3-Methylcrotonylglycinuria: a new common mutation in the Portuguese population?

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INTRODUCTION

3-Methylcrotonylglycinuria (MCG) is an inborn error of the leucine catabolism resulting from isolated biotin-insensitive deficiency of 3-methylcrotonyl-CoA carboxylase (3-MCC), the enzyme converting 3-methylcrotonoyl-CoA to 3-methylglutaconyl-CoA (1).

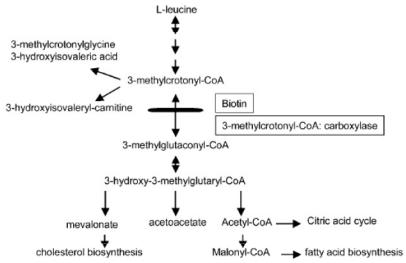

Figure 1: The L-Leucine catabolic pathway and the site of the defect in 3-methylcrotonyl-CoA carboxylase deficiency (MCC).

The metabolic phenotype characterizing MCC deficiency is the elevated excretion of the diagnostic compounds 3-methylcrotonylglycine and 3-hydroxyisovaleric acid, and the presence of abnormally elevated blood levels of 3-hydroxyisovalerylcarnitine (C5-OH), as determined by tandem mass spectrometry (MS/MS).

Expanded newborn screening for inborn errors of metabolism using MS/MS has demonstrated that 3-MCC deficiency is one of the most commonly detected inherited organic acidurias.

Patients with MCG, which is inherited as an autosomal recessive trait, show a highly variable clinical phenotype, ranging from asymptomatic to severe presentation. In the latter phenotype, episodes of acute metabolic decompensation can lead to coma, lethargy, and death (2).

PATIENT AND METHODS

The authors present six cases in a universe of thirty patients with an increase of C5-OH in the acylcarnitine profile.

Blood spot samples from newborns are collected between day 3 and 6 in Watman 903 filter paper. Acylcarnitines in samples are analysed by MS/MS (3). Molecular characterization of genes *MCCA* and *MCCB* that encodes the enzyme 3-MCC were studied by reported methods.

RESULTS

Table 1 presents the molecular results and C5-OH value obtained from the Guthrie card of the six cases.

Cases	C5-OH (N<1µM)	Nucleotide change	Predicted consequence	Reference
1	4,2	c.688A>G; c.641G>C	p.N230D/p.G214A	Not reported; rs277995
2	1	c.203G>T; c.688A>G	p.G68V/p.N230D	Not reported
3	3	c.688A>G; c.688A>G	p.N230D/p.N230D	Not reported
4	4,7	c.688A>G; c.641G>C	p.N230D/p.G214A	Not reported; rs277995
5	17	c.688A>G; c.1178A>C	p.N230D/p.Q393P	Not reported
6	3,9	c.688A>G; c.688A>G	p.N230D/p.N230D	Not reported

The six cases showed the same novel mutation p.N230D in the *MCCB* gene, proving that this is the most common new mutation in our population.

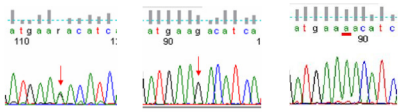

Figure 1- Mutation c.688A>G
Partial representations of the sequence evidence where the mutation c.688A> G heterozygosity in cases 1.2, 4 and 5 and cases 3 and 6 where it is present in homozygosity, compared with a normal control.

According to studies conducted to this new mutation using bioinformatic applications, it is considered benign, indicating that it is unlikely that protein structure or function are affected.

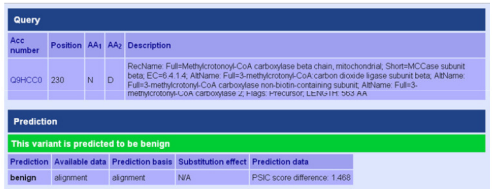

Figure 2 - Prediction of pathogenicity of the change c.688A> G MCCB gene (Polyphen).

The sequences of residues covering the codon changes were compared with other species, revealing that the residues in question are preserved in some species. The residue mutant, aspartic acid, is never present in homologous proteins.

Figure 3 - Alignment between species portion of protein sequence of the gene MCCB. Arrow specifies the site of the occurrence of replacement.

As this mutation creates a cutting site for the enzyme MboII, it was possible to study population for this change in 200 control alleles (100 control subjects). The fact that the change was not present in this sample suggests that this change has a low frequency in this population and a causal nature.

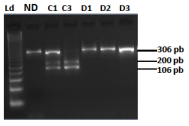

Figure 4 - Analysis of restriction of the PCR product of exon 7 in case 1 and 3 with the restriction enzyme MboII. The mutation creates a place of cutting which is demonstrated by the presence of two fragments (200 and 106 bp) in case 3 (C3-homozygous for the mutation) and three fragments (306, 200 and 106 bp) in case 1 (C1 -heterozygous for the mutation); ND - undigested control, D1, D2, D3 - digested controls.

DISCUSSION

Of the thirty MCC cases studied, p.N230D mutation revealed to be the most frequent new mutation. Bioinformatic analysis showed that this mutation is located in a non conserved area but the mutant residue was never present in the homologous proteins analyzed.

We can conclude that the alignment of the species and the population study conducted, showed that this mutation is responsible for the biochemical phenotype found in these cases.

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