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Coma Caused by Methylmalonic Acidemia in a Taiwanese Girl

Min-Hua Tseng[1], Chih-Chien Wang[2], Yeong-Seng Yuh[2], Chi-Chieh Lai[2], and Shih-Hua Lin[3*]

[1]Division of Pediatrics, Department of Medicine, Tri-Service General Hospital Peng-Hu branch, Peng-Hu;
[2]Department of Pediatrics, [3]Division of Nephrology, Department of Medicine,
Tri-service General Hospital, National Defense Medical Center, Taipei,
Taiwan, Republic of China

Methylmalonic acidemia (MMA) is an inborn error of metabolism caused by an accumulation of methylmalonic acid in blood and body tissue due to severe deficiency or a lack activity of the mitochondrial methylmelonyl-CoA mutase (MCM) enzyme. Affected individuals are typically presented during the newborn period with vomiting, failure to thrive, hyperammoninemia and ketoacidosis. Several mutations on MCM gene (*MUT* gene) were reported worldwide, but Taiwan. Here, we described a previously healthy 2-year-old Taiwanese girl, who had normal growth and developmental milestones, presented with abdominal pain, vomiting and was in coma. Physical examination revealed tachycardia, tachypnea and poor skin tugor. Laboratory investigations showed high anion gap (anion gap 35 mmol/L) metabolic acidosis (HCO_3^- 2.7 mmol/L), normal plasma osmolar gap (10 mosm/kgH_2O), β-hydroxybutyrate (4 mmol/l), ketonuria, hypoglycemia (2.6 mmol/l), hyperammoneemia (152.9 μmol/l), normal lactic acid level, normal renal function, suggesting a clinical diagnosis of organic acidemia. Urinary analysis for organic acid showed increased excretion of methylmalonic acid and in vitro MCM enzyme activity analysis by detecting the lymphoblast revealed trivial residual activity. Molecular analysis of MCM gene revealed that she was compound heterozygous for two mutation of *MUT* gene, a recurrent G427D and a novel mutation F307L; these two mutations are not identified of 100 alleles for 50 unrelated normal Taiwanese. MMA should be considered as a cause of high anion gap metabolic acidosis in children, even without previous episode and normal development milestone. Molecular study is needed to make a diagnosis of MMA under the trivial residual activity of MCM enzyme.

Key words: methylmalonic acidemia; methylmalonyl-CoA mutase; MUT

INTRODUCTION

Methylmalonic acidemia (MMA), a common form of organic acidemia, is a rare autosomal recessive metabolic disorders caused by a deficiency or a lack activity of the mitochondrial methylmalonyl-CoA mutase (MCM), either defect of MCM apoenzyme or adenosylcobalamin (AdoCbl), a cofactor of MCM.[1] Individuals with MMA commonly presented as failure to thrive, dehydration, hypotonia, respiratory distress and developed metabolic stroke with development delay if delayed treatment or misdiagnosis.[2] In human, the MCM is encoded by *MUT* gene which is mapped to chromosome 6p21, which consists of 13 exons spanning over 35 kilobase.[3] To date, more than 100 disease-causing mutations have been reported worldwide.[3-8]

Several *mut* mutations have been identified worldwide, including Japanese, but relatively little have been done in the rest of Asia. Moreover, most of the reported cases seem to be unique or restricted only a few pedigrees. In this report, we reported a 2-year-old girl with MMA with previously healthy and normal development. Initial investigations revealed high anion gap metabolic acidosis, hypoglycemia, hyperammonemia and presence of acetone in the urine. Organic acid analysis of urine by gas chromatography mass spectrometry demonstrated increased excretion of methymalonic acid and in vitro MCM enzyme activity analysis of her lymphoblast revealed trivial residual activity. Molecular analysis revealed a novel compound heterozygous *MUT* gene (F307L) mutation responsible for the genetic defect.

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*Corresponding author: Shih-Hua Lin, Division of Nephrology, Department of Medicine, Tri-Service General Hospital, National Defense Medical Center, No. 325, Sec. 2, Cheng-gong Road, Taipei 114, Taiwan, Republic of China. Tel: +886-2- 87927213; Fax: +886-2-87927134; E-mail: I521116@google.com

Table 1 Biochemical studies on admission

Blood	Normal range	
White blood count	(6,000-17,500/mm^3)	2,0700*
Hemoglobin	(11.5-15.5gm/dL)	11.1
Platelet count	(150,000-400,000/mm^3)	448,000
Plasma		
Na^+	(138-145 mmol/l)	133
K^+	(3.5-5.5 mmol/l)	4.9
Cl^-	(98-106 mmol/l)	95
pH	(7.38-7.42)	7.09*
$HCO3^-$	(21-28 mmol/l)	8.9*
Urea (BUN)	(1.8-6.4 mmol/l)	14.4*
Creatinine (Cr)	(27-62 μmol/l)	44.2
Glucose	(3.3-5.5 mmol/l)	2.6*
Ammonia	(17-68 μmol/l)	152.9*
Lactic acid	(0.8-1.5 mmol/l)	1.5
Plasma osmolal gap	(< 20 mosm/kg H2O)	10
β-hydroxybutyrate	(0.15-2 mmol/l)	4*
Urine		
Acetone	Negative	3 +*

* denotes an abnormal value.

CASE REPORT

This 2-year-old girl presented with 2-day history of abdominal pain and vomiting followed by progressive loss of consciousness on the day of admission. She was born to healthy non-consanguineous Taiwanese parents. She did not have any symptoms of metabolic decompensation after birth. During infancy, she did not have history of difficulty to thrive, irritability and lethargy. Her growth and development were well.

On physical examination, her blood pressure was 99/42 mmHg, heart rate, 159 beats/min, respiratory rate, 52 beats/min, and body temperature, 36.8°C. Her consciousness was drowsy, inspection of chest revealed intercostal retraction with accessory respiratory muscles use and skin turgor was poor.

The biochemistry studies are shown in Table 1. Arterial blood gas revealed metabolic acidosis (pH, 7.09, pCO_2, 8.9 mmHg and bicarbonate, 2.7 mmol/l, serum level of Sodium 133 mmol/l; K^+ 4.9 mmol/l; Cl- 95 mmol/l); high anion gap metabolic acidosis (anion gap 35 mmol/l) was noted. In order to differentiate the cause of high anion gap metabolic acidosis, several biochemistry studies were checked, including blood urea nitrogen 14.4 mmol/l, creatinine 44.2 μmo/l, blood sugar 2.6 mmol/l, lactic acid 1.2 mmol/l, β-hydroxybutyrate 4 mmol/l, urine ace-

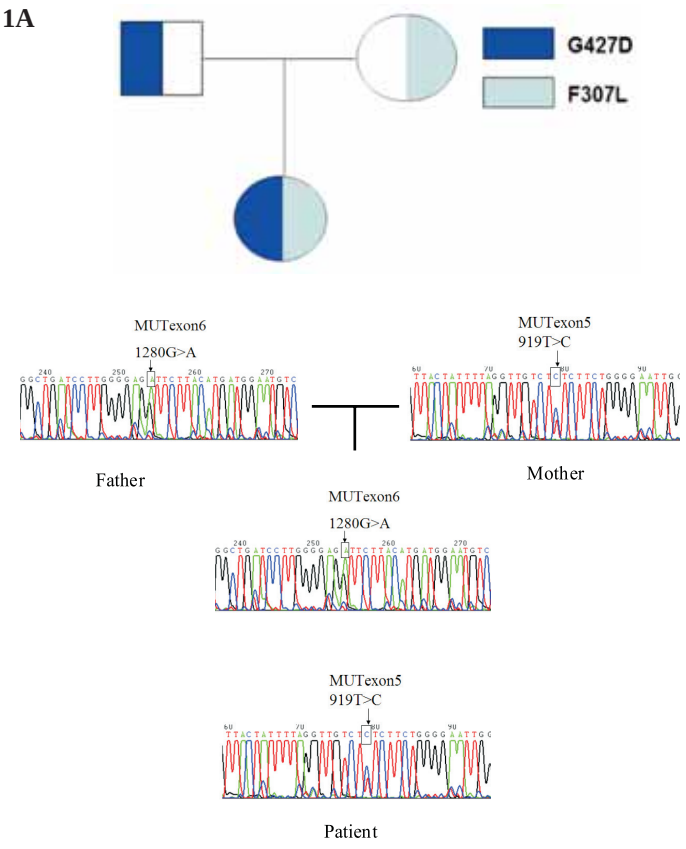

Fig. 1 Direct sequencing and restriction analysis of PCR fragment of exon 5 and 6 for MUT mutation in this family. Figure 1A: Right panel: arrow indicates the mutant base (919T>C, 1280G>A) and left row and right row indicate father and mother. Left panel: male and female indicated by squares and circles, respectively. Filled symbols represent affected individuals. Figure 1B: DNA of the proband bearing the homogeneous (F307L) mutation yields two fragment (242 bp and 105 bp) since the mutation creates a Bsm AI endonuclease restriction site. DNA of the proband bearing the homogeneous (G427D) mutation yields one fragment (275 bp) since the mutation prevents a EcoN I endonuclease restriction site (Figure 1B).

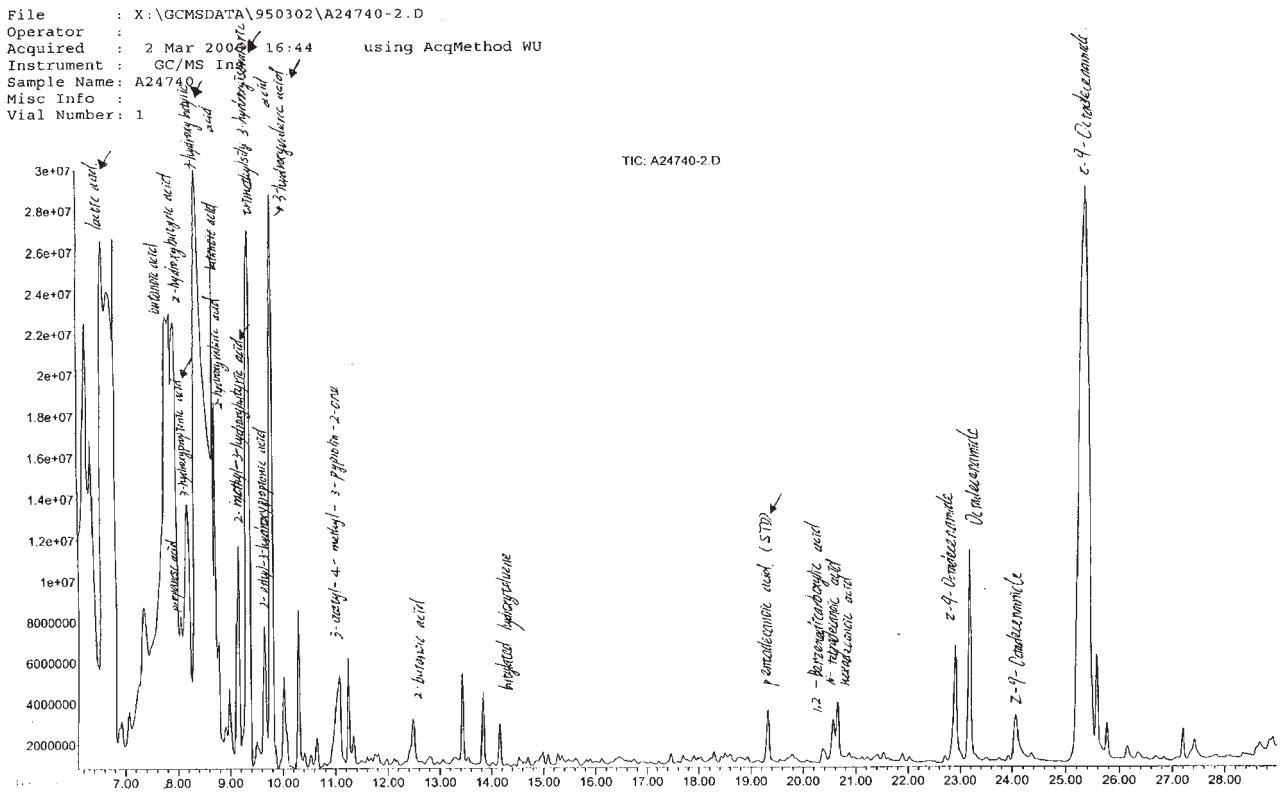

Fig. 2 Gas chromatography mass spectrometry revealed elevated level of lactic acid, 3-hydroxypropionic acid, 3-hydroxybutyric acid, 2-methyl-3-hydroxybutyric acid, Trimethylsily 3-hydroxyisovaleric acid, and 3-hydroxyvaleric acid. (Marked by arrow)

tone 3^{+}, plasma osmolar gap 10 mosm/kg H_2O, and blood ammonia 152.9 μmol/l, suggesting the diagnosis of organic acidemia. The patient being treated with parenteral sodium bicarbonate and L-carnitine, low protein and high energy diet, reducing production of methylmalonic acid from gastrointestinal tract with metronidazole. She has been stable and discharged couple of days.

Organic acid analysis of urine by gas chromatography mass spectrometry demonstrated increased excretion of methymalonic acid and elevated propionate derivative, 3-OH propionate derivatives. We measured MCM activity in lymphoblast draw from the patient's peripheral blood, and it revealed trivial residual activity (0.84 nmol/min/mg protein vs the normal control value of 11.2 nmol/min/mg protein; normal range 4.04-10.7) in the patient's lymphocytes, therefore, defining a mut- MMA phenotype.

Molecular analysis

As mentioned above, clinical diagnosis of MMA was made. We promptly arranged molecular analysis of MCM gene. We isolated and purified genomic DNA from peripheral blood of the patient and her parents for PCR amplification of the 13 exons of MCM gene, which encodes the MCM, with primer pairs corresponding to each exon, was performed subsequently. Direct sequencing of the genomic DNA showed a cytosine-to-thymine single base substitution at position 919 (C919T>C) in exon 5, and an adenine-to-guanine single base substitution at position 1280 (C1280G>A) in exon 6 of MCM cDNA, the patient's parents both carried the heterozygous mutation. Restriction fragment length polymorphism analysis (RFLP) showed that the G427D mutation in one allele was inherited from her father and the F307L mutation in the other allele was inherited from her mother. (Figure 1)

DISCUSSION

This reported patient had clinical symptoms and typical laboratory abnormalities, and these lead to the suspicion of inborn error of metabolism-related metabolic acidosis. Trivial residual MCM enzyme activity detected

by MCM activity analysis and one novel mutation was identified on *MUT* gene. Hence, the *mut*$^-$ phenoltype MMA was diagnosed in this 2 year-old Taiwanese girl. Our case had special presentations, late and abrupt onset, and novel genetic mutation on *MUT* gene. MMA should be considered as one of the differential diagnosis of children with metabolic acidosis despite of absence of previous episodes and normal developmental milestone.

Genetic analysis for the *MUT* gene defect, especially those reported previously, was essentially important in genetic counseling in family with member had MMA or equivocal in diagnosis of MMA. To our best knowledge, there were more than 100 disease-causing mutations have been reported[3-8] and about half of them were not located in the previously common reported exons, such as 2, 3, 11, and 12. Therefore, there were no mutation hotspots and this made the genetic counseling difficult, and especially there were no well-documented and specific mutations among Taiwanese population despite of previous oral reports. Two mutations, G427D and F307L, were identified in this reported case and not identified of 100 alleles for 50 unrelated normal Taiwanese were found to have these mutations. TheG427D mutation in one allele was from her father and F307L mutation, a novel one, was from her mother after direct sequencing of the genomic DNA. MCM activity test revealed mut- phenotype in our presented patient, and *mut*^{0} patients present early and have a poor neurological outcome compared to *mut*$^-$ patients generally.[9] With respect to genotype and phenotype correlations, it is important to try to correlate the phenotype with variant allelic variants because it could be able to predict clinical outcome and provide the decision making on prenatal intervention. However, only a few reports correlating genotypes and phenotypes.[10,11] In our presented case, nevertheless, the novel mutation, F307L, correlates to *mut*$^-$ phenotype, late onset with previously normal developmental milestone. Further, functional analysis of this mutant allele needed to be investigated to explain the molecular basis for the clinical course.

The mainstay treatment of *mut*$^-$ MMA were in preventing the formation and lowering the levels of accumulating metabolites including nutrition therapy with high energy and low protein diet, diminishing the precursors of propionyl-CoA, correction of acidosis with alkali therapy, and reducing the methylmalonic acid formation by administration of metronidazole and L-carnitine. In addition, patient with *mut*$^-$ type have an in vivo biochemical response to cobalamine supplements.[12] In our case demonstrated the efficacy of this treatment modality. In recent decades, the combined liver and renal transplantation provided an enzyme production from transplanted organ and against metabolic decompensation.[13]

There has been clear improvement in clinical outcome, long-term complication and survival rate in the last decades.[14] Hence, it is important that MMA should be diagnosed earlier when facing a child with high anion gap metabolic acidosis, ketonuria and hyperammoniemia even without previous episode. Early treatment to prevent possible complications, such as growth retardation, neurologic abnormalities (basal ganglioal lesion) and impairment of renal function.[14,15]

In conclusion, we presented an unusual initial presentation of MMA and have identified a novel *MUT* gene mutation in a Taiwanese girl with MMA. We hope that our reported novel mutation could be helpful in the further studies in DNA diagnosis of *mut*$^-$MMA within Taiwanese population.

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