# Molecular characterization of Methylmalonyl CoA mutase deficiency in patients identified through newborn screening

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## INTRODUCTION

Methylmalonic aciduria due to mutations in *MUT* gene (MMA *mut type*, OMIM 251000) is a rare metabolic disorder, with autosomal recessive inheritance, resulting from methylmalonyl CoA mutase (MCM, EC 5.4.99.2) apoenzyme deficiency.

MCM is a nuclear-encoded mitochondrial matrix homodimer, which uses adenosylcobalamin as an essential cofactor. Based on the complete or partial absence of functional MCM, and in the response to adenosylcobalamine, two distinct biochemical phenotypes can be associated with MMA *mut type*: $mut^0$ and $mut^-$ forms, respectively (Fenton et al., 2001).

*MUT* gene is located on chromosome 6 and consists of 13 exons spanning over 35 kb of genomic DNA (Jansen et al., 1989). The 2.7 kb expressed mRNA encodes a 750 amino acids protein with a 32 residues mitochondrial targeting sequence. Each MCM monomer comprises different functional domains (Figure 1): a N-terminal extended segment involved in dimerization, a N-terminal $(\beta\alpha)_8$ substrate binding domain and a C-terminal $(\beta\alpha)_5$ cobalamin binding domain, both linked through a long linker region (Fuchshuber et al., 2000).

## PATIENT AND METHODS

Since the inclusion of MMA *mut type* in the Portuguese Newborn Screening panel in 2004 (Vilarinho et al., 2010), approximately 715,000 newborns have been tested through tandem mass spectrometry analysis of acylcarnitines and amino acids as butyl esters (Rashed et al., 1995). MMA patients were identified through elevated C3 (propionylcarnitine) and C3/C2 ratio.

Suspected cases were confirmed through acylcarnitines analysis in a new blood spot sample, organic acid analysis in urine and total homocysteine determination in plasma.

Genomic DNA was extracted from dried blood spots in the BioRobot®EZ1, using the EZ1 DNA Tissue Kit from Qiagen. *MUT* gene whole coding sequence and exon-intron flanking regions were PCR amplified (Aquaviva, 2005) and directly sequenced on an ABI3130XL DNA Anallyzer, using the BigDye Terminator Cycle Sequencing Version 3.1 from Applied Biosystems.

## RESULTS AND DISCUSSION

Three cases confirmed to have MMA *mut type*, which reveals a low frequency for this disease in Portugal (1:238.333). In contrast to most other countries, MMA due to cobalamin C/D deficiency (1:80.000) is much more frequent. All three patients presented a severe clinical phenotype, with clinical symptoms already visible at the sampling day.

Four different mutations were identified (Table 1), all localizing in the 2 main MCM functional domains (Table 2, Figure 1), as reported for most mutations.

**Table 1 – MMA, *mut type* newborn screening patients**

| Patient | Sex | NBS day | NBS results | Genotype |
|---|---|---|---|---|
| 1 | M | 2 | C3=13.1 µM (N<6.2)<br>C3/C2=0.51 (N< 0.3) | p.G626Efs*18 /<br>p.G626Efs*18 |
| 2 | M | 3 | C3=12.4 µM (N<6.2)<br>C3/C2=0.84 (N< 0.3) | p.G717V / p.R108C |
| 3 | M | 5 | C3=7.0 µM (N<6.2)<br>C3/C2=0.70 (N<0.3) | p.N341Kfs*20 /<br>p.G626Efs*18 |

**Patient 1** is homozygous for a frameshift mutation previously reported in one $mut^0$ European patient (Aquaviva et al., 2005). Attending to the severity of the clinical symptoms, our case is probably also a $mut^0$ patient, although most mutations locating in the cobalamin binding domain are reported to be $mut^-$ mutations.

This mutation was also found in **patient 3**, despite the fact that their families don't seem to be related. Mutation c.1022dupA, also present in patient 3, is a frameshift mutation relatively frequent among Hispanic and Spanish patients (Martinez et al., 2005; Worgan et al., 2006) and reported to be associated to the $mut^0$ phenotype. This patient is probably also a $mut^0$ patient.

**Patient 2** is a compound heterozygote for two missense mutations. Mutation p.R108C, locates in the substrate-binding domain and is also a $mut^0$ mutation, frequent among Hispanic and Spanish patients.

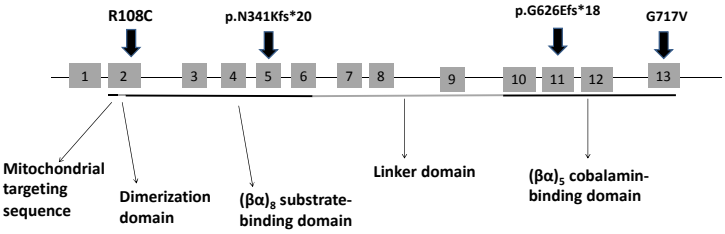
**Table 2 – Mutations identified in MMA *mut type* newborns**

| Amino acid substitution | Nucleotide substitution | Protein domain | *Mut* class* |
|---|---|---|---|
| p.R108C | c.322C>T | $(\beta\alpha)_8$ | $mut^0$ |
| p.N341Kfs*20 | c.1022dupA | $(\beta\alpha)_8$ | $mut^0$ |
| p.G626Efs*18 | c.1877-1889del13 | $(\beta\alpha)_5$ | $mut^0$ |
| p.G717V | c.2150G>T | $(\beta\alpha)_5$ | $mut^-$ |

Gene bank reference sequence NM_000255.2. *More frequently associated *mut* class.

p.G717V is typically a $mut^-$ mutation, frequent among Africa originating people (Adjalla et al., 1998; Worgan et al., 2006). The severity of the clinical phenotype and the fact that at least one $mut^0$ case was reported which was a compound heterozigote for p.G717V and a $mut^0$ mutation (Worgan et al., 2006), may suggest that this can also be a $mut^0$ patient. Nevertheless, although it seems to exist a correlation between the genotype and the biochemical phenotype, this correlation is not straightforward regarding the clinical phenotype and $mut^-$ patients can also exhibit severe clinical symptoms. This was reported for patients homozygous for mutation p.G717V (Worgan et al., 2006) and can also be the case for this patient.

**Figure 1 - Functional domains of *MUT* gene and localization of mutations found in Portuguese newborn screening patients**




## CONCLUSIONS

•MMA *mut* type is very rare in Portugal (approximate frequency < 1:200.000), but clinical severe forms seems to dominate, thus stressing the importance of early identification through newborn screening.

•Three newborn screening identified patients were studied at the molecular level and four different mutations were found: two of them are frequent among Spanish and Hispanic and another one, found in a Portuguese family with probable African origin, is frequent among black people. The fourth mutation is rare worldwide, but it was found in two non-related families, which may suggest some importance in the genetic epidemiology of the disease in Portugal.

•The molecular characterization of these patients contributed to the elucidation of the genetic epidemiology of the disease in Portugal, and is essential for genetic counseling and early prenatal diagnosis in at-risk families. The fact that novel antisense and antigenic therapies, based on the genotype, are being proposed for MCM deficiency also increases the importance of genotype determination.

## REFERENCES

Acquaviva et al., *Hum Mutat*, 2005; Adjalla et al., *Hum Mutat*, 1998; Fenton et al. *In Scriver CR, Beaudet AL, Sly WS, Valle D, editors*, 2001; Fuchshuber et al., *Hum Mutat*, 2000; Jansen et al., *Genomics*, 1989; Martínez et al., *Mol Genet Metab*, 2005; Rashed et al., *Pediatr Res,* 1995; Vilarinho et al., J Inherit Metab Dis, 2010; Worgan et al., *Hum Mutat*, 2006.