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PAH mutational spectrum: still expanding

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ABSTRACT

Phenylketonuria (PKU, MIM 261600) is the most common inborn error of amino acid metabolism. To date, a total of more than 500 mutations have been associated with the disease. In this report, the novel p.Glu182Lys mutation, found in a Portuguese family in combination with the previously reported p.Leu 348Val, is presented and its putative deleterious impact discussed.

Keywords: Phenylketonuria (PKU); Phenylalanine Hydroxylase; *PAH* Gene; Novel Mutation

1. INTRODUCTION

Phenylketonuria is the most common inborn error of amino acid metabolism, occurring in approximately 1 in 10,000 births among Caucasians [1]. It is caused by the impairment of the hepatic enzyme phenylalanine hydroxylase (*PAH*, EC 1.14.16.1) and inherited as a Mendelian autosomal recessive disease. Failure to convert phenylalanine to tyrosine leads to an increase of phenylalanine in body fluids which is responsible for growth failure, seizures, microcephaly, mental retardation and intellectual impairment [2]. As result of the neonatal screening programs, affected patients are identified early and phenylalanine-poor diets implemented to prevent the appearance of PKU-related symptoms.

At the genetic level, PKU is caused by mutations in *PAH* gene located on q22 - q24.1 region of chromosome 12 [3]. To date, a total of more than 500 mutations are listed in public databases [4,5]. The distribution and relative frequencies of the *PAH* gene mutations have been reported in various populations and current data reveal a large degree of heterogeneity in the *PAH* mutational spectrum [6-13]. Still, there are some mutations that occur more frequently associated with specific populations as is the case of p.Arg270Lys, p.Val388Met, p.Phe410Cys and IVS11 + 5 G > A, whose origin is frequently associated with the Iberian population. The most recent case refers to the p.Phe410Cys which was firstly detected in a Portuguese family [14] and thereafter also found in a Brazilian patient [15]. Because no haplotypic data were available, conclusive data on a common origin is not possible, although this hypothesis seems very likely taking into account the Brazilian population history.

Herein, we report a novel mutation detected in the *PAH* gene in a family of Portuguese ancestry. To evaluate the genotype-phenotype association we have combined data on the nature of amino-acid replacement and the conservation degree of the residue in non-human *PAH* homologues, which allowed us to predict the deleterious effect of the novel p.Glu182Lys replacement.

2. MATERIAL AND METHODS

2.1. Patient Screening

Until the end of 2010, 3.072.774 newborns were screened in National Neonatal Screening Program. Among those 280 were assigned as PKU patients. After obtained informed consent, 160 PKU patients were further studied at the molecular level. All the mutations found were previously identified and recorded in the literature, with the exception of the replacement here discussed.

The patient reported here is a female that was born in 1990 from non-consanguineous parents. At day seven of life, a blood sample was collected and the phenylalanine level was determined (600 µmol/L). The patient started a phenylalanine-poor diet protocol in order to avoid PKU symptoms.

2.2. Molecular Analyses

Genomic DNA was automatically extracted from dried blood spots (EZ1 DNA Tissue kit, QIAGEN). Polymerase chain reaction (PCR) amplification of the *PAH* exons using flanking intronic primers were performed

followed by automatic sequencing in an ABI PRISM 3130XL Genetic Analyser (Applied Biosystems). Primer sequences are detailed in **Table 1**.

PCR was performed as follows: initial denaturation (95˚C, 10 min); 30 cycles of denaturation (95˚C, 1 min), annealing (60˚C, 1 min) and extension (72˚C, 1 min), and a final extension at 72˚C for 5 min. All primers were designed with M13 F/R tail labeled. The sequence of M13F tail is 5'TGTAAAACGACGGCCAGT3' and the M13R tail is 5'CAGGAAACAGCTATGACC3'.

Amplified products were purified with ExoSAP-IT (USB Corporation, Ohio, USA) by incubation at 37˚C for 15 min and followed by enzyme inactivation for 15 min at 85˚C. The sequencing reaction was performed with BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) using the M13F and the M13R primers. Sequencing reaction conditions were the following: initial denaturation at 94˚C for 2 min followed by 25 cycles of denaturation (94˚C, 10 sec), annealing (50˚C, 10 sec) and extension (60˚C, 4 min). The fragments resulting from sequencing reaction were purified with DyeEx 96 kit (QIAGEN) and sequenced in an ABI3130 XL automated sequencer.

Table 1. Primers sequences and PCR conditions for the amplification of *PAH* gene.

Exon	Sequence (5'-3')	Amplicon size (bp)
1	F-CCTCCTGCGTCAGGACAAC R-AAAGCCACCGAGGACAGAT	454
2	F-TGATCATTTAATTGCCCTGGA R-TGGAAAAACTGAAGCTCAGA	490
3	F-TCTCCATTTTGTTGCGTTAGG R-AATCCCCCAAACAGTCTTCC	470
4	F-CCGGTTTCTAAGGAAAATG R-CCAGCCCTCGTGTAAATAG	407
5	F-GCCCCCATTCAAAGCATT R-CCATCCTCAACTGGATGA	233
6	F-TGAAATTCACTGTAGCAAGT R-TTCCTGGAGGAATCAACCTG	466
7	F-TGGGAATTTACTTGATCCAGAT R-GATGGAGCAAATCTCCAGAA	492
8	F-GCCTTTTATGATCCCAACC R-ACCACACACCCATTTCAGGT	383
9*	F-TTCTATAACATATGGGCA R-TGTGCAAATGTAACCCACCA	357
10	F-TCATCCCAGTCAAGGTGA R-ACTGGAGAATGAGTTCCC	297
11	F-GCATTTGGGCTGTGATGTAG R-GTGTGTGCAAAGGTCAGCAT	462
12	F-GCTGTTGAAGACCCTGCTCTA R-TGGAGTGGAATCTAGGAAGG	468
13	F-TGAGGCTGTAAGCTCCTTGAA R-CTTGAATGAAGCAGGTCCC	507

*Annealing temperature: 55˚C.

2.3. Interspecies Comparative Analysis

Sequences from vertebrate *PAH* homologs were obtained in the Ensembl database [16] and aligned in Muscle [17] running in the Geneious v5.0.4 software [18].

3. RESULTS AND DISCUSSION

Here we present the molecular diagnostic screening of a female PKU patient who revealed an increased value of phenylalanine level at birth during the routine newborn screening. The analysis was based on a PCR-based strategy that resulted in the amplification of all exons of the *PAH* gene. A novel p.Glu182Lys replacement was found in heterozygozity with p.Leu348Val and both are located in the catalytic domain of the protein. Although this mutation is not yet documented, a different replacement (p.Glu182Gly) was previously found in the same residue [19] reinforcing the hypothesis of a putative deleterious impact when the wild-type Glu182 is replaced by a differently charged residue. Although the change involves the same residue, in p.Glu182Lys the first nucleotide of 182 codon is affected (c.544 G > A), while in previously described p.Glu182Gly is the second position (c.545 A > G). When the novel p.Glu182Lys was analyzed in Polyphen and SIFT [20,21], a deleterious effect was predicted in both programs. In agreement, the conservation degree analysis across vertebrates (**Figure 1**) shows that although the residue 182 is not fully

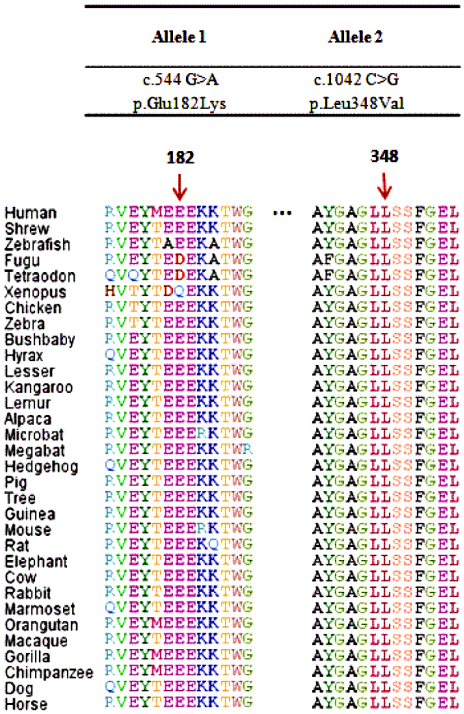

Figure 1. Comparative analysis record showing *PAH* residues 182 and 348 in vertebrate species.

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conserved across the entire vertebrate group (in fishes and amphibians Asp and Gln are wild-type alleles) the site is never occupied by a positive charged residue in either homolog. The second mutation (p.Leu348Val) lies in an invariant residue across the entire lineage. Altogether, molecular findings in combination with the biochemical PKU associated phenotype strongly support a deleterious effect of the novel p.Glu182Lys replacement.

4. CONCLUSIONS

In the era of genome-wide studies, reporting novel mutations in highly variable genes remains of extreme importance. Such information is expected to provide a better understanding of the mutational spectrum of the corresponding gene and also be useful when recurrent occurrence of the mutation allows phenotype comparisons among affected individuals. However, mutation identification alone is often not enough to allow predictions on the severity of observed phenotypes, that is, even for simple Mendelian disorders, such as PKU, genotype-phenotype correlations may be complex to infer. In fact, phenotypes are not only the result of the causal mutations but also environment factors and epistatic interactions between distinct alleles [22,23]. In this sense, other variants linked in *cis* to the mutation site may influence and even explain frequently observed inconsistencies in the genotype-phenotype relationship [24]. Phenylketonuria can be considered such a case. Thus far, more than 500 mutations are documented in the *PAH* gene. For over two years no novel mutations have been reported which may indicate that the large mutational spectrum of the *PAH* gene is reaching a limit. The novel mutation herein reported is contributing to enlarge the group of detectable mutations in the *PAH* gene.

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