Am. J. Hum. Genet. 46:1073–1081, 1990

A New Polymorphic Probe Which Defines the Region of Chromosome 19 Containing the Myotonic Dystrophy Locus

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Summary

The region of human chromosome 19 which includes the myotonic dystrophy locus (*DM*) has recently been redefined by the tight linkage between it and the gene for muscle-specific creatine kinase (CKMM), which lies just proximal to *DM*. Utilizing human/hamster hybrid cell lines containing defined breakpoints within this region, we have assigned a number of new probes close to *DM*. Two of these probes, p134B and p134C, were isolated from a single cosmid clone (D19S51) and detect the same *BglI* RFLP; p134C detects an additional RFLP with the enzyme *PstI*. Analysis of these probes in the Centre d'Etude du Polymorphisme Humain families demonstrates tight linkage with a number of markers known to be proximal to *DM*. A two-point lod score of 6.34 at $\theta = .025$ demonstrates the linkage of this probe to *DM*. Analysis of a *DM* individual previously shown to be recombinant for other tightly linked markers indicates that p134C is distal to *DM*. This result indicates that both the new probe and the existing group of proximal probes including CKMM and ERCC1 probably flank *DM* and define the genetic interval into which this mutation maps.

Introduction

Myotonic dystrophy (DM) is the commonest of the inherited adult dystrophies, with an estimated population frequency of between 1/5,000 and 1/15,000 (Todorov et al. 1970; Harper 1989). The disorder is characterized by sustained muscle contraction; a wide range of accompanying symptoms have been recorded. Often clinical diagnosis is hampered by the highly variable age at onset and severity that can occur within families, variability which must be considered when studying segregation of probes in families and scoring recombinants (Griggs et al. 1989). Despite the wide variation in symptoms, DM is genetically homogeneous in that, in every population so far studied, the disorder segregates as a single locus at 19q13.2 (Harper 1989; Takemoto et al. 1990). The new mutation rate in this disorder appears to be extremely low, with no known cases where genetic involvement has not been found (Harper 1989).

DM is inherited as an autosomal dominant trait and was shown to be linked to the Lutheran and ABH secretor blood group markers by Mohr (1954). The subsequent assignment of that linkage group to chromosome 19 (Whitehead et al. 1982) was the first such localization for a disease of totally unknown etiology by using the techniques of molecular genetics. Since that time a number of markers which are tightly linked to DM have been characterized and are utilized for prenatal and presymptomatic diagnosis of this disorder: the gene for apolipoprotein C2 (APOC2) (Humphries et al. 1984; Shaw et al. 1985; Pericak-Vance et al. 1986), the anonymous marker LDR152 (D19S19) (Bartlett et al.

Received October 25, 1989; final revision received January 25, 1990.

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0002-9297/90/4606-0007$02.00

1987), the translocation breakpoint clone BCL3 (Korneluk et al. 1989*b*), D19S50 (Korneluk et al. 1989*b*), and the gene for the muscle-specific form of creatine kinase (CKMM) (Brunner et al. 1989*a*).

The markers that are most tightly linked to DM have been mapped genetically, by utilizing the Centre d'Etude du Polymorphisme Humain (CEPH) panel of large sibship families (Nakamura et al. 1988; Harley et al. 1989) and physically by using a number of breakpoints within the 19q13.2-q13.3 region (Brook et al. 1986; Stallings et al. 1988; Schonk et al. 1989). In addition, a number of families which show recombination between *DM* and one or more of the closely linked markers have been identified, and *DM* has been located in the genetic map of 19q (Brunner et al. 1989*b;* Harley et al. 1989; Johnson et al. 1989; Korneluk et al. 1989*a*). The results of these studies strongly indicate a location for the DM gene distal to CKMM (fig. 1) (reviewed in Le Beau et al. 1989). This location places all of the most tightly linked probes to the proximal side of *DM*, with a relatively large genetic interval of 9 cM to D19S50, the nearest distal marker on 19q (fig. 1). The region of chromosome 19 into which *DM* maps has been more tightly defined by the demonstration of a recombination between it and ERCC1, placing *DM* distal to this marker (Smeets et al. 1989). This defines ERCC1 as the closest of the markers on the proximal side of *DM*.

The first priority for increasing the accuracy of prenatal and presymptomatic diagnosis in this disorder is to isolate a tightly linked marker distal to *DM* on 19q13.2. This is also a prerequisite for any strategy that aims to isolate the DM gene by reverse genetics. Physical linkage of the two flanking probes by using pulsed-field gel electrophoresis (PFGE) will define the physical size of the target region of the genome in which the mutation maps. Physical mapping of the region by PFGE shows that BCL3 and APOC2 are physically linked (Shaw et al. 1989), as are CKMM and the excision-repair genes ERCC1 and ERCC2 (Smeets et al. 1990). However, these two clusters, although tightly linked genetically, have not yet been physically linked to each other.

A series of hybrid cell lines has been identified which carry fragments of chromosome 19q13.2–13.3, generated by using the human ERCC1 gene as a dominant selectable marker in a DNA repair-deficient hamster background (Stallings et al. 1988). One of these hybrid lines, 20XP3542-1-4, contains the cluster of markers including ERCC1 which are on the proximal side of *DM*. In addition to these hybrids, a cell line (908K1) with a breakpoint within the CKMM gene has been described (Hulsebos et al. 1986; B. Wieringa, personal communication). By constructing a cosmid library from 20XP3542-1-4 DNA and by screening the human clones obtained against the 908K1 hybrid, we have obtained a series of clones which physically map distal to CKMM. We have isolated two single-copy, polymorphic probes from one of these cosmids, D19S51. The segregation of the alleles detected by these probes has been investigated in DM families. In addition, linkage analysis using CEPH families has helped to position these probes with respect to other markers in the region.

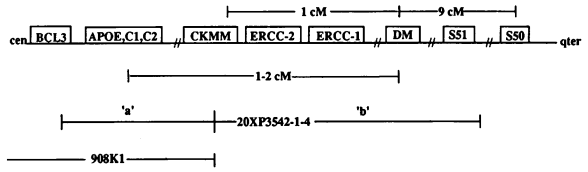

Figure 1 Genetic and physical map of chromosome 19q showing the hybrid breakpoints for 20XP3542-1-4 and 908K1, which divide the chromosome into useful physical divisions. The hybrid cell lines are described in the Introduction and Material and Methods sections. The key intervals are indicated for the 20XP3542-1-4 cell line. 'a' Indicates the interval proximal to CKMM, and 'b' indicates the interval distal to this marker. DM maps into interval 'b' and is flanked by ERCC1 on the proximal side and by D19S51 on the distal side. The map shows the putative position of D19S51, as determined from the recombination in family 120. The probe order is that determined from multipoint analyses in DM and CEPH families. Double slashes (//) indicate the positions of gaps in the physical map data between probes. This figure is a composite of data from Nakamura et al. (1988), Harley et al. (1989), Le Beau et al. (1989), Schonk et al. (1989), and Shaw et al. (1989).

Material and Methods

Hybrids

The hybrid cell lines used in the present work have all been described in detail elsewhere; they are the single human der(19) chromosome hybrid (908K1) (Hulsebos et al. 1986), the chromosome 19–only hybrid (5HL9–4) (Thompson et al. 1989), and the fragmented hybrid cell line (20XP3542-1-4), which was selected for by using ERCC1 as a dominant selectable marker for 19q13.2–13.3 (Stallings et al. 1988).

Cosmid Library Construction

A cosmid library was constructed in the vector Lorist X with DNA from the 20XP3542-1-4 cell line. This vector was provided by Dr. P. Little (Imperial College, London) and is a derivative of the vectors Lorist B and Lorist 6 (Little and Cross 1985; Gibson et al. 1987).

A cosmid library from the 5HL9–4 cell line was constructed in the vector Lorist B. High-molecular-weight (150–300 kb) DNA from confluent monolayer cultures of the hybrid cell line was prepared using the ABI automatic nucleic acid extractor (model 340A). Both libraries were constructed from partial *Hin*dIII digests of the cell line DNAs by using fragments of 40–45 kb in the final ligation.

Library Screening

The cosmid libraries from the hybrids 5HL9-4 and 20XP3542-1-4 were screened at low density (5,000 colonies/140-mm filter) on Hybond-N (Amersham) filters placed on NZCY plates containing 10 μg/ml of kanamycin. The filters were denatured and neutralized by standard procedures and hybridized with labeled total human DNA to identify the clones harboring human inserts in these libraries. The human clones were screened to purity and grown up in individual wells of 96-well microtiter dishes to facilitate the replication of the entire set of human clones. Each of the clones was stored as a glycerol stock in microtiter dishes, and all have been shown to remain viable for ≥1 year at −70°C.

Preparation and Labeling of Cosmid Clones

All of the positive clones from the 20XP3542-1-4 cell line library were grown up, and DNA was extracted from 3 ml of overnight culture by using the Birnboim and Doly (1979) procedure. The DNA obtained from these preparations was resuspended in 50 μl of TE. A 2.5-μl sample of the resuspended cosmid DNA was boiled for 10 min in a total volume of 32.5 μl and was radiolabeled using the method of Feinberg and Vogelstein (1983). Routinely this procedure gave probes labeled to a specific activity of $>10^8$ cpm/μg of DNA. All of the cosmid probes were prehybridized with sheared competitor DNA (human placental DNA of <1 kb) to compete out repetitive sequences (Sealey et al. 1985). The labeled cosmid DNA was boiled for 10 min in a total volume of 300 μl containing 100 μg of competitor DNA and a final concentration of 6 × SSC. The probe mixture was then placed at 65°C for 2 h prior to hybridization.

Family Material

Pedigree analysis was carried out on human DNA samples from a panel of DM families as well as from the 3-generation CEPH families. Wherever possible, the affected individuals in the DM panel satisfy the diagnostic criteria defined by the DM working group (Griggs et al. 1989), including paternity testing and repeat sampling. One important exception to the repeat-sampling clause is family 120 (fig. 2), which, despite repeated requests, has failed to assist us further in our research.

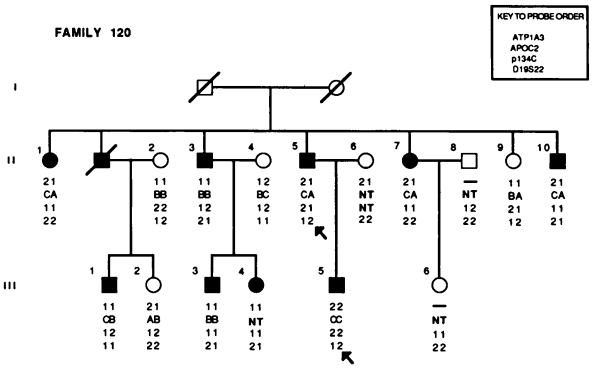

Figure 2 Pedigree and partial haplotypes of a family in which there is a recombinant with p134C. Typings are listed from top to bottom, corresponding to the descending (cen-to-ter) probe order on 19q. Typings are shown for the probes ATP1A3(*Pst*I), APOC2(*Taq*I, *Bgl*I), p134C(*Pst*I), and D19S22 (*Pst*I), respectively. Solid symbols indicate the presence of DM. NT indicates that a typing is not available. A dash (—) indicates that phase is unknown.

Our DM families include a number in which recombination between a tightly linked marker and the disease locus had been characterized in order to derive information on probe order. Linkage analysis was performed using the LINKAGE program (Lathrop et al. 1984).

DNA Techniques

DNA samples were prepared from peripheral blood or cell lines, and 2–3 μg were digested to completion by using the conditions specified by the supplier. Southern blotting was carried out using standard techniques (Maniatis et al. 1982). Hybridization probes were labeled to a specific activity of between 5×10^8 and 2×10^9 cpm/μg of DNA by using the method of Feinberg and Vogelstein (1983). The probes and restriction enzymes used to detect polymorphisms at loci on chromosome 19 are summarized in table 1. Insert DNA fragments were excised from their vector plasmids and isolated from low-melting-point agarose gels prior to labeling.

Results

Cosmid Screening and Mapping

The ligation of the 20XP3542-1-4 cosmid library yielded 5×10^5 recombinants from 1 μg of input DNA, and 1.5×10^5 clones from this library were screened

Table 1

Polymorphic Probes Used to Genotype DM Families

Locus and RFLP	Probe Source	Reference
PRKCG:		
*Msp*I	A. Ullrich	Johnson et al. 1988
CKMM:		
*Taq*I	B. Wieringa	Coerwinkel-Driessen et al. 1988
*Nco*I		Perryman et al. 1988
D19S22:		
*Pst*I	Y. Nakamura	Nakamura et al. 1988
*Pvu*II		
*Msp*I		
*Taq*I		
APOC2:		
*Taq*I	S. Humphries	Humphries et al. 1984
*Nco*I		Shaw et al. 1985
*Ban*I		Frossard et al. 1986
*Bam*HI		Meredith et al. 1986
*Ava*II		Frossard et al. 1987
pSC11:		
*Bgl*I	S. Humphries	Wallis et al. 1984
BCL3:		
*Ban*I	T. McKeithan	McKeithan et al. 1987
*Eco*RI/*Mlu*I		Shaw et al. 1989
D19S19:		
*Pst*I	R. Bartlett	Bartlett et al. 1987
*Msp*I		

with total human DNA. The 5HL9-4 cell line library yielded 4×10^5 recombinants, and 1,500 human positives were isolated from this library. These were gridded out on multiple replica filters and were stored as glycerols in 96-well microtiter plates. The number of human positive clones detected per filter for the 20XP3542-1-4 library varied between 2 and 10 at a plating density of 5,000 clones/filter, with a mean of 5. These figures agree well with the expected frequency, given that the cell line contains between 20 and 30 Mb of human DNA as determined by fluorescence using labeled Alu repeat-element probes (P. de Jong, personal communication).

Since the start of the attempt to construct the library from this cell line, fragments from at least one other autosome have been identified within this hybrid (M. Siciliano, personal communication). As a result, all of the human cosmids from the 20XP3542-1-4 library were screened against the chromosome 19–only hybrid, 5HL9–4, at the same time as they were screened against 908K1.

The current genetic map of the *DM* region of 19q (Le Beau et al. 1989) is shown in figure 1; *DM* is located in the interval between ERCC1 and D19S50. Shown on this map are the breakpoints determined for the proximal end of the 19q fragment (BCL3) in 20XP3542-1-4 and the breakpoint within the CKMM gene contained in the cell line 908K1 which has been confirmed by restriction mapping of this region (B. Wieringa, personal communication). Using these hybrids we have identified from 110 human positive cosmid clones from the 20XP3542-1-4 library those that map into the intervals shown in figure 1, as described above.

Typical data obtained with this type of mapping are shown in figure 3. Of the 110 human positives screened, 34 (31%) contain hamster inserts, and 76 (69%) contain human inserts; 32 (42%) of these 76 map to chromosome 19. Of these 32, 27 (84%) are proximal to CKMM (segment a of 20XP3542-1-4 chromosome 19 fragment in fig. 1), and the remaining 5 (16%) are distal to it (segment b in fig. 1).

Single-Copy Probes from Cosmids, and Mapping Data

The five cosmids which map distal to CKMM in the hybrid panel have been analyzed with a number of restriction enzymes, including rare-cutters, and the resulting fragments have been probed with total human DNA in order to identify single-copy sequences. Single-copy fragments were isolated in gel slices and were used to probe Southern blots of genomic DNA digested with a number of different enzymes to identify polymorphisms.

One of the five distal cosmids, 134 (D19S51), contains two small *Sac*II (*Sst*II) fragments (designated p134A and p134B) and a *Sac*II/*Hin*dIII fragment (designated p134C). These fragments are unique within the human genome. One of these fragments, p134C, identifies RFLPs with the enzymes *Pst*I (allele sizes 4.7 kb and 3.1 kb) and *Bgl*I (allele sizes 5.0 kb and 4.4 kb), shown in figure 4. The *Bgl*I polymorphism is also detected by p134B. These probes were not polymorphic for the enzymes *Taq*I, *Msp*I, *Eco*RI, *Bam*HI, *Hin*dIII, *Bgl*II, *Rsa*I, *Pvu*II, *Alu*I, or *Hin*fI. The CEPH reference panel was typed with the polymorphic probe p134C, and the lod scores obtained for two-point analyses of these data are summarized in table 2.

Analysis of chromosomes from 316 unrelated persons from a number of different ethnic backgrounds revealed the genotype frequencies in table 3 for the *Pst*I RFLP. Mendelian inheritance was demonstrated when segregation was studied in 3-generation families from CEPH. Hardy-Weinberg equilibria were satisfied by both RFLPs. The gene frequencies calculated from the data

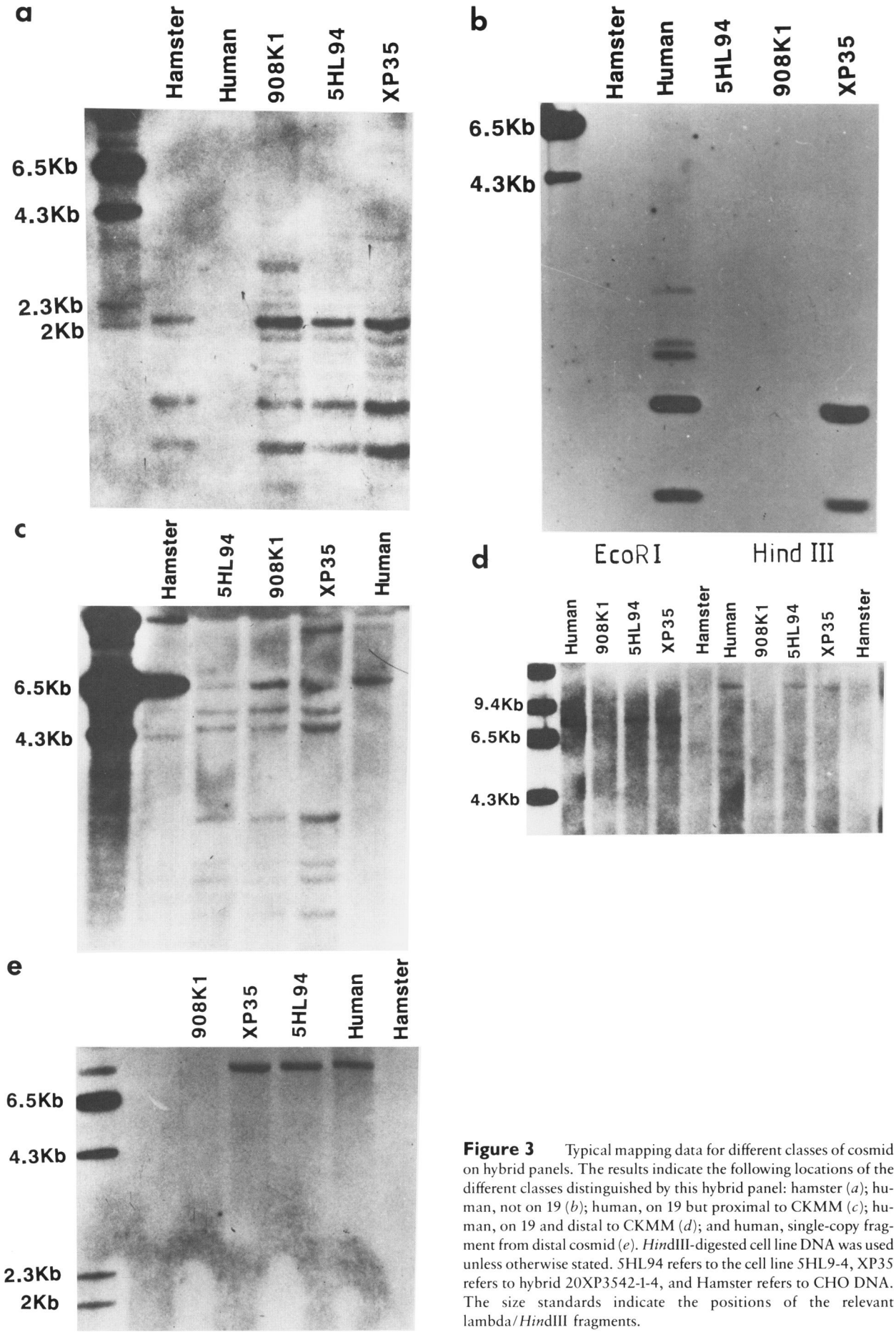

Figure 3 Typical mapping data for different classes of cosmid on hybrid panels. The results indicate the following locations of the different classes distinguished by this hybrid panel: hamster (*a*); human, not on 19 (*b*); human, on 19 but proximal to CKMM (*c*); human, on 19 and distal to CKMM (*d*); and human, single-copy fragment from distal cosmid (*e*). *Hin*dIII-digested cell line DNA was used unless otherwise stated. 5HL94 refers to the cell line 5HL9-4, XP35 refers to hybrid 20XP3542-1-4, and Hamster refers to CHO DNA. The size standards indicate the positions of the relevant lambda/*Hin*dIII fragments.

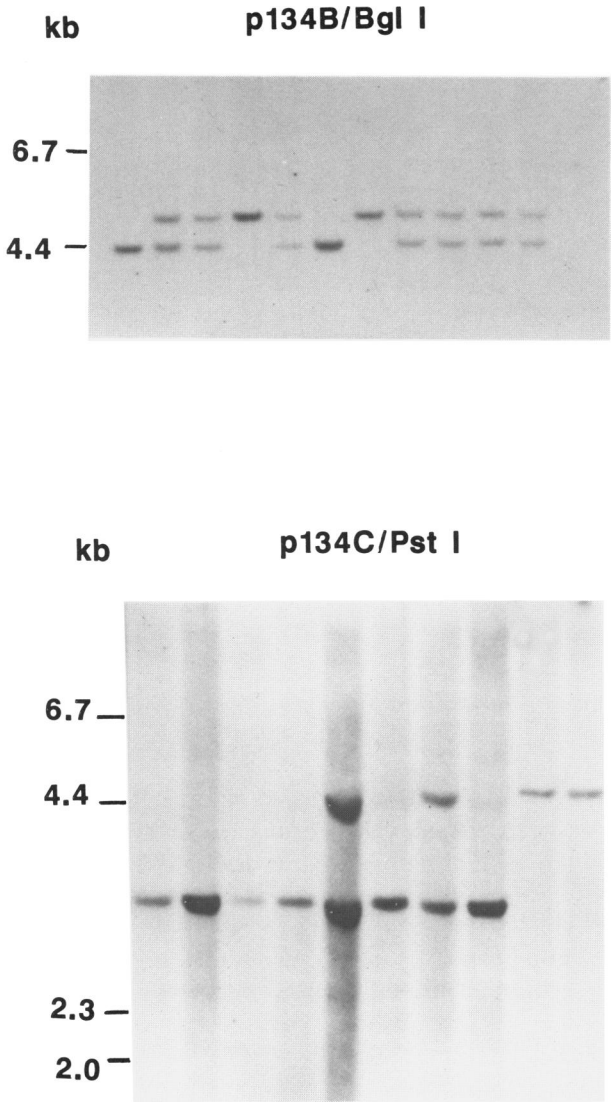

Figure 4 RFLPs detected with single-copy probes from cosmid 134 (D19S51) for the enzymes *Bgl*I and *Pst*I. Each lane contains 3 μg of genomic DNA digested with the enzyme indicated above the plate. The left-hand column indicates the relative positions of the lambda/*Hin*dIII size markers.

in table 3 were .20 and .80 for alleles 1 and 2, respectively (PIC .27), with no significant variation observed in 66 unrelated myotonic individuals. The only possible exception to these frequencies was suggested by data from 15 unrelated Japanese (seven with DM), all of whom were homozygous for the common allele.

Recombinant Family Data

The family shown in figure 2 has been haplotyped for the markers on 19q. Partial haplotypes for the most tightly linked probes that flank *DM* and which are informative in that family are shown, and the possible recombinants with D19S51 are indicated with arrows. All other tightly linked probes were not informative. In family 120 the recombination with D19S51 is either in individual II-5 or in individual III-5. This uncertainty is due to the fact that phase cannot be established in this family, as both parents are deceased; however, some reconstruction of their haplotypes is possible. It is most likely that the DM chromosome from the affected parent in generation I had the haplotype of ATP1A3-2, APOC2-C, D19S51-1, and D19S22-2. There are several recombinants with these markers in this pedigree; the most obvious is individual II-3 in whom *DM* and APOC2 recombine and from the phase assumed in figure 2. APOC2 segregates with the proximal marker ATP1A3 and DM with the distal markers D19S51 and D19S22. These results are completely in accordance with those reporting the location of DM distal to APOC2 (Brunner et al. 1989*b*; Johnson et al. 1989; Korneluk et al. 1989*a*).

Individual II-5 shows recombination within the DM haplotype between APOC2 and D19S51 with the disease locus segregating with the proximal marker and D19S51 segregating with the distal marker, D19S22. This phase would place D19S51 distal to DM and supports the order of the loci as being APOC2–DM–D19S51–D19S22. Other phases are possible but invoke recombinations which, on the basis of both the well-defined probe order and the distances that have been established for this part of 19q (Le Beau et al. 1989), are less likely.

Discussion

As the amount of human DNA within the hybrid 20XP3542-1-4 is of the order of 20–30 Mb, we can estimate, from our cosmid localization data, the amount of human DNA distal to CKMM on 19q contained in this cell line. If it is assumed that the human cosmid clones isolated from the library of 20XP3542-1-4 are distributed randomly throughout the human material within this hybrid, approximately 10 Mb of 19q13.2-13.3 is contained within 20XP3542-1-4, as 42% of the human clones map to chromosome 19. Of the chromosome 19–specific cosmids, 84% map proximal to CKMM, and the remaining 16% (2 Mb) map distal to CKMM. Therefore the five cosmids isolated from this distal region, which have average insert sizes of 40 kb, account for approximately 10% of the total DNA of this distal interval. Single-copy subclones from these cosmids will

Table 2

Pairwise Lod Scores for p134C Generated from the CEPH Panel Data

Loci	θ								θ_{max}	Z_{max}
	.01	.05	.10	.15	.20	.25	.30	.40		
p134C vs. D19S27	−1.27	1.17	1.89	2.10	2.07	2.07	1.61	.82	.17	2.11
p134C vs. D19S7	−13.0	−2.9	.51	1.88	2.37	2.37	2.05	.87	.22	2.42
p134C vs. D19S13	−2.12	2.68	4.02	4.33	4.17	3.74	3.10	1.47	.15	4.33
p134C vs. D19S9	−5.81	−.2	1.74	2.46	2.67	2.57	2.25	1.18	.21	2.67
p134C vs. CYP2A	8.96	11.2	11.1	10.2	9.00	7.54	5.89	2.28	.07	11.3
p134C vs. ATPA3	5.48	6.23	5.99	5.46	4.80	4.01	3.16	1.34	.05	6.23
p134C vs. D19S8	2.42	7.53	8.70	8.65	8.05	7.10	5.88	2.88	.12	8.78
p134C vs. D19S19	1.51	1.37	1.23	1.08	.93	.76	.59	.25	.00	1.51
p134C vs. BCL3	8.80	12.1	12.2	11.4	10.1	8.46	6.67	2.79	.08	12.4
p134C vs. APOC2	16.8	17.3	16.1	14.5	12.6	10.5	8.23	3.54	.03	17.4
p134C vs. CKMM	9.80	12.0	11.9	11.0	9.82	8.36	6.70	3.04	.07	12.1
p134C vs D19S22	−13.7	−5.1	−1.9	−.4	.45	.86	.99	.69	.30	.99
p134C vs. PRKCG	−17.7	−3.6	1.29	3.35	4.20	4.34	4.00	2.32	.24	4.36
p134c vs. *DM*	6.27	6.22	5.69	5.03	4.32	3.50	2.81	1.25	.025	6.34

NOTE.—The DM linkage data were generated from typings of families with the ethnic backgrounds given in table 3.

provide probes for pulsed-field mapping, enabling a physical map of the sequence between CKMM and the distal breakpoint on chromosome 19 to be constructed. The DM locus will lie within that physical map.

For the reasons detailed in the Material and Methods and Results sections, family 120 does not meet the criteria agreed by the MDA working group on DM for recombinant families. The substantial haplotype data available for this family imply a recombinant that places p134C distal to DM; it remains for further recombinants to be identified with this probe to establish absolutely the position of p134C relative to DM. To this end, the probe is available to all groups wishing to investigate linkage in DM families.

Prior to our showing that p134C is distal to DM, it was possible to consider that the region of 19q13.2 within the 20XP3542-1-4 hybrid did not extend far enough to include *DM*. For this reason we have identified polymorphic probes from this region to investigate their segregation in individuals known to be recombinant between *DM* and closely linked probes. We have identified a new polymorphic marker, p134C, which is probably flanking *DM* on the distal side. This defines the narrow genetic interval between two tightly linked probes into which *DM* maps, a step which is crucial in any strategy aimed at both isolating the DM gene and improving the accuracy of prenatal and presymptomatic diagnosis.

Our linkage data (table 1) show that the probe p134C is linked to *DM* with a lod score of 6.34 at $\theta = .025$. We see tight linkage between this marker and APOC2 in the CEPH families ($Z_{max} = 17.4$, $\theta = .03$). Although the recombination fraction between CKMM and p134C is higher than that between APOC2 and p134C, this difference is not statistically significant and underlines the limitations of two-point lod scores when the genetic interval between loci is small. For this reason we have analyzed well-characterized recombination events between probes that are within 5–10 cM of *DM* is a multipoint analysis which yields accurate information concerning probe orders (Johnson et al. 1989) and which agrees with other groups' data generated by simi-

Table 3

Genotype Frequencies of the *PstI* Polymorphism of p134C in Different Populations

	Unaffected Genotype			Myotonic Genotype		
	1/1	1/2	2/2	1/1	1/2	2/2
Swedish	1	7	10	1	2	3
Danish	0	15	42	0	0	0
Finnish	2	22	35	0	2	5
Japanese	0	0	8	0	0	7
French-Canadian	1	3	2	0	2	1
Other Europeans	6	30	52	1	16	25
CEPH parents	2	25	46	0	0	0
Cuban	0	1	6	0	0	1
Total	12	103	201	2	22	42

lar methods (Brunner et al. 1989*b*; Korneluk et al. 1989*a*). Unfortunately none of the established multigeneration recombinant families were found to be informative with D19S51.

We have not found significant difference between male and female recombination frequencies within this region. Allowing the male and female frequencies to vary did not significantly alter the *Z* values observed. Of the 66 unrelated myotonic individuals typed with p134C, we see segregation of DM with the rare allele in only two cases, where both individuals are homozygous for that allele. All of the heterozygotes in table 2 who are phase known show segregation of DM with the common allele. Estimates of disequilibrium between p134C and *DM* gave no significant disequilibrium values, because the disease is segregating with the common allele.

There are, at present, no convincing candidate genes for the mutation causing DM, although ion channels are implicated (Renaud 1987). At this time any genes mapping to the interval defined by the markers ERCC1 and p134C should be considered as candidate genes for the disorder. The tight delineation of this interval will minimize the number of coding genes that have to be characterized in this way.

Acknowledgments

This work was supported by the MDG of Great Britain through grant RA2/222/2 and by the MDA/Piton Foundation through a grant under its Task Force in Genetics. J.B. gratefully acknowledges the receipt of an SERC Ph.D. studentship. We appreciate the assistance of our colleague Dr. Martin Farrall in generating the Lod score data for the DM families. These studies were helped enormously by the major collaborative effort undertaken by the MDA/Piton Foundation working party for myotonic dystrophy. We would particularly like to thank Prof. Allen Roses and Dr. Margaret Pericak-Vance (supported by NINCDS grant NS19999), Profs. Peter Harper and Hans-Hilger Ropers, and Drs. Be Wieringa, Marie-Christine Thibault, Jean Mathieu, and Claud Laberge for providing family material from their respective group resources. We thank Drs. Duncan Shaw and David Brook for access to their CEPH data and Dr. Pieter de Jong for advice on cosmid techniques.

References

Bartlett R, Pericak-Vance M, Yamaoka L, Gilbert J, Herbstreith M, Hung W-Y, Lee J, et al (1987) A new probe for the diagnosis of myotonic muscular dystrophy. Science 235:1648–1650

Birnboim H, Doly J (1979) A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acids Res 7:1513–1523

Brook J, Shaw D, Thomas N, Meredith A, Cowell J, Harper P (1986) Mapping genetic markers on human chromosome 19 using subchromosomal fragments in somatic cell hybrids. Cytogenet Cell Genet 41:30–37

Brunner H, Korneluk R, Coerwinkel-Driessen M, Mackenzie A, Smeets H, Lambermon H, van Oost B, et al (1989*a*) Myotonic dystrophy is closely linked to the gene for muscle-type creatine kinase (CKMM). Hum Genet 81:308–310

Brunner H, Smeets H, Lambermon HMM, Coerwinkel-Driessen M, van Oost BA, Wieringa B, Ropers HH (1989*b*) A multipoint linkage map around the locus for myotonic dystrophy on chromosome 19. Genomics 5:589–595

Coerwinkel-Driessen M, Schepens J, Zandvoort P, van Oost B, Mariman E, Wieringa B (1988) NcoI RFLP at the human creatine kinase M gene locus. Nucleic Acids Res 16: 8743

Feinberg A, Vogelstein B (1983) A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. Anal Biochem 132:6–13

Frossard P, Coleman R, Assmann G (1986) NcoI RFLP at the apolipoprotein cII locus. Nucleic Acids Res 14:5120

Frossard P, Coleman R, Funke H, Assmann G (1987) Dimorphic markers for the human apolipoprotein CII gene locus. Gene 51:103–106

Gibson T, Rosenthal A, Waterston R (1987) Lorist 6, a cosmid vector with *Bam*HI, *Not*I, *Sca*I and *Hin*dIII cloning sites and altered neomycin phosphotransferase gene expression. Gene 53:283–286

Griggs R, Wood D, Moxley R, Ashizawa T, Epstein H, Hejtmancik F, Bartlett R, et al (1989) Criteria for establishing the validity of genetic recombination in myotonic dystrophy. Neurology 39:420–421

Harley H, Brook J, Rundle S, Walsh K, Sarfarazi M, Johnson K, Epstein H, et al (1989) A map of the long arm of chromosome 19: an order for fourteen polymorphic markers and the myotonic dystrophy gene. Human Gene Mapping 10. Cytogenet Cell Genet 51:1011

Harper P (ed) (1989) Myotonic dystrophy. Saunders, Philadelphia.

Hulsebos T, Wieringa B, Hochstenbach R, Smeets D, Schepens J, Oerlemans F, Zimmer J, et al (1986) Toward early diagnosis of myotonic dystrophy: construction and characterization of a somatic cell hybrid with a single human der(19) chromosome. Cytogenet Cell Genet 43:47–56

Humphries S, Jowett N, Williams L, Rees A, Vella M, Kessling A, Myklebost O, et al (1984) A DNA polymorphism adjacent to the human apolipoprotein C2 gene. Mol Biol Med 1:463–471

Johnson K, Jones P, Spurr N, Nimmo E, Davies J, Creed H, Weiss M, et al (1988) Linkage relationships for the protein kinase C gamma gene which exclude it as a candidate for myotonic dystrophy. Cytogenet Cell Genet 48:13–15

Johnson K, Shelbourne P, Davies J, Buxton J, Nimmo E, An-

vret M, Bonduelle M, et al (1989) Recombination events that locate myotonic dystrophy distal to APOC2 on 19q. Genomics 5:746–751

Korneluk R, MacKenzie AE, Nakamura Y, Dube I, Jacob P, Hunter AGW (1989*a*) A reordering of human chromosome 19 long-arm DNA markers and identification of markers flanking the myotonic dystrophy locus. Genomics 5: 596–604

Korneluk R, Macleod H, McKeithan T, Brooks J, MacKenzie A (1989*b*) A chromosome 19 clone from a translocation breakpoint shows close linkage and linkage disequilibrium with myotonic dystrophy. Genomics 4:146–151

Lathrop GM, Lalouel JM, Julien C, Ott J (1984) Strategies for multi-locus linkage analysis in humans. Proc Natl Acad Sci USA 81:3443–3446

Le Beau MM, Ryan D, Pericak-Vance MA (1989) Report of the Committee on the Genetic Constitution of Chromosomes 18 and 19. Human Gene Mapping 10. Cytogenet Cell Genet 51:338–357

Little P, Cross S (1985) A cosmid vector that facilitates restriction enzyme mapping. Proc Natl Acad Sci USA 82: 3159–3163

McKeithan T, Rowley J, Shows T, Diaz M (1987) Cloning of the chromosome translocation breakpoint junction of the t(14:19) in chronic lymphocytic leukemia. Proc Natl Acad Sci USA 84:9257–9260

Maniatis T, Fritsch EF, Sambrook J (eds) (1982) Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY

Meredith A, Huson S, Lunt P, Sarfarazi M, Harley H, Brook J, Shaw D, et al (1986) Application of a closely linked polymorphism of restriction fragment length to counselling and prenatal diagnosis in families with myotonic dystrophy. Br Med J 293:1353–1356

Mohr J (ed) (1954) A study of linkage in man. Munksgaard, Copenhagen

Nakamura Y, Lathrop M, O'Connell P, Leppert M, Lalouel J-M, White R (1988) A primary map of ten DNA markers and two serological markers for human chromosome 19. Genomics 3:67–71

Pericak-Vance M, Yakaoka L, Assinder R, Hung W-Y, Bartlett R, Stajch J, Gaskell P, et al (1986) Tight linkage of apolipoprotein C2 to myotonic dystrophy on chromosome 19. Neurology 36:1418–1423

Perryman B, Hejtmancik F, Ashizawa T, Armstrong R, Lin S, Roberts R, Epstein H (1988) *Nco*I and *Taq*I RFLPs for human M creatine kinase (CKM). Nucleic Acids Res 16: 8744

Renaud J (1987) Involvement of cation transporting systems in myotonic diseases. Biochimie 69:407–410

Schonk D, Coerwinkel-Driessen M, van Dalen I, Oerlemans F, Smeets B, Schepens J, Hulsebos T, et al (1989) Definition of subchromosomal intervals around the myotonic dystrophy gene region. Genomics 4:384–396

Sealey P, Whittaker P, Southern E (1985) Removal of repeated sequences from hybridization probes. Nucleic Acids Res 13:1905–1922

Shaw D, Harley H, Brook J, McKeithan T (1989) Long-range restriction map of a region of human chromosome 19 containing the apolipoprotein genes, a CLL-associated translocation breakpoint and two polymorphic MluI sites. Hum Genet 83:71–74

Shaw DJ, Meredith A, Sarfarazi M, Huson S, Brook J, Myklebost O, Harper P (1985) The apolipoprotein CII gene: subchromosomal localisation and linkage to the myotonic dystrophy locus. Hum Genet 70:271–273

Smeets H, Bachinski L, Coerwinkel M, Schepens J, Hoeijmakers J, van Duin M, Grzeschik K-H, et al (1990) A long-range restriction map of the human chromosome 19q13 region: close physical linkage between CKMM and the ERCC1 and ERCC2 genes. Am J Hum Genet 46:492–501

Smeets H, Coerwinkel M, Hermens R, Brunner H, Schonk D, Ropers H-H, Wieringa B (1989) Physical and genetic mapping of loci around the myotonic dystrophy (DM) mutation at 19q13. Human Gene Mapping 10. Cytogenet Cell Genet 51:1081

Stallings RL, Olsen E, Strauss AW, Thompson LH, Bachinski LL, Siciliano MJ (1988) Human creatine kinase genes on chromosome 15 and 19, and proximity of the gene for the muscle form to the genes for apolipoprotein C2 and excision repair. Am J Hum Genet 43:144–151

Takemoto Y, Miki T, Nishikawa K, Nakura J, Takai S, Honjo T, Ogihara T (1990) The locus of the Japanese myotonic dystrophy gene is also linked to D19S19 on the long arm of chromosome 19. Genomics 6:195–196

Thompson LH, Bachinski LL, Stallings RL, Dolf G, Weber CA, Westerveld A, Siciliano MJ (1989) Complementation of repair gene mutations on the hemizygous chromosome 9 in CHO: a third repair gene on human chromosome 19. Genomics 5:670–679

Todorov A, Jequier M, Klein D, Morton NE (1970) Analyse de la segregation dans la dystrophie myotonique. J Genet Hum 18:387–406

Wallis S, Donald J, Forrest L, Williamson R, Humphries S (1984) The isolation of a genomic clone containing the apolipoprotein CII gene and the detection of linkage disequilibrium between two common DNA polymorphisms around the gene. Hum Genet 68:286–289

Whitehead A, Solomon E, Chambers S, Bodmer W, Povey S, Fey G (1982) Assignment of the structural gene for the third component of complement to chromosome 19. Proc Natl Acad Sci USA 79:5021–5025