Molecular genetic analysis of X-linked recessive myotubular myopathy

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Abstract

The purposes of this study are to establish the effective and efficient screening protocol identifying the gene mutation, and to clarify the relationship between genotype and phenotype of X-linked myotubular myopathy (XLMTM) using the molecular genetic method. Five male patients in five families with XLMTM based on clinical features and muscle biopsy were analyzed according to the screening protocol.

Three types of abnormal band were identified by SSCP and HD analysis using genomic DNA from patients. In the direct sequence analysis, two frameshift mutations in exon 4 and 10 and one nonsense mutation in exon 3 were found. Frameshift mutation in exon 10 was a novel mutation.

RT-PCR method was performed to the other patients who showed no abnormal bands by SSCP and HD. In the direct sequence analysis, one missense mutation was found in exon 9. This mutation was a novel mutation. The relationship between phenotype and genotype was not clarified.

The screening protocol employed in this study detected the three mutations in genomic DNA and the one mutation in cDNA. The patient whom the mutation was not found was supposed to have the mutation in non-coding region of MTM1 gene. Present protocol is considered to be suitable to detect the mutations in XLMTM.

Key words: X-linked recessive myotubular myopathy, MTM1 gene, Mutation screening, Genetic analysis, RT-PCR method

1. Introduction

X-linked recessive myotubular myopathy (XLMTM) is a congenital myopathy characterized by severe hypotonia and generalized muscle weakness after birth in affected males[1, 2]. Polyhydraminos and weak fetal movement are observed in prenatal onset. The majority of patients died within the first few months of the life by respiratory insufficiency. The characteristics of muscle histopathology consist of small rounded muscle cells, these resemble fetal myotubes[3, 4]. XLMTM results from an arrest in the normal development of muscle fibers by impaired maturation[5].

MTM1 gene, the responsible gene for XLMTM, was isolated by a positional cloning strategy from proximal Xq28 region[6]. The MTM1 gene is composed

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of 15 exons and 603 amino acids and encodes myotubularin with a putative tyrosine phosphatase domain. Tyrosine phosphatases have been shown to be involved in transduction pathways controlling cell growth and differentiation.

Almost patients with XLMTM are severe, but a few patients survive for several years with medical complications and may show spontaneous improvement of the respiratory function after birth[7]. The clinical feature of XLMTM patients tends to be influenced by their genotype. Therefore, detecting the gene mutation rapidly and accurately is very important and provides valuable information for the later management.

The purposes of this study are to establish the effective and efficient screening protocol identifying the gene mutation of XLMTM, and to clarify the relationship between genotype and phenotype of XLMTM using the molecular genetic method. This study would contribute to the development of the rehabilitation science.

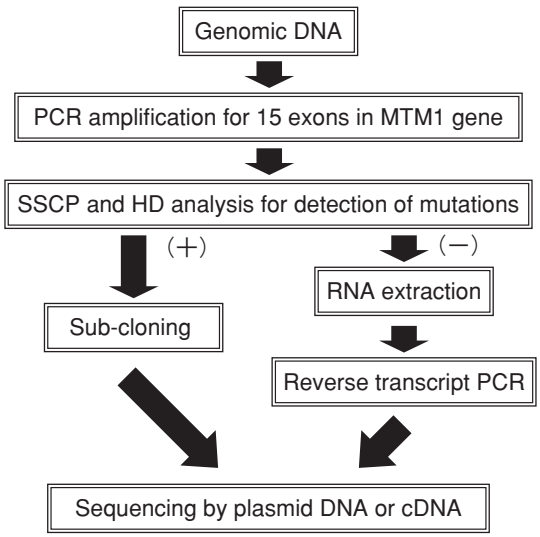


Fig. 1.　Screening protocol of X-linked myotubular myopathy

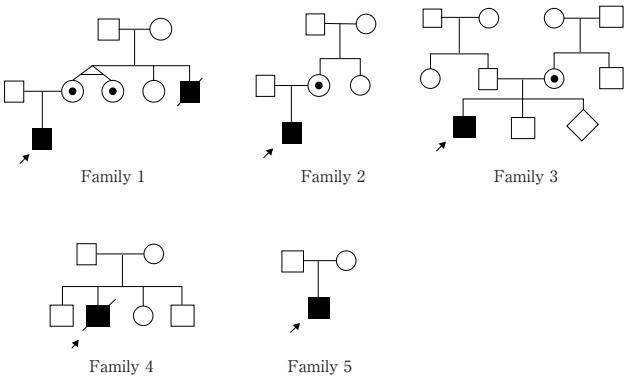


Fig. 2.　Pedigree of XLMTM families. Affected individuals are indicated in black and the carrier indicated the circle with dot. Deceased individuals are marked with slashes. Arrow indicates propositus.

2. Patients, materials and method (Fig. 1)

2. 1. Patients

Five male patients in five families with severe phenotype of XLMTM were analyzed based on clinical features and muscle biopsy (Fig. 2). Peripheral blood or muscle tissue was obtained from patients and their parents according to informed consent from those parents.

2. 2. Genomic mutation screening

Genomic DNA of the patients and their parents were extracted from peripheral blood lymphocytes or muscle tissues using Sepa Gene kit (Sanko Jun-yaku).

Fifteen exons of MTM1 gene were amplified by polymerase chain reaction (PCR). PCR amplification of all exons was carried out using GeneAmp PCR System 9600 (Applied Biosystems)[8]. Two types of PCR condition were designed to obtain the better PCR products in the quality. For exon 3, 5, 7, 9-14, initial

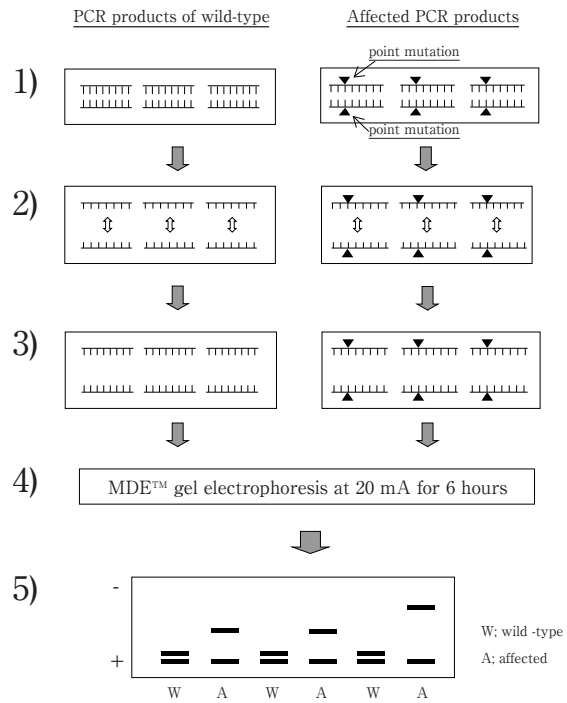


Fig. 3.　Principle of single-strand conformation polymorphism analysis.
1) PCR for target DNA.　2) Denaturing the each PCR product at 100 ˚C　for 10 min. 3) Quickly on-ice for 10 min.
4) Electrophoresis in MDE™ gel at 20 mA for 6 hours in a cold (4˚C) room.　5) Silver staining.

denaturation was at 94 ˚C for 1 min, followed by 30 cycles of 94 ˚C for 1 min, annealing at 60˚C for 1 min, and extension at 72˚C for 1min. For residual exons (1, 2, 4, 6, 8, and 15), initial denaturation was at 95 ˚C for 2 min, and 28 cycles with denaturation at 95 ˚C for 15 sec, annealing at 60 ˚C for 15 sec, and extension at 72 ˚C for 45 sec. The final extension was at 72 ˚C for 7 min in either case.

2. 3. Screening

2. 3. 1. Single-strand conformation polymorphism analysis (SSCP; Fig. 3)

One μl of PCR products added 15 μl of loading buffer was denatured at 100 ˚C for 10 min and quickly on ice for 10 min[9, 10]. The mixture loaded onto a gel in 1× TBE (Tris / Bolate / EDTA) buffer and electrophoresis was performed at 20 mA for 6 hours at 4 ˚C in a cold room[11].

The gel was silverstained in a room temperature. The gel was immersed in gultaraldehyde / double distilled water (DDW) (2:3) solution for 30 min initially. After washing the gel, it was placed in 1N NaOH / ammonia / 20 % silver nitrate / DDW (5:1:1:93) solution for 15 min, and again rinsed in water. The gel was put in 1 % citric acid / formaldehyde / DDW (10:1:200) solution[12].

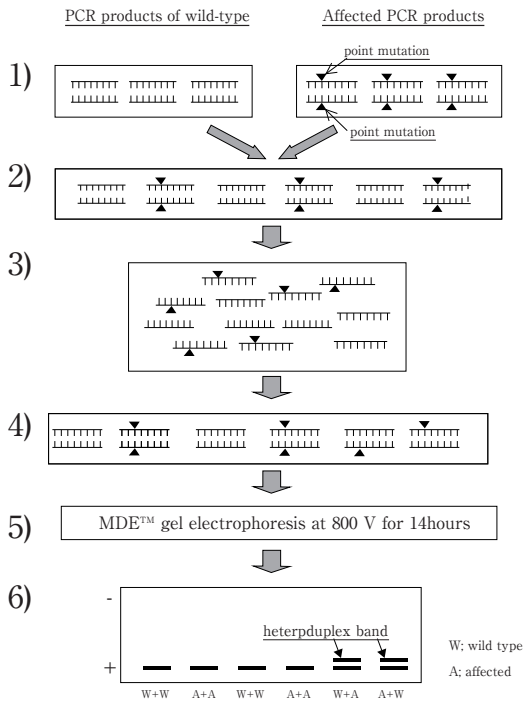


Fig. 4. Principle of heteroduplex analysis. 1) PCR for target DNA. 2) Mixed wild-type and affected PCR products. 3) Denaturing the mixture at 95 ˚C for 3 min. 4) Annealing at 37 ˚C for 60 min. 5) Electrophoresis in MDE™ gel at 800 V for 14hours at room temperature. 6) Staining in ethidium bromide.

2. 3. 2. Heteroduplex (HD) analysis (Fig. 4)

Five μl of PCR product of negative control and patient were mixed respectively and the mixtures were denatured at 95 ˚C for 3 min and annealed at 37 ˚C for 1 hour. One μl of loading buffer was added to 5 μl of the mixture and loaded onto a gel and electrophoresis was performed at 800 V for 14 hours at room temperature in 0.6×TBE buffer[13]. The gel was stained in ethidium bromide for 15 min[13, 14].

2. 4. Sub-cloning

Sub-cloning of mutant exon was performed using Original TA cloning Kit (Invitrogen) and to obtain the plasmid DNA, GFX™ Micro Plasmid Prep Kit (Amersham phalmacia biotech.) was used. The procedures were referred to manufacturer's instructions.

2. 5. RNA isolation and reverse transcript-PCR

In XLMTM patients having no specific bands by SSCP and HD analysis, reverse transcript-PCR (RT-PCR) was performed to detect the splicing mutations in exon/intron boundaries using their complementary DNA (cDNA) synthesized from total RNA (tRNA).

tRNA was isolated from frozen muscle specimens or peripheral blood lymphocytes on a guanidinium thiocynate based extraction[15]. First-strand cDNA were synthesized from tRNA using SUPERSCRIPT First-Strand Synthesis System for RT-PCR kit (Invitrogen). The full-length first-strand cDNA of MTM1 gene obtained in the previous procedure were amplified directly using PCR. The entire cDNA was divided into six fragments (RTS1-6) and the primers sets were designed referred to previous study (Table 1)[16]. PCR was performed for 40 cycles with denaturation at 94 ˚C for 1 min, annealing at 55 ˚C for 1 min, and extension

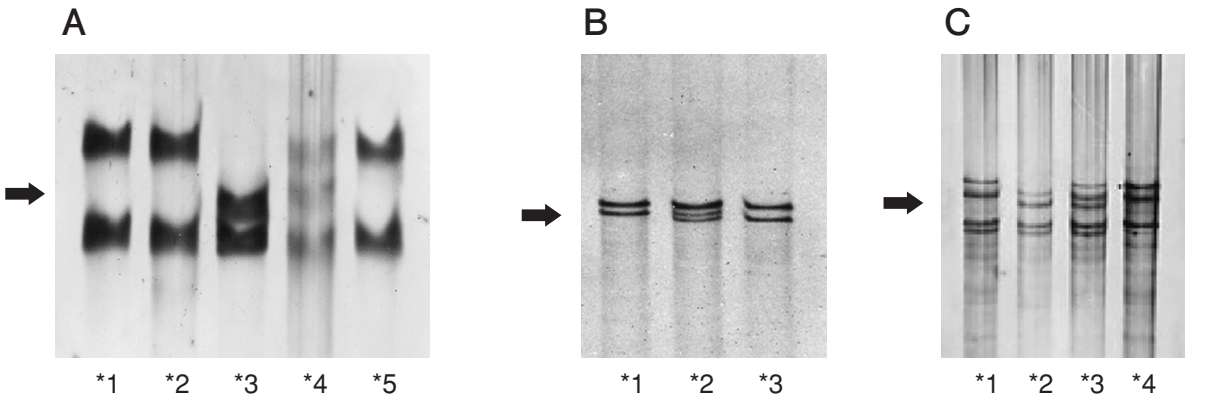
Table 1. Primers set for RT-PCR in each fragment of MTM1 gene

Name	Forward	Reverse
RTS1	5' -ATGGCTTCTGCATCAACTTC-3'	5' -CTCGAGATCACACCCAGAG-3'
RTS2	5' -GATTCTTCTCTAATACTTGATG-3'	5' -CTGCCTCCTGTATTCTTCC-3'
RTS3	5' -AGTGGAAGAATACAGGAGG-3'	5' -GAATAAACAATGTCCTTCAC-3'
RTS4	5' -GCATATCATAACGCCGAAC-3'	5' -TCGAGATGCAAATTTATGTCC-3'
RTS5	5' -TGAAGGGTTCGAAATACTGG-3'	5' -GAAACAGTCCTTTCTGTAAC-3'
RTS6	5' -CTGTTTCTTTATGGTCACTG-3'	5' -GAAGTGAGTTTGCACATGG-3

at 72 ˚C for 1min after denaturing at 94 ˚C for 2 min. Final extension was 72 ˚C for 7 min. 0.4 μl of Taq DNA Polymerase was added to the mixtures after initial denaturation[17]. The PCR products were purified, and subsequently direct sequence determination was performed about all fragments.

2. 6. Direct sequencing determination

DNA sequencer SQ5500E (Hitachi Electronics Engineering) was used for direct sequencing

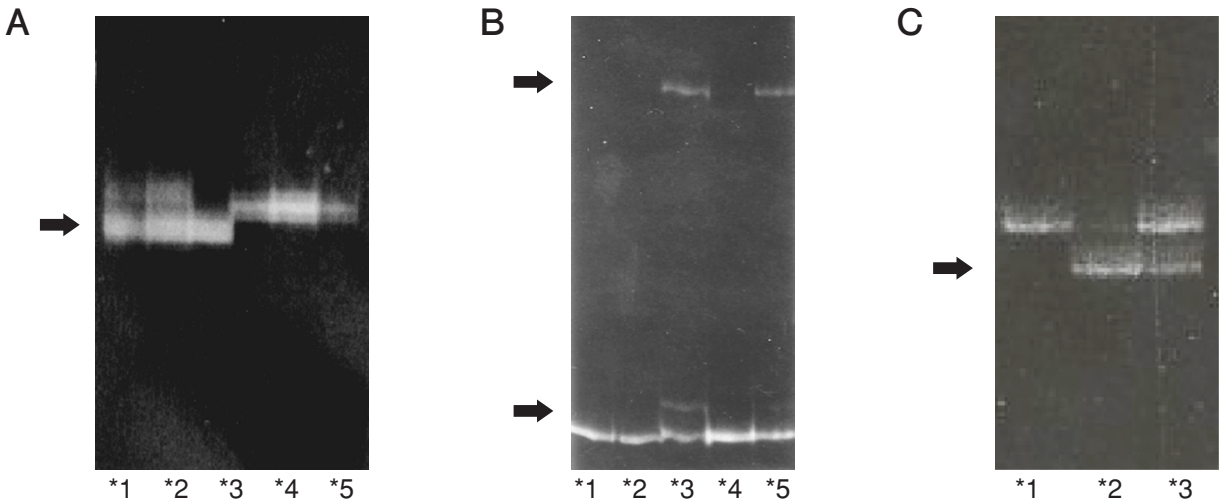


*1. control (male)
*2. father of patient 1
*3. patient 1
*4. mother of patient 1
*5. control (female)

*1. father of patient 2
*2. mother of patient 2
*3. patient 2

*1. father of patient 3
*2. patient 3
*3. mother of patient 3
*4. control

Fig.5. SSCP analysis in exon 3 (family 1, A), exon 4 (family 2, B) and exon 10 (family 3, C) of MTM1 gene in controls and XLMTM patients. A; Mutant bands are demonstrated in patient 1 (lane 3, arrowed) and his mother (lane 4). B; Mutant bands are demonstrated in patient 2 (lane 3, arrowed) and his mother (lane 2). C; Mutant bands are demonstrated in patient 3 (lane 2, arrowed) and his mother (lane 3).



*1. aunt of patient 1
*2. mother of patient 1
*3. patient 1
*4. father of patient 1
*5, *6 control

*1. control
*2. father of patient 2
*3. patient 2
*4. control
*5. mother of patient 2

*1. father of patient 3
*2. patient 3
*3. mother of patient 3

Fig.6. HD analysis in exon 3 (family 1, A), exon 4 (family 2, B) and exon 10 (family 3, C) of MTM1 gene in controls and XLMTM patients. A; Mutant bands are demonstrated in patient 1 (lane 3, arrowed) and his mother (lane 4). B; Mutant bands are demonstrated in patient 2 (lane 3, arrowed) and his mother (lane 2). C; Mutant bands are demonstrated in patient 3 (lane 2, arrowed) and his mother (lane 3).

determination. The sequence reaction was performed using the 500 ng of plasmid DNA obtained from sub cloning (template DNA), Texas Red M13 forward primer and Thermo Sequenase Core Seaquencing kit (Hitachi measurement apparatus service). Electrophoresis was carried out for 12 hours, checked the fluorogram and determined base arrangement by computer processing.

Sequence changes were identified by comparing with the MTM1 cDNA sequence (Genbank, U46024) or with the splice junction of MTM1 gene[18].

3. Results

3. 1. Detection of mutations in screening tests

The results of the SSCP analysis was shown in Fig. 5. In family 1 (Fig. 5, A), specific bands were observed in exon 3 of MTM1 gene. In patient (lane 3), specific band was observed (arrow). His mother (lane 4) had both normal and specific bands. In family 2 (Fig. 5, B), specific bands were observed in exon 4 of MTM1 gene. In patient 2 (lane 3), specific band was observed (arrow). His mother (lane 2) had both normal and specific bands.

In family 3 (Fig. 5, C), specific bands were observed in exon 10 of MTM1 gene. In patient 3 (lane 2), specific band was observed (arrow). His mother (lane 3) had both normal and specific bands. In patient 4 and 5, the specific bands were not found in the SSCP.

The results of the HD analysis was shown in Fig. 6. In family 1 (Fig. 6, A), patient 1 (lane 3) had a mutant homoduplex band (arrow). His aunt (lane 1) and mother (lane 2) had a mutant (arrow) and a normal band. In family 2 (Fig. 6, B), two abnormal bands were observed in the patient (lane 3) and one band in his mother (lane 5). In family 3 (Fig. 6, C), patient 3 (lane 2) had a mutant band, mother of patient 3 (lane 3) had a mutant and a normal band.

In patient 4 and 5, specific bands were not observed by the HD.

3. 2. Direct sequencing determination

The results of the direct sequence determinations were shown in Fig. 7. In patient 1 (Fig. 7, A), one base substitution (cDNA 109C-T), leading to an arginine to a stop codon, was identified (nonsense mutation). In patient 2 (Fig. 7, B), 4 bp deletion at cDNA 139-142 (AAAG) was identified, this mutation caused to shift the reading frame and terminated translation at the 25th codon from deleted site (frameshift mutation). In patient 3 (Fig. 7, C), 2 bp deletion of cDNA 1003-1004

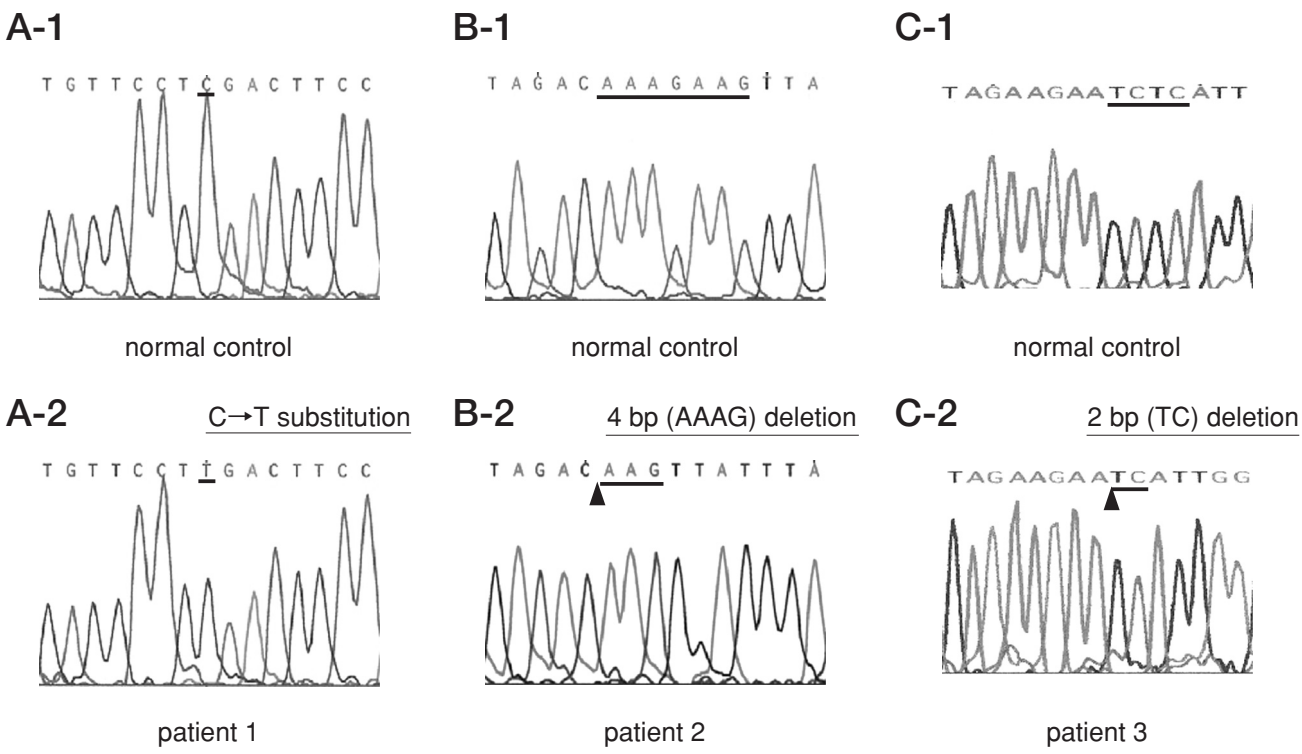


Fig. 7. Direct sequence analysis of MTM1 gene in genomic DNA in family 1(A-1; normal control in exon 3, A-2; patient 1), family 2 (B-1; normal control in exon 4, B-2; patient 2) and family 3(C-1; normal control in exon 10, C-2; patient 3) .

(TC) or 1005-1006 (TC) was identified. This mutation caused to shift the reading frame and terminated translation at the 42nd codon from deleted site (frameshift mutation). This mutation was not reported in the previous studies (novel mutation).

In patient 4 and 5, the specific bands were not observed by SSCP and HD. The direct sequence determinations were performed about six fragments of cDNA. In patient 4, mutations were not disclosed. In patient 5 (Fig. 8, D), one base substitution (cDNA 688T-A) was identified in RTS-3, corresponding to exon 9 in genomic DNA. This mutation led from a tryptophan to arginine (missense mutation). This mutation was not reported in the previous studies (novel mutation).

4. Discussion

MTM1 gene was mapped to Xq28. The MTM1 gene is about 100 kb and consisted of 15 exons. It encodes 603 amino acid protein[6]. Myotubularin, which is encoded by MTM1 gene, is highly conserved through the evolution. The function of myotubularin is not well known, but was clarified containing the consensus sequence of tyrosine phosphatases (PTP) active site in exon 11 and SET interaction domain (SID) in exon 12 and 13. This protein can dephosphorylate phosphorylated serine and phosphorylated tyrosine. It is considered to be a dual-specific phosphatase involved in signal transduction pathways which are implicated in muscle development[19]. The SET domain is epigenetic regulatory machinery concerned with the growth and differentiation of muscle[20]. As XLMTM seems to be due to a defect in late myogenesis, the regulation of the MTM1 gene transcription and/or regulation of myotubularin activity might be an essential step toward the complete maturation of muscle fiber[18]. The results of these previous studies

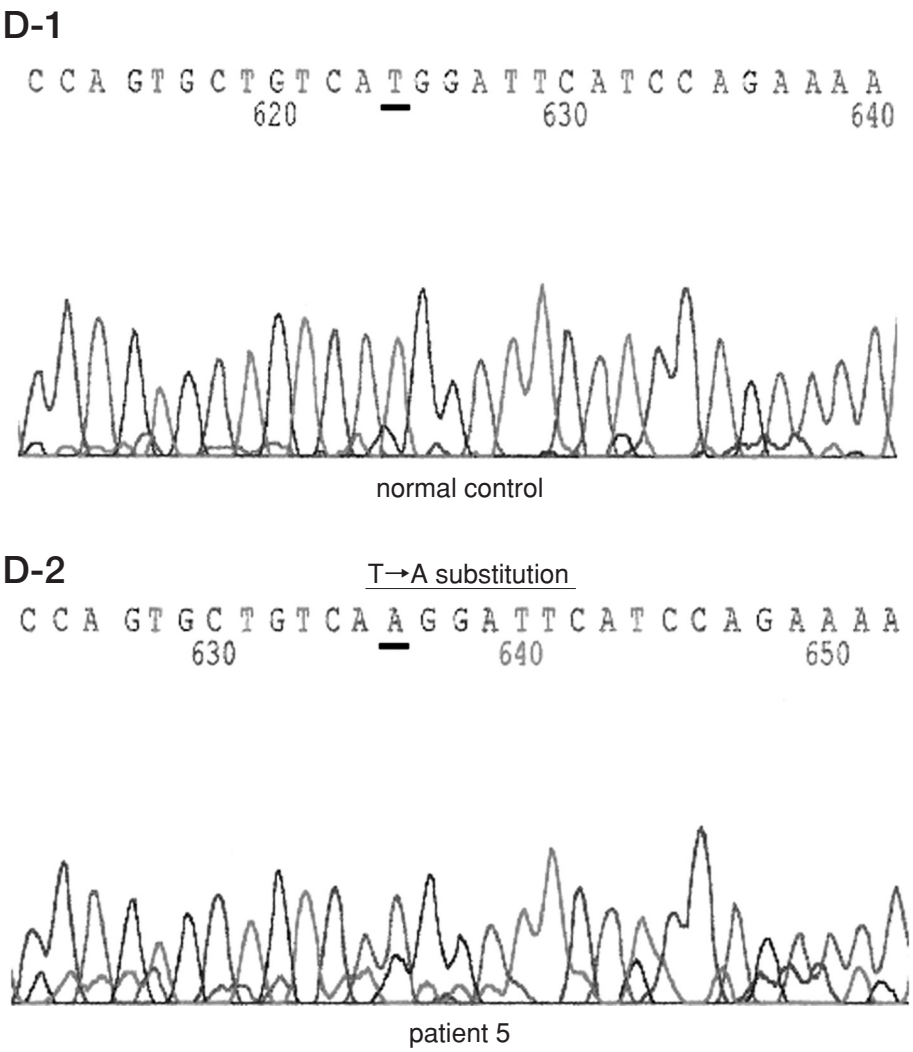


Fig. 8. Direct sequence analysis of MTM1 gene in cDNA in family 5 (D-1; normal control in RTS fragment 3, D-2; patient 5)

indicate that the PTP and SID domains play important roles in myotubularin. Truncated myotubularin might be unstable and/or may display altered interaction with other intracellular protein or subcellular localization[21].

In this study the mutations in MTM1 gene were screened for all exons in five unrelated XLMTM patients by the SSCP and the HD. The mutant band was detected in the three among the five patients by the SSCP and the HD but not in the two patients. In the mutation screening, the SSCP and the HD have a technical simplicity, a high sensitivity, validity and low cost for the detection of sequence mutations[11]. The level of sensitivity in SSCP and HD is 70 - 95 % and 80 - 90 %, respectively. The sensitivity is influenced by some factors, e.g. the length of fragment, the type of mutation and the matrix of running gel[22]. Rossetti et al.[13] reported that the combined efficiency of these two methods was estimated to 100%. These screening methods are thought to be effective techniques, but present data suggests that the SSCP and the HD could not detect mutations completely.

Next step, the RT-PCR was performed to two patients who were found no mutation in genomic DNA screening. The RT-PCR can be an alternative method in order to avoid analysis of individual exons, but this requires adequately stored cells[8]. The disadvantage of this method is the need for ready access to a suitable biological source from the patients. The mutation in patient 5 was detected by the RT-PCR analysis. The RT-PCR analysis must be performed in the cases that mutations were not found in genomic DNA screening. The screening protocol in the present study is considered to be suitable and rational to detect the mutations in coding region of MTM1 gene.

In the present study, the mutations were identified in the four patients and the two out of those were novel mutations (Table 2). Mutations have been restricted to the coding region of MTM1[8, 23]. Recently, highly homologous gene, MTMR1, was identified lying 20 kb distal to MTM1 gene[24]. Copley et al.[25] studied that no mutations were found in MTMR1 gene in fourteen XLMTM patients who had no mutations in MTM1 gene. These suggest that MTMR1 is not related to XLMTM. Kioschis et al.[24] suggests some of the mutations are located in non-coding regions (intron or promoter region) of MTM1. It is suggested that the mutations of one patient not identified in present study will be located in non-coding region.

Laporte et al.[26] reported the types and location of mutations in XLMTM. One third of patients with a missense mutation showed mild phenotype, most of the patients with other types of mutation showed severe phenotype.

Missense mutation is the most frequent in XLMTM. 85 % of the missense mutations are located from exon 8 to 12, includes PTP and SID domain. In present study, missense mutation of the patient 5 (cDNA 688T-A) was observed in exon 9 of MTM1 gene, and he showed severe phenotype. 7 /13 of missense mutations in exon 9 showed severe phenotype, but the change of amino acid from tryptophan to arginine was not reported in the previous study. It is supposed that this amino acid change may lead to severe phenotype.

The small deletion or insertion alters the reading frame of sequence. Two small deletions were found in present study. In patient 2, AGAA deletion at cDNA 141-144 was identified, and this mutation was observed in ten patients with previously reported XLMTM

Table 2. Clinical features and genotype

		clinical feature		genotype			
Patients	Age/Sex	hypotonia	respiration	exon / intron	nucleotide change	predicted protein alteration	mutational origin
patient 1	1 year / M	severe	ventilator	exon 3	C109T	frameshift truncation	mother heterozygote
patient 2	6 month / M	severe	ventilator	exon 4	139-142 del AAAG	Arg 37 Stop truncation	mother, aunt heterozygote
patient 3	5 year / M	severe	ventilator	exon 10	1003-1004 del TC	frameshift truncation	mother heterozygote
patient 4	1 month / M	severe	ventilator	—	—	—	unknown*
patient 5	1 year / M	severe	ventilator	exon 9	T688A	missense Trp230Arg	unknown**

* Mutation was not identified, so the mutation origin was not clarified.
** Blood sample was not obtained from the patient's family.

patients. Their phenotypes were all severe, and patient 2 showed also severe. In patient 3, TC deletion at cDNA 1003-1004 (exon 10) was identified, and this mutation was a novel mutation. His phenotype was severe. These deletions in present study truncated the myotubularin, and led to severe phenotype.

The nucleotide change from C to T at cDNA 109 in the present study was observed in five patients in previous study. Their phenotypes were all severe. Nonsense mutation truncated the myotubularin, and led to severe phenotype.

In medical management of rehabilitation, initial-data collection from the patient and family is the important information for the decision making and later treatment[27, 28]. The accurate data is required for establishing the appropriate plan to the goal, and the therapeutics on inadequate data may impose severe burden on the patient and the family. In addition, it is supposed to be difficult to perform some examinations as an initial-data collection in the disease which is severe and early onset. To specify the pathogenesis of a disease will estimate the prognosis, and support to design for the suitable program. In some hereditary diseases, previous studies indicate that the genotype and the phenotype are correlated. XLMTM is a lethal disease that most of the patients decease in a year, but some of the patients survive in long term. They show spontaneous improvement of the respiratory function. In present study, patient 2 survives with respirator now and is 5 years old. He has received respiratory physical therapy and aims at home care. These findings suggest the importance of effective care in neonatal period of XLMTM. To identify the genotype become an important and a reliable initial-data, and enable the early treatment to forestall the progress in hereditary diseases. Genotype analysis may lead to predict the prognosis in XLMTM. Genetic diagnosis supports the total management of hereditary disease in rehabilitation approach.

In genotype-phenotype correlation in present study, no obvious correlations are obtained between the type of mutation and the severity of the disease. The mutations truncate the essential function of this protein lead to the severe phenotype. Analysis of the genotype is considered to be particularly important in diagnosis of XLMTM. The results of this study should contribute to raise the quality of life of the patients and their family.

References

1) Joseph M, Pai GS, Holden KR et al.: X-linked myotubular myopathy: clinical observations in ten additional cases. Am. J. Med. Genet. 59：168－173, 1995.

2) Wallgren-Pettersson C, Clarke A, Samson F et al.: The myotubular myopathies: differential diagnosis of the X linked recessive, autosomal dominant, and autosomal recessive forms and present state of DNA studies. J. Med. Genet 32：673－679, 1995.

3) Soussi-Yanicostas N, Chevallay M, Laurent-Winter C et al.: Distinct contractile protein profile in congenital myotonic dystrophy and X-linked myotubular myopathy. Neuromuscular Disorders：1, 103－111, 1991.

4) Sawchak JA, Sher JH, Norman MG et al.: Centronuclear myopathy heterogeneity: distinction of clinical types by myosin isoform patterns. Neurology 41：135－140, 1991.

5) Sarnat HB: Myotubular myopathy: arrest of morphogenesis of myofibers associated with persistence of fetal vimentin and desmin: four cases compared with fetal and neonatal muscle. Can. J. Neurological Sci. 17：109－123, 1990.

6) Laporte J, Hu LJ, Kretz C et al.: A gene mutated in X-linked myotubular myopathy defines a new putative tyrosine phosphatase family conserved in yeast. Nature Genet. 13：175－182, 1996.

7) Herman G. E., Finegold M., Zhao W. et al.: Medical complications in long-term survivors with X-linked myotubular myopathy. J. Pediatr, 134：206－214, 1999.

8) Laporte J, Guiraud-Chanmeil C, Vincent MC et al.: Mutations in the MTM1 gene implicated in X-linked myotubular myopathy. Hum. Mol. Genet. 6：1505－1511, 1997.

9) Puck JM, Middelton L & Pepper AE: Carrier and prenatal diagnosis of X-linked severe combined immunodeficiency: mutation detection methods and utilization. Hum. Genet. 99：628－633, 1997.

10) Orban TI, Csokay B & Olah E: Sequence alterations can mask each other's presence during screening with SSCP or heteroduplex analysis: BRCA genes as examples. Bio Techniques 29：94－98, 2000.

11) Nataraj AJ, Olivos-Glander I, Kusukawa N et al.: Single-strand conformation polymorphism and heteroduplex analysis for gel-based mutation detection. Electrophoresis 20：1177－1185, 1999.

12) Nelis E, Simokovic S, Timmerman V et al.: Mutation analysis of connexin 32 (Cx32) gene in Charcot-Marie- Tooth neuropathy type 1: identification of five new mutations. Hum. Mutat. 9：47－52，1997.
13) Rossetti S, Corra S, Biasi MO et al.: Comparison of heteroduplex and single-strand conformation analyses, followed by ethidium fluorescence visualization, for detection of mutation in four human genes. Mol & Cell Probes 9：195－200，1995.
14) Kozuka N & Tachi N: Molecular analysis of hereditary motor and sensory neuropathy (Charcot-Marie-Tooth disease) associated with P0 mutation. The Sapporo Med. J. 67：39－49，1998. (in Japanese).
15) Chomczynski P & Sacchi N: Single-step method of isolation by acid guadinidium thiocynate-phenol-chloroform extraction. Analytical Biochem. 162：156－159，1987.
16) Tanner SM, Schneider V, Thomas NS et al.: Characterization of 34 novel and six known MTM1 gene mutations in 47 unrelated X-linked myotubular myopathy patients. Neuromuscular Disorders 9：41－49，1999.
17) Herman GE, Kopacz K, Zhao W et al.: Characterization of mutations in fifty North American patients with X-linked myotubular myopathy. Hum. Mutat. 19：114－121，2002.
18) Laporte J, Guiraud-Chanmeil C, Tanner SM et al.: Genomic organization of the MTM1 gene implicated in X-linked myotubular myopathy. Eur. J. Hum. Genet. 6：325－330，1998.
19) Laporte J, Blondeau F, Bui-Bello A et al.: Characterization of the myotubularin dual specificity phosphatases gene family yeast to human. Hum. Mol. Genet. 7：1703－1712，1998.
20) Cui X, De Vivo I, Slany R et al.: Association of SET domain and myotubularin-related proteins modulates growth control. Nature Genet. 12：331－337，1998.
21) Buj-Bello A, Biancalana V, Moutou C et al.: Identification of novel mutations in the MTM1 gene causing severe and mild forms of X-linked myotubular myopathy. Hum. Mutat. 14：320－325，1999.
22) Grompe M: The rapid detection of unknown mutations in nucleic acids. Nature Genet. 5：111－117，1993.
23) de Gouyon BM, Zhao W, Laporte J et al.: Characterization of mutations in the myotubularin gene in twenty six patients with X-linked myotubular myopathy. Hum. Mol. Genet. 6：1499－1504，1997.
24) Kioschis P, Wiemann S, Heiss NS et al.: Genomic organization of a 225-kb region in Xq28 containing the gene for X-linked myotubular myopathy (MTM1) and a related gene (MTMR1). Genomics 54：256－266，1998.
25) Copley LM, Zhao WD, Kopacz K et al.: Exclusion of mutations in the MTMR1 gene as a frequent cause of X-linked myotubular myopathy. Am. J. Med. Genet. 107：256－258，2002.
26) Laporte J, Biancalana V, Tanner SM et al.: MTM1 mutations in X-linked myotubular myopathy. Hum. Mutat. 15：393－409，2000.
27) Campbell SK: Models for decision making in pediatric neurologic physical therapy. Decision making in pediatric neurologic physical therapy, pp 1-22, Philadelphia, Churchill Livingstone, 1999.
28) Palisano RJ, Campbell SK & Harris SR: Decision making in pediatric physical therapy. In Campbell SK, Vander Linden DW & Palisano RJ (Eds.), Physical therapy for children (2nd ed.), pp 198-224, Philadelphia, W. B. Saunders, 2000.

X染色体劣性遺伝ミオチューブラーミオパチーの遺伝子解析

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要　　旨

本研究は遺伝性筋疾患であるX-linked myotubular myopathy（XLMTM）における遺伝子解析において、遺伝子変異を検出する効果的かつ効率的なスクリーニング方法を規定すること、および本疾患の表現型と遺伝子型の関連を明らかにすることを目的とした。対象は臨床症状および筋生検によって重症のXLMTMと診断された5家系5名の男児とし、スクリーニングプロトコルにしたがって解析を行った。

その結果、ゲノムDNAを用いたSSCPおよびHD解析において5名の患者のうち、3名について泳動変異を検出した。これらの直接塩基配列決定の結果、2名のフレームシフト変異（エクソン4および10）と1名のナンセンス変異（エクソン3）が確認された。エクソン10におけるフレームシフト変異は これまで報告例のない新しい変異であった。

ゲノムDNAを用いたスクリーニングで泳動変異が観察されなかった2名の患者に対してはRT－PCR法を用いたcDNAの直接塩基配列決定を行った。その結果、1名のミスセンス変異（エクソン9）を同定し、これまで報告例のない新しい変異であることを確認した。残り1名については変異が確認できなかった。表現型と遺伝子型の関係については、すべての患者が重症例であったこと、症例数が少なかったことから明らかにできなかった。

本研究ではゲノムDNAで3名、cDNAで1名の遺伝子変異を確認したが、変異を確認できなかった患者は非翻訳領域に変異が存在する可能性が考えられた。これらの結果から、本研究で実施したスクリーニングプロトコルはXLMTMにおける遺伝子変異検出に対し十分適切であることが考えられる。

＜索引用語＞X染色体劣性遺伝ミオチューブラーミオパチー、MTM1遺伝子、遺伝子変異スクリーニング、遺伝子解析、RT-PCR法