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GNE Myopathy and Duchenne Muscular Dystrophy in Two Moroccans Families

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SUMMARY

Background. Hereditary myopathies and muscular dystrophies encompass a diverse group of disorders sharing common clinical features, including muscle weakness, motor developmental delays, and respiratory and bulbar dysfunction. This study aimed to investigate the genetic basis of myopathy in two Moroccan families presenting with these clinical features.

Material and methods. Whole exome sequencing (WES) was employed as the primary method for genetic analysis in the two Moroccan families with myopathy symptoms. Candidate variants were confirmed by Sanger sequencing in all DNA available family members. In addition, computational analysis was utilized to assess the impact of the identified variant on the corresponding protein.

Results. In the first family, the identified variant in the *GNE* gene, a homozygous missense substitution (p.Arg246Trp), was associated with GNE myopathy. In the second family, a hemizygous nonsense variant (p.Arg2905Ter) in the *DMD* gene was identified in the proband, who exhibited symptoms consistent with Duchenne Muscular Dystrophy (DMD). The molecular analysis confirmed the pathogenicity of the identified variants in both GNE myopathy and DMD. The missense variant (p.Arg246Trp) in the *GNE* gene was found to affect the stability and amino acid interactions of the GNE protein, thus implicating it in GNE myopathy. Additionally, the nonsense variant (p.Arg2905Ter) in the *DMD* gene provided genetic confirmation of DMD in the second family.

Conclusions. This study represents the first genetically confirmed report of GNE myopathy in Morocco, emphasizing the significance of genetic analysis in diagnosing hereditary muscle disorders. The findings also underscore the importance of considering GNE myopathy as part of the differential diagnosis for patients presenting with slowly progressive distal lower extremity weakness. Overall, these results contribute to our understanding of the genetic basis of hereditary myopathies and muscular dystrophies in the Moroccan population.

KEY WORDS

GNE; myopathy; DMD; computational analysis; whole exome sequencing; Morocco.

INTRODUCTION

Hereditary myopathies are inherited disorders due to genetic defects in the contractile system of the muscle, and muscle biopsies show characteristic histochemical and ultrastructural alterations. It's characterized by common clinical features such as muscle weakness, motor delay, and respiratory and bulbar dysfunction. As regards muscular dystrophies, it's a disease of the muscle membrane or supporting proteins, which are usually marked by pathological signs of ongoing muscle degeneration and regeneration (1).

GNE myopathy (MIM: 605820), also known as distal myopathy with rimmed vacuoles or hereditary inclusion body myopathy, is an autosomal recessive neuromuscular disorder caused by pathogenic variants in *GNE* (MIM: 603824), which encodes a 722 amino acid bifunctional key enzyme in the sialic acid synthesis, UDP-N-acetylglucosamine 2-epimerase/N-acetyl mannosamine kinase (2). This enzyme catalyzes the first two successive steps in the biosynthesis of 5-N-acetylneuraminic acid (Neu5Ac), the common mammalian precursor of sialic acids (3).

GNE myopathy worldwide prevalence is estimated at 1 to 9 per 1,000,000 (4). The first symptoms are most common in the third decade of life (5). Clinical manifestations begin with a distal leg muscle weakness, followed by slowly progressing muscle weakness and lower and upper extremity muscles atrophy, with relative sparing of the quadriceps, ultimately leading to wheelchair dependence within 10 to 20 years, with a mild increase of serum creatine kinase (CK) levels (6). In addition, biopsies of affected muscles revealed numerous rimmed vacuoles are distinguishable in affected muscle fibers without inflammation (7). More than 200 variants are known to be associated with GNE myopathy which the majority are missense, insertion/deletion and intronic (splice site) variants (8).

Duchenne Muscular Dystrophy (DMD) (MIM:310200) is an X-linked recessive degenerative muscle disease, with a worldwide prevalence of 1 in 5,000 male births (9). Motor symptoms usually start at the age of three years old and aggravate quickly, most commonly weakness of pelvic and shoulder muscles occur in early childhood and serum creatine kinase (CK) levels are usually elevated (10). As the patient ages, cardiac or respiratory insufficiencies can manifest. Furthermore, DMD can be associated with cognitive impairment and autism spectrum disorder.

DMD is due to the absence or defect of dystrophin protein encoded by *DMD* gene (MIM: *300377). The dystrophin protein consists of 3,686 amino acid and comprises 4 main functional domains, an actin-binding amino-terminal domain (ABD1), a central rod domain, a cysteine-rich domain and a carboxyl-terminus. This protein is an important component of a dystrophin associated protein complex that involves reinforcing muscle fibers, and it stabilizes the muscle membrane during muscle contraction (11). The lack of dystrophin in DMD can cause abnormal muscle membranes, which can give rise to damage and finally death of muscle fiber (12). The DMD causing variants are very heterogeneous: the exons deletions are the most common and are associated with 60-65% of muscular dystrophies (13). There are 5 to 8% of patients that present exon duplications (14), while small variants account for 10% to 30% which one third are *de novo* (15). Pathogenic DMD variants were previously identified in Moroccan patients (16-19).

In this study, using whole exome sequencing (WES) for molecular analysis of two Moroccan families, we identified respectively a homozygous variant (p.Arg 246Trp) in *GNE* related myopathy and a hemizygous (p. Arg2905Ter) in *DMD* related muscular dystrophy.

PATIENTS AND METHODS

Patients

The study includes two Moroccan families with suspected genetically determined myopathy, based on the clinical presentation, electrophysiological studies, and absence of known acquired causes of myopathy. Family 1 was diagnosed in the Ibn Sina Hospital for clinical examination and both were addressed to the Genomics and Human Genetics Laboratory of Pasteur Institute of Morocco for the genetic testing. Written informed consent was obtained from all patients and the genetic study was approved by the Committee on Research Ethics of Faculty of Medicine and Pharmacy, Mohammed V University of Rabat (N° 09/19). This work was directed following the principles of the declaration of Helsinki. Informed consent was obtained from all patients included in the study.

Family 1

The index patient II:2 is a 32-year-old woman, offspring of healthy consanguineous parents (**figure 1A**). The disease began at the age of 18 years with a running difficulty at high speed, then at 22 years with climbing difficulty stairs, and walking problems that progressively set in over 8 years. For the last 2 years she presents a difficulty to comb one's hair, reduction of the walking perimeter and outdoor movements. Neurological evaluation revealed proximal paraparesis at 3/5 and scapular belts deficit at 4/5. The deep tendon reflexes were present. There was no cranial nerve dysfunction or sensory disturbance. Blood tests showed high CK levels (5,330 U/L), while other results were normal. ENMG results of the four members revealed a myogenic type of damage

more severe in the lower members in the absence of myositic signs. She has a normal mental and physical development.
Patient II-2, the older brother, at age 32, was confined to a wheelchair with complete distal leg muscle paralysis and severe proximal leg muscles paralysis, weakness of the hands is also observed. Whereas the quadriceps muscle was relatively unaffected. At 20 years, he has suffered from progressive muscle weakness, especially in the lower extremities. He had become unable to stand and walk without support at the age of 26.

Family 2

The patient II:1 is a 19-year-old boy from a consanguineous family (**figure 1B**), present with progressive lower muscle weakness, calf hypertrophy, and positive Gowers signs. His paternal cousin presented the same clinical features, and at the age of 20-year-old, he was unable to walk.

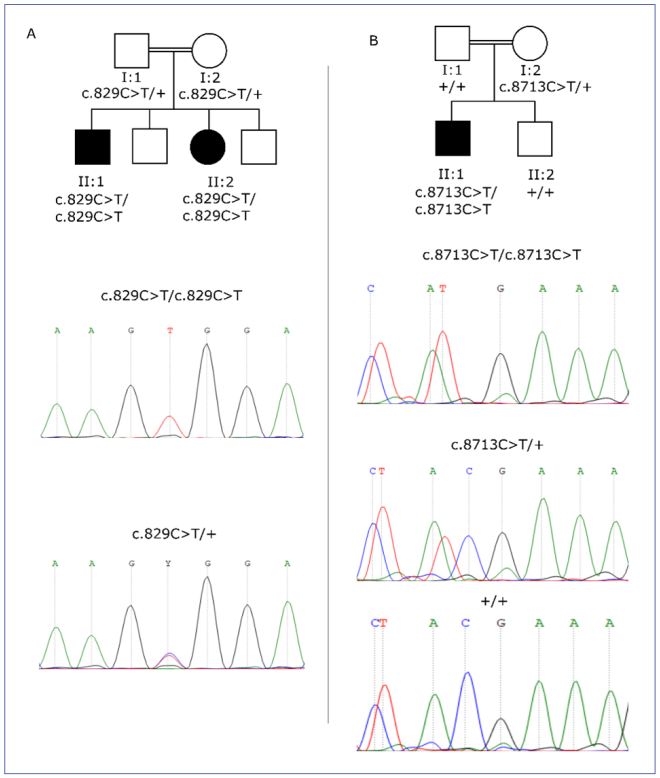

Figure 1. Pedigree and electropherograms.
(A) Family 1 pedigree and electropherograms of *GNE* variant. **(B)** Family 2 pedigree and electropherograms of *DMD* variant.

Genetic analysis

Genomic DNA of the probands and their parents was extracted from peripheral blood with the QIA amp DNA Blood Mini Kit (Qiagen, Germany). DNA of the probands underwent a WES at BGI Tech Solution (Hong Kong, China) as reported elsewhere (20).
Variants on genes known to cause hereditary myopathies and compatible with the autosomal recessive mode of inheritance were selected for analysis. We selected variants with an allele frequency < 0.01, and then we assessed their potential pathogenicity using PolyPhen-2, SIFT and Mutation Taster software.
The *GNE* and *DMD* identified candidate variants were confirmed by Sanger sequencing. PCR primers were designed by Primer3 software and PCR products were sequenced using ABI 3130 genetic Analyzer (Applied Biosystems) and analyzed using Ugene software.

Structure analysis

FASTA sequence of the GNE protein (Q9Y223) was retrieved from the UniProt database: https://www.uniprot.org/uniprotkb/Q9Y223.

Stability and conservation Analysis

The impact of the p.Arg 246trp variant in protein structure stability was predicted using I-Mutant 2.0 (http://folding.biofold.org/i-mutant/imutant2.0.html), MUpro (http://mupro.proteomics.ics.uci. edu/), and DUET (http://biosig.unimelb.edu. au/duet/) softwares.

Modeling of Native and Mutant Structure

The three-dimensional structure of native protein was obtained from the RCSB PDB server (https://www.rcsb.org/structure/4ZHT). The Arg246Trp change was introduced as a substitution in the obtained structure 4ZHT. Two variants of this structure were then generated: the first one corresponds to wildtype (considered as control structure) and the second one was the mutated form (that carries the Arg246Trp substitution). These two generated structures were minimized by YASARA Energy Minimization Server, and visualized using Yasara software, which examines the Arg246 residue and its mutant form, Trp246, for hydrogen and hydrophobic interactions. Root mean square deviation (RMSD) was calculated by superposing the native structure to the mutant using the same software.

RESULTS

In family 1, WES revealed the missense homozygous c.829C>T variant in exon 4 of the *GNE* gene (NM_001128227.3). This variant (rs773729410) generates a substitution of arginine to tryptophane (p. Arg246Trp) and referenced with an allele frequency of 1.19.e^{-5} in gnomAD Exomes databases and referenced as variant disease causing (CM024158) in the HGMD (Human Gene Mutation Database). The two

patients are homozygous, and the healthy parents were found as heterozygous by Sanger sequencing (**figure 1A**).
In family 2, the sequence analysis of the DMD associated genes revealed the nonsense variant c.8713C>T in exon 5 of *DMD* gene (NM_004006.3). This variant (rs128627256) generates a substitution of Arginine to stop codon (p. Arg2905Ter) and referenced as variant disease causing (CM950347) in the HGMD. Sanger sequencing show that the patient is hemizygous, the father and the brother are healthy homozygous, and the mother is heterozygous for this variant (**figure 1B**).

Stability analysis

The stability analysis indicated that the Arg 246trp variation decreases the stability of the protein (**table I**). RMSD analysis, the variant Arg246 Trp indicated a higher value than the native form.
After using YASARA software to visualize the structure of wild type and mutant proteins, we showed some differences in hydrophobic interactions. As represented in **table II** and **figure 2**, the Arg 246 of the native form of GNE protein showed a hydrophobic bond with Leu 205, Val 209 and Tyr 214, Asn 275 and a hydrogenic bonds with Ile 215 and Asn 275. In the mutated protein, there was an appearance of hydrophobic bond with Arg277 and disappearance of hydrophobic interaction with Asn 275. No changes were observed in position 205, 209, 214 and 215.

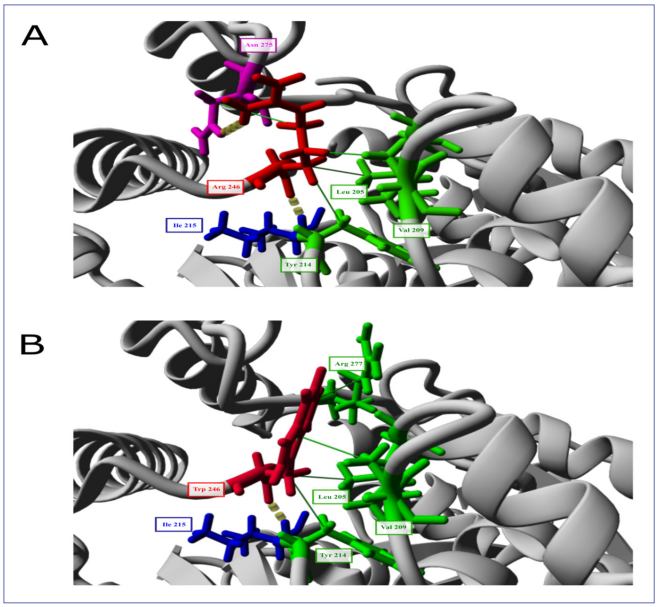

Figure 2. Comparison of the native GNE protein structure and a mutant form.
(A) The structural model of the wild-type protein (Arg246). **(B)** The structural model of the mutated form (Trp246).

DISCUSSION

The sibling for family 1 showed a severe progressive pattern with early adulthood onset, lower extremity muscle weakness and atrophy, proximal progression with preserved quadriceps, and was confined to a wheelchair within 10 years after disease onset. The clinical presentation is suggestive of hereditary inclusion body myopathy (hIBM, IBM2, OMIM 605820). GNE myopathy is an autosomal recessive myopathy of late onset in adults that is clinically marked by progressive atrophy and weakness of the distal legs, especially affecting the lower limbs but not affecting the quadriceps group. Therefore, it is also called distal myopathy with rimmed vacuoles (5). Genetic analysis identified the homozygous mutation Arg 246Trp in GNE gene, in the epimerase domain, in the index patient and her severely affected brother. To our knowledge, the Arg246Trp mutation has not been reported in GNE myopathies North African patients before. The heterozygous

Table I. Stability software predictions and DDG scores for the R246W of the *GNE* gene.

SNP	Mupro			DUET
Arg246trp	Prediction	DDG value	Prediction	DDG value
	Decrease	-1.012	Decrease	-0.889

Table II. Analysis of R246W effect on hydrogen bonds and hydrophobic interactions.

Substitution	Wild type	Mutated form	Wild type	Mutated form
	Hydrophobic interactions	Hydrophobic interactions	Hydrogenic interactions	Hydrogenic interactions
Arg246Trp	Leu 205	Leu 205	Ile215	Ile215
	Val 209	Val 209	Asn275	
	Tyr 214	Tyr 214		
	Asn275	Arg 277		

Arg246Trp mutation frequently reported in subjects of different ethnic origins Caucasian, Chinese, Japanese, Italian, USA (22-26) All Iranian affected individuals suffered from weak (strength < 3=5) tibialis anterior, iliopsoas, lower extremities adductors, and hamstrings muscle groups; all the patients had near normal strength quadriceps (strength > 4=5) in quadriceps. Clinical weakness of specific muscle groups occurred in all patients aged 19 to 44 years (22). The American patient of British/Irish/Scottish was 27 years old, similar in age to our patients at onset, recently required crutches to ambulate (23). Two Italian affected sisters presented disease onset in early adult life with weakness and atrophy of lower limb muscles that progressed proximally and spares the quadriceps. The older sister was wheelchair bound at 34 years of age (25). At the same amino acid position, the heterozygous variant Arg246Glu was previously described in patients from The Bahamas, Italy, and Taiwan (27-29). The Taiwanese patient, similar in age to our patient at onset, had sever HIBM with rapidly progressive muscular dystrophy course that also severely affected the quadriceps in the fourth decade of her life, and was confined to a wheelchair at age 32 years (29). An Italian patient had distal and proximal involvement of the upper and lower extremities, but still exhibited quadriceps sparing at age 50 years (28). The Arg246Trp variant in the epimerase domain cause loss of catalytic function of both the epimerase (< 2%) and kinase activities. These findings may explain by the missense mutation Arg246Trp produced misfolding of the enzyme, altering its secondary structure (23). GNE/MNK proteins are known to form homohexamers by oligomerization, and GNE mutation affect the oligomerization process and reduce GNE and MNK enzymatic activity (30). Furthermore, diminution in enzyme activity does not correlate with disease severity. Because GNE play a key role in sialic acid production, several studies concentrated on potential for changes in sialylation of cell surface glycoproteins and glycolipids. Whereas hyposialylation has been revealed, the linkage between disease severity and diminution of the overall sialylation of muscle cells is insignificant (31).

Since 1978, Duchenne Muscular Dystrophy or Becker Muscular Dystrophy (DMD/BMD) were described as caused by pathogenic variants in the dystrophin gene, which is the largest know human gene (2.4 MB and 79 exons) (32). Pathogenic variants in *DMD* gene were previously identified in Moroccan patients. A deletion of exons 45 to 49 of *DMD* gene was previously reported in a 9-year-old Moroccan boy (19). Molecular analysis results of dystrophin gene during 27 years in a large Moroccan cohort of 356 patients revealed different deletions of *DMD* exons and promoter, in addition to six novel variant and a novel large duplication from exon 48 to exon 63 (18).

In this study we reported, for the first time in a North African patient, a nonsense variant (p.Arg2905Ter) in actin binding domain 1 (ABD1) of DMD. The variant causes the production of a truncated protein or mRNA with a premature stop codon. This variant was previously reported in six patients, and it is identified as a CpG hotspot mutation with a rate extremely high, about 1.710^{-6} per nucleotide per generation (33). The somatic mosaicism of this nonsense variant is reported in a young patient, who presented with heart failure and not skeletal muscle symptoms (34). Therefore, the variant has been detected in a heterozygous female who exhibited muscle weakness, gait abnormalities, attention deficit hyperactivity disorder and bipolar disorder (35). Also, it was identified in a Hungarian patient with muscular dystrophy and autism spectrum disorders (ASDs) and reported to play a role in the ethology (34). Our patient exhibited with progressive lower muscle weakness, calf hypertrophy, and positive Gowers sing.

CONCLUSIONS

In summary, the two Moroccans families add to the wide phenotypic and genotypic spectrum of GNE myopathy and Duchenne muscular dystrophy.

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DATA AVAILABILITY

Data are available under reasonable request to the corresponding author.

CONTRIBUTIONS

NS: writing – original draft, software, writing – review & editing, data analysis. GA: software, writing – review & editing. AB, HN: writing – review & editing. MC: methodology, writing – review & editing, data analysis. GZ, RH, YK: data analysis. BE, GL, AB: supervision, validation. All authors: final approval.

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CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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