

Mutation analysis in Turkish phenylketonuria patients

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Abstract

Forty-four classical PKU patients have been screened for various mutations. The newly identified IVS 10 splicing mutation was found in 32% of the mutant alleles and comprises 74.5% of the mutations that could be typed: 261^{arg-gln} (6.8%), 158^{arg-sly} (2.3%), 252^{arg-trp} (1.1%), 280^{glu-lys} (—), and 272^{gly-stop} (—) were the other mutations that were screened.

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Phenylketonuria (PKU), which results from a deficiency of hepatic enzyme phenylalanine hydroxylase (PAH), is a common inborn error of metabolism. The average incidence of PKU is 1/10 000 in Caucasians.^{1,2} The frequency of PKU in Turkey, based on the results of a newborn screening programme in effect since 1983, is 1/4370.³ A high rate of consanguineous marriages (21.06%) in Turkey may be a contributing factor to this high incidence.⁴

Molecular genetic studies of the PAH gene began as a result of the cloning of the cDNA.⁵ The majority of PKU chromosomes are associated with haplotypes 1-4 as determined by restriction fragment length polymorphisms (RFLPs) created by seven restriction endonucleases.^{6,7}

A previous study of Turkish PKU alleles showed that about 40% of the mutant alleles are associated with haplotype 6, a rare haplotype in northern European populations.⁸ Various mutations associated with specific RFLP haplotypes have been determined.⁹

Methods

We have investigated 44 patients classified as having classical PKU on the basis of initial plasma phenylalanine concentrations and positive urine ferric chloride test for the following mutations: 280^{glu-lys}, 261^{arg-gln}, 252^{arg-trp}, 158^{arg-gln}, 272^{gly-stop}, and the recently published splicing defect in intron 10 which creates a *DdeI* restriction enzyme site.¹⁰⁻¹²

PREPARATION OF GENOMIC DNA SAMPLES

Peripheral blood was withdrawn in 13 mmol/l EDTA and leucocytes were separated by centrifugation and lysed in 10 mmol/l Tris, 10 mmol/l EDTA, 50 mmol/l sodium chloride, and 0.2% sodium dodecyl sulphate in the presence of proteinase K (200 µg/ml) overnight, followed by repeated phenol-chloroform extractions. DNA was then precipitated by ethanol and resuspended in 0.1 ml TE (10 mmol/l Tris-HCl, 0.1 mmol/l EDTA, pH 7.0).¹³

PREPARATION OF POLYMERASE CHAIN REACTION

The oligonucleotide primers (50 pmol/l of each) were used with genomic DNA (1 to 0.5 µg) in 1 × *Taq* DNA polymerase buffer (25 mmol/l Tris-HCl, pH 9.5, 50 mmol/l KCl, 10 mmol/l MgCl₂, 1 mmol/l dithiothreitol, 1 mg/ml bovine serum albumin, 300 µg/ml activated calf thymus DNA), 0.2 mmol/l each deoxynucleotide (dATP, dGTP, dTTP, dCTP), and 2.5 U *Taq* DNA Polymerase (Amersham) in a total volume of 0.1 ml reaction mixture (table 1).¹⁴

Results and discussion

Table 2 shows the distribution of various mutations in the 88 PKU mutant alleles. Forty-two percent of the alleles could be identified for a particular mutation and the IVS 10 splicing mutation by itself comprised 75.7% of the mutations identified.

Of 44 patients 11 were homozygous for the IVS 10 splicing mutation, two were homozygous for the codon 261 mutation, and seven

Table 2 Distribution of mutations in the mutant PKU alleles (n=88). IVS 10 splice mutation comprises 75.5% of the mutations that could be determined.

Mutation	No of alleles	% of total alleles
IVS 10 splice	28	32
261	6	6.8
158	2	2.3
252	1	1.1
280	0	—
272	0	—
Total identified	37	42.2

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Table 1 Conditions for mutation analysis: PCR primers and detection methods (A), allele specific oligonucleotides and hybridisation temperatures (B).

A Primers	Mutation	Detection methods
Exon 7 5'AGA TGA CGC TCA GTG TGC CT 3' 5'ATC CCA GCT TGC ACT GGT TTC CGC C 3'	R252W R261Q G272ter E280K	ASO ASO RFLP/ <i>Bam</i> HI ASO
R158Q 5'CCA TCC TCA ACT GGA TGA GG 3' 5'TCC TCT GTA CCG TGC AAG CC 3'	R158Q	RFLP/ <i>Msp</i> I
IVS 10 5'TGC AGC AGG GAA TAC TGA TC 3' 5'TAG ACA TTG GAG TCC ACT CTC 3'	IVS 10	RFLP/ <i>Dde</i> I
B Mutation	Oligonucleotides	Hybridisation temp
R252W	5'GCT TTC CTC TCG GGA TTT CTT 3' 5'GCT TTC CTC TTG GGA TTT CTT 3'	57°C
R261Q	5'GTG GAA GAC TCG GAA CGC CAG 3' 5'GTG GAA GAC TTG GAA CGC CAG 3'	62°C
E280K	5'TAT ACC CCC GAA CCG TGA 3' 5'TAT ACC CCC AAA CCG TGA 3'	54°C

patients were genetic compounds of whom only one had both alleles identified.

The IVS 10 splicing mutation has been reported to be linked to haplotype 6 in various ethnic groups.¹⁵ We have also found that 11 of the 12 haplotype 6 alleles are associated with this mutation indicating tight linkage between haplotype 6 and the IVS 10 splicing mutation in our population. Moreover, we found two patients homozygous for the IVS 10 splicing mutation who carried haplotype 16, a rare haplotype (figure).

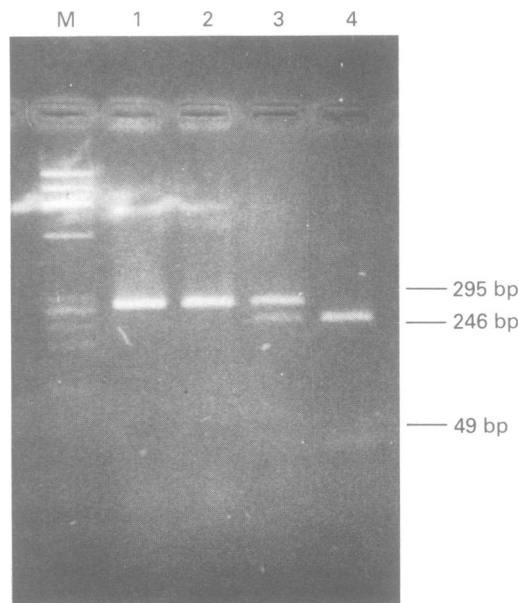
Interestingly, we have one patient with haplotype 2/5 who is a genetic compound of 261/IVS 10 splicing mutation. We also have one other patient of 2/12 haplotype background who is a genetic compound for 261/— mutations. Both patients' DNA have been checked previously and found not to carry the 408^{arg-trp} mutation which is tightly linked to haplotype 2 in northern European populations. The remaining four alleles bearing the codon 261 mutation are of haplotype 1 background. The codon 280 mutation reported to be found mostly in Mediterranean populations¹⁶ was not detected in any of the Turkish PKU alleles.

Since the detection of the IVS 10 splicing mutation can be done by a simple non-radioactive test, it is practical to screen our PKU

patients for this mutation. Currently over 600 classical PKU patients are being followed at the Department of Paediatric Metabolism. The low socioeconomic and educational level of the majority of the families, and the prohibitive cost of diet management for our population, are the main causes of demands for prenatal diagnosis. Thus, such direct testing is very valuable.

However, other mutations accounting for the remaining 60% of the mutant alleles have not been established yet and genotype analysis will still be done by RFLP analysis until more population specific mutations are determined.

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DdeI restriction digest results of amplified DNA from PKU patients. M = X174 *HaeIII* digest size marker DNA, 1 = PCR product, 2 = homozygous normal, 3 = heterozygote, 4 = homozygous PKU patient (carries *DdeI* site on both chromosomes).

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