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Mutations of the Phenylalanine Hydroxylase Gene in Iranian Azeri Turkish Patients with Phenylketonuria

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Aim: Phenylalanine hydroxylase (PAH) deficiency is caused by mutations in the *PAH* gene resulting in a primary deficiency of the PAH enzyme activity, intolerance to the dietary intake of phenylalanine (Phe), and production of the phenylketonuria disease. To date there have been no reports on the molecular analysis of phenylketonuria in the Iranian Azeri Turkish population. In this study, a total of 88 independent alleles from this ethnic group were investigated. *Results:* Thirteen different mutations have been detected, which account for 75% of the total mutant alleles. Two mutations were found at high frequencies: IVS10-11G>A (19.3%) and P281L (19.3%), possibly due to consanguinity and genetic drift, among other factors. The frequencies of the other mutations were c.590_612del (5.7%), R261Q (5.7%), R261X (4.5%), R243X (4.5%), IVS2+5G>C (3.4%), IVS4+1G>A (3.4%), R158Q (2.3%), E280K (2.3%), G247D (2.3%), IVS11+1G>C (1.1%), and R270K (1.1%). *Conclusions:* These intriguing preliminary findings confirm IVS10-11G>A as a major mutation among Mediterranean mutations. For this population, exons 7 and 11 and adjacent introns, which carry more than 75% of the mutations, would have to be primarily screened. However, the other exons must be studied when either one or no mutations are found in the primary screening. The mutation spectrum in the patients with Azeri Turkish ethnic origin differed from that observed in patients from other Mediterranean countries and further defined the molecular heterogeneity of this disease.

Introduction

Phenylketonuria (PKU) is the most prevalent disorder of amino acid metabolism, with a frequency of 1 in 10,000 Caucasians and 1 in 16,500 in Orientals, and is caused by a defect in the hepatic enzyme phenylalanine hydroxylase (PAH) (Scriver *et al*., 1995).

PAH catalyses the irreversible hydroxylation of phenylalanine to tyrosine and, when defective, leads to mental retardation because of the accumulation of phenylalanine. Low-phenylalanine dietary treatment prevents this effect if it is implemented at an early age, which has led to the establishment of neonatal screening programs worldwide. More than 500 disease-causing mutations in the *PAH* gene have so far been identified and are recorded in the *PAH* Mutation Analysis Consortium database (http://data.mch.mcgill.ca/pahdb_new/). Significant variation in the spectrum and prevalence rates of mutations in the *PAH* gene in different populations makes it important to define the PKU mutation profile in different distinct populations. Determination of mutation spectrum in Iranian Azeri Turkish patients may provide the design and early implementation of a patient-rational dietary regimen and facilitate genetic counseling in this ethnic group. Here, the molecular characterization of the *PAH* genes from Iranian Azeri Turkish PKU patients is reported.

Subjects and Methods

Patients

A total of 44 unrelated families with *PAH* deficiency with Azeri Turkish ethnic origin, corresponding to 88 independent alleles, were enrolled in this study. Phenotypes were defined on the basis of dietary phenylalanine tolerance and pretreatment blood phenylalanine levels (Güttler, 1980). Consanguinity among parents was proven in 30 (68%) cases.

Most samples reported in this study are from patients with classical PKU, which was detected after presentation of symptoms.

Mutation analysis

Each family was informed about the study and a written informed consent was obtained. Genomic DNA was extracted

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Table 1. Spectrum and Frequencies of Mutations in the Phenylalanine Hydroxylase Gene Identified in 88 Iranian Azeri Turkish Alleles

Mutation	*Exon/intron*	*Clinical severity (AV)*[a]	*Mutation type*	*Number of alleles*	*Frequency (%)*
IVS10-11G>A	I10	1	Splice	17	19.3
P281L	E7	1	Missense	17	19.3
c.590_612del	E6	1	Deletion	5	5.7
R261Q	E7	2	Missense	5	5.7
R261X	E7	1	Nonsense	4	4.5
R243X	E7	1	Nonsense	4	4.5
IVS2+5G>C	I2	1	Splice	3	3.4
IVS4+1G>A	I4		Splice	3	3.4
R158Q	E5	1	Missense	2	2.3
E280K	E7	1	Missense	2	2.3
G247D	E7		Missense	2	2.3
IVS11+1G>C	I11	1	Splice	1	1.1
R270K	E7	2	Missense	1	1.1
Number of alleles identified				66	75

[a]AV refers to the "assigned value" of individual mutations (Guldberg *et al*., 1998), differentiating between mutations causing severe PKU (AV = 1), moderate PKU (AV = 2), mild PKU (AV = 4), and mild hyperphenylalaninemia (MHP) (AV = 8), when associated with null mutations.

PKU, phenylketonuria.

from peripheral blood leukocytes according to standard protocols (Miller *et al*., 1988). Thirteen genomic fragments covering the entire coding sequence and the exon-flanking intron sequences of the *PAH* gene were amplified by polymerase chain reaction followed by single strand conformation polymorphism (SSCP) and subsequent direct sequence analysis (Santana da Silva *et al*., 2003). Some common mutations were confirmed by different methods such as restriction fragment length polymorphism and amplification refractory mutation system analysis if available (Eiken *et al*., 1991). The Mendelian inheritance was confirmed when possible by different methods and it was confirmed that all the mutations observed in the patients were inherited from their parents and the possibility of new mutations was excluded.

Results

Thirteen different mutations were detected on 66 of the 88 mutant alleles (diagnostic efficiency of 75%). These included six missense mutations (46%), four splice mutations (31%), two nonsense mutations (15%), and one deletion (8%) (Table 1). The two most common PKU mutations IVS10-11G>A and P281L accounted for more than 50% of mutant alleles. These results demonstrate that the PKU in this cohort is relatively homogeneous. Of the 44 unrelated families studied, 3 (6.8%) showed compound heterozygous mutant alleles, 26 (59.1%) were homozygotes (20 of them belonging to consanguineous families), 8 (18.2%) heterozygotes (only one mutant allele identified), and no PKU-causing mutations were detected in the remaining 7 families (16%). The following polymorphisms were observed in the *PAH* gene: V245V, IVS2+19 T>C, and L385L, with the frequencies of 6.8%, 5.7%, and 2.3%, respectively (Table 2). Genotypes of 44 PKU patients from this cohort are shown in Table 3.

Discussion

The 15–20 million Azeri Turks living in northwestern Iran, ethnically identical to Azeris and closely related to Turks, are believed to constitute 25% of the population. This is the first report about the mutation spectrum of the *PAH* gene in this ethnic group.

Analysis of the homozygosity of the mutations indicated that 59.1% (26/44) of the patients were homozygous and 25% (11/44) were heterozygous (8 heterozygotes and 3 compound heterozygotes) for the mutations that they carry. The high frequency of homozygosity, especially for rare mutations, can be explained by the high consanguinity (68%) and it is in agreement with what was found in other populations from Eastern and Central Europe (Pronina *et al*., 2003).

The most common Iranian Azeri Turkish mutations, IVS10-11G>A and P281L, account for more than 50% of the identified mutations. The relative predominance of IVS10-11G>A among this population has also been demonstrated in several other Mediterranean populations, including Spain, Italy, Greece, Turkey, and Palestinian, Moroccan, and other Jewish populations of the region (Kleiman *et al*., 1994). It would be interesting to expand this study to an investigation of haplotypes to clarify the geographic and ethnic origin of the mutations and also assess genetic admixture, the effects of migration and expansion, and founder effects in this ethnic group. The unexpectedly high frequency of mutation P281L might be explained by consanguinity (five homozygous patients are from consanguineous families) and other factors such as genetic drift operating on this population. The L48S mutation reported to be common among Turks (13.3%)

Table 2. Polymorphisms Identified in the Phenylalanine Hydroxylase Gene Among 88 Iranian Azeri Turkish Alleles

Polymorphism	*Exon/intron*	*Number of alleles*	*Frequency (%)*
V245V	E7	6	6.8
IVS2+19T>C	I2	5	5.7
L385L	E11	2	2.3
Number of alleles identified		13	14.8

Table 3. Genotypes of 44 Phenylketonuria Patients from Iranian Azeri Turkish Ethnic

Genotype	*Polymorphism*	*Number of patients*
IVS10-11G>A/IVS10-11G>A		7
P281L/P281L		7
R261X/R261X		2
R261Q/U		2
P281L/U		2
R243X/R243X	V245V/V245V	2
c.590_612del/c.590_612del		2
IVS11+1G>C/U	L385L/L385L	1
IVS10-11G>A/U		1
IVS10-11G>A/IVS4+1G>A		1
IVS10-11G>A/U	IVS2+19T>C/U	1
R158Q/R158Q		1
R261Q/R261Q		1
R261Q/R270K		1
P281L/IVS2+5G>C		1
E280K/E280K	V245V/V245V	1
G247D/G247/D		1
c.590_612del/U	IVS2+19T>C/ IVS2+19T>C	1
IVS2+5G>C/IVS2+5G>C	IVS2+19T>C/ IVS2+19T>C	1
IVS4+5G>A/IVS4+5G>A		1
U/U		7

U, unidentified.

(Aulehla-Scholz and Heilbronner, 2003) has an allelic frequency of null in our cohort. This indicates some differences between these two populations. This mutation is also less common in the other ethnic groups (Zschocke, 2003).

Some associations between mutations and polymorphisms were observed in this cohort. The IVS2+5G>C mutation occurred on the same allele with the IVS2+19T>C polymorphisms; similar results were previously reported in Egyptian and Brazilian patients (Hashem *et al*., 1996; Santana da Silva *et al*., 2003). Associations between V245V polymorphism and R243X and E280K mutations have also been detected in Azeri Turk patients.

Exons 7 and 11 and their adjacent intronic regions carry 78% of the mutant alleles. To achieve an efficient detection strategy, specimens will be tested first for mutations in these regions and the DNA testing will be considered complete if at least two mutations are identified. The other exons and boundaries will be studied only when either one or no mutations are detected in the initial screen.

The applied experimental approach failed to reveal any *PAH* gene mutations in 14 PKU chromosomes (16%). In these cases, mutant chromosomes may harbor other defects in the *PAH* locus, such as large deletions and mutations in intronic sequences not analyzed by SSCP or in the regulatory regions.

In summary, the analysis of the *PAH* mutations causing PKU in Iranian Azeri Turkish patients further documented the molecular homogeneity of this disease. In addition, the mutation spectrum found in our cohort was somewhat different from those identified in patients from other ethnic origins, reflecting the unique population mixture of Iranian Azeri Turks. In addition, these findings may contribute to future genotype–phenotype correlations.

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Disclosure Statement

No competing financial interests exist.

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