

Identification of 2 novel homozygous mutations in the methylmalonyl-CoA mutase gene in Saudi patients

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ABSTRACT

أهداف هذا التقرير هو تحليل المظاهر السريرية وطفرات الجين ميثيلمالونيل كواي (*MUT*) لإثنين من المرضى الذين يعانون من بيلة حمض الميثيلمالونيل (MMA). أحد الأطفال الذين تتراوح أعمارهم بين 6 أيام (المرضى 1)، و 3 أشهر (المرضى 2)، قدم مع صورة شبيهة الإنتان، الحمض الأيضي، وفرط أمونيا الدم. وكشفت التحقيقات ارتفاع (C3) propionylcarnitine وحمض الميثيل مالونيك البولي، 3-hydroxypropionic و methylcitrate، بما يتفق مع MMA. وكشفت دراسة الجين بواسطة تسلسل السانقر عن وجود طفرة متماثلة الألائل الطفرة (c.329A>G؛ p.Y110C) في الجين *MUT* عند المريض ومتغايرة الألائل عند الوالدين. ومن المتوقع أن يكون هذا التحول ضار على بنية البروتين ووظيفته. في المرضى 2، اكتشفنا طفرة جديدة متماثلة الألائل (c.2200C>T؛ p.Q734X) ومتغايرة الألائل في الوالدين. هذا التحول يؤدي إلى سابق لأوانه وقف رامزة في كودون 734 من الجين *MUT*. في هذه الدراسة، حددنا طفرتين جديدتين في الجين *MUT* سببت MMA.

The aim of this report is to analyze the clinical features, and mutations of the methylmalonyl CoA mutase (*MUT*) gene in 2 patients with methylmalonic aciduria (MMA) attending King Saud University Medical City, Riyadh, Saudi Arabia in January 2014. The infants aged 6 days (patient 1) and 3 months (patient 2) with sepsis-like picture, metabolic acidosis, and hyperammonemia were presented. Investigations revealed high propionylcarnitine (C3), elevated urinary methylmalonic acids, 3-hydroxypropionic acids and methylcitrate, consistent with MMA. Sanger-sequencing detected a homozygous novel mutation (c.329A>G; p.Y110C) in the *MUT* gene in patient 1 and a heterozygous in parents. This mutation is predicted to have a damaging effect on the protein structure and function. In patient 2, we detected a novel homozygous nonsense mutation (c.2200C>T; p.Q734X) and a heterozygous in parents. This mutation leads to a premature stop-codon at codon 734 of the *MUT* gene. We identified 2 novel mutations in the *MUT* gene causing isolated MMA.

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Methylmalonic aciduria (MMA) is a genetically heterogeneous disorder caused by an inborn error of methylmalonate and cobalamin metabolism.¹ The estimated prevalence of this condition is one in 50,000 to 100,000 people.² Most isolated MMA cases are caused by mutations in the methylmalonyl CoA mutase (*MUT*) gene (OMIM 251000). There are other genes involved in the pathogenesis of isolated MMA, such as *MMAA*, *MMAB*, *MMADHC*, and *MCEE*.² The *MUT* gene encodes the enzyme *MUT* that converts L-methylmalonyl-CoA to succinyl-CoA. Mutations in the *MUT* gene alter this enzyme's structure, or reduce its amount leading to partial or complete deficiency.^{3,4} As a result, methylmalonyl acid, and other potentially toxic compounds can accumulate in the tissues, causing signs and symptoms of MMA.² In contrast, patients diagnosed on newborn screening based on the detection of elevated concentration of blood propionylcarnitine (C3) are usually asymptomatic at the time of diagnosis.⁵ The MMA usually presents with variable severity, from mild to life-threatening during the neonatal period. Affected infants may experience poor feeding,

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vomiting, dehydration, seizures, hepatomegaly, and delayed milestones.¹ Without treatment, this disorder can lead to encephalopathy, coma, and death in some cases.¹ Long-term complications of MMA include feeding problems and growth failure, chronic renal disease, pancreatitis, movement disorders, and intellectual disability.⁵ As molecular studies of MMA in Saudi Arabia are limited, our objective was to analyze the clinical features and novel mutations of *MUT* gene in 2 patients with isolated MMA attending King Saud University Medical City (KSUMC) in Riyadh, Saudi Arabia in January 2014.

Case Report. Patient 1. A 6-day-old baby boy presented with decreased activity and poor feeding. He was delivered at term by an elective cesarean section due to breech presentation with a birth weight of 3.07 kg. His parents are first cousins with no family history of metabolic or genetic diseases (Figure 1). His initial evaluation showed a lethargic, sick looking baby with severe dehydration. The rest of the systemic examination was unremarkable. He received fluid resuscitation, covered with broad-spectrum antibiotics, and electively intubated and ventilated. His baseline investigations showed hypoglycemia, increased anion gap metabolic acidosis, ketonuria, and serum ammonia of 633 $\mu\text{mol/l}$ (normal value [NV] <80 $\mu\text{mol/l}$). The working diagnosis was organic acidemia and accordingly our patient was commenced on ammonia scavenger medications, namely, intravenous sodium benzoate and sodium phenylbutyrate, in addition to intravenous arginine hydrochloride, oral carnitine, and hydroxocobalamin intramuscular injections. Four hours later, ammonia was increased to 917 $\mu\text{mol/l}$ and hence, continuous renal replacement therapy was started, and the ammonia gradually decreased to reach 298 in less than 24 hours.

However, on the second day of admission our patient deteriorated, became hemodynamically unstable despite being on maximum inotropic support. He developed severe coagulopathy and died of presumed sepsis while the ammonia level was below 300 $\mu\text{mol/l}$. The result of urine organic acids was consistent with MMA. It showed highly elevated methylmalonic acids. It showed highly elevated 3-hydroxypropionic acids and methylcitrate, while acylcarnitine profile showed propionylcarnitine of 21 μM (cutoff 10 μM), and the ratio of propionylcarnitine to acylcarnitine (C3/C2) was 2.14 (cutoff 0.4) (Table 1). In addition, high glycine was observed in serum amino acid.

Patient 2. A 3-month-old boy presented with sepsis-like picture. He was born at term by spontaneous vaginal delivery with unremarkable pregnancy and

Table 1 - Characteristics of patients with methylmalonic aciduria.

Variables	Patient 1	Patient 2
Gender	Male	Male
Age at presentation	6 days	3 months
Consanguinity	First cousins	First cousins
pH	7.16	7.23
Ketonuria	Present	Present
Initial ammonia	633 $\mu\text{mol/L}$	161 $\mu\text{mol/L}$
Highest ammonia	917 $\mu\text{mol/L}$	161 $\mu\text{mol/L}$
Propionylcarnitine level	21 μM (<10 μM)	High (qualitative)
C3/C2 level	2.14 (<0.4)	not available
Serum methylmalonic acid	275.2 $\mu\text{mol/l}$ (<0.4)	not available
Urine organic acid	High methylmalonic acid, methylcitrate, 3-hydroxypropionate	High methylmalonic acid, methylcitrate, 3-hydroxypropionate
Mutation	Homozygous (c.329A>G; p.Y110C)	Homozygous (c.2200C>T; p.Q734X)

C3/C2 - ratio of propionyl carnitine to acylcarnitine

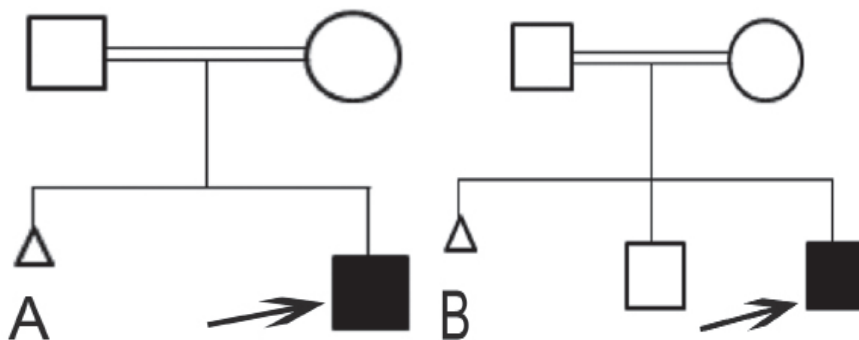


Figure 1 - Family pedigree of A) patient 1 and B) patient 2. □ - unaffected male, ○ - unaffected female, ■ - affected male △ - miscarriage

Table 2 - Primers used for polymerase chain reaction and sequencing of the methylmalonyl CoA mutase gene.³

Exon	Forward Primer	Reverse Primer	Annealing Temperature (Celsius)
Exon 1	CTGGCTGTGTGTGGATGTCTGA	TAAAGGACAGAGCGGGAGAG	59°
Exon 2	TCCCACCCCTCTTCTAAAT	ACAGAGATTAACCCCCAAAAA	55-65°
Exon 3	CATTTTACCTTGATTCCAGACTCTT	CCAGATTCTCGCAAGTAACGA	58°
Exon 4	CCAAGGTTTGGGTCTACAAC	AAAATGGTCTATGCATTTTC	55°
Exon 5	TTTCTTCCATATGCATAAAACTGT	GTGCCACATTGCTCAGAAAA	57°
Exon 6	TTGCTGTTTAATCATGTTGCTG	TCTGAAAACAAAGTTGCAAAGTG	58°
Exon 7	TTCTCCCAAGACTTAAGAGGTTTT	ACATATGCTTGCCTGTGTGC	58°
Exon 8	CCCCTTCTCAGATTGGGATT	TTAGCCAGAGCCCAGAACAC	58°
Exon 9	TGCATCAGGGTCTAATCTCTTG	TTGGGCTCACATGGTTTACA	58°
Exon 10	GATGCATAAAGGCATCCAGAA	TCAAGGGGATTGTGCTAACAG	59°
Exon 11	ACTTGAAGATTGCTGTG	TGTCGTGCATCATCATTTTACTAC	48°
Exon 12	CAGGGTTTTTATAGTCATTA	CAAGATTCCCATCACAGT	50°
Exon 13A	CCGTGAAAATGGAAATAGTGG	GCATGACACCAGGCCTATAA	58°
Exon 13B	CCGTTCAAGGTGCTTGATGAT	TGGTAGAATTTGGCTGAGGA	59°

neonatal history. His parents are first cousins with no family history of inherited disease, or early neonatal deaths (Figure 1). He was well until the age of 2 months when he was admitted to the hospital with respiratory distress treated for pneumonia for 5 days. Ten days later, he presented with fever, poor feeding, and decreased activity. Upon arrival at the hospital, he was sick, looking pale, and tachycardic. The rest of his systemic examination was normal apart from increased tone in the upper and lower limbs. All sepsis screening tests were negative including blood, urine, and cerebrospinal fluid cultures and viral serology. His initial blood gas showed increased anion gap metabolic acidosis and serum ammonia was 161 $\mu\text{mol/l}$ and serum methylmalonic acid was 275.2 (NV: $<0.4 \mu\text{mol/L}$). This biochemical profile was suggestive of methylmalonic aciduria that was also supported by the high urinary methylmalonic acid, elevated 3-hydroxypropionic acids and methylcitrate (Table 1). In addition, acylcarnitine showed high propionylcarnitine and high propionyl carnitine to acylcarnitine ratio. Our patient responded nicely to low protein formula, oral carnitine, and hydroxocobalamin intramuscular injections. His ammonia was maintained within the normal limits, and his growth and development were age-appropriate.

Genetic findings. Blood samples (5 mL) were collected in EDTA tubes from patient 1 and patient 2. The tubes were centrifuged at 5,500 \times g for 5 min. The DNA was extracted from the buffy layer using the illustrated blood genomic Prep Mini Spin kit (GE Healthcare, Buckinghamshire, UK) and stored at -20°C in aliquots until further use. The DNA samples from both patients were tested for mutations in *MUT* gene by

polymerase chain reaction and direct Sanger-sequencing. Full coding region covering all 13 exons and flanking region were amplified using previously described primers (Table 2).³ Successfully amplified fragments were sequenced in both directions using the M13 forward and reverse primers and the BigDye terminator v3.1 cycle sequencing kit (Applied Biosystems, Foster City, CA). Fragments were electrophoresed on the 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA) according to the manufacturer protocol. All the sequenced fragments were then analyzed using SeqScape software v2.6 (Applied Biosystems, Foster City, CA). Prediction of potential pathogenic effect of the detected mutation(s) were performed utilizing SIFT (<http://sift.jcvi.org/>) and PolyPhen-2 (<http://genetics.bwh.harvard.edu/pph2/>).^{6,7}

In patient 1, we detected a homozygous mutation (c.329A>G;p.Y110C) and heterozygous in both his parents. This mutation is novel (not reported previously). To study the effect of this mutation on the protein structure and/or function, we utilized SIFT (<http://sift.jcvi.org/>) and PolyPhen2 (<http://genetics.bwh.harvard.edu/pph2/>), both databases predicted that this mutation will have an effect on the protein structure and function.^{6,7} The SIFT predicted it to be not tolerated, and PolyPhen2 predicted that this mutation would be possibly damaging. This mutation was not detected in 100 chromosomes of matching ethnicity.

In patient 2, we detected a homozygous nonsense mutation (c.2200C>T;p.Q734X) in the patient and heterozygous in both his parents. The mutation will

lead to a premature stop-codon (X) at codon 734 of the *MUT* gene. This mutation is novel (not reported previously) and was not detected in 100 chromosomes of matching ethnicity

Discussion. Isolated methylmalonic aciduria is an autosomal recessive disorder caused mainly by a deficiency of the enzyme *MUT*.¹ It usually presents with non-specific symptoms in infancy.² In this report, we described 2 infants with severe isolated MMA presented early in infancy with sepsis-like picture, metabolic acidosis, and hyperammonemia. While the second patient responded to the therapeutic measures including intravenous fluid, carnitine, and low protein formula, the first patient died from presumed septic shock. Patients with MMA identified by neonatal screening benefit from early treatment that leads to the prevention of acute complications and death in infancy. However, the long-term outcome of screened patients is less well defined as data is scarce.⁵ Although newborn national screening for genetic diseases operates in most hospitals in Saudi Arabia, it is not available in the hospital where our patients were born. Furthermore, the early and severe presentation combined with the location of the mutation (middle of the *MUT* gene active-site) suggests our patients would likely be unresponsive to even appropriate early treatment. The clinical presentation of our 2 patients and the abnormal findings on amino acids, acylcarnitine profile, and organic acid were highly suggestive of MMA. We therefore opted to perform mutation study of the *MUT* gene. We sequenced the full coding regions and splice junctions of this gene in our 2 patients, and detected 2 homozygous mutations. After an extensive search of the literature and available database, we concluded that the 2 mutations described here are novel (not reported previously).

There are 4 other genes where mutations can cause MMA. The mutation rates for genes involved in MMA phenotype are: 60% for *MUT*; 25% for *MMAA*; 12% for *MMAB*; 2% for *MCEE*; and 1% for *MMADHC*. Due to the genetic heterogeneity of MMA, there are no specific mutation hotspots although specific mutations among various populations have been observed.^{2,3} The human genome mutation database, HGMD professional 2014.4, currently lists a total of 248 mutations in the *MUT* gene.⁸ Approximately 70% of the mutations detected in this gene are missense or nonsense mutations. PolyPhen and SIFT predicted that both mutations identified to be potentially pathogenic, and for this reason, it is highly likely that both mutations are responsible for the MMA

phenotype presented in both patients. In patient 1, the c.329A>G;p.Y110C mutation is located in the alpha/beta chain catalytic domain of the protein, whereas in patient 2, the (c.2200C>T;p.Q734X) is located in the cobalamin binding domain of the protein. This mutation is nonsense and leads to a premature stop-codon. In contrast, most but not all mutations reside in the cobalamin binding domain of the mutated protein were associated with residual enzyme activity.⁹ There are 2 recognized enzymatic subtypes of MMA caused by *MUT* gene mutations, mut⁰ with a complete absence of the enzyme activity and mut⁻ with residual enzyme activity.¹ We cannot make a strong statement regarding the enzymatic subtype of our patients and their B₁₂ responsiveness as the relevant cellular and biochemical studies required to characterize them precisely were not carried out. These studies include in vivo and in vitro vitamin B₁₂ responsiveness, cobalamin distribution assays, ¹⁴C propionate incorporation assays, and complementation analysis.^{1,2}

Compared to most patients with MMA from different ethnicities who are compound heterozygous, our 2 patients were homozygous for *MUT* gene mutation.^{1,4} This is not surprising as both parents are first cousins. Also, the prevalence of consanguinity in Saudi population reaches 56% that predisposes to the expression of homozygous mutant genes.¹⁰ Similarly, homozygous mutations in *MUT* gene were previously reported in both mut⁰ and mut⁻ patients.^{3,4} The predominant compound heterozygous genotype makes genotype/phenotype correlation difficult as some patients who carry 2 mutations may have one mut⁻ while the other is mut⁰. This leads to unpredicted enzyme level and possibly diverse clinical phenotype.²

In conclusion, we report 2 novel mutations in the *MUT* gene causing isolated MMA in 2 Saudi infants.

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Clinical Practice Guidelines

Clinical Practice Guidelines must include a short abstract. There should be an Introduction section addressing the objective in producing the guideline, what the guideline is about and who will benefit from the guideline. It should describe the population, conditions, health care setting and clinical management/diagnostic test. Authors should adequately describe the methods used to collect and analyze evidence, recommendations and validation. If it is adapted, authors should include the source, how, and why it is adapted? The guidelines should include not more than 50 references, 2-4 illustrations/tables, and an algorithm.