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Clinical Case Report

ATYPICAL MYOPATHY IN A YOUNG GIRL WITH 91 CTG REPEATS IN DM1 LOCUS AND A POSITIVE DM1 FAMILY HISTORY

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Myotonic dystrophy type 1 (DM1) is an autosomal dominant inheritable disease associated with an expansion of CTG repeats in the 3′ UTR of the *DMPK* gene. The subject is an 11-year-old girl with atypical myopathy. Because the proband's family has a positive DM1 history, a molecular-genetic analysis for DM1 was performed. This study showed that proband had a small *DMPK* expansion (91 CTG repeats) although the observed myopathy would not normally be associated with DM1. These results show how the phenotypic manifestation of DM1 can have unusual symptoms with a completely unexpected relationship to genotype.

Keywords   CTG repeats, *DMPK* gene, expansions, myotonic dystrophy type 1, myopathy, trinucleotides

INTRODUCTION

Myotonic dystrophy type 1 (DM1; MIM# 160900) is an autosomal dominant inheritable disease that represents the most common adult-onset muscular dystrophy with an estimated frequency of ~1:8000 individuals (Harper, 1989). It is accompanied by a broad spectrum of muscular and systemic symptoms. Muscular symptoms include progressive weakness and atrophy of facial, neck, and distal limb muscles associated with the myotonia affecting the distal limbs as well as cranial and axial muscles (Harper, 1989; de Die-Smulders et al., 1998). The most common systemic manifestations are cataract, frontal balding, cardiac conduction defects, endocrine dysfunctions, smooth muscle and respiratory involvement, neurobehavioral manifestations as well as cognitive impairment (Harper, 1989; de Die-Smulders et al., 1998; Delaporte, 1998). The phenotypic manifestation of DM1 is highly variable between and within affected pedigrees. According to the age at onset and severity of symptoms there are three main clinical forms of DM1: (1) late-onset, which manifests itself after the age of 50 with only mild symptoms, (2) juvenile-adult form, which develops usually between 10 and 50 years of age with typical DM1 symptoms, and (3) the

most severe form, congenital DM1 (CDM1), with symptoms already *in utero* or at birth (Harper, 1989).

DM1 is associated with an unstable expansion of (CTG) repeats in the 3′ untranslated region (3'UTR) of the dystrophia myotonica protein kinase, *DMPK* gene (Brook et al., 1992; Mahadevan et al., 1992; Fu et al., 1992). The CTG repeats are polymorphic in copy number in the general population, ranging from 5 to 35, and undergo a remarkable expansion in DM1 patients, from 50 to several thousand (IDMC, 2000). As in other trinucleotide repeat diseases, the size of the unstable CTG repeats is correlated with the age at onset of symptoms and the overall severity of the disease (Brook et al., 1992; Harley et al., 1993; Savić et al., 2002). In general, asymptomatic or late-onset DM1 adults have approximately 50 to 80 CTG repeats and these small expansions are termed protomutations (Barcelo et al., 1993). Juvenile-adult DM1 patients have a broad range of CTG repeats, numbering roughly between 100 and 1,000, while CDM1 patients have more than 1,000 repeats (IDMC, 2000). The expansions with more than 80 CTG repeats are known as disease-associated (full) mutations. Very rare *DMPK* alleles, which are between the normal and protomutation range (from 35 up to ∼50 repeats) and which are not associated with disease, are termed premutations (Yamagata et al., 1994).

An intergenerational increase of the CTG repeats length is seen in DM1 families (Brook et al., 1992; Harley et al., 1993) and underlies the phenomenon of anticipation—increased severity and earlier age at onset of symptoms in succeeding generations (Howeler et al., 1989). Molecular-genetic studies of DM1 pedigrees have revealed parent-of-origin differences in the intergenerational transmission of the CTG repeats. Pre- and protomutations can be inherited relatively stably for several generations if transmitted by the female (Barcelo et al., 1993; Simmons et al., 1998; Martorell et al., 2001). When transmitted by the male, premutations are more liable to expand, even reaching the full mutation size in a single generation, whereas protomutations passages through the male germ-line almost invariably result in a large increase in size to that of a disease-associated mutation (Barcelo et al., 1993; Simmons et al., 1998; Martorell et al., 2001). Disease-associated mutations are always unstably transmitted. Paternal transmission usually results in an intergenerational expansion, but also a contraction and very rarely a reversion can occur (Lavedan et al., 1993; Ashizawa et al., 1994; O'Hoy et al., 1993, Brunner et al., 1993). The largest expansions associated with CDM1 are almost exclusively maternally transmitted (Lavedan et al., 1993; Tsilfidis et al., 1992) although a few cases of paternally transmitted CDM1 have been reported (Bergoffen et al., 1994; de Die-Smulders et al., 1997). The molecular basis
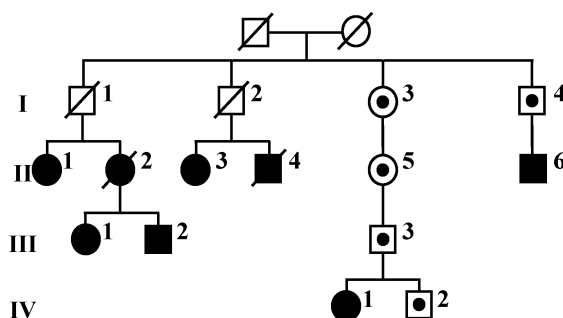
Figure 1. The pedigree of the reported DM1 family. Members with confirmed presence of DM1 expansions and unexamined DM1 symptomatic members (II 1–4) are shown. Males are represented by squares, females by circles. Diagonal lines indicate deceased persons. ■ and ⬤ = symptomatic DM1 mutation carrier; ◉ and = DM1 pre- and protomutation carrier and asymptomatic DM1 mutation carrier (Subject IV2). □ and ○ = healthy individuals.

of the parental bias on intergenerational transmission of trinucleotide repeat expansion is not known, but it has been suggested that the main factor affecting this phenomenon in DM1 is germ-line in origin (Martorell et al., 2000).

This article present a study on an 11-year-old girl with a phenotype that might be seen in some neuromuscular diseases and that would have normally not been expected in a DM1 patient. It also shows intergenerational transmission of the DM1 pre- and protomutations and generation of new disease-associated mutations through several generations in her family.

PATIENTS, METHODS, AND RESULTS

The pedigree of a DM1 family from Serbia and Montenegro is shown in Figure 1. Informed consent was obtained from all family members and for testing the minor, Subject IV2, informed consent was obtained from his parents. Two neurologists independently examined all individuals. *DMPK* molecular-genetic data and basic clinical data for the persons examined are shown in Table 1 and Figure 1.

Subject IV1, Proband

Proband is an 11-year-old girl born to healthy and young parents. When the girl was 10 years old she started complaining about pains in her calves after walking relatively short distances.

Table 1. Clinical data and *DMPK* molecular-genetic data of examined subjects

Subject	Age at sampling	Phenotype	Age at onset	Wt *DMPK* allele*	Mutant *DMPK* allele*
IV1	11	Atypical DM1	10	5	91
IV2	5	Normal	/	13	170–220 (190)**
III3	36	Normal	/	5	59
II5	55	Late-onset DM1	55	12	59
I3	75	Normal	/	12	54
II6	40	Juvenile-adult DM1	20	13	270–880 (460)**
I4	65	Normal	/	12	89
III1	26	Juvenile-adult DM1	16–17	13	580–1320 (900)**
III2	22	Juvenile-adult DM1	18	13	400–960 (680)**

*Number of CTG repeats. **Range and mean, in the brackets, of the number of CTG repeats in disease-associated *DMPK* alleles.

On neurological examination only a few findings were positive. Judging from the clinical and laboratory point of view, the Subject IV1 was considered within the differential diagnostic range of: (1) glycogenosis type V (*M. McArdle*); (2) congenital myopathy, or (3) spinal muscular atrophy type 3 (SMA3).

Histochemistry was performed on a muscle and it excluded McArdle's disease (phosphorylase was present in the muscle in normal amounts) and all forms of congenital myopathies. It revealed the presence of large groups of the fibers that belonged to the same type, without significant changes in the fiber structure. This picture could correspond to reinervation after denervation and might have been indicative of SMA3. Molecular genetic analysis did not confirm the homozygotic deletion of the SMN1 gene nor the deletion of the NAIP gene. As one of the proband's distant relatives (Subject II6) suffers from DM1, molecular-genetic studies of CTG repeats in the *DMPK* gene were undertaken and revealed the presence of a small expansion, which is in the borderline between proto- and disease-associated *DMPK* mutations (Table 1).

Subject II5

Proband's grandmother on the father's side was found to be another member of this family with a pathological phenotype. A neuroophthalmological examination showed the bilateral punctiform cataracts typical of DM1. Molecular genetic studies showed the presence of CTG expansion into the protomutation range (Table 1).

Subject I3

The proband's great-grandmother is a person of normal phenotype who has a CTG expansion in the borderline range between the pre- and protomutation (Table 1).

Subject I4

The father of Subject II6 has normal phenotype and has the CTG expansion in the borderline between the DM1 protomutation and full mutation (Table 1). There were 3 other branches in this family with typical DM1 diseased members in 3 generations (Figure 1).

Molecular-Genetic Analysis of CTG Repeats in *DMPK* Gene

Genomic DNA was isolated from white blood cells using proteinase K/SDS digestion and phenol-chloroform extraction (Sambrook et al., 1989). Wt, pre- and protomutation *DMPK* alleles were amplified by PCR (Čuljković et al., 2000), resolved on 6% denaturing polyacrylamide gels, detected by silver staining (Bassam et al., 1991) and the number of CTG repeats was determined using a DNA sequencing ladder. Small pool/long range-PCR (SP/LR-PCR) was used to amplify all types of expanded *DMPK* alleles (Savić et al., 2002). The products were analyzed by Southern blot hybridization using a $(CAG)_{12}$ oligonucleotide labeled at the 3' end with DIG-ddUTP (Figure 2). The number of CTG repeats was determined according to DNA molecular weight marker.

DISCUSSION

The phenotype exhibited by the 11-year-old girl could be considered an atypical myopathy. Possible different diagnoses (*M. McArdle*, congenital myopathy, and SMA3) were excluded by histochemical and molecular-genetic tests. Although the patient did not show the characteristics typical for DM1 patients, the presence of DM1 positive family members prompted the authors to analyze her *DMPK* gene. PCR-based diagnostics revealed that she had a small DM1 expansion. This small expansion in *DMPK* does not correlate well with the proband's phenotype nor with the results of the histochemical examinations (the result of histochemistry tests on muscle are completely different from the pathological changes expected in DM1 individuals). Further, according to the well-established correlation between age at onset of the symptoms and genotype
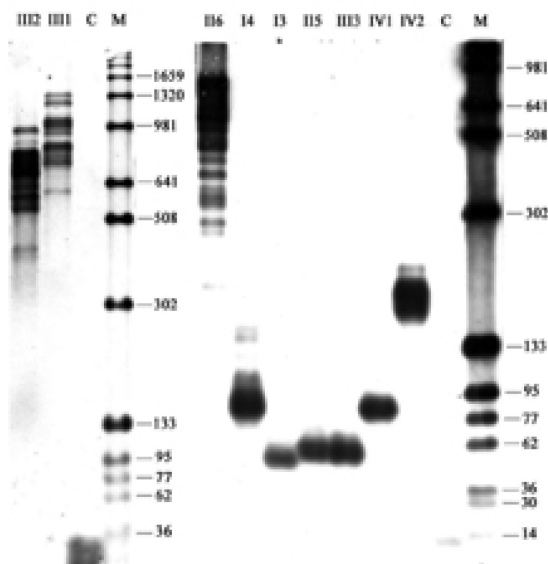
Figure 2. SP/LR-PCR based Southern blot analysis of *DMPK* expansion. PCR products of Subjects III1 and III2 are resolved on a 1% agarose gel, whereas PCR products of Subjects II6, I4, I3, II5, III3, IV1, and IV2 are resolved on a 1.5% agarose gel. C—negative DM1 control; M—DNA molecular weight marker (DNA marker X; Roche Molecular Biochemicals, Mannheim, Germany) represented by the number of CTG repeats.

for DM1, it was unexpected to find that an individual with 91 CTG repeats would develop any symptoms at 10 years of age. Comparing the genotype and phenotype in proband and Subject I4 (Table 1, Figure 1), who has a similar CTG expansion size in the *DMPK* gene in a similar genetic background, it can be concluded that some individual-specific factors are probably modifying the phenotypic manifestation of DM1 (e.g., a factor influencing the somatic instability of the CTG array in affected tissues, the status of neighboring genes to *DMPK* and/or other genes whose transcripts are abnormally processed due to a toxic gain-of-function of mutant *DMPK* transcript). At present there is no formal proof that the clinical phenotype is related to the CTG expansion in the *DMPK* gene. But if one considers that proband's phenotype could be caused by the *DMPK* expansion with 91 CTG repeats, albeit with possible interaction with modifing factors, this case report appears to demonstrate that the phenotypic manifestation of DM1 can have unusual symptoms that are completely unexpected according to the genotype. In that case the reported

family is a good example of a strongly expressed intrafamilial phenotypic variability of DM1.

Protomutation carriers can develop some mild DM1 symptoms late in life (e.g. Subject II5), and usually are not aware of them. The fact that Subject I3 (with only 5 CTG repeats less than Subject II5) and Subject I4 (with 89 CTG repeats) do not show any pathological signs, are evidence for the possible role of some other genetic and epigenetic factors in the DM1 phenotypic manifestation.

There are few families in which the intergenerational transmission of DM1 pre- and protomutation can be followed, because first generations harboring such small expansions are frequently missed due to the lack of symptoms. In the reported DM1 pedigree were found one premutation, 2 protomutatons, and one expansion with a size in the borderline between proto- and disease-associated alleles and their intergenerational transmissions were followed. Pre- and protomutations passage through the female germ-line is stabile or with only small changes in the repeat copy number at least across the two generations in the family examined (Figures 1 and 2). This is consistent with the previous results that pre- and protomutations can be stably maintained through several female generations. On the other hand a protomutation is more unstable in the male germ-line and almost invariably expands to the full disease range in the next generation. This is also true for the three branches of the reported pedigree (Figures 1 and 2). Different behavior of pre- and protomutation in the male and female transmissions indicates that the threshold for more pronounced instability of small DM1 expansions is lower in the male than in the female germ-line, as well as in lymphocytes. For a similar expansion size, the parent-of-origin effect, based on a greater tendency to expand in the male germ-line, is comparable for all the different trinucleotide repeat diseases.

REFERENCES

Ashizawa, T., Anvert, M., Baiget, M., et al. (1994). Characteristics of intergenerational contractions of the CTG repeat in myotonic dystrophy. *American Journal of Human Genetics*, *54*, 414–423.

Barcelo, J. M., Mahadevan, M. S., Tsilfidis, C., et al. (1993). Intergenerational stability of myotonic dydstrophy protomutation. *Human Molecular Genetics*, *2*, 705–709.

Bassam, B. J., Caetano-Anolles, G., & Gresshoff, P. M. (1991). Fast and sensitive silver staining of DNA in polyacrylamide gels. *Analytical Biochemistry*, *196*, 80–84.

Bergoffen, J., Kant, J., Sladky, J., et al. (1994). Paternal transmission of congenital myotonic dystrophy. *Journal of Medical Genetics*, *31*, 518–520.

Brook, J. D., McCurrach, M. E., Harley, H. G., et al. (1992). Molecular basis of myotonic dystrophy: Expansion of a trinucleotide (CTG) repeat at the 3′ end of a transcript encoding a protein kinase family member. *Cell*, *68*, 799–808.

Brunner, H. G., Jansen, G., Nillsen, W., et al. (1993). Reverse mutation in myotonic dystrophy. *The New England Journal of Medicine*, *328*, 476–480.

Čuljković, B., Stojković, O., Savić, D., et al. (2000). Comparison of the number of triplets in SCA1, MJD/SCA3, HD, SBMA, DRPLA, MD, FRAXA and FRDA genes in schizophrenic patients and a healthy population. *American Journal of Medical Genetics (Neuropsychiatric Genetics)*, *96*, 884–887.

de Die-Smulders, C. E., Smeets, H. J., Loots, W., et al. (1997). Paternal transmission of congenital myotonic dystrophy. *Journal of Medical Genetics*, *34*, 930–933.

de Die-Smulders, C. E., Howeler, C. J., Thijs, C., et al. (1998). Age and causes of death in adult-onset myotonic dystrophy. *Brain*, *8*, 1557–1563.

Delaporte, C. (1998). Personality patterns in patients with myotonic dystrophy. *Archives of Neurology*, *55*, 635–640.

Fu, Y.-H., Pizzuti, A., Fenwick, R. G., et al. (1992). An unstable triplet repeat in a gene related to myotonic muscular dystrophy. *Science*, *255*, 1256–1258.

Harley, H. G., Rundle, S. A., MacMillan, J. C., et al. (1993). Size of the unstable CTG repeat sequence in relation to phenotype and parental transmission in myotonic dystrophy. *American Journal of Human Genetics*, *52*, 1164–1174.

Harper, P. S. (1989). *Myotonic Dystrophy.* 2nd ed. London: Saunders.

Howeler, C. J., Busch, H. F. M., Geraedts, J. P. M., et al. (1989). Anticipation in myotonic dystrophy: Fact or fiction. *Brain*, *112*, 779–797.

IDMC (The International Myotonic Dystrophy Consortium). (2000). New nomenclature and DNA testing guidelines for myotonic dystrophy type 1 (DM1). *Neurology*, *54*, 1218–1221.

Lavedan, C., Hofmann-Radvanyi, H., Shelbourne, P., et al. (1993). Myotonic dystrophy: Size- and sex-dependent dynamics of CTG meiotic instability and somatic mosaicism. *American Journal of Human Genetics*, *52*, 75–883.

Mahadevan, M., Tsilfidis, C., Sabourin, L., et al. (1992). Myotonic dystrophy mutation: An unstable CTG repeat in the 3′ untranslated region of the gene. *Science*, *255*, 1253–1255.

Martorell, L., Monckton, D. G., Gamez, J., et al. (2000). Complex patterns of male germline instability and somatic mosaicism in myotonic dystrophy type 1. *European Journal of Human Genetics*, *8*, 423–430.

Martorell, L., Monckton, D. G., Sanchez, A., et al. (2001). Frequency and stability of the myotonic dystrophy type 1 premutation. *Neurology*, *56*, 328–335.

O'Hoy, K. L., Tsilfidis, C., Mahadevan, M. S., et al. (1993). Reduction in size of the myotonic dystrophy trinucleotide repeat mutation during transmission. *Science*, *259*, 809–812.

Sambrook, J., Fritsch, E. F., & Maniatis, T. (1989). *Molecular Cloning: A Laboratory Manual*. 2nd ed. New York: Cold Spring Harbor Laboratory Press.

Savić, D., Rakočević-Stojanović, V., Keckarević, D., et al. (2002). 250 CTG repeats in *DMPK* gene is a threshold for correlation of expansion size and age at onset of juvenile-adult DM1. *Human Mutation*, *19*, 131–139.

Simmons, Z., Thornton, C. A., Seltzer, W. K., et al. (1998). Relative stability of a minimal CTG repeat expansion in a large kindred with myotonic dystrophy. *Neurology*, *50*, 1501–1504.

Tsilfidis, C., MacKenzie, A. E., Mettler, G., et al. (1992). Correlation between CTG trinucleotide repeat length and frequency of severe congenital myotonic dystrophy. *Nature Genetics*, *1*, 192–195.

Yamagata, H., Miki, T., Sakoda, S. I., et al. (1994). Detection of a premutation in Japanese myotonic dystrophy. *Human Molecular Genetics*, *3*, 819–820.