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Molecular Analysis of The *MUT* Gene of an Egyptian Patient with Methylmalonic Acidemia: Case Report

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Abstract:

Methylmalonic acidemia (MMA) is an autosomal recessive inherited inborn error of metabolism that results from the functional impairment of methyl malonyl-CoA mutase (MCM). The impairment of MCM is due to either defect in the apoenzyme (mut-type MMA) or in biosynthesis of the cofactor, adenosylcobalamin (cbl type). In this study, we analyze MUT gene mutations in an Egyptian patient diagnosed with MMA. The molecular analysis shows a previously reported homozygous missense mutation c.2150G>T (p.Gly717Val) in exon 13. This mutation located in C-terminal $(\beta\alpha)_5$ B12-binding domain exhibiting a mut$^-$ MMA phenotype with pathogenic effect.

Keywords:

Methylmalonic academia; Methylmalonyl-CoA mutase; MUT; cbl; GC/MS; LC/MS-MS.

1. Introduction

Methylmalonic acidemia (MMA; MIM#251000) is an autosomal recessive inherited inborn error of metabolism that results from the functional impairment of methylmalonyl-CoA mutase (MCM, EC 5.4.99.2). MCM is a mitochondrial nuclear-encoded homodimer enzyme responsible for the conversion of L-methylmalonyl-CoA to succinyl-CoA in the propionyl-CoA catabolic pathway (Acquaviva et al., 2001). Isoleucine, threonine, methionine, valine, and odd-chain fatty acid catabolism produces propionyl-CoA, which is degraded via methyl- malonyl-CoA and enters the Krebs cycle (Acquaviva et al., 2005). An overview of the major pathway for propionylCoA metabolism into the Krebs cycle is depicted in Figure (1) (Chandler&Venditti, 2005).

Defects either in the apoenzyme (mut-type MMA) or in biosynthesis of the cofactor (adenosyl-cobalamin, a vitamin B12 derivative) impair MCM function and result in an accumulation of methylmalonate in the body fluids (Liu et al., 2012).

MMA is categorized as either apoenzyme deficiency (mut) type or coenzyme B12 metabolic disorder (Cbl) type. The mut type is subdivided into mut°, which results from complete deficiency, and mut$^-$, which results from partial deficiency. The mut° type, which is characterized by apoenzyme activity of not more than 0.1 %, often presents with metabolic acidosis within 1 week after birth, frequently resulting in death in early childhood. The mut$^-$ type presents with repeated attacks of ketoacidosis triggered by infection after the age of 1 year (Imataka et al., 2013)

Patients with this disorder present classically in the newborn period with nonspecific findings such as

recurrent vomiting, dehydration, lethargy, failure to thrive, severe metabolic acidosis, hepatomegaly, hyperammonemia, thrombocytopenia, neutropenia,

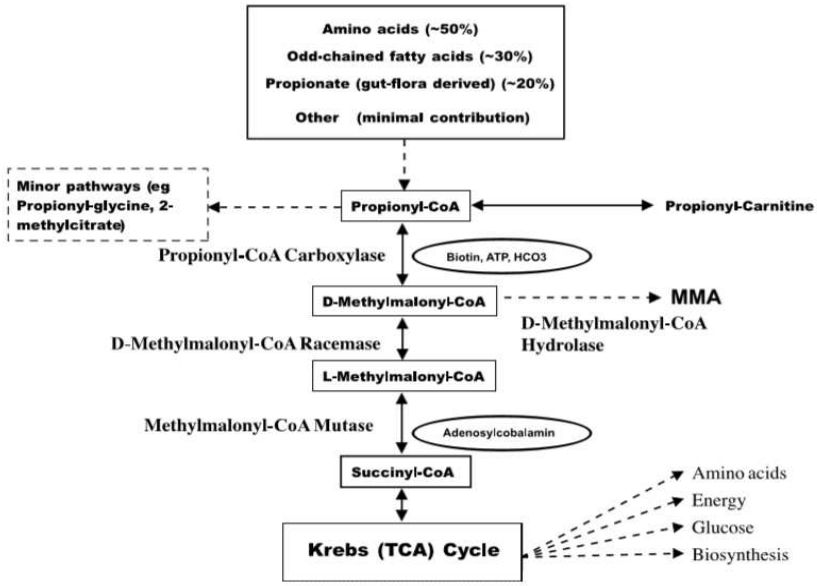


Figure 1. Major pathway of the conversion of propionyl-CoA into succinyl-CoA. The precursors are indicated with their approximated contribution to whole body propionate metabolism. The biotin-dependent enzyme, propionyl-CoA carboxylase, converts propionyl-CoA into D-methylmalonylCoA that is then racemized into L-methylmalonyl-CoA and isomerized into succinyl-CoA, a Krebs cycle intermediate. The location of the D-methylmalonyl-CoA hydrolase is indicated as are minor pathways of propionyl-CoA metabolism, such as the glycine conjugation pathway and 2-methylcitrate formation. The L-methylmalonyl-CoA mutase reaction requires adenosylcobalamin, an activated form of vitamin B12.

neurological deficit, coma and even death (Silao et al., 2009).

The treatment for MMA includes dietary protein restriction, L-carnitine supplementation, and oral antibiotics administration to reduce the production of propionate by intestinal bacteria. Supplementation of hydroxocobalamin or cyanocobalamin is used for cobalaminresponsive patients (Liu et al., 2012).

The MUT gene is located on chromosome 6p21.1(Ledley et al., 1988) and consists of 13 exons spanning over 35 kb (Fowler et al., 2008) and produces mRNA with a length of 3.886 kp (GenBank: NM_000255.3). The gene is expressed and encodes for a 750 amino acid precursor protein that contains an N-terminal mitochondrial targeting sequence of 32 amino acids and two functional domains, a (β/α)8 barrel (residues 88–422) substrate-binding site and a C-terminal (βα)5 B12-binding domain (residues 578–750) (Fowler et al., 2008). The leader sequence directs the precursor proapoenzyme into the mitochondria, where it is translocated and cleaved by a matrix peptidase to form the mature subunit (718 amino acids). Two subunits then assemble into a homodimer that binds two molecules of AdoCbl to form the active holoenzyme (Acquaviva et al., 2005).

To date, about two-hundred fifty five mutations have been described in the human MUT gene (Forny et al., 2016). The estimated worldwide incidence of MMA is between 1:50,000 to 1:100,000 (Silao et al., 2009) but it is more common in countries with high amount of consanguinity and countries with no systematic newborn screening, like developing countries (Saini et al., 2015).

In this study, we report and review the clinical and biochemical features of an Egyptian patient affected with MMA as result of a homozygous missense mutation c.2150G>T in exon 13 of the MUT gene.

2. Materials and Methods

2.1. Patient

The patient is a 7-year-old girl. She was born as the 6th order of birth from a first cousin consanguineous marriage. She had two sibling; the

first was diagnosed as a case of meningitis that led to death at the age of 1 year 8 months. The second was normal until 8 months when he developed acute vomiting, metabolic acidosis that progressed to coma and death.

At age of 50 days, she developed an attack of vomiting followed by a disturbed level of Conscious- ness, Atonic convulsions, and oculogyric crisis. Arterial blood gases showed compensated metabolic acidosis. Abdominal, chest and cardiac examination were normal. There were not hypertonia nor hyperreflexia. Her body weight was 5 kg. The diagnosis of MMA was accomplished by the analysis of acylcarnitine profiles by tandem mass spectro-metry MS/MS, measuring urinary nonvolatile organic acid patterns by gas chromatography-mass spectro-metry GC/MS and mutational analysis of the *MUT* gene.

The patient then received treatment in the form of protein restriction, XMTV1, Biomil1, B_{12} injection (depovit), Ducal and L-Carnitine on regular follow up in genetics clinic, Pediatric Hospital, Ain Shams University. Informed consent was obtained from the guardians of the patient. The study was approved by the ethics committee at Ain-Shams University.

2.2. Biochemical assays

The analysis of amino acids and acylcarnitine profiles was carried out by MassChrom® Amino Acids and Acylcarnitines from Dried Blood / Nonderivatised Kit. The blood samples were taken from patients by heel stick, spotted on Whatman filter paper cards (Schleicher and Schuell 903; Dassel, Germany) and left to dry before LC/MS-MS screening by tandem mass spectrometry (ACQUITY UPLC H-Class. Waters® Corporation, Massachusetts USA). Organic acids were determined in urine by gas chromato-graphy-mass spectrometry GC/MS (Shimadzu, GC/MS TQ8040 type Excellence in Science, Japan).

2.3. Mutation analysis

Total RNA was extracted from the peripheral blood of the patient by using GeneJET™ Whole Blood RNA Purification Mini Kit, #K0761 (Thermo Scientific, Germany), and was converted to cDNA by using the QuantiTect Reverse Transcription Kit; Cat. no.205314 (Qiagen, Belgium). cDNA was amplified by PCR using Go Taq® green master mix, 2X, cat. no. #M7112 (Promega, Fitchburg, WI). Five sets of primers (forward and reverse) were designed for amplification of specific sites covering the entire length of cDNA of MUT gene. These primers were designed by using the web-based primer-blast tool, NCBI (National Center for Biotechnology infor-mation), (http:/www.ncbi.nlm.nih.gov/tools/primer-blast /).

Table 1. Sequence of designed primers used for PCR.

Set	Primer	Start	End	Size (bp)	GC%	Tm	PCR product
1	F: 5`-CTACGCCCCCAGAGTCGTC-3`	66	84	19	68.42	60º	625
	R: 5`-GTCGCCAGATCAAAGGCAAC-3`	690	671	20	55.00		
2	F: 5`-CCTTTAGGCCCTGGACCATC-3`	567	586	20	60.00	61º	629
	R: 5`-TCCCCAGAAGAAAGACAACCT-3`	1195	1175	21	47.62		
3	F: 5`-GGCTGGCCTGACAATTGATG-3`	1144	1163	20	55.00	61º	666
	R: 5`-ATCTGCCTGTTTCGCACTG-3` A	1809	1790	20	50.00		
4	F: 5`-TCAGTGCGAAACAGGCAGAT-3`	1790	1809	20	50.00	61º	677

	R: 5`-TGAACGGCAGCCTTTGGAAT-3`	2466	2447	20	50.00		
5	F: 5`- CTTGTCATGTGTGGAGGGGT-3`	2357	2376	20	55.00	60º	647
	R: 5`-CCCAAAATCTCTTTTGCCACG-3`	3003	2983	21	47.62		

3. Results

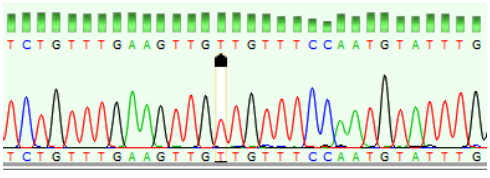
3.1. Biochemical investigations

At the age of 50 days, LC/MS-MS screening of her dried blood spot by tandem mass spectroscopy exhibited high levels of C0-Carnitine (=152µMol/l, normal 5.00 – 55.8 µMol/l) and C3-Carnitine (=21.6 µMol/l, normal <7.00 µ mol/l). Measuring of urinary organic acids by GC/MS showed highly elevated Methylmalonic acid and highly elevated methyl citrate. Concentration of methylmalonic acid in plasma was 1.17 mM (normal range = 0.4 mM).

3.2. Mutation analysis

Molecular analysis of the MUT gene of the patient showed a homozygous missense mutation c.2150G>T (p.Gly717Val) in exon 13 of the MUT gene (figure 2.a). This mutation has been reported previously for the first time by (Crane et al., 1992a).

a) Patient



b) control

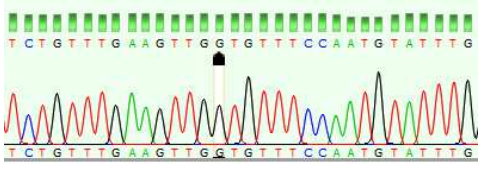


Figure 2. Sequencing analysis of the MUT gene showing (a) a homozygous missense mutation c.2150G>T as result of substitution of a single nucleotide G –to– T in the patient and (b) healthy control subject.

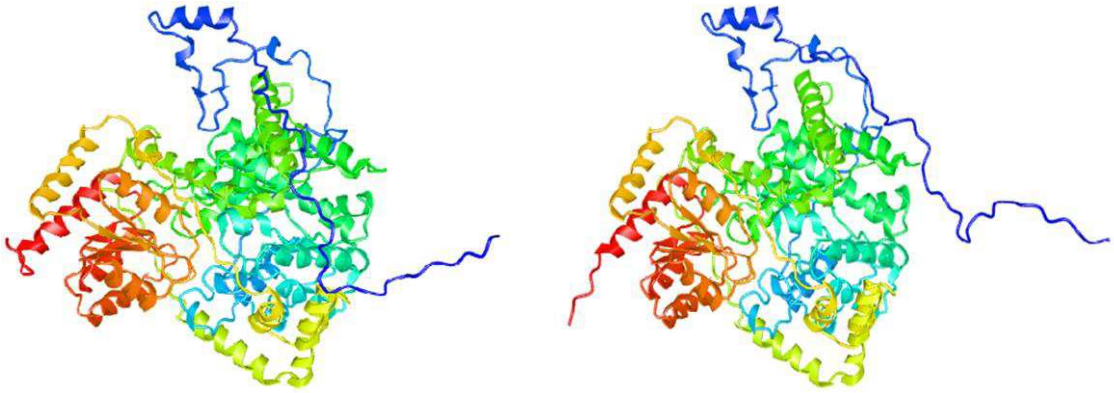
4. Discussion

In this study, the cDNA of previously diagnosed MMA patient was sequenced to identify the disease-causing mutations in her MUT gene. We identified a homozygous missense mutation c.2150G>T (p.Gly717Val) in exon 13 due to the substitution of a single nucleotide G –to– T (refSNP: rs121918252). This mutation has been identified as pathogenic mutation exhibiting a mut$^-$ MMA phenotype by Crane et al., (1992a). It was considered a common mutation in black patients (Adjalla et al., 1998; Worgan et al., 2006). This mutation is located in C-terminal (βα)5 B12-binding domain. The mut$^-$ MMA phenotype has in most cases been associated with mutations within the cobalamin-binding domain (Worgan et al., 2006). c.2150G>T mutation caused interfering with the binding of the deoxy adenosyl-lcobalamin cofactor to the apoenzyme producing a mutant holoenzyme that was defective, but not completely inactive, in vitro (Crane et al., 1992b). The enzyme specified by this mutation was shown to have a very high Km for adenosylcobalamin (Nyhan et al., 2005). The conserved residue Gly 717 is found in a sharp turn at the C-terminal end of helix IIα4 and mutation p.Gly717Val would be incompatible with this geometry (Thomä and Leadlay, 1996). This patient showed severe clinical phenotype and her biochemical findings showed an elevation of Methylmalonic acid and methyl citrate in her urine, elevation of Methylmalonic acid in plasma, and high levels of C0-Carnitine and C3-Carnitin in her metabolic screen. The MCM enzyme with p.Gly717Val mutation was stimulated by high concentrations of cobalamin in vitro (Crane and Ledley, 1994). Our clinical and biochemical manifestations of the patient support these observations where she was gradually improved by protein restriction and the medical treatment. She was responsive to pharmacological B12 when she received vitamin B12 in a dose of 1mg/day.

Reducing the dose to 1mg twice/week, led to a deterioration of the level of attention, increased sleepiness, and an increase in the level of C3-Carnitine increased from 5.13 to 23.9 µMol/l (normal <4.72 µMol/l) and C3-C2 ratio increased from 0.39 to 1.48 µMol/l (normal <0.2 µMol/l). She improved by returning the daily dose of intramuscular B12 injections.

5. Conclusion

We reported a patient with missense mutation (p.Gly717Val) in the mut locus that codes for methylmalonyl-CoA mutase enzyme. The reported clinical findings in our patient support earlier suggestions that the missense mutations, which are localized in the C-terminal domain, were more likely to belong to the mut⁻ class and therefore be responsive to cobalamin in vitro (Forny et al., 2016). Our patient has been responsive to pharmacological B12 in vivo and the clinical biochemical outcome was directly related to the intake of adequate pharcological dose of IM B12. Our report shows the importance of determining the nature of mutation in prediction of the expected phenotype and response to treatment.



(A) (B)

Figure 3. view of the three-dimensional structure of the human methyl malonyl-CoA mutase enzyme models built by the online program, RaptorX Structure Prediction (http://raptorx.uchicago.edu/ StructurePrediction/predict/) showing an altered geometry in C-terminal (βα)5 B12-binding domain due to the substitution of conserved residue glycine in position 717 by valine. A) Model of the normal subunit of the human MCM enzyme. B) Model of the subunit with p.Gly717Val mutation located in C-terminal (βα)5 B12-binding domain.

6. Acknowledgements

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