Novel Mutation of Methylmalonyl-CoA Mutase Gene in a Thai Infant with Methylmalonic Acidemia (mut°)


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ABSTRACT

Isolated methylmalonic acidemia is found in patients with mutations in the MUT gene causing partial methylmalonyl CoA mutase deficiency, mut-, or complete methylmalonyl CoA mutase deficiency, mut°. Most mut° patients have an earlier and more severe presentation than the other groups such as mut- and cbl defect. We report a 6-month-old Thai male presenting with wide-anion gap metabolic acidosis after acute lower respiratory infection. Urine organic acids analysis demonstrated excretions of methylmalonic acid and methylcitrate, consistent with methylmalonic acidemia. He was then started on low protein diet with an appropriate metabolic formula, L-carnitine (100 mg/kg/day), and oral vitamin B12 (1 mg/day). He had only one single metabolic episode at 2 years of age. At present, he is doing well with normal growth and development. His methylmalonyl-CoA mutase activity was undetectable compatible with mut°. He was found to be homozygous for a novel IVS11-2A>G mutation causing two aberrantly spliced transcripts. The identified mutation and enzyme activity of this patient should cause severe phenotype, although, our patient has milder clinical manifestations. Therefore we hypothesize that there are other factors that may determine the clinical phenotype of mutase deficiency in the present case.

Keywords: Methylmalonic acidemia, methylmalonyl-CoA mutase, MUT gene, novel mutation

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Methylmalonic acidemia (MMA) is a genetically heterogeneous disorder of methylmalonate and cobalamin (cbl; vitamin B12) metabolism. Methylmalonyl-CoA mutase (MCM) is a mitochondrial enzyme that catalyzes the isomerization of methylmalonyl-CoA to succinyl-CoA. MCM activity requires 5-prime-deoxyadenosylcobalamin (AdoCbl), a coenzyme form of vitamin B12. Isolated methylmalonic aciduria is found in patients with mutations in the MUT gene causing partial, mut-, or complete, mut°, MCM enzyme deficiency. This form is unresponsive to B12 therapy. Mut° patients have an early neonatal/infantile onset, compared to those of mut- and cbl defect. Most mut° patients have fatal neonatal disease, and die during the first few months of life or have poor neurologic outcomes.1,2 A few patients have been reported with benign conditions3,4 or mild symptoms.5 Although a broad correlation was found between mutase class and phenotype, survival with good outcome was possible among mut° patients and, conversely, significant morbidity occurred among mut- patients. Acidosis and metabolic imbalance are not necessary preconditions for significant morbidity.6 More than 150 MUT mutations have been reported.7 Most of them are private mutations. The genotype may be useful to determine the severity of
mut^0 patients. We here report a Thai mut^0 patient with a novel mutation of the MUT gene.

CASE REPORT

A 6-month-old Thai male, the first child from a non consanguineous couple, was born at term with weight of 2,780 g and APGAR scores 9 and 10 at 1 and 5 minutes respectively. In the first 6 months of life, he was developmentally and physically normal without any metabolic crisis. He developed tachypnea and mild metabolic acidosis (HCO_3 12 mmol/L) during the admission due to acute bronchiolitis at 6 months of age. Then he was referred to Siriraj Hospital for further management. His development was normal. Physical examination was normal except for mild tachypnea. Laboratory evaluation revealed wide anion gap metabolic acidosis (Na 137 mmol/L, K 4.7 mmol/L, Cl 101 mmol/L, HCO_3 14 mmol/L, anion gap 22), plasma lactate 4.3 mmol/L (normal < 2), NH_3 53 mmol/L (normal < 40), and plasma ketone 0 mmol/L. Plasma amino acid profiles revealed elevated glycine 336 mmol/L (normal 90-120). Urine organic acids analysis demonstrated markedly increased excretion of methylmalonate in urine. He was then started on a low protein diet and supplemented with the metabolic formula, L-carnitine (100 mg/kg/day) and oral vitamin B12 (1 mg/day). He had only one acute metabolic episode at 2 years of age. At present, he is doing well with normal growth and development.

MCM activity in the leukocytes was determined in vitro by measuring the isomerization of L-methylmalonyl-CoA to succinyl-CoA using high-performance liquid chromatography as previously described with reduced reagent volumes. Specific activities of MCM in the leukocyte extracts of the patient and his parents were determined. The patient showed undetectable MCM activity indicating that the patient is mut^0 MMA (patient = 0, patient’s father = 317.1, patient’s mother = 261.5 pmol/min/mg; normal controls = 121 ± 50 pmol/min/mg).

Genomic DNA and total RNA were isolated separately from whole blood. First-strand cDNA was generated from the total RNA using Superscript III Reverse Transcriptase (Invitrogen). A cDNA containing the entire coding region was amplified by PCR with the previously described method. Mutation analysis of the MUT gene was performed by direct sequencing in both directions using a BigDye terminator cycle sequencing kit v.3.1 (Applied Biosystems) and analyzed on an ABI 310 automated DNA sequencer (Applied Biosystems). One hundred control chromosomes from unrelated healthy Thai individuals were analyzed for the mutation found by sequencing. We identified one novel splice site mutation of MUT in the patient. The patient was homozygous for a novel IVS11-2 A>G mutation inherited from his father and mother. This mutation affects the 3′ splice site of intron 11. RT-PCR analysis showed that this mutation activates alternative splice sites, yielding two aberrantly spliced transcripts: one skipping the first 13 nucleotides of exon 12 and another incorporating a total of 76 bp of intron 11 (c.1957-974_c.1957-899) in addition to skipping the first 13 nucleotides of exon 12, thereby leading to frameshifts (Fig 1). This novel mutated allele was not found on screening 100 control chromosomes.

DISCUSSION

Our patient presented the first time at 6 months of age with severe metabolic acidosis following respiratory tract infection. After diagnosis and treatment, he has been doing well with normal growth and development. The late onset and mild clinical course in this patient are considered to be either mut^- or cobalamin A defect. Surprisingly, his mutase enzyme analysis revealed undetectable activity (mut^0). This made clinical and enzyme correlation in methylmalonyl-CoA mutase deficiency more difficult.

The IVS11-2A>G mutation identified in this patient results in 2 aberrant transcripts: the p.T653fsX667 (skipping the first 13 nucleotides of exon 12) causes a premature termination of the MCM amino acid chain and shortens the protein to 666 amino acids instead of 750 amino acids and the p.T653fsX772 incorporates a total of 76 bp of intron 11 (c.1957-974_c.1957-899) in addition to skipping the first 13 nucleotides of exon 12, which result in incorrect amino acid sequences from the start of the frameshifts. The absence of this mutation in the controls supports that this nucleotide change is a causative mutation.

This mutation causes termination of the MCM amino acid chain resulting in incomplete mutase enzyme deficiency, but the clinical manifestation is relatively mild. Our patient is another example of the inverse correlation of in vitro mutase enzyme activity, genotype, and clinical phenotype.

REFERENCES