Congenital Myotonic Dystrophy: Variability in Muscle Involvement and Histopathological Process

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Abstract- In order to understand the involvement of specific muscles in congenital myotonic dystrophy type 1 (DM1), we studied the clinical manifestations, and the genetic effects on various tissues in 2 siblings with congenital DM1. The distal leg muscles were more severely involved than the thigh muscles, as seen in the skeletal muscle magnetic resonance imaging. Molecular genetic analysis of the myotonic dystrophy type 1 protein kinase showed an elongation of the CTG triplet repeats between 850 and 1400 in the leukocytes, skin, fat, tendon, and muscles. Muscle biopsies showed a significant difference in the fiber type distribution between these two congenital DM1 patients. One revealed a prominent involvement of the tibialis anterior muscle with a predominance of type 1 fibers, similar to those muscle fiber distributions in older congenital or classic DM1 patients, suggesting a neurotrophic influence during muscle development. Another revealed a predominance of type 2 fibers in all muscle specimens, and dystrophic changes were observed in the peroneus longus muscle indicating a delayed differentiation or maturation of muscle fibers. We conclude that despite nearly the same number of CTG repeats in the leukocytes, highly individual variability of muscle differentiation may occur at teenagers of congenital DM1 in addition to different pathological findings in various skeletal muscles of patients with congenital DM1.

Key Words: Congenital myotonic dystrophy, Histopathologic findings, Skeletal muscle MRI, Somatic mosaicism, Myotonic dystrophy type 1 protein kinase

INTRODUCTION

Myotonic dystrophy type 1 (DM1) is a multi-system disorder that often involves the neuromuscular system.

Molecular genetic studies show a prolonged length of CTG trinucleotide repeats in the untranslated region of the myotonic dystrophy type 1 protein kinase (DM1PK) gene on chromosome 19[1-4]. Based on the clinical fea-

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tures, age at onset, and the number of CTG trinucleotide repeats, the disease can be categorized into mild, classic, and congenital forms[5,6]. The hereditary characteristics of this disease frequently show an anticipation phenomenon, that patients inherited from the affected mothers seem to have a longer CTG trinucleotide expansion than those from the affected fathers[3,4,7].

In 1960, Vanier[8] first reported a group of patients with a congenital variant of DM1 who suffered from many systemic disorders after birth, including hypotonia, difficulty in feeding and sucking, and respiratory distress. With few exceptions, almost all congenital DM1 patients are born to affected mothers with more than 500 CTG trinucleotide repeats[3-7]. Even if they have survived, they might suffer from delayed motor development, mental retardation, facial weakness, tented upper lip, and joint deformities[3-9].

The myopathic pattern in DM1 is distinctive from those of other types of muscular dystrophy. Besides myotonic phenomena, muscular weakness is more prominent in the cranial musculatures and distal limb muscles. The degree of abnormal CTG expansion in the leukocytes correlates with the severity of muscle weakness and central nervous system involvement[3-5,7].

We studied the clinical manifestations of congenital DM1 in 2 siblings who inherited from their affected mother, and analyzed the numbers of CTG triplet repeats of DM1PK in the muscle biopsies, leukocytes, tendon, skin and fat. The present study aimed to evaluate the developmental abnormalities and degeneration of skeletal muscles and to correlate among the numbers of CTG repeats, the magnetic resonance images (MRI) and muscle biopsies in congenital DM1.

MATERIALS AND METHODS

Patients

The patients were two female siblings (patients 1 and 2, aged 15 and 13, respectively) born from an affected mother with 170 CTG triplet repeats in the DM1PK. They had neonatal hypotonia, difficulty in swallowing and sucking, and respiratory distress with ventilator assistance for several days after birth. Premature labor

had not occurred. Subsequently, the siblings had delayed motor milestones and mental retardation. They had carp mouth, facial diplegia, dysarthria, limb weakness, muscle wasting, and talipes (Fig. 1). Table 1 showed the clinical manifestations of these two patients. However, neither cataracts nor dysphagia was found. The brain MRI showed cortical atrophy, ventriculomegaly and periventricular white matter lesions at posteriosuperior trigones in those 2 patients. Myotonia was evident in the clinical and electromyographic examinations (Fig. 2). Progressive deformities of the right foot of patient 1 and of both feet of patient 2 required surgical correction.

Figure 1. Facial diplegia, tented mouth and distal leg muscle wasting were noted in two patients with congenital DM1. (A) Patient 1 had equinovalgus in the right leg and (B) patient 2 had equinovarus in both legs.
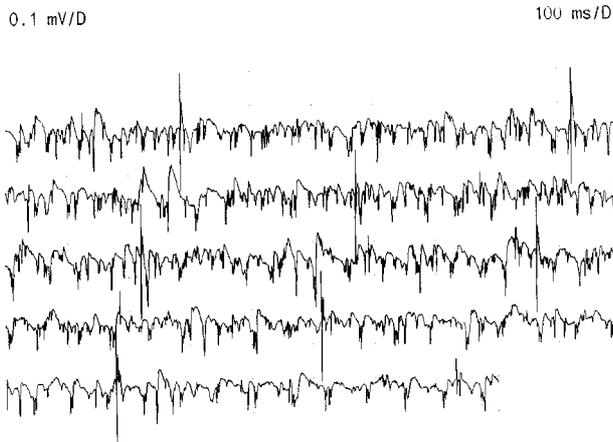
0.1 mV/D   100 ms/D

Figure 2. Myotonic discharges were found in the electromyographic studies of patient 1.

Table 1. *Clinical summary of 2 patients with congenital myotonic dystrophy*

Patient	1	2
Age (yrs)	15	13
Gender	F	F
Birth history		
Premature labor	−	−
Respiratory distress	+	+
Hypotonia	+	+
Difficulty sucking/feeding	+	+
Clinical manifestations		
Myotonia	+	+
Mental retardation	+	+
Heart disease	MR, TR	MVP
Carp mouth	+	+
Facial diplegia	+	+
Dysarthria	+	+
Hypersomnolence	+	−
Cataract	−	−
Dysphagia	−	−
Limb weakness	Distal leg	Distal leg
Muscle wasting	+	+
Talipes	R	R, L

F: female, MR: mitral regurgitation, TR: tricuspid regurgitation, MVP: mitral valve prolapse, +: presence, −: absence, R: right, L: left.

Biopsies of tissues

In order to correct the foot deformities, patient 1 received an anterior transfer of the peroneus longus tendon, posterior releasing with Achilles tendon lengthening, and plantar releasing of the right foot, while patient 2 underwent a bilateral anterior transfer of the tibialis posterior, plantar releasing, and Achilles tendon lengthening. Tissue biopsies were taken from these 2 patients with informed consent. Muscle biopsies were obtained during the surgical procedure from the right peroneus longus and lateral head of the gastrocnemius of patient 1 and from the left tibialis anterior and posterior muscles of patient 2. Other tissues included the Achilles tendon, skin, subcutaneous fat, and blood cells. A needle muscle biopsy was also taken from the right vastus lateralis muscle of patient 1 and the left one of patient 2. Specimens of the muscle biopsies were flash-frozen and stored at -70℃. Cryosections were cut in 8μm at -20℃. The muscles were stained with haematoxylin and eosin (H & E), modified Gomori-trichrome, nicotine adenine dinucleotide tetrazolium reductase, succinate dehydrogenase, periodic acid Schiff, and myofibrillar adenosine triphosphatase (ATPase) at pH 9.4, and after acid preincubations at pH 4.55, and 4.4.

Molecular genetic analysis

The template DNA was extracted from blood leukocytes using a Puregene DNA isolation kit (Genetra Systems, Minneapolis, MN), while other tissues, including the skeletal muscles, tendons, skin and fat were analyzed using a QIA amp DNA mini kit. The CTG repeat lengths in various tissues were determined by PCR-based Southern blot analysis as described previously[10].

Skeletal muscle MRI

The MRI of both legs was performed in the supine position using T1 (TR 665 / TE 12 ms) and T2 (TR 4000 / TE 96 ms) weighted spin echo images, with 1.5 Tesla of MRI (Siemens Vision). Non-contrast axial and coronal views of the muscles were obtained at the hip, thighs and lower legs. The interval of each slice was 15 mm. Semi-quantitation of fatty transformation was measured by 3 neurologists in each T1-weighted spine echo axial images individual muscles and the result was limited to less than 10% of inter-examiner variability.

RESULTS

Muscle biopsies
Patient 1

The peroneus longus muscle biopsies showed an irregularly distributed small, round fibers with large vesicular internal nuclei. Muscle fibers varied in size and clusters of internal nuclei were presented in some hypotrophic fibers. Long chains of internal nuclei were noted at a longitudinal section (Fig. 3A). Some large round fibers of type 1 and 2B muscle fibers were presented in myofibrillary ATPase stain, and heterogenous focal decrease of oxidative enzyme activity was noted (Fig. 3B). The phagocytosis and necrosis were less prominent in this specimen. In the gastrocnemius muscle, there was an increased variation of fiber size, small fibers with some clusters of internal nuclei, and presence of small angulated fibers. In the myofibrillary ATPase stain, most fibers were type 2 (Figs. 3C-D). In the vastus lateralis muscle, the majority of muscle fibers had a minimal degree of myopathy and a predominance of type 2 fibers except for a mosaic pattern in a fascicle.

Patient 2

The tibialis anterior muscle biopsies revealed a slight variation in fiber sizes with an increased number of internal nuclei. Predominant type 1 fibers, and some small angulated fibers were also noted (Figs. 3E-F). In the tibialis posterior, there were minimal changes, sporadic fibers with internal nuclei and some small angulated fibers. In the vastus lateralis, the histopathological studies were essentially normal.

The muscle strength, dystrophic changes, fiber type distribution, diameter of fiber type and numbers of CTG repeats are summarized in Table 1.

Molecular genetic analysis

The sizes of the CTG triplet repeats in the various skeletal muscles of these 2 patients with congenital DM1 are shown in Table 2. The CTG repeats in the DM1PK with PCR-based Southern blot analysis were 850 and 850 respectively, in the abnormal allele of the leukocytes in these 2 patients. The lengths of the CTG repeats of other tissues were relatively longer than those of the leukocytes, including fat: 980, skin: 1130, and tendon: 1400 in patient 1, and fat: 1300, skin: 1000, and tendon: 950 in patient 2.

Skeletal muscle MRI

In the hip girdle muscles, the findings of the skeletal MRI were essentially normal. In the thigh muscles, the T1 and T2 weighted MRI images showed moderately high signal intensities in the long head of both biceps and severe hyperintensities in the right sartorius of patient 2 (Fig. 4). The numbers of visible muscles were 10 in each thigh and 7 in each leg. The numbers of fatty
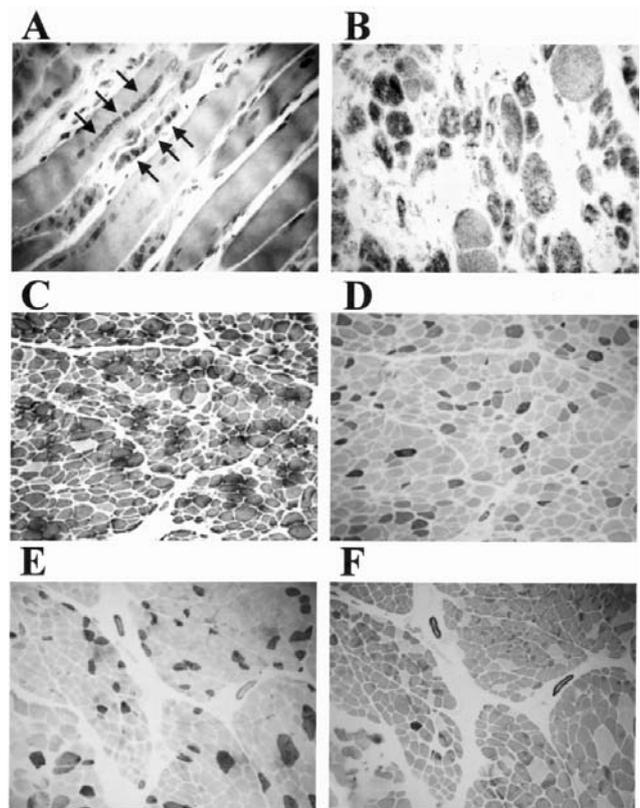

Figure 3. Muscle biopsies of patient 1 demonstrated (A) a linear aligment of internal nuclei (arrow) in a longitudinal section of H & E stain (400 ×), and (B) a hetergenous defect of oxidative enzyme activity on NADH stain (400 ×) in the peroneus longus muscles (C) ATPase at PH 9.5, and (D) ATPase stain at PH 4.55 (100 ×). Muscle biopsies from patient 2 showed a slight variation in fiber size with type 1 predominance in the tibialis anterior muscle. (E) ATPase stain at PH 9.5, and (F) ATPase stain at PH 4.55 (100 ×).

Table 2. *Muscle strength, myopathic changes, fiber type distribution, diameter of fiber type and CTG repeat number of muscles and leuko-cytes in congenital myotonic dystrophy*

Patient	Tissues	Muscle strength*	Dystrophic changes	Fiber type distribution			Diameter of fiber type (mean ±SD, μm)			Number of CTG repeats
				1	2A	2B	1	2A	2B	
1	Peroneus longus	1	†††	6%	83%	11%	26.2±7.9	9.7±3.3	24.0±9.2	1280
	Gastrocnemius	3	††	7%	80%	13%	21.9±5.5	25.6±8.1	26.4±8.1	920
	Vastus lateralis	4	±	17%	41%	42%	30.6±7.8	24.7±6.7	29.5±8.3	1300
	Leukocytes									850
2	Tibialis anterior	3	†	87%	7%	6%	26.0±7.1	27.2±7.0	22.0±13.7	1100
	Tibialis posterior	4	±	31%	50%	19%	23.4±4.3	18.9±5.2	21.6±3.5	1100
	Vastus lateralis	5	±	39%	26%	35%	22.2±5.2	20.7±4.1	19.8±3.9	950
	Leukocytes									850

*: Muscle strength according to the Scale of the Medical Research Council; ±: equivocal; †: mild; ††: moderate; †††: marked.
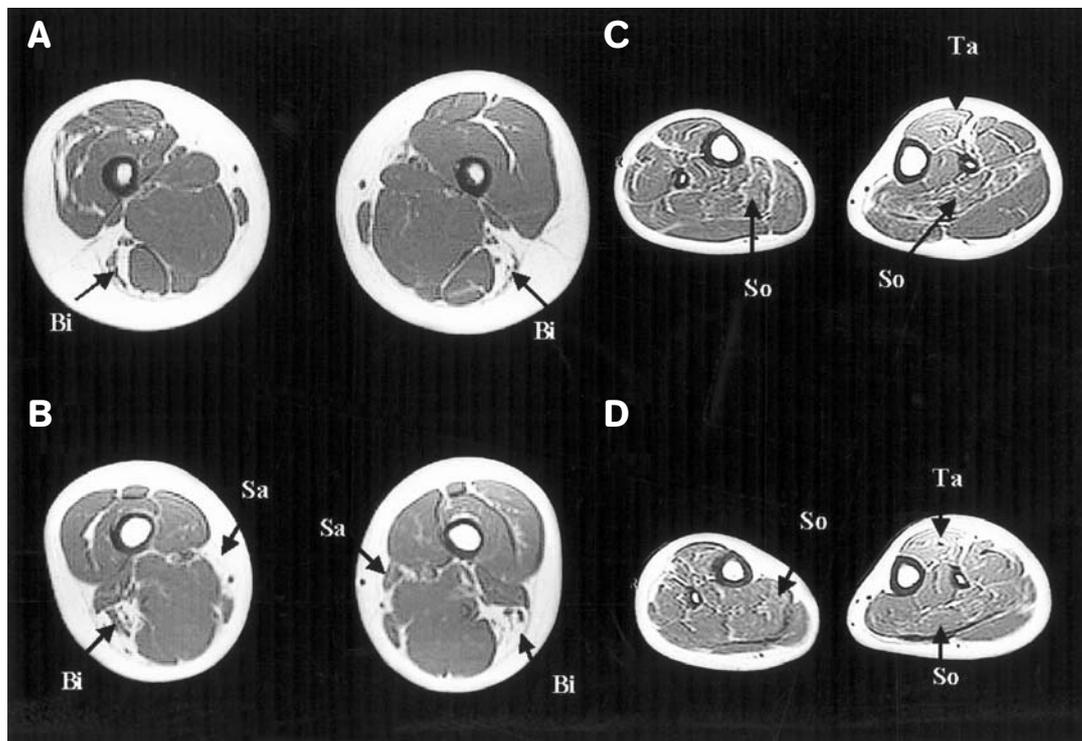

Figure 4. T1-weighted magnetic resonance images of the legs of patient 2 showed (A) at the upper thigh level and (B) at the lower thigh level: symmetrically moderate fatty degeneration in the long head of both biceps (Bi) muscles and symmetrical fatty replacement in the sartorius (Sa) muscles; (C) at the upper leg level and (D) at the lower leg level: mild fatty degeneration in bilateral soleus (So) and left tibialis anterior (Ta) muscles.

degeneration in muscles were 4 out of 20 thigh muscles and 9 out of 14 leg muscles in patient 1, and 8 out of 20 thigh muscles and 13 out of 14 leg muscles in patient 2 (Table 3). Generally, the distal leg muscles were more affected than the proximal one except for some thigh muscles of patient 2; asymmetric involvement was seen in the lower leg muscles of patient 1.

Table 3. *Skeletal muscle MRI findings of the lower limb muscles in 2 patients with congenital myotonic dystrophy*

Patient	1		2	
	R	L	R	L
Thigh muscles				
Rectus femoris	-	-	-	-
Vastus lateralis	-*	-	±	-*
Vastus intermedium	-	-	±	±
Vastus medialis	-	-	±	±
Adductors	-	-	-	-
Semitendinosus	-	-	-	-
Semimembranosus	-	-	-	-
Sartorius	±	±	†††	-
Gracilis	-	-	-	-
Biceps				
short	±	±	±	±
long	±	±	††	††
Lower leg muscles				
Tibialis anterior	±	±	±	†*
Extensor digiti & hallucis longus	±	-	±	±
Tibialis posterior	±	-	±	±*
Peroneus longus	†*	-	±	±
Flexor digiti longus	±	-	±	±
Gastrocnemius				
medial	†	†	-	±
lateral	±*	-	-	-
Soleus	†	-	†	†

R: right; L: left; -: normal; ±: equivocal fatty degeneration, †: mild fatty degeneration; ††: moderate fatty degeneration; †††: severe fatty degeneration; *: sites of muscle biopsy.

DISCUSSION

Previous studies have shown that the expansion of CTG repeats in skeletal muscles is usually 2-13 times higher than that in leukocytes[11-13], and that there is no significant difference in the different muscles of each individual[14]. In adult DM1, the degrees of histopathologic abnormalities and muscle weakness were not correlated with the sizes of CTG repeat expansion[15,16], but the sizes of CTG repeats were dynamic, and mitosis could have contributed to the degree of somatic mosaicism[11,12,17,18]. In contrast, congenital DM1 shows a minimal somatic instability of the CTG repeats in different tissues, particularly in the leukocytes and skeletal muscles of younger children or infants[11-13,18]. In our two patients with congenital DM1, there was a slight difference in the CTG repeats in various tissues and different parts of the muscles. Our data seem to confirm that minimal somatic instability of the CTG repeats might occur in congenital DM1[11,15,17,18].

The functional impairment was grossly correlated with the pathological findings of the muscle biopsies, but not with the skeletal MRI of both legs. There was no correlation between the MRI and pathologic findings of the peroneus longus muscle of patient 1, implying a mosaic pattern of intramuscular fatty replacement. In addition, the MRI abnormalities and an increase in signal intensity are unlikely to reflect the ultrastructural changes of skeletal muscles[19]. The most characteristic feature was the peculiar distribution of muscle involvement. Preferential involvement of the medial head of the gastrocnemius, soleus and tibialis anterior, but sparing the tibialis posterior muscles, has been reported in skeletal CT and MRI studies in adult DM1[20-22], but not in congenital DM1. In these 2 patients with congenital DM1, the muscle involvement was not always symmetrical such as the sartorius of patient 2 and the peroneus longus of patient 1. Moreover, such variable involvement among synergic muscles may lead to various types of joint deformities. In our patients, the degree and spatial pattern of the muscle abnormalities might have resulted from various degrees of immaturity and early degeneration of the muscles.

Tanabe and Nonaka[23] have suggested that in congenital DM1, the fiber type may transform from type 2 to type 1 with aging. Serial studies have also disclosed that muscle maturation in infants did occur, but never became normal[24,25]. The many type 2A hypotrophic fibers with internal nuclei in the peroneus longus muscle of patient 1 suggested a delayed differentiation or maturation of the muscle fibers. In addition, in the gastrocnemius and vastus lateralis muscles, the type 2 fiber predominance and type 1 fiber deficiency can be explained by some degrees of developmental abnormalities of the skeletal muscles. Although the severe hypoplastic fibers, which are related to talipes, can be noted in congenital DM1 patients, type 2 fiber predominance in muscle biopsies of proximal and distal leg has previously only

been reported in severe congenital DM1[24,26]. The present pathologic findings of the distal leg muscles of patient 1 seem to confirm that the disease might progress before the muscles become mature, whereas the proximal muscles might not well developed and then degenerate.The data is compatible with previous findings that there is no correlation between the degree of immaturity and the numbers of CTG triplet repeats in the skeletal muscles[15]. It also can be explained by the recent findings of reduced DM1PK and delayed slow fiber maturation in congenital DM1[27].

In contrast, the fiber differentiation in the tibialis anterior muscle of patient 2 had histopathological pictures similar to those found in older congenital or some classic DM1 patients. Impairments in muscle maturation and innervations of immature muscle fibers may result in the predominance of type 1 and the loss of type 2 fibers in older congenital DM1 patients[25,28]. The expansion of the CTG repeat in DM1 has been identified as altering the DM1PK transcription[27,29]. Recent studies also suggested that the decreased DM1PK transcription in type 2A fiber of DM1 patient might result in the progressive loss of type 2A fiber[30,31]. Our data showed type 1 fiber prepondance in the lower leg muscles of patient 2, supporting that DM1PK elongation in congenital DM1 patients do harm to the type 2A fiber through an unknown destructive mechanism or conversion type 2 to 1 fibers.

The observation that the pathological variability of muscle biopsies was prominent in these 2 siblings with similar genetic presentations and disease duration suggests that an undetermined pathogenesis other than the expanded CUG repeats of DM1PK mRNA may interfere with the differentiation of muscle fibers and then result in muscular immaturity in patients with congenital DM1. However, the interpretation should be very cautious because of the different sites of biopsied muscles in these two patients and the lack of reference values of fiber type differentiation in the lower leg muscles. In addition, the process of muscular dystrophy may have proceeded in our patients in a manner similar to other adult DM1 patients, if muscular development is more mature in congenital DM1. Furthermore, studies aimed at identifying expression levels of the DM1PK and other genes related to muscle maturation and differentiation in DM1 patients may provide insight into the pathogenetic basis of DM1 at the molecular level.

ACKNOWLEDGEMENTS

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