A Middle Eastern Founder Mutation Expands the Genotypic and Phenotypic Spectrum of Mitochondrial *MICU1* Deficiency: A Report of 13 Patients

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Abstract *MICU1* encodes a Ca^{2+} sensing, regulatory subunit of the mitochondrial uniporter, a selective calcium channel within the organelle's inner membrane. Ca^{2+} entry into mitochondria helps to buffer cytosolic Ca^{2+} transients and also activates ATP production within the organelle. Mutations in *MICU1* have previously been reported in 17 children from nine families with muscle weakness, fatigue, normal lactate, and persistently elevated creatine kinase,

as well as variable features that include progressive extrapyramidal signs, learning disabilities, nystagmus, and cataracts. In this study, we report the clinical features of an additional 13 patients from consanguineous Middle Eastern families with recessive mutations in *MICU1*. Of these patients, 12/13 are homozygous for a novel founder mutation c.553C>T (p.Q185*) that is predicted to lead to a complete loss of function of *MICU1*, while one patient is compound heterozygous for this mutation and an intragenic duplication of exons 9 and 10. The founder mutation occurs with a minor allele frequency of 1:60,000 in the ExAC database, but in ~1:500 individual in the Middle East. All 13 of these patients presented with developmental delay, learning disability, muscle weakness and easy fatigability, and failure to thrive, as well as additional variable features we tabulate. Consistent with previous cases, all of these patients had persistently elevated serum creatine kinase with normal lactate levels, but they also exhibited elevated transaminase enzymes. Our work helps to better define the clinical sequelae of *MICU1* deficiency. Furthermore, our work suggests that targeted analysis of the *MICU1* founder mutation in Middle Eastern patients may be warranted.

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Introduction

Mitochondrial Ca^{2+} homeostasis plays key roles in many aspects of cell physiology (Kamer and Mootha 2015). Physiological Ca^{2+} uptake into mitochondria helps to shape cytosolic signaling events and regulates aerobic metabolism, whereas pathological Ca^{2+} overload can lead to mitochondrial injury and cell death.

Mitochondrial Ca^{2+} uptake occurs primarily via the uniporter, a highly selective, calcium-activated calcium channel complex in the organelle's inner membrane (Kamer and Mootha 2015). The channel's pore forming subunit, MCU, is kept in an open state by a companion protein called EMRE. Mitochondrial calcium uptake 1 (*MICU1*), the founding member of the uniporter complex, is an EF hand containing calcium-binding protein which regulates the uniporter (Perocchi et al. 2010). Under resting conditions when cytosolic calcium levels are low, *MICU1* inhibits the uniporter, keeping it closed. Following a spike in cytosolic calcium exceeding a threshold, the EF hands of *MICU1* are engaged, leading to disinhibition of the pore, allowing calcium through the channel (Mallilankaraman et al. 2012; Csordas et al. 2013; Kamer and Mootha 2014; Kamer et al. 2017). Hence, *MICU1* is required to close MCU at low cytosolic $[\text{Ca}^{2+}]$ ($[\text{Ca}^{2+}]_c$) and facilitates its disinhibition at high $[\text{Ca}^{2+}]$.

Two previous studies have identified patients with recessive mutations in *MICU1*. Logan et al. (2013) have described the clinical presentation of 15 children with two different homozygous mutations in the *MICU1* gene (Dutch and Pakistani descent) who presented mainly with learning disabilities and proximal muscle weakness with elevated creatine kinase (CK) level. Most patients have subsequently developed progressive extrapyramidal signs, and some have debilitating disease. Lewis-Smith et al. (2016) have reported a homozygous deletion of exon 1 in *MICU1* in two cousins presenting with lethargy, fatigue, and persistently elevated CK levels.

In this report we present additional 13 patients from consanguineous Middle Eastern Arab families with a nonsense variant in the *MICU1* gene, c.553C>T (p. Q185*) (rs755651388), which has a reported minor allele frequency of 1:60,000 in ExAC (Lek et al. 2015). However, among 5,016 anonymous healthy individuals of Middle Eastern background, nine carried the p.Q185* variant (MAF = 0.0009), indicating a carrier rate as high as 1:557 (0.2%) in this population. This nonsense variant creates a premature stop early in the protein sequence, in the N-terminal domain before the calcium-binding EF hand domains but after the mitochondrial targeting signal. Thus, it is predicted to be a loss-of-function mutation. We describe their clinical, biochemical, and molecular data while comparing to the previously reported cases.

Clinical Description

Herein we describe 13 patients from ten different families, including one family with three affected boys and another family with two affected boys. A summary of the clinical data is presented in Table 1. Below we report in detail one

atypical case to highlight the vast spectrum of symptoms of *MICU1* deficiency, the progressive nature of the disease and to show the diagnostic odyssey that clinicians may encounter.

Case Report (Family 2)

This is a 10-year-old Qatari girl. She is the product of a full-term lower segment cesarean section. Pregnancy was complicated by gestational diabetes which was managed by diet. Birth weight was 2.9 kg. At 5 days of age she was diagnosed with a ventricular septal defect which was corrected surgically when she was 4 months old.

At the age of 2 years she was noted to have motor delay with incidental findings of elevated liver transaminases and plasma creatine kinase levels.

Examination at the age of 2 years showed weight on the 25th percentile, height on the 5th percentile, and occipito-frontal circumference on the 50th percentile. She has subtle dysmorphic features in the form of hypertelorism, low-set ears, and hypoplastic alae nasi. She had ptosis; however, there was no nystagmus and cranial nerves were intact. She had both axial and peripheral hypotonia. Deep tendon reflexes were elicited in all limbs. She had an unbalanced ataxic gait.

Developmentally, she crawled at the age of 1 year. She sat unsupported at 18 months. She walked at age 2 years and 10 months. She had delayed speech; her first words were at the age of 1 year and 9 months. Currently she can run and climb stairs. She talks and interacts with her family.

Her family history showed that the parents are not consanguineous but they are from the same tribe and they have three other healthy daughters and two healthy sons. There was no family history of a similar condition, nor of an inborn error of metabolism.

At the age of 3 years she complained of muscular cramps with walking long distances that improved with massaging. At the age of 6 years she presented with pallor, dark urine, jaundice, and positive coombs test. She was diagnosed with autoimmune hemolytic anemia. She also had history of recurrent chest infections requiring ICU admission. In addition she has recurrent lymphadenopathy and fever and thus was suspected to have immunodeficiency: investigations showed a low IgG level, low B and T cells, and absence of NK cells.

At the age of 7 years she presented with fever with pancytopenia, positive coombs test, reticulocytosis, and massive hepatosplenomegaly. She was suspected to have developed Evans syndrome. Bone marrow biopsy done later yielded no pathology. At the age of 10 years she developed involuntary movements that occur with startle and touch.

Table 1 Clinical features of individuals with *MICU1* variants

	Family 1	Family 2	Family 3	Family 4	Family 5	Family 6	Family 7	Family 8	Family 9	Family 10
Gender (age)	M (16)	F (10)	F (14)	F (14)	M (26) M (24) M (24)	F (7)	F (6)	M (6) M (14)	M (10)	M (4)
Age at presentation	9 years	2 years	10 years	11 years	23 years 21 years 26 years 24 years 24 years	2 years	4 years	6 years 14 years	10 years	3 years
Age at last examination	15 years	10 years	10 years	13 years	26 years 24 years 24 years	7 years	4 years	6 years 14 years	10 years	3 years
Variant	p.Q185* (HOM)	p.Q185* (HOM)	p.Q185* (HOM)	p.Q185* (HOM)	p.Q185* (HOM) p.Q185* (HOM)	p.Q185* (HOM)	p.Q185* (HET)/ Exon 9 and 10 dup (HET)	p.Q185* (HOM)	p.Q185* (HOM)	p.Q185* (HOM)
Developmental delay	+	+	+	+	+	+	+	+	+	+
Speech delay	+	+	+	+	+	+	n/a	n/a	+	+
Elevated liver transaminases	+	+	+	n/a	+	+	+	+	+	+
Elevated CK	+	+	+	n/a	+	+	+	+	+	+
Poor growth	+	+	+	—	n/a	+	—	—	—	—
Normal serum lactate levels	+	+	+	n/a	+++	+	+	n/a	+	+
Height (0.5–1.6 mmol/L)	5th	5th	<5th	50th	n/a	<5th	25th	25–50th	50th	10th
Weight (%ile)	5th	25th	n/a	50th	n/a	<5th	50th	25–50th	50th	10th
HC (%ile)	50th	50th	75th	n/a	n/a	n/a	+	25–50th	50th	25th
Hypotonia	+	+	—	—	—	—	+	+	—	+
Hepatomegaly	—	+ Hepatosplenomegaly	+	—	n/a	—	n/a	n/a	—	—
Abnormal gait	—	+	—	—	—	n/a	+	+	—	n/a
Frequent falls	—	—	—	—	+++	n/a	+	n/a	—	n/a
VSD	—	+	—	—	—	+	—	—	—	—
Learning disability	+	—	+	+	+	n/a	n/a	+	+	+
Extrapyrimal signs at which age	—	+10 years	—	—	+++ 25 years 23 years 23 years	—	—	—	—	+4 years
Other features	Muscular cramps Proximal myopathy	Dysmorphism, abnormal MRI showing white matter changes. Hemolytic anemia, low IgG, B, T, and NK cells. Massive hepatosplenomegaly	Easy fatigability and muscle pain with walking long distances Increased liver periportal echogenicity on ultrasound	Hyperactivity	Calf muscle hypertrophy Seizures Muscular cramps Tremors Postural dystonia	Dysmorphism: short neck, doughy skin, lax joints, down turned lower lip, tented upper lip, and mild syndactyly Coarse liver on ultrasound	Dysmorphism: short neck, doughy skin, lax joints, down turned lower lip, tented upper lip, and mild syndactyly Coarse liver on ultrasound	Positive Gower sign	Seizures Hyperactivity	Myopathic face Choreoathetoid movement of hands and legs and orofacial dyskinesia
Family history	CS parents	NCS parents (same tribe); three unaffected sisters, two unaffected brothers	CS parents (1st cousins); three unaffected siblings	CS parents;	CS parents; three similarly affected brothers	CS parents; one unaffected sister	CS parents; two unaffected siblings	CS parents; two similarly affected brothers; one sister (deceased at age 2); one unaffected sister	CS parents	CS parents

HOM homozygous, *F* female, *M* male, “+” present, “—” absent, *HC* head circumference, %ile percentile, *n/a* not available, *VSD* ventricular septal defect, *CS* consanguineous, *NCS* nonconsanguineous

Investigations

CK 4,829 U/L (6–137), ALT 119 U/L (5–20), and AST 137 U/L (0–37).

Ammonia, lactate, serum amino acids, and karyotyping all were normal.

Acylcarnitine profile, plasma carnitine, urine organic acids, lysosomal enzymes, peroxisomal studies, and transferrin isoelectric focusing for congenital disorders of glycosylation all were normal.

Molecular gene testing for glycogen storage disease type IV, V, VI, VII, IXa2, and XIII was performed for *GBE1*, *PYGM*, *PYGL*, *PFKM*, *PHKA2*, and *ENO3* gene sequencing yielded no mutations.

MRI study of the head at the age of 2 years: Increased periventricular white matter signal intensity along the occipital horns of the lateral ventricles. MR spectroscopy showed slight reduction of N-acetylaspartate with slight increase of choline in some areas of white matter with normal lactate and lipids.

Echocardiography at the age of 2 years: Well-patched VSD with no residual shunt. Tiny patent foramen ovale with left-to-right shunt.

Skin biopsy at the age of 3 years: Normal respiratory chain enzymes, PDH complex, and citrate synthase.

Muscle biopsy at the age of 3 years: Lipid storage myopathy, normal mitochondrial complex I, II, III, and IV.

Whole exome sequencing revealed that she has a homozygous p.Q185* variant in the *MICU1* gene.

Methods and Subjects

Thirteen patients were collected from three centers: from Qatar, Saudi Arabia, and Kuwait. Ten patients were from Hamad Medical Corporation in Qatar, two patients were from Saudi Arabia, and one patient was from Adan Hospital in Kuwait. Consent was obtained from patients. Data was collected retrospectively from the medical records.

MRI/MRS brain was done in 4 out of 13 patients. Echo was done in five patients and ultrasound abdomen was performed in four patients. The patients with abnormal findings are described in the table.

DNA extracted from peripheral blood samples was sent to a molecular diagnostic laboratory and clinical exome sequencing, bioinformatics analysis, and variant interpretation were performed as reported earlier (Yavarna et al. 2015). All reported variants were confirmed using capillary sequencing (p.Q185*) or CGH array (duplication) in the proband and parent or other family members if they were submitted for variant segregation analysis.

Clinical Findings

Thirteen patients (eight male and five female patients) diagnosed with *MICU1*-related mitochondrial disorder were included from ten unrelated families. The ages of the patients ranged from 4 to 26 years, with a mean age of 13 years. Consanguinity was reported in 11 cases (85%).

The most common presenting signs (>50%) were motor developmental delay in the younger patients and learning disability in the older patients, 100% ($n = 13$) and 77% ($n = 10$), respectively. Ten patients had speech delay (77%).

Four patients had poor growth (30%). Six patients had hypotonia on examination (46%). Four patients had history of frequent falls (30%). Three patients had facial dysmorphism; one of whom had a myopathic face (23%). One patient had an abnormal brain MRI (8%). Two patients had seizures (16%). Four patients complained of muscular cramps with walking for long distances (30%). Three patients from one family had calf hypertrophy (23%). Five patients developed extrapyramidal manifestations (38%).

Laboratory Findings

Markedly elevated creatine kinase was present in 12 patients (92%). One patient did not have a documented creatine kinase or liver function test measurement. Creatine kinase levels ranged from 2,203 to 7,852 U/L with an average level of 4,535 U/L. Liver transaminases were raised in 12 patients (92%). ALT levels ranged from 87 to 202 U/L with a mean level of 138 U/L. AST levels ranged from 73 to 203 U/L with a mean level of 122 U/L.

Ten patients were diagnosed by whole exome sequencing (77%). Three patients were diagnosed by targeted variant testing due to positive family history. Three patients had muscle biopsy done as part of the workup which was inconclusive in all three patients (23%).

Twelve patients yielded a homozygous p.Q185* variant in the *MICU1* gene (92%). One patient had a heterozygous p.Q185* variant (absent in mother) and a maternally inherited heterozygous intragenic duplication of exons 9 and 10 (8%). The duplication (arr[GRCh37] 10q22.1 (74167558_74183198)x3 mat) was detected by exome sequencing and confirmed in proband and her mother using an exon array.

Discussion

The most common reason for presentation was learning disability in the older patients and delayed motor skills in the younger patients. All of the patients who underwent

testing had significantly elevated creatine kinase level, and all of them had elevated liver transaminases and that was frequently an incentive to refer the patients for genetic evaluation. We speculate that the elevated transaminases are secondary to muscle involvement evidenced by the high creatine kinase. Liver involvement as evidenced by hepatomegaly was present in three patients, two of whom had changes in liver echotexture: coarse liver and increase in periportal echogenicity. Yet the synthetic functions of the liver (PT, PTT, bilirubin, and GGT) were within normal limits. None of the patients had any skin findings as previously described by Logan et al. (2013). All patients from Qatar, Saudi Arabia, and Kuwait have the same pathogenic variant (p.Q185*) suggesting that it may be a founder mutation with a carrier rate of 1 in 557 in the region. Interestingly five patients developed extrapyramidal signs; one at the young age of 4 years in the form of choreoathetoid movements in the arms and legs. Also three of our oldest patients, being in their mid-20s (Family 5), have only very recently developed extrapyramidal symptoms in the form of tremors and postural dystonia. These manifestations seem to be slowly progressive as described by Logan et al. (2013) in 10 out of their 15 patients. We will continue to follow the rest of the patients and observe them closely to see if any development over time.

Conclusions

Our report expands the phenotypic spectrum of *MICU1*-related mitochondrial disorder with two important features: intellectual disability and elevated liver enzymes. Awareness, lower index of suspicion, and more use of molecular testing may aid early diagnosis, appropriate genetic counseling, and management. This can also aid in the identification of more cases with this disease which can help in better estimation of its prevalence and understanding of the phenotype associated with it.

Compliance with Ethics Guidelines

Conflict of Interest Sara Musa, Vamsi K. Mootha, Wafaa Eyaied, Rehab Ali, Mariam Al-Mureikhi, Nawal Makhseed, Zakkariah Mohamed, Wafaa Ali AlShehhi, Kimberli J. Kamer, Jane Juusola, Fatma Al Mesaifri, Noora Shahbeck, and Tawfeg Ben-Omran declare that they have no conflict of interest.

Informed Consent All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki declaration of 1975. As revised in 2000 (5). Informed consent was obtained from all patients for being included in the study.

Author Contributions Sara Musa and Tawfeg Ben Omran designed the study and drafted the first article.

Sara Musa, Jane Juusola, and Tawfeg Ben Omran performed data analysis and interpretation.

Sara Musa, Vamsi Mootha, Rehab Ali, Mariam al-mureikhi, Wafaa Eyaied, Wafaa Shehhi, Nawal Makhseed, Zakkariah Mohamed, Kimberli J. Kamer, Fatma Al Mesaifri, Noora Shahbeck, and Tawfeg Ben Omran collected the data, performed critical review of the final manuscript, and approved the final version of the manuscript.

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