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SHORT REPORT

Mutational spectrum in ten Italian patients affected by methylmalonyl-CoA mutase deficiency

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Summary: We report seven novel mutations, including three amino acids substitutions (p.Glu286Lys, p.Cys560Tyr, p.Pro615Leu), two nonsense mutations (p.Arg31X, p.Glu 451X), one splicing defect (c.2125−1G>A), one small deletion (c.1758–1759delA) and nine previously described mutations identified in 10 unrelated Italian patients affected by mut MMA.

Mutations in the *MUT* gene, encoding mitochondrial methylmalonyl-CoA mutase (MCM) (EC 5.4.99.2), give rise to mut methylmalonic aciduria (mut MMA) (McKusick 251000). Mut MMA shows a wide clinical spectrum, ranging from severe acute neonatal onset and early death to late-onset mild forms. Two biochemical phenotypes have been reported: mut^0 with no detectable enzymatic activity and mut^- with residual activity. The mature MCM is organized in a barrel domain with the CoA-binding site, a cobalamin-binding domain and a linker region.

This study includes 10 unrelated Italian patients affected by mut MMA. Seven patients presented a neonatal onset and three showed the first symptoms between 3 months and 2 years of age. Diagnosis of MMA was performed by plasma amino acids and GC–MS urinary organic acids analyses. Complementation analysis, MCM activity assay and [^{14}C]propionate incorporation test with and without hydroxocobalamin (OHCbl) added to the culture medium were carried out in eight available patient fibroblast lines by Professor D.S. Rosenblatt (McGill University, Montreal Quebec, Canada; patients 2 and 6), Dr. J.F. Benoist (Robert Debre Hospital, Paris, France; patient 3) and Dr R. Baumgartner (University Children's Hospital, Basel, Switzerland; patients 1, 7, 8, 9 and 10). Complementation analysis and deficient MCM enzyme activity proved mut MMA in all patients. A very low uptake of [^{14}C]propionate, not responsive to OHCbl supplementation in the medium, classified six patients as mut^0. Two patients showed the typical mut^- phenotype, with a slight stimulation of [^{14}C]propionate incorporation in presence of OHCbl.

Mutation analysis was performed by direct sequencing of genomic DNA and/or total RNA isolated from peripheral blood.

Seven new mutations and nine previously reported mutations were detected (Table 1). In order to exclude the possibility of a benign polymorphism for the newly identified missense mutations, specific restriction analyses were carried out on 100 control gDNAs.

The new heterozygous c.856G>A and c.1351G>T changes, leading to substitution of glutamate by a lysine (p.Glu286Lys) and a stop codon (p.Glu451X), were identified in patient 1. She was affected by mut$^-$ MMA and had experienced only two acute metabolic crises before diagnosis, made at the age of 2 years. She is presently 21 years old and doing well. The p.Glu286Lys and p.Glu451X mutations map in the barrel domain and linker region, respectively. Since the p.Glu451X mutation leads to a truncated protein, containing only the barrel domain, the patient's mild clinical phenotype can be correlated to the p.Glu286Lys. Glu286 is a moderately conserved acid residue and its replacement with a basic codon may slightly affect the function of MCM.

In patient 2, affected by severe mut^0 MMA, who died during a metabolic crisis at the age of 6 years, the new c.1679G>A (p.Cys560Tyr) and c.2125−1G>A substitutions were identified. The c.1679G>A (p.Cys560Tyr) was detected as a homozygous change at cDNA level and as heterozygous in gDNA. This amino acid change involves a highly conserved residue of the linker region and it could alter the structural stability of MCM protein. Up to now, 11 known mutations mapping in the MCM linker region have been reported, but only the new p.Cys560Tyr and the known p.Ala535Pro (Fuchshuber et al 2000) are amino acid substitutions. Interestingly, in patient 2 the second c.2125−1G>A mutation was only revealed in genomic DNA. No aberrant transcript was detected in the agarose gel when we analysed the cDNA PCR products. Such a mutation affects the acceptor splicing site of the last exon (exon 13) of the MUT gene, and this could explain the non-detection of any extra band in cDNA, synthesized by oligo-dT and specific 3′ UTR primers and amplified using the specific 3′ UTR primer. Thus, the importance of a combined analysis on cDNA and gDNA should be stressed. In this patient, the pathogenic effect of such mutations proved consistent with the mut^0 phenotype determined biochemically.

The novel c.1844C>T transition, leading to p.Pro615Leu in the cobalamin-binding domain, and the known p.Ser262Asn, were identified in patient 3. A mutation affecting the same 615 codon (p.Pro615Thr) has been reported in a mut^0 patient and incorrect protein folding, resulting in an alterated cobalamin-binding domain function, was hypothesized (Peters et al 2002). Since the p.Ser262Asn mutation was previously correlated to mut^0 patients and our patient 3 (p.Pro615Leu/p.Ser262Asn compound) showed a mut$^-$ phenotype, it can be speculated that the p.Pro615Leu mutation associates with the mut$^-$ phenotype.

The new c.91C>T transition, introducing an early termination codon p.Arg31X within the mitochondrial target sequence, and the p.Arg108His change were identified in patient 4. The p.Arg108His and the new c.1758–1759delA were identified in patient 5. The latter small deletion, causing a frameshift with an early stop codon (p.Ala586fsX597), leads to a protein lacking the cobalamin-binding domain. Since the p.Arg108His change was correlated to the mut^0 phenotype (Acquaviva et al 2005) and both patients 4 and 5 are compound heterozygous for the p.Arg108His and a null allele, a mut^0 phenotype can be hypothesized also for them.

The known p.Asn219Tyr mutation was detected in three of our patients (20% of the screened alleles), confirming it to be a frequent mutation among Caucasian mut^0 MMA patients as previously reported by Acquaviva and colleagues (2001). Patient 8 was homozygous

Table 1 Summary of mutations and clinical features in MMA patients analysed

Patient	*Onset/Clinical and biochemical phenotype*	*Location*	*Nucleotide change*	*Effect on coding sequence*	*Restriction site alteration*	*Mutation reference*
1	Late (2 y)/2 AMC before 2 y, alive at 21 y; mut$^-$	Exon 4 Exon 7	**c.856G>A** **c.1351G>T**	**p.Glu286Lys** **p.Glu451X**	Taq I (+ACRS) –	This study This study
2	Neonatal (5 d)/died at 6 y, AMC, PD, CD; mut^0	Exon 10 Intron 12	**c.1679G>A** **c.2125−1G>A**	**p.Cys560Tyr** **Splicing defect**	Bst1107I (+ACRS) –	This study This study
3	Neonatal (2 d with HC)/died at 3 y during AMC; mut$^-$	Exon 4 Exon 11	c.785G>A **c.1844C>T**	p.Ser262Asn **p.Pro615Leu**	+AsnI StuI (−ACRS)	Acquaviva et al (2005) This study
4	Late (3 mo)/alive at 27 y, AMC, RI; –	Exon 2 Exon 2	**c.91C>T** c.323G>A	**p.Arg31X** p.Arg108His	– –AciI	This study Acquaviva et al (2005)
5	Neonatal (3 d)/alive at 8 y, AMC, RI, PD; –	Exon 2 Exon 10	c.323G>A **c.1758–1759delA**	p.Arg108His **p.Ala586fsX597**	–AciI –	Acquaviva et al (2005) This study
6	Neonatal (10 d)/alive at 6 y, PD, AMC; mut^0	Exon 3 Exon 5	c.643G>A c.1036–1038delCTT	p.Gly215Ser p.Leu346del	–KpnI –	Acquaviva et al (2005) Adjalla et al (1998)
7	Neonatal (1 d)/died at 6 y, AMC, PD; mut^0	Exon 3 Exon 13	c.655A>T c.2194–2197del4ins5	p.Asn219Tyr p.Ala732fsX737	– –	Berger et al (2001) Fuchshuber et al (2000)
8	Late(3 mo)/alive at 12 y AMC, MD, RI; mut^0	Exon 3 Exon 3	c.655A>T c.655A>T	p.Asn219Tyr p.Asn219Tyr	– –	Berger et al (2001)
9	Neonatal (2 d)/alive at 12 y, AMC, LT at 7 years; mut^0	Exon 3 Exon 7	c.655A>T c.1420C>T	p.Asn219Tyr p.Arg474X	– –	Berger et al (2001) Acquaviva et al (2005)
10	Neonatal (1 d)/alive at 6 y, AMC, mild PD; mut^0	Exon 6 Intron 8	c.1106G>A c.1560+1G>T	p.Arg369His –	– –	Mikami et al (1999) Acquaviva et al (2005)

New mutations are shown in bold type

d, day(s); mo, month(s); y, years(s); −, not determined; ACRS, amplification-created restriction site; AMC, acute metabolic crises; PD, psychomotor delay; CD, cardiac decompensation; HC, hyperammonaemic crisis; RI, renal involvement; MD, motor delay; LT, liver transplantation

for the p.Asn219Tyr amino acid change but, unfortunately, the possibility of a partial or complete deletion of the patient's MUT gene cannot be ruled out owing to the unavailability of her parents' DNA.

In conclusion, molecular analysis provides a reliable tool in confirming diagnosis of the proband, identifying carrier status and performing early prenatal diagnosis, and this study may contribute to increased knowledge about the spectrum of mutations leading to methylmalonyl-CoA mutase deficiency.

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