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CASE REPORT

Congenital myasthenic syndrome type 23 caused by a missense homozygous c.205G>T (p.Asp69Tyr) in *SLC25A1* gene in four Emirati patients from a single family

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ABSTRACT

Background: Congenital myasthenic syndromes (CMSs) are a clinically and genetically heterogeneous group of disorders caused by mutations that lead to altered neuromuscular junction transmissions. Recently, the solute carrier family 25 member 1 (*SLC25A1*) gene was described to cause CMS type 23. This gene encodes a mitochondrial citrate carrier, associated mainly with a severe neurometabolic disease like combined D-2- and L-2-hydroxyglutaric aciduria (D/L-2-HGA).

Case presentation: Here, we report four Emirati patients with a homozygous missense variant in *SLC25A1* with a phenotype restricted to relatively mild CMS. We performed whole exome sequencing (WES) in two relatives who presented with CMS to identify the underlying causative gene.

Conclusion: The WES analysis revealed the presence of a homozygous c.205G>T (p.Asp69Tyr) [(c.226G>T (p.Asp76Tyr)] in the *SLC25A1* gene; the same variant was identified in the other members in this family with the same phenotype. This suggests that c.205G>T (p.Asp69Tyr) [(c.226G>T p.(Asp76Tyr)] is associated with a relatively mild CMS phenotype and can be considered as a founder mutation in our region.

Keywords: Congenital myasthenic syndrome type 23, *SLC25A1* gene, whole exome sequencing.

Introduction

Congenital myasthenic syndromes (CMSs) are a heterogeneous group of disorders caused by mutations, leading to impaired functions of neuromuscular junction (NMJ) transmissions. This group is characterized by fatigable weakness on exertion of skeletal muscles (e.g., ocular, bulbar, and limb muscles) with onset at or shortly after birth or in early childhood; the symptoms may not manifest until later in childhood rarely (1-3). Cardiac and smooth muscles are usually not involved. The disease's severity and course are highly variable, ranging from minor symptoms to progressive disabling weakness. In some subtypes of CMS, myasthenic symptoms may be mild. Still, rapid and severe exacerbations of weakness or even sudden respiratory insufficiency episodes may be precipitated by fever, infections, or excitement, especially in individuals with CMS with episodic apnea or endplate rapsyn deficiency (4-6). Most often these disorders arise from mutations affecting postsynaptic components of the NMJ. Due to mutations affecting the motor nerve terminal, CMS comprises a rarer subset; they are increasingly recognized, and the majority of the recently discovered CMS genes result in presynaptic NMJ defects (7). *SLC25A1* is a mitochondrial citrate carrier that mediates the exchange of citrate/isocitrate with cytosolic malate (8). Variants in the *SLC25A1* gene are associated with severe neurometabolic diseases like combined D-2- and L-2-hydroxyglutaric aciduria (D/L-2-HGA) Online Mendelian Inheritance in Man (OMIM# 615182) (9-11). Here, we report four Emirati patients with a homozygous missense variant in *SLC25A1* with a phenotype restricted

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to a relatively mild CMS. We performed whole exome sequencing (WES) in two relatives presenting with CMS to identify the underlying causative gene. The WES analysis revealed the presence of a homozygous c.205G>T (p.Asp69Tyr) [(c.226G>T (p.Asp76Tyr)] in the *SLC25A1* gene. The same variant was identified in the other affected members in this family with this phenotype. This suggests that c.205G>T (p.Asp69Tyr) [(c.226G>T (p.Asp76Tyr)] is associated with a relatively mild CMS phenotype and can be considered a founder mutation in our region.

Case Presentation

Four Emirati patients from the same family with early infancy easy fatigability and bilateral ptosis were identified by the metabolic/genetic and neurology teams in Tawam Hospital (Al-Ain city) for clinical evaluation and follow-up. These patients were born to Emirati consanguineous parents (Figure 1). After obtaining informed consent, blood samples from the patients were collected in ethylenediaminetetraacetic acid tubes. According to the manufacturer's protocol, one of the affected individuals' deoxyribonucleic acid (DNA) was extracted from peripheral blood cells using the Flexigene DNA extraction kit (Qiagen Gmbh, Germany). The WES was carried out by the CENTOGENE AG laboratory in Rostock, Germany (www.centogene.com). The sample was processed on the Ion Proton platform (Life Technologies, Renfrew, United Kingdom). Approximately 36.5 Mb of coding exons were converted as described by consensus coding sequences. An in-house bioinformatics pipeline, including reading alignment to GRCh37/hg19 genome assembly, variant calling (single nucleotide and small deletion/insertion variants), annotation, and comprehensive variant filtering, was applied. All variants with minor allele frequencies of less than 1% in the gnomAD database and disease-causing variants reported in Human Gene Mutation Database ®, in ClinVar, or CentoMD® were considered. The investigation for relevant variants focused on coding exons and flanking ±20 intronic nucleotides of genes with clear gene-phenotype evidence (based on OMIM® information). Another one of the affected individuals' DNA was sent to the Prevention Genetics laboratory for PGxome, where they use next generation sequencing (NGS) technologies to cover the coding regions of targeted genes plus ~10 bases of non-coding DNA flanking each exon. Patient DNA corresponding to these regions was captured using Agilent Clinical Research Exome hybridization probes. The internally developed infinity pipeline carried out the data analysis and interpretation. Variant calls were made by the Genome analysis toolkit Haplotype caller and annotated using the in-house software and Jannovar. Copy number variants (CNVs) were also detected from NGS data. We utilized a CNV calling algorithm that compared mean read depth and distribution for each target in the test sample against multiple matched controls. Neighboring target read depth and distribution and zygosity of any variants within each target region reinforced CNV calls. All reported CNVs were confirmed using other technologies, such as Array comparative genomic hybridization, Multiplex ligation-dependent probe amplification, or Polymerase chain reaction.

Results

Patient 1: The index patient (IV-1) is currently a 6-year-old female child, seen initially at 2 years of age, referred to the metabolic clinic with a history of hypoglycemia after an episode of gastroenteritis, with incidental findings of failure to thrive and bilateral ptosis. The mother reported that she gets easy fatigued and gets quickly tired with walking for a short distance, to the extent that she cannot move, and all of this resolves after taking rest for a few minutes. Family history reported an affected maternal aunt and uncles (Figure 1). On examination at 2 years of age, the growth parameters showed that weight was below the 3rd percentile, head circumference was above the 97th centile for age and gender, and her developmental milestones were appropriate for her age. She was not dysmorphic, pale, or jaundiced. She had bilateral ptosis, with soft systolic murmur grade 2 in the left sternal border, not radiating. She has lumbar lordosis, lax knee joints with flat feet, and no other systemic abnormalities. The following laboratory investigations were unremarkable: complete blood count, renal function, liver function, ammonia, homocysteine, Creatine kinase, serum lactic acid, plasma

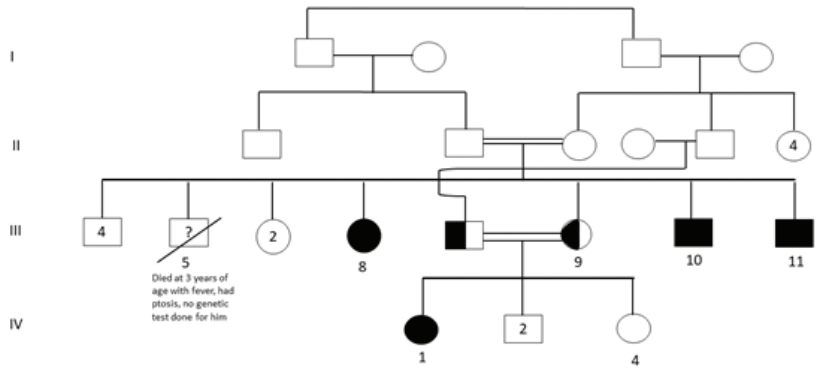

Figure 1. *Family pedigree. Circles and squares are females and males, respectively; filled symbols are affected members; half-filled symbols are carrier members; roman numbers indicate the generations; and Arabic numbers indicate offspring.*

and urine amino acid profiles, urine organic acid profile, carnitine, acylcarnitine profiles, and anti-acetylcholine receptor (AChR) antibodies. WES followed by whole genome sequencing showed a homozygous missense variant in *SLC25A1* gene: NM_001256534.1: c.226G>T (p.Asp76Tyr). The missense change was of uncertain significance, predicted by various *in silico* models: Sorting Intolerant From Tolerant (tolerated) and MutationTaster (disease-causing); it is classified as a variant of uncertain significance (class 3) according to recommendations of Centogene and The American College of Medical Genetics and Genomics. Her mitochondrial genome was negative.

Patient 2: The second patient (III-10) is currently a 24 year-old male, he was 21 years of age when he was initially presented with exertional fatigability that was effort-specific without diurnal variation, requesting a medical report to exempt him from compulsive military training. He had a long-term history of mild bilateral ptosis and easy fatigability since childhood. His symptoms were mainly related to rapid fatigue of his muscles. If he carried a heavy object, for example, a coffee pot, he would soon drop for some time, and his hands and fingers would become weak, and he cannot use them for 5-10 minutes. The same thing happens to his leg if he runs or walks for a long distance for more than 20 minutes. He has to stop frequently to rest and allow his muscles to work again. When he talks for a long time, his mouth would stop moving, and he would have slurring of speech. Keeping his mouth open at the dentist for a procedure would lead to the inability to close his mouth, and he would temporarily have to lift his jaw to shut his mouth. Climbing stairs worsened his symptoms. He denies any numbness, focal muscle pain, clear diurnal variation, double vision, blurring of vision, headache, dizziness, cognitive difficulties, trouble swallowing, trouble using a pen or writing, neck weakness, or shortness of breath. On examination, he was not dysmorphic, pale, or jaundiced. There were temporal muscle wasting, mild symmetrical facial weakness, and mild bilateral ptosis compared to his siblings, with other systemic examinations being unremarkable. He has negative AChR antibodies. Repetitive nerve stimulation and nerve conduction studies were reported as normal. His initial WES was carried out in 2018, which showed a heterozygous variant of unknown significance in the cholinergic receptor nicotinic epsilon (CHRNE) gene [c.1314G>C (p.Glu438Asp)]. His WES was re-analyzed after 18 months and showed a homozygous missense variant in *SLC25A1* gene: NM_001256534.1: c.205G>T (p.Asp69Tyr).

Patient 3: The third patient is currently 30-year-old female, who was seen along with her affected siblings in the genetic clinic. She had a long-term history of bilateral partial ptosis and rapid fatigability since she was 2 years of age. She would be tired and fatigued much earlier compared to her other healthy brothers and sisters. After 15-20 minutes of regular low-pace walking, she would feel that her legs are heavy, right side more than the left side, drag them, and sometimes she would just fall. After a few minutes of rest, she would regain her strength. Similar rapid fatigability occurs if she writes for a long time, studies, uses her hands for some mechanical skills, e.g., carrying a bag. After a few minutes of rest, she would return to normal. She learned to limit her activities to short phases and took frequent rests. When she gets a flu or cough, she would develop difficulty breathing and become rapidly short of breath with simple activities. She would go to the Emergency room and use puffers and feel better. She also has rapid fatigable eyelids and intrinsic eye muscles, which lead to bilateral ptosis, and her eyes would be in bilateral esotropia. Due to the chronicity of her symptoms without seeking medical help, her bilateral ptosis and esotropia are persistent. She denied any dysphagia, slurring, aphasia, focal weakness, or focal numbness. She has regular coordination and chewing. Her examination at presentation showed that she has a bilateral mild symmetrical facial weakness, eyelid ptosis, bilateral esotropia, and impaired lateral eye movement. Focusing her gaze on an object in front of her would worsen her eyelid ptosis, and her eyes would move to the midline. Other systemic examinations were unremarkable. There was no evidence of myotonia. She has negative AChR antibodies. Brain and spine magnetic resonance imagings (MRIs) were normal, with no evidence of demyelination. Nerve conduction studies of bilateral upper and bilateral lower extremities and repetitive nerve stimulation studies from right ulnar, right facial, and left accessory nerves were both reported as normal. A single-fiber electromyography (EMG) study was conducted in the right extensor digitorum communis muscle. All four pairs studied showed significantly abnormal jitter (>55 micro seconds). She had also a homozygous missense variant in the *SLC25A1* gene.

Patient 4: The last patient (III-11) is currently an 18-year-old male, who was seen along with his affected siblings in the genetic clinic. He had a long-term history of bilateral ptosis and easy fatigability since he was 1-2 years of age. His symptoms are mainly rapid fatigue of his muscles. If he carries a heavy object for a short time, his hands will become weak and he cannot use them for 5-10 minutes. The same thing happens to his leg if he runs or jogs. He has to stop frequently to rest and allow his muscles to work again. He can walk for around 20 minutes before he takes a rest. Frequent ptosis if he watches a movie or Television. He denies any numbness, focal muscle pain, clear diurnal variation, double vision, blurring of vision, headache, dizziness, cognitive difficulties, trouble swallowing, trouble using a pen or writing, neck weakness, or shortness of breath. On examination at presentation he has bilateral ptosis, and mild symmetrical facial weakness. Other systemic examinations were unremarkable. A repetitive nerve stimulation study was conducted from the right ulnar, right facial, and left spinal accessory nerves, which was reported as normal. Targeted familial mutation testing showed that he also has the homozygous missense variant in the *SLC25A1* gene.

Discussion

In this study, we reported four patients from one family (three of which are siblings) presenting with visual and generalized rapid fatigability with a clinical phenotype suggestive of myasthenia, confirmed by single-fiber

EMG. A homozygous missense variant in *SLC25A1* was the mutation identified, pointing to the diagnosis of congenital myasthenia syndrome type 23 (CMS type 23; OMIM # 618197). Pathogenic variants in the *SLC25A1* gene are well known to cause combined D-2- and L-2-hydroxyglutaric aciduria (D/L-2-HGA) autosomal recessive severe neurometabolic disorder characterized by neonatal onset encephalopathy with severe muscular weakness, intractable seizures, respiratory distress, and lack of psychomotor development, resulting in early death. Brain imaging showed enlarged ventricles, delayed myelination, and germinal layer cysts (OMIM # 615182) (9-11). Recently, reports on homozygous p. (Arg247Gln) missense variant in the *SLC25A1* gene caused a CMS phenotype, but without systemic manifestations of mitochondrial disease (12,13). Here, we report on four additional CMS individuals of Emirati ethnicity harboring a homozygous missense variant. Interestingly, the same variant was seen in a family from Oman (14), suggesting that this variant is seen as a result of a founder effect. Clinically, our patients had early onset, fatigable ocular, and proximal muscle weakness, which are clinical hallmarks of impaired neuromuscular transmission. Also, there was no cognitive impairment found in all our bedside evaluation cases (Table 1). Our findings support that mutations in *SLC25A1* can be associated with impaired neuromuscular transmission and causing CMS type 23, ranging from mild phenotypes to mild with intellectual disability, keeping in mind that mitochondrial DNA depletion syndromes can present with progressive external ophthalmoplegia, which is characterized by ptosis, impaired eye movements due to paralysis of the extraocular muscles (ophthalmoplegia), oropharyngeal weakness, and variably severe proximal limb weakness with exercise intolerance (15). In contrast to this, CMS is characterized by non-progressive muscle weakness (a feature found in all CMS reported cases with *SLC25A1* mutations) (12,13). Pathogenic variants in *SLC25A1*, which cause D/L-2-HGA, are typically associated with increased urine, plasma, and cerebrospinal fluid of D-2-hydroxyglutaric acid/ L-2-hydroxyglutaric acid. One of our patients was found to have normal urinary 2-hydroxyglutaric acid levels (done three times).

The diagnosis of CMS is based on clinical findings, a decremental EMG response of the compound muscle action potential (CMAP) on low-frequency (2-3 Hz) stimulation, a positive response to acetylcholinesterase (AchE) inhibitors, absence of AChR, and anti-muscle-specific kinase antibodies in the serum, and lack of improvement of clinical symptoms with immunosuppressive therapy. Conventional skeletal muscle biopsy and routine histochemical studies in individuals with CMS generally show no significant abnormalities, except type I fiber predominance and occasionally minor myopathic changes. Pathogenic variants in one of the known genes encoding proteins expressed at the neuromuscular junction are currently associated with CMS's subtypes (16). Regarding therapy, on reviewing reported cases, two patients had functional benefit from AchE inhibitor treatment, but in other patients, it was not beneficial despite adequate dosing. One patient was treated with 3, 4-diaminopyridine (DAP) and responded well to the treatment, in keeping with a presynaptic NMJ defect (13).

In patients with milder phenotype having minimum symptoms, treatment with AchE inhibitor or 3,4-DAP may not be necessary. However, a trial of the above drugs may be given to check for any benefits, especially if excessive lethargy and fatigability are significant concerns. Here, we want to stress that although WES provided a molecular diagnosis, the results required careful interpretations. Many additional investigations are required to prove/disapprove the pathogenicity of the detected variant. In summary, as this missense homozygous c.205G>T (p.Asp69Tyr) [(c.226G>T p. (Asp76Tyr))] in *SLC25A1* gene was described in another patient from Oman, we can consider it as a founder mutation in our region.

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List of Abbreviations

AchE	Acetylcholinesterase
AChR	Anti-acetylcholine receptor
CMS	Congenital myasthenic syndromes
CNVs	Copy number variants
D/L-2-HGA	Combined D-2- and L-2-hydroxyglutaric aciduria
DAP	3, 4-diaminopyridine
DNA	Deoxyribonucleic acid
EMG	electromyography
MRI	Magnetic resonance imaging
NGS	Next Generation Sequencing
NMJ	Neuromuscular junction
OMIM	Online Mendelian Inheritance in Man
SLC25A1	Solute carrier family 25 member 1
WES	Whole exome sequencing

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Declaration of conflicting interests
The authors of this article have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

Ethical approval
This article does not contain any studies with human participants or animals performed by any authors. This study is approved by Tawam Human Research Ethics Committee, Ref. No.: AA/AJ/732.

Consent for publication
Informed consent was obtained from all individual participants included in the study.

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Table 1. *Summary of the clinical, neurophysiological, and biochemical features of the reported cases.*

	Current study				Previous studies						
					Balaraju S. et al. (2019)				Chaouch A. et al (2014)		
	Patient 1 (IV-1)	Patient 2 (III-10)	Patient 3 (III-8)	Patient 4 (III-11)	Patient 1	Patient 2	Patient 3	Patient 4	Patient 1	Patient 2	Patient 3
Age at exam/ Gender	6 years/ F	24 years/M	30 years/F	18 years/M	26 years/M	25 years/F	14 years/M	14 years/F	33 years/M	19 years/F	18 months/F
Ethnic Origin	Emirati	Emirati	Emirati	Emirati	Indian	Indian	Greek Pomak	Pakistani	British	British	Ashkenazi-Jewish
Consanguinity	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	No
Age of Onset	Early childhood	Early childhood	Early childhood	Early childhood	Infancy	Infancy	Infancy	Infancy	Infancy	Infancy	Neonatal
Mutation in SLC25A1	c.205G>T (p.Asp69Tyr) (c.226G>T p.(Asp76Tyr))				c.740G>A; p.(Arg247Gln)				c.740G>A; p.(Arg247Gln)		p.G130D, p.R282H
CMS-associated features											
Ocular involvement	Marked ptosis, Inability to maintain sustained upward gaze	Mild ptosis	Marked ptosis, ophthal	Marked ptosis, mild ophthal	Mild ptosis, ophthal	Mild ptosis, ophthal	Marked ptosis, mild ophthal	Mild ptosis, diplopia	Mild ptosis, no ophthal	Diplopia, no Ptosis	Optic nerve hypoplasia
Bulbar involvement	No	Yes with exertion	No	No	No	No	Yes	Yes	Yes	No	Yes
Weakness distribution	Proximal, UL=LL	Proximal, UL=LL	Proximal, UL=LL	Proximal, UL=LL	Proximal, UL=LL	Proximal, UL=LL	Proximal, UL=LL	Proximal, LL>UL, NF weakness	Proximal, UL=LL	Proximal, UL=LL, NF weakness	Generalized hypotonia
Fatigability	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Treatment; response	Not tried	AChEI minimal response	AChEI some response	AChEI some response	AChEI and salbutamol - no response	AChEI and salbutamol - no response	AChEI some response	AChEI no response	AChEI no response, 3,4-DAP good response	AChEI some response	3,4-DAP, pyridostigmine and ephedrine – no response
Additional features											
Intellectual disability	None, attended mainstream school	None, attended mainstream school	None, attended mainstream school	None, attended mainstream school	Unable to read/write/ calculate Binet-Kamat IQ	Unable to read/ write	Yes, attended school with additional support needs	Mild, attended mainstream school	Mild, attended mainstream school	Yes, attended school with additional support needs	Yes

	Current study				Previous studies						
					Balaraju S. et al. (2019)				Chaouch A. et al (2014)		
	Patient 1 (IV-1)	Patient 2 (III-10)	Patient 3 (III-8)	Patient 4 (III-11)	Patient 1	Patient 2	Patient 3	Patient 4	Patient 1	Patient 2	Patient 3
Dysmorphism	Myopathic facies, high arched palate	No	No	No	Elongated facies, prominent ears, high arched palate	Elongated facies, prominent ears	No	No	No	No	No
Other	Hyperextensible joints	Weakness exacerbated by intense exercises.	Weakness exacerbated by infections.	Weakness exacerbated by intense exercises.	Hypermobility, increase in lactate with exercise	Hyperextensible joints, chin fasciculations	Pes planovalgus	Weakness exacerbated by heat and cold. Marked increase in lactate with exercises	Pes cavus	Bicuspid AV and MV prolapse	Apnea crisis, epilepsy, hearing loss, microcephaly
Neurophysiology											
Repetitive nerve stimulation	Not done	Normal	Normal	Not done	Decrement on 3 Hz (MVC not performed)	Decrement on 3 Hz (MVC not performed)	No dec on 3 Hz stimulation after 20s MVC	No dec on 3 Hz stimulation (MVC not performed)	No dec on 3 Hz stimulation, no inc after 20 s MVC	Not done	Not done
Single-fiber EMG	Not done	Not done	Significantly abnormal jitter (> 55 micro seconds).	Not done	Not done	Not done	Not done	Jitter within normal limits	Increased jitter and block	Not done	Increased jitter and block
Additional analysis											
Muscle biopsy	Not done	Not done	Not done	Not done	Not done	Not done	Fiber size variation, few central cores, sporadically accumulated mitochondria in EM	Mildly atrophic fibers, normal COX staining, mild reduction in complex 1 activity	Normal COX staining, enlarged mitochondria and increased in number on EM	Not done	Muscle biopsy was uninformative because of poor tissue quality. Cultured skin fibroblasts showed abnormal mitochondrial functions in form of decreased growth in glucose-free medium, increased ROS production and decreased mitochondrial membrane potential but normal ATP content.

	Current study				Previous studies								
					Balaraju S. et al. (2019)					Chaouch A. et al (2014)			
	Patient 1 (IV-1)	Patient 2 (III-10)	Patient 3 (III-8)	Patient 4 (III-11)	Patient 1	Patient 2	Patient 3	Patient 4	Patient 1	Patient 2	Patient 3		
Urine organic acid	Normal	Not done	Not done	Not done	Not done	Not done	Normal	Not done	Normal	Normal	Large peak of 2-hydroxyglutaric acid and Krebs cycle intermediates.		
Brain MRI	Not performed	Not performed	Normal	Not performed	Not performed	Normal	Not performed	Normal	Normal	Normal	Agenesis of the corpus callosum		

3,4-DAP:3,4-aminopyridine, AchEI: acetylcholinesterase inhibitor, ATP: Adenosine triphosphate, AV: aortic valve, COX: cytochrome c oxidase, Dec: decrement of the CMAP, EM: electron microscopy, EMG: electromyography, inc: increment of the CMAP, LL: lower limb, MRI: magnetic resonance imaging, MV: a mitral valve, MVC: maximum voluntary contraction, NF: neck flexor, Ophtha: ophthalmoplegia, ROS: reactive oxygen species, UL: lower limb.

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