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Case report

A novel *DOK7* mutation causing congenital myasthenic syndrome with limb-girdle weakness: case series of three family members

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ABSTRACT

Congenital myasthenia syndrome (CMS) is a group of heterogeneous diseases affecting the neuromuscular end-plate. CMS has a considerably different phenotypic presentations, with the onset time ranging from early infancy to late adulthood. Here, we report a case of a CMS due to a new DOK7 mutation in a 28-year-old man and two of his sisters, who have a pure limb-girdle weakness. DOK7 CMS has a varying presentation. Typically, the onset occurs in childhood with ptosis, bulbar symptoms, difficulty walking, weakness, and gait abnormality. This case sheds light on a novel DOK7 gene mutation with a unique presentation of CMS and provides insight into its unique phenotypic presentation.

1. Introduction

Congenital myasthenia syndrome (CMS) is a spectrum of hereditary disorders with the clinical features of fluctuating, often exercise-induced transient muscle weakness and easy fatigability. It is characterized by genetic, phenotypic, pathophysiologic, and histopathologic variabilities [1]. The age of onset of CMS greatly varies, from presentation, commonly, in early infancy with apneic attacks and respiratory difficulties to that in adulthood with limb-girdle weakness and ptosis [2]. The mode of inheritance of CMS can be autosomal dominant or recessive. The neuromuscular defect of CMS can be presynaptic, synaptic basal laminar, or postsynaptic [1]. The deficient protein varies with the type of CMS and includes Dok-7, Musk, b2-Laminin, AChR, GFPT1, or Plectin [3].

The Dok-7 protein is encoded by the DOK7 gene on chromosome 4p16.2. The clinical presentation of DOK7 mutations varies, with the typical onset in childhood, usually after the completion of motor milestones with progressive weakness and difficulty in ambulation. DOK7 mutations can be associated with ptosis, hypotonia, and bulbar symptoms such as dysphagia and dysarthria. Proximal muscle weakness is greater than distal weakness in most of the studied populations [4]. Truncal weakness is common in this population and can lead to spinal deformity [5]. Forced vital capacity is reduced in most studied DOK7 mutations but respiratory symptoms are rarely presented. Here, we report a case of isolated limb-griddle CMS owing to a novel DOK7 gene mutation in a 28-year-old man and two of his sisters from Saudi Arabia.

2. Cases

2.1. Case#1

A 30-year-old otherwise healthy man presented with a history of bilateral proximal weakness involving both upper and lower limbs since 12 years of age. The patient described his weakness as intermittent, ranging from apparently symptom-free days to days with difficulty raising his arms above the shoulder, easy fatigue while climbing stairs, difficulty combing his hair, difficulty holding objects for a long time, increased difficulty in standing from a

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seated position, and difficulty jogging irrespective of the time of the day.

His symptoms worsened during family and social events. He suffered from generalized fatigue with inability to drive for long periods of time and staying up late. Symptoms did not show diurnal variation, and he did not have the history of bulbar weakness, diplopia, ptosis, facial weakness, sensory complains, cognitive impairments, seizures, joint pain, ulcers, or rashes. There was no family history of malignancy. However, both sisters showed similar symptoms at 20 years of age. Both parents were first cousins. The patient had no prenatal, natal, or postnatal history as well as no past surgical history. He worked as an accountant. Several other family members are symptomatic (Figure 1).

Upon examination, there was a limb-girdle pattern of muscle weakness, involving both upper and lower limbs, with inability to raise arms above the shoulder level. He had normal neck muscles power, reflexes, sensations in all limbs, and a down-going planter response. There were no bulbar signs, tongue atrophy, extraocular muscle weakness, ptosis, ocular misalignment, or abnormalities in other cranial nerves. The patient had a positive Gower's sign but no fatigable weakness, myotonia, change in phonation, or gait abnormalities.

All laboratory investigations were within normal ranges, including AChR antibody, anti-MUSK antibodies, thyroid function, creatine kinase enzyme level, bone profile, and vitamin D level. Nerve conduction studies were within normal limits for the following recorded nerves: right median, ulnar, tibial, peroneal, sural, and superficial peroneal nerves. Electromyography of the right deltoid and iliopsoas muscles showed normal spontaneous activity and normal motor unit potential duration, amplitude, and recruitment. Repetitive nerve stimulation of the ulnar nerve revealed normal response in the abductor digiti minimi muscle, while that of the axillary nerve revealed decremental responses in the deltoid muscle (>50%) (Figure 2).

Muscle biopsy of the right deltoid revealed normal histology with no evidence of myopathic or neurogenic pathology. Furthermore, genetic analysis and exome sequencing of the patient

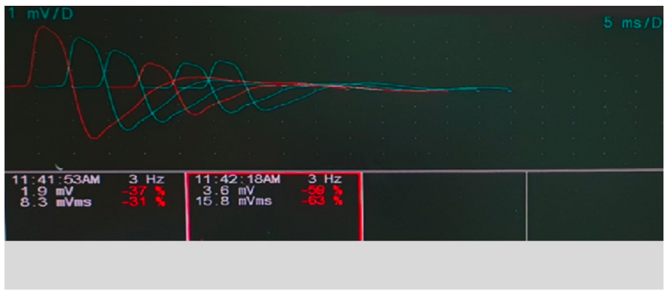

Figure 2. Repetitive nerve stimulation of the right deltoid revealed decremental response.

(proband III-8) using clinical exome sequencing of clinically-relevant 6698 genes was performed using the TruSight One Expanded Exome panel from Illumina. The mean region coverage depth achieved was 166X, and 95% of the variants identified were covered at 50X or more. We identified a homozygous missense mutation in the DOK7 gene, which resulted in the replacement of glycine residue at position 127 with a serine with the designation NM_173660.4: c.379G > A, p.(Gly127Ser) (Figure 3). This variant is predicted as a variant of unknown significance according to ACMG guidelines [6]. However, the GnomAD exomes allele frequency of this variant is rare (0.00004), and it is predicted to be damaging by SIFT, PolyPhen, and Mutation Taster with a DANN score of 0.9985. This variant was confirmed by Sanger sequencing and identified in the homozygous state in two additional siblings of the proband (proband III-3, III-9). Two other third generation family members (proband IV-1, IV-2) exhibit symptoms of weakness and fatigue but have not been tested yet. The Gly127 residue is highly conserved in evolution (Figure 4) and 3D modeling of the Gly127Ser change is predicted to cause structural damage to the protein based on The Missense3D application (Figure 5).

The patient and his two symptomatic sisters were started on oral salbutamol (4 mg) twice per day. He reported and showed a considerable improvement during a follow-up.

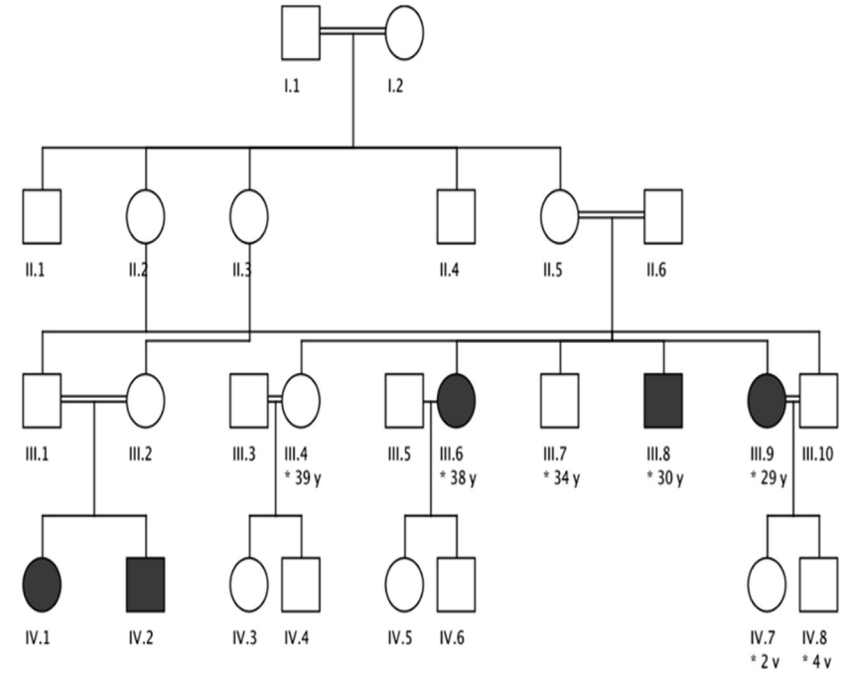

Figure 1. Pedigree of the proband family. (III.8) is the proband. Square are the males while circle are the female. The colored figures are the ones who exhibited symptoms.

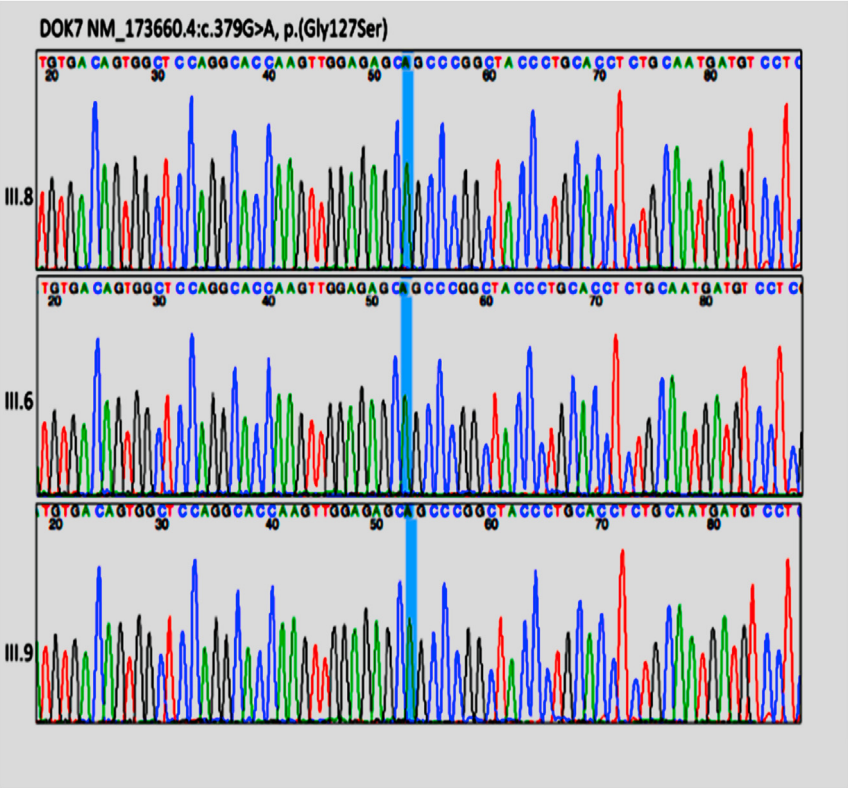

Figure 3. Gene sequence of the three active cases.

2.2. Case #2

A 38-year-old healthy woman presented with similar symptoms of limb-griddle weakness, which started at 24 years of age. She reported difficulty in standing up and lifting heavy objects and, on some days, needing to use both hands to lift a glass of water. There was no diurnal variation, but her symptoms worsened when she was under heavy physical stress, reaching a state where she could not even raise herself up from a chair. She worked as a school principal and reported that on some days she could not attend her work owing to the weakness. She has a daughter and a son (6 and 10 years old, respectively), and they did not develop any weakness. She was started on the same regimen as case 1. She reported a considerable improvement during a follow up.

2.3. Case#3

A 29-year-old healthy woman presented with similar symptoms of limb-griddle weakness, which started at 19 years of age and was similar to her two siblings. She reported difficulty in standing up and lifting heavy objects, requiring all her effort to lift a spoon to eat with; on some days, both her hands were required to complete a task. It was difficult for her to use stairs; on some days, she required the assistance of other people. There was no diurnal variation, but her symptoms worsened when she was under heavy physical stress. She does not work. She has a daughter and a son (2 and 5 years old, respectively), and they did not develop any weakness. She was started on the same regimen of oral salbutamol (4 mg) twice per day. She reported only marginal improvement during a follow up. Patient could take stairs independently, but she still had difficulty with upper limb activity, and it was challenging for her to hold a glass of water.

Informed consent was obtained from each participant before recruiting them in the case series. The consent included the study purpose and confidentiality of the participants. Anonymity was assured to all the participants. Moreover, approval from IRB in King Abdulaziz University was also obtained before commencing this study, No of Registration at National Committee of Bio.& Med. Ethics (HA-02-J-008).

3. Discussion

We reported a family from Saudi Arabia diagnosed with CMS, which was secondary to a novel DOK7 missense mutation, presenting with isolated limb-griddle weakness. For DOK7 mutation, ptosis is a common sign [5]; however, it was absent in our patients. The limb-griddle pattern of weakness can be associated with tubular aggregation on muscle biopsy [7], which was not present in our patients. CMS with tubular aggregation tends to exclusively affect proximal muscles over the distal muscle similar to DOK7 CMS. Our patient had only proximal weakness, which implied that the

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HUMAN    GTKLESGPATLHLCNDVLVLARDIPPAVTGQWKLSDLRRYGAVPSGFIFEGGTRCGYWAG 180
MOUSE    GTKLESGPATLHLCNDILVLARDIPPTVMGQWKLSDLRRYGAVPNGFIFEGGTRCGYWAG 180
RAT      GTKLESGPATLHLCNDILVLARDIPPTVTGQWKLSDLRRYGAVPNGFIFEGGTRCGYWAG 143
CHICKEN  GTKLESGPATLHFCNDILVLAKDLPPSVMGQWKLSDLRRYGAVPNGFIFEGGTRCGFWAG 180
XENOPUS  GTKLESGLATLHLCNDILVLARDIPPVVIGQWKLTDLRRYGAVANGFVFEGGTRCGYWAG 180
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Figure 4. CLUSTALW alignment of DOK7 amino acid sequences shows a high level of conservation at the Gly127 residue.

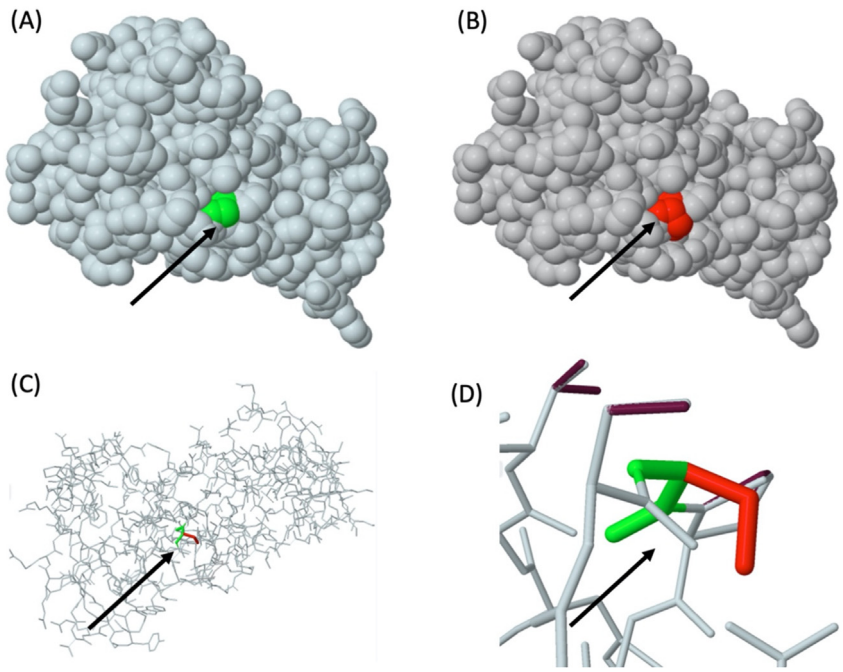

Figure 5. 3D modelling of the effect of the Gly127Ser variation on the DOK7 structure. (A) wild-type model (B) mutant DOK7 (C) wild-type (green) and Gly127Ser (red) shown on a wire diagram of the DOK7 protein model (D) Enlarged residue 127 shown in (C). Black arrow indicates the variant identified in this study. The Missense3D application was used to model the variant effect on the DOK7 structure.

phenotypic presentation of DOK7 mutation significantly varied across patients; this observation could be attributed to different pathophysiology of different mutations and to how many amino acids were affected in the gene sequence of DOK7 [2,4,8].

One characteristic of DOK7 CMS is its poor or absent response to cholinesterase inhibitors, or the illness can even worsen, as has been reported by Ben Ammar et al., where a 43-year-old patient with a DOK7 mutation deteriorated within 10 days of taking cholinesterase inhibitors, which led to tetraplegia and loss of ambulation that have lasted for a year [2]. Another study showed patients who improved transiently on cholinesterase inhibitors, before deteriorating in a short period of time [7]. 3,4-diaminopyridine was used on some patient in combination with cholinesterase inhibitors, which showed only temporary improvement before its effect was lost [7].

DOK7 CMS may benefit from sympathomimetics such as salbutamol and ephedrine [9]. Another genetic entity, such as limb-gridle CMS with tubular aggregate, showed good response to cholinesterase inhibitors [2]. Ephedrine, salbutamol, and similar sympathomimetics increase neuromuscular transmission at the end-plate junction. A proposed theory for the mechanism is the stimulation of presynaptic α1-and β-adrenoceptors and postsynaptic β2-adrenoceptors [10]. The effect of Sympathomimetic on the postsynaptic Stabilization of the membrane of the NMJ was demonstrated on mice models with DOK7 CMS, Showing Increased number of active NMJs after administration of Salbutamol, even though it may take several months to see any effect on the NMJ [11]. This effect is consistent even in Anti-MuSK MG, which is an Autoimmune entity with dysfunction in the post synaptic part of NMJ due to Antibodies against it [12].

According to GenBank, this missense mutation with the replacement of glycine residue at position 127 with serine c.379G > A, p.(Gly127Ser) is novel. The disease has a pure limb-girdle distribution of weakness, without bulbar weakness or spinal deformity, and has a variable age of onset, ranging from early adolescence to early adulthood. In general, this genotype of DOK7 CMS has a mild course compared with other mutations of the DOK7 gene, with some patients having antenatal onset, and others exhibiting progressive proximal muscle and bulbar weakness, and eventually respiratory compromise [4]. Analysis of the predicated protein structure of the variant showed its pathogenicity, this is a reliable method to predict the pathogenicity of missense mutations [13]. the resultant crystal structure of the mutated DOK7 protein may shed some light on some of the phenotypic characteristics seen in our patients, such as the response to sympathomimetics and the primary presentation of limb-girdle weakness, as it was shown in two patients with solely N-terminal missense mutations [14, 15]. The Gly127Ser variant is in the phosphotyrosine-binding domain (PTB) at the N-terminus of the protein which is required for binding to and activation of the effector MuSK protein in myotubes [16]. The structural damage predicted as a result of this mutation is likely to interfere with MusK activation as shown previously for a neighboring missense change (Arg158Gln) [16]. The location of the mutation in the N-terminus may explain its milder phenotype.

A case of heterozygous DOK7 frameshift gene mutation was described in a 67-year-old female [17], in addition to multiple cases of patients with heterozygous genes presenting with symptoms [8, 18]. This variability between genotype-phenotype could be explained by the method of genetic analysis employed, as exome sequencing, in comparison to whole genome sequencing, leaves out introns and regulatory regions outside the protein-coding sequence, thus it may miss contributing abnormalities in the after mentioned sites [19]. This may lead to incomplete understanding of the possible pathogenicity of the genes, especially the heterozygous mutations. This method may help identify paradoxically symptomatic patients with a heterozygous genotype.

Our patients had a fluctuating course of disease, with minimal weakness on some days and debilitating weakness on others. The fluctuating nature of the phenotype is similar to some DOK7 mutations [2]. No cases of DOK7 CMS was reported in Saudi Arabia to draw comparison to. The disease was responsive to sympathomimetics, as expected with DOK7 CMS in the literature; however, one patient had only minor improvement with it.

4. Conclusion

We reported a novel DOK7 gene mutation with an adolescent-young adult presentation of pure limb-girdle weakness with response to sympathomimetics. Our aim was to demonstrate the phenotypic and

genotypic variability of DOK7 congenital myasthenic syndrome, and how gene analysis could guide the physicians to use the most appropriate medication for the patient's genotype. The observation of variable phenotype associated with the variant could be attributed to the fact that it is a missense change with variable effects on protein structure and thus function. Generating a disease models (e.g. stem cells) harboring this variant could serve as valuable tool to understand the disease as well as identify therapeutics that could reduce the negative impact of the variant.

Declarations

Author contribution statement

All authors listed have significantly contributed to the investigation, development and writing of this article.

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Data availability statement

Data will be made available on request.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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