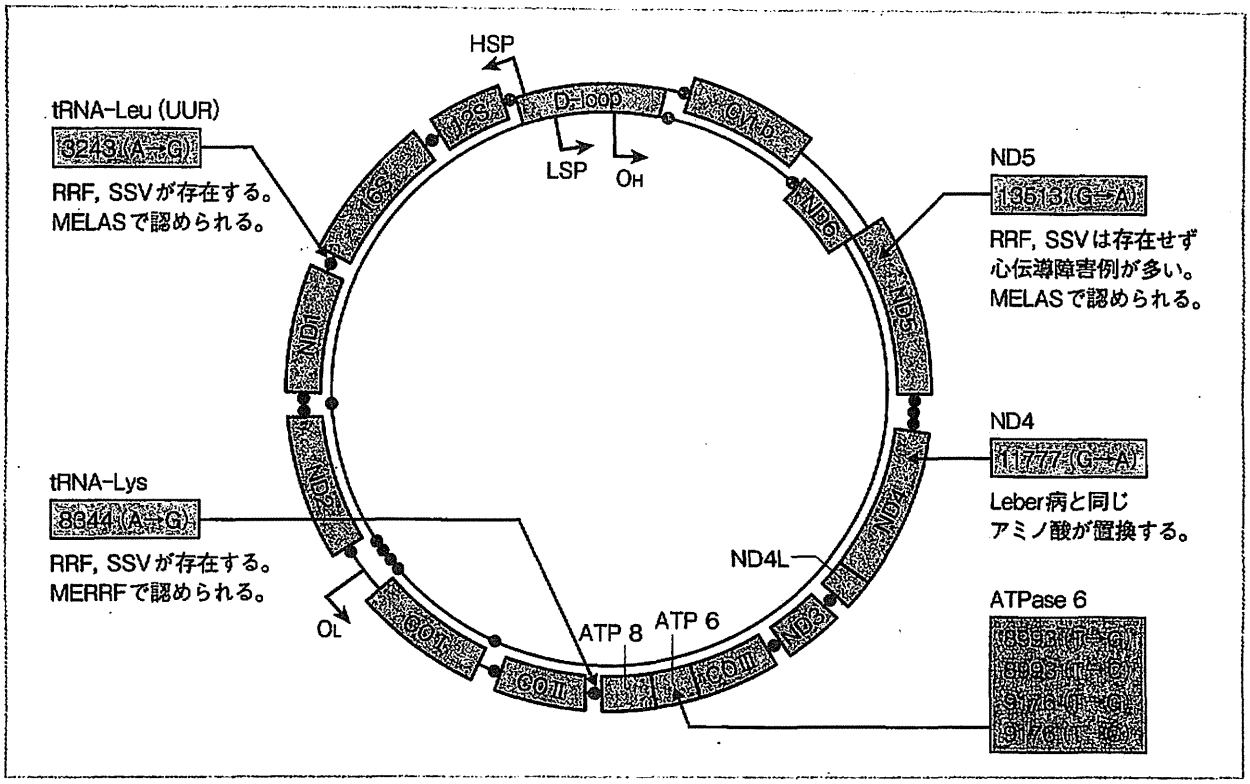


図7-12　Leigh脳症で認められるmtDNAの点変異

対応がまったく変わってくるといえます。

臨床の場において ミトコンドリア病がみつかったら

私たちが最も慎重を要しなければならないのは，検査をしてミトコンドリアに遺伝子変異がみつかった場合，それをどのように両親にお話しするかということです。母系遺伝という言葉から，母親が負担を感じるような話し方をしてはいけないわけで，加えて変異をもってるからといって即病気になるわけではないということを詳しく話す必要があります。説明したとおり，比率が低ければ，まったく病気にかかわらない可能性もあるわけです。それからもう1つは，mtDNAにも突然変異と考えられる変異がみつかってきているということです。患者には8993変異がほぼ100%存在しているのに，その母親や姉の血液をみたらまったく変異が存在していないという事例もあります。おそらくこれはたまたま母親の卵子のなかに起こった点変異が増えて，それがたまたま子に伝わったのではないかと考えられます。要するに *de novo*（新生）の突然変異ではないかということが推定されるわけです。こういう例は8993変異以外でも報告されています。何度もいいますが，診断がついたからといって，それが即母系遺伝であるとはいえない場合があるということに注意しましょう。

表7-7は最近のデータになりますが，新生児3168人の臍帯血を調べた例です。3168人のmtDNAのいろいろな病的点変異をチェックしたところ，15人で点変異がみつかったという結果になっています。たとえば3243変異をみていた

表7-7　臍帯血における主要なmtDNA点変異（新生児3168人の臍帯血）（文献1より）

変異	陽性例	母の血液で陰性の例
1555A→G	2	0
3243A→G*	4	2
3460G→A	3	0
7445A→G	0	0
8344A→G	0	0
8993T→G	0	0
11778G→A	3	1
13513G→A	0	0
14459G→A	0	0
14484T→C	3	0
合計	15（0.47%）	3

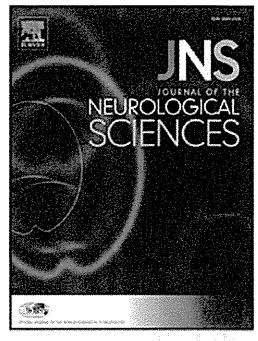
last-cycle fluorescent PCR：測定感度1.8%
*少なくとも出生200人に1人には点変異が存在。3243変異はその内の33%を占める。新生変異は10万人に107人の割合（1000人弱に1人）である。

だくと，陽性例が4人，その母親の血液では陰性が2例となっています。これは母親は変異をもっておらず，卵子のなかで起こった突然変異でお子さんが変異をもったということです。3460変異では3例とも母親では変異がみつかっていません。このように，おそらく相当程度，新生突然変異が起こっているだろうということが，このデータから推定されます。診断してお話しする際には，比率が低い場合には何も起こらないし，突然変異の場合もあることをきちんと説明することが重要かと思います。

最後になりますが，これが最も本講でお話ししたかったことになります。mtDNAの変異が，実際の臨床症状としてあらわれるまでにはいくつもの段階があります。mtDNAに変異がみつかったというだけではまったく意味がなくて，それがどの臓器，どの細胞でどれくらい存在しているかということまでわからないと臨床症状の説明ができませんし，ヘテロプラスミーで起こる病気の場合は，その比率がわかったからといって1つの細胞からでは全身のことは到底わかりません。そういうことをきちんと理解したうえで，病理検査，生化学検査などを総合的に組み合わせながら，ミトコンドリアの遺伝子検査を行っていくべきだろうと思います。

◉文献

1) Elliott HR, Samuels DC, et al: Pathogenic mitochondrial DNA mutations are common in the general population. Am J Hum Genet 2008; 83: 254-60.



Congenital fiber type disproportion myopathy caused by *LMNA* mutations

Sachiko Kajino, Kayo Ishihara, Kanako Goto, Keiko Ishigaki, Satoru Noguchi, Ikuya Nonaka, Makiko Osawa, Ichizo Nishino, Yukiko K. Hayashi

PII: S0022-510X(14)00141-5
DOI: doi: 10.1016/j.jns.2014.02.036
Reference: JNS 13075

To appear in: *Journal of the Neurological Sciences*

Received date: 5 December 2013
Revised date: 24 February 2014
Accepted date: 26 February 2014

Please cite this article as: Kajino Sachiko, Ishihara Kayo, Goto Kanako, Ishigaki Keiko, Noguchi Satoru, Nonaka Ikuya, Osawa Makiko, Nishino Ichizo, Hayashi Yukiko K., Congenital fiber type disproportion myopathy caused by *LMNA* mutations, *Journal of the Neurological Sciences* (2014), doi: 10.1016/j.jns.2014.02.036

Congenital fiber type disproportion myopathy caused by *LMNA* mutations

Sachiko Kajino [a,b], Kayo Ishihara [a], Kanako Goto [a], Keiko Ishigaki [b], Satoru Noguchi [a,c], Ikuya Nonaka [a], Makiko Osawa [b] , Ichizo Nishino [a,c] , and Yukiko K Hayashi [a,c,d]

a. Department of Neuromuscular Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry, Tokyo, Japan

b. Department of Pediatrics, Tokyo Women's Medical University, School of Medicine, Tokyo, Japan

c. Department of Clinical Development, Translational Medical Center, National Center of Neurology and Psychiatry, Tokyo, Japan

d. Department of Neurophysiology, Tokyo Medical University, Tokyo, Japan

Corresponding author; Yukiko K. Hayashi, MD, PhD

Mailing address: Department of Neurophysiology Tokyo Medical University, 6-1-1 Shinjuku, Shinjuku-ku, Tokyo 160-8402, JAPAN

Tel.:+81-3-3351-6141, Fax:+81-3-3351-6544

E-mail: yhayashi@tokyo-med.ac.jp

Keywords: LMNA-myopathy, CFTD, fiber type disproportion (FTD), *ACTA1*, *TPM3*, muscular dystrophy, congenital myopathy

Abstract

A boy, who had shown muscle weakness and hypotonia from early childhood and fiber type disproportion (FTD) with no dystrophic changes on muscle biopsy, was initially diagnosed as having congenital fiber type disproportion (CFTD). Subsequently, he developed cardiac conduction blocks. We reconsidered the diagnosis as possible LMNA-myopathy and found a heterozygous mutation in the *LMNA* gene. This encouraged us to search for *LMNA* mutations on 80 patients who met the diagnostic criteria of CFTD with unknown cause. Two patients including the above index case had heterozygous in-frame deletion mutations of c.367_369delAAG and c.99_101delGGA in *LMNA,* respectively. Four of 23 muscular dystrophy patients with *LMNA* mutation also showed fiber type disproportion (FTD). Importantly, all FTD associated with LMNA-myopathy were caused by hypertrophy of type 2 fibers as compared with age-matched controls, whereas CFTD with mutations in *ACTA1* or *TPM3* showed selective type 1 fiber atrophy but no type 2 fiber hypertrophy. Although FTD is not a constant pathological feature of LMNA-myopathy, we should consider the possibility of LMNA-myopathy whenever a diagnosis of CFTD is made and take steps to prevent cardiac insufficiency.

1. Introduction

Mutations in the gene encoding nuclear envelope proteins of A-type lamins (*LMNA*) cause several disorders referred to as laminopathies, which include skeletal and cardiac muscle disorders, lipodystrophy, peripheral neuropathy, and premature aging syndromes. Laminopathies predominantly affecting skeletal muscles (LMNA-myopathy) are clinically classified into three different phenotypes; Emery-Dreifuss muscular dystrophy (AD-, AR-EDMD), limb girdle muscular dystrophy type 1B (LGMD1B), and LMNA-related congenital muscular dystrophy (L-CMD). EDMD has distinctive clinical features including early joint contractures, humero-peroneal muscle weakness and dilated cardiomyopathy with conduction defects. LGMD1B is characterized by proximal muscle involvement and cardiomyopathy with conduction defects, but joint contracture is not prominent. L-CMD is an early onset form showing severe weakness of respiratory and neck muscles from infancy. Serum CK levels in LMNA-myopathy are normal to moderately elevated (2-20 times the upper limit of the normal range). Cardiac involvement, such as conduction blocks, dilated cardiomyopathy and sudden death, usually appears after the second decade of life. To minimize the risk of sudden cardiac death, early diagnosis and appropriate cardiac defibrillator implantation is recommended [1, 2, 3].

Pathologically, LMNA-myopathy is usually characterized by nonspecific dystrophic changes with variation in fiber size, mild necrotic and regenerating processes, and an

increased number of muscle fibers with internalized nuclei. Both type 1 and type 2 fibers are affected. Nuclear abnormalities are common [4]. Interestingly, marked mononuclear cellular infiltrations mimicking inflammatory myopathy can be seen in some patients with the infantile onset form of LMNA-myopathy [5].

We recently experienced a patient with a *LMNA* mutation whose initial diagnosis was congenital fiber type disproportion (CFTD). This patient had shown muscle weakness, hypotonia, and unstable gait from early childhood with no dystrophic changes, but prominent fiber type disproportion (FTD) on his muscle biopsy performed at 4 years of age. At his age of 16 years, he was pointed out to have atrial-ventricular conduction block and incomplete right bundle branch block. We thus reconsidered a possible diagnosis of LMNA-myopathy and identified a mutation in the *LMNA* gene.

CFTD is one of the congenital myopathies pathologically defined by smaller type 1 fibers, by at least 12%, than type 2 fibers without structural abnormalities such as nemaline bodies, cores, and central nuclei. Clinically, CFTD patients show generalized muscle hypotonia and weakness from infancy, multiple joint contractures, scoliosis, long thin face, and high arched palate. Approximately 30% of individuals with CFTD have mild-to-severe respiratory involvement. Cardiac involvement is seen in less than 10% of affected individuals [6, 7]. Six causative genes for CFTD have been identified: *ACTA1* [8], *TPM3* [9], *RYR1* [10], *TPM2* [11], *MYH7* [12] and *SEPN1* [13] encoding α-skeletal actin, α-tropomyosin slow, ryanodine receptor type 1, ß-tropomyosin, slow

ß-myosin heavy chain and selenoprotein N1, respectively.

In this study, we genetically screened CFTD patients for mutations in *LMNA*. We also re-evaluated clinical and pathological findings in patients previously diagnosed as having LMNA-myopathy to ascertain whether these patients have features similar to those of CFTD.

2. Materials and methods

All clinical materials used in this study were obtained for diagnostic purposes with written informed consent. This work was approved by the Ethics Committee of the National Center of Neurology and Psychiatry (NCNP).

2.1. Patients

We examined 80 unrelated muscle biopsies from the NCNP muscle repository. All specimens were from patients who had been diagnosed as having CFTD based on pathological findings as well as clinical features. All cases satisfied the pathological criteria for CFTD; mean type 1 fiber diameter is at least 12% smaller than the mean type 2 fiber diameter, with no structural abnormalities such as nemaline bodies, cores, and increased number of fibers with internal nuclei. In addition, we re-evaluated muscle pathology findings from 23 unrelated patients who had previously been diagnosed as having LMNA-myopathy. We chose genetically confirmed CFTD patients

including 7 with *ACTA1* mutation and 2 with *TPM3* mutation for comparison of clinicopathological features. Clinically, all of the patients including in this study had muscle weakness and/or hypotonia from the preschool years (onset age; <6 years).

2.2 Mutation analysis

Genomic DNA was extracted from peripheral lymphocytes or frozen muscle specimens using standard techniques. For mutation screening of *LMNA*, *ACTA1* and *TPM3*, all exons and their flanking intronic regions were amplified by PCR and directly sequenced using an ABI PRISM 3100 automated sequencer (PE Applied Biosystems, Foster City, CA). Primer sequences are available on request.

2.3 Histochemical analysis of biopsied muscles

Biopsied skeletal muscles were frozen with isopentane cooled in liquid nitrogen. Serial frozen sections, 10 μm in thickness, were stained employing histochemical methods including hematoxylin and eosin (H&E), modified Gomori-trichrome (mGT), NADH-tetrazolium reductase (NADH-TR), and ATPases (pH 10.6, pH 4.6 and pH 4.3). For each muscle specimen, the mean fiber diameter was calculated by obtaining the shortest anteroposterior diameters of 100 type 1 and type 2 (A+B) fibers each using ATPase stains. Fiber size disproportion (FSD) was computed as; difference between type 2 fiber diameter (mean) and type 1 fiber diameter (mean) divided by type 2 fiber

diameter (mean) × 100%. To obtain muscle fiber size information for age-matched controls, a total of 18 muscle specimens with minimal pathological changes from each age were examined.

2.4. Electron microscopic observation

Muscle specimens were fixed with 2% glutaraldehyde in 0.1 M cacodylate buffer. After shaking with a mixture of 4% osmium tetroxide, 1.5% lanthanum nitrate, and 0.2 M s-collidine for 2-3 h, samples were embedded in epoxy resin. Semi-thin sections (1 μm-thickness) were stained with toluidine blue. Ultrathin sections, 50 nm in thickness, were stained with uranyl acetate and lead citrate, and then examined under a tecnai spirit transmission electron microscope (FEI, Japan) at 120 kV.

2.5. Statistical analysis

All data are presented as means ± SD. Comparisons among groups were made using Student's *t* test and analysis of variance (ANOVA). A difference was considered to be statistically significant at a p value less than 0.05.

3. Results

3.1 Mutation analysis

Among the 80 unrelated patients who were diagnosed as having CFTD based on

clinical and pathological findings, a heterozygous *LMNA* mutation was identified in two; a previously reported c.367_369delAAG (p.Lys123del) in Patient 1 and a novel c.99_101delGGA (p.Glu33del) in Patient 2 [14]. *ACTA1* mutations found in the 7 CFTD patients were c.16G>A (p.Glu6Lys), c.142G>T (p.Gly48Cys), c.668T>C (p.Leu223Pro), c.682G>C (p.Glu228Gln), c.981T>A (p.Met326Lys), and c.1000C>T (p.Pro334Ser). Two CFTD patients had the same heterozygous c.502C>T (p.Arg168Cys) mutation in *TPM3.* The novel mutations of *LMNA* c.99_101delGGA (p.Glu33del) and *ACTA1* c.981T>A (p.Met326Lys), were not found in either 100 Japanese control chromosomes or the dbSNP and 1000 Genomes databases.

3.2 Histological findings

Histologically, type 1 fiber predominance (more than 55% of type 1 fibers) and type 2B fiber deficiency (less than 5% of type 2B fibers) were observed in 61% and 28%, respectively, of our 80 CFTD cohort. These results are consistent with those of a previous report [7].

Two patients with *LMNA* mutations showed a marked difference in the sizes of type 1 and type 2 fibers, resulting in FSD of 57% and 13%, respectively (Figure 1). Neither type 1 fiber predominance nor type 2B fiber deficiency was seen (Table 1).

Re-evaluation of genetically confirmed LMNA-myopathy revealed that 4 of 23 patients (17%) had fiber type disproportion (FTD). Their FSD was ranged from 15 to

42%. All 4 patients with FTD also showed some necrotic and/or regenerating fibers in their muscle biopsy and had a diagnosed of muscular dystrophy. These 4 patients with FTD had 3 different mutations. Two mutations of c.1583C>A (p.Thr528Lys) and c.1357C>T (p.Arg453Trp) have already been reported [15, 16], whereas the c.907T>C (p.Ser303Pro) mutation was not reported previously. These mutations were distributed in both central rod and tail domains, but not in the head domain (Table 1).

To clarify whether LMNA-myopathy patients with FTD have specific pathological findings different from those affecting CFTD muscles with known gene mutations, we carefully re-evaluated the muscle pathologies of the 6 LMNA-myopathy patients with FTD, 7 CFTD patients with *ACTA1* mutations, and 2 CFTD patients with *TPM3* mutations. FSD in LMNA-myopathy with FTD, and in CFTD with *ACTA1* and *TPM3* mutations were calculated to be 27.8 ± 17.9% (mean ± SD), 37.7 ± 12.1%, and 54.1 ± 13.1%, respectively. No significant differences were seen in FSD among the 3 groups. We also compared fiber sizes among LMNA-myopathy with FTD, CFTD with *ACTA1* or *TPM3* mutations and age-matched controls. Surprisingly, CFTD with *ACTA1* and *TPM3* mutations showed type 1 fiber atrophy, whereas LMNA-myopathy with FTD showed type 2 fiber hypertrophy with lack of type 1 fiber atrophy (Figure 2).

In this study, type 1 fiber predominance was seen in 86% of CFTD patients with *ACTA1* mutations and in 100% of those with *TPM3* mutations, but in only 33% of LMNA-myopathy patients with FTD. The percentage of type 1 fibers in

LMNA-myopathy was calculated to be 44.6 ± 12.8 (mean ± SD), which was significantly lower than that in CFTD with *ACTA1* mutations (64.1 ± 7.1%) and that with *TPM3* mutations (57.0 ± 1.4%) ($p < 0.05$). Type 2B fiber deficiency was not seen in LMNA-myopathy with FTD (Table 1, 3), whereas 4 of 7 (57%) patients with *ACTA1* mutations and one (50%) with *TPM3* mutation showed type 2B fiber deficiency.

On electron microscopic (EM) observations, nuclear changes are important pathological findings in skeletal muscles of LMNA-myopathy [4]. We examined the nuclear changes in Patients 2, 4 and 5 on EM, and found a few myonuclei showing abnormal shapes and chromatin disorganization (Figure 3). Smaller nuclei arranged in a row, giving the appearance of a 'nuclear chain', were also seen (data not shown). However, nuclear abnormalities in patients who had LMNA-myopathy with FTD were milder and less frequent than previously reported for AD-EDMD and LGMD1B muscles [4].

3.3 Clinical findings

Table 2 summarizes the characteristics of the 6 LMNA-myopathy patients with FTD. Patients 1 and 2 were initially diagnosed as having CFTD, and the 4 remaining patients (patients 3 to 6) showed FTD together with dystrophic changes on muscle pathology. All patients had normal antenatal courses and uneventful births. All patients had started walking without delay, but showed a waddling gait and muscle

weakness and/or hypotonia from the preschool years. None had a high arched palate or respiratory dysfunction. Four of the 6 (67%) patients had contractures of the ankles and/or elbows which had not been present at birth but appeared with age. Serum creatine kinase (CK) was mildly elevated in all patients.

Sixteen of the 78 (21%) CFTD patients with unknown cause had high CK levels (>200 IU/l), and four of these 16 showed a high arched palate and respiratory involvement.

4. Discussion

FTD can be seen in a single muscle biopsy from patients with several diseases including congenital myotonic dystrophy and centronuclear myopathy [17-20]. Here we identified 2 LMNA-myopathy among patients diagnosed as CFTD. We also found FTD in 17% of muscular dystrophy patients with LMNA mutations. These results suggest that FTD may not be rare in LMNA-myopathy. None of these patients had either a high arched palate or respiratory insufficiency, and serum CK levels were mildly elevated. Pathologically, FTD in LMNA-myopathy is associated with type 2 fiber hypertrophy with lack of type 1 fiber atrophy, whereas type 1 fiber atrophy is seen in CFTD with *ACTA1* or *TPM3* mutations. Unlike CFTD due to *ACTA1* or *TPM3* mutations, type 1 fiber predominance and type 2B fiber deficiency are absent in LMNA-myopathy. These results suggest that *LMNA* analysis should be performed in CFTD patients who has the clinical features such as no high arched palate, no respiratory insufficiency and

high CKemia, and has pathological features such as type 2 fiber hypertrophy and lack of type 1 fiber atrophy, type 1 fiber predominance, and type 2B fiber deficiency.

LMNA-myopathy is categorized as muscular dystrophy, and mild necrotic and regenerating processes are usually seen. However, no dystrophic features can be seen as reported herein. Higher CK levels raise the possibility of LMNA-myopathy being dystrophic in nature. On the other hand, in our series, 16 of the 78 (21%) CFTD patients with unknown cause had high CKemia. This result suggests a difficulty in making a differential diagnosis between congenital myopathy and muscular dystrophy in some cases.

Clinically, respiratory insufficiency is common, reportedly being seen in 30% of CFTD patients [7], and in 73% of L-CMD patients [4]. However, 2 CFTD patients with *LMNA* mutations in this study showed no respiratory involvement. Furthermore, in CFTD associated with *LMNA* mutations, FTD is the only pathological abnormality, while prominent dystrophic and/or inflammatory changes are seen in L-CMD. There results suggest that CFTD is the milder form of early onset LMNA-myopathy.

Acknowledgments

This study was supported partly by Research on Intractable Diseases, Comprehensive Research on Disability Health and Welfare, and Applying Health Technology from the Ministry of Health Labour and Welfare; partly by Intramural

Research Grant 23-5 for Neurological and Psychiatric Disorders of NCNP; and partly by JSPS KAKENHI Grant Number of 12839666 and 12929543.

References

[1] Emery AE. Emery-Dreifuss muscular dystrophy - a 40 year retrospective. Neuromuscular Disord 2000; 10: 228-232

[2] Muchir A, Bonne G, van der Kooi AJ, et al. Identification of mutations in the gene encoding lamins A/C in autosomal dominant limb girdle muscular dystrophy with atrioventricular conduction disturbances (LGMD1B). Hum Mol Genet 2000; 9: 1453-1459

[3] Quijano-Roy S, Mbieleu B, Bönnemann CG, et al. De novo LMNA mutations cause a new form of congenital muscular dystrophy. Ann Neurol 2008; 64: 177-186

[4] Park YE, Hayashi YK, Goto K, et al. Nuclear changes in skeletal muscle extend to satellite cells in autosomal dominant Emery-Dreifuss muscular dystrophy/limb-girdle muscular dystrophy 1B. Neuromuscular Disord 2009; 19: 29-36

[5] Komaki H, Hayashi YK, Tuburaya R, et al. Inflammatory changes in infantile-onset LMNA-associated myopathy. Neuromuscular Disord 2011; 21: 563-568

[6] Banwell BL, Becker LE, Jay V, et al. Cardiac manifestations of congenital fiber-type disproportion myopathy. J Child Neurol 1999; 14: 83-87

[7] Clarke NF, North KN. Congenital fiber type disproportion - 30 years on. J Neuropathol Exp Neurol 2003; 62: 977-989

[8] Laing NG, Clarke NF, Dye DE, et al. Actin mutations are one cause of congenital fiber type disproportion. Ann Neurol 2004; 56: 689-694

[9] Clarke NF, Kolski H, Dye DE, et al. Mutations in TPM3 are a common cause of congenital fiber type disproportion. Ann Neurol 2008; 63: 329-337

[10] Clarke NF, Waddell LB, Cooper ST, et al. Recessive mutations in RYR1 are a common cause of congenital fiber type disproportion. Hum Mutat 2010; 31: E1544-50

[11] Clarke NF, Waddell LB, Sie LT, et al. Mutation in TPM2 and congenital fiber type disproportion. Neuromuscular Disord 2012; 22: 955-8

[12] Ortolano S, Tarrio R, Blanco-Arias P, et al. A novel MYH7 mutation links congenital fiber type disproportion and myosin storage myopathy. Neuromuscular Disord. 2011; 21: 254-62

[13] Clarke NF, Kidson W, Quijano-Roy S, et al. SEPN1: associated with congenital fiber-type disproportion and insulin resistance. Ann Neurol 2006; 59: 546-552

[14] Keller H, Finsterer J, Steger C, et al. Novel c.367_369del LMNA mutation manifesting as severe arrythmias, dilated cardiomyopathy, and myopathy. Heart Lung 2012; 41: 382-386

[15] Bonne G, Mercuri E, Muchir A, et al. Clinical and molecular genetic spectrum of autosomal dominant Emery-Dreifuss muscular dystrophy due to mutations of lamin A/C gene. Ann Neurol 2000; 48 : 170-80

[16] Brown CA, Lanning RW, McKinney KQ, et al. Novel and recurrent mutation in lamin A/C in patients with Emery-Dreifuss muscular dystrophy. Am J Med Genet 2001; 102: 359-67

[17] Tominaga K, Hayashi YK, Goto K, et al. Congenital myotonic dystrophy can show congenital fiber type disproportion pathology. Acta Neuropatholog 2010 ;119: 481-6

[18] Danon MJ, Giometti CS, Manaligod JR, Swisher C. Sequential muscle biopsy changes in a case of congenital myopathy. Muscle Nerve. 1997; 20: 561-9

[19] Shishikura K, Osawa M, Suzuki H, et al. Clinical variability of congenital myopathy with type 1 fiber atrophy: a long-term observation of three cases. Acta Paediatr Jpn. 1994; 36: 186-93

[20] Okamoto N, Toribe Y, Nakajima T, et al. A girl with 1p36 deletion syndrome and congenital fiber type disproportion myopathy. J Hum Genet. 2002; 47: 556-9