A novel MUSK mutation in a patient with CMS9

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Introduction

Congenital myasthenic syndromes (CMS) are a relatively rare cause of fatigable muscle weakness, often with significant ocular, bulbar and respiratory impairment1. Mutations in the gene encoding muscle-specific tyrosine kinase (MuSK) can lead to abnormal endplate and acetylcholine receptor functioning and cause an autosomal recessive post-synaptic CMS (CMS9). Only 23 patients with CMS9 have been characterized in the literature since the initial description in 20042. Here, we report a newly diagnosed case of CMS9 in a 23-year-old female who harbored a novel c.296G>T (Cys99Phe) mutation in the MUSK gene, thereby expanding the phenotypic/genotypic characterization of this rare disorder.

Case Report

A 23-year-old woman presented with a 13-month history of fluctuating limb weakness, fatigue, ptosis, double vision, dysphagia, and orthopnea. She had been consequently diagnosed with autoimmune myasthenia gravis, although AChR binding/modulating antibodies were negative, and treated with mestinon (60mg TID) and prednisone 30mg daily. She had also had one prior hospitalization for worsening bulbar /respiratory symptoms treated with a single IVIG course. She did not have a satisfactory response to overall therapy and was consequently referred for further neuromuscular evaluation. However, she developed worsening dysphagia and orthopnea, requiring local ER visitation, intubation, and transfer to our medical center. On exam, she had limited horizontal and vertical gaze with bilateral ptosis, bifacial weakness, and proximal (4/5) weakness in neck flexion, shoulder abduction, and hip flexion. Reflexes and sensations were normal. She was able to be extubated quickly (prior to PLEX) and then underwent six cycles of plasma exchange. She was discharged on mestinon and prednisone (50 mg daily) and scheduled for IVIG 2 grams/kg infusion with a follow-up in the NM clinic. Admission antibodies, including AChR, MuSK, LRP-4, and VGCC, were drawn and resulted in a negative.

She presented for an initial NM clinic visit about one-month post-discharge, just before the initial IVIG infusion. She had discontinued Mestinon as it provided no benefit. Her exam was notable for bilateral asymmetric ptosis, limited vertical and horizontal gaze, and bifacial weakness. Flaccid dysarthria, fatigable proximal weakness, and finger extension weakness. She was also still bothered by orthopnea with morning headaches. Additional workup included a low CK (23 U/l), normal TSH (3.17 mcIU/ml), normal lactate (2.1 mmole/l), and normal pyruvate (1mg/dl). Three hz repetitive nerve stimulation (RNS) of the trapezius showed a maximum baseline decrement of 22% with immediate post-exercise repair (Figures 1A and B). SFEMG on the frontalis showed increased jitter with an increased MCD of 87 (Table 1). These results confirmed a defect in NMJ transmission, and a diagnosis of seronegative myasthenia gravis was given. Cellcept was added to her prednisone and IVIG therapy. Pulmonary function testing showed a restrictive pattern with significant worsening in the supine position (Table 2). Nocturnal BiPAP was started, which helped with her morning headaches.

At a follow-up NM visit, the patient reported no significant improvement in symptoms, and her exam remained unchanged. Given her seronegative status and lack of response to necessary immunomodulatory therapy, we sent a genetic panel for congenital myasthenic syndromes through Invitae Genetics. Analysis showed a known pathogenic c2368G>A (p.Val790Met) mutation in MUSK as well as a variant c.296G>T (p.Cys99Phe) of unclear significance. To help determine the phase of these mutations (cis/trans), we sequenced the MUSK gene in both parents. Father harbored the c.296G>T (p.Cys99Phe) mutation while mother possessed the c2368G>A (p.Val790Met) mutation proving the MUSK mutations in the proband were in trans. Both parents were asymptomatic and had normal exams. There was no family history of anyone with similar symptoms. With a diagnosis of CMS9, immunosuppressive medications were stopped without any change in her symptoms. She had no benefit from a trial of 3.4 aminopyridine (Firdapse), discontinued at the dose of 15 mg TID due to side effects. She was then started on Albuterol with modest improvement.

Discussion

Here we present a 23-year-old female with CMS9 who harbors an established pathological c2368G>A mutation in compound heterozygosity with. a novel .c296G>T mutation in exon 3. This novel sequence change replaces cysteine with phenylalanine at codon 99 of the MuSK protein (p.Cys99Phe), which resides in the first three immunoglobulins (Ig)-like domains of the protein. The cysteine 99 residue is highly conserved across species, and a marked physicochemical difference exists between the uncharged polar cysteine and uncharged non-polar phenylalanine (Figure 2A). Such a mutation would be

expected to disrupt normal protein function based on a functional modeling platform (Sherloc) performed at Invitae[3].

MuSK is a postsynaptic muscle-specific tyrosine kinase receptor comprised of three extracellular immunoglobulin-like domains, a frizzled cysteine-rich domain, a transmembrane-spanning region, and an intracellular region including a juxta-membrane domain, a kinase domain, and a short C-terminal tail (Figure 2B). It is part of a signaling pathway critical for the clustering of acetylcholine receptors and maintenance 4,5,6. Disease-causing mutations have been reported in all MuSK domains, save the transmembrane and c-terminal tail regions, with a majority occurring in the kinase domain 2,4-14. These mutations can reduce MuSK protein stability and expression and interfere with its ability to interact with other critical postsynaptic junction proteins, including LRP-4 and Dok7. As LRP-4 binds to MuSK in the first Ig-like domain, it is reasonable to hypothesize that the novel Cys99 Phe mutation presented here may impair normal LRP4-MuSK interaction.

Although there is some variability in the clinical presentation of CMS9, a review of the published cases suggests two distinct phenotypic presentations (Table 3). The most common is neonatal onset, with an overwhelming respiratory presentation either from diaphragm weakness or vocal cord dysfunction. All patients carrying one truncation or stop codon mutation and one KD mutation, save one, had this severe phenotype. A minority of patients presented in adolescence or early adulthood with proximal limb girdle weakness and ocular symptoms. Patents harboring at least one Ig-like or JM domain mutation presented with this later-onset phenotype. Our patient is the first case reported in the literature carrying one Ig-like and 1 KD mutation. Interestingly, she appears to have a phenotype characterized by later onset presentation but with significant respiratory complaints requiring nocturnal non-invasive ventilation. She has had a partial response to albuterol with improvement in fatigue, limb strength ptosis, and double vision, similar to other published CMS9 patients.

In summary, we report a patient with a novel (C99F) mutation and an established M790V mutation in the MUSK gene, causing a rare form of CMS9. She has a later age presentation phenotype with a significant respiratory component. Her case emphasizes the importance of genetic testing in seronegative myasthenia patients so that exposure to immunosuppressive medications can be minimized with a correct genetic diagnosis.

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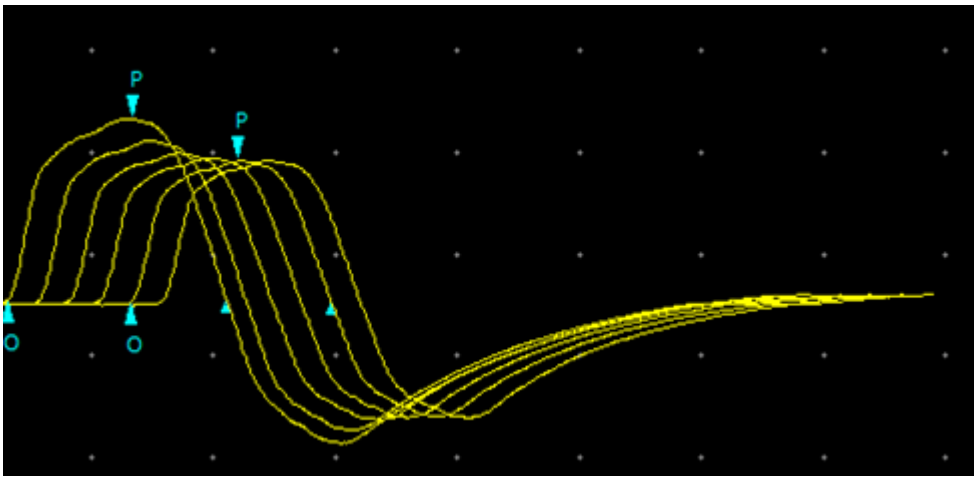

Figure 1A. 3Hz repetitive nerve stimulation of the spinal accessory nerve recording from the trapezius at baseline.

Trace #	Amp (mV)	Amp % Dif	Area (mV·ms)	Area % Dif
Right Trapezius				
Tr 1: Baseline: Trace 1	3.54	100	34.15	100
Tr 1: Baseline: Trace 2	3.16	-10.9	29.15	-14.6
Tr 1: Baseline: Trace 3	2.90	-18.1	26.33	-22.9
Tr 1: Baseline: Trace 4	2.82	-20.4	25.38	-25.7
Tr 1: Baseline: Trace 5	2.76	-22.0	24.72	-27.6
Tr 1: Baseline: Trace 6	2.78	-21.5	24.81	-27.3
Tr 2: Post Exercise: Trace 1	3.68	100	34.46	100
Tr 2: Post Exercise: Trace 2	3.45	-6.4	31.02	-10.0
Tr 2: Post Exercise: Trace 3	3.22	-12.4	28.68	-16.8
Tr 2: Post Exercise: Trace 4	3.15	-14.6	27.86	-19.2
Tr 2: Post Exercise: Trace 5	3.15	-14.4	27.66	-19.7
Tr 2: Post Exercise: Trace 6	3.17	-13.8	27.83	-19.3

Figure 1B. Data from 3 Hz RNS at baseline and after 1 minute of exercise.

Table 1. Single Fiber EMG Frontalis study

Run	Samples	Blocks	IPI	Jitter	Jitter Norm	MCD	MSCD	Freq.	Stored
Right Frontalis									
1.1	63	0	1818.4	33.4		34.9	33.4	23.5	Yes
2.1	90	0	2801.5	127.8		127.8	132.9	27.0	Yes
3.1	69	0	1467.3	133.0		133.0	138.4	17.3	Yes
4.1	94	0	1256.3	50.0		50.0	50.6	17.1	Yes
5.1	91	0	1220.6	55.5		56.6	55.5	16.3	Yes
5.2	91	0	2054.2	77.1		77.1	77.1	16.3	Yes
6.1	95	0	2948.7	45.4		45.4	46.2	23.3	Yes
6.2	96	0	1309.5	26.8		28.4	26.8	23.3	Yes
7.1	83	0	1583.6	77.3		79.7	77.3	15.3	Yes
7.2	65	0	442.1	136.6		136.6	152.0	15.8	Yes
8.1	94	0	1884.9	37.6		39.2	37.6	23.1	Yes
8.2	34	0	853.6	211.9		234.9	211.9	23.3	Yes
Mean			1636.7	84.4	<34	**87.0**	86.6	20.1	
StdDev			697.8	54.0		58.2	56.0	3.9	

Block Ratio: 0% Fiber Density: 0.00

Table 2. Pulmonary Function Testing

		Pred	Pred LL	Pre	Pre%Ref	Post	Post%Ref	%Chg
FVC	**L**	4.38	3.50	3.24	74	2.85	65	-12
FEV 1	**L**	3.74	3.01	2.73	73	2.48	66	-9

		Pred	Pred LL	Pre	Pre%Ref	Post	Post%Ref	%chg
MIP	**cmH2O**	72.9	31.1	56.4	77	50.4	69	-11
MEP	**cmH2O**	93.3	41.8	46.6	50	50.3	54	8

Pre = sitting position; Post = supine position.

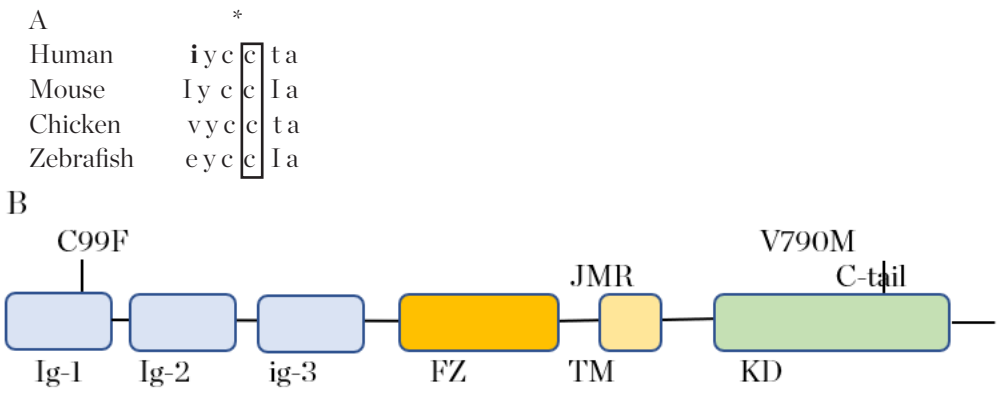

Figure 2 A) Alignment of amino acid sequences across multiple species encompassing human C99 (*). B) Schematic representation of MuSK including the various functional domains. The two mutations of this case report are shown. Ig= immunoglobulin-like; Fz=frazzled domain; TM= transmembrane; JMR = juxtamembranous region ; KD = kinase domain

Table 3 Genotypic/phenotypic correlations

Genotype	Domains	Presentation	Weakness
M605I/A727V	KD/KD	Neonatal	Respiratory
V722A/ c.79 +2T	KD/#	Neonatal	Vocal cord, Respiratory
Q688X/F775S	KD/KD	Neonatal	Respiratory
N103S/R166X	Ig/*	Neonatal	Respiratory
C317R/ A617V	FZ/KD	Neonatal	Respiratory, Hypotonia
V790M/Lys156Argfsstop20	KD/*	Neonatal or adult	Respiratory or Ocular
V790M/ c.220insC	KD/#	Neonatal	Respiratory, Ocular
K720E/c.79 +2T	KD/ #	Neonatal	Vocal cord, Respiratory
A763T/R816X	KD/ *	Neonatal	Vocal cord, Respiratory
P344R/P344R	FZ/FZ	Early childhood	Ocular, Limb-girdle
D38E/ genomic deletion encompassing exons 2–3	Ig/#	Early childhood	Limb-girdle
M835V/M835V	KD/KD	Childhood	Ocular, Limb-girdle
P650T/ I795S	KD/KD	Childhood	Limb-girdle
I581P/I581P	KD/KD	Adolescence	Limb-girdle
L545P /R166X	JM/*	Adolescence or adult	Limb-girdle
C99F/V790M	Ig/KD	Adult	Ocular, Respiratory, LG

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