

Identification of new DNA markers close to the myotonic dystrophy locus

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Abstract

The most useful markers for the prenatal diagnosis of myotonic dystrophy (DM) are *APOC2* and *CKM*, both of which map proximal to *DM*. In order to produce other markers useful for *DM*, we have screened genomic DNA libraries constructed from cell line 20XP3542-1-4, which contains 20 to 30 Mb of human material including *APOC2* and *CKM*. Of 51 human clones identified, seven map to chromosome 17, four to chromosome 8, and nine to chromosome 19, and the remaining 31 were excluded from chromosome 19 but not localised further. Four of the clones from chromosome 19 map distal to *CKM* and two of these clones (*D19S62* and *D19S63*) are closely linked to *DM*. Analysis of a family in which a crossover between *CKM* and *DM* has occurred shows that neither *D19S62* nor *D19S63* and *DM* have recombined, suggesting that *D19S62* and *D19S63* are either closer to or flanking *DM* in relation to *CKM*. Pulsed field gel analysis showed that *CKM*, *D19S62*, and *D19S63* map to a region of at least 1500 kb.

Myotonic dystrophy (DM) is the most common adult form of muscular dystrophy.¹ It is an autosomal

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Note: *APOC2*=apolipoprotein C2. *BCL3*=B cell chronic lymphocytic leukaemia/lymphoma 3 related sequence. *CKM*=creatine kinase—muscle form (3' subclone). *CGB*=Chorionic gonadotrophin B. *ERCC1*=excision repair complementing defective repair in Chinese hamster cells 1, complementation group 2. *FTL*=ferritin light chain.

dominant condition with a prevalence of 1 in 8000. The *DM* gene is located on the long arm of chromosome 19 within the interval 19q13.2-19q13.3. Recently, a consensus order for markers in this region has emerged, which reads from centromere to telomere: *BCL3-APOC2-CKM-ERCC1-DM-D19S50-D19S22*.²⁻⁵ On the proximal side of *DM*, *CKM* and *ERCC1* map to the same 300 kb *NotI* fragment.⁶ For *ERCC1* few linkage data are available, as no conventional RFLPs have been documented. *CKM* is closely linked to *DM* with a θ_{max} of 0.02 ($Z_{max}=22.20$) and a -1 lod support interval of 0 to 0.05. On the distal side of *DM* the closest linked marker is *D19S50* ($\theta_{max}=0.09$, $Z_{max}=15.45^4$). Clearly there is a need for more closely linked markers on the distal side of *DM*, in order to define the smallest genomic region in which the gene must be located.

Somatic cell hybrids WILFM2⁷ and 908K1⁸ have been used to make genomic libraries from which many DNA markers on chromosome 19 have been identified.^{2,9,10} Neither of these cell lines contains *ERCC1* nor markers mapping distal to it, and are thus unlikely to contain the *DM* locus. Hybrid cell line 20XP3542-1-4¹¹ contains approximately 20 to 30 megabases of human DNA present as one contiguous piece, translocated onto a hamster chromosome.¹² Markers *BCL3*, *APOC2*, *CKM*, and *ERCC1* are present in this hybrid whereas distal 19q markers *CGB* and *FTL* are absent.¹¹ In view of the small amount of human material present in 20XP3542-1-4 and because it contains markers which are closely linked to *DM*, we have constructed and screened libraries from this cell line. Nine phage which map to chromosome 19 were isolated and, of these, four map distal to *ERCC1*. We have identified restriction fragment length polymorphisms (RFLPs) with three of the markers and report linkage analysis between two of these (*D19S62* and *D19S63*) and markers on 19q including *DM*. The relationships between the new markers and *CKM* and *ERCC1* were also investigated by pulsed field gel electrophoresis.

Methods

DNA TECHNIQUES

Southern blotting and filter hybridisations were performed according to standard procedures.¹³

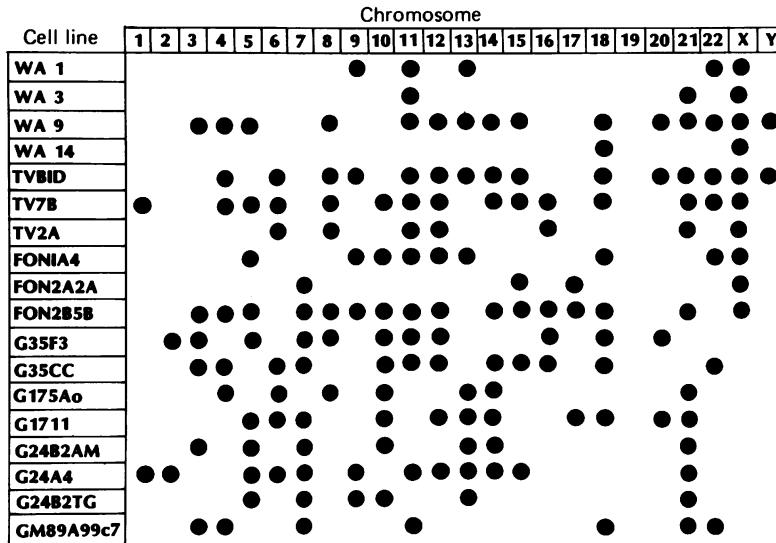


Figure 1 The chromosome content of the somatic cell hybrid panel used in this study. Hybrids in the bottom half (from G35F3 down) contain t(X;19) translocations and the X chromosome content is Xq24–Xqter for G35F3, G35CC, G175Ao, and G1711, Xpter–Xqter for GM89A99c7, and Xq12–Xqter for G24B2AM. G24B2TG lacks the t(X;19) chromosome. G35F3, G35CC, G175Ao, and G1711 contain the pter–q13.2 region of chromosome 19, and GM89A99c7 contains the reciprocal part of the same translocation (19q13.3–19qter). G24B2AM contains 19p13.2–19qter. TVB1D contains a t(1;19) retaining the region 19q12–19qter; TV7B contains the reciprocal part of the same translocation (19pter–19q12) and TV2A contains a fragment of 19 derived from the translocation chromosome.²⁰ FON1A4 contains a different t(1;19) and retains most of 19q including D19S9 and all markers distal; FON2B5B contains the reciprocal part of the same translocation (Brook *et al*, submitted).

Hybond-N (Amersham) nylon membrane was treated according to the manufacturer's instructions. Labelling of probes was performed according to the method of Feinberg and Vogelstein¹⁴ using a commercial kit (Amersham). Probes containing human repeat sequences were preannealed with a 1000-fold excess of sheared, unlabelled, human DNA.

SOMATIC CELL HYBRIDS

The chromosome content of the somatic cell hybrids used in this study is illustrated diagrammatically in fig 1. Hybrids WA1, WA3, WA9, and WA14 are described in Worwood *et al*,¹⁵ and hybrids TVB1D, TV7B, and TV2A are described in Brook *et al*.⁷ Hybrids G35F3, G35CC, G24B2AM, G175AOXi, G24B2TG, G1711, and G24A4 were kindly provided by Dr Gail Bruns, Boston, MA, and are described by Brook *et al*.¹⁶ Hybrid GM89A99c7¹⁶ was a gift from Dr B Hellkuhl (Munster, Germany). Hybrids FON1A4, FON2A2A, and FON2B5B are described in Brook *et al* (submitted). Hybrid WILFM2 is described by Brook *et al*⁷ and 5HL9-4 was kindly provided by Dr L Thompson (Lawrence Livermore Laboratory, USA) and contains a single human chromosome 19. For rodent controls, RAG (mouse) and Wg3h (hamster) cell lines were used.

LIBRARY CONSTRUCTION AND SCREENING

Two libraries were constructed with DNA from hybrid cell line 20XP3542-1-4 and screened for human sequences. For library I cell line DNA was digested to completion with *EcoRI*, fractionated on a 0.6% low melting point agarose gel, and the 15 to 22 kb fraction excised. This DNA was recovered and ligated into the *EcoRI* site of lambda EMBL3. Library II was constructed in a similar manner except that the cell line DNA was partially digested with *MboI* such that most of the DNA was in the 15 to 22 kb size range. This was recovered and ligated into the *BamHI* site of lambda EMBL3. Each library was plated out on bacterial strain NM646, blotted onto Hybond-N (Amersham) nylon filters, and screened with labelled total human DNA. Positive clones were rescreened, plaque purified, and DNA minipreps performed.¹³ For each clone single copy fragments were identified and used as probes in hybridisations to Southern blots of hybrid cell line DNA for chromosomal localisation.

PULSED FIELD GEL ELECTROPHORESIS

DNA samples were prepared in agarose blocks, digested with rare cutter restriction enzymes, and

electrophoresed on a Biorad CHEF pulsed field gel apparatus, as described previously.¹⁷

LINKAGE ANALYSIS

The linkage relationship of *D19S62*, *D19S63*, and

Table 1 The primary location of clones from 20XP3542-1-4 libraries.

Hybrid	Group				
	A	B	C	D	E
G35F3	-	+	+	-	-
WILFM2	-	-	+	-	+
GM89A99c7	-	-	-	+	-
Library I (<i>Eco</i> RI)	12	3	4	3	0
Library II (<i>Mbo</i> I)	19	6	2	1	1
Total phage	31	9	6	4	1

+ indicates the presence of human clone in a particular hybrid.
- indicates that it is absent.

The chromosome content of G35F3 and GM89A99c7 is given in fig 1. WILFM2 contains a single chromosome of human origin, with material derived from 19q12-19q13.2 and two other chromosomes.

Table 2 Further localisation of markers in each of the primary panel groups.

Group	Probe lab name	Chromosomal localisation	D segment No
A	λ D26	17	<i>D17S243</i>
A	λ D11	17	<i>D17S244</i>
A	λ D13	17	<i>D17S245</i>
A	λ D23	17	<i>D17S246</i>
A	λ D50	17	<i>D17S247</i>
A	λ D74	17	<i>D17S249</i>
A	λ D55	1 or 2*	
B	λ D19	8	<i>D8S80</i>
B	λ D38	8	<i>D8S81</i>
B	λ D48	8	<i>D8S82</i>
B	λ D47	8	<i>D8S83</i>
B	λ D43	1 or 2*	
C	λ D6	19	<i>D19S59</i>
C	λ D14	19	<i>D19S40</i>
C	λ D16	19	<i>D19S41</i>
C	λ D21	19	<i>D19S42</i>
C	λ D56	19	<i>D19S60</i>
C	λ D54	16	<i>D16S203</i>
D	λ D3	19	<i>D19S61</i>
D	λ D8	19	<i>D19S62</i>
D	λ D10	19	<i>D19S63</i>
D	λ D36	19	<i>D19S64</i>
E	λ D62	17	<i>D17S248</i>

*Indicates provisional localisation.

Table 3 Linkage analysis of *D19S62* and *D19S63* to DM (sexes combined).

Marker loci	Lod scores (θ)											-1 lod support interval	
	0	0.05	0.1	0.15	0.2	0.25	0.3	0.35	0.4	0.45	θ_{max}		Z_{max}
<i>DM-D19S62</i>	8.27	9.75	8.80	7.68	6.49	5.24	3.97	2.69	1.50	0.52	0.02	10.09	0.005 -0.08
<i>DM-D19S63</i>	14.13	19.82	17.87	15.47	12.87	10.18	7.50	4.94	2.66	0.91	0.02	20.28	0.01 -0.06

myotonic dystrophy (*DM*) was analysed using the panel of *DM* families that has formed the basis of previous studies.¹⁸ Generally, only definitely affected subjects were used for the calculation of recombination fractions between *DM* and marker loci; age at onset corrections were not used. Standard clinical criteria for the diagnosis of *DM* were used. Lod scores and recombination fractions were calculated using the LINKAGE programs.¹⁹

Results

Fifty-one lambda clones that contain human inserts were identified. These were divided into five groups based on their hybridisation patterns against a primary panel of three hybrid cell lines, G35F3, WILFM2, and GM89A99c7, and these data are summarised in table 1. A selection of clones from each group were hybridised against the full panel of cell lines shown in fig 1. These fully localised clones with D numbers assigned are listed in table 2. Clones that map to groups A, B, and E of the primary panel are derived from chromosomes other than 19.

The clones on chromosome 19 localise to both sides of a breakpoint at the interface of 19q13.2-19q13.3. Fig 2 shows that clones in group C (λ D6, λ D14,

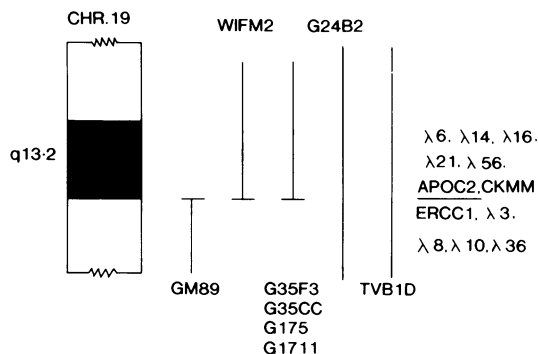


Figure 2 This shows the subchromosomal localisation on chromosome 19 of nine clones reported here, in relation to previously reported markers close to DM. The chromosome 19 content of these hybrids is 19pter-19q13.2 for G35F3, G35CC, G175, and G1711 and 19q13.3-19qter for GM89. G24B2AM retains 19q13.2-19qter. TVB1D retains 19q12-19qter. WILFM2 retains a fragment of 19q including some of 19q13.1 and 19q13.2. (GM89=GM89A99c7; WIFM2=WILFM2; G175=G175oXi=G175Ao).

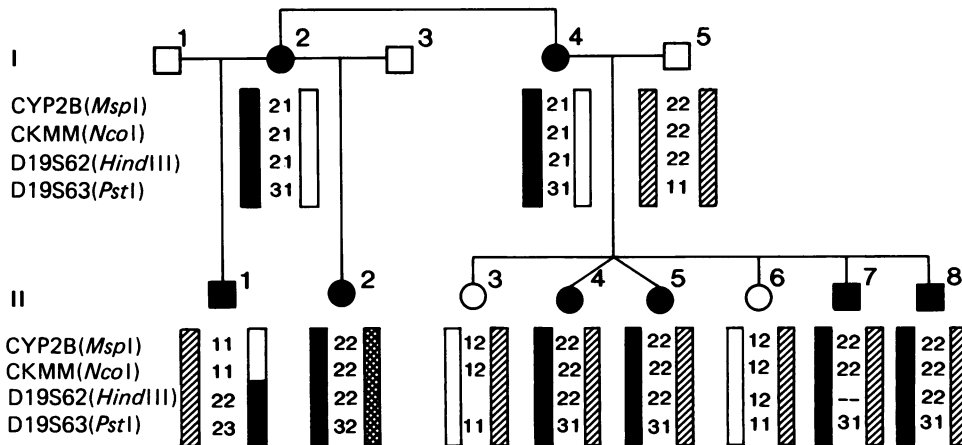


Figure 3 Pedigree of a myotonic dystrophy family, showing that in subject II·1 the markers CYP2B and CKM have recombined with DM, D19S62, and D19S63. Chromosomes carrying the DM disease allele are shown in black; normal chromosomes from the affected parent in white; and chromosomes from unaffected spouses are hatched.

λ D16, λ D21, and λ D56) are at 19q12–19q13.2 whereas those in group D (λ D3, λ D8, λ D10, and λ D36) are at 19q13.3–19qter. Clones λ D3, λ D8, λ D10, and λ D36 were screened for RFLPs with a variety of enzymes and polymorphisms identified for λ D8 (*D19S62*), λ D10 (*D19S63*), and λ D36 (*D19S64*). In this study, a three allele *Pst*I RFLP for *D19S63* and a two allele *Hind*III RFLP for *D19S62* were used.^{21 22}

Linkage analysis was performed with *D19S62*, *D19S63*, and *DM* in DM families. These data are shown in table 3. *D19S62* is closely linked to *DM* ($\theta_{max}=0.02$, $Z_{max}=10.09$), as is *D19S63* ($\theta_{max}=0.02$, $Z_{max}=20.28$). No obligate recombinants with either marker were observed. *D19S62* and *D19S63* are also closely linked to both *APOC2* and *CKM*, but less closely linked to *D19S22*. The data relating to the linkage of the new markers to other RFLPs on chromosome 19q will be presented in full elsewhere (Harley *et al*, in preparation).

The segregation of *D19S62* and *D19S63* was examined in a family for which a crossover between *CKM* and *DM* has been identified. This family is shown in fig 3. Subject II·1, who is recombinant for *CKM-DM*, is also recombinant for *CKM-D19S62* and *CKM-D19S63*, but not for *DM-D19S62* or *DM-D19S63*.

Human and somatic cell hybrid DNA was digested with *Not*I or *Mlu*I, separated by CHEF electrophoresis, and probed with *ERCC1*, *CKM*, *D19S62*, and *D19S63*. *ERCC1* and *CKM* hybridise to the same 300 kb *Not*I fragment.⁶ *D19S62* and *D19S63* did not hybridise to any fragments in common with each other or with *ERCC1* or *CKM*. *D19S63* hybridised to a 200 kb *Not*I fragment, and *D19S62* to a series of *Not*I partial digest fragments, the largest of

which was 1000 kb. Recent studies using further probes from this region have linked *D19S63* to *ERCC1* and *CKM*, and show that the order of markers is *CKM-ERCC1-D19S63-D19S62* (manuscript in preparation).

Discussion

The markers most closely linked to *DM* are *APOC1*, *APOC2*, *BCL3*, and *CKM*^{5 2 23} on the proximal side and *pEWRB1* (*D19S50*) on the distal side.⁴ *CKM* and *APOC2* are closely linked to *DM* at a θ_{max} of 0.02 and 0.03 respectively. *pEWRB1* (*D19S50*), which is linked to *DM* with a θ_{max} of 0.09,⁴ is the closest marker on the distal side. In order to identify more human sequences which are useful for linkage analysis and prenatal diagnosis of *DM*, in particular closely linked markers on the distal side of this locus, we have constructed and screened genomic libraries from cell line 20XP3542-1-4.¹¹ The nine clones reported here that map to chromosome 19 should be useful for further studies on *DM*. Four of these clones, D3 (*D19S61*), D8 (*D19S62*), D10 (*D19S63*), and D36 (*D19S64*) map distal to *CKM* in the interval 19q13.3–19qter, and are therefore potentially flanking the *DM* locus.

Analysis of *DM* families showed that *D19S62* and *D19S63* are closely linked to *APOC2*, *CKM*, and *DM*. No obvious recombinants have been observed between *D19S62* or *D19S63* and *DM*. In one family (fig 3) for which a *CKM-DM* crossover has been observed, *D19S62* and *D19S63* have also recombined with *CKM*, and there is no crossover between *D19S62* or *D19S63* and *DM*. This family suggests that *D19S62* and *D19S63* are either closer to, or

flanking, *DM* in relation to *CKM*, but until recombination is detected between *DM* and these new markers, it will not be possible to determine if either marker flanks *DM* on the distal side.

Cell line 20XP3542-1-4 contains 20 to 30 megabases of human material.¹² We estimate that slightly less than 20% of this is derived from human chromosome 19, since in the present study nine out of 51 clones mapped to 19. This 4 to 6 Mb of chromosome 19 material can be divided into two intervals using available cell lines. Of the nine clones on chromosome 19, five map to the same interval as *CKM* and four map distal. Thus, we estimate that cell line 20XP3542-1-4 contains 2 Mb of material from chromosome 19 distal to *CKM*. Pulsed field gel analysis has shown that four of the markers studied here (*CKM*, *ERCC1*, *D19S62*, and *D19S63*) detect *NotI* fragments with a total length of 1.5 Mb. If we assume 1 cM is equivalent to about 1 Mb we would predict from the linkage analysis that *DM* maps not more than 2 Mb from *CKM*, quite likely within the chromosome 19 derived material in 20XP3542-1-4.

In a recent study, Johnson *et al.*²⁴ reported the construction of a cosmid library from the hybrid 20XP3542-1-4. They isolated 76 human cosmids of which 32 (42%) mapped to chromosome 19 and, of these 32, five (16%) were derived from the region distal to *CKM*. The difference between these proportions and those obtained in the present study may be because of one or more of the following factors: different vectors and host strains; different partial digestion conditions; different hybrid cell lines used for chromosomal localisation; or random statistical fluctuation.

From existing information it is not possible to determine whether 20XP3542-1-4 contains the *DM* locus. Nevertheless the four clones reported here mapping distal to *CKM* should be useful for the prenatal diagnosis of *DM* and in jumping and linking strategies to flank and identify the *DM* gene. It will also be possible to construct a complete physical map of the region and to identify HTF islands and coding sequences which would be candidates for *DM*.

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