Instability of normal (CTG)\textsubscript{n} alleles in the DM kinase gene

David J Dow, David C Rubinsztein, John R W Yates, David E Barton, Malcolm A Ferguson-Smith

Abstract

We report on a myotonic dystrophy (DM) family exhibiting instability of normal sized (CTG)\textsubscript{n} alleles in the DM kinase gene on the non-DM chromosome. At least two mutational events involving normal DM alleles must have occurred in this family; one was characterised as a 34-35 (CTG)\textsubscript{n} repeat mutation. These findings represent a dissociation between (CTG)\textsubscript{n} repeat instability and myotonic dystrophy. Furthermore, this family highlights genetic counselling issues relating to the pathogenicity of alleles at the upper end of the normal size range and the risk of further expansion into the disease range. (*J Med Genet 1997;34:871-873)

Keywords: myotonic dystrophy; instability; normal alleles

Myotonic dystrophy (DM) is the most common form of adult muscular dystrophy with an incidence of 1 in 8000. It is characterised by impaired muscle relaxation and progressive weakness of the limbs and other muscles. Clinical manifestations include systemic features such as myotonia, cardiac conduction defects, frontal balding, and cataracts. Mildly affected gene carriers may show abnormalities on electromyography (EMG) or slit lamp examination of the eyes. The mode of inheritance is autosomal dominant with variable expression.

The abnormal expansion of a (CTG)\textsubscript{n} sequence in the 3′ untranslated region of a gene encoding a protein kinase has been found to be associated with DM. The number of (CTG)\textsubscript{n} repeats is polymorphic with repeat numbers of 3 to 37 in normal subjects and generally greater than 50 copies in affected subjects. Normal alleles are usually stably transmitted, while DM alleles are unstable and exhibit both somatic and germline mosaicism. The boundary between mutationally stable normal alleles and disease associated or unstable alleles has not been precisely defined, owing to the paucity of alleles in the 40-50 range. Here we report on a family in which routine testing for diagnostic purposes led to the chance finding of a large normal (CTG)\textsubscript{n} allele (unrelated to the DM disease allele), which showed instability of the (CTG) copy number upon transmission.

The family is shown in fig 1. The diagnosis of DM was made in the index case II.5 at 24 years

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**Markers**

- **X75vSSM**: 1, 2, 3, 4, 5, 6, 7, 8
- **D18S85 ins/del**: 9 kb, 10 kb, exp
- **(CTG)\textsubscript{n}**: 5, 12, 16, 30, 35, 36, 39, 40, ?
- **Apo AC VSSM**: 1, 2, 3, 4, 5, 6, 7, 8

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**Figure 1** Pedigree and molecular data for the family. The (CTG)\textsubscript{n} sequence in all subjects was examined using EcoRI and BamHI digests of lymphocyte DNA probed with pM10M-4. (CTG)\textsubscript{n} sequences were also examined by PCR using primers DM101 and DM102 as previously described. In addition all family members were typed for the microsatellite markers X75b and Apo AC which flank the DM gene.
of age when he presented with myotonia. Subsequent progression of the muscle disease was very slow. On review at 46 years of age, he was noted to have weakness of the hands and baldness. The second case in the family, II.6, complained of myotonia and weakness of grip from 20 years of age and the diagnosis of DM was made at the age of 33. Their sibs, II.1 and II.4, were asymptomatic and clinically normal at 52 and 44 years of age, respectively. Their father, I.1, was killed at 47 years of age. He had no symptoms of muscle disease. In adult life his eyesight deteriorated but the cause is not known. Their mother, I.2, had no symptoms of DM and died from Parkinson's disease at 64 years of age. There was no relevant family history to third degree relatives.

Molecular genetic analysis was carried out to exclude II.1 and II.4 from being carriers of the DM mutation. Confirmation of the diagnosis in the affected brothers, II.5 and II.6, was provided by the demonstration of enlarged fragments of DM by Southern blot analysis with EcoRI and the probe M10M-6' (data not shown). II.1 and II.4 gave normal results on Southern blot analysis but PCR analysis of the (CTG)n sequence showed repeat numbers of 34 and 40, respectively (fig 2A). Haplotyping by the flanking microsatellite markers Apo AC' and X75b' showed that these large (CTG)n repeats were associated with the same haplotype, which was distinct from the disease haplotype (fig 1). It would appear that one allele in I.1 either was reduced in size upon transmission to II.1 (40 to 34) and then expanded on transmission to his daughter (34 to 35), or it expanded twice upon transmission from I.1 (34 to 40 in II.4 and 34 to 35 in II.1). Since III.2 is homozygous for all markers tested, our data do not exclude the possibility that the 35 repeat allele is of maternal origin and that the 16 repeat allele has arisen through a reduction in repeat number of the 34 repeat paternal allele. However, the most likely explanation is that the 34 repeat allele has increased in size to 35 repeats on transmission from II.1 to his daughter III.2 (fig 1). The 40 repeat allele had been passed on unchanged from II.4 to her daughter III.4. All four subjects carrying the large repeats were asymptomatic. In II.1, II.4, and III.4, neurological examination, slit lamp examination of the eyes, and EMG were normal at 42, 44, and 12 years of age respectively. We did not have the opportunity to examine III.2 who was in good health at 24 years of age.

Weber and Wong previously reported instability of a normal DM allele in a CEPH family, which we have further characterised as a 26 repeat to a 27 repeat mutation (fig 2B). This mutation was confirmed to be an in vivo mutation by the authors who determined the genotypes in DNA from untransformed cells. Yamagata et al described a Japanese family which was ascertained through its sole symptomatic case, a boy aged 14 years. Besides the expansion mutation leading to disease in this boy, two other mutations were described in this family, 44 to 46 repeats and 46 to 70 repeats. Also, recently, a family was described in which a 38 to 44 repeat mutation had occurred. Thus, we know of five mutations of DM alleles with fewer than 50 repeats, excluding cases where expansion led to new cases with disease in a family. Four of the five mutations showed an increase in repeat number, consistent with the proposition that microsatellites have a tendency to expand more often than contract when they mutate. The other mutation (I.1, fig 1) could either be an expansion or a contraction (see above).

The family we have described raises several diagnostic issues. Firstly, the risk of disease symptoms associated with an allele of 40 repeats is unclear. There is a lack of clinical data on such people and the issue is confounded by the variable phenotype associated with particular repeat sizes. This issue has recently been highlighted by the overlap between the disease and normal size ranges in Huntington's disease. In this study, a few nonsymptomatic elderly subjects were found to have 36-39 repeats while the HD chromosomes had 36 or more CAG repeats. In addition, the repeat size of disease alleles tends to increase with age and varies in different tissues. Thus, the repeat number found in lymphocytes may not be representative of the tissues which are most affected in DM. While any disease associated with 40 repeats is likely
to be mild, the risk to future generations is more of an issue. There is some evidence to suggest that 35 repeats can expand to give DM and precedents exist in other triplet repeat diseases for mutation from large normal alleles into the disease range. Yamagata et al have highlighted this problem in DM by showing the mutation of a 46 repeat "premutation" allele in a normal subject to an expanded allele causing childhood onset DM. Further studies of the behaviour of large normal alleles in trinucleotide disease genes are needed to aid counselling in such cases and to understand the evolution of these diseases.

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