Molecular Analysis of the MUT gene in Filipino Patients with Methylmalonic Acidemia

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ABSTRACT

Introduction. Methylmalonic acidemia (MMA) is an autosomal recessive inborn error of metabolism resulting from defects in the nuclear encoded mitochondrial enzyme methylmalonyl-CoA mutase. This study characterizes for the first time the genotype of Filipino patients with MMA.

Methods. Clinical data were collected from 3 patients diagnosed with MMA at the Department of Pediatrics of the Philippine General Hospital from January 2002 to June 2008. The diagnosis was confirmed by urine organic acid analysis using gas chromatography – mass spectrometry (GC-MS). Molecular analysis of the MUT gene was subsequently performed using DNA from dried blood spots or peripheral blood of patients, PCR amplification and direct sequence analysis.

Results. The patients presented classically with progressive encephalopathy, metabolic acidosis and secondary hyperammonemia in the early neonatal period. Urine amino and organic acid screens showed increased glycine, methylmalonic acid and other secondary metabolites for methylmalonic aciduria. Mutations detected in the MUT gene analysis [c.1595G>A (p.R532H), c.2011G>A (p.V671I), c.322C>T (p.R108C), c.982C>T (p.L328F) and c.1280G>A (p.G427D)] were compound heterozygous in all patients.

Conclusion. Our results show the genetic heterogeneity in Filipino MMA patients and helped emphasize the importance of molecular diagnosis particularly in the genetic counseling of the patients and their families.

Key Words: Methylmalonic Acidemia, Metabolic Disorders, Methylmalonyl-CoA mutase, MMA, MUT

Introduction

Methylmalonic acidemia (MMA; OMIM 251000) is an autosomal recessive disorder caused by inadequate function of the methylmalonyl-CoA mutase, a mitochondrial enzyme involved in the metabolism of certain amino acids, odd chain fats and cholesterol (Figure 1).[1] Two biochemical phenotypes have been established with *mut*^{0} cells having very low or non-detectable enzyme activity and *mut*$^-$ having residual enzyme activity that is increased by addition of hydroxycobalamin during cell culture.[2] Patients with this disorder present classically in the newborn period with non-specific findings such as recurrent vomiting, dehydration, lethargy, failure to thrive, severe metabolic acidosis, hepatomegaly, hyperammonemia, thrombocytopenia, neutropenia, neurological deficit, coma and even death.[3]

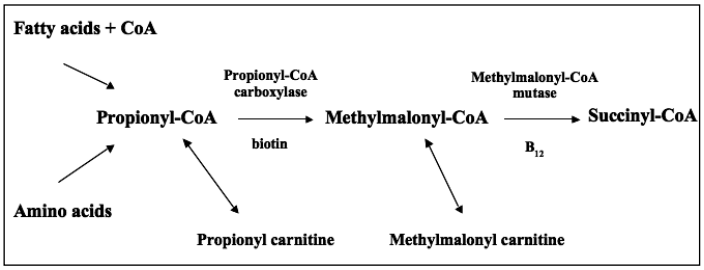

Figure 1. Simplified pathway illustrating the metabolic blockage points of MMA. The mut^0 form of MMA results in a complete blockage of methylmalonyl-CoA mutase producing a toxic build-up of acyl-CoA precursors and their corresponding acylcarnitines, propionyl-carnitine, and methylmalonyl-carnitine.[1]

Methylmalonyl-CoA mutase is encoded by the MUT gene (GenBank NG_007100) which maps to chromosome 6p12-21.2.[4] MUT has 13 exons spanning over 35 kb.[5] The open reading frame consists of 2.7 kb, 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.[6-8]

The estimated worldwide incidence of MMA is between 1:50,000 to 1:100,000.[9-14] In countries offering expanded neonatal metabolic screening, cases can be detected using tandem mass spectrometry. In the Philippines, there is routine testing for 5 disorders such as congenital hypothyroidism, congenital adrenal hyperplasia, phenylketonuria, galactosemia and G6PD deficiency. Expanded neonatal metabolic screening is not yet available locally. Eight (8) patients have, however, been clinically diagnosed. Seven of these eight patients died secondary to infection and

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intractable metabolic acidosis. The only surviving patient is presently being closely followed up at the Metabolic Clinic of the Department of Pediatrics, Philippine General Hospital (PGH).

This study describes the genotype of three Filipino MMA patients drawing attention to the importance of molecular diagnosis.

Methods

Cases

Three unrelated patients of Filipino descent clinically diagnosed with MMA by the Section of Genetics, Department of Pediatrics, PGH from January 2002 to June 2008 were enrolled in this present investigation. There were no consanguineous marriages among the parents of these families. All families provided informed consent for the study.

Biochemical data clinched the diagnosis of MMA for these patients. Urine amino acid screen using High Voltage Electrophoresis profiles showed increased glycine and increased methymalonic acid on fast blue-B staining. Urine organic acid analysis further supported the diagnosis of MMA with increased levels of methylmalonic acid and other secondary metabolites such as methylcitrate.

Molecular Analysis

Genomic DNA was extracted from dried blood spots or peripheral blood, in accordance with standard protocols, using the QIAamp Blood DNA Mini Kit (QIAGEN Inc., Valencia, Calif.).

The primers used to sequence the MUT gene were as described with minor modifications to the reaction conditions.[14] This allowed the analysis of the whole coding sequence including intron-exon borders and untranslated regions. PCR products were bi-directionally sequenced using the big dyeTM terminator cycle sequencing ready reaction kit and the products were subsequently separated on an ABI PRISM 3730xl electrophoresis system (Applied Biosystems, USA).

Results and Discussion

Clinical data was available for all patients. Common clinical and biochemical features showed methylmalonic aciduria, acute episodes of severe metabolic acidosis, lethargy, poor suck/cry and activity, vomiting, encephalopathy, anemia, leukopenia and hyperammonemia. All clinical presentations appeared at 2 days of age and were initially managed as infection. Despite marked improvement with medical management, two of the patients included in this study died after 2 months to one year of age due to sepsis secondary to severe pneumonia and intractable metabolic acidosis. The only surviving patient is presently being closely followed up and given a low protein diet, carnitine supplementation, and metronidazole to reduce methymalonic acid production from gut flora. This patient is also being given supportive management during periods of disease (Table 1). Clinical data on age at onset and diagnosis, clinical findings, management and outcome were

Table 1. Clinical features of the three MMA patients

Patient	Body Weight (kg)	Age of Gestation (weeks)	Age at onset/ diagnosis	Clinical Course
MMA-1 (5mo/M)	2.5	28	2d / 18d	(+)FHx: 2 spontaneous abortions; 2nd liveborn died at 10d – irritability, vomiting, hypotonia – unknown diagnosis At birth: poor cry/activity, hypotonia, vomiting, leukopenia, thrombocytopenia, uncompensated metabolic acidosis. At 2 mos: alert, active, good tone, development at par with age. Died at 5mos: sepsis due to severe pneumonia, intractable metabolic acidosis after 48 hours.
MMA-2 (3yo/M)	2.59	38	2d / 11d	At 11d: vomiting, poor suck/cry, progressive encephalopathy, severe metabolic acidosis, hyperammonemia, ketonuria, anemia, leukopenia, hypotonia At 2 mos: infectious diarrhea, severe metabolic acidosis At 4 mos: seizures, CT scan – corpus callosum dysgenesis, developmental delay. Died at 3 yrs: sepsis due to severe pneumonia, severe metabolic acidosis after 72 hrs
MMA-3 (4yo/F)	2.7	38	2d / 1mo	At 2 d: poor suck/ activity, severe metabolic acidosis At 1 mo: difficulty of breathing At 3 yrs: sepsis due to gastroenteritis, ketonuria, hyperammonemia, severe metabolic acidosis At present: development at par, carnitine, vit B12, protein restriction diet, metronidazole, regular monitoring

further analyzed and have been detailed elsewhere.[15]

To elucidate the molecular background of MMA in the 3 unrelated patients, sequence analyses of the MUT gene was done. The identified mutations were c.1595G>A, c.2011A>G, c.322C>T, c.982C>T and c.1280G>A. All the five mutations identified were compound heterozygous in all patients (Figures 2-3). Three (c.322C>T, c.982C>T and c.1280G>A) of the five identified mutations were previously reported to be disease-causing alleles (Table 2A).[8,16, 17]

Table 2A. List of pathogenic mutations identified

	Mutation	Consequence	Reference
Patient 1	?	?	?
Patient 2	c.322C>T	p.R108C	Worgan et al 2006[17]
	c.982C>T	p.L328F	Acquaviva et al 2005[18]
Patient 3	c.1280G>A	p.G427D	Worgan et al 2006[17]
	?	?	?

Table 2B. List of polymorphisms identified

	SNP	Allele(s) Present	AA Change	Reference(s)
Patient 1	c.1595G>A	Hetero G/A	Nonconservative (p.R532H)	Crane & Ledley 1992[16]; Worgan et al 2006[17]
	c.2011G>A	Hetero A/G	Conservative (p.V671I)	Crane & Ledley 1992[16]; Worgan et al 2006[17]
Patient 2	?	?	?	?
Patient 3	c.1595G>A	Hetero G/A	Nonconservative (p.R532H)	Crane & Ledley 1992[16]; Worgan et al 2006[17]

The c.322C>T nucleotide substitution was identified in patient 2, causing an arginine to cysteine change at amino acid 108 (p.R108C) in one allele of the MUT gene. This mutation has been identified in 60% of Hispanic patients which is not surprising since Philippine history tells us of 3 centuries of Spanish colonization (1521-1898) with European missionaries and immigrants steadily flowing to the colony during the Spanish conquest in the 16th century.[17,18,19]

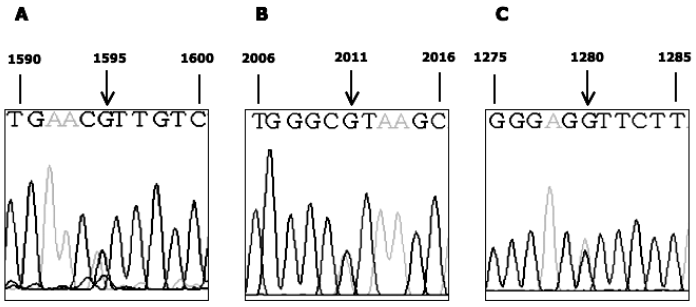

Figure 2. Chromatograms showing A. the previously reported polymorphisms c.1595G>A responsible for p.R532H and B. c.2011G>A responsible for p.V671I; and C. the previously reported missense mutation c.1280G>A responsible for p.G427D (GenBank M65131.1).

The c.982C>T change producing a leucine to phenylalanine change at amino acid 328 (p.L328F) was also found in patient 2 (Figure 3). This mutation was previously reported in Europeans and is said to alter the folding or the structural stability of the protein.[8]

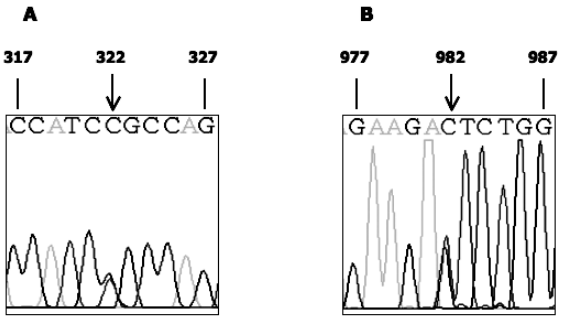

Figure 3. Direct sequencing results of MMA Patient 2 showing the pathogenic mutations A. c.322C>T responsible for p.R108C; and B. c.982C>T responsible for p.L328F (GenBank M65131.1).

In 2006, Worgan et al. identified only in Asian patients, the missense mutation c.1280G>A, changing glycine to aspartic acid at amino acid 427 (p.G427D), to affect a highly conserved amino acid in the linker region that connects the substrate-binding N-terminal β/α barrel domain and the cofactor (adenosylcobalamin) binding C-terminal (α,β) domain. Patient 3 was identified to be heterozygous for this mutation (Figure 2). This finding is interesting since this suggests that Filipinos may have a similar mutation spectrum as our Asian neighbors.

The c.1595G>A transition changing the amino acid arginine to histidine at position 532 (p.R532H) of the MUT gene was found in patients 1 and 3. The p.R532H, however, is a non-conservative substitution that does not interfere with enzyme activity suggesting that it occurs in a region of the protein not intimately involved with function (Figure 2).[16,20] Patient 1 also presented with a c.2011G>A change causing a valine to isoleucine change at amino acid 671 of the MUT gene (p.V671I). This mutation was found to be conservative by Crane et al in 1992. Both these mutations, c.1595G>A and c.2011G>A, have been found to be common polymorphisms identified in the coding exons of the MUT gene (Table 2B).[17]

The study, therefore, was only able to identify both disease causing mutations in patient 2 and only one in patient 3. It is likely that the other mutations not identified in patients 1 and 3 are present in non-coding or regulatory regions of the MUT gene or in genes required for the provision of cofactor B12 since these areas were not analyzed.[21]

Conclusion

The frequency of metabolic disorders in the Philippines is unknown with these diseases often presenting as a diagnostic challenge for pediatricians. Therefore, a substantial proportion of cases remain undiagnosed or

misdiagnosed with serious consequences such as disability or death even before a diagnosis is made. Since genetic heterogeneity is high for MMA with several disease causing alleles repeatedly reported in different populations,direct DNA analysis therefore, allows directed mutational analyses for our own population.[17]

Although there is no genotype-phenotype correlation, this study still helps show the genetic heterogeneity for our patients with MMA. The molecular findings allow proper genetic counseling for these patients and highlight the potential for early prenatal diagnosis for at risk families.

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