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A rare mutation in MYH7 gene occurs with overlapping phenotype

Lucia Ruggiero [a, *], Chiara Fiorillo [b], Sara Gibertini [c], Francesco De Stefano [d], Fiore Manganelli [a], Rosa Iodice [a], Floriana Vitale [a], Simona Zanotti [c], Maurizio Galderisi [d], Marina Mora [c], Lucio Santoro [a]

[a] Department of Neurosciences, Reproductive and Odontostomatological Sciences, University Federico II of Naples, Via Sergio Pansini 5, 80131 Naples, Italy
[b] Laboratory of Molecular Medicine and Neuromuscolar Disorder IRCCS Stella Maris Calambrone, Pisa, Italy
[c] IRCCS Foundation Neurological Institute, Isituto C. Besta, Via Giovanni Celoria 11, 20133 Milan, Italy
[d] Department of Medical Translational Sciences, University Federico II of Naples, Via Sergio Pansini 5, 80131 Naples, Italy

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ABSTRACT

Mutations in the beta-myosin heavy chain gene (MYH7) cause different muscle disorders. The specific molecular pathobiological processes that cause these different phenotypes remains unexplained. We describe three members of a family with an autosomal dominant mutation in the distal rod of MYH7 [c.5401G> A (p.Glu1801Lys)] displaying a complex phenotype characterized by Laing Distal Myopathy like phenotype, left ventricular non compaction cardiomyopathy and Fiber Type Disproportion picture at muscle biopsy. We suggest that this overlapping presentation confirm the phenotypic variability of *MYH7* myopathy and may be helpful to improve the genotype phenotype correlation.

1. Introduction

Myosin 7 gene (*MYH7*) encodes slow/beta-cardiac myosin heavy chain (MHC-β), a class II myosin expressed primarily in the heart, but also in skeletal muscles and in particular in type I fibers [1]. *MYH7* gene mutations are known to cause three different muscle disorders: Laing Distal Myopathy (LDM), Myosin Storage Myopathy (MSM) and Familial Hypertrophic Cardiomyopathy (FHCM) [2–4]. Clinical phenotypes have been related to the site of mutations; in fact, mutations in the globular head of the protein have been linked to FHCM, mutations in distal rod to MSM, whereas mutations in middle or proximal rod cause LDM [5].

Very recently mutations in distal rod of protein have been associated with a form of Congenital Myopathy with Fiber Type Disproportion (CFTD) [6]. In CFTD cases, the majority of patients have congenital onset and present a LDM phenotype or MSM phenotype without specific cardiac impairment [7,8]. In addition, several families with mutations in the *MYH7 gene* have been described presenting exclusively a heart impairment, consisting in ventricular non compaction (LVNC). LVNC is detected by an echocardiographic examination: the apical region of the heart shows wide trabeculae of the muscular tissue, separated by lacunae. This abnormality leads to contractile dysfunction, as well as arrhythmias and embolic complications, due to the blood stasis within the lacunae [9].

The specific molecular pathobiological processes that cause these different phenotypes remains unexplained [10].

We describe three members of an Italian family with a mutation of the *MYH7* gene c.5401G> A (p.Glu1801Lys) in the distal rod of the protein. This mutation is for the first time described in an italian family whose clinical presentation includes non compaction cardiomyopathy, LDM like phenotype and FTD picture at muscle biopsy. We speculate that the complex phenotype of the family confirms the wide phenotypic variability of MYH7 mutations,

Abbreviations: ACTA1, actin 1; CPK, creatine phosphokinase; CFTD, Congenital Fiber Type Disproportion; COX, cytochrome C oxidase; *DES*, desmin; EMG, electromyography; FHCM, Familial Hypertrophic Cardiomyopathy; IF, immunofluorescence; *ITGA7*, integrin 7; LDM, Laing Distal Myopathy; *LMNA*, lamin; LVNC, left ventricular non compaction; MHC-β, slow/beta-cardiac myosin heavy chain; MSM, Myosin Storage Myopathy; *MYH7*, *Myosin 7* gene; NADH, nicotinamide adenine dinucleotide dehydrogenase; PM, pacemaker; SDH, succinate dehydrogenase.

* Corresponding author. Fax: +39 0817462667.
E-mail addresses: ruggilucia@gmail.com (L. Ruggiero), Chi.fiorillo@gmail.com (C. Fiorillo), Sara.Gibertini@istituto-besta.it (S. Gibertini), fradestefano@alice.it (F. De Stefano), fioremanganelli@gmail.com (F. Manganelli), rosaiodice81@gmail.com (R. Iodice), florisea@tin.it (F. Vitale), simona.zanotti@istituto-besta.it (S. Zanotti), mgalderi@unina.it (M. Galderisi), mmora@istituto-besta.it (M. Mora), lusantor@unina.it (L. Santoro).

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which can not be fully explained by mutation sites, and may be helpful to improve the genotype phenotype correlation.

2. Materials and methods

We investigated three members of a family from southern Italy (Fig. 1). The evaluation of patients included neurological examination, blood tests, electrophysiological study, cardiac assessment, muscle biopsy and molecular analysis.

2.1. Cardiac evaluation

Patients underwent a comprehensive cardiac evaluation, including physical examination, search for cardiac symptoms (such as fatigue, dyspnoe and exercise intolerance), a standard 12-lead electrocardiogram and a bidimensional echocardiogram with speckle-tracking analysis of longitudinal myocardial contractile function, as well as a real-time 3D echocardiography. This technique is the only one able to measure the contractile function of the three different layers of myocardium: subendocardial (value of longitudinal strain), midwall (circumferential strain) and subepicardial fibers (radial strain).

2.2. Muscle biopsy

Two patients (case II2 and case III1) had an open biopsy of biceps brachii muscle and specimens were frozen in isopentane cooled in liquid nitrogen. Unfixed cryostat sections (10 μm) were stained with a panel of histological, histochemical and immunohistological techniques according to standard procedures [11].

2.3. Molecular analyses

Genomic DNA was extracted by standard methods from peripheral blood lymphocytes. For mutation screening, primers flanking the intron-exon junctions of each *MYH7* exon, the 5′ and 3′ UTR, were designed for the genes *MYH7* based on published sequences (GenBank accession number: *MYH7* NM_000257.3). PCRs were performed with Mega Mix Double (Microzone, Haywards Heath, West Sussex, UK). The products were purified using microCLEAN (Microzone, Haywards Heath, West Sussex, UK) and sequenced directly with the BigDye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems, CA, USA). Sequences were analyzed on ABI Prism 3100 Genetic Analyzer (Applied Biosystems, CA, USA).

3. Results

Case 1 (II2): is a 65 year old man, who reported, at the age of 25 years, sudden onset of symptoms with shortness of breath during exertion and orthopnea. A subsequent echocardiographic assessment revealed the presence of a cardiomyopathy, characterized by incomplete compaction of myocardium (Fig. 2) for which the patient started a specific pharmacological treatment. At the age of 60 years, during the routine cardiac follow up, heart rhythm abnormalities were detected and the patient underwent a pacemaker (PM) implantation. Neurological deficits had appeared in the third decade with waddling gait and foot steppage. Over the years, the clinical picture has been slowly progressive and currently the patient requires a support to climb the stairs and to ambulate. Last neurological evaluation showed waddling gait, foot drop (right > left), inability to stand on the heels and tips, hyperlordosis and positive Gowers manouver. In addition, chest muscles, neck flexor, triceps and biceps muscles showed a moderate hypotrophy and weakness, which were marked for the muscles of the shoulder girdle. At lower limbs weakness and hypotrophy were severe in distal muscles and moderate in proximal muscles (quadriceps, iliopsoas and hamstring muscles). Tendon reflex were absent. Creatine phosphokinase (CPK) was increased to 400–500 UI/L [normal value 180 UI/L], electromyography (EMG) showed myopathic changes while the nerve conduction study was within normal limits. For the presence of PM the muscle MRI was not carried out.

A muscle biopsy, performed at the age of 43 years, revealed two separate populations of fibers: hypotrophic and rounded type 1 fibers and normal type 2 fibers. Type 1 fibers were clearly more numerous than type 2 (nearly 80% of all fibers) and their average diameter was 33.8 +/−9.1 μ, while the average diameter of type 2 fibers was 62.5 +/−13.3 μ. Mild increase of nuclear centralizations and splitting phenomena were also found (Fig. 3). COX, SDH, NADH stainings were normal and no accumulation of glycogen and lipids was observed. Immunofluorescence (IF) showed normal expression of dystrophin, sarcoglycans, caveolin-3, dysferlin and merosin. Since the histological findings were consistent with a FTD,

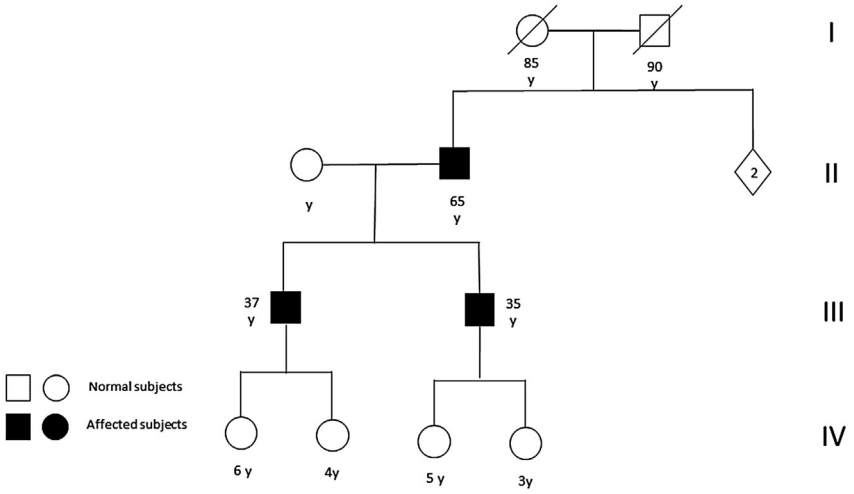

Fig. 1. Family tree Complete pedigree of family with heterozygous mutation p.Glu 1801Lys in *MHY 7 gene*. Squares represent males; circles represent females; blank symbols represent normal subjects; black filled symbols represent affected subjects.

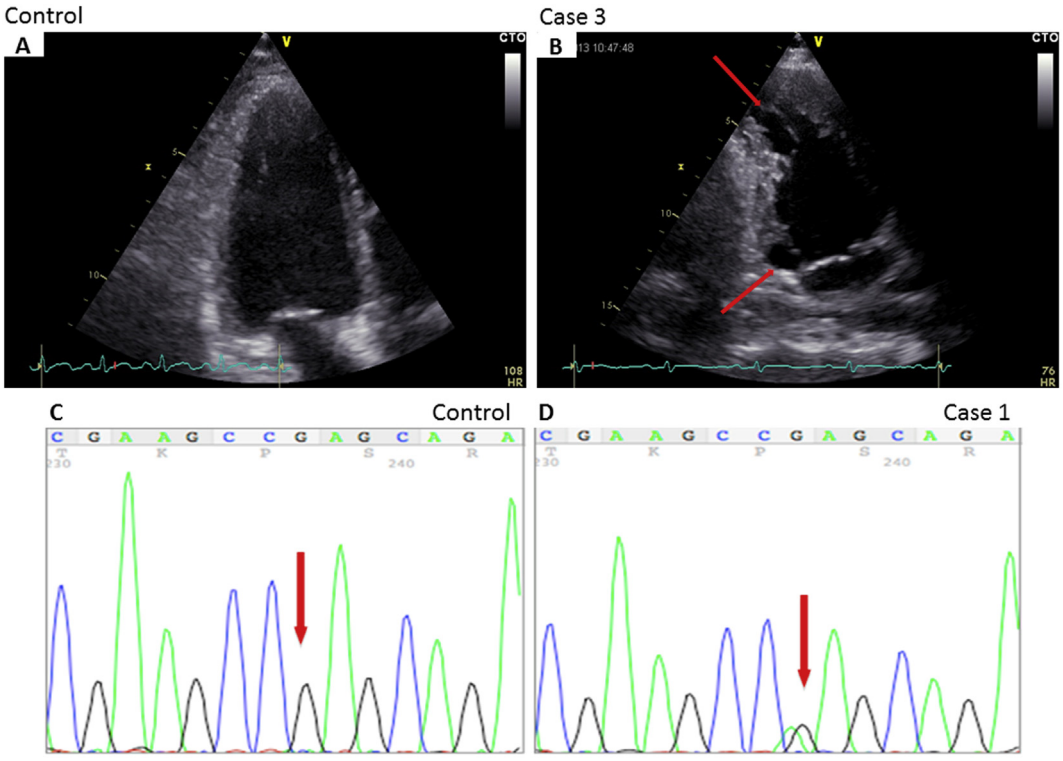

Fig. 2. Echocardiography and genetic analysis (A–B) 2D-echocardiography of control (A) and patient 1 (B). A non- compacted endocardial layer, with wide trabeculations and lacunae dividing the muscular tissue is evident especially in the midventricular and apical zones (red arrows in B). (C–D) Electropherograms of the *MYH7* sequence in control (C) and patient 1 (D). The mutation c.5401G>A is highlighted by arrow in D. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

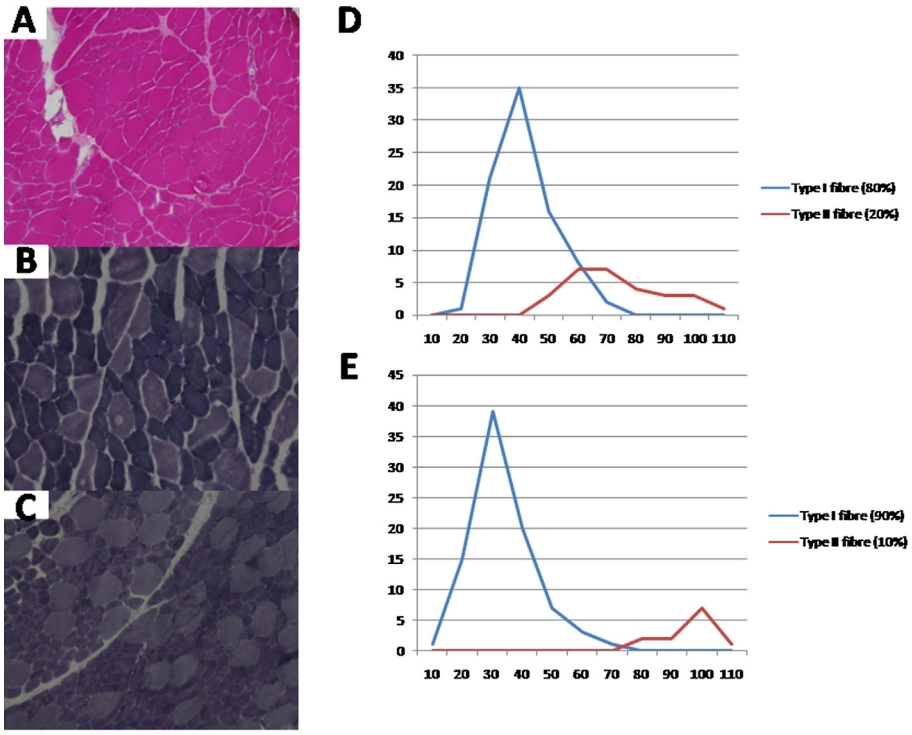

Fig. 3. Muscle biopsy (A) HE, (B and C) NADH stained muscle biopsies from Case 1 (A and B) and Case 2 (C) showing consistent fiber size disproportion between type 1 fibers (dark in B and C) and type 2 fibers (pale in B and C). (D and E) distribution graphs of the number and diameter of the fibers for case 1 (D) and case 2 (E).

molecular analysis for *ACTA1* and *ITGA7* was performed and resulted negative. In consideration of the cardiac phenotype molecular analysis for *DES*, *LMNA* and *MYH7* genes were carried out and resulted positive for a heterozygous mutation, c.5401*G>A* (p.Glu1801Lys), in the *MHY7* gene (Fig. 2). No mutation were found in *DES* and *LMNA*.

Case 2 (III1): a 37 years old man, eldest son of case 1, presented by the age of 17–18 years with gait problems such as waddling and steppage; he was able to stay on the toes but no on heels. The patient got worse over the years and at last neurological evaluation he had a hyperlordotic posture with flared abdomen and needed support to climb stairs and get up from a chair. Gowers sign was positive. The patient showed marked hypotrophy and weakness of the shoulder girdle muscles and mild weakness of the neck flexors. In the upper limb muscles the strength was normal. At lower limbs there was a severe bilateral weakness of the tibialis anterior muscles, and marked weakness of the iliopsoas and quadriceps. Reflexes and sensation were normal. The CPK was moderately elevated (about 500 UI/L). For the presence of PM the muscle MRI was not carried out. At the age of 18 years an electromyography showed mild myopathic changes in all explored muscles with normal conduction study. At that time a muscle biopsy (biceps brachii muscle) was performed and showed a FTD picture, very similar to that of father (case 1). Type 1 fibers were clearly prevalent (about 90%) and their average diameter was of 22.6 +/− 6.45 μ while the average diameter of type 2 fibers was 65.9 +/− 13.8 μ (Fig. 3). Molecular analysis confirmed the presence of the c.5401*G>A MHY7* mutation. Over the years the echocardiographic assessment showed diagnostic features of a not compacted myocardium (Fig. 2). Analogously to patient II2 it was necessary to implant a PM for the appearance of significant electrocardiographic abnormalities, such as first degree atrioventricular block, left bundle branch block, and ventricular extrasystoles.

Case 3 (III2): a 35 year old man, the second son of case 1, showed onset of symptoms at the age of 18 years with gait difficulty, such as waddling and steppage. As for the affected relatives the clinical picture has been slowly evolving and at last neurological evaluation he had difficulty to climb stairs and to get up from a chair. Gowers manouver was positive. A slight weakness of the shoulder girdle muscles, pectoral muscles moderate wasting and minimal weakness of the neck flexor muscles, were present. Muscle strength and mass were normal at upper limb. At lower limbs there was weakness of tibialis anterior and iliopsoas muscles. However, muscle involvement was less severe, compared to the relatives. As in case 2 reflexes and sensitivity were normal and the CPK was moderately increased (about 500 UI/L). The cardiac evaluation showed a non-compact myocardium (Fig. 2) without clinical and electrocardiographic relevant abnormalities. The patient refused muscle biopsy, electrophysiological study and muscle MRI. Genetic tests detected the same *MYH7* mutation already reported in the family (case 1 and 2).

4. Discussion

The clinical spectrum of phenotypes associated with *MYH7 gene* mutations can be very wide and includes LDM, MSM and FHCM. The phenotype variability has been interpreted as depending from gene mutation site. We identified an Italian family with a mutation of the *MYH7* gene sited in the distal rod of the protein, which presents with a very complex overlapping syndrome characterized by non compaction cardiomyopathy, LDM like phenotype with early and markedly proximal involvement and FTD picture at muscle biopsy. The mutation c.5401G > A (p.Glu1801Lys) has never been described in an Italian family whereas has been reported by Dubourg O et al. in two families, one from France and another from Norway, and in a Finnish sporadic case [12]. All the affected patients have similar clinical pictures, compatible with LDM phenotype. The muscle biopsy was obtained only from three patients and showed different findings. The French proband (age 12 years) showed variation of fiber size and fiber atrophy (more type 1 than type 2 fibers), rarely necrosis and regeneration, excess of internalized nuclei, nuclear clump fibers, and few rimmed vacuolated fibers. The Finnish patient (age 17 years) showed a CFTD picture without rimmed vacuoles or others specific pathological changes. In the Norwegian proband, there were core and multiple mini-core changes together with type 1 atrophy and a couple of subtle rimmed vacuoles.

Interesting, one of the patients showed FTD similar to our cases. Overall, the presence of FTD with mutation of *MYH7* gene has been reported several times before but the frequency of the association is not clear [6]. The FTD muscle picture has been reported associated with both LDM [7] and, less frequently, with MSM phenotype [8], but in all cases the disease onset was congenital or in childhood [13]. Differently, in our family muscle symptoms developed in adulthood. We think it is useful to stress here this clinical aspect as previously we reported in *LMNA* gene mutations [14]. Another interesting aspect is that, our patients have an early impairment of both proximal and distal muscles with sparing of finger and wrist extensor muscles. This clinical figure could be considered an overlapping phenotype between MSM and LDM. A possible explanation could be the location of mutation in the exon 37, a border region between middle rod associated with LDM and the distal rod associated with the MSM.

The association of cardiac and skeletal muscle involvement has often been reported in *MYH7* mutations [10]. However the specific LVNC has always been described in absence of skeletal muscle impairment [9]. Therefore, the association of LVNC and muscle involvement is another unique aspect of present family. In addition, at the best of our knowledge, this is the first report with FTD at muscle biopsy and confirmed cardiac impairment.

Overall FTD, LVNC and LDM have been previously described in *MYH7* mutations but the present family is peculiar for the contemporary presence of all these three clinical aspects. Our report confirms the possibility to observe very complex phenotypes associated with *MYH7* mutations. The variability of phenotypes, even in presence of identical mutations suggest that genotype–-phenotype correlations can be partially explained by mutation sites on the gene. Epigenetic factors have probably an important role and further studies are needed.

Informed consent

Written informed consent was obtained from all patients for the publication of this case report and any accompanying images.

Conflict of interest

All the authors disclose any conflicts of interest including any financial (grant or fundings), personal or other relationships with other people or organizations within three years of beginning the work submitted that could inappropriately influence or bias their work.

References

[1] T. Jaenicke, K.W. Diederich, W. Haas, J. Schleich, P. Lichter, M. Pfordt, A. Bach, H.P. Vosberg, The complete sequence of the human beta-myosin heavy chain

gene and a comparative analysis of its product, Genomics 8 (2) (1990) 194–206.
[2] C. Meredith, R. Herrmann, C. Parry, K. Liyanage, D.E. Dye, H.J. Durling, R.M. Duff, K. Beckman, M. de Visser, M.M. van der Graaff, P. Hedera, J.K. Fink, E.M. Petty, P. Lamont, V. Fabian, L. Bridges, T. Voit, F.L. Mastaglia, N.G. Laing, Mutations in the slow skeletal muscle fiber myosin heavy chain gene (MYH7) cause laing early-onsetdistal myopathy (MPD1), Am. J. Hum. Genet. 75 (4) (2004) 703–708.
[3] H.1 Tajsharghi, L.E. Thornell, C. Lindberg, B. Lindvall, K.G. Henriksson, A. Oldfors, Myosin storage myopathy associated with a heterozygous missense mutation in MYH7, Ann. Neurol. 54 (4) (2003) 494–500.
[4] R. Walsh, C. Rutland, R. Thomas, S. Loughna, Cardiomyopathy: a systematic review of disease-causing mutations in myosin heavy chain 7 and their phenotypic manifestations, Cardiology 115 (1) (2010) 49–60.
[5] N.F. Clarke, K. Amburgey, J. Teener, S. Camelo-Piragua, A. Kesari, J. Punetha, L.B. Waddell, M. Davis, N.G. Laing, N. Monnier, K.N. North, E.P. Hoffman, J.J. Dowling, A novel mutation expands the genetic and clinical spectrum of MYH7-related myopathies, Neuromuscul. Disord. 23 (5) (2013) 432–436.
[6] E.T. DeChene, P.B. Kang, A.H. Beggs, Congenital fiber-type disproportion, GeneReviews 11 (2013).
[7] N. Muelas, P. Hackman, H. Luque, M. Garcés-Sánchez, I. Azorín, T. Suominen, T. Sevilla, F. Mayordomo, L. Gómez, P. Martí, J. María Millán, B. Udd, J.J. Vílchez, MYH7 gene tail mutation causing myopathic profiles beyond Laing distal myopathy, Neurology 75 (8) (2010) 732–741.
[8] S. Ortolano, R. Tarrío, P. Blanco-Arias, S. Teijeira, F. Rodríguez-Trelles, M. García-Murias, V. Delague, N. Lévy, J.M. Fernández, B. Quintáns, B.S. Millán, A. Carracedo, C. Navarro, M.J. Sobrido, A novel MYH7mutation links congenital fiber type disproportion and myosin storage myopathy, Neuromuscul. Disord. 21 (4) (2011) 254–262.
[9] S. Klaassen, S. Probst, E. Oechslin, B. Gerull, G. Krings, P. Schuler, M. Greutmann, D. Hürlimann, M. Yegitbasi, L. Pons, M. Gramlich, J.D. Drenckhahn, A. Heuser, F. Berger, R. Jenni, L. Thierfelder, Mutations in sarcomere protein genes in left ventricular non compaction, Circulation 117 (22) (2008) 2893–2901.
[10] Lamont PJ, W. Wallefeld, D. Hilton-Jones, B. Udd, Z. Argov, A.C. Barboi, C. Bonneman, K.M. Boycott, K. Bushby, A.M. Connolly, N. Davies, A.H. Beggs, G.F. Cox, J. Dastgir, E.T. DeChene, R. Gooding, H. Jungbluth, N. Muelas, J. Palmio, S. Penttilä, E. Schmedding, T. Suominen, V. Straub, C. Staples, P.Y. Van den Bergh, J.J. Vilchez, K.R. Wagner, P.G. Wheeler, E. Wraige, N.G. Laing, Novel mutations widen the phenotypic spectrum of slow skeletal/β-cardiac myosin (MYH7) distal myopathy, Hum. Mutat. 35 (7) (2014) 868–879.
[11] V. Dubowitz, Muscle Biopsy. A Practical Approach, BaillieÁre Tindall, London, 1985.
[12] O. Dubourg, T. Maisonobe, A. Behin, T. Suominen, O. Raheem, S. Penttilä, M. Parton, B. Eymard, A. Dahl, B. Udd, A novel MYH7 mutation occurring independently in French and Norwegian Laing distal myopathy families and de novo in one Finnish patient, J. Neurol. 258 (6) (2011) 1157–1163.
[13] N.F. Clarke, Congenital fiber type disproportion—a syndrome at the crossroads of the congenital myopathies, Neuromuscul. Disord. 21 (4) (2011) 252–253.
[14] Ruggiero L1, C. Fiorillo, A. Tessa, F. Manganelli, R. Iodice, R. Dubbioso, F. Vitale, E. Storti, E. Soscia, F. Santorelli, L. Santoro, Muscle fiber type disproportion (FTD) in a family with mutations in the LMNA gene, Muscle Nerve (2014).