A novel DOK7 mutation causing autosomal recessive limb-girdle congenital myasthenic syndrome

Adam S. Reynolds MD, Ava Y. Lin MD PhD, Leo H. Wang MD PhD

Department of Neurology, University of Washington Medical Center, Seattle, Washington

ABSTRACT

We report a case series of 5 Latino patients with limb-girdle pattern weakness; four patients are sisters, with one patient unrelated. Repetitive nerve stimulation showed a significant decrement in all cases. Targeted genetic testing for congenital myasthenic syndromes demonstrated a known DOK7 pathogenic mutation in each case, and in all five cases also revealed a novel DOK7 missense mutation in exon 7 with c.94G>A; providing strong evidence this mutation is pathogenic. DOK7-related congenital myasthenic syndrome often lacks significant oculobulbar involvement, and may present with limb-girdle weakness, mimicking limb-girdle muscular dystrophy.

Key words: Congenital myasthenia gravis, Limb-girdle weakness, Downstream of tyrosine kinase 7 (DOK7)

Introduction

Congenital myasthenic syndromes (CMS) are a growing group of rare, genetic disorders affecting neuromuscular transmission at the neuromuscular junction.[1] Clinical features of CMS are highly variable compared to auto-immune-mediated myasthenia gravis, and may not have significant oculobulbar involvement. Limb-girdle CMS is a subgroup that features prominent proximal and sometimes distal appendicular weakness, and can present similarly to limb girdle muscular dystrophy.[2] Limb-girdle CMS can be further subdivided into defects in glycosylation or MUSK-AGRN complex.

DOK7 CMS is characterized clinically by limb-girdle pattern weakness with childhood onset and variable clinical course ranging from pediatric respiratory failure to mild weakness.[3-8] Treatment of DOK7 CMS with anticholinesterase therapy usually results in worsening of symptoms [9,10]. One large case series reported that 94% of adult-onset CMS patients were initially misdiagnosed, most often with myopathy or seronegative myasthenia gravis, with an average delay of 26 years from symptom onset to diagnosis.[11] DOK7 CMS is highly treatable with β-agonist therapy, and to a lesser extent with 3,4-diaminopyridine (3,4-DAP). Herein, we describe a five patient case series with novel DOK7 mutation, characterize their clinical course, and discuss their relevant electrodiagnostic findings, and their subsequent response to albuterol therapy.

Case Report

Patient 1

A 22-year-old right-handed female referred for weakness since childhood. She endorsed normal motor development until age three, when she developed diffuse weakness. Her weakness fluctuated throughout the day and was slightly worse at the end of the day. She denied any falls, dysphagia, ptosis, diplopia, dark colored urine, weight loss, muscle atrophy, or history of respiratory failure.

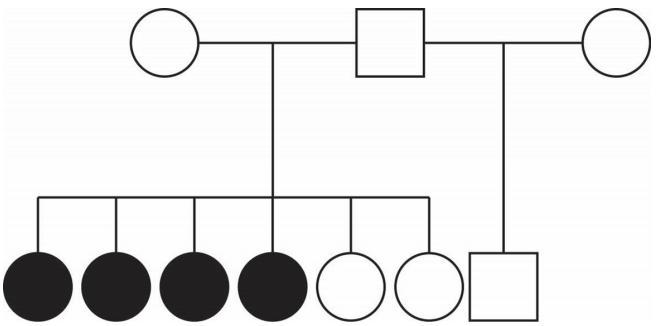


Figure 1. Pedigree of the proband. Circles are female, squares are male. Shaded shapes indicate symptomatic individuals.

She has five sisters and one half-brother. Three of her five sisters had a very similar pattern of weakness. None of her other family members including her parents, grandparents and other siblings were affected.

Her exam was notable for normal facial strength, no ptosis, no diplopia, normal sustained up-gaze, with moderate, diffuse and symmetric weakness, worst proximally.

Repetitive stimulation at 2Hz of the spinal accessory nerve showed a significant decrement. Pyridostigmine was then prescribed empirically at 60mg daily for possible congenital myasthenic syndrome, resulting in a dramatic worsening of weakness within two days, and was subsequently stopped. DOK7 congenital myasthenia was then suspected based on phenotype and worsening with pyridostigmine. Empiric treatment with albuterol 2 milligrams three times a day resulted in significant improvement.

After starting albuterol, she could use the stairs, raise her arms above her head and walk on her toes; none of which she could do prior. A Mayo Congenital Myasthenic Syndrome panel demonstrated a known pathological

heterozygous c.1124_1127dupTGCC frameshift mutation (p.Pro376ProfsX30), which is the most common disease causing mutation in DOK7-related CMS from European studies,[4,6,7,12] in addition to a novel missense c.94G>A mutation that causes a change from valine to methionine in codon 32.

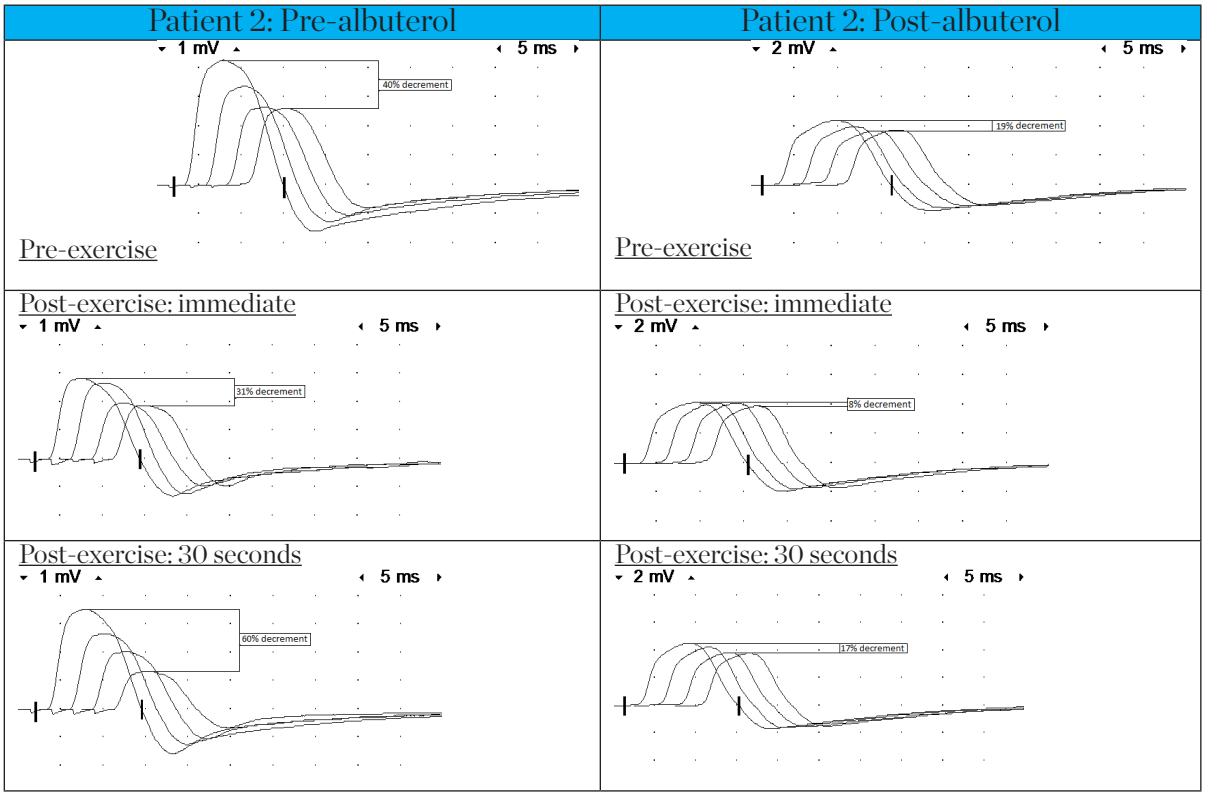
Patients 2-4

All presented similarly to the index patient, with proximal greater than distal weakness, minimal to no fatigable weakness, no oculobulbar involvement, and no history of respiratory failure. All improved substantially after albuterol. Targeted genetic testing for DOK7 in patients 2-4 was the same as in patient 1.

Table 1. Clinical characteristics and treatment responses of 5 patients with DOK7-related CMS in our kinship. SAN: spinal accessory nerve. Rep stim: repetitive stimulation.

	Pre-albuterol treatment							Post-albuterol	
Patient	Age at symptom onset	Ptosis/ diplopia	Distal/ proximal weakness	Fatiguable weakness/ temporal variability	Average grip strength (lbs)	Max % decrement on SAN/SAN max post exercise decrement	Pyridostigmine response	Albuterol response	Average grip strength (lbs)
1	3	-/-	-/+	-/-	N/A	19/23	Worsening	Excellent	72
2	4	-/-	+/+	-/-	25	40/60	N/A	Excellent	80
3	4	-/-	+/+	-/-	26	17/71	N/A	Excellent	35
4	4	-/-	-/+	-/-	57	10/56	N/A	Excellent	N/A
5	1	+/-	+/+	-/-	N/A	6/18	Respiratory arrest	Excellent	87

Figure 2. Patient 2, Electrodiagnostic data pre- and post-albuterol treatment. Note the electrodecrement is significantly lessened post-albuterol.



Patient 5

A 23-year-old right-handed male referred for weakness. A weak suck was noted at birth. He had mild respiratory insufficiency, diplopia and dysphagia. He walked at age 3, then became non-ambulatory at age 7. He was diagnosed with presumptive congenital myasthenia gravis at age 8. He was treated with empiric pyridostigmine which triggered respiratory failure and received no further pharmacologic treatment. No other family members had weakness, including a son, three half-brothers, and one half-sister.

His exam was notable for bilateral ptosis and moderate diffuse weakness, worst in the proximal upper extremities and distal lower extremities. Repetitive stimulation at 2Hz of the spinal accessory nerve showed a significant decrement.

Genetic testing with the Invitae Comprehensive Neuromuscular Disorders Panel yielded a known pathologic, nonsense, c.957 del (p.Lys320Serfs*136) DOK7 mutation, as well as the same novel missense c.94G>A DOK7 mutation seen in Patients 1-4.

Discussion

There are more than 100 different disease causing mutations in DOK7, with the most common mutation being the c.1124_1127dupTGCC frameshift mutation.[4,6,7,12] The 1124_1127dupTGCC has been shown to result in a truncated C-terminal region. The C-terminal truncations impair activation of MuSK in specific situations. Proteins with these mutations are able to induce MuSK activation during differentiation of C2C12 cells into myotubes but not when the myotubes are fully differentiated.[13]

In our cohort, we report a novel DOK7 mutation that is likely pathogenic. The clinical phenotype in our cohort is similar to that previously reported for a patient carrying mutation from alanine to valine on residue 33, immediately neighboring our unique mutation site. (Figure 3) Residues 30 to 33 were previously shown to be responsible for dimerization of DOK7 to allow appropriate interaction with phosphorylated MuSK to form the Agrin-MuSK-DOK7 complex.[5,14,15]

The novel DOK7 missense c.94G>A mutation seen in our cohort was reported as a variant of unknown significance. Algorithms predicting protein structure disagree on the mutation's effect. PolyPhen-2: benign, SIFT: deleterious. The gnomAD exome allele frequency is rare (0.00003) and 3 of the 4 reported alleles were in Latino patients, with a correspondingly higher frequency in this population. Our cohort of five patients are all Latino. Latino populations have lower rates of genetic testing than other racial groups, and this variant may be under reported as a result.

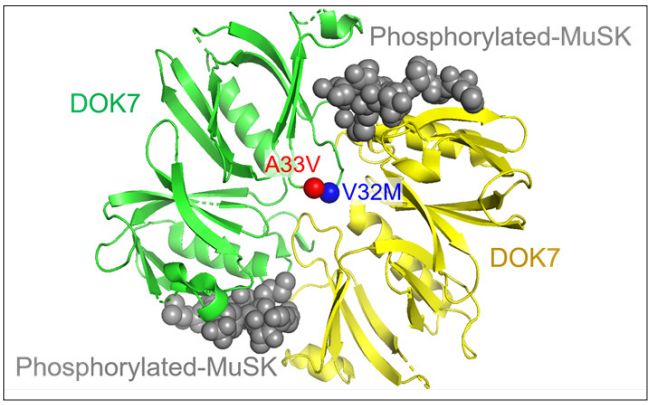


Figure 3. Structural depiction of novel point mutation of V32M (blue dot). DOK7 dimers with molecule 1 in green and molecule 2 in yellow. Phosphorylated tyrosine 553 of its binding partner MuSK identified in grey spheres. A33V is a previously reported disease-causing mutation. Both V32M and A33V are in a highly conserved loop and are key components of proper formation of the DOK7 dimers.[16] This region is proposed to be highly dependent on tight structural coupling and sensitive to steric sizes of these hydrophobic residues. Both mutations of V32M and A33V caused a mutation into a larger hydrophobic residue which would presumably cause steric clash and interference with dimerization.

The functional absence of DOK7 leads to the defective formation of the neuromuscular junction, which has been described in the biopsies of patients with DOK7-related CMS.[13,16-20] We hypothesize that the combination of DOK7 mutations that affect dimerization and activation of MuSK results in a more severe defect in neuromuscular junction formation in mature muscle fibers that results in a more severe CMAP decrement on repetitive nerve stimulation compared with mutations that result in truncation of the C-terminus alone.

Limb-girdle weakness with onset in early childhood may be a form of treatable CMS. Several tests have proven to be helpful in assisting diagnosis of DOK7-related CMS, including electro-microscopic structure of post-synaptic cleft,[21] trial of pyridostigmine,[9,22,23] and repetitive stimulation of proximal nerves. Therefore, repetitive nerve stimulation of proximal nerves should be performed in patients with early-onset unexplained limb-girdle weakness. In a case series of 179 patients with myasthenia gravis with significant decrement on low frequency repetitive nerve stimulation at rest, the average worsening of decrement of the spinal accessory nerve post exercise was 1.9%, and the maximal worsening seen in any patient was 12%.[24] In our series, the post exercise maximal absolute worsening of decrement in the spinal accessory nerve was equal to or more than 12% in patients 2, 3, 4 and 5. (Table 1) This unusually severe pattern of decrement could be related to the combination of the two mutations—missense c.94G>A mutation and nonsense

mutation in the C terminus (c.1124_1127dupTGCC,p.Pro376ProfsX30 or (c.957 del,p.Lys320Serfs*136).

To our knowledge, this is the first published CMS post-exercise repetitive nerve stimulation data, and further study of CMS electrodiagnostic data will be needed to confirm this trend. Our suggestion is that any patient with a post exercise maximal worsening of decrement greater than 10% be considered for CMS; this may have the greatest utility in differentiating CMS from sero-negative myasthenia gravis.

In summary, we report a five patient case series of patients with a limb girdle pattern of weakness, across two unrelated Latino families, all with homozygous DOK7 mutations with a known pathogenic DOK7 mutation in addition to a novel missense c.94G>A variant of unknown significance. This provides strong evidence that DOK7 c.94G>A is pathogenic. Furthermore, this case series illustrates the importance of performing repetitive nerve stimulation in patients with suspected limb-girdle muscular dystrophy, as decrement on repetitive stimulation may indicate limb-girdle CMS.

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