Origin of a regressed myotonic dystrophy allele

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Abstract
A new case of regression of the CTG copy number in the myotonic dystrophy allele was observed in a 7 year old boy. His affected father had an expanded allele of about 100 repeats in his lymphocyte DNA while the child showed a 60 repeat allele, of the same size as that present in the grandfather. Analysis of the father's sperm DNA allowed us to detect an expanded fragment of approximately the same size (62 repeats) as that present in the child's and grandfather's lymphocytes. This fragment was not detectable in the father's lymphocytes. Thus the regression is constitutive in the child, being already present in his father's germline. It is therefore likely that the regressed allele is present in all the tissues of the child, allowing a favourable prognosis.

(Med Genet 1994;31:130-132)

The molecular basis of myotonic dystrophy (DM) has been correlated with the instability of a CTG trinucleotide repeat that shows a variable degree of expansion in patients, ranging from 80 to 2000 repeats. Subjects with minimal amplification of the repeat, ranging from 50 to 80 copies, often go undetected or present very mild signs of the disease. This class of DM mutation has been therefore defined as a protomutation.

The variable degree of expansion of the DM gene can be the consequence both of meiotic and mitotic instability. While the first can be detected as an intergenerational increase or, more rarely, decrease in the CTG copy number, mitotic instability gives rise to somatic mosaicism for the length of the unstable sequence in affected subjects.

Here we report a case of regression of the CTG copy number from about 100 repeats in an affected father to 60 repeats, in the protomutation range, in his 7 year old son. The reduction detected in the subject could be the consequence of a meiotic event in the father's germline. Alternatively the regression present in the child's lymphocyte DNA could have occurred in the early stages of development, as a consequence of mitotic instability. Consequently he could carry a more severe expansion in other tissues. In order to investigate the origin of the triplet repeat reduction and to provide more accurate genetic counselling for this child, the CTG copy number was analysed in the father's germline.

Materials and methods

PEDIGREE

The proband of the family shown in fig 1 (II 1) was a 38 year old man, diagnosed as affected according to the published criteria for DM, who requested genetic counselling for his son. The brother of the proband (II 3), aged 21, was also affected with definite DM in a more severe form. The parents of the proband (I 1 and I 2, aged 57 and 66), without any clinical symptoms of DM, gave consent for genetic analysis but refused to undergo clinical or other investigation (electromyography and slit lamp examination). The proband's son (III 1) was a 7 year old boy with a negative neurological examination (absence of clinical myotonia in grip on percussion of thenar eminence or tongue or muscle weakness). The child has not been subjected to electromyography and slit lamp examination because genetic analysis is more accurate and less invasive, while penetrance of the DM gene is low before 10 years of age.11 12

MOLECULAR GENETIC ANALYSIS

Chromosomal DNA was extracted from peripheral lymphocytes and sperm according to standard procedures. For Southern blot analysis, 5 μg of DNA were digested with the restriction enzyme EcoRI following the manufacturer's instructions (Boehringer), run on a 0.8%-agarose gel, and blotted on a nylon membrane (Hybond-N, Amersham). The filter was probed overnight with a 32P radio-labelled 1-4 kb fragment from cosmid MDY11 (a

Figure 1. Southern blot analysis of EcoRI digested genomic DNA in the DM family. The proband (II 1) and his affected brother (II 3) show a normal 10 kb fragment plus a second larger fragment indicated by the arrow. The son (III 1) shows only a 10 kb band.
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Figure 2 Southern blot analysis of PCR products resolved on a 2.5% agarose gel and hybridised with the (CTG)_n oligonucleotide probe. A 100 bp ladder was used as size marker. Lymphocyte DNA samples from the different subjects tested are indicated as in figure 1. C = control sperm DNA; S = sperm DNA of II-1.

kind gift from Dr A Pizziuti) which identifies a two allele polymorphism in normal subjects (9 and 10 kb).

Amplification of the DM locus was performed with primers 101 and 102 flanking the variable CTG triplet, as reported by Brook et al.3 The five repeat allele amplified with these primers yields a 129 bp fragment. PCR products were resolved on a 2.5% agarose gel or on a 6% denaturing polyacrylamide (sequencing) gel. Both gels were further blotted onto Hybond membranes (PCR-Southern blot) and probed with a 32P labelled (CTG)_n oligonucleotide for two hours at 58°C. This allows the identification of only specific DNA fragments. Membranes were washed twice in 6 × SSPE and 0.5% SDS at 65°C. The hybridising fragments were detected after exposure of the filters to a Kodak XAR film for a half to two hours. The length of PCR fragments run on the 6% denaturing polyacrylamide gel was estimated by loading as markers on the same gel a sequence and a five repeat control sample both labelled with 35S. The marker lanes were cut from the gel before blotting and directly exposed on to a Kodak film. Linkage analysis was performed with the highly polymorphic microsatellite marker within the apolipoprotein C2 (ApoC2) locus, according to Mulley et al.14

Results

All members of the family shown in fig 1 were analysed for triplet repeats at the DM locus in peripheral blood cell DNA. Southern blot analysis of genomic DNA showed in both affected brothers (II-1 and II-3) an expanded fragment of approximately 10-3 kb corres-

ponding to about 100 repeats of the CTG triplet. No visible expansion was present in their parents (I-1 and I-2) or in the child III-1. However, PCR amplified DNA run on a 2.5% agarose gel and stained with ethidium bromide showed a band of approximately 290 bp, corresponding to about 60 repeats, in both I-2 and III-1 (data not shown). Thus the size of the DM allele had increased from the first to the second generation, but regressed in III-1. Linkage analysis with the ApoC2 marker confirmed that the same DM chromosome had been transmitted from the first to the third generation.

To test whether the regressed allele was present in the father’s germline, sperm DNA from subject II-1 was also analysed. Fig 2 shows the result of Southern blot analysis followed by hybridisation with a (CTG)_n probe performed on the CTG sequence amplified from lymphocyte DNA of all members of the family and from sperm DNA from II-1. Notably a band of the same expansion as the one detected in lymphocytes of I-2 and III-1 was present in the germline of II-1.

Electrophoresis on a sequencing gel of the same PCR products followed by hybridisation with the (CTG)_n probe allowed us to estimate precisely the length of the expanded allele in lymphocytes of I-2 and III-1 and in sperm of II-1. This showed the presence of a 60 repeat allele in I-2 and III-1 and of 62 repeats in the sperm of II-1. Thus the regressed allele was present in the germline of II-1, though not detectable in his lymphocyte DNA.

Discussion

The new case of regression of the DM allele reported here presents some special points of interest. Several previous families showing cases of regression have been documented.5-9 Four cases had regressed to the normal range. Among the other reported subjects the regressed allele remained in the expansion range that is generally associated with the presence of serious clinical symptoms.

The contraction described here of the number of repeats falls in the protomutation range. This kind of reduction event, though not previously observed, has been hypothesised to be one of the mechanisms allowing maintenance of the DM allele in the population.3

Genetic counselling is difficult in this case. The reduction of the unstable sequence could be the consequence of a regression of the CTG triplet which occurred during the transmission of the DM allele from II-1 to III-1; however, a mitotic event restricted to the haematopoietic tissues of III-1 cannot be excluded. If the reduction in size of the CTG element in III-1 occurred during the first stages of embryogenesis this subject could be a mosaic for reduced and enlarged fragments, with the risk of developing serious symptoms if an increased copy number is present in tissues involved in the disease and the risk of having affected offspring if a large expansion is present in his germline.
To discriminate between a meiotic event in the germline of II-1 and a mitotic one in the first stages of development of III-1, the length of the unstable triplet was analysed in the father’s germline. In a previously reported case of regression to the normal size range, the regressed allele was not detectable in the affected father’s germline. The case presented here shows, for the first time, the presence of a contracted DM allele in the germline of an affected subject who carried a more expanded allele in his lymphocytes. The number of triplet repeats present in the father’s germline was almost identical (62 v 60) to that found in the son’s lymphocytes. This slight variation in the CTG repeat number is comparable to that reported for the transmission of a 60 repeat protomutation through successive generations and could be because of slippage of the replication complex during gamete production, changing the allele length by a few units. Notably an allele of the same size (60 repeats) was also present in the grandfather’s (I-2) lymphocyte DNA. We could hypothesise that the allele received by III-1 had never expanded and was transmitted unchanged from I-2 through the germline of II-1. In this case the expansion detected in lymphocytes of II-1 would be explained by a postzygotic event. A similar phenomenon could also have occurred in his brother, II-3. Alternatively the 62 repeat allele present in the germline of II-1 may be a consequence of a contraction during gametogenesis of a more expanded allele inherited from I-2. Expansions of this extent from a 60 repeat protomutation have been reported with a frequency of 57%. Whatever the case, our results suggest that the 60 repeat allele in III-1 is constitutive, being already present in his father’s germline. It is therefore likely that the triplet is reduced in all tissues of III-1, allowing a favourable prognosis. Preliminary results show that this is indeed the case in three different tissues besides lymphocytes, namely buccal epithelium cells, cells from hair roots, and epidermal cells (data not shown).

The clinical phenotype of the subject under study will eventually depend on how superimposed mitotic instability modifies the degree of expansion in relevant tissues. Thus clinical follow up of this case and in the future accompanied by molecular analysis of various tissues, including muscle, is planned.

This work was supported by grants from Telethon, Italy, and from MURST 40%. M Giordano was financed by a fellowship from “Fondazione per le Biotechnologie”, Torino, and M S DeAngelis by a fellowship from “Associazione Malattie Rare Mauro Beschirrato”. We are grateful to Professor B Dallapiccola and his collaborators and to Dr A Pizzuti for helpful suggestions.

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doi: 10.1136/jmg.31.2.130

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