

Congenital myopathy associated with a novel mutation in *MEGF10* gene, myofibrillar alteration and progressive course

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Early-onset myopathy, areflexia, respiratory distress, and dysphagia (EMARDD) is caused by homozygous or compound heterozygous mutation in the *MEGF10* gene (OMIM#614399). Phenotypic spectrum of EMARDD is variable, ranging from severe infantile forms in which patients are ventilator-dependent and die in childhood, to milder chronic disorders with a more favorable course (mild variant, mvEMARDD). Here we describe a 22 years old boy, offspring of consanguineous parents, presenting a congenital myopathic phenotype since infancy with elbow contractures and scoliosis. The patient developed a slowly progressive muscle weakness with impaired walking, rhinolalia, dysphagia, and respiratory involvement, which required noninvasive ventilation therapy since the age of 16 years. First muscle biopsy revealed unspecific muscle damage, with fiber size variation, internal nuclei and fibrosis. Myofibrillar alterations were noted at a second muscle biopsy including whorled fibres, cytoplasmic inclusion and minicores. Exome sequencing identified a homozygous mutation in *MEGF10* gene, c.2096G > C (p.Cys699Ser), inherited by both parents. This variant, not reported in public databases of mutations, is expected to alter the structure of the protein and is therefore predicted to be probably damaging according to ACMG classification. In conclusion, we found a new likely pathogenic mutation in *MEGF10*, which is responsible for a progressive form of mvEMARDD with myofibrillar alterations at muscle biopsy. Interestingly, the presence of *MEGF10* mutations has not been reported in Italian population. Early diagnosis of *MEGF10* myopathy is essential in light of recent results from in vivo testing demonstrating a potential therapeutic effect of SSRIs compounds.

Key words: congenital myopathy, *MEGF10*, myofibrillar, respiratory defect

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Introduction

Early-onset myopathy, areflexia, respiratory distress, and dysphagia (EMARDD) is a congenital myopathy caused by homozygous or compound heterozygous mutation in the *MEGF10* gene (OMIM #614399)^{1,2}. Patients with EMARDD often present severe childhood weakness and hypotonia with respiratory distress caused by diaphragmatic paralysis where-

by they become ventilator-dependent, and die in infancy. Within MEGF10 myopathies, milder chronic disorders with a more favourable course and later onset have been described and classified as mild variant or mvEMARDD. These forms can manifest only very mild muscle weakness and mild respiratory dysfunction. Of note, minicores on muscle biopsy are described albeit not in all mvEMARDD patients³⁻⁶.

In 2011, pathogenic variants in the multiple epidermal growth factor-like domains 10 (MEGF10) gene were found to be causative of EMARDD in five independent index patients¹. Since then, just over 20 patients have been reported with variants in *MEGF10*⁶. MEGF10 is a single transmembrane protein with 17-EGF-like domains in the extracellular portion and multiple tyrosine phosphorylation residues in the cytoplasmic domain. It has been proposed that MEGF10 mediates cell-cell adhesion, acts as a phagocytosis receptor for apoptotic cells^{1,7,8}, and mediates signalling such as cell proliferation and differentiation^{9,10}. In skeletal muscle, MEGF10 is mainly expressed in the satellite cell, which is a mononuclear muscle stem cell¹⁰. Thus, MEGF10 appears to be a key regulator of muscle development and repair. Expression of the human MEGF10 transcript is restricted to the adult and foetal brain, spinal cord, and skeletal muscle. High concentrations are also present in the neuromuscular junction¹.

In this study, we report the first Italian patient harbouring a novel homozygous *MEGF10* variant. This subject showed early disease onset with progressive course and muscle biopsy revealed the presence of myofibrillar alterations.

Methods

Tissue and blood samples were obtained after written informed consent from the patient parents and stored in the Muscle Pathology Laboratory for diagnostic and research purposes as approved by Local Ethic Committee.

Exome sequencing (ES) was performed as previously described¹¹ and the computational analysis of candidate variants was performed according to allele frequency, conservation of affected residues, and predicted impact on protein function and structure¹². The candidate pathogenic variant in *MEGF10* was confirmed by Sanger sequencing and segregation was performed in the healthy parents. Web-based softwares were used to predict the pathogenicity of mutations, as reported in the Results section.

Muscle biopsies were obtained from patient quadriceps muscle after local anaesthetic injection and snap frozen in isopentane. Routine histological procedures were performed according to standard protocols and

included Hematoxylin and eosin (H&E), Modified Gomori Trichrome, Cytochrome Oxidase (COX), Succinate Dehydrogenase (SDH), Nicotinamide adenine dehydrogenase (NADH), Adenosine triphosphatase (ATPase), Adenosine triphosphatase (ATPase), Periodic Acid Schiff (PAS), Oil red O.

Case report

This patient is a 22-year-old boy, born to healthy consanguineous parents without family history of neuromuscular diseases. The patient was born at 33 weeks gestation by planned caesarean delivery due to foetal tachycardia, after a pregnancy complicated by threatened miscarriage requiring drug therapy and rest. Apgar to minute 1 was 3, so he underwent primary resuscitation for asphyxia and was admitted to the NICU for 48 hours with good recovery. He had weak suction and presented with a congenital myopathic phenotype with elbow contractures and scoliosis since the first months of life.

No motor developmental delay or intellectual disability were observed, but he developed a slowly progressive muscle weakness with impaired walking, rhinolalia, dysphagia, and respiratory involvement, which required nocturnal non-invasive ventilation therapy since the age of 16 years. Brain and spine MRI, ECG, echocardiogram and serum creatine kinase (CK) level test were performed, resulting normal. Electrophysiological investigation documented motor and sensory conduction parameters within normal limits, with the exception of a reduction in amplitude of the muscle evoked potential. A first muscle biopsy at age of 5 years, revealed unspecific muscle damage, with fibre size variation, internal nuclei and fibrosis. In a second muscle biopsy performed years later, myofibrillar alterations were noted, including whorled fibres, cytoplasmic inclusion and minicores (Fig. 1).

Follow-up neurological examination showed axial hypotonia, generalised muscular hypotrophy and weakness (Fig. 2), preserved facial expressions, cleft palate, and global areflexia. Joint retractions at the elbows and ankles bilaterally were also observed. The patient was able to walk without support with equinus-varo-supinate foot stance, hyperlordosis, and tilting of the pelvis. Sensation was not impaired. In the following years, there was a slow worsening of the neuromuscular picture, requiring the use of a manual wheelchair for longer trips. The patient also underwent bilateral Achilles tendon tenotomy and vertebral arthrodesis surgery. Since age 17, he developed swallowing difficulties and recurrent pulmonary infections due to aspiration of food.

Exome sequencing led to the identification of the homozygous variant (NM_001256545.2): c.2096G > C (p. Cys699Ser) in the *MEGF10* gene. This variant is absent

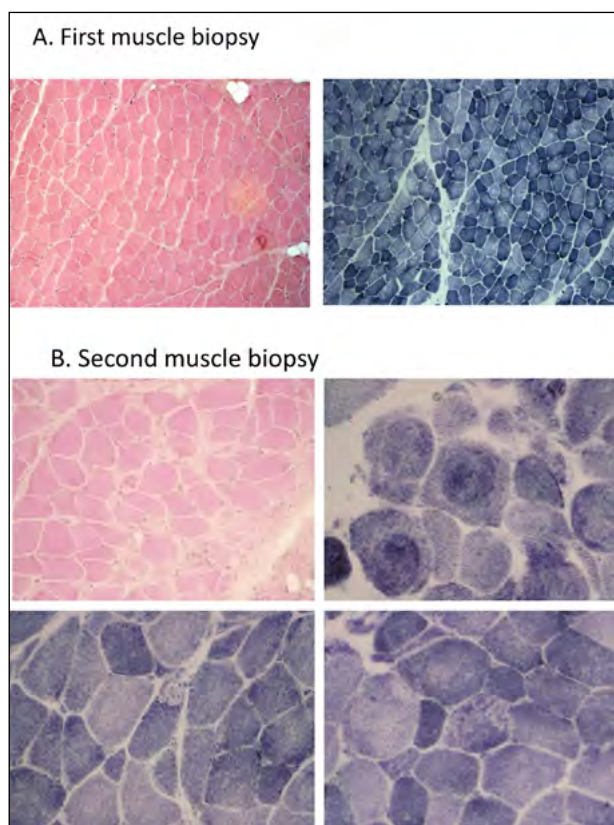


Figure 1. Muscle biopsy with HE and NADH staining. A) first muscle biopsy revealed unspecific muscle damage, with fibre size variation, internal nuclei and fibrosis; B) in a second muscle biopsy performed years later, myofibrillar alterations including whorled fibres, cytoplasmic inclusion and minicores, were evident.



Figure 2. A) patient at the age of six; B) patient at 17 years old. A progression of generalised muscular hypotrophy, scoliosis and hyperlordosis is evident; joint retractions at the elbows and tibio-tarsus bilaterally are also present. Ambulation is still possible for less than 10 meters.

in genomic databases (such as ClinVar and gnomAD), affects a conserved residue (GERP score = 5.85), and is predicted to be pathogenic by several in silico tools, including CADD (27.9), Mutation Taster (disease causing, score = 1), SIFT4G (damaging, score = 0.001), and Polyphen-2 HVAR (damaging, score = 0.99). This amino acid change is further classified as likely pathogenic according to the ACMG criteria (PM2, PP3). The variant is not predicted to alter the splicing (SpliceAI scores = 0). Sanger sequencing was also performed and confirmed that the patient was homozygous for the variant, and both parents were carriers.

Discussion

We describe a novel mutation in *MEGF10* in a patient presenting with a progressive form of myopathy characterised by generalised muscle weakness, scoliosis and respiratory involvement, in whom a muscle biopsy showed features suggestive of myofibrillar myopathy.

To date, 23 patients with *MEGF10* myopathy have been reported in the literature and these cases have been recently reviewed by Fujii et al.⁶ We summarised the clinical characteristics of the current and reported patients, according to the classification in one of the two forms (classic early onset EMARDD or mild variant mvEMARDD) (Fig. 3). The age of onset was established as the age at which patients manifested respiratory distress or progressive muscle weakness.

Initially, mutations in the *MEGF10* gene were associated with a severe phenotype of congenital myopathy termed EMARDD, characterized by generalised muscle weakness, breathing difficulties, joint contractures and scoliosis^{1,2}. More recently, it became apparent that *MEGF10* mutations may result in a wide spectrum of disease in terms of clinic pathological features, ranging from severe infantile to milder chronic disorders with a more favourable course^{1-6,10,13}. In a study reported for EMARDD and mvEMARDD, it was shown that there is considerable overlap in clinical findings between the EMARDD and minicore myopathy diagnostic groups, although certain features are more frequently reported according to clinical/genetic diagnosis⁵.

Genotype–phenotype correlations have been also postulated^{6,10}. Indeed, individuals with more damaging variants (homozygous null mutations/frameshift mutations)^{1,2} show the EMARDD phenotype with respiratory failure starting in infancy and non-specific changes on muscle biopsy. In contrast, patients displaying a less severe phenotype, with later respiratory failure and minicores on muscle biopsy (mvEMARDD or with minicore myopathy)^{3,10} harbour homozygous or compound heterozygous missense variants affecting the cysteine in

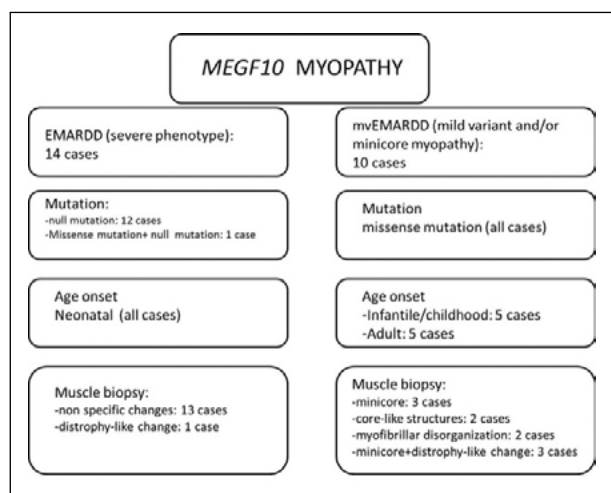


Figure 3. Clinical and genetic characteristics of patients with *MEGF10* mutations reported in literature according to the classification in early onset severe form and mild variant form.

the EGF-like domain¹⁰. Additionally, missense changes are statistically associated with a later onset and benign course, whereas truncating variants are expected to cause an early onset severe phenotype⁶.

Although our patient carried a missense variant, he displayed an early-onset myopathy with progressive course. Hypotonia at birth was followed by scoliosis, retraction and respiratory involvement, requiring respiratory support at age 6 and swallowing problems since at age 17. Two consecutive muscle biopsies confirmed the progression of the disease over the years. The first muscle biopsy performed at age 5 years revealed unspecific muscle damage, whereas the second biopsy performed one year later showed more definite and significant alterations, suggestive of a myofibrillar myopathy with presence of core like areas, inclusions, and whorled fibre (Fig. 1). Interestingly, another reported patient with late onset *MEGF10* myopathy phenotype had only nonspecific findings at the first and second biopsies, and then exhibited cores at the third biopsy¹³. Thus, we propose that minicores that mark the late onset group, can actually be a consequence of a longstanding myofibrillar pathological processes starting in infancy.

Myofibrillar alterations have been already reported in late onset mvEMARDD^{3,4}. However, not all the mvEMARDD patients present minicores^{5,6} and these features are not typical for early-onset forms. This seems to confirm the hypothesis that minicores may only occur at a later stage. Indeed, myofibrillar myopathies (MFM) are a group muscle disorders usually with adult onset of distal weakness. Onset in infancy or childhood is very uncommon. In addition, the association of *MEGF10* defect and

deterioration of the myofibrillar network has not been formally validated and functional studies are needed to explore the putative role of *MEGF10* in maintenance of the myofibrillar network.

From a genetic perspective, *MEGF10* variants have never been reported in subjects of Italian ancestry. In our patient, we identified the novel homozygous variant p.Cys699S affecting a very conserved cysteine residue in a crucial EGF-like functional domain. Cysteine substitutions in the extracellular EGF-like domains of *MEGF10* are the most commonly reported missense variants^{1,3-5,10} and they have been shown to result in a significant decrease in the tyrosine phosphorylation activity in the intracellular domain^{9,10}. These findings thus support the pathogenic relevance of the p.Cys699Ser variant in our patient. Furthermore, the degree of functional impairment of *MEGF10* has been demonstrated to depend on which EGF-like domain is mutated^{9,14}. This suggests that different EGF-like domains may be relatively more important for *MEGF10* signalling than others⁵. Functional studies are warranted to confirm the role and pathogenetic association of *MEGF10* mutation in our patient.

It has been reported that *MEGF10* regulates the proliferation and differentiation of muscle stem cells by promoting the activation of satellite cell proliferation and, simultaneously, inhibiting the myoblast differentiation^{1,10,15}. Centrally located nuclei are also a substantial feature in the histopathological picture of our case. Albeit unspecific, central nuclei can be linked to a possible involvement of the regeneration pathway which is so far the most corroborated patho-mechanism of the *MEGF10* defect. *MEGF10* also interacts with the intracellular domain of NOTCH. The action of *MEGF10* on myoblasts appears to be mediated, at least in part, by interactions with components of the Notch signalling pathway. The NOTCH pathway in general and NOTCH1 in particular, is a key regulator of satellite cell and myoblast physiology¹⁶. Notch1 interacts with both *MEGF10*¹⁷ and the serotonin pathway¹⁸ and defects in these interactions may participate in the pathogenesis of EMARDD¹⁷. Recent findings indicate that sertraline, an SSRI, ameliorates the phenotype of *MEGF10* myopathy in different disease model, including myoblasts (mouse-derived and human-derived), *Drosophila* and zebrafish, revealing its potential as a novel therapy for *MEGF10* myopathy¹³.

Conclusions

In summary, our report proposes a continuum in the phenotypes associated with *MEGF10* variants, which include a clinical and histopathological progression of the disease and represent a novelty from the dichotomy between the early onset classic severe EMARDD and mvE-

MARDD. We also suggest that myofibrillar alterations are a common finding, and that *MEGF10* gene should be tested in suspected MFM patients with childhood onset of progressive myopathy, scoliosis and retraction, and respiratory defect. Accumulation of further cases will be important to confirm the current genotype-phenotype correlations and for early diagnosis, which will play a relevant role in the management of patients and the use of potential therapeutic strategies.

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Conflict of interest statement

The Authors declare no conflict of interest.

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Author contributions

CC: acquisition and analysis of data, drafting of the manuscript; MT, MI, FC: acquisition and analysis of genetic data; SB: acquisition and analysis of muscle biopsy data; MP, CB: acquisition and analysis of clinical data; MS: drafting the manuscript; CF: conception and design of the study, drafting the manuscript.

Ethical consideration

This study has been approved by local Ethic Committee within a project of WES in Neuromuscular Disorders. Patient and family signed informed consent for research use of clinical data and publication of photographic material.

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