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RESEARCH REPORT

# Methionine Adenosyltransferase I/III Deficiency in Portugal: High Frequency of a Dominantly Inherited Form in a Small Area of Douro High Lands

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**Abstract** Methionine adenosyltransferase deficiency (MAT I/III deficiency) is an inborn error of metabolism resulting in isolated hypermethioninemia, and usually inherited as an autosomal recessive trait, although a dominant form has been reported in several families.

During the last 6 years, approximately 520,000 newborns were screened in the Portuguese Newborn Screening Laboratory by MS/MS, and 21 cases of persistent hypermethioninemia were found. One case was confirmed to be a deficiency of cystathionine β-synthase and 20 cases were confirmed by *MAT1A* gene analysis to have an elevation of methionine due to MAT I/III deficiency, which indicates an incidence for this condition of 1/26,000. Twelve of the MAT I/III deficient newborns, belonging to 11 families, were identified in the northern region of Portugal and sent to the same treatment center, where they are under follow-up. Clinical, biochemical, and genetic characteristics of individuals from these 11 families are presented. Plasma methionine and homocysteine concentrations were found to be moderately increased in all newborns, and molecular analysis revealed that they all were heterozygous for R264H mutation. Normal growth, development, and neurological examination were observed in all cases, and cerebral MRI performed in six cases revealed myelination abnormalities in one case. Plasma methionine concentration for all 12 cases was always below 300 μM, and they are all on a normal diet for their age.

**Abbreviations**

| | |
|---|---|
| AdoMet | *S*-adenosylmethionine |
| CNS | Central nervous system |
| MAT | Methionine adenosyltransferase |
| MRI | Magnetic resonance imaging |
| MS/MS | Tandem mass spectrometry |

## Introduction

Over the last decade, high-throughput methods for multi-component analyses, especially tandem mass spectrometry (MS/MS) have transformed newborn population screening. The overall frequency of disorders detected by MS/MS in Portugal is about 1 out of every 2,396 (Vilarinho et al. 2010).

Methionine adenosyltransferase deficiency (MAT I/III deficiency, OMIM 250850) is an inborn error of metabolism resulting in isolated hypermethioninemia and 1 of the 25 diseases integrated into the panel of the Portuguese National Newborn Screening Program. This enzyme (MAT, EC 2.5.1.6) catalyzes the biosynthesis of *S*-adenosylmethionine from methionine and ATP, and both forms of hepatic MAT (MAT I and III) are encoded by *MAT1A* gene (Mudd et al. 2001). In the majority of the cases, MAT I/III deficiency is inherited as an autosomal recessive trait, although a dominant form has been reported in several families, associated with the R264H mutation (Chamberlin et al. 1997; Chou 2000; Chien et al. 2005; Couce et al. 2008).

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Pathogenesis in this disease is not clearly elucidated and it might result from different factors: extraordinarily high plasma methionine levels can directly contribute to neurological abnormalities (Mudd 2011), the lack of synthesis of *S*-adenosylmethionine (AdoMet)-dependent methylated products can cause demyelination (Chamberlin et al. 1996), and hyperhomocysteinemia might bring about an elevated risk of vascular and thrombotic diseases (Stabler et al. 2002).

Clinical manifestations in individuals with isolated persistent hypermethioninemia due to deficient MAT I/III activity are variable, depending on the extent of the methionine elevation which, on the other hand, seems to be related to the specific *MAT1A* gene mutation (Mudd 2011). In cases associated with R264H mutation, approximately 30% of residual enzyme activity can be found (Chamberlin et al. 1997) and in accord with this, clinical outcome is usually favorable (Pérez Mato et al. 2001). Nevertheless, caution is recommended regarding the establishment of genotype/phenotype correlations to predict the development of CNS problems (Mudd 2011). The optimal management of MAT I/III deficiency also remains to be defined, and the clinical and biochemical follow-up of more patients and during more years is required to achieve definitive conclusions. Before the introduction, in 2004, of the MS/MS technology in the Portuguese Newborn Screening Laboratory, no cases of MAT I/III deficiency were reported in our population. Since then, 20 newborns and several related individuals were identified, revealing the under-diagnosis of this condition in Portugal.

We describe the clinical, biochemical, and genetic characteristics of 11 families with MAT I/III deficiency, identified by newborn screening, and who are being followed in the Oporto Hospital Center. The aims of this work were: (a) to determine the frequency of hypermethioninemia due to MAT I/III deficiency in the Portuguese population, (b) to characterize the genotype of affected individuals and try to establish genotype/phenotype correlations to predict the clinical outcome, and (c) to study the families in order to identify other individuals at risk.

## Patients and Methods

During the last 6 years, approximately 520,000 newborns, originating from the whole country, were screened in the Portuguese Newborn Screening Laboratory by MS/MS.

Blood samples were collected between the third and sixth days of life on Whatman 903 filter paper, and the analysis of amino acids and acylcarnitines, as butyl esters (Rashed et al. 1995), was performed using two API 2000 triple quadrupole tandem mass spectrometers. Methionine quantification allows the detection of cystathionine β-synthase deficiency, as well as hypermethioninemia due to MAT I/III deficiency. The positive screening criterion used for both these conditions was a methionine concentration above 50 μM. The cases of transitory hypermethioninemia were ruled out by asking for a second sample for confirmation, and only newborns with persistently high methionine concentrations were sent to a treatment center for further evaluation; plasma and urinary amino acid chromatography, plasma total homocysteine, urinary organic-acid analysis as well as hepatic function evaluation were performed.

In cases with a persistently high methionine level, MAT I/III deficiency was confirmed by *MAT1A* gene analysis. Genomic DNA was extracted from dried blood spots in the BioRobot®EZ1, using the EZ1 DNA Tissue Kit from Qiagen. *MAT1A* gene exons I–IX and the corresponding intronic flanking areas were PCR amplified, directly sequenced using the BigDye Terminator Cycle Sequencing Version 3.1 from Applied Biosystems and analyzed on an ABI3130XL DNA Analyzer.

## Results

Since 2004, 21 cases of persistent hypermethioninemia have been confirmed in 520,000 newborns screened in Portugal. One case was diagnosed as cystathionine beta-synthase deficiency and 20 cases (14 females and 6 males) were confirmed to have an isolated elevation of methionine due to MAT I/III deficiency, which indicates an incidence for this condition of 1/26,000 newborns. Twelve of these individuals, belonging to 11 families, were identified in the northern region of Portugal and sent to the same treatment center (Table 1). Patients 1–5 were previously reported (Martins et al. 2007). As far as we could investigate from family histories, these families are not related. These patients had methionine concentrations at screening ranging from 52 to 124 μM (average: 85 μM; normal $< 50$ μM). On confirmation (sampling day between 12 days and 1 month), methionine concentration rose in most patients and ranged from 79 to 247 μM (average: 151 μM; normal $< 50$ μM). Simultaneously, plasma total homocysteine was determined and all patients but one were found to have a mildly increased concentration (range: 13–21 μM; normal $< 9$ μM). Patient 12 was found to have a moderate homocysteinemia (64 μM; normal $< 9$ μM), and a possible diagnosis of cystathionine β-synthase deficiency was suspected. While waiting for confirmation, a low protein diet was immediately started, and after 48 h homocysteine levels came down to 17 μM. After the exclusion of this diagnosis, a normal protein diet was resumed, and total homocysteine concentration never went again above 20 μM.

**Table 1** Characterization of MAT I/III deficient patients followed in Oporto Hospital Center

| Patient | Sex | Current age (y/m) | Screening/confirmation sampling day | Methionine at screening (normal < 50 μM) | Methionine at confirmation (normal < 50 μM) | Homocysteine (normal < 9.0 μM) |
|---|---|---|---|---|---|---|
| 1 | F | 6 y | 6/12 | 85 | 80 | 13 |
| 2 | M | 4 y | 4/19 | 77 | 121 | 16 |
| 3 | M | 4 y | 4/19 | 52 | 168 | 16 |
| 4 | F | 4 y | 4/16 | 103 | 85 | 13 |
| 5 | M | 3 y | 3/29 | 58 | 247 | 18 |
| 6 | F | 3 y | 4/30 | 80 | 195 | 14 |
| 7 | F | 29 *m* | 4/24 | 85 | 245 | 20 |
| 8 | M | 23 *m* | 6/23 | 73 | 145 | 14 |
| 9 | F | 23 *m* | 5/24 | 124 | 155 | 15 |
| 10 | F | 23 *m* | 5/30 | 91 | 79 | 11 |
| 11 | F | 22 *m* | 5/18 | 117 | 182 | 21 |
| 12 | F | 19 *m* | 4/20 | 79 | 111 | 64 |

*m* months, *y* years

Molecular analysis from the 12 cases revealed that they all were heterozygous for the R264H mutation, and no other mutation was identified in the *MAT1A* gene. The sequencing of *MAT1A* exon seven in all patients and their parents confirmed the association of mutation R264H and the 870G (rs10887711) and 882C (rs10788546) *MAT1A* allelic variants, as previously reported (Chamberlin et al. 1997). The parents of the index cases were studied and, as expected, one in each family was found to be heterozygous for the R264H mutation. Parents' plasma methionine concentration was also measured (Table 2), and except for the mother of patients 2 and 3, with normal plasma methionine, and the mother of patient 12, for whom it was not possible to measure the methionine, all other parents with R264H mutation had moderately elevated methionine concentrations. Except for families of patients 1, 4, and 11, the parents' methionine concentration was lower than that of their children on confirmation. For some of the parents, the plasma total homocysteine levels were also analyzed and found to be mildly increased. In several families, methionine was quantified by MS/MS not only in parents but also in other relatives and those with elevated methionine levels were all confirmed to be R264H carriers (Table 2). Except for the father of patient 10, all the other relatives found to be R264H heterozygotes are clinically well.

**Table 2** Characterization of MAT I/III deficient patients' relatives

| Family | Patient | Affected relatives | Methionine (normal < 50 μM) | Homocysteine (normal < 15.0 μM) |
|---|---|---|---|---|
| 1 | 1 | Mother | 114 | 15.5 |
| 2 | 2, 3 | Mother | 32 | 21.9 |
| 3 | 4 | Father | 119 | 54.5 |
| 4 | 5 | Mother | 146 | 13.9 |
| 5 | 6 | Mother | 111 | *ND* |
| | | Maternal aunt | 65 | *ND* |
| | | Maternal uncle | 112 | *ND* |
| | | Maternal grandmother | 92 | *ND* |
| 6 | 7 | Mother | 57 | *ND* |
| 7 | 8 | Father | 68 | *ND* |
| | | Brother | 102 | *ND* |
| 8 | 9 | Mother | 79 | 26 |
| 9 | 10 | Father | 65 | 28 |
| 10 | 11 | Father | 187 | *ND* |
| 11 | 12 | Mother | *ND* | 30.5 |

*ND* not determined

These 12 cases (ages between 1 and 6 years old) are under biochemical and clinical follow-up in Oporto Hospital Center and until now all have normal growth, development, and neurological examination. Cerebral MRI was performed in the six index cases aged more than 2 years, and only in patient 6 myelination abnormalities were observed. No other heterozygotes in this family have neurological signs or symptoms suggestive of myelination abnormalities.

In families 1 and 4 (from patients 1 and 5), there was a history of severe vascular disease. In these families, the mothers both carried the MAT I/III mutation R264H, and both maternal grandfathers died, at ages 40 and 44 years, respectively, of thromboembolic events without any other risk factors being identified. One of these grandfathers

(family 1) did carry the mutation, but for the other no mutation analysis was possible. The father of patient 10 also had a myocardial infarction at a young age, but in this family a history of mild hypercholesterolemia also exists.

## Discussion

Screening programs must be adapted to the ethnic and genetic background, customs, social characteristics, medical environment, and economic status of a country. Based on our previous experience in the diagnosis of metabolic disorders in Portugal, our program includes some diseases which are prevalent in our population, namely arginase deficiency, 3-hydroxy-3-methylglutarylCoA lyase deficiency, and cystathionine β-synthase deficiency. Ten cases of this later disease had been previously diagnosed in our center, and because of that, methionine measurement was integrated into our MS/MS screening approach. Unexpectedly, during these 6 years of MS/MS screening, only one case of cystathionine β-synthase deficiency was found and the predominant cause of hypermethioninemia in Portuguese newborns was revealed to be MAT I/III deficiency (20 out of 21 cases), although no cases were previously reported in our country.

Most reports of this condition are recent since they are subsequent to the introduction of the MS/MS technology in newborn-screening programs. Its true incidence worldwide is not known, but it is surely under diagnosed (Baric 2009). The frequency found in our population (1:26,000) is similar to the one reported in Galicia (Couce et al. 2008), which may indicate a similar frequency in the whole Iberian Peninsula.

The molecular study of *MAT1A* gene allowed the confirmation of all suspected MAT I/III deficient cases. Twelve of these cases originate from a small area of Douro high lands in the northern region of Portugal (Fig. 1), and all of them were revealed to be heterozygotes for R264H mutation. Despite the relatively high frequency in the general population of 870G and 882C *MAT1A* allelic variants (58% for both forms in a population of European ancestry, according to the International HapMap Project information), the fact that this mutation was found in all individuals associated with these variants may indicate a single origin for this allele. At least 37 mutations have so far been reported in *MAT1A* gene (Mudd 2011), R264H exceptionally behaves as a dominant mutation and causes relatively mild hypermethioninemia, even in heterozygotes. This behavior is explained by the fact that the R264H mutated subunit can form inactive dimers with the normal subunit (Chamberlin et al. 2000; Pérez Mato et al. 2001). R264H heterozygosity seems to be relatively frequent among the patients identified by screening for methionine (Chien et al. 2005; Couce et al. 2008), and it is usually considered to be clinically benign (Pérez Mato et al. 2001). The complete lack of MAT I/III activity can represent a risk for development of brain demyelination, but some residual activity seems to be sufficient to maintain clinical well-being (Chamberlin et al. 1996). R264H heterozygotes' parents and grandparents were found to be generally healthy, which is in accordance with the residual enzyme activity reported to be associated with this mutation (Chamberlin et al. 1997) and which is reflected in the moderately high methionine levels previously reported for these individuals (Couce et al. 2008) and confirmed in this study. Several MAT I/III deficient patients have been reported who presented with CNS problems, and although most of them had high plasma methionine levels, certainly indicating severe MAT I/III deficiencies, genotype/phenotype correlations to predict the development of CNS involvement are still not identified (Mudd 2011). Myelination alterations do not seem to be directly related to methionine levels (Chamberlin et al 1996) and can occur before changes in neurological examination and so, besides the regular clinical and biochemical monitoring, and in spite of normal growth, development, and neurological examination, cerebral MRI was performed in all children over 2 years old. Before 18 months of age, due to a high degree of immaturity in myelination, observed alterations are difficult to evaluate.

The finding of a 3-year-old girl with myelination abnormalities (patient 6) confirms that care must be taken when assuming that none of the R264H heterozygote


**Fig. 1** Hot spot for MAT I/III deficiency due to R264H mutation in a small area of Douro high lands

individuals will develop clinical signs. One possibility is that the observed alterations do not result from the elevated methionine but from AdoMet depletion, and to confirm this possibility we intend to perform AdoMet quantification. In spite of the few experiences reported, and depending on the individual AdoMet levels observed, AdoMet supplementation will be considered for all the patients, and especially for the case presenting brain MRI abnormalities.

Abnormal elevations of plasma total homocysteine have been reported among more severely affected MAT I/III deficient patients with the most markedly elevated levels of plasma methionine (Stabler et al. 2002; Linnebank et al. 2005) and might possibly increase the long-term risk for strokes (Linnebank et al. 2005). Despite the moderately increased levels of methionine, all our cases have increased levels of total homocysteine, and we found two positive family histories for severe vascular disease episodes without other risk factors (families 1 and 4). This fact may indicate that also in cases presenting with moderately increased plasma methionine and total homocysteine levels, as are usually the cases associated with R264H mutation, the risk for vascular diseases may exist. Mild hyperhomocysteinemia as a risk factor for cardiovascular disease is a possibility that, although not yet definitely proved, should still be kept in mind (Smulders and Blom 2011). All the families are informed regarding the possible risks associated with R264H heterozygosity. The *MAT1A* gene study is offered to all the possible heterozygotes, which are then advised to do careful regular clinical monitoring.

Since exceptionally high methionine levels were never observed in our patients, and a low methionine diet can aggravate AdoMet deficiency and contribute to neurological abnormalities (Chien et al. 2005), for the pediatric cases only regular clinical monitoring and periodical control of methionine and homocysteine levels are being undertaken.

## Conclusion

The neonatal screening community and health care policy makers have to balance the pros and cons of integrating some diseases into newborn screening panels. However, everything that can improve the quality of life of our children and contribute to the elucidation of the families should be considered.

The unexpected findings of such a high frequency for MAT I/III deficiency, and of an hot spot for R264H mutation in a small region from the north of the country, brought us new insights into this condition in our country. A similar high frequency for MAT I/III deficiency due to R264H was only reported in Galicia, which is not surprising due to the close historical origins of populations from Galicia and from the north of Portugal. The long-term benefit of the screening of this condition will be evaluated thoroughly.

## Synopsis

High frequency of isolated hypermethioninemia detected in Portuguese newborn screening due to a dominantly inherited MAT I/III deficiency form associated with R264H mutation.

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