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ORIGINAL ARTICLES

Familial cardiomyopathy caused by a novel heterozygous mutation in the gene *LMNA* (c.1434dupG): a cardiac MRI-augmented segregation study

Mashael Alfarih[1 2 3], Petros Syrris[2], Eloisa Arbustini[4], João B. Augusto[1 2], Alun Hughes[2 5], Guy Lloyd[1 2], Luis R. Lopes[1 2], James C. Moon[1 2], Saidi Mohiddin[1] and Gabriella Captur[1 2 5 6]

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In a five-generation family carrying a novel frameshift *LMNA* variant (c.1434dupG, p.Leu479AlafsX72), imaging-augmented segregation analysis supports its association with lamin heart disease. Affected members exhibit conduction abnormalities, supraventricular and ventricular arrythmias, dilated cardiomyopathy with non-infarct pattern midwall septal fibrosis, heart failure and thromboembolic complications.

Key words: familial dilated cardiomyopathy, lamin A/C, cardiolaminopathies

Introduction

Lamin A/C are structural intermediate filaments encoded by the *LMNA* gene. *LMNA* gene mutations are responsible for various multi-system laminopathies including lamin heart disease (LHD) which is characterized by cardiac conduction system disease (CCD), dilated cardiomyopathy (DCM), heart failure, malignant ventricular arrythmias (VA) and sudden cardiac death (1). Given the high arrhythmogenic risk, early recognition and intervention by implantable cardioverter defibrillator (ICD) can be life-saving.

Defining pathogenicity of novel *LMNA* variants remains a challenge, but long-term surveillance of mutation-positive families permits segregation studies that are essential contributors to the validation of pathogenic mutations. Advanced tissue characterisation by cardiovascular magnetic resonance (CMR) may help clinicians better understand the potential pathogenicity of variants, especially when segregation studies include family members with borderline/subclinical phenotypes by other imaging modalities.

Here we describe the deep clinical phenotypes associated with a previously unreported *LMNA* variant: c.1434dupG.

Case presentation

We report a family in which all affected members with cardiomyopathy (Fig. 1, left panel) carry the heterozygous *LMNA* variant, c.1434dupG (Tab. 1). The variant is predicted to disrupt protein reading frame creating a premature termination codon confirmed by Alamut® software. As variant pathogenicity was unknown we performed an imaging-assisted segregation study, demonstrating a link between variant and LHD.

Within this five-generation family, four members are confirmed carriers of the variant by DNA testing, expressing a clinical phenotype that includes DCM, myocardial septal fibrosis [anecdotally found in 88% of *LMNA* gene mutation carriers (2)], supraventricular and ventricular arrhythmias, CCD, heart failure and throm-

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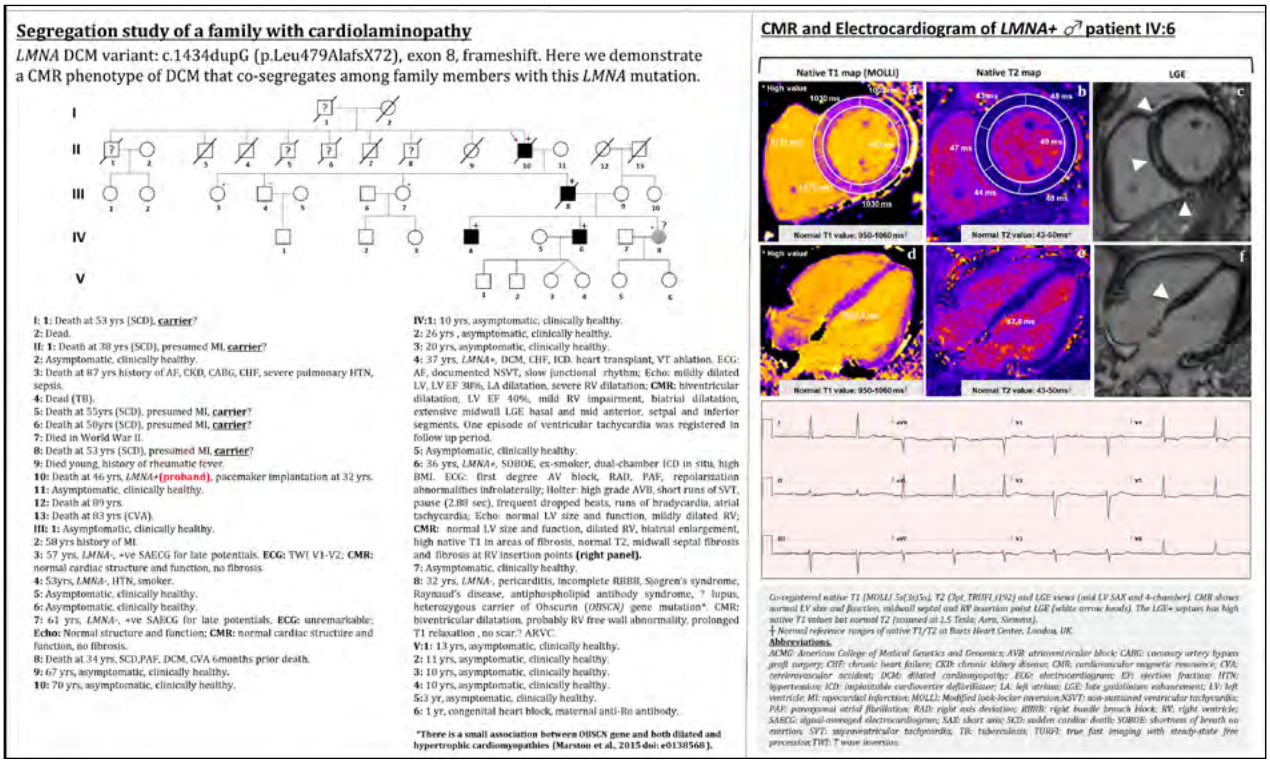

Figure 1. CMR-assisted segregation study for a family with novel LMNA frameshift variant c.1434dupG, p.Leu479AlafsX72 [Classification by ClinVar "not provided"; ACMG: "pathogenic (Ia)"; gnomAD: "absent"].

Table 1. Novel *LMNA* genetic variant summary.

Variant	c.1434dupG, p.Leu479AlafsX72
Mutation status	Autosomal dominant, heterozygous
Variant type	Frameshift (truncation predicting mutation)
Molecular consequence	NM_170707: c.1434dupG: loss-of-function variant
Genomic location	Chr 1: 156,114,707-156,140,089
Variant location	Exon 8: Single nucleotide duplication, premature termination codon; downstream of the nuclear localization signal (NLS, exon 7) but upstream of the C-terminal tail* ** Recent data suggests that there is an association between more adverse cardiac phenotype and LMNA mutations upstream of the NLS or upstream of the tail (Captur et al., 2018. Doi: 10.1136/openhrt-2018-000915).*
Phenotypic group	Dilated cardiomyopathy-conduction disease (DCM-CD)
GnomAD database	Absent
ClinVar clinical significance	Not reported
ACMG assertion of pathogenicity	Pathogenic (Ia) *Using American College of Medical Genetics and Genomics/Association for Molecular Pathology (ACMG/AMP) criteria: the variant fulfills PVS1, PP1, PM2 and PP4 (1 x very strong, 1x moderate and 2 x supporting) implying pathogenicity.*
Type of analysis	Direct fluorescent DNA sequencing for exon 8

boembolic complications (Tab. 2). There was a history of sudden death in seven members, interestingly all males [male gender is an adverse prognostic marker in LHD (3)]: proband II-10 died suddenly in his 40s likely from paternal inheritance of the variant as his father (I-1) died abruptly in his 50s (no genetic data available for

Table 2. Family study-disease progression and results of electrocardiogram, 24-hour Holter and CMR.

Family code	Sex	Genetic status	Adverse events					ECG/holter					CMR
			mVA	SCD	CHF	CVA	DCM	Brady	SVT	AVB	A.Fib/F	PAC/ PVC	LGE
I-1	M	NK(?)		+									
II-1	M	NK(?)		+									
II-5	M	NK(?)		+									
II-6	M	NK(?)		+									
II-8	M	NK(?)		+									
II-10	M	LMNA+		+									
III-3	F	LMNA–	–	–	–	–	–	–	–	–	–	–	–
III-4	M	LMNA–	–	–	–	–	–	–	–	–	–	–	–
III-7	F	LMNA–	–	–	–	–	–	–	–	–	–	–	–
III-8	M	LMNA+		+		+	+				+		
IV-4	M	LMNA+	+		+		+	+			+		+
IV-6	M	LMNA+						+	+	+	+		+
IV-8	F	LMNA–	–	–	–	–	–	–	–	–	–	–	–

+/– Indicates presence/absence of the trait; blanks represent missing data. Abbreviations: A.Fib/F: atrial fibrillation/flutter; AVB: Atrioventricular block; Brady: bradyarrhythmia; CHF: congestive heart failure; CMR: cardiovascular magnetic resonance; DCM: dilated cardiomyopathy; ECG: electrocardiogram; F: female; LGE: late gadolinium enhancement; M: male; NK(?): Not known (genetic testing not done); PAC: Premature atrial contraction; PVC: premature ventricular contraction; SCD: sudden cardiac death; SVT: supraventricular tachycardia; mVA: malignant ventricular arrhythmia; *LMNA+: LMNA* gene mutation present; *LMNA-:* negative for *LMNA* gene mutation.

the latter). Members I-1, II-1, II-5, II-6 and II-8 died young and unexpectedly (no genetic data available). Patient III-8 carries the variant and expresses an overt DCM phenotype together with atrial fibrillation (AF). Two of his children were also found to be carriers: IV-4 expressed DCM with heart failure, advanced CCD and ventricular arrhythmias that required an ICD. A DCM-pattern of extensive myocardial midwall septal fibrosis was noted by cardiovascular magnetic resonance (CMR) imaging. His brother IV-6, exhibited isolated right ventricular enlargement and biatrial dilatation in the context of AF, supraventricular arrhythmias and progressive CCD. Following multidisciplinary team (MDT) meeting discussion family member IV-6 received a primary prevention dual chamber ICD given his two risk factors for sudden cardiac death [male gender and non-missense *LMNA* mutation (4)]. His CMR similarly revealed midwall septal fibrosis matched by elevated native myocardial T_1 times (Fig. 1, right panel). On follow-up, post-ICD, we detected a self-terminating ten-beat salvo of non-sustained ventricular tachycardia (VT).

DNA testing in their sister IV-8 with normal CMR, excluded the presence of an *LMNA* mutation but identified a variant of uncertain significance (VUS) in the *Obscurin* gene (*OBSCN*, c.21011C > G, p.Ser7004Cys). Although a few *OBSCN* mutations have been reported in the context of DCM and hypertrophic cardiomyopathy (5), their occurrence in heterozygous states in individuals from the general population argue against their pathogenicity.

Discussion

Our report describes a family in which the proband and affected family member harbour a novel potentially pathogenic mutation in *LMNA* gene (c.1434dupG). The mutation was not previously described in the literature, however the clinical course and ominous outcomes resemble those reported in cardiolaminopathy. In this family there were seven premature sudden deaths (6). In a multicentre study of 269 *LMNA* mutation carriers male gender, non-missense mutation, left ventricular ejection fraction < 45% and presence of non-sustained VT were found to be independent predictors of malignant VA (4). In this regard, member IV-4 with ICD satisfies all four risk factors whilst member IV-6 with progressive CCD and ICD scored positive for two risk factors (male, non-missense mutation).

Pharmacological therapy in our *LMNA* gene mutation carriers with heart failure consisted of usual heart failure medications although in patients with bradyarrythmias, beta blockers were reserved till after device implantation or else discussed in a dedicated cardiomyopathy MDT meeting. Arrhythmias were managed according to standard clinical practice, and all decisions related to device implantation were reached after considering SCD risk factors and broader MDT discussion.

This pedigree analysis highlights the added value of CMR in segregation studies of LHD. For example, CMR potentially enables clinicians to better: 1) differentiate *LMNA*-DCM from other phenotypic mimics such

as arrhythmogenic cardiomyopathy; 2) exclude ischemic DCM using quantitative perfusion mapping approaches; 3) monitor LHD progression over time; 4) detect subclinical phenotypes otherwise missed by other imaging modalities; 5) plan optimal timing of device implantation in patients with borderline phenotypes as part of a multidisciplinary team meeting discussion.

Previous CMR work reported that non-ischaemic (midwall) scar in patients with LHD and VA predominantly involved the basal septum, basal inferior wall, and sub-aortic mitral continuity (7), which tallies with our current data. Member IV-6 had non-infarct pattern midwall LGE in the basal-to-mid septum matched by high myocardial T1 but normal T2 times, suggesting true myocardial septal fibrosis. Indeed he had QRS fragmentation on ECG (Fig. 1, right panel).

Limitations include that other genomic changes in this family cannot be definitively excluded, CMR was not performed on all family members, and genetic data unavailable for the five family members in generations I/II with premature sudden deaths.

Conclusions

LMNA frameshift variant (c.1434dupG) seems to be causative of lamin heart disease on the basis of this CMR-augmented segregation analysis however further studies are necessary to confirm our hypothesis.

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Conflicts of interest

The Authors declare to have no conflict of interest.

References

1. Peretto G, Sala S, Benedetti S, et al. Updated clinical overview on cardiac laminopathies: an electrical and mechanical disease. Nucleus 2018;9:380-91. https://doi.org/10.1080/19491034.2018.1489195.
2. Holmström M, Kivistö S, Heliö T, et al. Late gadolinium enhanced cardiovascular magnetic resonance of lamin A/C gene mutation related dilated cardiomyopathy. J Cardiovasc Magn Reson 2011;13:30. https://doi.org/10.1186/1532-429X-13-30.
3. Pasotti M, Klersy C, Pilotto A, et al. Long-term outcome and risk stratification in dilated cardiolaminopathies. J Am Coll Cardiol 2008;52:1250-60. https://doi.org/10.1016/j.jacc.2008.06.044
4. Priori S, Lundqvist C, Mazzant A, et al. ESC Guidelines for the management of patients with ventricular arrhythmias and the prevention of sudden cardiac death. Eur Heart J 2015;36:2793-867. https://doi.org/:10.1093/eurheartj/ehv316
5. Marston S, Montgiraud C, Munster AB, et al. OBSCN mutations associated with dilated cardiomyopathy and haploinsufficiency. PloS One 2015;10:e0138568.
6. Bécane H, Bonne G, Varnous S, et al. High incidence of sudden death with conduction system and myocardial disease due to lamins A and C gene mutation Pacing Clin Electrophysiol 2000;23:1661-6. https://doi.org/10.1046/j.1460-9592.2000.01661.x
7. Kumar S, Androulakis AF, Sellal JM, et al. Multicenter experience with catheter ablation for ventricular tachycardia in lamin A/C cardiomyopathy. Circ Arrhythm Electrophysiol 2016;9: pii: e004357. https://doi.org/10.1161/CIRCEP.116.004357.

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Phenotypic and genetic spectrum of patients with limb-girdle muscular dystrophy type 2A from Serbia

Stojan Peric[1*], Jelena Stevanovic[1*], Katherine Johnson[2], Ana Kosac[3], Marina Peric[4], Marija Brankovic[1], Ana Marjanovic[1], Milena Jankovic[1], Bojan Banko[5], Sanja Milenkovic[6], Milica Durdic[5], Ivo Bozovic[1], Jelena Nikodinovic Glumac[3], Dragana Lavrnic[2], Ruzica Maksimovic[5], Vedrana Milic-Rasic[3] and Vidosava Rakocevic-Stojanovic[1]

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Limb-girdle muscular dystrophy (LGMD) type 2A (calpainopathy) is an autosomal recessive disease caused by mutation in the *CAPN3* gene. The aim of this study was to examine genetic and phenotypic features of Serbian patients with calpainopathy. The study comprised 19 patients with genetically confirmed calpainopathy diagnosed at the Neurology Clinic, Clinical Center of Serbia and the Clinic for Neurology and Psychiatry for Children and Youth in Belgrade, Serbia during a ten-year period. Eighteen patients in this cohort had c.550delA mutation, with nine of them being homozygous. In majority of the patients, disease started in childhood or early adulthood. The disease affected shoulder girdle – upper arm and pelvic girdle – thigh muscles with similar frequency, with muscles of lower extremities being more severely impaired. Facial and bulbar muscles were spared. All patients in this cohort, except two, remained ambulant. None of the patients had cardiomyopathy, while 21% showed mild conduction defects. Respiratory function was mildly impaired in 21% of patients. Standard muscle histopathology showed myopathic and dystrophic pattern. In conclusion, the majority of Serbian LGMD2A patients have the same mutation and similar phenotype.

Key words: calpainopathy, c.550delA mutation, muscle magnetic resonance imaging, muscle histopathology

Abbreviations

AS: asymmetry in muscle strength, CK: creatine kinase, Cont.: contractures, EE: elbow extensors, DF: dorsal flexors, DTF: dorsal toe flexors, ECG: electrocardiography, EF: elbow flexors, EMR: electromyography, FVC: forced vital capacity, H.Abd: hip abductors, H.Add: hip adductors, HE: hip extensors, HF: hip flexors, KE: knee extensors, KF: knee flexors, LGMD: limb girdle muscular dystrophy, MRC: Medical Research Council Muscle Grading Scale, MRI: magnetic resonance imaging, PF: plantar flexors, PTF: plantar toe flexors, RBBB: right bundle branch block, S.Add: shoulder adductor

Introduction

Limb-girdle muscular dystrophy (LGMD) type 2A (calpainopathy) is an autosomal recessive disease caused by mutation in the *CAPN3* gene (1). Calpain 3 is a skeletal muscle-specific isoform of the Ca^{2+}-dependent non-lysosomal calpain cysteine protease, essential for normal muscle function (1). It plays a role in numerous functions in muscle cells, such as muscle regeneration, sarcolemmal repair, cytoskeleton regulation and calcium homeostasis (2-5). Calpainopathy is the most common form of LGMD in majority of countries worldwide (6, 7).

Based on the distribution of muscle weakness and age at onset, three main phenotypes of LGMD2A have been identified: 1) pelvi-femoral phenotype is the most common one, with muscle wasting and weakness starting

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in pelvic girdle, later affecting shoulder girdle; 2) scapulo-humeral phenotype with affection of the shoulder girdle from the beginning; and 3) hyperCKemia, which may be considered as a presymptomatic stage of calpainopathy, most commonly exhibited in children and young adults (8-10).

Aim of this research was to analyze genetic and phenotypic features of Serbian patients with calpainopathy.

Methods

The study was approved by the Ethical Board of the Neurology Clinic, Clinical Center of Serbia. It comprises patients that have been genetically diagnosed with LGMD2A at the Neurology Clinic, Clinical Center of Serbia and the Clinic for Neurology and Psychiatry for Children and Youth in Belgrade, Serbia during a ten-year period from 2008-2017. These are the two largest neuromuscular centers diagnosing patients with LGMD from the whole country and the region. Genetic analysis was performed through the whole exome sequencing in 15 subjects (confirmed with Sanger), sequencing of the whole *CAPN3* gene in one patient and analysis of four (exons 4, 6, 10 and 13) out of 24 coding exons in three subjects. Selection of these four exons was based on the previous genetic results of LGMD patients in the region and was done in order to lower the financial costs (11). Sequence variants were numbered according to the reference sequence NM_000070.3/ NP_000061.1.

Follow-up visits were regularly scheduled once or twice per year for all patients. Clinical assessment included a detailed medical and family history and neurological examination, performed by an experienced neurologist. First degree relatives of patients were assessed where possible. Muscle strength was scored using the Medical Research Council (MRC) scale (12). Electromyography (EMG) and nerve conduction studies were performed in 11 patients. Serum creatine kinase levels were measured in all patients at multiple time points during the course of the disease. Respiratory function was assessed by spirometry annually, while regular cardiac examinations included ECG each year and echocardiogram every second year. Muscle biopsies, usually of deltoid muscle, were performed in nine patients. Calpain 3 immunohistochemistry and immunoblot were not performed.

Muscle magnetic resonance imaging (MRI) was performed in four patients in axial and coronal planes of the lower limbs using the following sequences: T1-weighted (T1w), T2-weighted (T2w), proton-density weighted (PDw), and 3-point Dixon (13, 14). Images were assessed on an individual muscle basis and graded according to the five-point scale published by Mercuri et al. (15).

Methods of descriptive statistics were used: mean, standard deviation, median. Chi-square test, Mann-Whitney U test and Student t test were used for comparisons between two groups, as appropriate. Level of statistical significance was 0.05.

Results

We identified 19 LGMD2A patients from 18 families. This accounts for approximately 30% of all LGMD patients diagnosed in our two centers in observed period. Eighteen (95%) patients in this cohort had c.550delA (p.Thr184ArgfsTer36) mutation on at least one allele, with 9 (47%) patients being homozygous for this mutation (Tab. 1). Clinical presentation did not differ between c.550delA homozygous and heterozygous patients.

Majority of our patients were sporadic. Three of them had positive family history for LGMD2A (patients #6, #7 and #18). LGMD2A was diagnosed in patient #7 at the age of 12. Thus, his one-year older brother (#6) went through the full clinical examination – CK level was elevated with symptomatology showing mild wasting of the muscles in scapular region, contractures of the ankles, and inability to walk on heels.

We observed equal distribution regarding gender with eight (42%) patients being male (Tab. 2). The age at disease onset ranged between 7 to 40 years old (mean 16.4 ± 7.6, median 14.5 years). In one patient (#10) the disease started at the age of 40, while all of the others had disease onset between the age of 7 and 22. Almost half of the patients (42%) had multiple symptoms at the disease onset. In majority (74%) of the patients, the first symptom was proximal muscle weakness in lower limbs. Another common initial symptom was gait on tiptoes observed in 26% of patients. In two (11%) patients the disease started with proximal arm weakness. Two patients were asymptomatic, and they were accidentally diagnosed with hyperCKemia at age 9 and 11. Although patient #10 had late symptom onset, the disease showed more rapid progression, and the patient started to use a cane only after two years from the disease onset.

Average age at the last examination was 25.4 ± 10.4 years (median 26) (Tab. 2). Mean duration of disease was 11.6 ± 4.3 years (median 11.5). The facial and bulbar muscles still were not affected. Muscle atrophy and weakness was observed in pelvic girdle and thighs in 84% patients and the same number had shoulder girdle and proximal arm muscle wasting. Lower leg muscles were affected in 79% of patients and only one patient had distal muscle weakness in arms. Pseudohypertrophy of muscles was detected in ten (53%) patients, with seven of them exhibiting hypertrophy in calves, two in proximal muscles of lower limbs, and one in lower arms.

Table 1. Genetic findings in *CAPN3* gene in investigated patients with LGMD2A.

#	Mutation in one allele	Mutation in another allele	Genetic method
01	p.Thr184ArgfsTer36	p.Glu566Lys	Gene sequencing
02	p.Thr184ArgfsTer36	p.Thr184ArgfsTer36	Targeted exons sequencing
03	p.Thr184ArgfsTer36	p.Arg440Trp	Whole exome sequencing
04	p.Thr184ArgfsTer36	c.1194-9A > G	Whole exome sequencing
05	p.Thr184ArgfsTer36	p.Thr184ArgfsTer36	Whole exome sequencing
06	p.Thr184ArgfsTer36	c.1746-20C > G	Whole exome sequencing
07	p.Thr184ArgfsTer36	c.1746-20C > G	Whole exome sequencing
08	p.Thr184ArgfsTer36	c.2380+1G > A	Whole exome sequencing
09	p.Thr184ArgfsTer36	p.Thr184ArgfsTer36	Whole exome sequencing
10	p.Thr184ArgfsTer36	p.Thr417Met	Whole exome sequencing
11	p.Thr184ArgfsTer36	p.Asp295LeufsTer57	Whole exome sequencing
12	p.Thr184ArgfsTer36	p.Thr184ArgfsTer36	Whole exome sequencing
13	Deletion of 10362 kb (genomic coordinates 15:42676429-42686791)[a]	p.Gly441ValfsTer22	Whole exome sequencing
14	p.Thr184ArgfsTer36	p.Asn434LysfsTer37	Whole exome sequencing
15	p.Thr184ArgfsTer36	p.Thr184ArgfsTer36	Whole exome sequencing
16	p.Thr184ArgfsTer36	p.Thr184ArgfsTer36	Whole exome sequencing
17	p.Thr184ArgfsTer36	p.Thr184ArgfsTer36	Whole exome sequencing
18	p.Thr184ArgfsTer36	p.Thr184ArgfsTer36	Targeted exons sequencing
19	p.Thr184ArgfsTer36	p.Thr184ArgfsTer36	Targeted exons sequencing

[a] Genomic variants are based on build37/hg19.

Table 2. Sociodemographic, clinical and laboratory features of our patients with LGMD2A.

#	Sex	Age at onset	Symptoms at onset	Creatin kinase increase	Age at the last visit	Ambulant at the last visit
01	M	20	Difficulties climbing stairs and rising up from squatting	10x	30	Yes
02	F	9	Increased CK	10-50x	9	Yes
03	M	19	Difficulties climbing stairs	10x	37	Yes
04	M	11	Gait on tiptoes	10-50x	21	Yes
05	F	11	Gait on tiptoes, difficulties climbing stairs and rising up from squatting	50x	16	Yes
06	M	14	Proximal arm weakness	> 50x	14	Yes
07	M	12	Gait on tiptoes, elbow contractures	50x	15	Yes
08	M	22	Difficulties climbing stairs and walking uphill	> 5x	35	Yes
09	F	14	Gait on tiptoes, difficulties climbing stairs	10x	26	Yes
10	F	40	Wasting of proximal leg muscles, muscle pain	> 50x	51	Yes
11	M	7	Weakness of proximal leg muscles	> 50x	20	no (from age 19)
12	M	NA*	Increased CK	> 50x	11	Yes
13	F	14	Weakness of proximal leg muscles, difficulties walking on heels	10x	29	no (from age 27)
14	F	13	Gait on tiptoes, difficulties rising up from squatting	10x	29	Yes
15	F	18	Difficulties climbing stairs	10-50x	26	Yes
16	F	15	Proximal arm and leg weakness	< 5x	34	Yes
17	M	20	Difficulties climbing stairs	10x	33	Yes
18	F	10	Difficulties climbing stairs	10-50x	21	Yes
19	F	16	Difficulties climbing stairs	10-50x	25	Yes

M: male; F: female; NA: not applicable

Muscle strength was most severely reduced in the lower limbs, especially proximally with hip flexors and adductors being the most affected, and knee extensors relatively spared (Tab. 3). Dorsal flexors of foot and toes were significantly more affected than plantar ones. Shoulder abductors and adductors and elbow flexors were the most impaired upper limb muscle, with distal arm muscle strength being preserved in almost all patients. Scapular winging was present in 16 (84%) patients. Contractures of the ankles were present in all patients, with two of them as a result of surgery to prevent foot drop. Three (16%) patients had elbow contractures. All patients, except two, remained ambulant at the last examination. One patient started to use a wheelchair at the age of 19 after 12 years of disease duration, and another one at age of 27 after 13 years of having the disease. In the group of ambulant patients, two of them were using assisting walking device – one cane, and the other one four-wheel walker.

Serum CK levels during the course of the disease were elevated in all of the patients and were 4-80 times higher than upper border of reference values (normal value < 150IU/l). EMG was performed in 11/19 patients and it showed myopathic pattern in all patients with no spontaneous activity at rest. As for cardiology findings, none of the patients had cardiomyopathy. Electrocardiography showed incomplete right bundle branch block (RBBB) in one patient at age 11 when he was diagnosed with calpainopathy. Three other patients had mildly prolonged PQ interval observed at regular cardiologic checkups between age 25 and 30. Neither of them had any cardiac complaints. Respiratory function was impaired in 4 (21%) patients who had restriction (FVC < 90%) with the lowest FVC being 72% in patient who had a long disease duration of 19 years. It is of interest that patient #2 had signs of mild restriction (FVC 85%) from the beginning of the disease.

Seven patients underwent biopsy of the deltoid muscle. One of them (#7) showed no pathological changes at age 15, three years after disease started. In all other patients, dystrophic pattern was observed, including fiber size variations (6/6), necrosis (4/6), and connective and/or fat tissue infiltration (6/6). Some patients had additional myopathic signs: split fibers (3/6) and internal nuclei (3/6). In four out of six patients, inflammation was observed: two of them had rare T lymphocytes and hystiocytes, one rare eosinophiles and macrophages, and one significant eosinophilic infiltration (Fig. 1). Patient with significant eosinophilic infiltration was at age 12 at the time of the biopsy.

Muscle MRI of thighs and legs was performed in eight patients (Fig. 2). Uniform pattern of affection of the thigh was observed with gluteal muscles, posterior thigh compartment, hip adductors and iliopsoas muscles being severely affected. Adductors, semitendinosus and semi-

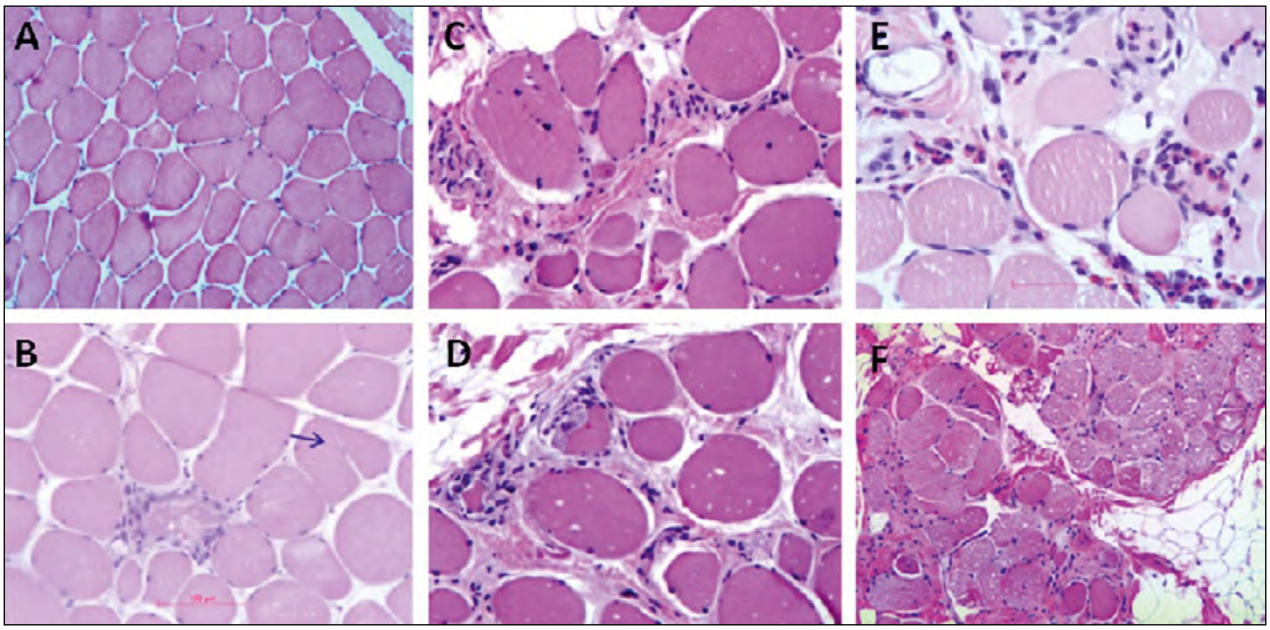

Figure 1. Histopathological findings in patients with calpainopathy.
A: normal muscle architecture in patient #7; B: fiber size variation, necrotic fiber with macrophage infiltration and split fiber (arrow) in patient #3; C,D: fiber size variation, internal nuclei, necrotic fibers with macrophage infiltration in patient #16; E: significant eosinophilic infiltrate in patient #12; F: connective and fat tissue infiltration in patient #15

Table 3. Pattern of muscle involvement in our patients with LGMD2A.

#	Upper extremities – proximal muscles	Upper extremities – distal muscles	Lower extremities – proximal muscles	Lower extremities – distal muscles	As.	Scapular winging	Spine deformities	Cont.
01	S.Abd 4, S.Add 4, EF 3	Normal	HF 4, HE 4, H.Abd 4, H.Add 2, KF 4, KE 3	DF 4, DTF 4	-	+	Hyperlordosis	Ankles
02	Normal	Normal	Normal	DF 4, DTF 4	-	+	None	Ankles
03	S.Abd 4, S.Add 4, EF 3	Normal	HF 1, HE 4, H.Abd 3, H.Add 2, KF 3, KE 4	normal	+	+	Hyperlordosis	Ankles
04	S.Abd 3, S.Add 4, EF 3, EE 4	Normal	HF 2, HE 4, H.Abd 4, H.Add 3, KF 4, KE 4	DF 4, DTF 4, PTF 4	+	+	Hyperlordosis	Ankles
05	S.Abd 4, EF 4	Normal	HF 3, HE 3, H.Abd 3, H.Add 3, KF 4, KE 4	DF 3	-	+	Scoliosis	Ankles
06	Normal	Normal	Normal	DF 4	-	+	None	Ankles, elbows
07	S.Abd 4	Normal	HF 4, HE 4, H.Abd 4, H.Add 4	DF 4	-	+	None	Ankles, elbows
08	S.Abd 3, S.Add 2, EF 3, EE 3	Normal	HF 1, HE 3, H.Abd 2, H.Add 2, KF 3, KE 3	Normal	-	+	None	Ankles
09	S.Abd 4, S.Add 3, EF 3, EE 4	Normal	HF 2, HE 3, H.Abd 2, H.Add 2, KF 3, KE 3	DF 4, PF 4	+	+	Hyperlordosis	Ankles (surgery)
10	EF 3	Normal	HF 4, HE 3, H.Abd 3, H.Add 3, KF 3, KE 4	Normal	-	-	Hyperlordosis	Ankles
11	S.Abd 2, S.Add 3, EF 3, EE 3	Normal	HF 2, HE 2, H.Abd 2, H.Add 2, KF 2, KE 2	DF 2, PF 2, DTF 2, PTF 2	-	+	Hyperlordosis	Ankles
12	Normal	Normal	Normal	Normal	-	-	None	Ankles
13	S.Abd 2, S.Add 2, EF 2, EE 2	WE 4, FF 4, FE 4	HF 2, HE 2, H.Abd 2, H.Add 2, KF 3, KE 3	DF 2, PF 4, DTF 2, PTF 4	-	+	None	Ankles, elbows
14	S.Abd 3, S.Add 3, EF 3, EE 4	Normal	HF 2, HE 2, H.Abd 2, H.Add 2, KF 2, KE 2	DF 3, PF 4, DTF 3, PTF 4	+	+	Hyperlordosis	Ankles (surgery)
15	S.Abd 4, S.Add 4, EF 4, EE 4	Normal	HF 2, HE 3, H.Abd 4, H.Add 3, KF 3, KE 4	DF 4, DTF 4,	-	+	Scoliosis	Ankles
16	S.Abd 2, S.Add 2, EF 3, EE 3	Normal	HF 2, HE 2, H.Abd 2, H.Add 2, KF 3, KE 3	DF 3, PF 4, DTF 3, PTF 4	-	+	Scoliosis	Ankles
17	S.Abd 3, S.Add 3, EF 3, EE 3	Normal	HF 3, HE 2, H.Abd 2, H.Add 2, KF 2, KE 2	DF 3, PF 4, DTF 3, PTF 4	-	-	Hyperlordosis	Ankles
18	S.Abd 3, S.Add 3, EF 3, EE 4	Normal	HF 2, HE 2, H.Abd 2, H.Add 2, KF 3, KE 4	DF 2, PF 3, DTF 2, PTF 3	-	+	Hyperlordosis	Ankles
19	S.Abd 4, S.Add 4, EF 4	Normal	HF 2, HE 2, H.Abd 4, H.Add 3, KF 3, KE 4	DF 4, PF 4, DTF 3, PTF 4	+	+	Scoliosis	Ankles

AS: asymmetry in muscle strength; Cont.: contractures; DF: dorsal flexors; DTF: dorsal toe flexors; EE: elbow extensors; EF: elbow flexors; H.Abd: hip abductors; H.Add: hip adductors; HE: hip extensors; HF: hip flexors; KE: knee extensors; KF: knee flexors; PF: plantar flexors; PTF: plantar toe flexors; S.Abd: shoulder abductor; S.Add: shoulder adductor; '+' present; '-' absent; Muscle strength was graded according to the Medical Research Council Scale

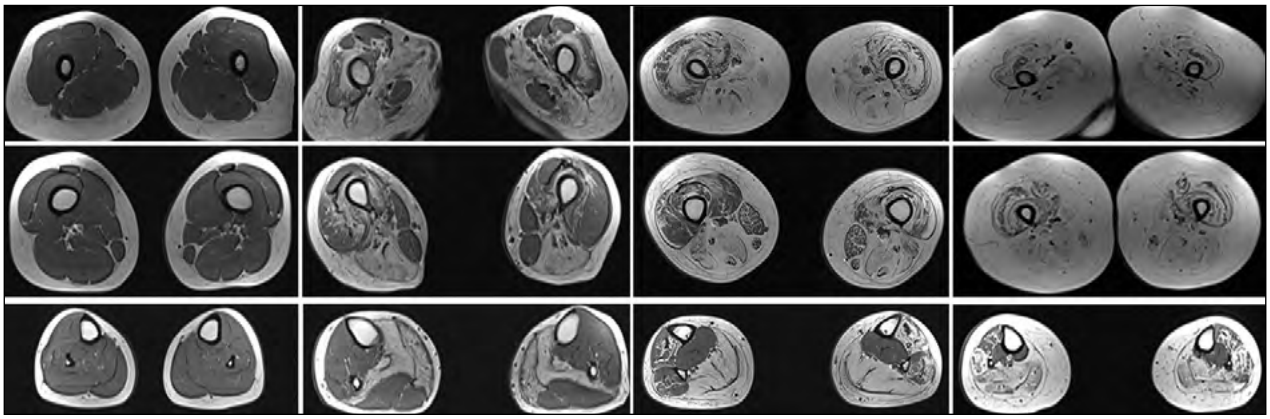

Figure 2. Muscle MRI findings in patients with calpainopathy.
Columns from left to right: normal MRI findings in healthy person, patient #10, #3, #14; rows from top to bottom: upper thighs, lower thighs, legs; more description is given in the text

membranosus muscles were more affected then biceps femoris. It seems that sartorius (from the anterior compartment) and gracilis (from the medial compartment) were the most spared muscles of the thighs. In all patients the most commonly affected muscles of lower legs were of the posterior compartment, with medial head of the gastrocnemius and soleus being more affected than lateral head of the gastrocnemius. Anterior and especially deep posterior compartments were long preserved.

Discussion

Calpainopathy was diagnosed in one third of Serbian patients with LGMD and c.550delA was the most common mutation found in 95% of subjects (in half of them in homozygous state). Depending on the geographic region, LGMD2A represents approximately 30% of all LGMD (16, 17). The c.550delA mutation accounts for up to 75% of LGMD2A patients in Slavic countries, Turkey, Italy and Germany (18-23). It was marked as the Eastern Mediterranean founder mutation (18). One in 133 patients from Croatian general population carries c.550delA mutation (11).

In our patients, disease usually started from childhood to early adulthood (median 14.5 years) which is in accordance with previous data (18). First symptoms in our group were proximal muscle weakness in lower limbs, walking on tiptoes, and in some cases proximal arm weakness which is in line with the literature data (18). Due to the presence of proximal arm atrophy and weakness and scapular winging these patients may be misdiagnosed as facioscapulohumeral muscular dystrophy although the second disease can be differentiated because of the facial muscle weakness and asymmetric atrophy and weakness in upper limbs.

Two of our patients were diagnosed with LGMD2A due to incidentally discovered hyperCKemia. Asymptomatic hyperCKemia is usually seen in children or young adults with calpainopathy and it may persist for decades (18). Serum CK levels during the course of the disease were 4-80 times elevated in all of our patients and this is the most common and unvarying feature of the LGMD2A from early infancy (19, 24).

During the disease course, pelvic girdle/thighs and shoulder girdle/proximal arms were clinically affected with similar frequency although muscle weakness was more severe in lower limbs. MRI results in proximal leg muscles were in accordance with the clinical findings: hip extensors and adductors and knee flexors were the most affected, while knee extensors were relatively spared. This was clinically described even in the first papers on calpainopathy (25, 26). Similarly, Straub and colleagues described prominent involvement of the gluteal and posterior compartment of the thigh in LGMD2A (27). According to them, pathology seems to start in the adductor magnus muscle and spreads to the semitendinosus and thereafter to all the hamstring muscles which is similar to our findings. This typical and selective pattern may be of diagnostic significance (18).

Lower leg muscles were affected in around 80% of patients during the course of the illness. Dorsal flexors of foot and toes were clinically more affected than plantar. On the contrary, MRI results showed that the most commonly affected muscles of lower legs were of the posterior compartment which is in accordance with previous reports (27). Sparing of the tibialis posterior muscle was observed in our cohort and previously reported in the literature (27, 28).

Muscle pseudohypertrophy was detected in half of our patients, usually in calves. This has been previously

reported only as an occasional sign in LGMD2A, although 86% of Brazilian LGMD2A patients had this finding (18, 29). This may be of diagnostic importance since male patients with hyperCKemia and calf pseudohypertrophy may be misdiagnosed with dystrophinopathy. It was reported that up to 20% of LGMD patients and 7% of LGMD2A actually have dystrophinopathy (30, 31). Ankle contractures were present in all of the patients in our cohort, and three had elbow contractures. Literature data suggest that joint contractures are typical for LGMD2A and may be common even in early disease stages (18, 28).

After ten or more years of disease duration some of our patients became non-ambulant. Loss of ambulation in LGMD2A occurs about 10-30 years after the disease onset (32). Cardiomyopathy was not observed, and only minor cardiac conduction impairments were found in our cohort. Heart involvement is generally rare in LGMD2A, but there are some cases that had cardiomyopathy and cardiac arrest (33-37). Mild respiratory restriction was diagnosed in 21% of our LGMD2A patients, and in one patient it was present from the beginning of the disease. Reduced forced vital capacity is frequently reported in LGMD2A (38, 39) due to the diaphragm weakness, but usually after longer disease duration.

Standard muscle histopathology in our patients was nonspecific, showing a dystrophic (and myopathic) pattern. Nevertheless, biopsy may be of importance to exclude myofibrillar myopathies and myositis (18). Due to the progress in genetic methods, the American Academy of Neurology suggest muscle biopsy only if genetic testing is inconclusive (40). Some of our patients had inflammation in muscle samples, one of them had significant eosinophilic infiltration. This was previously described in young patients with LGMD2A (41-43). Some of these patients were previously treated with steroids for years without any effect (44). Krahn and colleagues reported that eosinophilic myositis is an early and transient feature of the calpainopathy since it was not found in older patients with LGMD2A (42, 43).

Conclusions

Almost all Serbian patients with calpainopathy had c.550delA mutation. In most of the patients, disease started in the childhood or early adulthood. The disease affected both shoulder girdle – upper arm and pelvic girdle – thigh muscles with similar frequency, although muscles of lower limbs are more severely impaired. None of the patients had cardiomyopathy, while 21% showed mild conduction defects. Respiratory function was slightly impaired in 21% of patients.

Funding

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Conflict of interest

The Authors declare to have no conflict of interest.

Ethical approval

All procedures performed in studies were in accordance with the ethical standards of the Ethical Board of the Neurology Clinic, Clinical Center of Serbia, and with the 1964 Helsinki declaration and its later amendments.

Informed consent

Informed consent was obtained from all individual participants included in the study.

References

1. Richard I, Broux O, Allamand V, et al. Mutations in the proteolytic enzyme calpain 3 cause limb-girdle muscular dystrophy type 2A. Cell 1995;81:27-40. https://doi.org/10.1016/0092-8674(95)90368-2
2. Taveau M, Bourg N, Sillon G, et al. Calpain 3 is activated through autolysis within the active site and lyses sarcomeric and sarcolemmal components. Mol Cell Biol 2003;23:9127-35. https://doi.org/10.1128/mcb.23.24.9127-9135.2003
3. Toral-Ojeda I, Aldanondo G, Lasa-Elgarresta J, et al. Calpain 3 deficiency affects SERCA expression and function in the skeletal muscle. Expert Rev Mol Med 2016;18:e7. https://doi.org/10.1017/erm.2016.9
4. Huang Y, de Morrée A, van Remoortere A, et al. Calpain 3 is a modulator of the dysferlin protein complex in skeletal muscle. Hum Mol Genet 2008;17:1855-66. https://doi.org/10.1093/hmg/ddn081
5. Hauerslev S, Sveen ML, Duno M, et al. Calpain 3 is important for muscle regeneration: evidence from patients with limb girdle muscular dystrophies. BMC Musculoskelet Disord 2012;13:43. https://doi.org/10.1186/1471-2474-13-43
6. Bushby KMD, Beckmann JS. The 105th ENMC sponsored workshop: pathogenesis in the non-sarcoglycan limb-girdle muscular dystrophies (Naarden, April 12-14, 2002). Neuromuscul Disord 2003;13:80-90.
7. Guglieri M, Magri F, D'Angelo MG, et al. Clinical, molecular, and protein correlations in a large sample of genetically diagnosed Italian limb girdle muscular dystrophy patients. Hum Mutat 2008;29:258-66. https://doi.org/10.1002/humu.20642

8. Angelini C, Fanin M. Calpainopathy. Seattle: University of Washington, 1993.
9. Fanin M, Nascimbeni AC, Aurino S, et al. Frequency of LGMD gene mutations in Italian patients with distinct clinical phenotypes. Neurology 2009;72:1432-5. https://doi.org/10.1212/WNL.0b013e3181a1885e
10. Kyriakides T, Angelini C, Schaefer J, et al. EFNS guidelines on the diagnostic approach to pauci – or asymptomatic hyperCKemia. Eur J Neurol 2010;17:767-73. https://doi.org/10.1111/j.1468-1331.2010.03012.x
11. Canki-Klain N, Milic A, Kovac B, et al. Prevalence of the 550delA mutation in calpainopathy (LGMD 2A) in Croatia. Am J Med Genet 2004;125A:152-6. https://doi.org/10.1002/ajmg.a.20408
12. Personius KE, Pandya S, King WM, et al. Facioscapulohumeral dystrophy natural history study: standardization of testing procedures and reliability of measurements. Phys Ther 1994;74:253-63. https://doi.org/10.1093/ptj/74.3.253
13. Willis TA, Hollingsworth KG, Coombs A, et al. Quantitative magnetic resonance imaging in limb-girdle muscular dystrophy 2I: a multinational cross-sectional study. PLoS One 2014;9:e90377. https://doi.org/10.1371/journal.pone.0090377
14. Hollingsworth KG, de Sousa PL, Straub V, et al. Towards harmonization of protocols for MRI outcome measures in skeletal muscle studies: Consensus recommendations from two TREAT-NMD NMR workshops (2 May 2010, Stockholm, Sweden, 1-2 October 2009, Paris, France). Neuromuscul Disord 2012;22:S54-67. https://doi.org/10.1016/j.nmd.2012.06.005
15. Mercuri E, Pichiecchio A, Counsell S, et al. A short protocol for muscle MRI in children with muscular dystrophies. Eur J Paediatr Neurol 2002;6:305-7.
16. Chou FL, Angelini C, Daentl D, et al. Calpain III mutation analysis of a heterogeneous limb-girdle muscular dystrophy population. Neurology 1999;52:1015-20. https://doi.org/10.1212/wnl.52.5.1015
17. Zatz M, Vainzof M, Passos-Bueno MR. Limb-girdle muscular dystrophy: one gene with different phenotypes, one phenotype with different genes. Curr Opin Neurol 2000;13:511-7.
18. Fanin M, Angelini C. Protein and genetic diagnosis of limb girdle muscular dystrophy type 2A: the yield and the pitfalls. Muscle Nerve 2015;52:163-73. https://doi.org/10.1002/mus.24682
19. Fanin M, Nascimbeni AC, Fulizio L, et al. The frequency of limb girdle muscular dystrophy 2A in northeastern Italy. Neuromuscul Disord 2005;15:218-24. https://doi.org/10.1016/j.nmd.2004.11.003
20. Pogoda TV, Krakhmaleva IN, Lipatova NA, et al. High incidence of 550delA mutation of *CAPN3* in LGMD2 patients from Russia. Hum Mutat 2000;15:295. https://doi.org/10.1002/(SICI)1098-1004(200003)15:3%3C295::AID-HUMU15%3E3.0.CO;2-8
21. Milic A, Canki-Klain N. Calpainopathy (LGMD2A) in Croatia: molecular and haplotype analysis. Croat Med J 2005;46:657-63.
22. Hanisch F, Müller CR, Grimm D, et al. Frequency of calpain-3 c.550delA mutation in limb girdle muscular dystrophy type 2 and isolated hyperCKemia in German patients. Clin Neuropathol 2007;26:157-63.
23. Balci B, Aurino S, Haliloglu G, et al. Calpain-3 mutations in Turkey. Eur J Pediatr 2006;165:293-8. https://doi.org/10.1007/s00431-005-0046-3
24. Piluso G, Politano L, Aurino S, et al. Extensive scanning of the calpain-3 gene broadens the spectrum of LGMD2A phenotypes. J Med Genet 2005;42:686-93. https://doi.org/10.1136/jmg.2004.028738
25. Fardeau M, Eymard B, Mignard C, et al. Chromosome 15-linked limb-girdle muscular dystrophy: clinical phenotypes in Reunion Island and French metropolitan communities. Neuromuscul Disord 1996;6:447-53.
26. Richard I, Roudaut C, Saenz A, et al. Calpainopathy – a survey of mutations and polymorphisms. Am J Hum Genet 1999;64:1524-40. https://doi.org/10.1086/302426
27. Straub V, Carlier PG, Mercuri E. TREAT-NMD workshop: pattern recognition in genetic muscle diseases using muscle MRI (Rome, Italy, February 25-26, 2011). Neuromuscul Disord 2012;22:S42-53. https://doi.org/10.1016/j.nmd.2012.08.002
28. Mercuri E, Bushby K, Ricci E, et al. Muscle MRI findings in patients with limb girdle muscular dystrophy with calpain 3 deficiency (LGMD2A) and early contractures. Neuromuscul Disord 2005;15:164-71. https://doi.org/10.1016/j.nmd.2004.10.008
29. Passos-Bueno MR, Vainzof M, Moreira ES, et al. Seven autosomal recessive limb-girdle muscular dystrophies in the Brazilian population: from LGMD2A to LGMD2G. Am J Med Genet 1999;82:392-8.
30. Arikawa E, Hoffman EP, Kaido M, et al. The frequency of patients with dystrophin abnormalities in a limb-girdle patient population. Neurology 1991;41:1491-6. https://doi.org/10.1212/wnl.41.9.1491
31. Georgieva B, Todorova A, Tournev I, et al. 550delA mutation in the calpain 3 (CAPN3) gene: *DMD/BMD, SMA*, or LGMD2A – Clinically misdiagnosed cases. Am J Med Genet 2005;136 A:399-400. https://doi.org/10.1002/ajmg.a.30809
32. Angelini C, Nardetto L, Borsato C, et al. The clinical course of calpainopathy (LGMD2A) and dysferlinopathy (LGMD2B). Neurol Res 2010;32:41-6 https://doi.org/10.1179/174313209X380847
33. Dirik E, Aydin A, Kurul S, et al. Limb girdle muscular dystrophy type 2A presenting with cardiac arrest. Pediatr Neurol 2001;24:235-7.
34. Sveen ML, Thune JJ, Køber L, et al. Cardiac involvement in patients with limb-girdle muscular dystrophy type 2 and becker muscular dystrophy. Arch Neurol 2008;65:1196-201. https://doi.org/10.1001/archneur.65.9.1196
35. Todorova A, Georgieva B, Tournev I, et al. A large deletion and novel point mutations in the calpain 3 gene (*CAPN3*) in Bulgarian LGMD2A patients. Neurogenetics 2007;8:225-9. https://doi.org/10.1007/s10048-007-0083-3
36. Okere A, Reddy SS, Gupta S, et al. A cardiomyopathy in a patient with limb girdle muscular dystrophy type 2A. Circ Hear

Fail 2013 6:e12-3. https://doi.org/10.1161/CIRCHEARTFAILURE.112.971424

37. Hashiguchi S, Adachi K, Inui T, et al. A clinicopathological investigation of two autopsy cases of calpainopathy (LGMD2A). Brain Nerve 2014;66:1097-102.
38. Pollitt C, Anderson LV, Pogue R, et al. The phenotype of calpainopathy: diagnosis based on a multidisciplinary approach. Neuromuscul Disord 2001;11:287-96.
39. Urtasun M, Sáenz A, Roudaut C, et al. Limb-girdle muscular dystrophy in Guipúzcoa (Basque Country, Spain). Brain 1998;121 (Pt 9):1735-47. https://doi.org/10.1093/brain/121.9.1735
40. Narayanaswami P, Weiss M, Selcen D, et al. Evidence-based guideline summary: diagnosis and treatment of limb-girdle and distal dystrophies: report of the Guideline Development Subcommittee of the American Academy of Neurology and the Practice Issues Review Panel of the American Association of Neuromuscular & Electrodiagnostic Medicine. Neurology 2014;83:1453-63. https://doi.org/10.1212/WNL.0000000000000892
41. Brown RH, Amato A. Calpainopathy and eosinophilic myositis. Ann Neurol 2006;59:875-7. https://doi.org/10.1002/ana.20900
42. Krahn M, Goicoechea M, Hanisch F, et al. Eosinophilic infiltration related to *CAPN3* mutations: a pathophysiological component of primary calpainopathy? Clin Genet 2010;80:398-402. https://doi.org/10.1111/j.1399-0004.2010.01620.x
43. Krahn M, Lopez De Munain A, Streichenberger N, et al. *CAPN3* mutations in patients with idiopathic eosinophilic myositis. Ann Neurol 2006;59:905-11. https://doi.org/10.1002/ana.20833
44. Richard I, Brenguier L, Dinçer P, et al. Multiple independent molecular etiology for limb-girdle muscular dystrophy type 2A patients from various geographical origins. Am J Hum Genet 1997;60:1128-38.

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Current and emerging therapies in Becker muscular dystrophy (BMD)

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Becker muscular dystrophy (BMD) has onset usually in childhood, frequently by 11 years. BMD can present in several ways such as waddling gait, exercise related cramps with or without myoglobinuria. Rarely cardiomyopathy might be the presenting feature. The evolution is variable. BMD is caused by dystrophin deficiency due to inframe deletions, mutations or duplications in dystrophin gene (Xp21.2) We review here the evolution and current therapy presenting a personal series of cases followed for over two decades, with multifactorial treatment regimen. Early treatment includes steroid treatment that has been analized and personalized for each case. Early treatment of cardiomyopathy with ACE inhibitors is recommended and referral for cardiac transplantation is appropriate in severe cases. Management includes multidisciplinary care with physiotherapy to reduce joint contractures and prolong walking. BMD is slowly progressive with phenotypic variability. Despite childhood onset, independent walking is never lost before the third decade. Personalized medicine is required to tailor treatment to individual cases.

Key words: Becker muscular dystrophy, BMD, steroids

Introduction

After the description by Becker and Kiener in 1955 in affected families, the discovery by Monaco et al. (1) of the dystrophin gene (*DMD*) and the diagnostic use of dystrophin protein testing led to a drastic re-consideration of clinical phenotypes associated with deletion or duplication of the dystrophin gene: several different clinical entities were described associated with different prognosis according to the localization of mutation and residual amount of dystrophin protein (2, 3). Among these phenotypes, there are patients with cramps and myalgia, myoglobinuria, mild myopathy, quadriceps myopathy, late-onset myopathy, X-linked dilated cardiomyopathy (2, 3). It is not uncommon to find in diagnostic procedures patients with a deletion in the dystrophin gene that have normal muscle strength and endurance, but present high CK, and so far their follow-up and treatment recommendations are still a matter of debate. Patients with early cardiomyopathy are also a possible variant of BMD (4, 5) and may be susceptible either to specific drug therapy and/or to cardiac transplantation (6-8). Here we cover emerging therapies considering follow-up, and exemplifying some phenotypes and treatments by a few study cases.

Pathophysiology and rationale of therapeutic targets

All patients with dystrophin of abnormal quantity or size have clinical manifestations compatible with BMD range of phenotypes and their diagnostic traits have been identified (9). In addition to classical BMD cases, on the basis of *DMD* gene mutations or dystrophin protein abnormality it is possible to diagnose a number of "preclinical" or "asymptomatic" cases.

Following them for decades as atypical cases, most "asymptomatic" BMD patients show less ability in term to run or perform Gowers' maneuver, episodes of myoglobinuria or fatigability. This progression is mainly to be attributed to two patho-mechanism factors:

- **inflammatory cytokines** and TNF-alpha-induced microRNAs control dystrophin expression (10). Inflammatory cells could play a role in microRNAs induction and cross talk, and likely the microRNA pattern could not only be a signaling of NF-kB pathway but also play a role in promoting myogenesis, differentiation and muscle regeneration (11);
- **oxidative stress** refers to an imbalance between the generation of free radicals- chemical species with a high reactivity and instability of oxygen (Reactive Oxygen Species, ROS) and nitrogen (Reactive Nitro-

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gen Species, RNS), and the activity of the antioxidant defense systems (12). Among the ROS that are mainly generated during the mitochondrial electron transport chain, the superoxide anion, the hydrogen peroxide, and the hydroxyl radicals are the most studied.

The nitric oxide (NO) and the peroxynitrite are the most known among the RNS. The NO, a low reactive molecule that can become toxic forming peroxynitrite in the presence of superoxide anion, is synthesized by the enzyme NO synthase (NOS), among which isoforms there is the neuronal NOS (nNOS), localized in the sarcolemma of muscle fibers and deemed to be the main producer of RNS (12). Increasing levels of both ROS and RNS can damage different intracellular macromolecules, such as lipids, proteins, and nucleic acids (13). In particular, lipids of the sarcolemma are frequently attacked in a process called lipid peroxidation (14) and, for this reason, the products of lipid peroxidation are often used as biomarkers of oxidative stress (12).

Among therapeutic targets of metabolic pathways involved in muscle plasticity, there are increasing utrophin (15), NO (16), and inhibiting ROS and RNS (17, 18).

Case reports (Tab. 1)

Case 1

This 15-year-old boy presented with muscle weakness, high CK levels, recurrent myoglobinuric episodes, scoliosis, calf hypertrophy, waddling gait, pes cavus, and thigh hypertrophy. He was found to carry a novel missense variant p.Thr160Pro in exon 6 of *DMD* gene,

Table 1. Clinical and molecular features in the present BMD patient series.

Clinical feature	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7
Dystrophin protein	427 kDa, 10%	600 kDa, 100%	n.d.	n.d.	427 kDa, 5%	370 kDa, 30%	380 kDa, 60%
Dystrophin gene mutation	Missense p.T160P	Dupl. ex.14-42	Del. ex.48-49	Del. ex.47-49	Point 5'-UTR	Del. ex.45-47	del. ex.45-49
Age at onset (yrs)	15	4	9	Childhood	16	5	17
Cramps/myalgia	No	Yes	No	No	Yes	No	No
Myoglobinuria	Yes	No	No	No	Yes	No	No
Fatigability	Yes	No	Yes	No	No	Yes	No
Calf hypertrophy	Yes	Yes	No	Yes	No	Yes	Yes
Pes cavus	Yes	No	No	No	No	No	Yes
Joint contractures	No	No	Yes	No	No	Yes	Yes
Cardiac involvement	+++	+/-	+	+/++	+++	++/+	+
Left ventricular ejection fraction	n.r.	n.r.	54-55%	50-51%	34%	45-55%	55%
Cardiac transplantation	At 25 yrs	No	No	No	at 32 yrs	No	No
CK levels (U/L)	2930-3479	1400-8630	1003-3600	656-919	488-1700	1106-2794	459
Forced vital capacity	n.d.	n.d.	89%	n.d.	n.d.	Normal	76%
Mental/ behavioral changes	Yes	No	Yes	No	No	No	Yes
Steroid treatment	Prednisone	Prednisone	No	No	No	Deflazacort	Deflazacort
Duration of steroid treatment	10 years	5 months	-	-	-	26 years	20 years
Effects of steroid treatment	Initial benefit	No benefit	-	-	-	Functional stabilization on long-term	Initial benefit, functional stabilization on long-term

N.d.: not determined; N.r.: not reported

dystrophin protein was of normal molecular weight but reduced to 10% of controls. CK levels ranged between 2930-3479 U/L. He developed an early and severe form of dilated cardiomyopathy, which required cardiac transplantation at age 25 years (Fig. 1A). He was then closely followed both by cardiologist and neurologist. Since the heart transplant, he has been treated with 160 mg cyclosporin, 75 mg azathioprine, 20 mg prednisone daily, and has been followed once a year. He first presented some gait improvement and followed aerobic rehabilitation at home, as well as in a rehabilitative hospital. At age 35 he was able to perform a 6-meter walking test (6MWT): at two minutes he walked 50 m, and at 6 minutes 160 m. He had some behavioral problems, but was still able to work as a telephone operator. At last examination, he could stand, but walked only few steps; he was referred by family relatives to present rage tempers, but then he started coping with disease limitations and required drugs.

Case 2

At 4 years of age this child had onset of calf myalgia with cramps and CK levels ranging between 1400-8630 U/L. He was treated for 5 months with 50 mg prednisone without a clear benefit during his early teens. A muscle biopsy performed at 10 years of age showed active degeneration and regeneration foci, and a few inflammatory cells. By immunohistochemistry, there were many fetal myosin positive fibers (regenerating fibers). Immunoblot analysis showed normal quantity of an abnormally large dystrophin (600 kDa) as compared to normal size (427 kDa), originated by a duplication in the *DMD* gene involving exons 14-42 (19). Clinical severity was mild. In the subsequent follow-ups, he discontinued prednisone treatment, was able to walk, but presented a definite deterioration, was apparently weak with persistent cramps and walking difficulty. He presented also ECG changes, such as right bundle branch block, increased R/S ratio and T-wave abnormalities. This case is interesting because it demonstrates that an enlarged dystrophin molecule is only partially functional and causes progressive weakness.

Case 3

This patient was an hyperactive child. He started walking at 14 months of age. He suffered from stuttering and headache and was seen as "lazy" respect to peers. A muscle biopsy showed abnormal dystrophin, due to a deletion of exons 48-49 in the *DMD* gene. He then presented restrictive ventilatory dysfunction and elevated CK (3600 U/L), and had to leave elementary school for his poor performance. At 9 years he was able to raise from floor with one hand. At age 19 he was still reported as "nervous" and presented joint contractures, normal limb strength and grip, but weakness of extrarotator upper limb muscles and curved shoulders. On spirometry, forced vital capacity was 89%. He presented easy fatigability during cycling and elevated CK levels (1003 U/L). On echocardiography, left ventricular ejection fraction was 54-55%. This pauci-symptomatic patient did not follow any drug treatment.

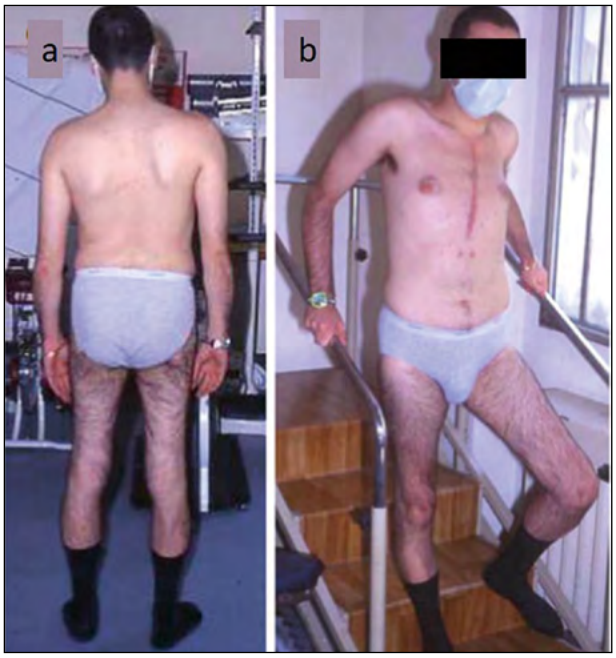

Figure 1. The patient (Case 1) after cardiac transplantation uses the handrail in descending stairs. Note hypotrophy of quadriceps muscle.

Case 4

This child had high CK but presented normal muscle strength, and had only slight scapular winging and calf hypertrophy. A muscle biopsy showed few hypercontracted fibers. Genetic analysis showed a deletion of exons 47-49 in *DMD* gene. At last examination, CK levels were moderately increased (656-919 U/L), and he could perform normal sporting ability and occasionally mountain climbing. Echocardiography showed altered diastolic relaxation and left ventricular ejection fraction was 50-51%.

Case 5

This 16-years-old boy (with a brother affected with severe cardiomyopathy who had died two years after cardiac transplantation) suffered from myalgia, recurrent myoglobinuric episodes, CK = 1700 U/L, and dilated cardiomyopathy. At age 32 years he had no muscle weakness and muscle biopsy showed dystrophin of normal

molecular weight but severely reduced amount (5% of controls) caused by a splicing mutation in 5'-UTR region of *DMD* gene (20). The severity of cardiomyopathy progressed and he required a cardiac transplantation. This is a rare case of X-linked dilated cardiomyopathy caused by a mutation in the 5'-end of the *DMD* gene, leading to the absence of the muscle-promoter and the first exon of *DMD* gene, resulting in no dystrophin transcript in the cardiac muscle alone, whereas two alternative promoters were active in skeletal muscle (brain and Purkinje-cells promoters) and expressed sufficient dystrophin to prevent muscle symptoms (21).

Case 6

Since age 5 years this boy presented difficulty running and at age 11 years he had difficulty walking. Spirometry and echocardiography were normal. At age 14 he had a waddling gait, proximal lower limbs hypotrophy, Achilles tendon retractions, and calf pseudo-hypertrophy. CK level was 1106 U/L (n.v. 10-80), EMG was myogenic. Muscle biopsy showed dystrophin protein of 370 kDa, 30% of controls, due to a deletion in exons 45-47 of *DMD* gene.

Since age 16 years he presented difficulty climbing stairs and since age 18 years he had difficulty performing aerobic exercises. He was then started on deflazacort 30 mg/10 days/month, that at age 28 was increased to 60 mg/10 days/month and associated to diphosphonate for bone osteoporosis.

At 28 years of age he was found to have a left ventricular enlargement, with ejection fraction 45%. He was started on ACE-inhibitors (2.5 mg Triatec) and β-blockers (Congestor 1.25 mg): after 15 years of this drug regimen, his left ventricular ejection fraction improved to 55%, and CK was 2794 U/L. At age 39 years, echocardiography showed left ventricle hypokinesia with dyastolic dysfunction (altered relaxation).

At age 44 years (after 26 years of steroid treatment), he presented calf pseudo-hypertrophy, was able to rise from floor putting hands on the table, he had proximal muscle weakness in iliopsoas, semitendinosus, semimembranosus 3/5, quadriceps 4/5, triceps 4/5, biceps 3+/5 (MRC), his overall muscle functions were stable, walking ability was preserved but waddling. Muscle MRI demonstrated fibro-fatty replacement of posterior thigh and calf muscles.

Case 7

At 17 years of age he started having difficulty walking, had pes cavus and was first mis-diagnosed as affected with spinal muscular atrophy (Kugelberg-Welander syndrome). A muscle biopsy showed myopathic changes, dystrophin protein of 380 kDa, 60% of controls, due to a deletion in exons 45-49 of *DMD* gene. Since age 18 he was put on deflazacort (60 mg/alternate day) with improvement for about 7 years. Since age 25 he has difficulty climbing stairs requiring the use of handrail. At age 28 years he underwent cataract surgery. At age 24 echocardiography showed hypokinesia of left ventricle, with ejection fraction 55%.

Spirometry showed respiratory insufficiency, with FVC = 76%, compatible with slight restrictive ventilatory dysfunction. At age 33 years he had waddling gait, calf hypertrophy, weakness in ileopsoas, quadriceps and extrarotator muscles. CK was 459 U/L.

At age 37 he complained of limb pain: a spine MRI evidenced disc compression at L3-L4 and L4-L5 roots. The patient had also panic attacks and was treated with benzodiazepines, but continued alternate-day deflazacort with strength stabilization.

At age 38 years (after 20 years steroid treatment) he had waddling gait with lordotic posture, was able to raise from a chair using one hand, was able to raise from floor in 5-8 secs, muscle strength of ileopsoas and quadriceps was 4/5.

Genotype-phenotype correlations and prognostic features

The cases above reported show that there is a wide clinical variability for BMD, ranging from asymptomatic hyperCKemia with cramps/myalgia syndrome and myoglobinuria to proximal myopathy with cardiomyopathy.

Mutations in the *DMD* gene that do not alter the reading-frame (in-frame deletions/duplications) are usually associated with BMD phenotypes (22). In general, mutations in the proximal region of the dystrophin gene (exons 2-10) are associated with early-onset and severe phenotype, while those in the proximal region of central rod domain (exons 11-43) are associated with mild or asymptomatic phenotype, and those in the distal region of central rod domain (exons 44-55) are associated with the classical phenotype (23); we also observed a possible relationship between the presence of dilated cardiomyopathy and mutations in particular regions (exons 48-49, 5′-region) (4-6, 20). Dystrophin immunoblot is the most important biochemical tool to diagnose patients even in the preclinical stage of the disease; this analysis is able to demonstrate the abnormality of dystrophin quantity and molecular weight, usually due to in-frame deletion/duplications in the *DMD* gene. For example, in young patients with high CK, a good prognosis can be entertained by dystrophin protein quantity over 70%. The clinician should not restrict the research to DNA genetic analysis, but prognostic assessment might need a muscle biopsy. Severe cases usually have less than 20% dystrophin protein amount, while mild cases with

over 70% dystrophin would be late-onset ambulatory or even asymptomatic (2, 3).

Management of clinical manifestations

Cardiac involvement

Heart failure from dilated cardiomyopathy is a common cause of morbidity and the most common cause of death in BMD patients. Early diagnosis and treatment of cardiomyopathy is important for improving quality of life and maximizing patients' survival.

ENMC (European Neuro Muscular Center) recommendations include a yearly monitoring by ECG and echocardiography starting soon after diagnosis. The importance of a frequent cardiac surveillance also in BMD patients without or with minor muscle symptoms has been highlighted by the observation that a subclinical cardiac involvement is very frequent in such patients (5, 6), who may later develop an overt dilated cardiomyopathy requiring proper treatment.

The frequency of cardiac monitoring should be increased as directed by the cardiologist, with the onset of heart failure symptoms. Treatment of cardiac involvement in dystrophinopathy patients was pioneered by Nigro (24, 25) and described by Melacini (4).

The consensus guidelines recommend initiation of angiotensin converting enzyme (ACE) inhibitors therapy with or without beta-blockers. Most cardiologists start treatment when the left ventricular ejection fraction drops below 55%. The possible preventive efficacy of ACE inhibitors was evaluated in a randomized trial of 57 children with BMD (mean age 10.7 years) who had a mean left ventricular ejection fraction of 65% (26): the children were assigned to perindopril or placebo and treated up others. Survival for the perindopril and placebo groups was 93 and 66%, respectively.

The use of eplerenone in a combined cardioprotective therapy, was able to attenuate the progressive decline of left ventricular systolic function in Duchenne patients (27), and, due to the absence of important contraindications, it has been suggested as a new drug to be used in future trials also in BMD patients, aimed at investigate its possible benefit as a cardiomyopathy treatment (28, 29).

When dilated cardiomyopathy evolves towards the stage of heart failure, this rapidly becomes intractable and cardiac transplantation is the only life-saving option. Heart transplant has so far only been reserved to BMD patients with both severe dilated cardiomyopathy and limited evidence of skeletal muscle disease (7). Long-term follow-up of transplanted patients suggested that this procedure is able to consistently prolong life expectancy (8), provided that the exact modulation of immunosuppressive dosage to avoid worsening of myopathy and the adoption of proper respiratory and muscle functional rehabilitation are pursued.

In the present series of cases, cardiac involvement was present in Cases 1, 3, 4, 5, 6 and it appeared as a prominent clinical feature in Cases 1 and 5, who successfully underwent cardiac transplantation.

Intellectual disability

Mental retardation (IQ < 75) has been rarely reported in BMD patients (30), possibly related to deletion mutations removing the Dp140 regulatory region of the *DMD* gene. However, some patients may present behavioral problems, panic attacks, language problem, hyperactivity and sometimes difficulty on fining adequate jobs (as in the present Cases 1, 3 and 7). Some of these difficulties might be attributed to social problems (31), but the study of central nervous system involvement in BMD would need a large cooperative study.

Rehabilitative therapy

A relevant aspect to consider when advising physical exercise in BMD patients is related their muscle metabolism. As known, the muscle fibers can be distinguished into two main categories (17): type I fibers (slow), which predominantly use an oxidative metabolism and are predisposed for an aerobic work; and type II fibers (fast), which have a glycolytic metabolism and therefore are predisposed for an anaerobic work. There are also the type IIa fibers, that have intermediate characteristics between the oxidative fibers and the glycolytic fibers.

Furthermore, a characteristic of the muscle fiber is that it is endowed with plasticity, i.e. the ability to modify metabolism and physiological properties depending on external stimuli, especially exercise and drugs (18). The transition from the fast fibers to the slow fibers of healthy muscles is not complete, and intermediate fibers characteristics predominate.

This transition may have crucial implications from a therapeutic perspective, since it has been demonstrated that the slow muscle fibers are more resistant to necrosis and, therefore, seem to be more spared in muscular dystrophies (17).

Several researches are underway to identify which properties make the slow fibers more resistant to the dystrophic process. To date, it is known that these fibers: a) are characterized by an increase in the expression of utrophin, a protein with a structure similar to dystrophin and that can partially compensate for its deficiency (15); b)

releases less ROS; c) are more resistant to fatigue and, consequently, can be more resistant to the injury caused by extreme contractions; d) have a higher expression of the adenosine monophosphate activated protein kinase (AMPK), which protects the cells through different mechanisms, including the reduction of fibrosis by increasing the number of mitochondria. Overall, a better understanding of how the transition of fast fibers to slow fibers changes the metabolism and physiological properties of muscle will play an important role in the development of new treatment options for muscular dystrophies.

A better understanding of the disease process and progression, including comorbidities and complications, is mandatory for a comprehensive and individually-tailored planning and monitoring of rehabilitative activities. This will also shed lights on the intrinsic properties of the dystrophic muscle to respond to different interventions, possibly involving the enhancement of the residual motor activities and adaptive plastic changes in response to fiber loss. Interestingly, multimodal integrated interventions targeting both perception abilities and motor skills have been shown to be effective also in other clinical contexts (32). In particular, it has been demonstrated that motor learning and memory mediated mechanisms of plasticity might underlie the improvement of hand motor coordination, speed-accuracy, and fine motor performance in some neurological disorders.

The loss of nNOS may cause functional ischemia contributing to skeletal and cardiac muscle cell injury. The effects of NO is augmented (by inhibiting degradation of cGMD) by the use of sildenafil and tadanafil. Although promising results have been observed using these drugs in dystrophinopathy animal models, the effects in Duchenne and Becker patients have been disappointing, with minor effects on upper limb performance and none on ambulation (16).

Endurance training has been demonstrated to be a safe method to increase exercise performance and daily function in BMD patients (33), suggesting the usefulness of an active approach to rehabilitation (34, 35).

Prevention of other secondary complications

To avoid respiratory complications, pneumococcal and influenza annual immunization, as well as annual spirometry is recommended. In patients presenting a decline of forced vital capacity leading to respiratory insufficiency, supportive respiratory aids should be offered when necessary.

Other complications in BMD are joint contractures (pes cavus was reported in 15-70% of cases, and it was present in Cases 1 and 7), which can be prevented by physical therapy to promote mobility and reduce cramps and muscle fatigue.

A proper control of dietary intake is necessary to avoid obesity and reduce the risk of bone fractures (diet rich in vitamin D and calcium).

Current and future therapies

Medical treatments

Anti-inflammatory steroid therapy have been so far adopted in BMD patients, since steroids improve muscle strength and function. Steroids are the most relevant available treatment, and we present our experience in this patients series.

Long-term treatment with deflazacort on intermittent schedule has been used in two patients in the present series (Cases 6 and 7), in whom we have been able to obtain an initial improvement, followed by stabilization of the patient condition, with preservation of muscle function and prolongation of walking ability. Furthermore, this therapy is expected to maintain upper limb muscle strength, and delay the decline of respiratory and cardiac function, although this field needs further investigations. Prednisone was less successful, since both patients (Cases 1 and 2) had muscle strength deterioration, although other comorbidities such as cardiomyopathy were relevant clinical features. The two drugs had different cardiological and side effects. Steroid treatment may be continued on a long-term basis if side effects (weight gain, increased risk of bone fractures, cataract, behavioral changes) are not severe, and the schedule and dosage of treatment should be changed and personalized if necessary.

Givinostat, anti-fibrotic agent, is under trial with a morphological outcome.

Emerging molecular therapies: personalized medicine and future directions

The field of dystrophinopathies has seen an unprecedented advancement in the field of new molecular therapies in the last two decades. Most of them aim to the molecular correction of dystrophin deficiency in selected series of patients with specific mutations. A series of antisense oligonucleotides (AON), such as eteplirsen, have been demonstrated to be able to induce specific exon skipping during splicing, to correct the open-reading-frame, and to restore dystrophin production (36). This potential of this therapy has been so far investigated in Duchenne muscular dystrophy patients, showing beneficial results (37), but not in BMD patients. Unfortunately, most of the molecular defects in BMD concern the quantity and quality of dystrophin and are not amenable to correction.

The CRISP/Cas9 technique, which has been shown to partially restore dystrophin expression in cardiac and skeletal muscle in dystrophinopathy mouse model by removing the noncoding introns that flank the mutation-containing exon (38), could be applied in BMD patients, especially in those bearing a gene duplication.

Conclusions

The clinical heterogeneity of BMD is extreme, with patients presenting only with cramps/myalgia syndrome and high CK, other recurrent myoglobinuric episodes, while loss of strength is usually seen in the typical cases. A good term to define this heterogeneous group of disorders/phenotypes is *mild dystrophinopathy*.

Since some cases have a predominant heart phenotype, their cardiac prognosis is crucially determined by an adequate treatment aimed to prevent cardiac failure and death. In selected patients, cardiac transplantation may be offered as a life-saving therapy, which is able to considerably prolong life expectancy.

Although new molecular therapies are emerging as a future way to systemically treat dystrophin deficiency, at the moment no curative treatments are available, and we can only treat symptoms, such as muscle functional impairment with steroids, heart failure with specific drugs and transplantation, joint contractures with physical therapy.

However, to obtain a long survival, it is of crucial importance that patients with BMD stay in shape and continue to use their muscles. This can include physical therapy treatment and aerobic exercise, which are directed to maximize muscle function.

Conflict of interest

The Authors declare to have no conflict of interest.

References

1. Monaco AP, Bertelson CJ, Middlesworth W, et al. Detection of deletions spanning the Duchenne muscular dystrophy locus using a tightly linked DNA segment. Nature 1985;316:842-5.
2. Angelini C, Fanin M, Pegoraro E, et al. Clinical-molecular correlation in 104 mild X-linked muscular dystrophy patients: characterization of subclinical phenotypes. Neuromusc Disord 1994;4:349-58.
3. Angelini C, Fanin M, Freda MP, et al. Prognostic factors in mild dystrophinopathies. J Neurol Sci 1996;142:70-8.
4. Melacini P, Fanin M, Danieli GA, et al. Cardiac involvement in Becker muscular dystrophy. J Am Coll Cardiol 1993;22:1927-34.
5. Melacini P, Fanin M, Danieli GA, et al. Myocardial involvement is very frequent among patients affected with subclinical Becker muscular dystrophy. Circulation 1996;94:3168-75.
6. Nigro G, Comi LI, Politano L, et al. Evaluation of the cardiomyopathy in Becker muscular dystrophy. Muscle Nerve 1995;18:283-91.
7. Melacini P, Gambino A, Caforio A, et al. Heart transplantation in patients with inherited myopathies associated with end-stage cardiomyopathy: molecular and biochemical defects on cardiac and skeletal muscle. Transplant Proc 2001;33:1596-9.
8. Papa AA, D'Ambrosio P, Petillo R, et al. Heart transplantation in patients with dystrophinopathic cardiomyopathy: review of the literature and personal series. Intract Rare Dis Res 2017;6:95-101.
9. Hoffman EP, Kunkel LM, Angelini C, et al. Improved diagnosis of Becker muscular dystrophy by dystrophin testing. Neurology 1989;39:1011-7.
10. Fiorillo AA, Heier CR, Novak JS, et al. TNF-α-Induced microRNAs control dystrophin expression in Becker muscular dystrophy. Cell Rep 2015;12:1-13.
11. Roberts TC, Godfrey C, McClorey G, et al. Extracellular microRNAs are dynamic non-vesicular biomarkers of muscle turnover. Nucleic Acids Res 2013;41:9500-13.
12. Chico L, Ricci G, Cosci O, et al. Physical exercise and oxidative stress in muscular dystrophies: is there a good balance? Arch Ital Biol 2017;155:11-24.
13. Phaniendra A, Jestadi DB, Periyasamy L. Free radicals: properties, sources, targets, and their implication in various diseases. Indian J Clin Biochem 2015;30:11-26.
14. Markert CD, Ambrosio F, Call JA, et al. Exercise and Duchenne muscular dystrophy: toward evidence-based exercise prescription. Muscle Nerve 2011;43:463-78.
15. Blake DJ, Tinsley JM, Davies KE. Utrophin: a structural and functional comparison to dystrophin. Brain Pathol 1996;6:37-47.
16. Dombernowsky NW, Olmestig JNE, Witting N, et al. Role of neuronal nitric oxide synthase (nNOS) in Duchenne and Becker muscular dystrophies. Still a possible treatment modality? Neuromusc Disord 2018;28:914-26.
17. Heydemann A. Skeletal muscle metabolism in Duchenne and Becker muscular dystrophy – implications for therapies. Nutrients 2018;10. pii: E796.
18. Ferraro E, Giammarioli AM, Chiandotto S, et al. Exercise-induced skeletal muscle remodeling and metabolic adaptation: redox signaling and role of autophagy. Antioxid Redox Signal 2014;21:154-76.
19. Angelini C, Beggs AH, Hoffman EP, et al. Enormous dystrophin in a patient with Becker muscular dystrophy. Neurology 1990;40:808-12.
20. Milasin J, Muntoni F, Severini GM, et al. A point mutation in the 5' splice site of the dystrophin gene first intron responsible for X-linked dilated cardiomyopathy. Hum Molec Genet 1996;5:73-9.
21. Towbin JA, Hejtmancik JF, Brink P, et al. X-linked dilated cardiomyopathy. Molecular genetic evidence of linkage to the Duchenne

muscular dystrophy (dystrophin) gene at the Xp21 locus. Circulation 1993;87:1854-65.

22. Monaco AP, Bertelson CJ, Liechti-Gallati S, et al. An explanation for the phenotypic differences between patients bearing partial deletions of the DMD locus. Genomics 1988;2:90-5.
23. Bello L, Campadello P, Barp A, et al. Functional changes in Becker muscular dystrophy: implications for clinical trials in dystrophinopathies. Scient Rep 2016;6:32439.
24. Nigro G, Comi L, Politano L, et al. The incidence and evolution of cardiomyopathy in Duchenne muscular dystrophy. Int J Cardiol 1990;26:271-7.
25. Politano L, Nigro G. Treatment of dystrophinopathic cardiomyopathy: review of the literature and personal results. Acta Myol 2012;31:24-30.
26. Duboc D, Meune C, Lerebours G, et al. Effect of perindopril on the onset and progression of left ventricular dysfunction in Duchenne muscular dystrophy. J Am Coll Cardiol 2005;45:855-7.
27. Raman SV, Hor KN, Mazur W, et al. Eplerenone for early cardiomyopathy in Duchenne muscular dystrophy: a randomized, double-blind, placebo-controlled trial. Lancet Neurol 2015;14:153-61.
28. Breitenbach S, Lehmann-Horn F, Jurkat-Rott K. Eplerenone repolarizes muscle membrane through Na,K-ATPase activation by Tyr10 dephosphorylation. Acta Myol 2016;35:86-9.
29. Angelini C. Prevention of cardiomyopathy in Duchenne muscular dystrophy. Lancet Neurol 2015;14:127-8.
30. Bardoni A, Sironi M, Felisari G, et al. Absence of brain Dp140 isoform and cognitive impairment in Becker muscular dystrophy. Lancet 1999;353:897-8.
31. Magliano L, Scutifero M, Patalano M, et al. Integrated care of muscular dystrophies in Italy. Part 2. Psychological treatments, social and welfare support, and financial costs. Acta Myol 2017;36:41-5.
32. Cantone M, Catalano MA, Lanza G, et al. Motor and perceptual recovery in adult patients with mild intellectual disability. Neural Plast 2018;2018:3273246.
33. Sveen ML, Jeppesen TD, Hauerslev S, et al. Endurance training improves fitness and strength in patients with Becker muscular dystrophy. Brain 2008;131:2824-31.
34. Sveen ML, Andersen SP, Ingelsrud LH, et al. Resistance training in patients with limb-girdle and Becker muscular dystrophies. Muscle Nerve 2013;47:163-9.
35. Jensen BR, Berthelsen MP, Husu E, et al. Body weight-supported training in Becker and limb girdle 2I muscular dystrophy. Muscle Nerve 2016;54:239-43.
36. Goemans NM, Tulinius M, Van den Akker JT, et al. Systemic administration of PRO051 in Duchenne's muscular dystrophy. N Engl J Med 2011;364:1513-22.
37. Mendell JR, Goemans N, Lowes LP, et al. Longitudinal effect of eteplirsen versus historical control on ambulation in Duchenne muscular dystrophy. Ann Neurol 2016;79:257-71.
38. Long C, Amoasii L, Mireault AA, et al. Postnatal genome editing partially restores dystrophin expression in a mouse model of muscular dystrophy. Science 2016;351:400-3.

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The first Portuguese family with NEFL-related Charcot-Marie-Tooth type 2 disease

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CMT disease caused by *NEFL* gene mutations is rare. The mode of inheritance can be dominant or recessive and nerve conduction velocities can be normal, reduced (demyelinating) or presenting intermediate values. Two Portuguese adult related members in two successive generations were affected by peripheral neuropathy, one with a chronic ataxic peripheral neuropathy and the other with a classical Charcot-Marie-Tooth phenotype. An axonal sensorimotor peripheral neuropathy was described at neurophysiology. A missense heterozygous mutation, c.794A > G (p.Tyr265Cys), in the *NEFL* gene was found in both patients. This is the first Portuguese family reported with NEFL-related CMT type 2.

Key words: CMT type 2E, *Neurofilament light* gene mutation, *NEFL* gene, NEFL Tyr265Cys mutation

Introduction

The neurofilament light-chain polypeptide (NEFL) is a constituent of neurofilaments, the major intermediate filament of neurons and axons, playing a pivotal role in the maintenance of the cytoskeleton (1). Mutations in the neurofilament light-chain polypeptide gene (*NEFL*) are responsible for 2% of all cases of CMT (2). Dominant axonal (CMT 2E) (3) and demyelinating phenotypes (CMT 1F) (2), and rarely recessive axonal CMT (4), caused by *NEFL* gene mutations have been described. CMT caused by *NEFL* gene mutations is clinical and electrophysiological heterogenous (2, 3, 5, 6).

Herein, we report the clinical, neurophysiologic and molecular findings of the first Portuguese kindred with CMT type 2E, caused by a missense heterozygous mutation, variant c.794A > G (p.Tyr265Cys), in the *NEFL* gene.

Clinical cases

Patient 1

The patient is a 68-year-old woman, born of a second-degree consanguineous marriage. Her mother and one maternal aunt were suspected of having a similar neuromuscular condition, but were not available to examination (Fig. 1). She had a normal motor and intellectual childhood development and experienced an active professional life until retirement.

At the age of 42, she reported the beginning of gait difficulties and numbness in her feet. A few years later, the gait difficulties became worse with occasional falls and she began experiencing pain in the feet with sporadic exacerbations.

Neurological examination at the age of 66, revealed a wide-based ataxic gait, needing support in turns. Walking on heels and tiptoes was difficult, mainly due to ataxia. The Romberg sign was positive. Manual muscle examination did not reveal distal or proximal muscle weakness in the upper and lower limbs. Myotatic reflexes were abolished in the lower limbs and reduced distally in the upper limbs. There was a stocking and glove pattern of diminished tactile and pain sensation, with absent vibratory sensation in the feet and reduced in the upper limbs (10 seconds). Pseudo-athetosis was absent. No cerebellar signs were noted and cranial nerves evaluation was unremarkable. Serum vitamin B12 values were normal and there was no megaloblastic anemia.

Patient 2

The patient is a 47-year-old woman, the single offspring of a non-consanguineous marriage, daughter of Patient 1 (see Figure 1). She presented a normal motor and intellectual development in childhood. At the age of

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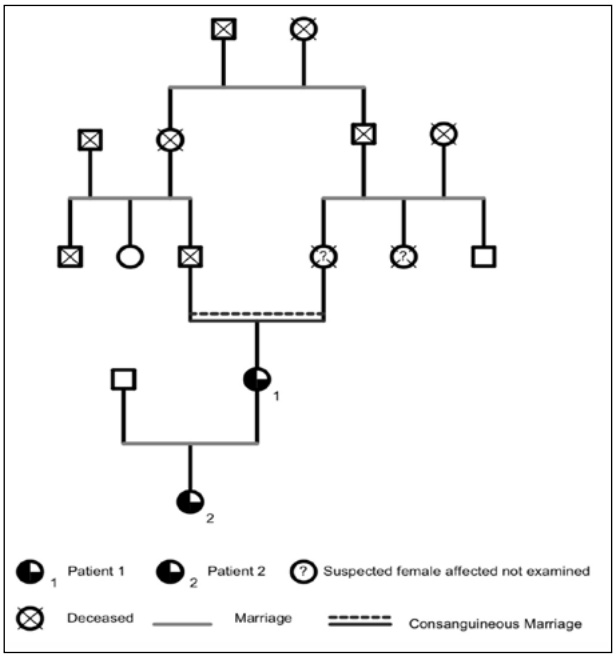

Figure 1. Pedigree of family 1.

33, she reported increasing gait difficulties, without any sensory or balance complaints. At 43 years of age, she underwent bilateral corrective orthopedic surgery of *pes cavus*.

At the age of 46, neurologic examination revealed bilateral *pes cavus*, hammer toes deformity on the left foot and an inverted champagne appearance of the legs. She walked with a steppage gait and walking on tiptoes was possible. In the lower limbs the extensor muscles of the feet were weak bilaterally (3-/5 MRC). No muscle weakness was present in the upper limbs. Myotatic reflexes were abolished throughout. Tactile and pain sensations were apparently normal and vibratory sensation was slightly reduced distally in the lower limbs (10 seconds). No cerebellar signs were observed and cranial nerves evaluation was unremarkable.

Neurophysiological assessment

In Patient 1, motor and sensory nerve conduction studies disclosed a length-dependent axonal sensorimotor neuropathy, with bilateral absent sural and peroneal motor responses (recorded in the feet). Median motor nerve conduction velocity and distal motor amplitude values were of 50 m/s and 4.6 mV, respectively. Somatosensory evoked potentials after median and tibial nerves stimulation did not show sensory responses at peripheral and central levels, which was interpreted as a result of profound peripheral nerve sensory involvement. In Patient 2, the values of median motor nerve conduction velocity and distal motor amplitude were of 56 m/s and 4 mV, respectively. Needle examination of the tibial anterior muscle showed signs of chronic denervation, with a mild reduced muscle recruitment pattern in Patient 1 and severe in Patient 2.

Molecular study

The next generation sequencing (NGS) panel for hereditary peripheral neuropathies, including CMT, was performed through a custom targeted NGS panel. Enrichment was performed by hybrid capture (exons and flanking intronic regions of the 74 target genes) and, after library preparation, the DNA library was subjected to NGS.

A missense heterozygous variant, c.794A > G (p.Tyr265Cys), was detected in the *NEFL* gene in both patients (Fig. 2).

This *NEFL* gene sequence variant was not registered in the Single Nucleotide Polymorphism (dbSNP) or the Genome Aggregation Database (gnomAD), but it is reported as a likely pathogenic variant in ClinVar database (Acession: RCV0001438101). This residue is highly conserved and bioinformatic analysis suggests that this variant is deleterious. Additionally, it has been previously reported in another family (5).

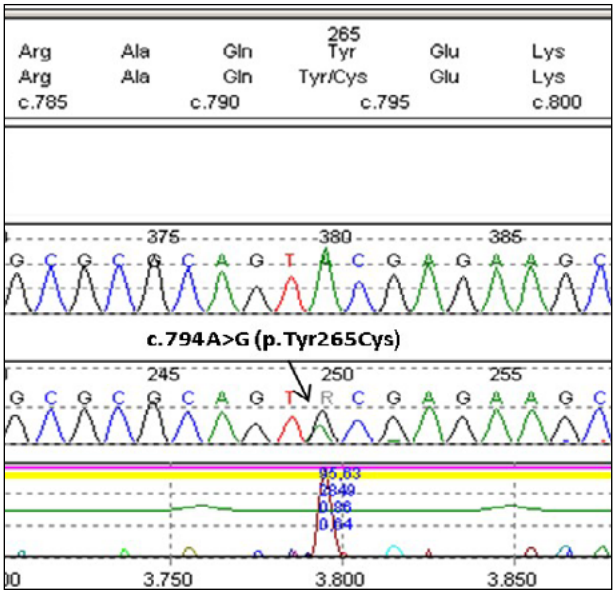

Figure 2. Electropherogram: Molecular study detected a missense heterozygous variant, c.794A > G (p.Tyr265Cys), in the *NEFL* gene in both patients (black arrow).

Discussion

Charcot-Marie-Tooth disease (CMT) is the most common inherited motor and sensory neuropathy and is divided into demyelinating (CMT1) and axonal (CMT2) forms using electrophysiological and pathological criteria. CMT1 is characterized by demyelination and slow nerve conduction velocities (NCVs), whereas CMT2 is characterized by signs of axonal regeneration and normal or slightly reduced NCVs.

To date, mutations in as many as 14 different genes have been implicated in CMT2 (9), including KIF1B (CMT2A1), MFN2 (CMT2A2), RAB7 (CMT2B), TRPV4 (CMT2C), GARS (CMT2D), NEFL (CMT2E), HSPB1 (CMT2F), MPZ (CMT2I/J), GDAP1 (CMT2K), HSPB8 (CMT2L), DNM2 (CMT2M), AARS (CMT2N), LAMIN A (AR-CMT2A) and MED25 (AR-CMT2B). Among them, mutations in MFN2 have been found in approximately 11-24.2% of CMT2 patients, whereas AARS and TRPV4 mutations were only recently identified in limited CMT2 families, and mutations in other genes were found in only a few patients.

Mutations in the neurofilament light chain polypeptide (*NEFL*) gene are present in CMT2E and CMT1F neuropathies, with variable clinical and pathological expressions. Codon 22 is one of the mutational hot spots in the *NEFL* gene. Three types of Pro22 mutations have been previously reported: Pro22Ser in CMT2E with giant axons, Pro22Thr in CMT1F and Pro22Arg in a Korean CMT1 family, associated with demyelinating neuropathy features in CMT1F. Histopathological findings showed onion bulb formations but no giant axons (10-15). Pro22 mutations may influence not only the Thr-Pro phosphorylation site by proline-directed protein kinases but also impact the structure of the NEFL protein in a different way.

We report the first Portuguese family with CMT type 2E. The identified mutation, already described in an Australian family (5), promotes an amino acid exchange of tyrosine by cysteine (Tyr265Cys) and occurs in a highly conserved residue of the *NEFL* gene. Two related affected subjects in two generations carried the mutation. Moreover, it is probable that two more family members were clinically affected by this mutation (Patient 1's mother and maternal aunt).

It has long been recognized that *NEFL* gene mutations are associated with intra-familial phenotypic variability (5, 6), which is present in this Portuguese family, with the oldest patient presenting an ataxic sensory peripheral neuropathy and the youngest one a classical CMT phenotype.

Significant inter-familial phenotypic variability regarding onset, clinical presentation and severity of the disease has already been described (3, 7, 8) and it is obvious when comparing the Australian and Portuguese families with the same *NEFL* gene mutation. The proband of the Australian family presented a more complex and severe neurological involvement, combining central and peripheral nervous system symptomatology with significant clinical disability. In the Australian and Portuguese families, the mode of inheritance was dominant and the peripheral nerve involvement was of the axonal type. Cases of peripheral nerve involvement of the demyelinating type (CMT1F) (2), as well as of the intermediate type (6), have been described, and the mode of inheritance is dominant in the majority of cases, but recessive inheritance has already been reported (4).

Some components of the peripheral nervous system can be clinically more severely affected than others and some patients present central nervous system involvement, with compromise of the pyramidal tract (5) or of the brainstem and cerebellum (6). NEFL-associated CMT nerve pathology is primarily of the axonal type, with focal accumulations of neurofilaments, axonal swellings and significant secondary demyelination (9) and electrophysiology can present axonal, demyelinating and intermediate nerve conductions values, to which correspond CMT type 2E, CMT type 1F and Intermediate NEFL-associated CMT, respectively.

Conflicts of interest

The Authors declare to have no conflict of interest.

References

1. Grant P, Pant HC. Neurofilament protein synthesis and phosphorylation. J Neurocytol 2000;29:843-72.
2. Jordanova A, De Jonghe P, Boerkoel CF, et al. Mutations in the neurofilament light chain gene (*NEFL*) cause early onset severe Charcot-Marie-Tooth disease Brain 2003126:590-7.
3. Mersiyanova IV, Perepelov AV, Polyakov AV, et al. A New variant of Charcot-Marie-Tooth disease type 2 is probably the result of a mutation in the neurofilament-light gene. Am J Hum Genet 2002;67:37-46.
4. Abe A, Numakura C, Saito K, et al. Neurofilament light chain polypeptide gene mutations in Charcot-Marie-Tooth disease: nonsense mutation probably causes a recessive phenotype. J Hum Genet 2009;54:94-7.
5. Drew AP, Zhu D, Kidambi A, et al. Improved inherited peripheral neuropathy genetic diagnosis by whole-exome sequencing. Mol Genet Genomic Med 2015;3:143-54.
6. Berciano, Peeters K, Garcia A, et al. NEFL N98S mutation: another cause of dominant intermediate Charcot-Marie-Tooth disease with heterogeneous early onset phenotype. J Neurol 2016;263:361-9.

7. De Jonghe P, Mersivanova I, Nelis E, et al. Further evidence that neurofilament ligt chain gene mutations can cause Charcot-Marie-Tooth disease type 2E. Ann Neurol 2001;49:245-9.
8. Zuchner S, Vorgerd M, Sindern E, et al. The novel neurofilament light (NEFL) mutation Glu397Lys is associated with a clinically and morphologically heterogeneous type of Charcot-Marie-Tooth neuropathy. Neuromusc Disord 2004;14:147-57.
9. Hoyle JC, Isfort MC, Roggenbuck J, et al. The genetics of Charcot-Marie-Tooth disease: current trends and future implications for diagnosis and management. Appl Clin Genet 2015;8:235-43.
10. Fabrizi GM, Cavallaro T, Angiari C, et al. Giant axon and neurofilament accumulation in Charcot-Marie-Tooth disease type 2E. Neurology 2004;62:1429-31.
11. Fabrizi GM, Cavallaro T, Angiari C, et al. Charcot-Marie-Tooth disease type 2E, a disorder of the cytoskeleton. Brain 2007;130:394-403.
12. Georgiou DM, Zidar J, Korosec M, et al. A novel NF-L mutation Pro22Ser is associated with CMT2 in a large Slovenian family. Neurogenetics 2002;4:93-6.
13. Yoshihara T, Yamamoto M, Hattori N, et al. Identification of novel sequence variant in the neurofilament light gene in a Japanese population: analysis of Charcot-Marie-Tooth disease patients and normal individuals. J Peripher Nerv Syst 2002;7:221-4.
14. Shin JS, Chung KW, Cho SY, et al. NEFL Pro22Arg mutation in Charcot-Marie-Tooth disease type 1. J Hum Genet 2008; 53:936-40.

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CASE REPORT

Acute sensorimotor polyneuropathy as an early sign of polyarteritis nodosa. A case report

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We examined a patient aged 31 who had a sudden burning paraesthesia, pain and numbness in the lower legs together with an increased temperature of 39°C. Clinical examination showed asymmetrical sensory polyneuropathy more clearly seen in the lower legs and the left wrist, with high ESR (up to 44 mm/h), leukocytosis, slight anemia and proteinuria. CSF was normal. After three weeks the temperature suddenly increased again up to 39°C and severe flaccid distal tetraparesis was seen more clearly with foot drop in the left lower leg and dense oedema in the left wrist, purple cyanosis and haemorrhagic foci appeared on the skin of the toes, feet, lower legs and left wrist. ESP increased up to 65 mm/h, CK was 200 IU (normal ≤ 190 IU) and hypergammaglobulinaemia developed. An EMG study showed sensorimotor, mainly axonal, polyneuropathy with different degrees of involvement of some nerves and with conduction block in the left ulnar nerve. Muscle biopsy revealed findings of inflammatory vasculitis that resembled polyartheritis nodosa with secondary denervation atrophy and non-specific myositis. The patient was treated with high doses of prednisolone, dexamethasone and cyclophosphamide with plasmapheresis. Motor disturbances and pain decreased and the patient began walking with a stick. However, the necrosis of the toes gradually progressed to dry gangrene and amputations of the toes were carried out three months after the disease began. At that time the patient had the clinical features of multisystem disease with progressive heart, lung, liver and kidney failure. The patient died suddenly of pulmonary artery thrombo-embolism a year after the onset of the disease. An autopsy confirmed the diagnosis of polyarteritis nodosa (PN). Thus, in this patient the asymmetrical sensory polyneuropathy progressed rapidly in symmetrical sensorimotor peripheral polyneuropathy which preceded the clinical multisystem involvement in polyarteritis nodosa.

Key words: asymmetrical sensory polyneuropathy, burning paraesthesia, necrosis of the toes, progressive heart, lung, liver and kidney, polyarteritis nodosa

Note. This article was presented as Abstract under name " Neurological complication on periarteritis nodosa" 1999,pp.117-119 in Russian medical Collection "Questions of clinical neurology" ed. By Prof. N.M. Zulev (Moscow) and as Abstract under the name "Acute sensorimotor polyneuropathy as an early sign of polyarteritis nodosa" in the Collection valled "XXIVTH Oxford Symposium on Muscle Disease", Oxford 1999, p. 24.

Introduction

Polyarteritis nodosa (PN) is one of the systemic necrotic vasculitis. The characteristic pathological feature is inflammation and necrosis of vascular walls of small and middle sizes arteries. These changes are the basis for the infarction and ischemia which can be seen in different tissues and organs. Lesion of the nervous system occurs in 50% of cases with PN (1) The classical example of a peripheral nervous system lesion in PN is mononeuritis multiplex or asymmetrical peripheral motorsensory polyneuropathy. Often neurological signs appear after polyorganic pathology in PN patients, but in some cases lesions of peripheral or central nervous systems may be the first sign of the disease (2-4).

Case report

A male patient aged 31 was admitted to the Neurological Department complaining of burning pains and numbness of the distal parts of the legs, and difficulty in walking due to severe pain. His temperature rose to 39°C and symptoms of nasal congestion appeared. Four days after the onset he was hospitalized with the diagnosis of

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Guillain-Barre Syndrome. However two weeks after admission his general conditions deteriorated with increasing neurological abnormalities. His temperature suddenly increased again to 39ºC. Pain burning and numbness increased in the legs and the same symptoms appeared in the hands. He complained of asymmetrical weakness of the distal parts of the legs and small hand muscles, worse on the left. Marked oedema of feet and toes appeared which spread over the lower legs. There was a change of skin of feet toes and lower legs.

The pulse was 96, the blood pressure increased to 160/90 mmHg. The patient was normally orientated in place and time. Cranial nerves were normal. Chest and abdomen were normal. The flexors of the neck were spared. All the small muscles of the hand were severely atrophied and a moderate atrophy of the distal part of forearms was seen. Muscular strength was measured by MRC scale (grade 1-5): shoulder girdle, deltoid, upper arms and extensors of the wrists, grade 5; extensors digitorum communis and flexors of the wrists, grade 3; interossei volaris and dorsalis and lumbricales, grade 1-2, with both side involved, more clearly on the left. Muscle tone in the upper limbs was decreased. Deep reflexes of the arms were reduced. Abdominal reflexes were absent. There was a moderate atrophy of the thigh and a severe atrophy of shin muscles. Proximal muscle power including hip and knee flexion and extension was normal. There was no movement of the ankle and toes on the left, and power was reduced to 1-2 grades on the right. Muscle tone and knee reflexes were reduced. The ankle jerks and plantar responses were absent on both sides.

There was dysesthesia, hyperpathia and hyperesthesia of all kinds of sensation in the toes, feet and lower third of the lower legs. Lasègue sign was positive. Palpation of ulnar, radial and especially peroneal communis, tibial and dorsal pedis nerves was painful. Palpation of arm and leg muscles was painless. Dense oedema of the lower legs and feet worsen on the left. Purple cyanosis and hemorrhagic foci were seen on the skin of the feet, lower legs, back of the hands and distal part of the left arm. There were necrotic changes of the distal phalanges of the first and third toes of the left foot.

Blood analysis showed a decrease of Hb (to 117 g/L) and erythrocytes (to 3.5 x 10/1), moderate leukocytosis up to 9.3 x 10/1. ESV increased to 69 mm/h. The blood levels of sugar, bilirubin, creatine, urea, cholesterol, serum protein, potassium, calcium and phosphorus were within the normal limits; alpha-1 and alpha-2 globulins were increased up the normal levels. Blood levels of ALT and AST were increased up to 97 U/I (normal, less than 43 U/I) and 156 U/I (normal, less than 34 U); CK was greatly increased up to 2190 U/I (normal below 190 U/I). There was proteinuria 0.39 g/L.

ECG showed a sinus tachycardia of 110 per min. Fibrogastroscopy was normal. Repeated chest X-ray radiographies showed signs of slight oedema of the lungs and possible pericarditis. Ultrasonography showed slight hepatosplenomegaly.

Needle EMG (gastrocnemius, tibialis anterior, extensor digitorum communis and abductor pollicis brevis muscles) showed many fibrillation and single/moderate fasciculation potentials, with positive sharp waves at rest. Many long duration and increased amplitude of MUPs appeared on minimal voluntary contraction. On maximum contraction a discrete pattern was evident. 25-33% of the action potentials were polyphasic, suggesting a neurogenic process.

On electric stimulation of the saphenous and afferent fibres of median nerves the motor responses were absent. Sensory right ulnar nerve conduction velocities were greatly decreased in distal and mildly slowed in proximal parts (14 and 44 m/sec, respectively). Terminal latency was markedly increased up to 7.9 msec.

Motor nerve conduction velocities in the distal parts of right and left ulnar nerves were mildly slowed (44 and 38 m/sec, respectively), but in the proximal parts of ulnar and in the distal and proximal parts of the median nerves were within normal limits. The terminal latencies were increased (in median nerves 6.6 and 4.3 m/sec, and in the ulnar nerves 3/7 and 4 m/sec). The compound muscle action potentials (CMAPs) were markedly decreased in amplitude on stimulation of the ulnar (1 and 0.1 mV) and median (0.5 and 1.2 mV) nerves. On stimulation of the right peroneal nerve the very lower amplitude (0.7 mV) response was seen only in the tibial anterior muscle. Motor conduction velocity was greatly decreased to 24 m/sec (below fibular head-popliteal fossa).

Left motor ulnar nerve conduction showed partial conduction block between the elbow and wrist. The amplitude of the CMAPs was reduced by 79% and the area by 88%. The criterion for partial conduction block was a 50% or greater reduction in amplitude and a 40% or greater reduction in negative peak area of surface-recorded CMAPs obtained with proximal compared with distal stimulation of ulnar nerve, in the absence of increased duration of CMAPs more than 20%, according (3).

These ENMG findings showed mainly axonal sensorimotor polyneuropathy with different degrees of affection of some nerves, more clearly seen in the lower limbs with partial motor conduction block in the left ulnar nerve (see Tables 1-2).

The biopsy (stain H.E. and Van Gieson) performed at the right gastrocnemius muscle showed various changes of the majority of epimysial, perimysial and endomysial arteries of medium and small sizes. Simultaneous atrophy of almost all muscle fibres with knots and chains of

Table 1. Electrophysiological data in the affected motor nerves.

Nerve	Amplitude (mV)	Duration (ms)	CV m/s	Terminal latency (ms)	Are mV ms
Ulnar R.	1.0	9.5	**44/69**	4.0	5.0
Ulnar L.	**0.1**	**3.8**	**38/55**	**3.7**	**0.2**
Median R.	0.5	8.4	**56/68**	4.3	1.5
Median L.	1.2	17.6	**54/64**	6.6	10.5
Peroneal R.	0.7	18.6	**24** (below fibular head-popleteal fossa)	2.9	-

Table 2. Site and value of the conduction block and electrophysiological data in the left ulnar nerve.

Level	Amplitude (mV)	R1	Area (mV ms)	R2	Duration (ms)	CV m/s	Terminal latency (ms)
	Prox. Distal		Prox. Distal		Prox. Distal		
Below elbow	0.13 0.60	0.2 (79%)	0.23 2.08	0.1 (88%)	3.7 6.5	38/55	3.7

dark sarcolemmal striations were invisible and sarcolemmal nuclei disoriented. In the transverse section, in some regions uniform small fibers with degenerative changes, dark cluster nuclei and mononuclear infiltration were also seen. In other regions of the sections increased variation of the diameter of muscle fibres, loss of few fibers, focal necrosis of some muscles with phagocytosis, increased of connection tissue in the endomysium and cellular infiltration were evident. The skin biopsy from right lower leg, at the site of muscle biopsy, showed smoothing of the papillar layer and oedema with inflammatory mononuclear infiltration in the arterial and venous walls (Figs. 1-6).

Cross section of biopsy of right gastrocnemius muscle (formol-calcium, Van Gieson stain) (Figs. 1-6).

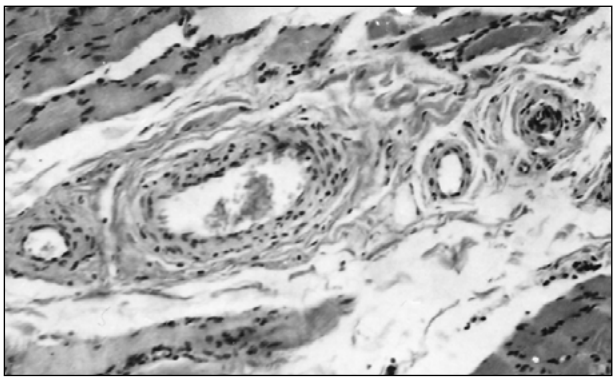

Figure 2. Thickening and inflammatory cellular infiltration in the arterial walls with trombosis of a small muscle artetia. X 200

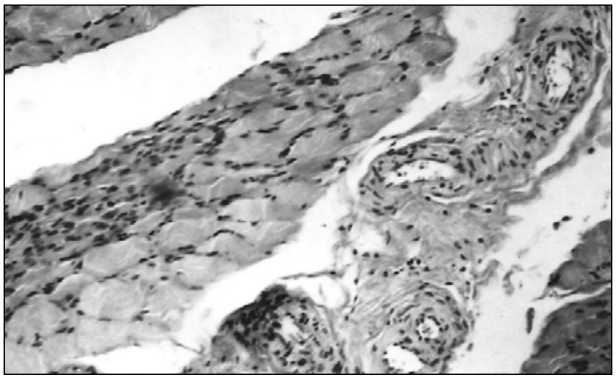

Figure 1. Thickening in the arterial walls with mononuclear cellular infiltration in the arterial walls and around arteries with stenosis of their lumens. Cellular infiltration of the muscle is seen, as well. X 80

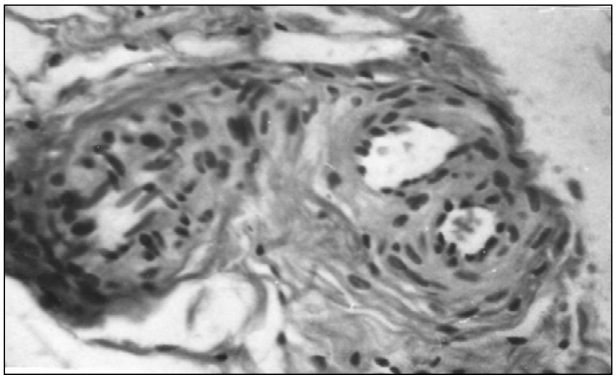

Figure 3. The same section. Foci of fibrinoid necrosis with inflammatory infiltration and with marked thickening of the arterial walls with marked stenosis of the lumen of arteries. X 400

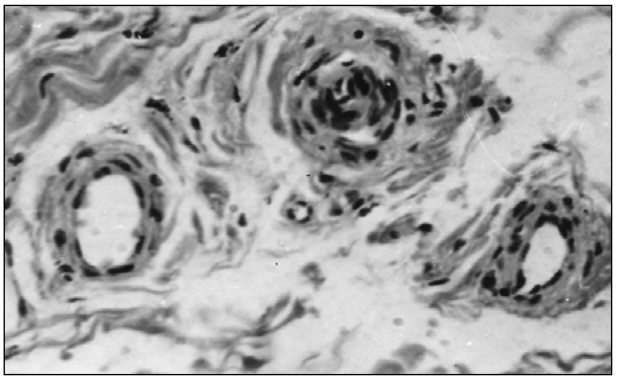

Figure 4. The same section. Almost full occlusion of the small muscle artery with trombosis. Foci of fibrinoid necrosis with mononuclear cellular infiltration is seen. X 400

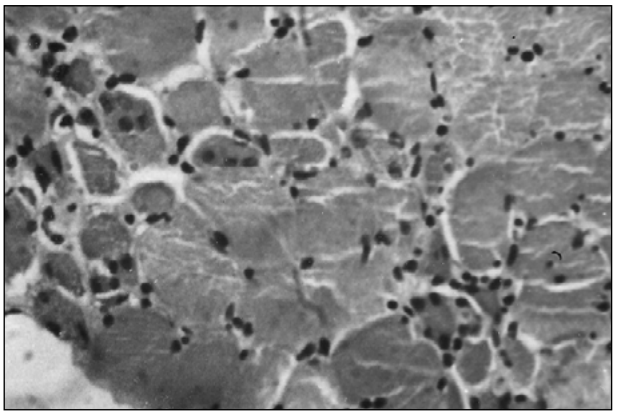

Figure 5. Two small foci of infarction with necrosis and hyalinosis of muscle fibres and phagocytosis. X 200

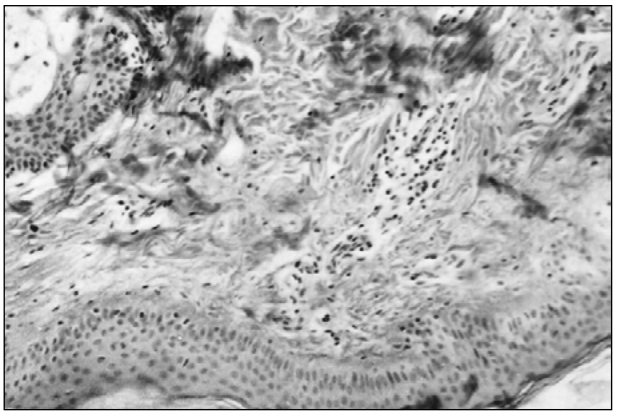

Figure 6. The skin biopsy (Van Gieson stain). The smooth papillar layer and oedema of dermis with inflammatory cellular infiltration in the vascular walls.

The muscle biopsy findings were compatible with productive inflammatory vasculitis that resembled polyarteriitis nodosa with secondary neural muscular atrophy and nonspecific myositis.

The patient was treated with intravenous infusions of dexamethasone 150 mg daily for 5 days, then prednisolone – 500 mg daily – for 5 days and later with prednisolone, 90 mg/day per os in association with intravenous infusion of cyclophosphamide, 200 mg/day for 15 days, followed by cyclophosphamide, 150 mg/day per os, and plasmapharesis.

Sensory and motor disturbances decreased and the patient began walking with a cane. The skin swelling decreased, ESR and leucocytosis fell, but a pronounced trophic and vegetative vascular disorders persisted in toes. The necrosis of the toes gradually progressed to dry gangrene. The patient was transferred to a surgical department; further he developed a progressive heart, lung, liver and kidney insufficiency, despite a moderate regression of the neurological symptoms. The patient suddenly died of thromboembolism of the pulmonary artery one year after the onset of the disease. The autopsy confirmed the diagnosis of polyarteritis nodosa.

Discussion

The first symptoms of the disease indicated a peripheral nervous system involvement, namely, an asymmetrical sensory neuropathy, without signs of systemic or polyorganic involvement. Furthermore, positive sensory disorders (pain, paresthesia, hyperpathia,hyperalgesia) in legs were the main clinical signs in the early stages of the disease, before the appearance of motor disorders. The asymmetrical sensory polyneuropathy rapidly progressed to a symmetrical sensorimotor peripheral polyneuropathy which anticipated the multisystemic manifestastions.

The ENMG and needle EMG findings confirmed the typical manifestation of sensorimotor peripheral polyneuropathy showing the characteristic axonal nerve lesions. However, one of the peculiarities of the ischemic neuropathy in our patient was the signs of partial conduction block in the left ulnar nerve (3, 4). At our knowledge, there are only a few descriptions of the appearances of conduction block in ischemic peripheral nerve lesion in medical literature (1).

The unusual feature of the severe trophic disorder of the distal parts of the legs not responding to specific therapy and leading to dry gangrene of the toes, remains unexplained.

Conflict of interest

The Authors declare to have no conflict of interest.

References

1. Ropert A, Metral S. Conduction block in neuropathies with necrotizing vasculitis. Muscle Nerve 1990;13:102-5.
2. Daube JR, Dyck P. Neuropathy due to peripheral vascular discas. In: Dyk PJ, Thomas PK, Lambert EH, Eds. Peripheral neuropathy. Philadelphia: Saunders 1984, pp. 1458-78.
3. Feasby TE, Brown WF, Gilbert JL. The pathological basis of conduction block in human neuropathies. J Neurol Neurosurg Psychiatry 1985;48:239-44.
4. Vital A, Vital C. Polyarteritis nodosa and peripheral neuropathy. Intrastructural study of 13 cases. Acta Neuropathol 1985;67:136-41.
5. Daniels I, Williams M, Worthingham C. Muscle testing. Techniques of manual examination, 2nd ed. Philadelphia: Saunders 1949.

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NEWS FROM AROUND THE WORLD

AIM

The XIX Congress of the Italian Association of Myology (AIM) was held in Bergamo from 5 to 8 June 2019; the event saw eighty speakers alternate on stage in front of a group of 189 participants.

The congress was preceded by a satellite Symposium the 5 June, organized by Prof. D'Antona of the "Voghera Sports Medicine Center" of the University of Pavia, Italy, and by Dr. Angela Berardinelli, of the "Neurological Institute Casimiro Mondino" of Pavia, Italy. The Symposium entitled: "Physical exercise: pros and cons of taking care of myopathic patients", saw the presence of more than 100 participants, who attended the reports held by international experts of the highest level, including Prof. Vissing, (President of the upcoming 24th International Annual Congress of the World Muscle Society in Copenhagen), who illustrated data from basic research and clinical experience, pros and against physical activity as part of the process of taking care of patients with neuromuscular disease.

Immediately after the Symposium, work continued in Bergamo, "Città Alta", where, in the scenic "Aula Magna" of the University of Bergamo, Prof. Carlo Minetti – President of AIM – and Dr. Angela Berardinelli – President of the Congress – opened the AIM Conference. On the same day of June 5, a round table was also held open to Patient Associations.

The second day the Congress moved to the Social Theatre, with a rich program of contributions. Also this year various aspects of hereditary and acquired diseases of muscles and of neuromuscular junction were discussed, in line with the most recent and updated data of the scientific literature. In particular, Prof. Bjarne Udd from Helsinki reported on "Titin Role in NMDs: overview and new phenotypes". In the third day of the Congress Prof. Toscano reported on the results of the "Neuromuscular Days 2019", held in last March; Dr. Adele D'Amico (AIM) and Dr. Anna Ambrosini for Telethon Foundation, Italy. Prof. Gabriele Siciliano and Prof. Alessandra Ferlini reported on ERNs of interest for neuromuscular field. A prominent guest from Copenhagen, Prof. John Vissing, spoke about "MRI in muscle disorders".

The last day was an occasion to celebrate the Prizes for the best contributions and the farewell to Matera site of the next XX National Congress. Young researchers were honoured for their contributions. The "Giovanni Nigro" award was assigned to dr. A. Genazzani from Vercelli, while the AIM award was conferred to Dr. M. Sframeli from Messina. Doctors M.S. Falzarano from Ferrara and S. Baratto from Genoa were the winners of the Poster prizes. In conclusion, the exciting "passing the bell" between Dr. Angela Berardinelli, President of the Congress of Bergamo and Dr. Pietro Masciandaro, President of the next AIM National Congress to be held in Matera in June 2020. (Information from the Association's Secretariat)

MSM

The 14th Meeting of the Mediterranean Society of Myology (MSM) will probably be held in 2020 in Sicily, organised by prof. Antonio Toscano.

WMS

The 24th annual congress of the World Muscle Society will be held in Copenhagen from 1st to 5th October, in the old Tivoli Garden Concert Hall and adjoining buildings. Join WMS for the networking reception to be held on Tuesday 1st October in the theatre, Det Ny Teater, located a 5-minute walk from Tivoli gardens. This will follow the long tradition of WMS to facilitate networking and catch up on the latest developments in myology around the world during this 4-day meeting.

As usual, the meeting will be preceded by a teaching course, which will be held in Copenhagen on September 30th and October 1st 2019.

The Copenhagen Neuromuscular Center at the National Hospital, Rigshospitalet, led by John Vissing, will host and organise this meeting. The main thematic topics that will be addressed in the plenary sessions will be:

1. Metabolic disturbances in neuromuscular diseases
2. Extra-muscular manifestations in neuromuscular diseases
3. Advances in the treatment of neuromuscular disorders

FORTHCOMING MEETINGS

2019

August 30 – September 2

National LGMD Conference 2019, Chicago, USA. Information: *https://nationallimbgirdlemusculardystrophyconference.com*

August 31- September 4

ESC Congress 2019 together with World Congress of Cardiology. Paris, France. Information: website: *https://escardio.org*

September 2-5

9th UK Nuclear Envelope and Chromatin Organization Meeting - 3rd International Meeting on Laminopathies. London, UK. Information: website: *https://www.laminopathiesmeeting2017.com*

September 13-14

The Myotonic Dystrophy Foundation Annual Conference, Philadelphia, PA, USA. Information: *https://www.myotonic.org›2019-myotonic-annual-conference*

September 20-22

Muscle Study Group Annual Scientific Meeting, Snowbird, UT, USA. Information: website: *https://musclestudygroup.org_*

September 23-25

2nd Annual NMD Summit 2019 | Neuromuscular Drug Development, Boston, MA, USA. Information: website: *https://nmd-summit.com*

October 1-5

24th Congress of World Muscle Society. Copenhagen, Denmark. Information: website: *https://www.worldmusclesociety.org*

October 12-15

50th Congress of Italian Society of Neurology, Bologna, Italy. Information: website: *http://www.neuro.it*

October 15-19

2919 ASHG Annual Meeting. Houston, TX, USA. Information: website: *www.ashg.org*

October 16-19

American Association of Neuromuscular & Electrodiagnostic Medicine, Austin, TX, USA. Information: website: *https://www.aanem.org*

October 24-27

Asia Pacific Heart Rhythm Society (APHRS). Centara Grand & Bangkok Convention Centre at CentralWorld. Bangkok, Thailand. Information: website: *https://www.aphrs.org*

October 28-30

20th Italian Telethon Convention. Riva del Garda, Italy. Information: website: *https://www.telethon.it*

October 29-30

TREAT-NMD SMA Expert Masterclass. London, UK. Information: website: *https://treat-nmd.org*

November 13-15

3rd International Conference on Genomic Medicine (GeneMed-2019), Baltimore, USA. Information: website: *http://unitedscientificgroup.com/conferences/genemed*

November 15-16

The Action Duchenne International 2019 conference, Hinckley Island, Birmingham, UK. Information: website: *https://www.actionduchenne.org*

November 28 - November 30

XLIV National Congress of Pediatric Neurology Naples, Italy. Information: website: *www.neurologiapediatrica.it*

November 29

Metabolic Myopathies. Meeting in Memory of Stefano di Donato. Milan, Italy. Information: First Class S.r.l. Meetings and Conferences; elettra.marchegiani@fclassevents.com

December 9-11

6th TREAT-NMD International Conference. Leiden, The Netherlands. Information: website: *www.treat-nmd-cnference.org*

2020

February 5-7

2nd International Scientific & Clinical Congress on Spinal Muscular Atrophy. SMA Europe - Evry, France. Information: website: *https://evry2020.sma-europe.eu*

March 11-14

8th Dysferlin conference, Jain Foundation. Orlando, Florida, USA. Information: website: *https://www.jain-foundation.org*

March 16-17

International Conference on Orphan Drugs & Rare Diseases. Berlin, Germany. Information: website: *https://www.meetingsint.com/conferences/orphandrugs-raredisease*

April 25 - May 1

72nd Annual Meeting American Academy of Neurology, Toronto, Ontario, Canada. Information: website: *https://www.aan.com*

June 3-6

XX Congresso Nazionale AIM Giugno 2020. Matera, Italy. Information: website: *https://www.miologia.org*

June 6-9

The European Human Genetics Conference. Berlin, Germany. Information: website: *https:// www.eshg.org*

July 6-9

New directions in Biology and Disease of Skeletal Muscle Conference, New York, NY, USA.
Information: website: *https://myology.institute.ufl.edu/conferences/new-directions*

September 25-29

Muscle Study Group Annual Scientific Meeting, Washington, USA. Information: website: *https://musclestudygroup.org/events/2020-annual-meeting*

September 30 - October 4

25th Congress of World Muscle Society. Halifax, Toronto, Canada. Information: website: *www.worldmusclesociety.org*

October 27-31

ASHG Annual Meeting. San Diego, CA, USA. Information: website: *www.ashg.org*

2021

June 12-15

The European Human Genetics Conference. Glasgow, United Kingdom. Information: website: *https:// www.eshg.org*

September 21-25

26th Congress of World Muscle Society. Prague, Czech Republic. Information: website: *www.worldmusclesociety.org*

October 19-23

ASHG Annual Meeting. Montreal, Canada. Information: website: *www.ashg.org*

For application or renewal to MSM

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Manuscript submission must be effected on line: **www.actamyologica.it** according to the following categories:
Original articles (maximum 5000 words, 8 figures or tables). A structured abstract of no more than 250 words should be included.
Reviews, Editorials (maximum 4000 words for Reviews and 1600 words for Editorials). These are usually commissioned by the Editors. Before spontaneously writing an Editorial or Review, it is advisable to contact the Editor to make sure that an article on the same or similar topic is not already in preparation.
Case Reports, Scientific Letters (maximum 1500 words, 10 references, 3 figures or tables, maximum 4 authors). A summary of 150 words may be included.
Letters to the Editor (maximum 600 words, 5 references). Letters commenting upon papers published in the journal during the previous year or concerning news in the myologic, cardio-myologic or neuro-myologic field, will be welcome. All Authors must sign the letter.
Rapid Reports (maximum 400 words, 5 references, 2 figures or tables). A letter should be included explaining why the author considers the paper justifies rapid processing.
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Standard journal article: Figarella-Branger D, Bartoli C, Civatte M, et al. Cytokines, chemokines and cell adhesion molecules in idiopathic inflammatory myopathies. Acta Myol 2000;19:207-8.
Books and other monographs: Dubowitz V. Muscle disorders in childhood. London: WB Saunders Company Ltd; 1978.
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