclerotic process and linkage to other genes we have used previously described cDNA probes (13,14) to localize the genes for apo AII and apo CI.

## MATERIALS AND METHODS

Somatic Cell Hybrids. Human-mouse somatic cell hybrids were constructed by fusing human fibroblasts or leukocytes (with normal karyotypes or containing translocation chromosomes) with mouse cell lines ($LMTK^-$ and RAG). The construction and characterization of these hybrid cell lines (WIL, REW, EXR, XER, ATR, JSR, JWR, DUA, DUM, NSL, REX, SIR, JVR and TSL) have been described extensively elsewhere (15). A total of 31 cell hybrids from these independent hybrid sets involving twelve unrelated human parental cells were utilized for these studies.

Chromosome Composition of Cell Hybrids. Human-mouse somatic cell hybrids were characterized for their human chromosome content as previously described (16,17). Human chromosomes were identified in cell hybrids by the Giemsa-trypsin chromosome banding technique (17). On the same cell passage, cell hybrids were examined for 31 human chromosome-specific enzyme markers as previously described to confirm the chromosome analysis and to recognize regions of human DNA not identifiable by microscopy (16).

Filter hybridisation. The apo AII and apo CI probes were previously described cDNAs of 410 and 372bp respectively (13,14). The cDNAs were isolated as restriction fragments and labelled with $^{32}P$ to a specific activity of 0.8 to 1.0 x $10^9$ cpm/µg DNA by primed synthesis (18). Apo AII and apo CI gene sequences were detected by filter hybridization (19). DNA was isolated from hybrid cells at the same cell passage used for karyotyping and marker enzyme analysis (20). DNA isolation, restriction enzyme digestion, agarose gel electrophoresis, and filter hybridization were performed as we previously reported (15,16,20).

## RESULTS

Chromosomal localization of apo AII and apo CI genes. The apo AII cDNA hybridizes to a 15 Kb Eco RI fragment of human DNA but not to mouse DNA

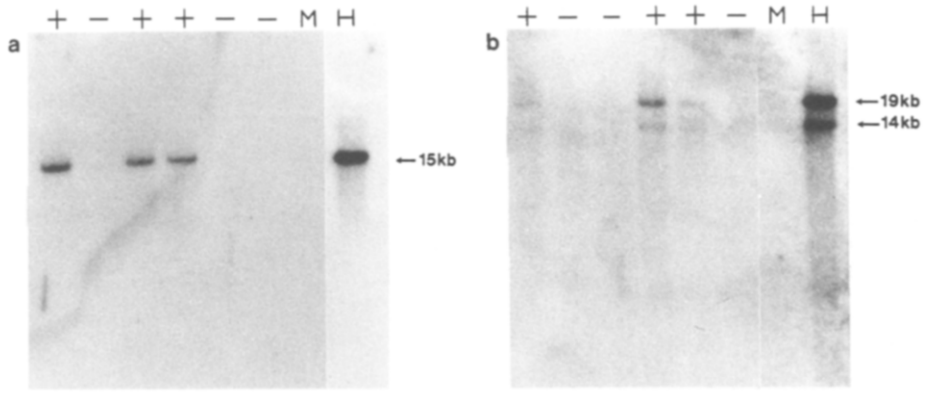


Figure 1. Southern hybridization of $^{32}$P-labelled cDNA probes for apo AII and apo CI to restriction digests of human, mouse and somatic cell hybrid DNA. The length of bands in kilobases is shown at the right of the figures. (a) Hybridization of apo AII probe and (b) apo CI probe to human Wi38 DNA (H) mouse LM/TK$^-$ DNA (M) and cell hybrid DNA digested with Eco Rl. Positive hybridization is denoted (+) and negative hybridization (−).

under similar conditions (Fig. 1a). In an analysis of DNA prepared from 31 cell hybrids containing various human chromosomes the apo AII sequences consistently segregated only with chromosome 1 (Fig. 1a and Table 1).

The apo CI cDNA hybridized to Eco RI fragments of 19 and 14 kb in human DNA but no mouse band was visible (Fig. 1b). In DNA from human-mouse hybrids human apo CI sequences cosegregated with chromosome 19.

Regional assignment of apo AII

The hybrid cell line JWR-22H retains the, 2pter-2q37::1p21-1pter, translocation chromosome containing the 1p21-pter region of chromosome 1 (21). This hybrid was negative for the apo AII gene (Table 1). Conversely JWR 26C retains the 2qter-2q37::1p21-1qter translocation chromosome containing the 1p21-1qter region of chromosome 1 (21) and was positive for the apo AII gene. Thus the human apo AII gene is located in the 1p21-1qter region and is most likely located on the long arm.

Restriction fragment length polymorphism of the apo AII gene

Analysis of the human apo AII gene in DNA from 10 unrelated individuals revealed a restriction fragment length polymorphism after digestion with MspI. A 2.5 kb DNA fragment hybridising strongly to the AII probe was detected in all cases but three individuals displayed an additional strongly hybridizing DNA fragment of 3.0 kb (Fig. 2). DNA sequence analysis of the gene reveals MspI sites 175 bp downstream in the 3' flanking sequence and in one of the introns 5' to the region coding for the mature protein (T.J. Knott and J. Scott, unpublished results). Whether the mutated site is

Table 1. Segregation of APOA2 and APOC1 with Human Chromosomes in Mouse-Human Somatic Cell Hybrids

| | | | Human Chromosomes | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Cell Hybrids | APOA2 | APOC1 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | X | Translocation |
| WIL-2 | − | − | − | − | − | − | − | − | − | + | − | + | − | + | − | − | + | − | + | − | − | − | + | − | + | |
| WIL-5 | − | − | − | − | − | + | − | − | − | − | − | + | − | − | − | − | − | − | + | + | − | − | + | − | + | |
| WIL-6 | − | + | − | + | − | + | + | + | + | + | − | + | + | − | − | + | − | − | + | − | + | + | + | − | + | |
| WIL-7 | − | − | − | + | + | − | + | + | − | + | − | + | + | − | + | + | − | − | + | + | − | − | + | − | + | |
| WIL-13 | − | − | − | − | − | − | + | − | − | − | − | − | − | − | + | − | − | − | + | + | − | − | + | + | − | |
| WIL-14 | + | − | + | − | + | − | − | − | + | + | − | + | − | + | − | + | + | − | + | − | − | − | − | − | + | |
| WIL-15 | − | − | − | + | + | + | − | − | + | − | − | + | + | + | + | + | − | − | + | + | − | + | + | − | + | |
| REW-5 | + | + | + | + | + | + | + | + | + | + | − | + | + | + | + | + | + | − | + | + | + | − | + | + | + | |
| REW-8D | − | − | − | − | − | + | − | − | − | + | − | − | − | − | − | + | − | − | + | − | − | + | + | + | + | |
| REW-10 | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | − | + | + | + | + | + | − | + | |
| REW-11 | − | − | − | − | − | + | − | − | − | − | − | − | + | + | + | − | − | + | − | − | − | + | + | − | + | |
| REW-15 | + | + | + | + | + | + | + | + | + | + | − | + | + | + | + | + | + | − | + | + | + | + | + | + | + | |
| TSL-1 | − | − | − | − | + | + | − | − | − | − | − | + | + | − | + | + | − | + | + | + | − | + | − | − | − | |
| TSL-2 | − | − | − | + | − | − | + | + | − | − | − | + | − | + | − | − | − | − | − | + | − | + | + | − | + | 17/3 |
| NSL-9 | − | − | − | − | − | − | + | − | − | + | − | + | − | + | + | + | + | + | + | − | − | + | + | + | − | 17/9 |
| JSR-17S | + | − | + | + | + | − | + | − | − | + | + | + | + | + | + | + | + | + | + | + | − | + | + | + | − | 7/9 |
| XER-7 | + | + | + | + | + | + | + | + | + | + | + | + | − | + | + | + | + | − | − | + | + | − | − | − | + | 11/X |
| XER-11 | + | + | + | − | + | + | − | + | + | + | − | + | − | + | + | − | + | + | + | + | + | + | + | + | − | 11/X X/11 |
| DUA-1 CSAzF | − | − | − | − | − | − | − | − | + | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | − | |
| DUA-3 BSAgA | − | − | − | + | − | − | − | − | + | + | − | − | − | − | + | + | − | − | + | − | − | − | − | − | − | |
| DUA-5 BSAgA | − | − | − | − | + | − | + | − | − | − | − | − | + | − | − | + | − | − | + | + | − | − | + | − | − | |
| DUM-13 | + | + | + | + | + | − | − | + | + | − | − | + | + | + | − | + | − | + | + | + | + | + | + | + | − | 15/X X/15 |
| EXR-5 CSAz | + | + | + | − | + | + | + | + | + | + | + | + | − | + | + | + | + | − | + | + | + | + | + | + | − | X/11 |
| SIR-8 | + | − | + | + | + | + | + | − | + | + | + | + | + | + | + | + | + | + | + | + | − | − | + | + | + | |
| ATR-13 | + | + | + | + | + | + | + | + | + | + | − | + | − | + | + | + | + | + | + | + | + | − | − | − | − | 5/X |
| XTR-2 | − | − | − | − | − | − | + | − | − | + | − | + | − | + | + | − | − | − | − | + | − | + | + | − | − | 3/X |
| XTR-22 | − | + | − | + | − | + | + | + | − | + | − | + | + | − | − | − | + | − | − | + | + | + | + | + | − | X/3 |
| REX-11 BSHF | − | − | − | − | + | − | − | − | − | − | − | + | − | − | − | + | − | − | − | + | − | − | − | − | − | 22/X |
| JVR-22 | + | + | + | + | + | + | + | + | + | + | − | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 2/1 |
| JWR-22H | − | − | − | − | − | + | − | + | − | − | − | + | + | + | − | + | + | − | + | + | − | + | + | − | − | 2/1 |
| JWR-26C | + | − | − | + | + | + | + | + | + | − | + | + | + | + | − | + | + | + | + | + | − | + | + | − | + | 1/2 |
| % Discordancy APOA2 | | | 0 | 32 | 23 | 35 | 35 | 26 | 13 | 32 | 26 | 39 | 32 | 23 | 35 | 35 | 13 | 29 | 45 | 39 | 19 | 48 | 55 | 32 | 39 | |
| % Discordancy APOC1 | | 1 | 19 | 29 | 35 | 29 | 35 | 13 | 26 | 32 | 39 | 45 | 32 | 42 | 42 | 48 | 26 | 42 | 58 | 45 | 0 | 42 | 55 | 29 | 39 | |

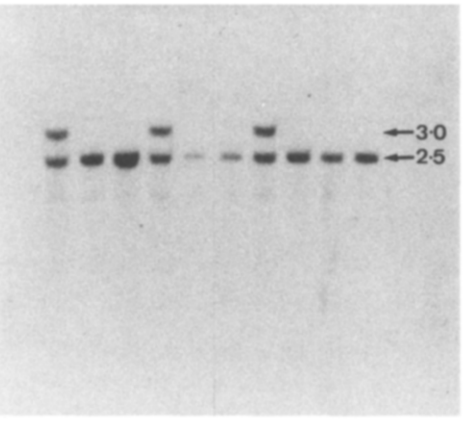


Figure 2. Hybridisation of apo AII cDNA to MspI digested human DNA.

linked to a perturbation of lipid metabolism such as the hypertriglycidaemia associated with an Sst I polymorphism in the apo AI - CIII gene complex remains to be established (9).

## DISCUSSION

We have localized the genes for human apo AII (APOA2) and apo CI (APOCI) to the long arm of chromosome 1 and to chromosome 19 respectively. The other apolipoprotein genes (except for apo B) have been mapped to chromosomes 11 and 19 (22-25). The clustering of the apoprotein genes together with their functional and structural homologies favours the apoproteins as a multigene family with a common ancestor rather than the products of convergent evolution. The demonstration of APOA2 on the long arm of chromosome 1 is not inconsistent with this suggestion because gene families may show clustering and also have members dispersed throughout the genome (26).

The genes for LDL receptor (LDLR), apo CII (APOC2) and apo E (APOE), have already been mapped to chromosome 19 (22,27). APOC1 is the fourth gene for a protein involved in lipid metabolism to be localised to this chromosome. Family studies indicate that LDLR and APOE are both linked to the gene for complement component C3 (C3) but are not themselves closely linked (22) suggesting that they lie on opposite sides of C3. The regional localization of APOC1 and APOC2 is not yet known, however, the genes are not as closely linked as the APOA1-APOC3 complex since neither APOC2 nor APOE are found on the Eco RI fragments carrying APOC1.

The mouse apo AII gene (Alp-2) has been mapped to the distal part of mouse chromosome 1 in a linkage group with the genes specifying the lymphocyte antigens Ly-9 and Ly-20 and the loop tail phenotype (Lp) (28,29). More proximal on mouse chromosome 1 is a peptidase gene (Pep-3) which is linked to Lp at a distance of 18.4 centimorgans (30). Pep-3 is closely linked to the genes for the acid proteases renin (Ren 1,2) and urinary pepsinogen (Upg-2) (29). Comparison of this distal portion of mouse chromosome 1 with human chromosome 1 reveals similarities; namely a peptidase gene (PEPC), the equivalent of Pep-3, between q25 and q42 and the human renin gene (REN) (22,31,32). By analogy with the mouse map, APOA2 may be located distal to PEPC and REN on the long arm of chromosome 1. One interesting possibility suggested by Lusis et al. for the mouse is that HDL regulator genes might be linked to Alp-2 (28). Similar regulatory sequences may be close to APOA2 on human chromosome 1.

The mouse apo AI (Alp-1) and apo AII genes are located on different chromosomes as are the human genes. Mouse Alp-1 is on chromosome 9 in a linkage group with an esterase gene and the uroporphyrinogen 1 synthetase gene (31). This relationship has been preserved on human chromosome 11, where the apo A1 gene (APOA1) has been mapped to q13 by somatic cell hybrid studies (23-25,31). The apo CIII (APOC3) gene is located 3 kb downstream of APOA1 and family studies link the apo AIV (APOA4) gene with this complex (7,22).

Comparison of human chromosomes 1 and 11 reveals the presence of conserved groups of genes involving APOA1 and APOA2. The c-Ha-ras1 proto-oncogene and the insulin and insulin-like growth factor (IGF) II genes are located on the short arm of chromosome 11 and near the centromere on the long arm is the APOA1-APOC3 complex (22,33). Similarly, on the short arm of chromosome 1 the NRAS proto-oncogene and the β-nerve growth factor gene, a distant relative of the insulin-IGF gene family, are situated in a region near the centromere. These conserved genes in regions that span the centromere suggest an evolutionary relationship between human chromosomes 1 and 11 and provide further comparative evidence that APOA2 will be found on the long arm of chromosome 1. Moreover, the physical closeness of members of the ras, insulin and apoprotein gene families may have evolutionary or functional significance since lipoproteins are known to mediate nutrient uptake and regulate cell growth (34). The recently described sequence homology between the epidermal growth factor precursor and the LDL receptor further suggests relationship between lipid metabolism and growth control (35,36).

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