Linkage relationships of the gene for apolipoprotein CII with loci on chromosome 19

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Summary. Two common restriction fragment length polymorphisms detected with cloned gene probes for apolipoprotein CII (apo CII) have been used to study the inheritance of the gene in families segregating for loci on chromosome 19. Lod scores for APOC2 with the gene for complement component 3 (C3) exclude close linkage and give a maximum at a male recombination fraction of 0.25–0.30. Lod scores for APOC2 and FHC, the gene causing familial hypercholesterolaemia, are negative in males and suggest the genes may not be linked. However, it appears that APOC2 may be closely linked to the blood group loci Lutheran (Lu) and Secretor (Se), and probably less closely linked to Lewis (Le). These data are consistent with the gene order:

\[ \text{FHC} \rightarrow \text{C3} \rightarrow \text{(Lu, Se, APOC2)} \]

Introduction

Human apolipoprotein CII (apo CII) is a peptide of 79 amino acids (Jackson et al. 1977) which is associated with circulating triglyceride-rich lipoproteins, chylomicrons, and very low density lipoprotein (VLDL) (Kostner and Holasek 1972). Apo CII has been shown to activate lipoprotein lipase (La Rosa et al. 1970; Breckenridge et al. 1978), which hydrolyses the triglycerides of chylomicrons and VLDL (Brown et al. 1981). Individuals with a deficiency of apo CII have severe hypertriglyceridaemia and other lipoprotein abnormalities (Breckenridge et al. 1978; Stalenhoef et al. 1981). Recently cDNA clones containing the gene for human apo CII have been isolated (Myklebost et al. 1984b; Jackson et al. 1984). By using these clones to analyse panels of somatic cell hybrids, the gene for apo CII has been assigned to chromosome 19 (Jackson et al. 1984; Jeunpierre et al. to be published).

It has now been established that there exists a linkage group of considerable size on chromosome 19. Recent data are summarized in the report of the 7th Human Gene Mapping Meeting held in Los Angeles in 1983 (Westerveld and Naylor 1984). Linkage between the blood group markers Lutheran (Lu) and Secretor (Se) has been established for some time (Mohr 1951), as well as linkage of these loci to the locus for myotonic dystrophy (DM) (Mohr 1954; Renwick 1971). The locus for the Lewis blood group (Le) was shown to be linked to the gene for complement component 3 (C3) (Weitkamp 1974) and DM (Simola et al. 1982). With the assignment of C3 to chromosome 19 by Whitehead et al. (1982), this linkage group was also assigned to that chromosome. Additional loci assigned to this linkage group on chromosome 19 include the loci for peptidase D (PEPD) (McAlpine et al. 1975; O'Brien et al. 1983), familial hypercholesterolaemia (FHC) (Berg and Heiberg 1978), and another apolipoprotein, APOE (Olaisen et al. 1982).

We are interested in determining the position of the APOC2 locus in relation to this linkage group. We have used two restriction fragment length polymorphisms (RFLPs) of the Apo CII gene (Humphries et al. 1983; Wallis et al. 1984) to study the inheritance of this gene in families segregating for a number of loci on chromosome 19, and report here our linkage analysis.

Materials and methods

Families

The families used were collected as part of studies of other genetic markers and disease conditions.

Familial hypercholesterolaemia families: MRC reference numbers 4893/1, 2, 4, 8, 10, 14, 15, 18, and 4979; Oslo FH2. The criteria for diagnosis of familial hypercholesterolaemia are given in Humphries et al. (1984a).

Hyperlipidaemia families: MRC 5068, 5084, St. Mary's HTG1.

Families segregating for ApoE: Oslo 41157; Aberdeen 238 = MRC 5024, Aberdeen 183 = MRC 4870, Aberdeen 395 = MRC 5048.

Families segregating for PEPD: MRC 1022, 1091, 1121.
Gene probe for apo CII

The apo CII cDNA probe consists of a 440 bp sequence cloned in the PstI site of pKT218. The sequence contains the entire 237 bp coding region for the protein plus 53 bp of a signal peptide sequence at the 5' end and 150 bp of untranslated sequence at the 3' end (Myklebost et al. 1984b). The apo CII genomic subclone (designated pSC11, Wallis et al. 1984) is a 400 bp sequence located 1 kb to the 5' direction of the gene, cloned in the EcoRI site of pUC8 (Vieira and Messing 1982).

Plasmid DNA was prepared by a modified cleared lysis method (Birnboim and Doly 1979). DNA was labelled in vitro to a specific activity of $2\times10^7$ cpm/µg by nick translation using an enzyme kit and $^{32}$p-DCTP at 400 Ci mmol (Radiacal Centre, Amersham, Ltd.).

DNA analysis

Blood samples were taken in EDTA tubes and frozen at $-70^\circ$C. Total genomic DNA was prepared from the leucocytes of 10 ml of blood by a Triton X100 lysis method (Kunkel et al. 1977). Five µg of DNA were digested with TaqI or BglI (BRL Ltd., Cambridge) using conditions recommended by the manufacturers, and the fragments separated by size using a gel of 1% agarose (Sekem). "Southern blots" (Southern 1975) were performed using the modification of Jeffreys and Flavell (1977). Hybridizations were carried out for 2-3 days at 65°C, with 0.5-1 $\times 10^5$ cpm/ml in 4 x SSC. Nitrocellulose filters (Schleicher and Schuell, BA85, 0.45µm) were washed to stringency of 1 x SSC and were exposed at $-70^\circ$C to preflashed Fuji X-ray film for 2-5 days using an X-ograph intensifying screen.

C3 protein and DNA analysis

The protein polymorphism was typed by high voltage agarose gel electrophoresis using plasma stored at $-20^\circ$C for up to one week, as previously described (Alper and Propp 1968). Analysis of the C3 DNA polymorphism detected with SstI was carried out as previously described (Davies et al. 1983).

Lewis, Lutheran, and secretor analysis

Standard serological techniques were used for Lutheran and secretor tests (Race and Sanger 1975). The Lewis phenotyping was done by testing papain-treated red cells against two anti-Le$^b$ (one human and one made in a rabbit) and two anti-Le$^b$ (one human and one of animal origin) antisera. Typing for Le-APOC2, Le-BglI and two anti-Le-b (one human and one made in a rabbit) antisera. Typing for C3-APOC2, FHC, and SstI was carried out as previously described (Davies et al. 1983).

<table>
<thead>
<tr>
<th>Locus</th>
<th>Allele frequencies</th>
<th>Reference</th>
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<tbody>
<tr>
<td>FHC</td>
<td>FH = 0.01</td>
<td>N = 0.999</td>
</tr>
<tr>
<td>C3 Sst RFLP</td>
<td>A1 = 0.64</td>
<td>A2 = 0.36</td>
</tr>
<tr>
<td></td>
<td>S = 0.77</td>
<td>F = 0.23</td>
</tr>
<tr>
<td>Lutheran</td>
<td>Lu$^a$ = 0.04</td>
<td>Lu$^b$ = 0.96</td>
</tr>
<tr>
<td>Lewis</td>
<td>Le = 0.82</td>
<td>le = 0.18</td>
</tr>
<tr>
<td>Secretor</td>
<td>Sc = 0.52</td>
<td>se = 0.48</td>
</tr>
<tr>
<td>APOC2 BglI RFLP</td>
<td>B12 = 0.47</td>
<td>B9 = 0.53</td>
</tr>
<tr>
<td>TaqI RFLP</td>
<td>T3.8 = 0.59</td>
<td>T3.5 = 0.41</td>
</tr>
</tbody>
</table>

Table 1. Allele frequencies for loci on chromosome 19

Lod score analysis of family data

Lod scores were determined using the LIPED computer program (Ott 1974) using the allele frequencies shown in Table 1. The data were entered as haplotypes for C3 and APOC2, for which two polymorphisms exist. In the case of C3, where there is no significant linkage disequilibrium (Donald and Ball 1984), the haplotype frequencies were used to calculate the lod scores for the genes. However, as there is linkage disequilibrium between the two RFLP sites for APOC2 (Wallis et al. 1984), the observed haplotype frequencies were used: $B12\ T3.8\ 0.097,\ B12\ T3.5\ 0.348,\ B9\ T3.8\ 0.533,\ B9\ T3.5\ 0.022$

Results

Two restriction fragment length polymorphisms are detected with gene probes for apo CII. The frequency of heterozygotes is 0.58 for the TaqI polymorphism and 0.46 for the BglI polymorphism. However, because of the linkage disequilibrium between the two polymorphic sites (Wallis et al. 1984), only a small amount of additional linkage information has been obtained by using both RFLPs.

We have studied 23 families segregating for APOC2 and at least one other genetic marker on chromosome 19. One such family (MRC 5068, part of a study of familial hyperlipidaemia) is shown in Fig. 1. The right-hand part of this pedigree indicates that APOC2 is on the same side of C3 as Lu and Se, an observation supported in other families showing crossovers between C3 and APOC2.

The lod scores obtained from the family data using the LIPED computer programme are presented in Table 2. The lod scores for C3-APOC2 exclude linkage at recombination fractions less than 0.05 ($z (m+f) = -5.25, \Theta = 0.05$) and give a small positive maximum value at 0.25-0.30. The values for FHC and APOC2 are negative at all values of $\Theta$ in males, suggesting that the genes are far apart. On the other hand, APOC2 does appear to be closely linked to Se and Lu, with lod score maxima (male and female combined) at $\Theta = 0.0$ of $+3.93$ for Lu and Se. The lod scores for Le-APOC2 in males ($z = +0.05, \Theta = 0.25$), although very small, suggest that the two genes are not closely linked.

Discussion

The C3-APOC2 region appears to be loosely linked to C3, unlinked or only very loosely linked to FHC, but quite closely linked to Lu and Se. While the lod score maxima for the individual comparisons do not always