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Short Communication – The PAH Gene

Frequency of 12 Mutations in 114 Children with Phenylketonuria in the Midwest Region of the USA

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Phenylalanine hydroxylase (PAH) is a liver-specific enzyme involved in the hydroxylation of phenylalanine (phe) to tyrosine. The deficiency of PAH results in excessive accumulation of phe in body fluids that leads to phenylketonuria (PKU). Among Caucasians, PKU affects 1 in 10000 live births and the carrier frequency of this inherited metabolic disorder is 1 in 50 (Scriver et al 1989). Clinically, this disorder is accompanied by neurological impairment and mental retardation of varying degree that seem to bear a relationship to the residual PAH activity in these patients (Okano et al 1991; Ramus et al 1993). Even though the mechanism of neurological involvement is not known at present, it has been observed that regulating the dietary phenylalanine intake of PKU patients will minimize their CNS involvement (Matalon and Michals 1991). The mass screening of newborns in the industrialized world has led to early identification and dietary intervention to modulate the outcome of PAH deficiency in such individuals. The clinical variation in the severity of PKU is believed to be determined by the genotype of the PKU alleles inherited by the affected individuals (Okano et al 1991; Ramus et al 1993).

Phenylalanine hydroxylase maps to 12q22-q24.1 in the human genome; and spans about 90 kb of genomic DNA with 13 exons and 12 introns of varying sizes (Kwok et al 1985; Lidsky et al 1985; DiLella et al 1986). The heterogeneity observed in PKU populations results from more than 60 mutations characterized so far that affect the expression of PAH gene (Eisensmith and Woo 1992). The nature of these mutations determines the level of expression and the residual PAH activity observed *in vitro* or *in vivo*. Higher levels of residual activity should lead to milder forms of PKU. The genetics of PKU is, however, complicated by the dimeric subunit nature of the PAH protein. Therefore, the phenotypic outcome of a mutation in a compound heterozygote will be determined by the effect of these mutations on the overall conformation of the ultimate PAH dimer formed. It would therefore be helpful to genotype the PKU

alleles so that dietary management and genetic counselling would be more meaningful to PKU patients.

The population in the USA is of diverse ethnic backgrounds with the majority being of European descent. Therefore, the frequency of PKU mutations observed in the USA would vary depending upon the ethnic origin of population under study. This study was undertaken to determine the frequency of 12 PKU mutations in the midwest region of the USA. The panel of 12 mutations studied has been found to be prevalent in the European population (Guldberg et al 1993).

MATERIALS AND METHODS

Patients: The PKU patients and their family members enrolled in this study were from Illinois, Michigan and Minnesota from the midwest region of the USA. Affected siblings of the PKU patients, with the same genotype, were not included in determining the frequency of each mutation. The PKU patients were diagnosed and classified according to the standard clinical criteria.

Mutation analysis: Dried blood spots were received from the participating clinics and were used as a source of DNA for polymerase chain reaction (PCR)-based amplification of specific regions of the PAH gene. PCR was carried out in 100 μl volume under standard conditions that included 30 cycles of denaturation at 94°C for 30 s, annealing at T_m −4°C, and extension at 72°C for 30 s. At the end of 30 cycles a final extension reaction was carried out at 72°C for 7 min. The amplified DNA fragments were denatured and blotted in duplicate on nitrocellulose membrane using slot-blot or dot-blot apparatus. The presence of specific PKU mutations was analysed by hybridization of duplicate blots to ^{32}P-labelled allele-specific oligonucleotides under stringent conditions. The hybridized blots were exposed to X-ray film and autoradiographed.

RESULTS AND DISCUSSION

Genotype analysis of PKU patients has resulted in identification of more than 60 mutations so far and new mutations are constantly being identified. Recently, Guldberg et al (1993) have reported identification of 99% of the mutations in 308 Danish PKU chromosomes. Four mutations, IVS-12nt 1 (g → a), arg408 → trp, tyr414 → cys and IVS-10 nt546 (gg → ag) accounted for 70% of all the PKU mutations in the Danish study. The homogeneous nature of the population thus facilitates mutation analysis. The results of mutation analysis in the midwest region of the USA are listed in Table 1. Analysis of 12 mutations in PKU patients revealed that we could detect 56.5% (129/228) of all the mutant chromosomes. In terms of determining both mutant alleles, 35% of the PKU patients were completely genotyped. About 9.4% of the patients were homozygous for arg408 → trp mutation, whereas others with arg408 → trp mutation on one allele were compound heterozygotes for another PKU mutation. The genotype observed in PKU patients was also tested in other family members when available.

The genotype analysis of PKU patients from the midwest region has offered a

Table 1 Frequency of 12 mutations[a] in 228 PKU chromosomes in the midwest region of the USA

Mutation	*No. of alleles*	*Frequency*	*Danish study*[b]
Arg408 → Trp	51	0.224	0.182
IVS10 G → A	29	0.127	0.052
IVS12 G → A	15	0.066	0.373
Tyr414 → Cys	11	0.048	0.101
Arg261 → Gln	10	0.044	0.016
Pro281 → Leu	5	0.021	0.013
Glu280 → Lys	3	0.013	0.029
Arg111 → Ter	2	0.008	0.0003
Gly272 → Ter	2	0.008	0.016
Ile94delATC	1	0.004	Not reported

[a]None of the alleles tested carried arg158 → gln or leu311 → pro mutation
[b]Guldberg et al (1993)

different experience from that involving mutation analysis in PKU subjects from across the US subcontinent (Kaul et al, unpublished data from maternal PKU collaborative study). Only 36% of the PKU chromosomes could be genotyped in the collaborative study compared to the 56.5% characterized in the present study. Our data suggest that the PKU mutation analysis in the USA population will be important but more difficult than the Danish experience. We are currently in the process of characterizing other mutations in PKU chromosomes that did not carry any of the 12 mutations. Such a study will be important in establishing the genotype–phenotype correlation in these patients.

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