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FREQUENCY OF THE IVS-10nt546 MUTATION IN 44 TURKISH PHENYLKETONURIA PATIENTS*

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SUMMARY: Özgüç M, Özalp İ, Coşkun T, Yılmaz E, Erdem H, Ayter Ş. (Departments of Medical Biology and Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey). Frequency of the IVS-10nt546 mutation in 44 Turkish phenylketonuria patients. Turk J Pediatr 35: 11-14, 1993.

The newly identified point mutation in intron 10 of the phenylalanine hydroxylase gene activates a cryptic splice site and results in an in-frame insertion of nine nucleotides between exons 10 and 11 of the processed transcript. This mutation is observed in association with haplotype 6 of phenylketonuria chromosomes. Since in Turkey, haplotype 6 is observed in 40 percent of mutant phenylketonuria alleles, the aim of this study was to establish the incidence of this particular mutation. Forty-four classical phenylketonuria patients were studied and the frequency of the intron 10 splicing mutation was determined to be 31 percent. *Key words: phenylketonuria, IVS-10nt546 mutation.*

Classical phenylketonuria (PKU) is an autosomal recessive disease which results from a deficiency of the hepatic enzyme phenylalanine hydroxylase (PAH). Since the isolation of the full-length cDNA for PAH[1], a multiple of different mutations have been reported in the PAH gene[2]. However, the mutant genotypes thus far reported account for only a fraction of all the mutant alleles and most of the mutations causing the disease, especially in the Mediterranean countries, remain unknown[3].

A particular pattern emerged from studies of PKU families in southern Europe, where haplotype 6 was found to be frequent[4]. In a previous study, it was found that 40 percent of the mutant Turkish PKU alleles are of haplotype 6 origin[4].

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Haplotype 6 is associated with the severe form of phenylalanine hydroxylase deficiency.

Recently a G to A transition in 3' of intron 10 of the phenylalanine hydroxylase gene was identified[5]. This mutation is in linkage with mutant RFLP (Restriction Fragment Length Polymorphism) haplotype 6 and is also seen on the rare haplotypes 10 and 36 in Bulgarians and Bulgarian Turks[6].

The mutation activates a cryptic splice site that leads to an in-frame insertion of nine nucleotides between exons 10 and 11 of the processed transcript. Moreover, this mutation creates a Dde I restriction endonuclease site that is easily detectable in a simple, nonradioactive assay based on PCR (Polymerase Chain Reaction) amplification and restriction analysis.

Since haplotype 6 is a common haplotype of mutant PKU chromosomes in Turkey, we chose to screen for the Dde I site mutation in Turkish PKU patients.

Material and Methods

Forty-four classical PKU patients followed at the Metabolism and Nutrition Unit of the Department of Pediatrics at Hacettepe Children's Hospital were studied for this mutation. Genomic DNA was extracted from peripheral blood samples by standard procedures[7]. One microgram of genomic DNA was amplified by using oligonucleotide primers (AP 255: 5' TGC AGC AGG GAA TAG TGA TC: 62-43 bp upstream of exon 11 and AP 264: 5' TAG ACA TTG GAG TCC ACT CTC: 79-99 bp downstream of exon 11 and 30 cycles were completed. One cycle of amplification consisted of denaturation (94 °C, 30 sec.) annealing (57 °C, 1 min.) and extension (72 °C, 1 min.).

PCR products were checked by 1.5% agarose gel electrophoresis and the amplified fragments were visualized with ethidium bromide under ultraviolet light. Ten microliters of the amplified product were digested by Dde I restriction enzyme (20 U of the enzyme incubated at 37 °C for a period of 16 hours). Restriction fragments were observed using ethidium bromide stained with 4% Nusieve agarose gel[8].

Results

When the PCR products are digested with Dde I enzyme, electrophoretically separable fragments of different lengths are produced which represent different alleles (Fig. 1).

The amplification results in a product of 295 bp. In the presence of the mutation, the 295 bp PCR product is cleaved into two fragments of 246 bp and 49 bp. Of the 44 PKU subjects, 11 (25%) were homozygous for this mutation and 6 (6.8%) were heterozygous. The total frequency was (28/88 alleles) 31 percent.

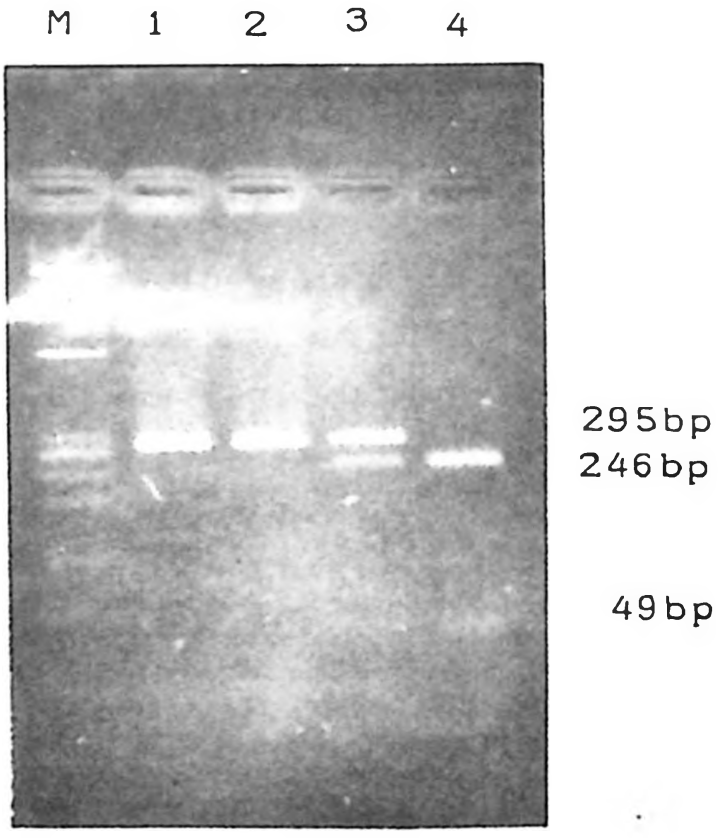

Fig. 1: Dde I restriction digestion results of amplified DNA from PKU patients. M: X174 Hae III digest size marker DNA, 1: PCR product, 2: homozygous normal, 3: heterozygous individual, 4: homozygous PKU patient (carries Dde I site on both chromosomes).

Discussion

The newly identified mutation in intron 10 of the phenylalanine hydroxylase gene activates a cryptic splice site and results in an in-frame insertion of 9 nucleotides between exons 10 and 11 of the processed transcript.

Molecular analysis in different European countries has revealed that the majority of PKU alleles are associated with a limited number of haplotypes. However, significant differences in the geographical distribution of particular haplotypes have been observed[9].

In this northern part of Europe, haplotype 3 accounts for a large proportion of PKU alleles, while it is nearly absent in Mediterranean populations[10]. However in the southern part of Europe, haplotype 6 is more dominant[4,11].

The splicing defect in intron 10 (IVS-10nt546) of the phenylalanine hydroxylase gene is strongly linked to haplotype 6 in different ethnic groups[6].

In addition, this mutation was present in PKU alleles of some rare haplotypes confined to specific populations (haplotype 10 in Bulgarians and haplotype 36 in Turks). These findings, together with the previously reported data on PKU haplotype distribution, suggest that in southern Europe, the splicing defect in

intron 10 of the phenylalanine hydroxylase gene is a common cause of PKU. The simple nonradioactive diagnostic test described here can be used for prenatal analysis and for carrier detection of PKU. Until more mutations specific to the Turkish population can be determined, haplotype 6 mutation can be used as an initial direct screening test.

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