

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

Sissel Rogne, Ola Myklebost, Johan H Olving, Heidi Tomter Kyrkjebø, Reidun Jonassen, Bjørnar Olaisen, Tobias Gedde-Dahl Jr

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-Eberhard¹).

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.^{2,3} 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.⁴⁻¹⁴ Recently C6 and C7 have also been assigned to chromosome 5.¹⁵ 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 C9.¹⁶ After separation on an agarose gel, the fragment was purified by the use of a DEAE membrane (Schleicher & Shuell 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.¹⁷ 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.¹⁸ 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.^{19,20} Bands of C6 activity were visualised using the techniques described by Hobart *et al*²¹

Department of Animal Science, Agricultural University, PO Box 25, N-1432 Ås-NLH, Norway.
S Rogne

Department of Tumor Biology, Institute for Cancer Research, Norwegian Radium Hospital, 0310 Oslo 3, Norway.
O Myklebost

Institute of Forensic Medicine, University of Oslo, Rikshospitalet, 0027 Oslo 1, Norway.
J H Olving, R Jonassen, B Olaisen

Department of Genetics, Institute for Cancer Research, Norwegian Radium Hospital, 0310 Oslo 3, Norway.
H T Kyrkjebø, T Gedde-Dahl Jr

Polar Institute of Medical Genetics, Regional Hospital and University of Tromsø, N-9000 Tromsø, Norway.
T Gedde-Dahl Jr

Correspondence to Dr Rogne.

Received for publication 21 December 1990.
Accepted for publication 3 April 1991.

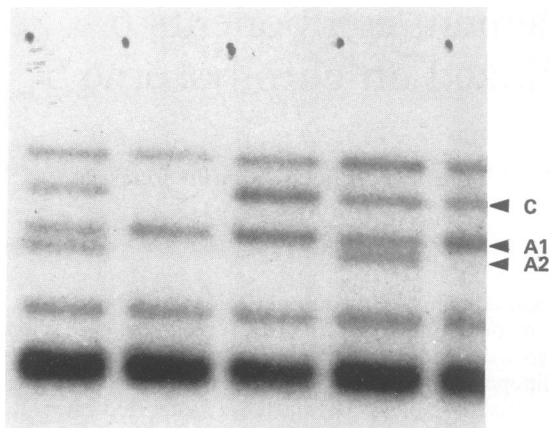


Figure 1 Autoradiograph showing gene fragments hybridising to the C9 cDNA probe after genomic DNA from different subjects were digested with *TaqI*, separated on an agarose gel, and blotted onto a nylon membrane.

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 computer program developed by Dan Wøien (Norwegian 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.

Sex	No of families	No of children	Lod score at recombination fraction						
			0.00	0.01	0.05	0.10	0.20	0.30	0.40
Males	5	20	4.52	4.43	4.07	3.61	2.64	1.60	0.51
Females	3	22	4.77	4.70	4.44	4.09	3.26	2.28	1.11
Both*	7	35	9.28	9.13	8.51	7.70	5.90	3.88	1.68
C6:A1A2†	3	26	6.92	6.81	6.37	5.78	4.50	3.05	1.40
C6:B1B2	2	5	0.90	0.88	0.79	0.68	0.45	0.23	0.07
C6:C+†	3	22	2.25	2.20	2.00	1.74	1.18	0.62	0.16

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

† Same families contributing.

Results

The restriction enzyme *TaqI* detects multiple RFLPs. Two of these (A and B) have been characterised previously.¹⁸ The third RFLP has been verified 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 according 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 recombination fraction can be derived the simplest as $\ln 10$ divided by the number of informative meioses.²⁴ When accepting 20 informative male and 18 informative female meioses as non-recombinant for the C6:C9 relation (two χ_2 score families²³ each with two children not being scorable), the limit is $\theta = 0.06$ ($\ln 10/38$). 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 inheritance 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 recombination 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).

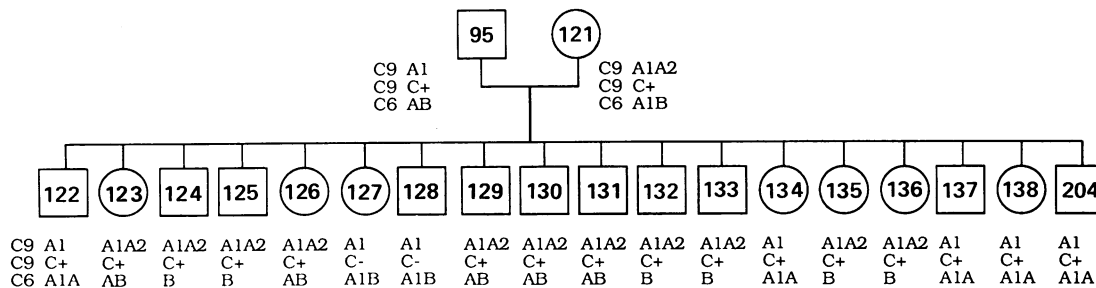


Figure 2 Pedigree of a family informative for C6 protein polymorphism (A, A1 and B) and both the C9 A and C RFLPs with TaqI (A1, A2 and C+, C-).

Table 2 C6:C9 haplotypes in unrelated subjects.

Allele	C9 A1	C9 A2
C6 A	15	8
C6 A1	1	
C6 B	11	7

Discussion

The genes for the terminal complement components C6, C7, and C9 have recently been localised to chromosome 5,^{14,15} whereas those for C8A and B are on chromosome 1.^{12,13} 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.^{27,28} 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.^{29,30} 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.

1 Müller-Eberhard HJ. The membrane attack complex of complement. *Annu Rev Immunol* 1986;4:503-28.
 2 Stanley KK, Luzio P. A family of killer proteins. *Nature* 1988;334:475-6.
 3 Herz J, Hamann U, Rogne S, Myklebost O, Gausepohl H, Stanley KK. Surface location and high affinity for calcium of a 500 kDa liver membrane protein closely related to the LDL-

receptor suggest a physiological role as lipoprotein receptor. *EMBO J* 1988;7:4119-27.
 4 Hedge PJ, Sellaer GC, Reid KBM, Solomon E. Assignment of the A chain of C1q (C1QA) to the short arm of chromosome 1. *Cytogenet Cell Genet* 1987;46:627.
 5 Solomon E, Skok J, Griffin J, Reid KBM. Human C1qB chain (C1QB) is on chromosome 1p. *Cytogenet Cell Genet* 1985;40:749.
 6 Cohen-Haguenaer O, Serero S, Tosi M, et al. Chromosomal assignment of human C1-R, C1-S genes on chromosome 12 and C1 inhibitor gene on chromosome 11. *Int Congr Hum Genet* 1986;7:617A.
 7 Day HK, L'Esperance P, Good RA, et al. Hereditary C2 deficiency: genetic studies and association with the HLA system. *J Exp Med* 1975;141:1464-9.
 8 Whitehead AS, Solomon E, Chambers S, Bodmer WF, Povey S, Fey GH. Assignment of a structural gene for the third component of human complement to chromosome 19. *Proc Natl Acad Sci USA* 1982;79:5021-5.
 9 Teisberg P, Akesson I, Olaisen B, Gedde-Dahl T Jr, Thorsby E. Genetic polymorphism of C4 in man and localisation of a structural C4 locus to the HLA gene complex of chromosome 6. *Nature* 1976;264:253-4.
 10 Jeremiah SJ, West LF, Davis MB, Povey S, Carritt B, Fey GH. Assignment of human complement component C5 to chromosome 9. *Cytogenet Cell Genet* 1987;46:634.
 11 Lemons RS, Le Beau MM, Tack BF, Wetsel RA. Chromosomal mapping of the gene encoding the fifth component of human complement. *Cytogenet Cell Genet* 1987;46:647.
 12 Rogde S, Mevåg B, Olaisen B, Gedde-Dahl T Jr, Teisberg P. Structural gene(s) for complement factor C8 (C8) on chromosome 1. *Cytogenet Cell Genet* 1983;37:571.
 13 Rogde S, Olaisen B, Gedde-Dahl T Jr, Teisberg P. Two complement component C8 loci are localized between PGM1 and RH on chromosome 2. *Cytogenet Cell Genet* 1985;40:734-5.
 14 Rogne S, Myklebost O, Stanley KK, Geurts van Kessel AHM. The gene for human complement C9 is on chromosome 5. *Genomics* 1989;5:149-52.
 15 Jeremiah SJ, Abbott CM, Murad Z, et al. The assignment of the genes coding for human complement components C6 and C7 to chromosome 5. *Ann Hum Genet* 1990;54:141-7.
 16 Stanley KK, Kocher HP, Luzio JP, Jackson P, Tschopp J. The sequence and topology of human complement component C9. *EMBO J* 1985;4:375-82.
 17 Rogne S, Skretting G, Larsen F, et al. The isolation of a cDNA clone for human lecithin:cholesterol acyl transferase and its use to analyse the genes in patients with LCAT deficiency and fish eye disease. *Biochem Biophys Res Commun* 1987;148:161-9.
 18 Rogne S, Mevåg B, Gedde-Dahl T Jr, Myklebost O. Multiple RFLPs of human complement component C9 (C9) detected by TaqI. *Nucleic Acids Res* 1990;18:3112.
 19 Olving JH, Olaisen B, Teisberg P, Gedde-Dahl T Jr, Thorsby E. Nonlinkage between C6 and chromosome 6 markers. *Hum Genet* 1977;37:125-9.
 20 Olving JH, Olaisen B, Gedde-Dahl T Jr, Teisberg P. Genetic linkage relations of the sixth component of complement (C6). *Hum Genet* 1979;46:181-92.

- 21 Hobart MJ, Lachmann PJ, Alper CA. Polymorphism of human C6. In: *Properties of the biological fluids*. Oxford: Pergamon Press, 1975: 575.
- 22 Olaisen B, Teisberg P, Gedde-Dahl T Jr, Moen T, Thorsby E. Complement loci of the HLA complex. Studies on families with intra-HLA cross-overs and haplotype associations. *Hum Immunol* 1981;2:247-54.
- 23 Morton NE. Sequential tests for the detection of linkage. *Am J Hum Genet* 1955;7:277-318.
- 24 Morton NE. Further scoring types in sequential linkage tests with a critical review of autosomal and partial sex-linkage in man. *Am J Hum Genet* 1957;9:55-7.
- 25 Skolnick MH, Willard HF, Menlove LA. Report of the committee on gene mapping by recombinant DNA techniques. *Cytogenet Cell Genet* 1984;37:210-48.
- 26 Conneally PM, Edwards JH, Kidd KK, Lalouel J-M, Ott J, White R. Report of the committee on methods of linkage analysis and reporting. *Cytogenet Cell Genet* 1985;40:356-9.
- 27 Hobart MJ, Joysey V, Lachmann PJ. Inherited structural variation and linkage relationships of C7. *J Immunogenet* 1978;5:157-63.
- 28 Lachmann PJ, Hobart MJ, Woo P. Combined genetic deficiency of C6 and C7 in man. *Clin Exp Immunol* 1978;33:193-203.
- 29 Karathanasis SK. Apolipoprotein multigene family: tandem organization of human apolipoprotein AI, CIII, and AIV genes. *Proc Natl Acad Sci USA* 1985;82:6374-8.
- 30 Myklebost O, Rogne S. A physical map of the apolipoprotein gene cluster on human chromosome 19. *Hum Genet* 1988;78:244-7.