

*MUTATION IN BRIEF*

# Ten Novel Mutations in the Phenylalanine Hydroxylase Gene (PAH) Observed in Brazilian Patients With Phenylketonuria

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**In the present study we report on the identification of ten novel mutations in the phenylalanine hydroxylase (PAH) gene of Brazilian patients with phenylketonuria (PKU): IVS5-54A>G, IVS6+17G>T, E205A, F240S, K274E, I318T, L321L, C357G, IVS11+17G>A and S411X. These mutations were detected during the characterization of the PAH genotypes of 115 patients with PKU from the southeast region of Brazil. The results obtained confirm the high heterogeneity of the PAH gene and provide information about the distribution of PKU mutations in the Brazilian population. © 2000 Wiley-Liss, Inc.**

KEY WORDS: Phenylalanine hydroxylase; PAH; phenylketonuria; PKU; Brazil

## INTRODUCTION

Phenylketonuria (PKU) is the most prevalent disorder of amino acid metabolism, with frequency of 1 in 10,000 Caucasians, and is caused by a defect in the hepatic enzyme phenylalanine hydroxylase (PAH; MIM# 261600), which converts phenylalanine to tyrosine (Scriver et al., 1995). PKU is highly heterogeneous at the molecular level. To date more than 400 different mutations in the PAH gene (Nowacki et al., 1998; Scriver et al., 2000) have been described associated with different geographical and ethnic groups.

In the present study we report on the identification of ten novel mutations in Brazilian patients with hyperphenylalaninemia using PCR, DGGE analysis and direct sequencing of PCR products, including both disease-associated mutations and single nucleotide polymorphisms.

## PATIENTS AND METHODS

### Patients

A total of 115 PKU families (292 individuals, 126 of them being patients and 166 relatives) mainly from the southeast region of Brazil were investigated. The patients were identified by a neonatal screening program and were considered to have PKU when the phenylalanine levels were above 4 mg/dl.

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## Methods

DNA from each individual was extracted from peripheral blood leukocytes by standard techniques (Kunkel et al., 1977) and screened for mutations by PCR amplification of the entire PAH gene, including all 13 exons, splice regions and promoter region, using DGGE, SSCP and sequence analysis. To evaluate the prevalence of single nucleotide polymorphisms (L321L, N426N and IVS11n+17G>A) we performed a population study on 50 Caucasian, 40 Asian and 50 African-American individuals. For the disease-associated mutations we tested a control sample of 50 individuals without PKU. Haplotypes and VNTRs were determined as described by Goltsov et al. (1992).

## RESULTS AND DISCUSSION

In this study we detected ten novel mutations including both disease-associated mutations and single nucleotide polymorphisms: IVS5-54A>G, IVS6+17G>T, E205A, F240S, K274E, I318T, L321L, C357G, IVS11+17G>A and S411X (Table 1).

The novel polymorphism IVS5-54A/G (c.510-54A>G) was observed in PKU patients from three independent families. The disease was identified during neonatal diagnosis and the patients received diet therapy promptly, and are asymptomatic. In all families, IVS5-54A/G (frequency of 8 chromosomes) occurred in *cis* with two other reported polymorphisms of the PAH gene: Q232Q, localized in exon 6, and V245V localized in exon 7, and a well known substitution - R176L – localized in exon 6. IVS5-54A/G was in homozygous state in the patient of family 2 and in the patient's mother of family 3. IVS6+17G/T (c.706+17G>T) was observed only in the patient's mother of family 1. The novel polymorphisms (IVS5-54A/G and IVS6+17G/T) present the 4.3 haplotype due the other known associated substitutions. Although we did not perform a population study we can assume that these mutations are polymorphisms because all the patients of all families have two other well known substitutions in the PAH gene that cause PKU, and the patient's mother of family 3 does not have PKU, in spite of having the IVS5 in homozygous state.

**Table 1: Novel mutations identified in PKU Brazilian patients**

Intron/ Exon	Nucleotide change	Amino acid change	Mutation type	Haplotype association	Clinical phenotype	Homologous allele
Intron 5	510-54A>G	-	substitution/ polymorphism	4.3	mild PKU family 1 classic PKU families 2, 3	R408W families 1, 3 R252W; L385L; Q232Q family 2
Intron 6	706+17G>T	-	substitution/ polymorphism	4.3	patient's mother, family 1	IVS5-54A>G; V245V; R176L; Q232Q
Exon 6	614A>C	E205A	missense	4.3	moderate PKU	R252W; Q232Q
Exon 7	719T>C	F240S	missense	6.9	classic PKU	R261Q
Exon 7	820A>G	K274E	missense/ polymorphism	15.9 or 37.9 or 80.9	*classic PKU	R252W; V245V
Exon 9	953T>C	I318T	missense	15.9 or 37.9 or 80.9	*classic PKU	R252W; V245V
Exon 9	963C>T	L321L	silent/ polymorphism	15.9 or 37.9 or 80.9	*classic PKU	R252W; V245V
Exon 11	1069T>G	C357G	missense	4.3	classic PKU	R176X
Intron 11	1199+17G>A	-	substitution/ polymorphism	7.8	moderate PKU	R261Q
Exon 12	1232C>G	S411X	nonsense	15.9 or 37.9	classic PKU	V388M

\* Same patient

In one family a PKU patient (sporadic case) presented the E205A mutation, which was a 614A>C substitution in the PAH gene in exon 6 at amino acid 205, resulting in a missense mutation. The patient had a neonatal diagnosis, with phenylalanine level indicating moderate PKU, and received diet therapy soon after, being currently asymptomatic. This mutation was not observed in the 50 normal subjects tested. This mutation was inherited from her father and occurred in *cis* with a N426N polymorphism (Aulehla-Scholz, 1999). A population analysis

suggests that this polymorphism is limited to black populations, with a frequency of 13% (13/100 chromosomes), and was not found in a control population of Caucasians (0/100) and Asians (0/80). The 4.3 haplotype was linked with the E205A mutation and the N426N polymorphism.

The 719T>C substitution in exon 7 of the PAH gene at amino acid 240 results in a missense mutation. The F240S mutation creates a restriction site for the *Nla*IV enzyme, and the RFLP study of 50 normal individuals did not reveal any sample with this mutation. This substitution was found in a PKU patient (sporadic case) and her mother. One reported IVS2+19T>C polymorphism occurred in *cis* to the F240S mutation, as the mother had these substitutions in the same status. The presence of this polymorphism was important to help the definition of the linked haplotype, since according to Lichter-Konecki et al. (1994) some polymorphisms confirm with high probability the linked haplotype, which in this case was 6.9. The patient is asymptomatic due to early diagnosis and diet therapy started at the age of 3 months.

The substitutions K274E (c.820A>G), L321L (c.963C>T) and I318T (c.953T>C) were detected in only one PKU family transmitted in *cis* (Gjetting et al., *in press*), probably associated with the 15.9, 37.9 or 80.9 haplotype. A population study of the polymorphism L321L showed a frequency of 8% (8/100 chromosomes) in a black population.. The K274E is an amino acid polymorphism, and the substitution I318T is a disease-causing mutation (Gjetting et al., *in press*).

The 1069T>G substitution in the PAH gene in exon 11 at amino acid 357 results in a missense mutation. This transversion abolishes a restriction site for the *Sca*I enzyme. The normal individuals tested did not reveal this mutation. This mutation was found in a PKU patient (sporadic case) and his father. Two reported V245V and Q232Q polymorphisms occurred in *cis* to the C357G mutation, as the father had these substitutions in homozygous state. The presence of these polymorphisms was important to define the associated haplotype, which in this case was 4.3 (Lichter-Konecki et al., 1994). The patient has mild mental retardation due to a late diagnosis, with diet therapy started after the age of 10 years.

The 1232C>G substitution in the PAH gene interrupts the reading frame in exon 12 at amino acid 411, resulting in a stop codon. Two possible haplotypes, 15.9 or 37.9, can be associated with S411X, with the need to study the *Eco*RI and *Eco*RV restriction sites to define the correct haplotype. The substitution was found in a PKU patient (sporadic case) and her mother. A reported IVS2nt19t/c polymorphism occurred in *cis* to the S411X mutation. The patient has been receiving diet therapy since the neonatal diagnosis and is asymptomatic. The normal individuals tested for this mutation did not present it.

The c.1199+17G>A substitution in intron 11 of the PAH gene results in a mutation. IVS11+17G>A abolishes a restriction site for the *Hph*I enzyme. This substitution was found in a PKU patient (sporadic case) and her mother. Two reported L385L and Q232Q polymorphisms occurred in *cis* to the IVS11+17G>A mutation, as the mother had these polymorphisms (Q232Q and L385L) in homozygous state. The presence of these polymorphisms was important to help the definition of the linked haplotype, which in this case was 7.8 (Lichter-Konecki et al., 1994). The patient is asymptomatic due to early diagnosis and diet therapy started at the age of 2 months. In the population study with Caucasians, Asians and African-Americans, this mutation was not detected.

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