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IDENTIFICATION OF MUTATIONS IN *MUT* GENE OF A PATIENT WITH METHYLMALONIC ACIDURIA

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Abstract: ***Objective*** To identify and analyze the mutation of *MUT* gene in a patient with methylmalonic aciduria. ***Methods*** A patient with methylmalonic aciduria was examined. Genomic DNA was amplified by PCR. The PCR products of mut gene were analyzed by sequencing. The three-dimensional structure of human methylmalonyl-CoA model was deduced. ***Results*** Four mutations carried by mother including c. 636G>A, c. 1106 G>A, c.1595 G>C and c. 2011 A>G at exon3, exon 6, exon 9, exon 12, respectively were detected. The mutation of IVS3+1 Del G INS TGGTTATTA from father in Intron 3-4 results in premature translation termination. ***Conclusions*** c. 1106 G>A, c.1595 G>C and IVS3+1 del G ins TGGTTATTA mutations of *MUT* gene are novel mutations which have never been reported.
Key words: Methylmalonic Aciduria; Mut Gene; Gene Mutation

INTRODUCTION

Methylmalonic aciduria (MMA) is an autosomal-recessive disorder of propionate metabolism caused by a defect in the isomerization ofL-methylmalonyl-CoA to succinyl-CoA. The reaction is catalyzed by L-meth-ylmalonyl-CoA mutase (MCM, EC 5.4.99.2), an enzyme which requires adenosylcobalamin (AdoCbl) as a cofactor [12]. Typical MMA is characterized clinically by feeding difficulties, failure to thrive, hematologic (e.g.,anemia, thrombocytopenia, and microthrombi)[1], neurologic (e.g,developmental delay, microcephaly, hypotonia, seizures, macrocephaly, dementia, and myelopathy), metabolic (acidosis), ophthalmologic (e.g., pigmentary retino-pathy, decreased visual acuity, and nystagmus), and dermatologic abnormalities[9]. In general, late-onset patients have better survival and response to treatment, and fewer neurologic sequelae compared with early-onset patients [11].

MMA is classified into two forms: one resulting from a defect in the MCM apoenzyme (mutMMA or vitamin B12-unresponsive MMA; MIM 251000) and another resulting from a defect in the steps leading to AdoCbl synthesis (cblMMA or vitamin B12-responsive MMA). MCM is encoded by MUT gene (GenBank NG_007100) which maps to chromosome 6p12-21.2[8]. MUT consists of 13 exons spanning 35 kb [10]. It produces a 2.7-kb mRNA, encoding 750 amino acids and has a 32-amino-acid N-terminal mitochondrial leader sequence that forms the mitochondrial targeting sequence. To date, about 200 disease-causing mutations in the human MUT gene have been reported [2-3].

Two groups of patients with MMA that have mutations in mut gene have been identified. Mut0 patients have very low or undetectable levels of enzyme activity. Mut-patients have residual enzyme activity that is increased by the addition of hydroxycobalamin during cell culture, and some of these cells have been shown to have a reduced affinity for adenosylcobalamin [7].

METHODS

Patient Clinical Report

A 2-month-old infant was admitted to our hospital for repeated vomiting. He was the second child of parents. There was no consanguineous marriage among the parent of patient. The infant with available clinical information had been symptomatic during their neonatal or infantile periods. Physical examination revealed blood, urine and liver and kidney function were normal. However, the patient had elevated methylmalonic acid level of 2680.6 mg/g creatinine (upper limit, 0.2～3.6), propionyl carnitine level of 12.34 mg/g (upper limit, 0.3 ～ 3). Therefore, MMA was highly suspected. Responsiveness to vitamin B12 was not found in patient.

Direct sequencing of the MUT genes

Genomic DNA was extracted from the patient and parents after obtaining written informed consent using

Qiamp Blood DNA mini Kit (Qiagen, Hilden, Germany). All coding exons of *MUT* gene were amplified by PCR using specific primers (Table **1**). PCR amplification was performed in Bio-Rad PTC-200PCR system under the conditions: 4 minutes at 94 ℃; 30 cycles of 30 s at 94 ℃, 30 s at 60 ℃, and 30 s at 72 ℃. PCR products were separated on agarose gels and purified using AxyPrep DNA Kit and directly sequenced on ABI PRISM 3730 automated sequence.

RESULTS

The sequencing analysis of patient resulted in the identification four heterozygous mutation in the coding exons of MTU gene. c. 636G>A, c. 1106 G>A, c.1595 G>C and c. 2011 A>G at exon3, exon 6, exon 9, exon 12, respectively (Fig. 1a-d). Mother was found to be a carrier of mutations (Figure. 1e-h). The c. 636G>A is a same sense mutation (p. Lys 212 Lys), while the other three mutations were missense mutation (p. Arg 369 His, p. Arg 532 Pro, p. Ile 671 Val). More importantly, we found a mutation of IVS3+1 del G ins TGGTTATTA in Intron 3-4 (Figure. 1m). The del G results the deficiency of RNA shearing sequence and premature translation termination. The father was found to be the carrier of this mutation (Figure. 1n). The three-dimensional structure of patient methylmalonyl-CoA model indicated the conformation and fictional dysfunction (Figure. 2).

Table 1 Primers for the amplification of the MUT gene

Mut-F2	5' TCTTATGAGTAGCTCCTATTTCCCA 3'
Mut-R2	5' CTAATTCCTTTTAAACAACGTGCA 3'
Mut-F3	5' AGATAGATACGAGATGATTGAGATTT 3'
Mut-R3	5' CTGCAAGTAACGACAGAACATAA 3'
Mut-F4	5' AAAGTAGGTACAGTCCTGATGATGGT 3'
Mut-R4	5' TGGCTTTTTCTCTCATTATCACTCA 3'
Mut-F5	5' TATATGCCTAGACCAATGCACAATC 3'
Mut-R5	5' TCGCCCAGCCTTGTTTACTT 3'
Mut-F6	5' TTGCAATATCTATCACCTGTTTCTT 3'
Mut-R6	5' AAACTGCTGTTCTTTGTATGAGCT 3'
Mut-F7	5' AAGAGGTTTTGTGTTTTTTGGAATATA 3'
Mut-R7	5' ATGGTTAGACATCCACACACACTTT 3'
Mut-F8	5' GATTGGGATTTGCTGATCTATATTG 3'
Mut-R8	5' ACTGAGTCCTGGTTGTCTATTTATCC 3'
Mut-F9	5' AGGGTCTAATCTCTTGATCTCTGTTT 3'
Mut-R9	5' TCAACTTTTAGTCTTTGGAAACCTC 3'
Mut-F10	5' AGTGTTGCTGTTTATCCATTTGTGT 3'
Mut-R10	5' TGTAAGGAAATTAAGCTCCCAGTAGA 3'
Mut-F11	5' AATAAACTTGAAAGATTTGCTGTGAA 3'
Mut-R11	5' AGAGAGTTAATAAACCCCTGCAGTA 3'
Mut-F12	5' AGTCTTTGCCCATTAGTATGTTCTG 3'
Mut-R12	5' AACCACAAATAAGCTATCATTACTCAA 3'
Mut-F13	5' AGAAGGTTTTGGGTAATTGAGTGAC 3'
Mut-R13	5' ATACCATTGTCCAGAGTTCTTGATAAA 3'

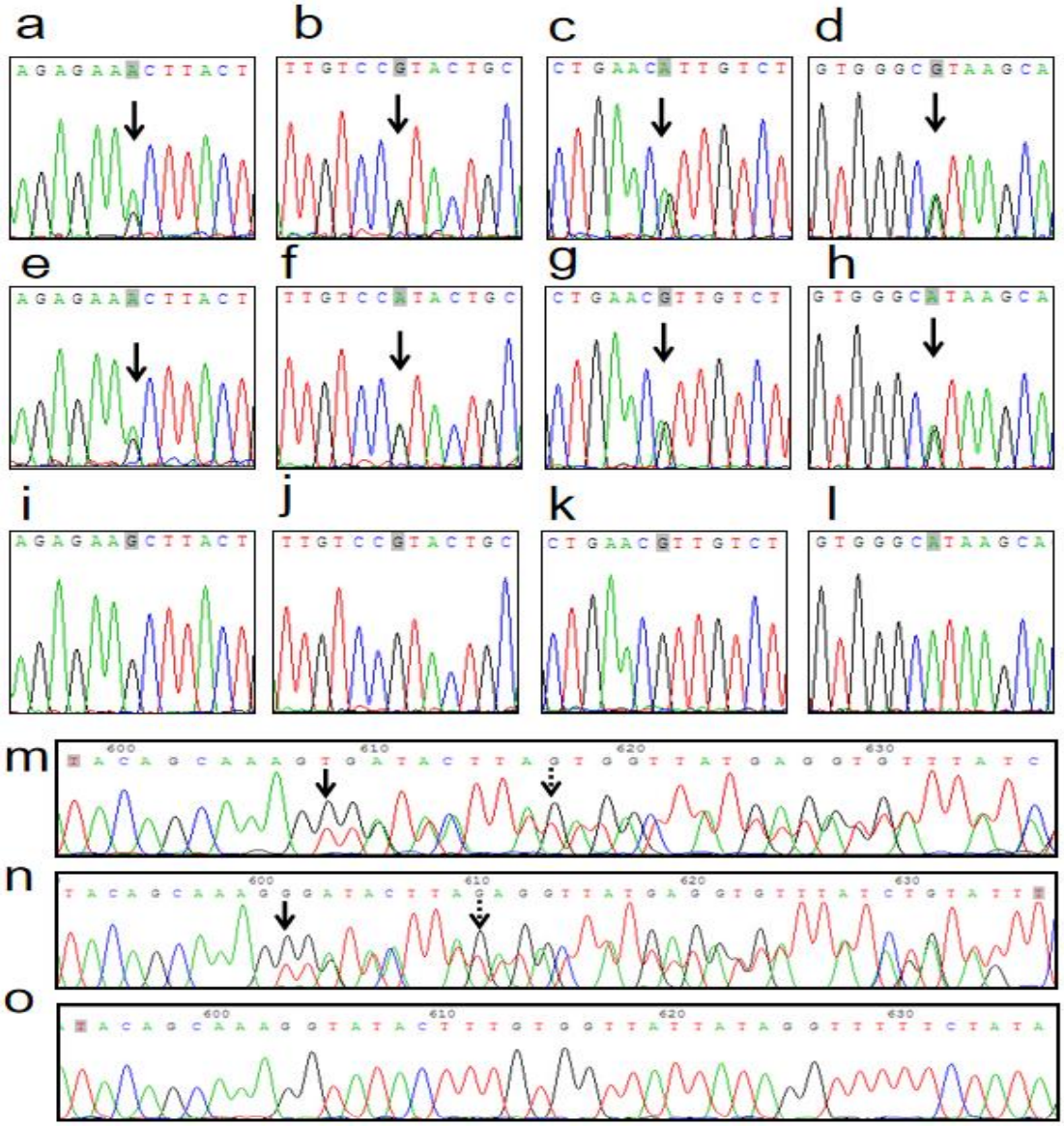

Fig.1 Direct sequencing results of MUT gene. (a-d), sequence of MUT gene of exon3, exon 6, exon 9 and exon 12, respectively in patient. (e-h), sequence of MUT gene of exon3, exon 6, exon 9 and exon 12, respectively in patient's mother. (i-l), sequence of MUT gene of exon3, exon 6, exon 9 and exon 12, respectively in patient's father. The mutated site is indicated by a line with an arrowhead. (m-o), sequence of MUT gene of Intron 3-4 in patient, father and mother, respectively. The solid arrow indicates insert sites, and the dotted arrow indicates deletion sites.

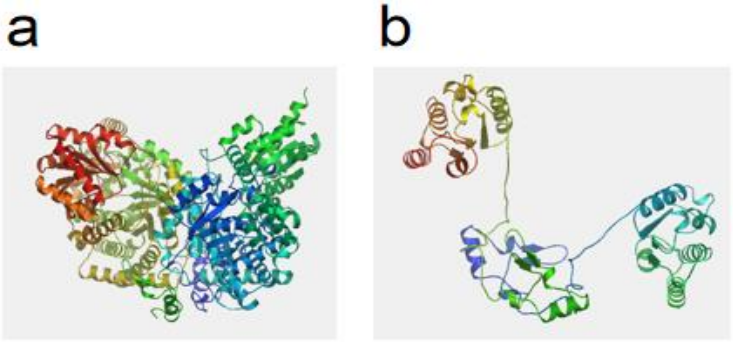

Fig.2 The three-dimensional structure of methylmalonyl-CoA model.

The normal (a) and patient (b) mehtymolonyl-coA model were established in **http://swissmodel.expasy.org/interactive**

DISCUSSION

Our study reported a MMA disease patient with three novel heterozygous mutations, c. 1106 G>A, c.1595 G>C and IVS3+1 del G ins TGGTTATTA. The laboratory findings, including elevated methylmalonic acid level led us to the possible diagnosis of MMA disease which was confirmed by mutation analysis. The central message of this study is to emphasize the contribution of sequencing to the diagnosis of complex human disease, especially in the context of prenatal screenings. In addition, the *MUT* gene mutation demonstrated in this study expanded the mutation spectrum of MMA disease.

With the cloning of a number of cobalamin-dependent enzymes[5-6], an effort has been made to determine both how these enzymes bind cobalamin cofactor and substrates and the similarities among the binding pattern of these apoenzymes with their prosthetic groups[4,10]. Comparison of the amino acid sequence of different vitamin B12-dependent enzymes from prokaryotic as well as eukaryotic organisms, including human MCM, allowed the discovery of a common binding motif for vitamin B12 cofactors. The active site is buried deep within the enzyme and access of methylmalonyl CoA substrate to this active site is through a narrow tunne along the axis of a β/α barrel. Binding of substrate occurs within the β/αbarrel, which may also serve to protect the reactive intermediates generated during enzyme activity. There is marked amino acid sequence conservation within the respective regions of MCM from P. shermaniiand humans suggesting that MCM activity may be seriously affected by changes in conserved amino acids in these domains.

We have now identified three additional mutations; these results expand the number of mutations known to affect MCM function in humans

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