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BRIEF REPORT

Identification of a de-novo variant of the MEGF10 gene associated with EMARDD

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KEYWORDS
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Abstract Early-onset Myopathy, Areflexia, Respiratory Distress and Dysphagia (EMARDD) is a congenital neuromuscular disease with a progressive muscle weakness, respiratory failure, joint contractures, and scoliosis without any symptoms of functional brain anomalies caused by variants in the *MEGF10* gene. Here, we report the clinical phenotype and genetic features of a Moroccan patient who carries a novel variant associated with EMARDD on the *MEGF10* gene. The Whole Exome Sequencing analysis conducted on a 11 year old boy with respiratory and swallowing difficulties revealed the presence of the novel variant c.978T>A (p.Cys326Ter) on exon 9 of the *MEGF10* gene; this variant is thought to be associated with EMARDD. Our study reports the first nonsense pathogenic de novo variant in *MEGF10* associated with EMARDD worldwide, identified in a Moroccan patient.

PALABRAS CLAVE
MEGF10;
EMARDD;
Secuenciación del exoma completo;
Paciente de Marruecos

Identificación de una variante *de novo* en el gen *MEGF10* asociado a EMARDD

Resumen La miopatía de inicio precoz, arreflexia, dificultad respiratoria y disfagia (EMARDD, por sus siglas en inglés) es una enfermedad neuromuscular congénita caracterizada por una debilidad muscular progresiva, insuficiencia respiratoria, contracturas articulares y escoliosis, sin síntomas de anomalías funcionales en el cerebro, causada por variantes en el gen *MEGF10*. En este estudio presentamos el fenotipo clínico y las características genéticas de un paciente de Marruecos que porta una variante nueva asociada a EMARDD en el gen *MEGF10*. El análisis de secuenciación del exoma completo realizado en un niño de 11 años de edad con dificultades

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respiratorias y de deglución reveló la presencia de la nueva variante c.978T>A (p.Cys326Ter) en el exón 9 del gen *MEGF10*; se cree que esta variante está asociada a la enfermedad EMARDD. Nuestro estudio presenta el primer caso en todo el mundo de una variante patogénica *de novo* sin sentido en *MEGF10* asociada a EMARDD, identificada en un paciente de Marruecos.

Introduction

Early-onset Myopathy, Areflexia, Respiratory Distress and Dysphagia (EMARDD), also known as ''*MEGF10* myopathy'', is a rare autosomal recessive human congenital myopathy/muscular dystrophy caused by homozygous or compound heterozygous mutations in the multiple epidermal growth factor (EGF)-like domains 10 (*MEGF10*) (OMIM #614399).[1,2] This type of myopathies is characterized by severe progressive congenital muscle weakness, severe respiratory failure, joint contractures, and scoliosis without any signs of structural or functional brain abnormalities.[3]

The MEGF10 gene, located on the long arm of chromosome 5q23.2, codes for a type I transmembrane orphan receptor protein with significant roles in muscle development and repair. In skeletal muscle, it is predominantly expressed in satellite cells, developing myoblasts, and the central nervous system, indicating its crucial role in mediating signal transduction. The expression of the MEGF10 transcript is confined to the adult and foetal brain, spinal cord, and skeletal muscle, with high concentrations at the neuromuscular junction.[4–7]

In this report, we present the clinical features of a Moroccan patient exhibiting signs of EMARDD, and whom Whole Exome Sequencing results have revealed the presence of a novel heterozygous de novo variant in exon 9 of the *MEGF10* gene.

Patient and methods

Study case

The patient is an 11-year-old Moroccan boy with no abnormal events during the perinatal period or any developmental delay. He is the second child of non-consanguineous healthy parents with no family history of neuromuscular disorders. His brother and sister are both healthy and do not exhibit any abnormalities.

At the beginning of her pregnancy, the mother experienced bleeding and loss of consciousness due to psychological shock, which required medical intervention and pregnancy stabilizing pills for ten days. The clinical presentation of the patient is shown in Table 1.

Physical examination revealed bilateral pseudohypertrophy of the calf muscles, motor deficit of the scapular and pelvic girdles, positive Gower's sign and a decrease observed in the osteotendinous reflexes (ROT) of the lower limbs. Nerve conduction study (NCS) showed normal nerve conduction velocity (NCV). However, needle electromyography (EMG) examination revealed myopathic pattern in the four limbs.

Biochemistry analysis indicated significantly increased levels of creatine kinase, alanine aminotransferase, aspartate aminotransferase, and lactate dehydrogenase (19 698 U/L, 93 U/L, 124 U/L and 230 mg/L, respectively). The main clinical features of the patient at the age of 11 years are shown in Fig. 1.

Sequencing study

Genomic DNA of all the family members was extracted from peripheral blood and the DNA of the proband II:1 underwent a WES (whole-exome sequencing) at BGI Tech Solution (Hong Kong, China). To select the pathogenic candidate variants, we prioritized genes known to be associated with myopathies. Assuming a consanguineous autosomal recessive pattern of inheritance, we first screened for homozygous and heterozygous compound variants, and then for heterozygous variants having allele frequencies <0.01 using SNP databases. The pathogenicity of the remaining variants was evaluated using PolyPhen-2, Mutation Taster, and SIFT.

To confirm the segregation of the variant, we performed Sanger sequencing on all available family members using MEGF10 exon 9 primers (Forward 5′-TTTCACATGGCACTAACTAATCTTTC and Reverse 5′-CCTGCCCAGAGTAGCCTAGA).

Results

The heterozygous variant c.978T>A (p.Cys326Ter) of the *MEGF10 was predicted to be pathogenic* using *in-silico* software. This variant is absent from both the gnomAD and the dbSNP databases. Sanger sequencing confirmed that the variant is de novo, as none of the other family members carry the variant (Fig. 2).

Moreover, the alignment of multiple orthologous MEGF10 protein sequences from different species showed that the p.Cys326Ter variant affects a conserved residue (Fig. 3).

Discussion

The gene causing EMARDD is the *MEGF10*, located on chromosome 5q23. It encodes for a 1140 amino acid protein, that

Table 1 Clinical presentation of the patient.

Age	Clinical presentation
Birth	No clinical or medical concerns.
0–6 months	Difficulty breast-feeding, weak sucking ability. At 6 months, difficulty swallowing solid foods, resulting in rapid vomiting.
19 months	Frequent stumbling, falling without using hands to break the fall, high activity and energy levels, strong visual and intellectual intelligence.
2 years	Able to climb stairs, play, and run, but occasionally experienced dizziness and walked in the opposite direction of his gaze.
3 years	Significant speech difficulties and learning challenges, remarkable emotional intelligence.
5 years	Fall during recreational activity, toe-walking on the right leg, hip hypertonicity, upward gaze strabismus, mild myopia.
9 years	Unsteady gait due to Achilles tendon contracture in the left foot, became unable to rise from bed, breathing difficulties.
10 years	Continued deterioration, forcefully walking on the left foot due to right leg tendon contracture, difficulty lifting right leg, numbness in both feet, facial pallor, reduced appetite, intestinal pain, laughter-induced urinary incontinence.
11 years	Challenges handling heavy objects, difficulty moving hands backward or forward, scoliosis, dyspnoea, significant cognitive impairment, wheelchair use.

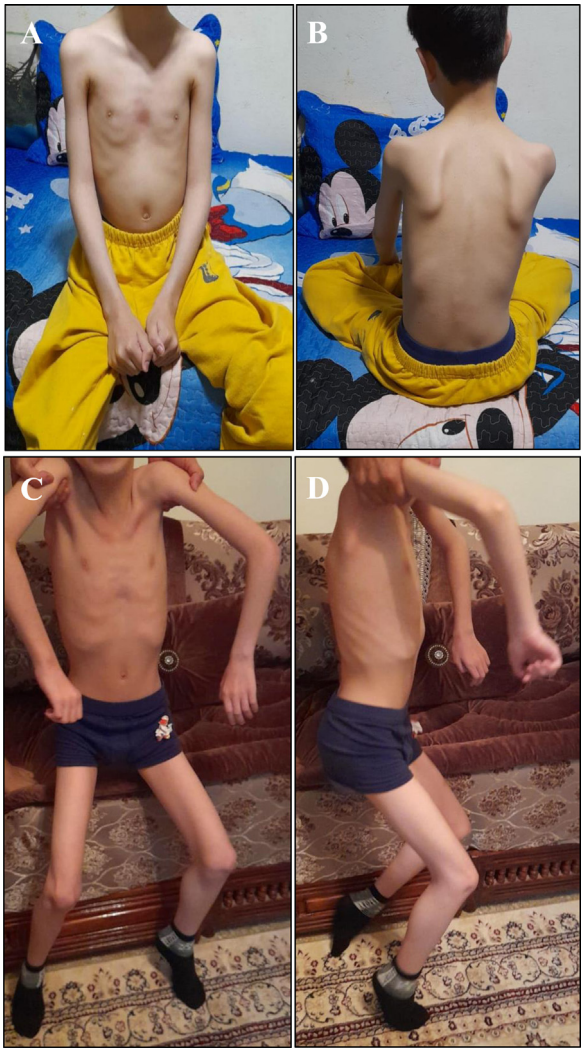

Figure 1 Clinical phenotype of the patient at the age of 11 years. (A, B) Note the wasting of shoulder girdle muscles, loss of normal spinal curvature and minimal scoliosis. (C, D) Generalized poor muscle mass, global wasting of thigh muscles, and inability to walk or stand independently, requiring continuous use of a wheelchair for all mobility needs.

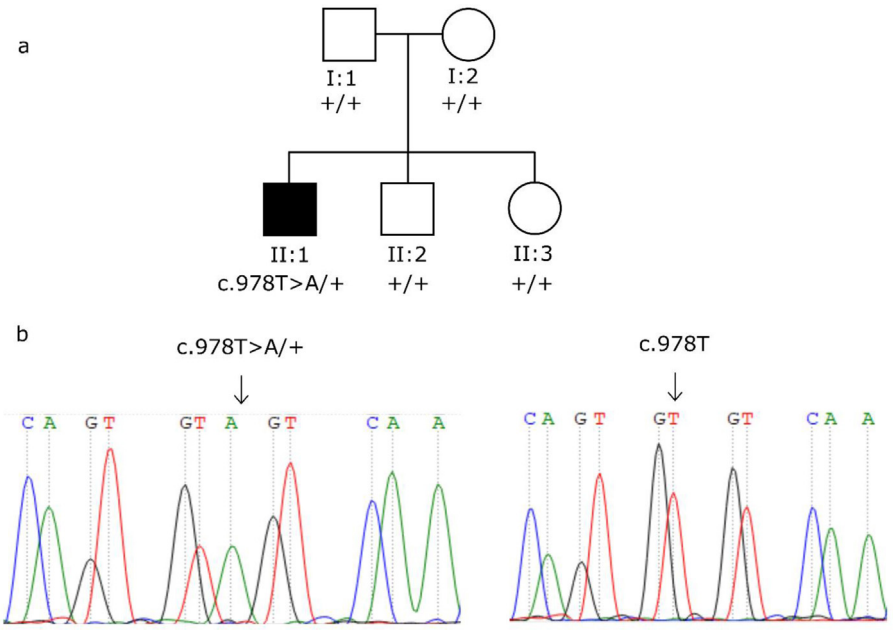

Figure 2 (a) Pedigree of EMARDD family studied. Among the two generations, only the proband had the syndrome and his parents had no relevant medical history. (b) The results of Sanger sequencing of the patient.

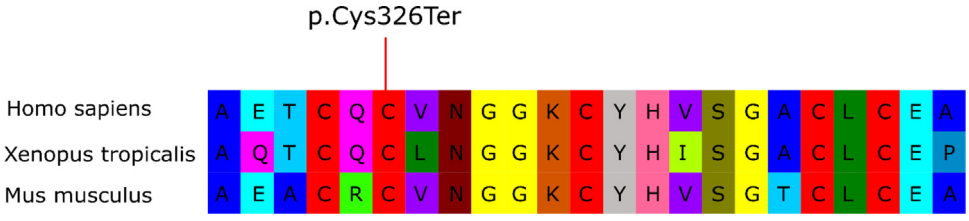

Figure 3 An analysis of conservation reveals the alignment of multiple amino acid sequences for the MEGF10 protein and its orthologues.

has been shown to play an important role in satellite cell proliferation and differentiation.[5] Satellite cells, also known as Muscle stem cells (MuSCs), are located peripherally in an asymmetric niche between the myofiber membrane and the basal lamina. In vivo studies in humans have shown that satellite cells are crucial for skeletal muscle fibre regeneration, homeostasis, repair, growth and hypertrophy.[8–10] Skeletal muscle regeneration is impacted in muscle-wasting diseases like EMARDD dystrophy, a severe congenital muscular disorder that causes severe progressive congenital muscle weakness and respiratory failure in affected individuals.

A study suggests that the configuration of specific epidermal growth factor (EGF)-like domains in the *MEGF10* gene plays a pivotal role in the transmission of extracellular signals. The ''C326R'' variant is predicted to disrupt the structural integrity of the second disulfide bond in its epidermal growth factor (EGF)-like domain. This study reveals an association between the C326R variant in the extracellular EGF-like domains of MEGF10 and a reduction in the tyrosine phosphorylation of the affected protein. The findings suggest that disruptions in the MEGF10 signalling cascade may result in a reduction of muscle satellite cells, potentially contributing to the development of MEGF10 myopathy.[11]

In our study, we identified the new heterozygous de novo substitution C326R (c.978T>A) in exon 9 of the *MEGF10* gene in a Moroccan patient, which is thought to be associated with EMARDD. This nonsense variant leads to a premature stop codon at position 326, resulting in the loss of 814 C-terminal amino acids, including a very conserved cysteine residue within a crucial EGF-like functional domain. *MEGF10* comprises 17 unconventional epidermal growth factor-like domains, each featuring eight cysteine residues, presumed to create disulfide bonds. The C326T variant specifically modifies one of these residues, which are uniformly preserved across vertebrates (Fig. 2). This modification causes truncation of the MEGF10 protein, leading to the clinical presentation observed in the affected child in this study, such as progressive muscle weakness and respiratory failure. Cysteine substitutions in the extracellular EGF-like domains of *MEGF10* are the most commonly reported missense variants and have been shown to result in a significant decrease in the tyrosine phosphorylation activity in the intracellular domain.[1,3,11–13] These results substantiate the pathogenic significance of the C326T variant in our patient.

To our knowledge, this variant has not been reported in the literature or in the HGMD database, and it is a novel variant associated with severe EMARDD phenotypes.

Clinically, the patient in this study has symptoms similar to those observed in other previously reported cases of *MEGF10*-related EMARDD.[1,14] Thus, it is essential to conduct functional studies in order to confirm the pathogenic association of this variant to the phenotypic profile of our patient.

Conclusion

In conclusion, this study presents the first reported case of a rare novel de novo pathogenic variant not previously described in the *MEGF10* gene. Our results enlarged the mutation spectrum of *MEGF10* in patients with EMARDD and proves the important role of the Whole Exome Sequencing technology in the genetic diagnosis of neuromuscular disease.

Informed consent

This study is covered by the local research ethics committee of the Institut Pasteur du Maroc. All family members who were included in this study gave their informed consent.

Ethics approval

The genetic study was approved by the medical ethics committee of Institut Pasteur du Maroc.

Conflict of interest

None.

Data availability

Data will be provided by the authors upon request.

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