Linkage analysis of myotonic dystrophy and sequences on chromosome 19 using a cloned complement 3 gene probe

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SUMMARY Variations in DNA sequence generate polymorphisms which can be followed through families. A cloned gene specific probe for human complement 3 (C3) was hybridised to DNA samples digested with restriction endonucleases. The C3 probe detects several restriction fragment length polymorphisms (RFLPs) that occur frequently in the general population. These DNA alleles can be readily used in linkage analyses of loci on chromosome 19, since most families studied are informative. The inheritance of one such polymorphism was followed through myotonic dystrophy families. The segregation data for both the C3 protein polymorphism and the C3 RFLP support the linkage of myotonic dystrophy (DM) and C3.

Differences in DNA sequence owing to single base changes, deletions, or insertions occur approximately once in every 100 to 200 base pairs in the normal population¹ and may be detected as changes in the size of fragments generated after digestion of the DNA with restriction endonucleases.² These restriction fragment length polymorphisms (RFLPs) show Mendelian inheritance and so can be used in family linkage studies to localise a specific gene of interest.³⁻⁷

If a linked restriction site polymorphism shows disequilibrium with a phenotype, as in sickle cell anaemia, then an analysis of the inheritance of the RFLP may also allow antenatal diagnosis of the disease.⁸ Diagnosis will also be possible if the mutation causing a disease generates a change in restriction pattern,⁹ or if a family study reveals a RFLP which shows linkage to a disease within the family even if in equilibrium in the population, as for some cases of thalassaemia.¹⁰

Myotonic dystrophy (DM) is a dominantly inherited disorder affecting about 1 in 8000 people in well studied populations.¹¹ Although the age of onset of the disease is very variable, it manifests its symptoms mostly in adult life after the carriers have reached reproductive age. As yet there is no direct antenatal

diagnosis. Linkage analysis using protein polymorphisms has established that the DM locus lies in the linkage group on chromosome 19 containing the loci for the Lutheran blood group and the ABH secretor system,¹²⁻¹⁴ the Lewis blood group,¹⁵ the C3 complement component,^{16 17} and the enzyme peptidase D.¹⁸⁻²⁰ In this paper, we describe the characterisation of common RFLPs detected by the DNA probe for C3 that can be used in the genetic mapping of chromosome 19, one of which gives a positive lod score with DM in the first few families studied.

Materials and methods

FAMILY STUDIES

Families with myotonic dystrophy likely to be suitable for linkage investigations were selected from a large number of kindreds previously studied throughout the UK. All affected persons and relatives were examined clinically to confirm the diagnosis, including ophthalmoscopic examination using a portable slit lamp (KOWA instruments). In the case of unaffected relatives, only those over 20 years old were included in the DM linkage analysis to minimise the misidentification of late onset subjects.

GENE PROBE FOR C3

The C3 DNA probe consists of a non-repetitive sequence, most likely an exon of the structural gene coding for this protein.¹⁷ The sequence, a PstI fragment 1.39 kb in length (1 kilobase (kb) equals 1000 base pairs of DNA), was cloned into pxf3, a derivative of pBR322. All recombinants were grown under conditions advised by GMAG.

HYBRIDISATION

DNA was isolated from blood samples taken from patients and their families.²¹ DNA was digested to completion with restriction enzymes and electrophoresed in 0.8% agarose gels. The gels were blotted onto nitrocellulose filters²² and hybridised to nick-translated C3 probe as described previously.²³ The filters were autoradiographed for 18 hours at -70°C .

C3 PROTEIN ANALYSIS

The C3 plasma protein polymorphism was typed by high voltage agarose gel electrophoresis using fresh plasma or plasma stored at -20°C for up to one week. The bridge buffer was 50 mmol/l barbital buffer pH 8.6 containing 1.8 mmol/l calcium lactate and then diluted 1 : 1 with distilled water for the gel buffer. One percent agarose gel was prepared using Litex agarose (Denmark) type HSB and poured onto alcohol washed $220 \times 150 \times 3$ mm glass plates to form a layer 1.25 mm thick. Up to 12 slots for plasma application were cut using 4 mm wide strips of Whatman No 3 paper and placed about 40 mm from the cathode end of the gel. A total of 3 μl of plasma was placed in each slot. Electrophoresis was carried out at 20 V/cm for 2 hours at 5°C . Gels were stained for protein using 0.1% Coomassie Blue.²⁴

FIG 1 Restriction enzyme fragment length polymorphisms detected by a C3 probe with the enzymes *SstI* (a), *TaqI* (c), and *BglII* (d). Southern blots were washed to $3 \times \text{SSC}$ and autoradiographed for 18 hours at -70°C . Fig 1b shows the stained C3 protein gel.

Results

DNA samples from both normal subjects and members of myotonic dystrophy families show DNA sequence polymorphisms with the gene specific probe for C3, using the restriction endonucleases SstI, TaqI, and BglII (fig 1a, c, d). The polymorphism used in this study, detected after digestion by the enzyme SstI, gives three patterns: a single 12.0 kb fragment, two fragments of 9.0 kb+3.0 kb, or all three fragments (lanes 6, 1, and 2 in fig 1a). These patterns are interpreted to be the result of the products of two alleles, A1 and A2. The frequency of the rarer allele (A2) in the random population is 0.36, which is similar to the frequency found in the myotonic dystrophy patients (table 1). Fig 1b shows the three C3 phenotypes FS, S, and F (lanes 1, 2, and 4) detected by electrophoresis. The frequency of the rarer allele (F) in the random population is 0.22.

Fig 2 shows a myotonic dystrophy pedigree in which both DNA and protein polymorphism are

MRC 4937, M92

FIG 2 Pedigree of family MRC 4937, M92 showing the segregation of myotonic dystrophy, the C3 protein polymorphism, and the C3 RFLP. (The numbering is consistent with that used in ref 20.)

TABLE 1 Comparison of gene frequencies of the C3 protein polymorphism and RFLP in the random UK population and unrelated myotonic dystrophy patients. (*Galton Laboratory, unpublished data.)

C3 RFLP	Phenotypes			Gene frequencies	
	A1	A1A2	A2	A1	A2
Normal population	19	21	6	0.64	0.36
DM patients	2	8	2	0.50	0.50
$\chi^2_{(1)} = 1.6, p > 0.20$					
C3 protein polymorphism*	Phenotypes			Gene frequencies	
	S	FS	F	S	F
Normal population	380	216	34	0.77	0.23
DM patients	26	14	2	0.79	0.21
$\chi^2_{(1)} = 0.05, p > 0.70$					

segregating; both show codominant inheritance. We have found no evidence for recombination between the C3 RFLP and the protein polymorphism.

Linkage between the C3 RFLP, the protein polymorphism, and myotonic dystrophy was analysed by the computer programme LIPED,²⁵ using the variable age of onset factor curve¹¹ (table 2). In calculating the total lod score, subjects heterozygous for both the RFLP and protein polymorphisms were included only once. For example, in family M92 (fig 2), I.1 was scored for the C3 protein polymorphism, and II.2 and II.3 were both scored for the C3 RFLP as they are phase known, whereas phase is not known for the C3 protein polymorphism.

TABLE 2 Lod scores between myotonic dystrophy and the C3-RFLP and protein polymorphisms.

	Sex	Children			Lod scores at various values of 0					
		No	rc	nrc	0.01	0.05	0.10	0.20	0.30	0.40
DM:C3—RFLP	P	9	0	4	+0.90	+0.83	+0.74	+0.57	+0.39	+0.20
	M	13	2	3	-4.60	-1.93	-0.98	-0.22	+0.03	+0.08
DM:C3—protein	P	22	0	1	+0.36	+0.98	+1.10	+0.93	+0.58	+0.21
	M	19	0	1	-1.94	-0.80	-0.26	+0.12	+0.17	+0.10
DM:C3 total	P	22	0	4	+1.22	+1.68	+1.68	+1.34	+0.86	+0.38
	M	29	2	4	-6.62	-2.84	-1.30	-0.13	+0.18	+0.17
DM:C3 Eiberg <i>et al</i> ¹⁶	P	16	0	0		+1.68	+1.63	+1.21	+0.67	+0.06
	M	6	0	0		-1.18	-0.67	-0.25	-0.09	-0.02
Total	P	38	0	4		+3.36	+3.31	+2.55	+1.53	+0.44
	M	35	2	4		-4.02	-1.97	-0.38	+0.09	+0.15

P = paternal double heterozygote.
M = maternal double heterozygote.
No = number scored.
rc = recombinants.
nrc = non-recombinants.

Discussion

Our study is in agreement with the linkage between C3 and myotonic dystrophy previously suggested by Eiberg and Mohr¹⁶ based on the protein polymorphism alone. So far we have not studied all the families for the C3 RFLP, but such a combined approach will considerably increase the proportion of families from which linkage data can be obtained. As expected, no example of recombination was found between the site defined by the C3 DNA probe and the C3 protein.

Our data show a marked difference in recombination fraction for male and female recombination (table 2), a rather general finding in human linkage, also found previously for the C3 protein polymorphism.¹⁶ For a marker to be of general use for antenatal diagnosis of myotonic dystrophy it must show linkage in both males and females. The genetic length of chromosome 19 in males is about 100 cM^{26,27} and the female genetic length may well be greater than 200 cM. Therefore, at least ten equally spaced markers will be required to permit the construction of a total genetic linkage map of chromosome 19.

The C3 and DM loci are at a considerable distance (of the order of 7 cM in males and much greater in females). Since the total DNA sequence length of the human haploid genome is approximately 3×10^9 bp (or 3×10^6 kb),²⁷ and since the male genetic length is 33 Morgans,^{28,29} one centiMorgan is approximately equal to 1000 kb for males. Therefore the C3 probe is at least 7000 kb from the DM defect, too far to 'walk' along the chromosome.³⁰ It is necessary to isolate further probes defining sites closer to myotonic dystrophy before 'walking' from a close-by site to the disease locus becomes feasible.

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