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Prenatal diagnosis of myotonic dystrophy using closely linked flanking markers

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Abstract

We report on two cases of prenatal diagnosis of myotonic dystrophy (DM), using flanking markers APOC2 or CKMM on the proximal side and *D19S51* on the distal side. By double digestion (*TaqI* and *NcoI*) of PCR amplified CKMM, the informativeness was increased from a PIC value of 0·57 to 0·69. Altogether, with a PIC value of 0·64 for APOC2, 0·69 for CKMM, and 0·27 for *D19S51* (*BglII*), presymptomatic and prenatal diagnosis can thus be offered to approximately 24% of persons with a risk between 0·0004 and 0·0008 using these flanking markers.

Myotonic dystrophy (DM), the most common form of adult muscular dystrophy, is an autosomal dominant disorder characterised by a marked variability in both age at onset and clinical severity.[1] DM ranges in expression from a congenital form that is frequently fatal in the newborn period to an asymptomatic condition associated with normal longevity. The major clinical features include myotonia, muscle weakness, lens opacities, and intellectual impairment.[1] Congenital DM is characterised by neonatal hypotonia and respiratory difficulties with mental retardation. Carrier mothers, whether affected or not, have a high risk of giving birth to congenitally affected infants. Determination of the carrier status for asymptomatic subjects and prenatal diagnosis for fetuses at risk is therefore requested by an increasing number of families.

Family studies have shown that the locus for DM maps to chromosome 19q13.2–13.3.[2] The locus for apolipoprotein CII is closely linked to *DM* (θ=2 to 4%). More recently, the gene for creatine kinase muscle type (CKMM) has been shown to be even closer on the proximal side of *DM* (θ=0 to 2%). This marker has already been used for presymptomatic detection and prenatal diagnosis of DM.[3] However, owing to the absence of a closely flanking marker on the telomeric side, the maximal risk was still around 0·02. The first distal marker, pEWRB1 (*D19S50*), was shown to be at a recombination distance of 10 to 15%.[4] Recently, one of us has cloned a genomic probe p134C (*D19S51*) which detects a *BglI* polymorphic site. This clone maps distal to *DM* at a recombination distance of 0 to 2%.[5] We report here on the first prenatal diagnosis of DM, using two closely flanking markers, APOC2 or CKMM on the proximal side and *D19S51* on the distal side.

Clinical details

The pedigree of family 1 is shown in fig 1. A 32 year old woman (II.3) with manifestations of DM, including typical myopathic facies and characteristic abnormalities of muscle fibres (biopsy), was referred to us at 12 weeks' gestation. Her 62 year old father (I.1) was also affected, presenting with lens opacities on slit lamp examination and the same abnormalities of muscle fibres. One year before, her sister (II.2) had given birth to a child (III.1) with a severe neonatal form of DM.

The pedigree of family 2 is shown in fig 2. A 30 year old woman (II.7) presenting with characteristic myotonic potentials on EMG was seen at 9 weeks' gestation. Four of her five brothers and her sister (II.1 to 5) were also affected with different degrees of severity. Their dead father (I.1) displayed typical clinical features.

Methods

DNA was extracted from lymphocytes and from chorionic villi obtained from the at risk fetuses following procedures already described.[7] We used the polymerase chain reaction to amplify the CKMM region encompassing the *NcoI* and *TaqI* polymorphisms,[6 8] and Southern blot analysis as

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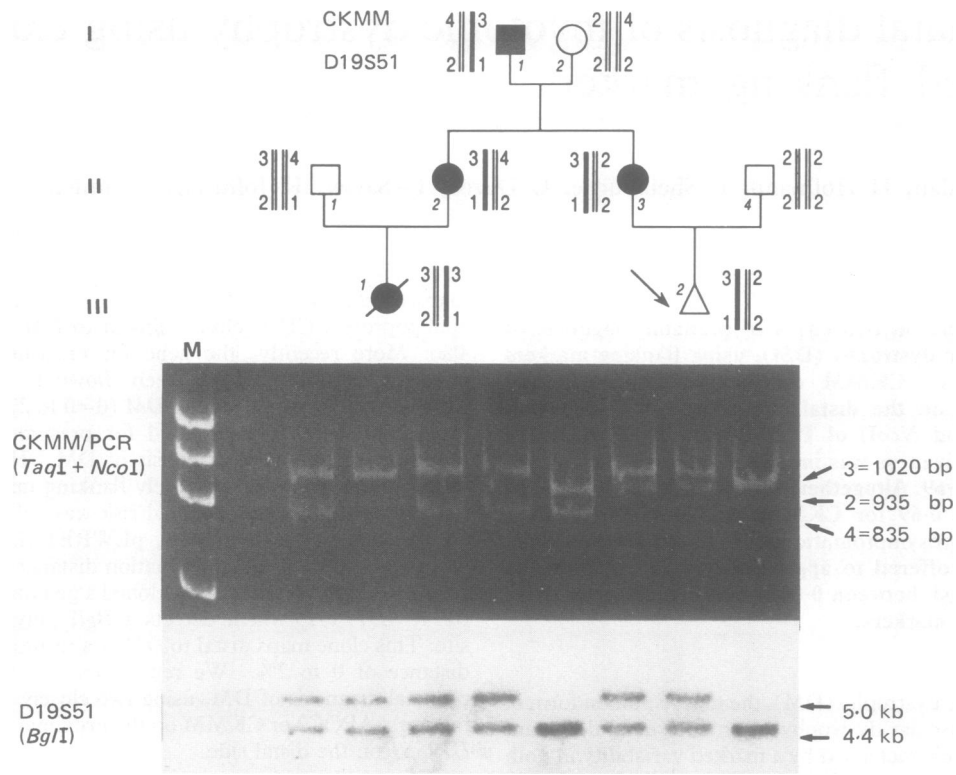

Figure 1 Genotyping of family 1 with CKMM and D19S51. Top: oligonucleotide primers 5′ (A) and 3′ (B) were used to amplify the CKMM sequence encompassing the NcoI and TaqI RFLPs. Four distinct haplotypes can be observed after migration on 3·5% polyacrylamide gels stained with ethidium bromide: TaqI, NcoI: 1=−/−, 2=−/+, 3=+/−, 4=+/+.[6] Bottom: Southern blot analysis using ^{32}P labelled D19S51 on BglI digested DNA.

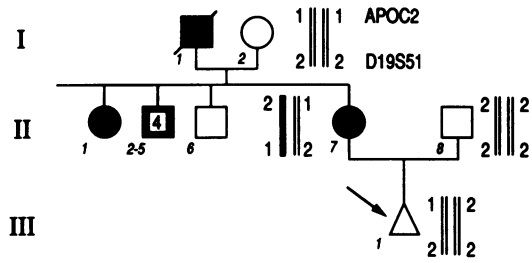

Figure 2 Genotyping of family 2 with APOC2 and D19S51.

previously described[7] to detect *BglI* polymorphisms with probes pSC11 (*APOC2*) and p134C (*D19S51*). PCR was carried out in a total volume of 100 µl with 1 µg of DNA, primers at 0·1 µmol/l in 50 mmol/l KCl, 10 mmol/l Tris, pH 8·3, 1·5 mmol/l MgCl2, 0·01% gelatin, dATP, dCTP, dGTP, and dTTP at 300 µmol/l each (Pharmacia) and 2 units of *Taq* polymerase (Perkin Elmer Cetus) for 25 cycles at 94°C denaturation (one minute), 63°C annealing (one minute), and 74°C extension (one minute) in an automated thermal cycler (Perkin Elmer Cetus). Initial denaturation was for seven minutes at 94°C and last extension seven minutes at 74°C. PCR primer sequences were as follows: A: 5′ATCGGCTGGGCTCGTCCGAAGTA 3′, designed from published data.[8] B: 5′CAGCTTGGTCAAAGACATTGAGG 3′, derived from the sequencing of genomic CKMM clones as previously reported.[9]

After quick phenol extraction, PCR products were ethanol precipitated and resuspended in water. An aliquot was successively digested with 10 units of the restriction endonucleases *TaqI* (Boehringer Mannheim) and *NcoI* (New England Biolabs) for two hours each at 65°C and 37°C respectively. DNA was visualised with ethidium bromide after electrophoresis through a 3·5% acrylamide gel for 60 minutes at 25V/cm. In order to avoid sampling discrepancies, aliquots from the appropriate mixture of buffer, enzyme, and other reagents were used.

Results

We analysed eight members of family 1 and four

members of family 2. As shown in fig 1, the fetus in family 1 (III.2) displayed the CKMM haplotype 3 and the *D19S51* allele 1. The mother elected to terminate the pregnancy since the fetus had the haplotype associated with DM with a high risk of being affected. In family 2 (fig 2) the mother (II.7) did not transmit the alleles associated with DM to her fetus (III.1), 2 for APOC2 and 1 for *D19S51* (Southern blots not shown).

Discussion

In family 1, the risk of the fetus being affected was estimated at 0·980 with CKMM as the only marker, using the LINKAGE program.[10] The risk was significantly increased to 0·9996, using both CKMM and *D19S51* for the calculation. In family 2, the risk was estimated at 0·0008. Altogether, with a PIC value of 0·64 for APOC2, 0·69 for CKMM, and 0·27 for *D19S51* (*BglI*), presymptomatic and prenatal diagnosis can be offered to approximately 24% of persons with a risk between 0·0004 and 0·0008, which is now quite acceptable, using these flanking markers. However, the assignment of *D19S51* distal to the DM locus is based on only one informative crossover event[5] and should therefore be regarded as provisional until confirmed by further family studies.

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