CASE STUDY

The association of MMUT mutation (NM_000255.4:c.976A>G) with wide spectrum clinical manifestations in a child affected with methylmalonic acidemia

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ABSTRACT

Methylmalonic acidemia (MMA) is a rare autosomal recessive disorder caused by methylmalonyl CoA mutase (MMUT) deficiency which converts methylmalonyl-coenzyme A (CoA) to succinyl-CoA. Patients with MMA present a wide spectrum of clinical manifestations including lethargy, vomiting, hypotonia, seizure, acid-base disturbances, and tachycardia. Here, we report the case of a six-day-old infant suffering from poor feeding, acid-base imbalance, weak reflexes (grasping, sucking, and moro reflex), seizures, hypotonia, tachypnea, and tachycardia. Results obtained from whole exome sequencing (WES) for the proband identified a homozygous mutation in MMUT (NM_000255.4:c.976A>G (p.Arg326Gly)). The mutation was confirmed in both parents by Sanger sequencing. Biochemical analysis demonstrated significantly increased levels of methylmalonic acid and 3-hydroxypropionic acid excretions as well as elevated levels of glycine, tyrosine, lysine, and decreased levels of methionine in serum (elevated propinylcarnitine). Both his non-consanguineous parents were identified to be heterozygous for this mutation.

Keywords: Methylmalonic acidemia; MMUT; Methylmalonyl-CoA mutase; Neonatal Screening; Hyperammonemia.

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INTRODUCTION

Methylmalonic acidemia (MMA) is due to methylmalonyl CoA mutase (MMUT) deficiency and is an autosomal recessive inherited metabolic disorder which mainly appears in early infancy with variable symptoms including lethargy, hypotonia, seizure, acid-base disturbances, and tachypnea and death. MMA may be caused by a defect in either MMUT or genes involved in the metabolism of 5′-deoxyadenosylcobalamin (AdoCbl) including the metabolism of cobalamin associated A, B, C, D (MMAA, B, C, D) (1). MMA often results from MMUT mutation and can be divided into $MMUT^0$ with no enzyme activity and $MMUT^-$ with insufficient enzyme activity for converting methylmalonyl-coenzyme A (CoA) to succinyl-CoA. The clinical symptoms are variable among patients with MMA based on the type of mutations. Initial diagnosis of MMA is performed by assessing metabolites including urine organic acids (UOA), plasma acylcarnitines, plasma methylmalonic acid, plasma amino acids (PAA), total homocysteine, and vitamin B12 levels in suspected patients. Although additional tests such as electrolytes, blood glucose, ammonia, carnitine, and urine ketones analysis could pave the diagnosis, it eventually should be confirmed by molecular testing.

CASE REPORT

This case, a six-day-old boy was born at 40 weeks of gestation with a normal delivery from non-consanguineous parents and was the first child of the family. He weighed 3kg and his height and head circumference at birth were 50 and 36 cm, respectively. His mother showed fever during delivery but she was negative for the SARS-Cov-19 (COVID) test. The subject was admitted in Amirkola Children's Hospital with several symptoms including restlessness, poor feeding, and tachypnea the third day after birth. On examination he was conscious while his reflexes including moro, grasping, and sucking were very poor. Furthermore, icteric sclera and lethargy were diagnosed during the first days of hospitalisation.

During the first week of admission he was intubated and had several seizure episodes. Although seizure was mainly improved by administration of phenobarbital and phenytoin, and anticonvulsant drugs, he was lethargic and intubation was maintained for two days. He received supplements including L-carnitine, vitamin-B12, biotin, calcium, and intralipid infusion during hospitalization. After the first week of admission, tone, reflexes, consciousness, and response to external stimuli improved following feeding with his mother's milk. Cardiac examination showed no abnormality over a one-month period. Oral thrush caused by *Candida albicans* was diagnosed at the end of the first month, and was treated with antifungals including oral fluconazole, nystatin, and clotrimazole. Biochemical and haematological parameters demonstrated several metabolic disturbances including metabolic acidosis (pH7.21, bicarbonate: 5.2mmol/L, pCO_2: 12.8mmHg), hyperammonemia (792µmol/L), decreased levels of alkaline phosphatase (244U/L), hyperglycemia (fasting blood sugar 10.1 mmol/L), hypothyroidism, aminoacidemia, G6PD deficiency, severe anaemia, and neutropenia (Table 1-3). In contrast to most MMA patients, the present case showed no enlarged liver, hypoglycemia, ketoacidosis, or decreased WBC, and platelets.

Whole exome sequencing (WES) was performed for the proband. In this regard, 1µg of genomic DNA was sheared into 300–500 bp fragments. Fragments were isolated by bead purification. The targeted DNA was captured. Captured DNA libraries were sequenced on Illumina HiSeq 2500 (Illumina Inc., San Diego, CA) as 150 bp paired-end reads at a depth of 100× according to the manufacturer's protocols. The results revealed a homozygous missense mutation in MMUT (NM_000255.4:c.976A>G (p.Arg326Gly)). The results were confirmed by Sanger sequencing for the proband and his parents. Although non-consanguineous, the parents were from the same village and were both carriers for this mutation (Figure 1A, B, and C). The subject recovered after about one month, but he was readmitted two months later for tachypnea, tachycardia, and disrupted arterial blood gases and severe anaemia. At the time of the article submission, the proband was 26 months old.

DISCUSSION

MMA is a rare hereditary metabolic disorder in which affected subjects are not able to breakdown certain amino acids and lipids due to MMUT deficiency. Patients with MMA present a wide spectrum of clinical manifestations from lethargy, hypotonia, seizure, acid-base disturbances, and tachypnea to death dependent on mutation in MUT or genes involved in AdoCbl metabolism. We reported a six-day-old case who has inherited $MMUT^0$ of NM_000255.4:c.976A>G (p.Arg326Gly) from both his carrier parents. Importantly, analysing of missense mutation using several powerful tools such as PolyPhen-2, PROVEAN, Fathmm, PANTHER, and SIFT showed that it could probably influence the structure and function of MMUT (Table 4). It is important to note that lethargy, restlessness, poor feeding, and tachypnea were the significant symptoms at the referral time. Similar symptoms have been reported in an Indian boy diagnosed with MMA carrying two variants in exon 3 and 5 who

was admitted with complaints of fever, vomiting, poor feeding, lethargy, and tachypnea (2, 3). Furthermore, reflexes including grasp, suck, moro, and root have been reported in infants with MMA to be poor, consistent with our case (4). Icteric sclera and lethargy were the first signs of the condition in the proband in confirmation with other studies (5, 6).

Accumulating evidence has been shown that acid-base imbalance, hyperammonemia, and defect in the metabolism of different amino acids such as methionine, threonine, isoleucine and valine are well-known symptoms in most MMA infants regardless of the mutation type. In this context, our case presented decreased levels of blood bicarbonate and pH indicating metabolic acidosis, as well as hyperammonemia and disturbances in the metabolism of glycine, tyrosine, methionine, and lysine. The complete blood count (CBC) analysis revealed decreased haemoglobin (Hb), mean corpuscular volume (MCV), and mean corpuscular haemoglobin concentration (MCHC). Also, the blood film showed polychromasia, hypochromia, and anisocytosis suggesting the presence of severe anaemia. Although he was transfused twice during his hospitalisation period, he was referred again two months later to the hospital with severe anaemia indices including low MCV, MCHC, and Hb level. Furthermore, he was regularly followed up for psychomotor skills including cognitive, motor, and language development. Also, brain magnetic resonance imaging (MRI) was performed and showed no abnormality. Notably, in such cases plasma and/or urine analysis is conducted by using several approaches including mass spectrometry, and gas-liquid chromatography and final diagnosis is confirmed by recruiting next generation sequencing (NGS) for mutation detection (7-9). Immediate management of manifestations by administration of hydroxocobalamin and L-carnitine significantly improves the survival of infants affected by MMA (10-12). Patients are also advised to have a dietary protein intake free from leucine, valine, threonine, and methionine (13). Importantly, physicians should be cautious about seizures which may lead to serious brain damage if left uncontrolled (14). Affected patients should be regularly followed up and screened for urine organic acids levels, amino acidemia, growth and development, psychomotor skills, feeding ability, and haematological indices (10, 15, 16). Although recruiting different strategies including management of levels of organic acids, and amino acids could be helpful for diminishing the disease manifestations and survival improvement, up to now there is still no certain treatment for MMA. Therefore, genetic counselling in the context of premarital and pre-pregnancy screening in those families with an affected or carrier member should be undertaken for siblings and at-risk relatives to prevent the birth of affected new-borns.

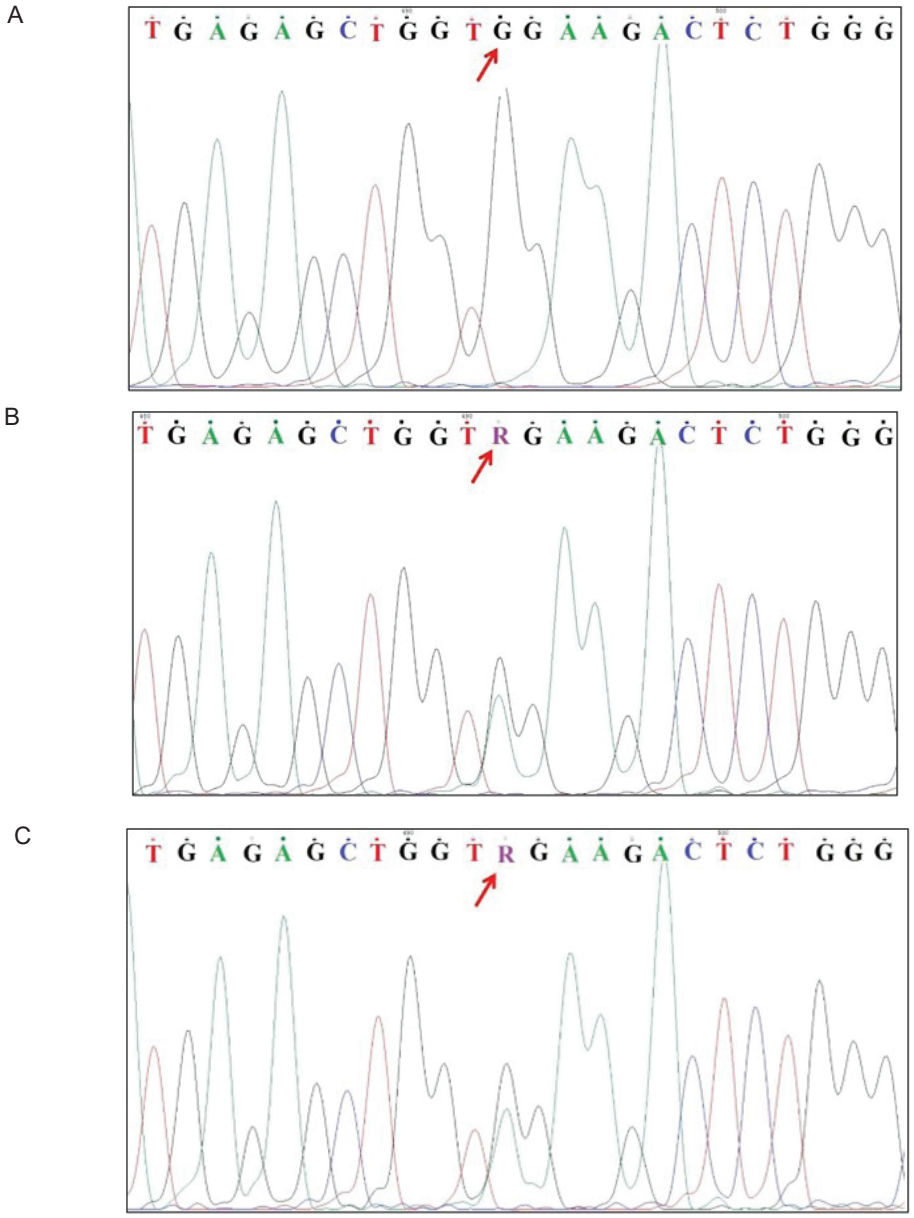

Figure 1. Electrophoregrams of MMUT after Sanger sequencing. (A) sequencing result of the child shows a missense homozygous mutation in MMUT (NM_000255.4:c.976A>G (p.Arg326Gly)); (B and C) sequencing results of the father and mother show a missense heterozygous mutation in MMUT(NM_000255.4:c.976A>G (p.Arg326Gly)).

Table 1. Biochemical analyses.

Test	Result	Unit	Normal range	Flag
SGOT	69	U/L	Up to 75	
SGPT	42	U/L	Up to 45	
Alkaline phosphatase	244	U/L	297-1178	Low
BS	124	mg/dL	New-born: <115	
Other: <200	High			
FBS	10.1	mmol/L	3.9-6.9	High
Reducing Substance Urine	Benedict and oxidase positive			
Lactate	13.32	mmol/L	0.5 to 2.2	High
Pyruvate	3.2	mg/dL	0.4-1.2	High
Ammonia	792	µmol/L	11-32	High
Homocysteine (Enzymatic)	6.6	µmol/L	3.7-10.3	
Lactate/Pyruvate	37.5	Ratio	6-20	High
BUN	0.82	mmol/L	2.1-8.5	Low
Creatinine	34.48	µmol/L	53-97.2	
Ca	1.98	mmol/L	1.75-3	Low
Na	135	mmol/L	130-148	
K	5	mmol/L	3.5-5.5	
Mg	0.78	mmol/L	0.66-1	
CRP (quantitive)	110	mg/L	Up to 60	High

SGOT: serum glutamic oxaloacetic transaminase; SGPT: serum glutamic pyruvic transaminase; BS: blood sugar; FBS: fast blood sugar; BUN: blood urea nitrogen; CRP: C-reactive protein.

Table 2. Liquid chromatography–mass spectrometry for amino acid analysis.

Name	Concentration (µmol/L)	Normal range (µmol/L)
Aspartic Acid	6.18	2-20
Glutamic Acid	65.75	31-302
Asparagine	36.41	25-91
Serine	114.87	69-271
Glutamine	323.89	316-1020
Histidine	82.77	10-116
Internal Std	0.00	I.S.
Glycine	430.12	111-426
Threonine	101.39	47-437
Citruline	13.01	9-38
Arginine	65.55	29-134
Taurine	57.14	10-167
Alanine	139.40	139-474
Tyrosine	150.71	26-115
Proline	149.57	85-303
Methionine	5.03	11-35
Valine	171.04	93-300
Phenyl alanine	56.72	28-80
Isoleucine	63.46	31-105
Allo-Isoleucine	...	<2
Leucine	114.05	48-175
Ornitine	49.55	20-130
Lysine	301.35	49-204

Table 3. Haematological indices analysis.

Test	Result	Unit	Normal range	Flag
W.B.C	7.7	10^9/L	6-17.5	
R.B.C	2.6	10^9/L	2.7-4.9	Low
Hb	68	g/L	138-172	Low
H.C.T	21.1	Ratio	28-42	Low
M.C.V	82.1	Fl	77-115	
M.C.H	26.5	Pg	26-34	
M.C.H.C	322	g/L	320-360	
Platelet	525	10^9/L	150-400	High
RDW	13.9			
Differential				
Poly	9.090%	10^9/L	55-70%	Low
Lymphocytes	83.73%	10^9/L	20-40%	High
Eosinophils	4.56%	10^9/L	1-4%	
Monocytes	2.22%	10^9/L	2-8%	
Basophils	0.4%	10^9/L	0.5-1%	
Polychromia	+			
Hypochromia	+			
Anisocytosis	+			

MCV: mean corpuscular volume; MCH: mean corpuscular haemoglobin; MCHC; mean corpuscular haemoglobin concentration; RBC: red blood cells; WBC: white blood cells; RDW: red cell distribution width, Hb: haemoglobin

Table 4. Prediction of function consequences of (NM_000255.4:c.976A>G (p.Arg326Gly) on MMUT structure and function by several powerful tools.

Database	Prediction	Score
PolyPhen-2 http://genetics.bwh.harvard.edu/pph2/	Damaging	1.000
PROVEAN http://provean.jcvi.org/index.php	Deleterious	-6.991
Fathmm https://fathmm.biocompute.org.uk/	Damaging	-6.29
PANTHER https://www.pantherdb.org/	Probably damaging	0.95
SIFT https://sift.bii.a-star.edu.sg/	Deleterious	0.05

CONCLUSION

Research on MMUT have shown that disruption of protein structure followed by a mutation in MMUT dysregulates several critical cellular processes including catabolism of odd-chain fatty acids, branched amino acids (valine, isoleucine, methionine, and threonine) and cholesterol. Of note, the clinical manifestations of MMA could be very variable and dependent on mutation in MMUT or genes involved in the metabolism of AdoCbl including MMAA, MMAB, MMACHC, and MMADHC. Thus, mutation detection could be helpful for predicting the protein function and genotype-phenotype correlation, and also prevent the birth of affected future new-borns through genetic counselling and prenatal diagnosis. In conclusion, we reported a homozygous case with NM_000255.4:c.976A>G (p.Arg326Gly) mutation in MMUT from non-consanguineous parents with a wide spectrum of severe manifestations including poor feeding, tachypnea, vomiting, methyl-malonic acidemia, hyperammonemia, disturbance in the metabolism of some branched amino acids, hypothyroidism, and severe anaemia.

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The authors have no conflicts of interest to declare.

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