Case Report

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Identification of a novel frameshift mutation (L345Sfs*15) in a Korean neonate with methylmalonic acidemia

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Methylmalonic acidemia (MMA) is an autosomal recessive metabolic disorder characterized by an abnormal accumulation of methylmalonyl-CoA and methylmalonate in body fluids without hyperhomocysteinemia. Cardiac disease is a rarely known lethal complication of MMA, herein, we report a Korean neonate diagnosed with MMA on the basis of biochemical and genetic findings, who developed cardiomyopathy, resulting in sudden death. The patient presented vomiting and lethargy at 3 days of age. Initially, the patient had an increased plasma propionylcarnitine/acetylcarnitine concentration ratio of 0.49 in a tandem mass spectrometry analysis and an elevated ammonia level of 537 μmol/L. Urine organic acid analysis showed increased excretion of methylmalonate. Subsequent sequence analysis of the methylmalonyl-CoA mutase (*MUT*) gene revealed compound heterozygous mutations c.323G>A (p.Arg108His) in exon 1 and c.1033_1034del (p. Leu345Serfs*15) in exon 4, the latter being a novel mutation. In summary, this is the first case of MMA and cardiomyopathy in Korea that was confirmed by genetic analysis to involve a novel *MUT* mutation.

Key words: Methylmalonic acidemia, Cardiomyopathies, *MUT* gene.

Introduction

Methylmalonic acidemia (MMA, MIM #251000) is an inborn error of metabolism that is biochemically characterized by accumulation of methylmalonate in urine and other body fluids [1,2]. MMA can be caused by a defect either in the activity of the methylmalonyl-CoA mutase (MCM, MIM#51000), which catalyzes the reversible isomerization of L-methylmalonyl-CoA to succinyl-CoA, or in the synthesis of its cofactor, 5-deoxyadenosyl-cobalamin (cblA, cblB, cblC, cblD variant-2 complementation groups coded by *MMAA, MMAB, MMACHC* and *MMADHC*) [1,3]. The deficiencies of MCM are further subdivided into mut^0, which indicates complete deficiency, and mut^-, which indicates partial deficiency [4]. MMA has been associated with various clinical phenotypes ranging from a benign condition to fatal neonatal disease [5]. Patients with mut^0 often present ketoacidosis, lethargy, repeated vomiting, coma or even death, in the newborn period, and suffer from severe long-term complications such as renal failure and neurological impairments. On the other hand, patients with mut^- have a lower occurrence of mortality, morbidity, and long-term complications [5]. Cardiac disease is a known, yet rare, lethal complication of MMA [6,7] and it has been scarcely reported in the Asian population [7]. We report herein a novel *MUT* gene mutation in a Korean newborn with

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MMA and cardiomyopathy, and review the relevant literature.

Case

A 3-day-old, female newborn presented vomiting and lethargy. The patient was born to healthy, non-consanguineous Korean parents as their first baby at 40 weeks of gestation. Pregnancy was uneventful and she was delivered spontaneously with a birth weight of 3,090 g (25th-50th percentile). Head circumference was 38 cm (>97th percentile) and height was 53 cm (75th-90th percentile). At 3 days of age, she was transferred to our hospital. With deterioration of self-respiration, she underwent ventilator care. Initial investigations showed she had a total leukocyte count of 3,400/mm^3, hemoglobin levels of 17.9 g/dL, and a platelet count of 210,000/mm^3. Arterial blood gas analysis revealed a pH of 7.29, $PaCO_2$ of 15 mmHg, HCO^{3-} of 12.4 mmol/L, and a base excess of −18.2, suggesting metabolic acidosis. Other laboratory results were ammonia of 537 (reference range [RR], <50) μmol/L, glycine of 320.4 (RR, 232-740) nmol/mL, aspartate transaminase of 111 (RR, <40) IU/L, alanine transaminase of 27 (RR, <40) IU/L, and lactic acid of 4 (RR, 0.5-2.2) mmol/L. Chest radiography showed no specific findings with cardiothoracic (CT) ratio of 0.41 (Fig. 1A). Echocardiography demonstrated good ventricular function with a small secundum atrial septal defect. Initial tandem mass spectrometry (MS/MS) analysis revealed an increased propionylcarnitine (C3)/acetylcarnitine (C2) concentration ratio of 0.49 (RR, 0.00-0.40). A urinary organic acid test revealed a marked increase in methylmalonate excretion of 884.9 (RR, <5) mmol/mol Cr, with an increased 3-hydroxypropionate excretion of 44.4 (RR, <19) mmol/mol Cr. Based on the MS/MS and organic acid analysis, a presumptive diagnosis of MMA was made. Emergency treatment was performed during the acute metabolic crisis, including administration of sodium benzoate, phenylbutyrate, and continuous renal replacement therapy (CRRT) on the day of admission. On the 5th day of age, the level of blood ammonia had decreased to 130 μmol/L, so CRRT was stopped. Having completely recovered from the acute metabolic crisis, the patient was released at 2 weeks age with a low protein diet and medications including sodium benzoate (450 mg/kg/day), phenylbutyrate (450 mg/kg/day) and L-carnitine (100 mg/kg/day). After discharge, she did not experience any further attacks. However, she was unexpectedly found cardiopulmonary arrest at 4 months of age without any signs of illness or metabolic crisis. Echocardiography was performed at the emergency room and revealed dilated, hypokinetic cardiac chambers, indicating cardiomyopathy. Chest radiography showed cardiomegaly with CT ratio of 0.56, which was suspected cardiomyopathy (Fig. 1B). Because of the patient's condition, laboratory evaluation could not be performed. Seven days before the event, ammonia level was 95 μmol/L on routine check-up. Despite aggressive resuscitation, her condition deteriorated rapidly, and she died in a day.

Genomic DNA was extracted from the family trio. Direct sequencing of all the coding exons, including flanking introns of *MUT*, was performed. Informed consent was obtained from the parents of the patient. Sequence analysis of the *MUT* gene revealed a G⟶A transition mutation at *MUT* cDNA nucleotide position 323 (p.Arg108His) in exon 1, which has already been reported. The other mutation, a 2-bp small deletion mutation in exon 4 (c.1033_1034del), caused a frameshift starting at codon 345, leading to a premature stop codon, which has never been reported (Fig. 2A). The former mutation was derived from the

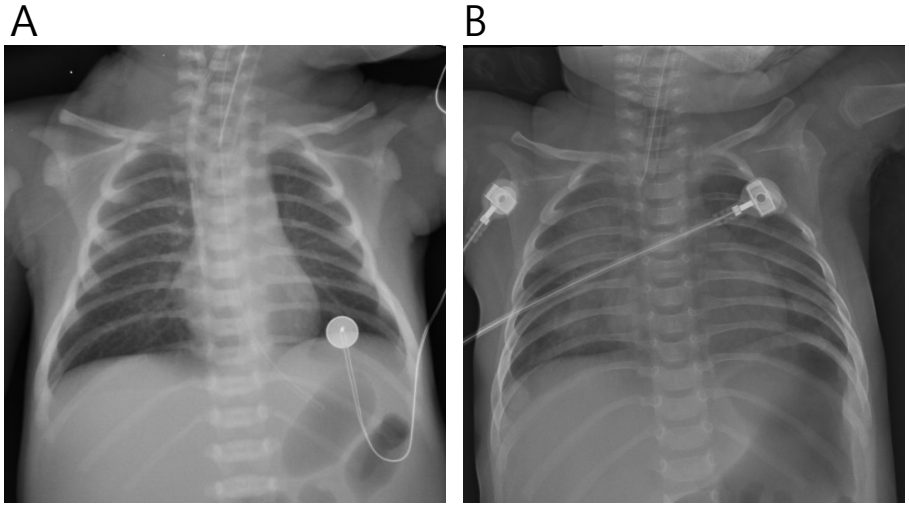

Fig. 1. Antero-posterior chest radiographs of the patient. (A) Initial chest radiograph shows no abnormal findings with cardiothoracic ratio of 0.41 at 3 days of age. (B) On the day of cardiopulmonary arrest, chest radiograph demonstrates cardiomegaly with cardiothoracic ratio of 0.56 and pulmonary infiltrates on both lung fields, which were thought to be secondary changes due to resuscitation.

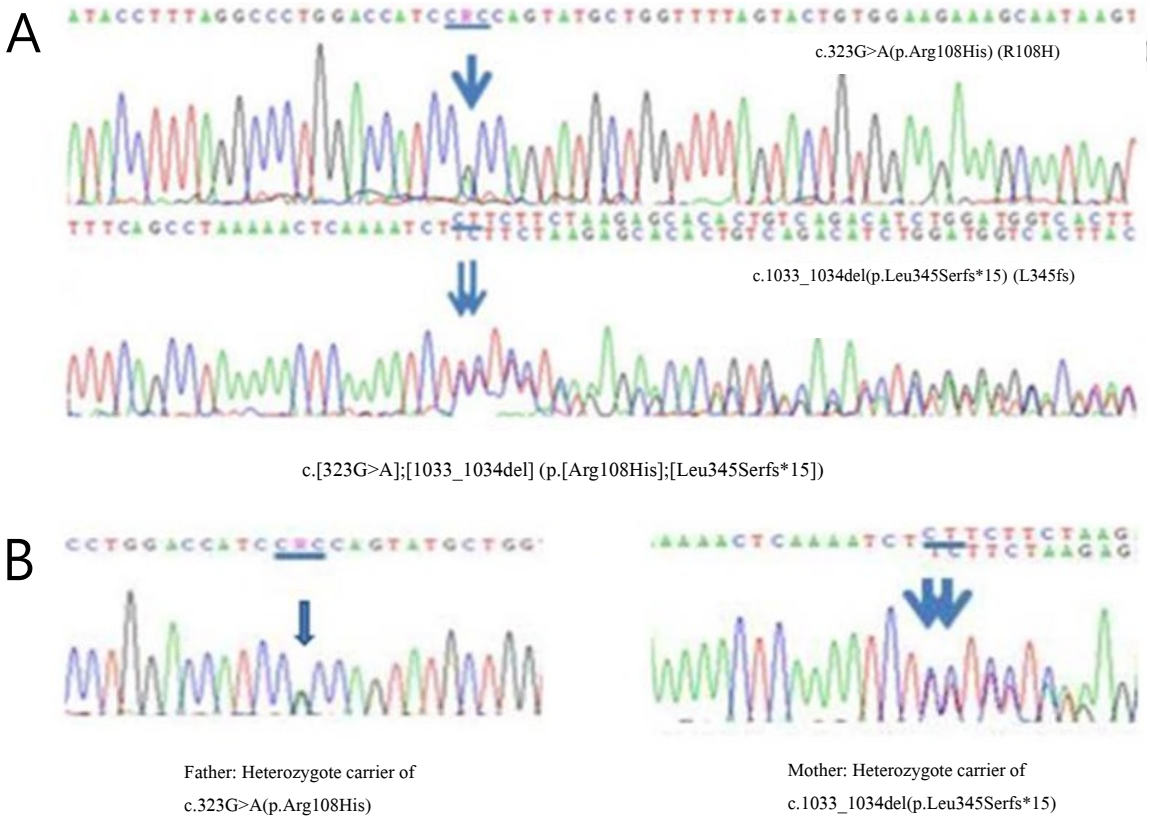

Fig. 2. Partial genomic DNA sequence of the *MUT* gene of the patient and her parents. (A) The patient had compound heterozygous mutations including a missense mutation (p.Arg108His) and a frameshift mutation (p.Leu345Serfs*15). (B) Patient's father is a heterozygous carrier of p.Arg108His and his mother is a heterozygous carrier of p.Leu345Serfs*15.

father, and the latter mutation was derived from the mother (Fig. 2B).

Discussion

The described case was diagnosed as early-onset MMA exhibiting a mut^0 phenotype based on clinical manifestations, and biochemical and genetic analysis. Initially, the patient had an increased C3/C2 concentration ratio of 0.49, as revealed by tandem MS/MS analysis, and an elevated ammonia level of 537 μmol/L, which is suggestive of MMA. It is very important to identify affected neonates immediately, when there are abnormal laboratory results regarding MMA in newborn screening. Cheng et al. [8] reported that referred newborns with elevated plasma C3/C2 ratios >0.4, and ammonia levels >200 μmol/L, should be highly suspected of having MMA.

Unfortunately, we could not check the patient's enzyme activity owing to technical limitations. Therefore, we performed the *MUT* genetic analysis. Ultimately, we identified a compound heterozygous missense mutation, c.323G>A (p.Arg108His), in exon 1, and a frameshift mutation, c.1033_1034del (p.Glu228Lys), in exon 4. To date, a total of 201 different mutations of the *MUT* gene have been listed on the Human Gene Mutation Database (http://www.hgmd.org), demonstrating the highly pleiomorphic nature of this condition; there are 138 missense/nonsense mutations, 27 small deletion mutations, 20 mis-splicing mutations, 12 small insertion mutations, 3 small INDELs mutations, and 1 gross deletion mutation. Mutations in the *MUT* gene have been identified in 17 Korean patients, and these were comprised of 17 different mutations (Table 1) [9-12]. The *MUT* mutations that show poor outcome in the Korean population are p.[Arg108Cys]; [Leu345Serfs*15], p. [Gly94Glu];[Arg369Cys], p.[Arg369His];[Arg369His], p.[Arg108Cys]; [Arg108His], p.[Arg228*];[Leu494*], p.[Arg31*];[p.Glu117*], and p.[Leu494*]; [Arg108His] [9,10]. The p.Arg108His mutation is relatively common in Korean patients [10], which causes severe metabolic crisis or developmental delay. The p.Gly94Glu mutation was only found in Korean patients with MMA [9]. The p.Arg369His mutation was identified in Korean patients with MMA, Japanese patients with MMA, and an American patient with MMA [9,13,14]. The

Table 1. Summary of mutation analysis in patients with methylmalonic acidemia in the Korean population

Case	Classification	Gene	Exon	Nucleotide change	Protein change	Outcome	Reference
1	NA	*MUT*	IV	c.1033_1034del[a]	p.Leu345Serfs*15	Cardiomyopathy → expired	This case
			I	c.323G>A	p.Arg108His		
2	NA	*MUT*	VI	c.1106G>A	p.Arg369His	NA	Song et al. [12] (2015)
			II	c.362_368dupAGTTCTA	p.Tyr123*		
3	NA	*MUT*	II	c.323G>A	p.Arg108His	Asymptomatic → normal development	Kwak and Kim [11] (2014)
			VIII	c.1672+2T>C (IVS8(+2)T>C			
4	NA	*MUT*	V	c.1031T>A	p.Ser344Tyr	Normal development	Lee et al. [10] (2008)
			VIII	c.1481T>A	p.Leu494*		
5	NA	*MUT*	II	c.356G>A	p.Ser119Asn	Normal development	Lee et al. [10] (2008)
			XIII	c.2179C>T	p.Arg727*		
6	NA	*MUT*	III	c.682C>T	p.Arg228*	Severe metabolic crisis → developmental delay	Lee et al. [10] (2008)
			VIII	c.1481T>A	p.Leu494*		
7	NA	*MUT*	II	c.91C>T	p.Arg31*	Developmental delay	Lee et al. [10] (2008)
			II	c.349G>T	p.Glu117*		
8	NA	*MUT*	II	c.91C>T	p.Arg31*	Normal development	Lee et al. [10] (2008)
			II	c.349G>T	p.Glu117*		
9	NA	*MUT*	II	c.322C>T	p.Arg108Cys	Severe metabolic crisis → expired	Lee et al. [10] (2008)
			II	c.323G>A	p.Arg108His		
10	NA	*MUT*	II	c.349G>T	p.Glu117*	Normal development	Lee et al. [10] (2008)
			VI	c.1105C>T	p.Arg369Cys		
11	NA	*MUT*	II	c.322C>T	p.Arg108Cys	Severe metabolic crisis → expired	Lee et al. [10] (2008)
			II	c.323G>A	p.Arg108His		
12	NA	*MUT*	II	c.349G>T	p.Glu117*	Normal development	Lee et al. [10] (2008)
			VIII	c.1505_61del	p.Val502Aspfs*11		
13	NA	*MUT*	VIII	c.1481T>A	p.Leu494*	Developmental delay	Lee et al. [10] (2008)
			II	c.323G>A	p.Arg108His		
14	NA	*MMACHC*	IV	c.482G>A	p.Arg161Gln	Severe metabolic crisis → normal development	Lee et al. [10] (2008)
				c.566_574del	p.Arg189_Ala191del		
15	NA	*MMACHC*	IV	c.482G>A	p.Arg161Gln	Developmental delay → normal development	Lee et al. [10] (2008)
			IV	c.609G>A	p.Trp203*		
16	Mut^0	*MUT*	II	c.357G>A	p.Gly94Glu	Severe metabolic crisis → expired	Jung et al. [9] (2005)
			VI	c.1181C>T	p.Arg369Cys		
17	Mut^0	*MUT*	II	c.357G>A	p.Gly94Glu	Severe metabolic crisis → normal development	Jung et al. [9] (2005)
			VI	c.1181C>T	p.Arg369Cys		
18	Mut^0	*MUT*	V	c.1117C>A	p.Ser344Tyr	Mild developmental delay	Jung et al. [9] (2005)
19	NA	*MUT*	III	c.643T>G	p.Asn189Lys	Mild developmental delay	Jung et al. [9] (2005)
			III	c.765C>T	p.Thr230Ile		
20	NA	*MUT*	VI	c.1182G>A	p.Arg369His	Severe metabolic crisis → expired	Jung et al. [9] (2005)

[a]Novel mutation.
NA, not available.

p.Leu494* mutation was found in compound heterozygous Japanese and Korean patients with MMA [10,15]. The p.Glu117* has been found with a high prevalence in Japanese and Korean patients with MMA [16,17]. A novel p.Leu345Serfs*15 mutation identified in the coding region in the present study occurs within the β/α barrel domain and may produce a truncated polypeptide that contains only the barrel domain and thus yield an inactive protein. Structural analyses of the mutated protein showed missing amino acids in the truncated protein (Fig. 3) and revealed major changes in its tertiary structure due to the deleted region (THR359-VAL750) and cutting C-terminus that will affect the cofactor 5-deoxyadenosyl-cobalamin-binding site, which causes the protein to be dysfunctional. It seems that the vast alteration in the structure of the protein causes great impacts on its function owing to its strong correlation with the protein tertiary structure.

A few report of cardiac disease in MMA patients have been found in the literature. To date, seven patients (2 Caucasian, 1 Moroccan and 4 Japanese) with MMA and cardiomyopathy have been reported, and 4 of them died because of cardiomyopathy [6,7]. And one Arab patient with MMA was reported to have been treated for heart failure due to suspected carnitine deficiency [18]. Our patient was supplemented with adequate dose of L-carnitine and presented good metabolic control, but ultimately died from cardiomyopathy. The pathogenesis of cardiomyopathy in our case remains unclear. The patient did not show any infection signs such as fever and grunting. However, de Keyzer et al. [19] suggested mitochondrial oxidative phosphorylation impairment as an additional mechanism to intoxication causes energetic-dependent cardiomyopathy in patients with MMA. Therefore, physicians should consider the potential of cardiac complication in these patients with MMA.

In summary, this is the first case with MMA and cardiomyopathy in Korea that was confirmed by genetic analysis to involve a novel *MUT* gene mutation. Further studies are required to understand the functional changes of proteins involved in this disorder and their associations with the phenotypic spectrum.

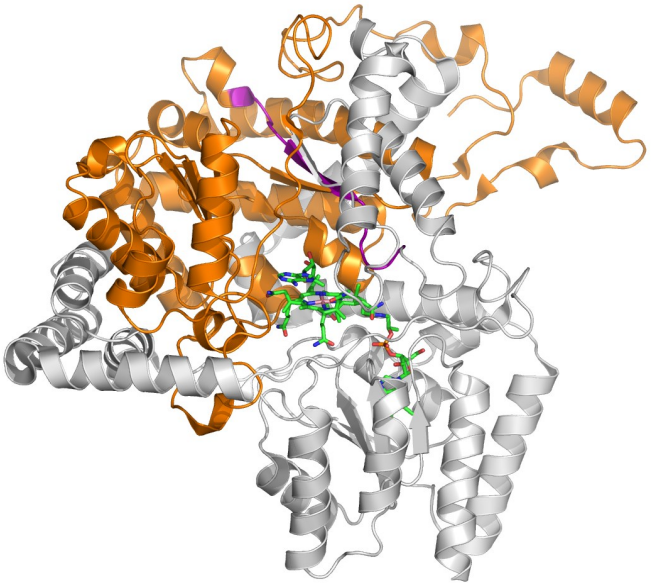

Fig. 3. X-ray crystal structure of human methylmalonyl-coA mutase (MCM) model built on the basis of the experimental structure of the A chain of the *Escherichia coli* enzyme (PDB 3BIC), showing missing amino acids in the truncated protein. MCM is shown as a ribbon model in orange, and missing amino acids are shown in light gray. The MCM nucleotide-binding site is shown as a cartoon representation, bound with guanosine diphosphate (in sticks). Case mutated residues discussed in this paper are shown as a ribbon model in violet.

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