The human genes for complement components 6 (C6) and 9 (C9) are closely linked on chromosome 5

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Abstract
We have used a cDNA probe for human complement component 9 (C9), which detects three DNA polymorphisms, to analyse the inheritance of C9 in families informative for C6 protein variants. We found that these genes are closely linked with a lod score of 9.28 at recombination fraction 0.00. There is no indication of allelic association.

Cytolysis mediated by the complement factors is a complex cascade reaction where the final membrane lesion is caused by the polymerisation of complement component 9 (C9) into cylindrical pores. The C9 pore formation can only take place in the presence of a receptor-like complex, the membrane attack complex (MAC), which is formed after the cleavage of C5 into C5a and C5b, where C5b reacts with C6 followed by C7 and C8 (for review, see Müller-Eberhard).

The terminal complement components belong to a family of proteins with highly conserved cysteine rich domains, related to perforin, thrombospondin, low density lipoprotein (LDL) receptor, epidermal growth factor (EGF) precursor, and low density lipoprotein receptor related protein (LRP). The shared homology between the genes indicates an evolutionary cut-and-pasting.

The complement component genes are located on several of the chromosomes. The genes for the two subcomponents of C1 are on chromosomes 1 and 12, whereas the genes for C2, C3, C4, C5, C8 (A and B), and C9 are on chromosomes 6, 19, 6, 9, 1, and 5, respectively. Recently C6 and C7 have also been assigned to chromosome 5. We now report close linkage between C6 and C9.

Materials and methods
C9 ANALYSIS
A fragment reconstituted from cloned cDNAs was used as a probe for the human C9 gene. The probe spanned from the 5' end of clone C9–30 to the 3' end of clone C9–26 and contained the entire coding sequence for human C9. After separation on an agarose gel, the fragment was purified by the use of a DEAE membrane (Schleicher & Shull NA45) and labelled by random primed synthesis.

DNA was isolated from leucocytes, and 7 µg samples were digested with TaqI, separated electrophoretically on 0.8% agarose gels, and blotted onto nylon membranes as previously described. The blots were hybridised to the probe in 5 x SSC, 100 µg/ml salmon sperm DNA, 5% dextran sulphate, and 1% SDS overnight at 65°C and washed down to 0.2 x SSC (30 mmol/l Na+) at 65°C. Autoradiographs were made by exposure of x ray film (Kodak XAR-5) to the filters for 16 to 40 hours at −70°C with intensifying screens (Agfa MR400). The TaqI polymorphisms with the alleles A1,A2 (7.6 kb, 6.6 kb) and B1,B2 (14 kb, 16 kb) have been described previously. We report here a 9.5 kb fragment (allele C+) scored as a Mendelian dominant.

C6 ANALYSIS
C6 typing was performed by high voltage agarose gel electrophoresis. Bands of C6 activity were visualised using the techniques described by Hobart et al.
with a haemolysis-in-gel assay with sensitised sheep
red cells and C6 deficient rabbit serum. Parts of the
material (including all subjects informative for C9)
have also been typed by isoelectric focusing gel
electrophoresis.21

FAMILY MATERIAL
The families studied are part of the Oslo NHIK
family material, which has been tested for more than
40 polymorphic markers.22 Families informative for
the C6 protein polymorphism have been tested for
the C9 RFLPs.

LOD SCORE ANALYSIS OF FAMILY DATA
The lod scores were calculated according to Morton,23,24 and lod scores for all recombination fractions
0-00, 0-01 to 0-49 calculated by the MOSM com-
puter program developed by Dan Weien (Norwe-
gian Computing Centre, Oslo 3, Norway) in
1970.

Table 1  Linkage relationships between C6 and C9. The female and male meioses informative for C6 and C9 were counted
separately and combined for the linkage analysis.

<table>
<thead>
<tr>
<th>Sex</th>
<th>No of families</th>
<th>No of children</th>
<th>lod score at recombination fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>0-00</td>
</tr>
<tr>
<td>Males</td>
<td>5</td>
<td>20</td>
<td>4-52</td>
</tr>
<tr>
<td>Females</td>
<td>3</td>
<td>22</td>
<td>4-77</td>
</tr>
<tr>
<td>Both*</td>
<td>7</td>
<td>35</td>
<td>9-28</td>
</tr>
<tr>
<td>C6:A1A2t</td>
<td>3</td>
<td>26</td>
<td>6-92</td>
</tr>
<tr>
<td>C6:B1B2</td>
<td>2</td>
<td>5</td>
<td>0-90</td>
</tr>
<tr>
<td>C6:C+†</td>
<td>3</td>
<td>22</td>
<td>2-25</td>
</tr>
</tbody>
</table>

* One family (fig 2) scored with seven children from the father and 18 children from the mother.
† Same families contributing.

Results
The restriction enzyme Taq1 detects multiple
RFLPs. Two of these (A and B) have been charac-
terised previously.18 The third RFLP has been veri-
fied as a Mendelian dominant trait (allele C+) in
this study. The other allele probably appears as
recessive because its position on the blot overlaps
one of the invariant bands. The RFLPs used here
are therefore numbered A1A2, B1B2, and C ac-

gording to the recommendations of Skolnick et al.25 The
RFLPs are shown in fig 1 and, a priori, the three
RFLPs are in the same locus. Consistent with this,
all give positive lod scores without recombination
with C6 (table 1). When no recombination is found,
the upper 90% confidence limit of the recombin-
ation fraction can be derived as the simplest as ln10
divided by the number of informative meioses.24

When accepting 20 informative male and 18 informa-
tive female meioses as non-recombinant for the
C6-C9 relation (two z2 score families22 each with
two children not being scorable), the limit is θ = 0-06
(ln10/38). This coincides with the confidence limit
obtained as the θ at which the lod score is equal to
the maximum minus one.26

Fig 2 shows the inheritance of the C6 protein and
C9 DNA polymorphisms in a large family. Apart
from the C9 C+ allele discussed above, the C6 and
C9 polymorphisms show normal codominant in-
eritance and no recombination has been found so
far. The combined results from the seven families
give a lod score for linkage of 9-28 at recombina-
tion fraction 0-00 (table 1). The C6–C9 haplotypes
of the independent subjects are presented in table 2. There
is no suggestion of allelic association.

Between the A1A2 alleles and the presence or
absence of the 9-5 kb C+ band there is strong
linkage disequilibrium. So far no subject with the
haplotype A2C— has been found (absent C band
= C—), 16 have A1C—, three have A1C+, and
seven have A2C+. By adding up the data from
Rogne et al,18 we get allele frequencies of A1 = 58%,
A2 = 42%, B1 = 93%, B2 = 7%, C+ = 58%, and
C— = 42% (calculated as the square root of
the number of subjects with C+ divided by the total
number of informative subjects).
The human genes for complement components 6 (C6) and 9 (C9) are closely linked on chromosome 5.

![Pedigree diagram](http://jmg.bmj.com/)

Figure 2: Pedigree of a family informative for C6 protein polymorphism (A1, A2 and B) and both the C9 A and C RFLPs with TaqI (A1, A2 and C+ , C-).

**Table 2 C6:C9 haplotypes in unrelated subjects.**

<table>
<thead>
<tr>
<th>Allele</th>
<th>C9 A1</th>
<th>C9 A2</th>
</tr>
</thead>
<tbody>
<tr>
<td>C6 A</td>
<td>15</td>
<td>8</td>
</tr>
<tr>
<td>C6 A1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>C6 B</td>
<td>11</td>
<td>7</td>
</tr>
</tbody>
</table>

**Discussion**

The genes for the terminal complement components C6, C7, and C9 have recently been localised to chromosome 5, whereas those for C8A and B are on chromosome 1. We now report close linkage between C6 and C9, with a maximum lod score of 9.3 at zero recombination frequency, well above the value of 3 which is ordinarily accepted as proof of linkage. The number of unrelated subjects is too low to draw any firm conclusions about linkage disequilibrium between C6:C9 haplotypes. However, the data indicate linkage equilibrium between the loci, and may be added to data from other groups.

The C6 and C7 genes have previously been shown to be closely linked. The close linkage of these two genes and C9 suggests a cluster of complement genes within a small area of chromosome 5, as has similarly been found for some structurally and functionally related genes, for example, some of the apolipoprotein genes. Assuming the genes are localised in a gene cluster, it would be of great interest to construct a physical map of the region spanning the C6, C7, and C9 genes with the aid of pulsed field gel electrophoresis.

We are grateful to Dr Keith Stanley for providing the C9 probe.

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