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A FAMILY WITH AN UNUSUAL MYOTONIC AND MYOPATHIC PHENOTYPE AND NO CTG EXPANSION (PROXIMAL MYOTONIC MYOPATHY SYNDROME): A CHALLENGE FOR FUTURE MOLECULAR STUDIES

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Abstract—Myotonic dystrophy (DM) is a well-defined autosomal dominant disorder characterized by myotonia, muscle weakness, cardiac conduction defects, cataracts, and endocrine abnormalities. Recently a newly recognized disorder, similar to but distinct from DM, has been observed with multisystem findings including intermittent myotonia, proximal myopathy, and occasional cardiac conduction disturbances. This disorder has been called proximal myotonic myopathy (PROMM). No history of anticipation is present and there is no linkage to the gene locus for DM or to the loci for the muscle sodium or chloride channels. This report describes a family with a normal size of the CTG trinucleotide repeat expansion of the DM gene in which affected individuals have myotonia (intermittent, exacerbated by cold), bilateral cataracts, mild hypogonadism and mild temporal atrophy. Affected individuals also have proximal muscle weakness, facial involvement, nonspecific abnormalities on muscle biopsy, normal cardiac conduction, and no glucose intolerance. The absence of trinucleotide repeat expansion in the DM gene is consistent with this family being affected by a disorder distinct from DM, possibly a form of PROMM. Copyright © 1996 Elsevier Science Ltd.

Key words: Myotonic dystrophy, trinucleotide expansion, amplification, ion channel disorders, myotonia.

INTRODUCTION

Myotonic dystrophy (DM) is a multisystem, autosomal, dominant disorder caused by an abnormal, unstable expansion of a trinucleotide CTG repeat on chromosome 19 in a gene that encodes a protein kinase [1–7]. Almost all patients (98–100%) with classical clinical features of DM (myotonia, cognitive impairment, distal weakness, endocrine and cardiac conduction abnormalities) [1–4] have the abnormal CTG repeat expansion. An inverse relationship exists between age at onset and size of the repeat, providing a genetic explanation for the appearance of more severe manifestations occurring at an earlier onset in successive generations, e.g. anticipation [8, 9]. Although the underlying molecular mechanisms of DM and the exact cause for the great variability in clinical presentation are still unknown, the size of the CTG repeat expansion in leucocyte DNA appears to correlate reasonably well with the age of onset and the severity of skeletal and cardiac muscle disease [3, 10–13]. However there are some families with affected individuals who have certain phenotypic similarities to DM in whom there is no abnormal CTG repeat expansion of the DM gene [4].

Recently a myotonic myopathy has been described in which affected individuals have a normal CTG expansion of the DM gene associated with a particular phenotype, characterized by myotonia, proximal weakness and cataracts [14]. These patients have only some of the usual features of DM [7, 15, 16]. The underlying genetic defect in this newly described disorder is

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still unknown. This syndrome has been called proximal myotonic myopathy (PROMM) and is now recognized as a new clinical disorder that is similar to but distinct from DM [14, 16]. In this report we highlight the clinical and genetic features in our family and have compared our findings to those seen in both DM and PROMM.

We evaluated four patients from one family and obtained historical information about five members spanning five generations. The affected individuals have a dominantly inherited disorder which has similarities to DM but differs in several essential features: (i) there is no anticipation throughout five generations; (ii) there are only mild myotonic symptoms in a few patients, and this varies in severity, decreasing as the outside temperature increases; (iii) weakness is mainly proximal and is variably present; (iv) muscle histopathology is mild and nonspecific; (v) there are no cardiac conduction abnormalities; and (vi) the size of the trinucleotide CTG expansion on the DM gene is normal.

Our findings suggest that PROMM may include patients with different clinical features, similar to, but distinct from DM or benign myotonic disorders, and that as more PROMM families are evaluated, it is possible that more extensive clinical and genetic heterogeneity will be identified.

PATIENTS AND METHODS

Clinical investigations were performed in four patients (mean age 47.6 yr, range 32–71 yr) and genetic linkage analysis was carried out in all known affected members (see pedigree, Fig. 1). No information was available for patients from the first generation. Patient II-2 died at 60 yr of age from a heart attack and had bilateral cataracts. Patient II-5 was wheelchair confined when she died, had diabetes and cataracts. Patient III-1 also had bilateral cataracts and hypogonadism while patient III-4 was known to have bilateral cataracts and diabetes. Patient III-9 works as a farmer and complains of no weakness at all; he had slightly elevated fasting glucose levels and mild glucose intolerance. Patient III-11 is healthy and has never consulted a physician other than for the presence of multiple lipomas over his arms. Patient IV-4 is a physical education instructor at a competitive level and only has mild bilateral cataracts. Patients from the fifth generation are in good health. Details about patients III-13 and IV-2, IV-6 and IV-7 are listed in Tables 1 and 2 and Fig. 2. Clinical examination was performed by one of the authors (GM). Age at onset was recorded as the age when symptoms were first recalled (such as distal weakness, myotonia, cardiac conduction abnormalities, the presence of cataracts and the presence of endocrine disturbances, especially diabetes).

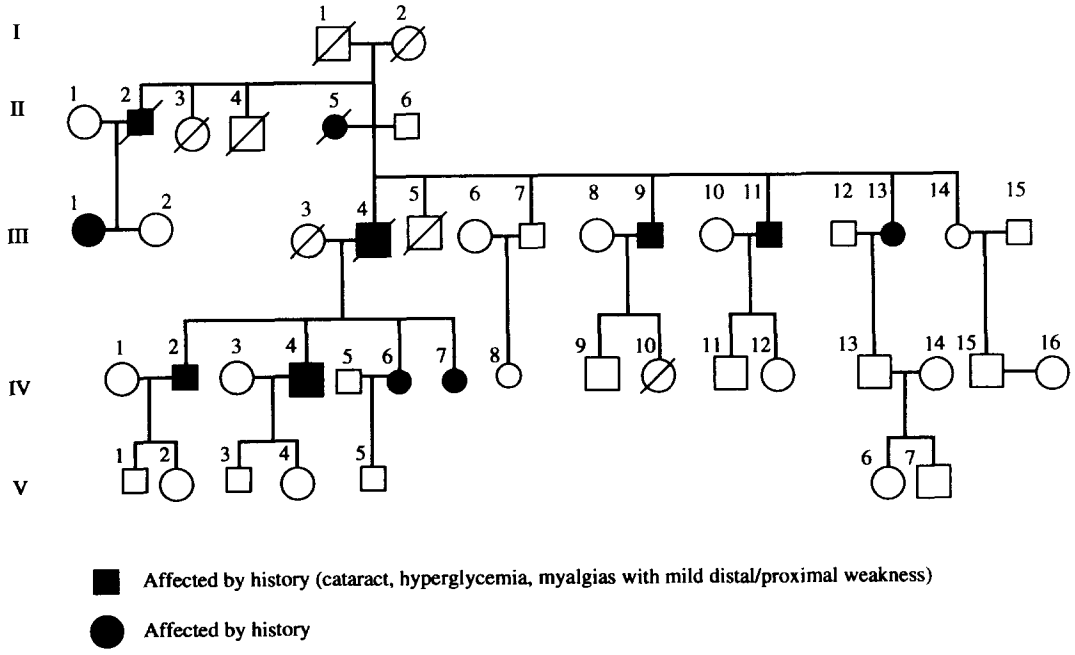

Fig. 1. Family pedigree.

Muscle strength was evaluated according to a five-point disability scale (MRC) in which the highest score signifies no muscle impairment. The MDRS scale [17] for DM patients was not adopted to classify our patients because proximal involvement is considered in this scale as a progression of severity of disease following distal involvement.

Needle EMG was performed in all patients. Percussion myotonia (thenar muscle) and grip myotonia, taking into account the times taken to bring the fingers from a flexed to an extended position in 1 min, were also evaluated (Table 2).

After obtaining informed consent, a muscle biopsy was obtained from the right biceps muscle under local anaesthesia in all patients. Cryostat sections 10 μm thick were processed and histological and histochemical reactions was performed as previously described [18].

Gonadal function was assessed by analysis of FSH, LH, LIH, GnRH, and thyroid function by T_3, T_4, and TSH levels. Glucose metabolism was studied by measurement of fasting glucose level, response to oral glucose tolerance testing and by determination of islet cell antibodies [19].

Cardiac evaluation included standard ECG recording, Holter ECG, echocardiography and cardiac MRI [20].

Brain MRI was performed in all patients to identify any possible white or grey matter involvement as previously described in DM patients [21].

DNA was extracted from peripheral blood leukocytes and skeletal muscle from all patients to determine the size of CTG repeat by

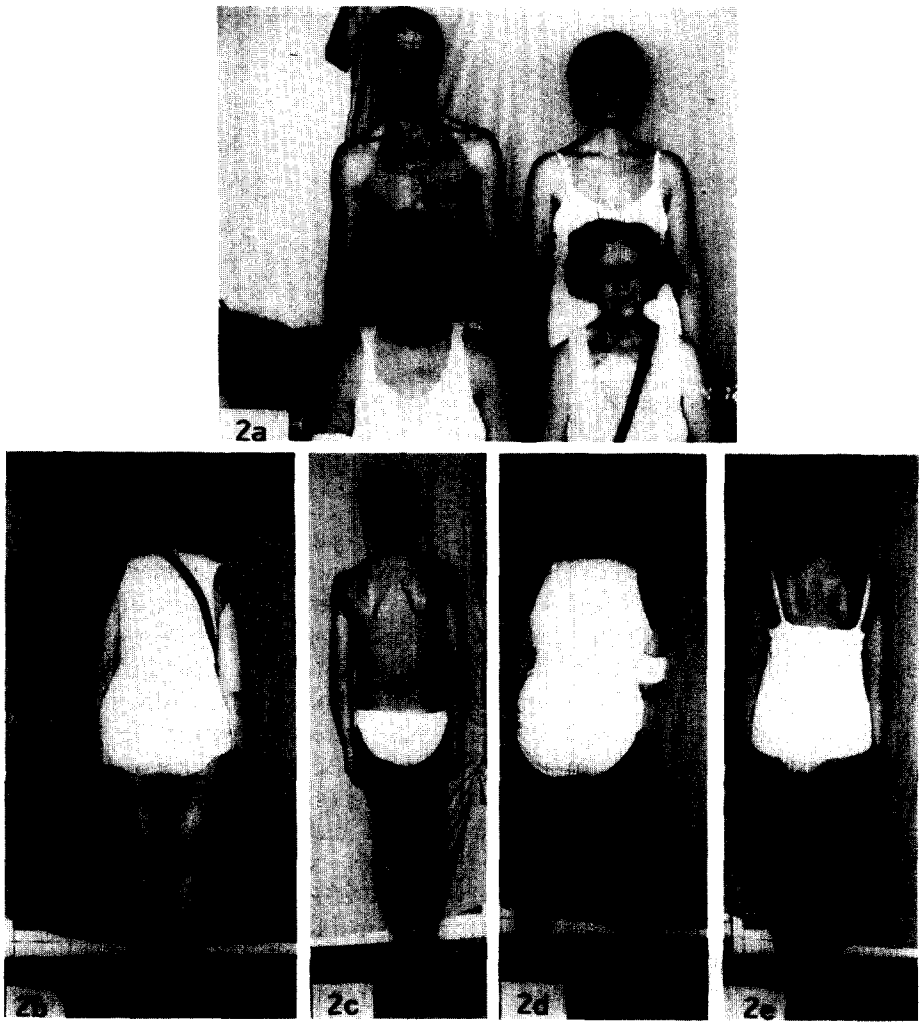

Fig. 2. (a) Facial appearance with mild temporal atrophy of the four patients examined; (b) III-13 slight atrophy with proximal weakness; (c) IV-2 no muscle weakness except a proximal atrophy with scapular winging and calf hypertrophy; (d) IV-6 proximal weakness; (e) IV-7 no muscle weakness except a slight proximal atrophy and calf hypertrophy.

Table 1. Clinical and genetic data of four PROMM patients

Patient	III-13	IV-2	IV-6	IV-7
Age (yr)	71	43	37	32
Age at onset (yr)	27	23	16	25
Inheritance	Paternal	Maternal	Paternal	Paternal
Cataracts	+	Lens opacity	+	Lens opacity
Baldness	–	–	–	–
Thyroid function	–	–	–	–
Gonadal function	–	Mild hypogonadism	Mild hypogonadism	–
Gamma-GT	–	–	–	–
Fasting glycemia	–	–	–	–
Oral glucose tolerance test	–	–	–	–
Standard ECG	–	–	–	Accelerated A-V conduction
Holter ECG	Supraventricular extrasyst.	–	–	–
Ecochardiogr.	EF 50% Mild LVH Mild aortic insuff.	–	–	Mitral prolapse
Cardiac MRI	–	–	–	–
Brain MRI	Multiple ischemic lesions	–	–	–
(CTG) *n* repeats	5/12	12/21	5/20	10/13

EF: ejection fraction; LVH: left ventricular hypertrophy; normal data –; presence +. The number of CTG repeats is indicated for both alleles. Detailed information concerning different symptoms and tests is given in the Patients and Methods section.

Table 2. Neuromuscular involvement

Patient	III-13	IV-2	IV-6	IV-7
Calf hypertrophy	–	+	–	+
Distal atrophy	+	–	–	–
Proximal atrophy	+	++	+	+
Distal weakness	4–5 bilat	5 bilat	3–4 bilat	5 bilat
Proximal weakness	3 bilat	5 bilat	2–3 bilat	4–5 bilat
Deep tendon reflexes	Normal	Normal	Normal	Normal
Temperature-sens. myotonia	–	+	+	–
Grip myotonia R	87	87	18	90
L	85	86	16	86
CK levels (U/L)	<195	321	<195	<195
EMG myopathic signs	–	–	–	–
myotonic signs	+	+	+	+
Ring-binden fibres	–	–	–	–
Sarcoplasmic masses	–	–	–	–
Central nuclei	+	+	++	+
Necrotic fibres	–	–	–	–

Absence of clinical signs –; presence of clinical signs +; degree of weakness is expressed according to MRC*. See text for clinical and methodological details. mildly present = +; moderately present = ++.

* Medical Research Council. Aids to the Examination of the Peripheral Nervous System. Memorandum 45, Pendragon House, London, 1976.

Southern blot and PCR reactions [22]. The DNA samples were digested with ECORI and electrophoresed on an 0.8% or 1% agarose gel. The DNA was transferred from the gel to a nylon membrane and hybridized with the ^{32}P-pMDY1 probe, a 1.4 kb Bam H1 fragment containing the unstable repeat region. The membrane was washed and analysed by autoradiography. PCR was performed using the genomic DNA samples as templates and a pair of oligonucleotide primers closely flanking the triplet region. The PCR products were analysed using 6% polyacrylamide gel electrophoresis followed by autoradiography [22]. Genetic linkage analysis was performed to test the hypothesis that the disease allele is linked to the myotonic dystrophy locus (Marker Mfd5). A marker tightly linked to the chromosome 17 voltage-gated sodium channel locus (HGH2) known to cause two other myotonic disorders, hyperkaliemic periodic paralysis and paramyotonia congenita [23–26] was analysed. Primers were end-labelled, used in PCR reactions, and electrophoresed using standard protocols [27]. Gels were transferred to filter paper and exposed to X-ray film for 1–4 days at –80°C. Genotypes were analysed using maximum likelihood methods to perform pairwise linkage analysis with MLINK program of the LINKAGE system [28]. Two-point analysis of each marker was performed using an autosomal dominant model, a penetrance of 0.95, and gene frequencies of 0.001 for the disease allele and 0.999 for the normal allele. Analysis was also performed with assumed penetrance ranging from 0.7 to 0.99.

RESULTS

Three of the four affected individuals that we have examined originally appeared to have a very mild form of DM. Most of the family members have undergone linkage analysis but have never had a physical examination because they have had no complaints. The onset of symptoms in symptomatic individuals falls between 16 and 27 yr of age and the disease is passed through both paternal and maternal transmission. Of the four patients examined, two showed no muscular impairment. None of these affected members complained of muscle weakness or recalled a prior history of weakness. Most patients are farmers and are able to perform strenuous exercise and manual labour. In particular patient IV-4 is a physical education trainer at a competitive level. Patient III-13 (Fig. 2b) has mainly proximal weakness with normal deep tendon reflexes. Patient IV-6 (Fig. 2d) only has more proximal weakness; patient IV-2 (Fig. 2c) has no weakness with proximal atrophy, scapular winging and calf hypertrophy. Patient IV-7 (Fig. 2e) has mild proximal weakness with slight proximal atrophy and calf hypertrophy. One (IV-6) of the four affected patients has prominent grip myotonia, which clearly improves with repeated muscle contractions (warm-up) (see Table 2). All four patients examined with mild temporal atrophy (Fig. 2a) have lens opacities and two of the four have mild hypogonadism.

Cardiac conduction is normal in the four affected individuals examined and no history of cardiac arrhythmias is present in the affected family members not examined. There are nonspecific alterations on echocardiography in the 71-yr-old patient and patient IV-7 has mitral valve prolapse. Cardiac MRI showed no structural abnormality.

Brain MRI was normal in all patients except for the 71-yr-old patient who had multiple nonspecific ischaemic lesions.

CK levels were normal in all members diagnosed as affected based upon their history and in three out of the four patients examined. The CK values showed no correlation with the presence of proximal or distal weakness.

EMG studies revealed myotonic discharges in all the muscles sampled in all four patients who were investigated.

Muscle biopsy analysis showed a mild to moderate increase in central nuclei. There were no ring-binden, sarcoplasmic masses, or necrotic fibres.

Leucocyte and skeletal muscle DNA testing showed normal size for the CTG repeat of the DM gene in all affected individuals studied. Linkage analysis between this kindred and markers tightly linked to the myotonic dystrophy and iperkaliemic periodic paralysis loci (Mfd5 and HGH2) demonstrated obligate recombinational events. Lod scores were less than for recombination values of 0–0.05. Because of the small size of the pedigree, it is not possible to exclude a large region flanking the markers with high statistical significance. However, these results suggest that the disease locus in this family is distinct from the myotonic dystrophy locus and the chromosome 17 sodium channel gene.

DISCUSSION

The clinical diagnosis of DM has previously been based upon a distinct pattern of distal muscle wasting, myotonia and multi-organ involvement in familes having a clear autosomal dominant transmission [7, 15]. An expansion of 50–2000 CTG repeats in the 3′ untranslated region of a putative protein kinase gene on chromosome 19 occurs in 98–100% of patients [4] and seems to define true cases of DM. Anticipation (i.e. increasing clinical severity with earlier age at onset throughout different generations) is inversely correlated with size of repeat and is considered one of the typical features of DM [8, 9].

CTG expansion varies between different tissues and only recently have some authors correlated repeat size to severity of skeletal and cardiac involvement [12, 13]. There is in general a reasonable correlation between CTG repeat size measured in leucocyte DNA and the phenotypic manifestations in DM patients [22, 29]. However, Thornton *et al.* [4] have described three patients with several clinical features compatible with myotonic dystrophy (myotonia, lens opacities, cardiac conduction defects, endocrine abnormalities, and muscle histopathology compatible with DM) and several totally atypical features. The affected individuals had weakness that was mainly proximal, instead of the typical distal wasting in DM. Their limb muscles were large or hypertrophied. No CTG repeat expansion was found in leucocyte or muscle DNA and there was no

Table 3a. Clinical and genetic heterogeneity in DM

Features of DM	Atypical DM without CTG expansion	PROMM	Our family
Autosomal dominant pattern	Yes	Yes	Yes
Anticipation	No	No	No
Age at onset (yr)	30–44	20–40	32–71
Baldness	Yes	No	No
Cataracts	Yes	Yes	Yes
Long lean face	No	No	No
Small sternocl. muscles	Yes	No	No
Myotonic symptoms	Yes	Yes	Mild, temperature-sensitive
Grasp myotonia	Yes	Yes	Intermittent
Percussion myotonia	Yes	Intermittent, asymmetrical	Intermittent, asymmetrical
EMG myotonia	Yes	Yes	Yes
Myopathic EMG	Yes	No	No
Ring fibres, sarcoplasmic masses, type I atrophy	Increase in central nuclei, bland myopathy, pyknotic nuclear clumps	No	No
Heart block	Yes	No	No
Liver gamma-GT elevation	Yes	Yes	No
Intellectual impairment	?	No	No
Hypersomnia	Yes	No	No

Table 3b. Clinical and genetic heterogeneity in DM

Features unlike DM	Atypical DM without CTG expansion	PROMM	Our family
Proximal limb weakness	Yes	Yes	Yes
Calf hypertrophy	Yes	Yes	Yes
Trinucleotide repeat	No	No	No
Linkage to CICN1*	Not tested	Not present	Not tested
DM1**	Not tested	Not present	Not present
SCN4A***	Not tested	Not present	Not present

* Gene locus on chromosome 7q for muscle chloride channel disorders.
** Gene locus on chromosome 19q for myotonic dystrophy.
*** Gene locus on chromosome 17q for muscle sodium channel disorders.

history to indicate anticipation. Similar to the patients described by Thornton *et al.* [4], there are reports of a new dominant disorder with intermittent, asymmetrical myotonia, muscle weakness and cataracts (proximal myotonic myopathy, PROMM) which can masquerade as a mild DM variant or as a peculiar form of myotonia congenita [16]. Again in these patients there was no abnormal CTG expansion of the DM gene. In this report we describe patients from one family showing a clear autosomal dominant inheritance, proximal weakness, hypogonadism, cataracts, and no anticipation. However there were no cardiac conduction abnormalities. No elevation of serum gamma-GT was found in our patients whereas this was a finding in 14 out of 18 patients described by Ricker *et al.* [16]. There was moderate hypertrophy of calf muscles in two of our four patients examined in accordance with the four patients described in other PROMM patients [16].

In two out of the four patients examined there was mild to moderate atrophy of proximal muscles. The mild to moderate proximal weakness that we have observed was not accompanied by significant muscle wasting or severe structural muscle damage. Patients had normal deep tendon reflexes and intermittent temperature-related myotonia. There was no cardiac involvement even at an anatomical level as indicated by magnetic resonance imaging [20]. These are features which are more consistent with the proximal myotonic myopathy described by Ricker *et al.* [16], although they described two patients with early onset cardiac arrhythmias and one of these patients required treatment with permanent pacemaker for heart block. Our patients had intermittent myotonia which was present in only two out of four affected patients examined. None of the affected individuals by history complained of myotonic symptoms. Myotonia was mild, never interfering with everyday activities. A 'warm-up' phenomenon was present.

Muscle biopsies taken from the biceps brachii of our four patients revealed a nonspecific mild myopathy. There was a mild to moderate increase in the number of central nuclei as in patient IV-6. There was no fibre type grouping,

no selective atrophy of type I or type IIb fibres, no ring-binden or sarcoplasmic masses. The muscle histopathology was not typical for DM [7], myotonia congenita and paramyotonia congenita [26, 30–33].

As in the cases so far described by other authors [4, 7, 14, 16], our patients have several features compatible with the clinical phenotype of DM and several features of PROMM, suggesting clinical and genetic heterogeneity of patients classified as having atypical DM without CTG repeat enlargement or as having PROMM. It is possible that the clinical spectrum of PROMM encompasses the families reported by Thornton *et al.* which were called atypical DM without CTG repeat expansion. At present these patients and those described by Ricker *et al.* [16] might best be classifed as having the PROMM syndrome (Table 3a, b). From a clinical point of view, it is important to distinguish atypical DM and PROMM patients from those with DM. Based on the presently reported cases, the long-term prognosis for patients with PROMM is more favourable than for DM. Respiratory failure, mental disturbances and a severe congenital form of PROMM have not been described.

The genetic locus responsible for the clinical abnormalities in affected individuals in the family we have described in this report is unknown. The presence of a normal size of the CTG repeat in the DM gene and the absence of genetic linkage to the loci responsible for myotonic dystrophy and sodium channel myotonic diseases exclude these genes as candidates. The study of this family and further studies of families with PROMM will increase our knowledge of the spectrum of its clinical manifestations and will give the opportunity to localize and identify the gene locus. Further investigations will establish whether the PROMM syndrome is a heterogeneous disorder caused by more than one gene lesion.

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