Mutation analysis of the phenylalanine hydroxylase gene in Azerbaijani population, a report from West Azerbaijan province of Iran

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ABSTRACT

Objectives: Phenylketonuria (PKU) is a genetic inborn error of phenylalanine (Phe) metabolism (1). Insufficiency in the hepatic specific enzyme, phenylalanine-4-hydroxylase (PAH), (EC 1.14.16.1) leads to hyperphenylalaninemia that is associated with the PKU (1). Deficiency of the PAH enzyme results in the elevation of Phe concentration in blood and biological fluids that is approximately above 2 mg/dl (120 μmol/l) in the pre-treatment condition (1-4). The prevalence of PKU among patients institutionalized for mental retardation varies from 1% to 3% (4-6). The PAH gene is located on q22-q24.1 regions of chromosome 12 (7). More than 530 PAH gene mutations have been identified (PAHdb; http://www.mcgill.ca/pahdb). The frequency of PKU varies from high incidence in Turkey (about 1 in 2600 births) to low incidence in Japan (about 1 in 125000 births) (7). Overall, the incidence of PKU among Caucasians is about 1 in 10,000, giving a carrier frequency of about 1 in 50 to 1 in 70 (7). The incidence of PKU in Iranian population has been expected at 1 in 3627 live births (8). Genetic structure of Iranian population is highly heterogeneous (8). Genetic overall diversity in Iranian populations is very high and comparable to the other populations from the South Caucasus region, Anatolia and Europe (9). It has been shown that Iranian Azerbaijanis with a population of about 15 to 20 million are more related to the Georgians in comparison to other Iranian groups (9). The finding of Derenko et al (2013) is based on maternal genetic structure on the mitochondrial DNA studies (9). However, this result may change based on paternal genetic structure and Y-chromosome tracing. West Azerbaijan province with a population of about 3 million is in North-West of Iran and closely related to Turks. Regarding to a relatively high incidence of PKU alleles in Iranian population as well as high rate of consanguineous marriages in Iran (10), this study was carried out for mutation analysis of the PAH gene in West Azerbaijan province of Iran.

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Table 1. Tested mutations in phenylketonuria families from West Azerbaijan province of Iran

| Mutation name     | Systematic name   | Region   | Mutation type | Restriction enzyme |
|-------------------|-------------------|----------|---------------|--------------------|
| IVS10nt-11        | c.1066-11g>a      | intron 10| splicing      | +Ddel              |
| s67p              | c.199 t>c         | exon 3   | missense      | -XbaI              |
| r261q             | c.782 g>a        | exon 7   | missense      | -HinII             |
| r252w             | c.754 c>t        | exon 7   | missense      | -Aval              |
| Ivs1nt-1 g>c      | c.1199+1 g>c     | intron 11| splicing      | +Ddel              |
| r408q             | c.1223 g>a       | exon 12  | missense      | -Sau96I            |
| q232q             | c.696 a>g        | exon 6   | silent        | +Ddel              |
| r434q             | c.728 g>a        | exon 7   | missense      | +PflmI             |
| I364del           | c.1090-1092dekt  | exon 11  | deletion      | -HindIII           |
| I333f             | c.997 c>t        | exon 10  | missense      | -BanII             |
| r261x             | c.781 t>c        | exon 7   | nonense       | +Ddel              |
| I65t              | c.194 t>c        | exon 3   | missense      | +Ddel              |
| r408w             | c.1222 c>t       | exon 12  | missense      | +Styl              |

Creation (+) and removal of (-) of a restriction site for related restriction enzyme by a mutation is shown.

Materials and Methods

This study was performed regarding the ethical guideline in human genetic research as described by Ethics Committee of Urmia University of Medical Sciences (West-Azerbaijan, Urmia, Iran) and was conducted in accordance with Declaration of Helsinki. We performed this investigation during 2012 to 2014 in Urmia University of Medical Sciences. The procedure of the present study was explained to all families. PKU patients were diagnosed and sequentially selected among patients referred to Motahari Hospital of Urmia University of Medical Sciences. The West Azerbaijan province ethnically has mixed population of Kurdish and Azeri. We studied Azeri cases that were resident in the West Azerbaijan Province of Iran. A total of 109 individuals from 40 PKU families including 40 PKU patients (16 males and 24 females) and their parents entered in the study. All cases were diagnosed by a pediatric neurologist in the department of pediatrics at the University Hospital of Motahary (Urmia, Iran). Medical data recordings, and tests assessments were carried out by the same physician for all cases using criteria for diagnosis of PKU (7,11). Patients with dihydrobiopterin reductase deficiency were excluded from the study. After obtaining an informed written consent from the parents of the kids for research study, 3 to 5 ml whole blood was taken from patients and their parents and collected in ethylenediaminetetraacetic acid (EDTA) tube. Genomic DNA was isolated from blood samples using ‘salting out’ method (12). A total of 218 alleles from 109 individuals from 40 PKU families (40 PKU patients and their parents) were tested for IVS10-11, S67P, R261Q, R252W, IVS11nt-1 g>c, R408Q, and Q232Q mutations were 28(35), 17(21.25), 15(18.75), 3(3.75), 3(3.75), 2(2.5), and 1(1.25), in case group and 51(23.4), 31(14.2), 27(12.4), 6(2.75), 6(2.75), 4(1.83), and 2(0.92) in total group, respectively. Observed mutations in the patients were inherited from their parents. The mutations of R243Q, 364delG, L333F, 261X, I65T, and R408W were not found in this study. Of the alleles studied, the most frequent mutation was IVS10nt546 (35%). Seven mutations represent approximately 86.25% and 83% of PKU chromosomes analyzed in cases and total groups, respectively. Our analysis showed that 37.5% (15/40) and 62.5% (25/40) of the cases have homozygote and compound heterozygote genotypes regarding the studied mutations (Table 3). The most common mutations of IVS10-11, S67P, and R261Q can be as a result of the high rate of consanguineous marriages. The frequencies of missense, splice, and silent mutations were 37 (46.25), 31 (38.75), and 1 (1.25), in cases and 68 (31.18), 57 (26.15), and 2 (0.92) in total groups.
Table 2. Mutation analysis in the phenylalanine hydroxylase gene of 40 cases and total (patients and parents) groups in West Azerbaijan province of Iran

| Mutation          | Total a, n=109, f (%) | Cases b, n=40, f (%) |
|-------------------|-----------------------|----------------------|
| ivs10nt-11       | 51 (23.4)             | 28 (35)              |
| s67p              | 31 (14.2)             | 17 (21.25)           |
| r261q             | 27 (12.4)             | 15 (18.75)           |
| r252w             | 6 (2.75)              | 3 (3.75)             |
| ivs11nt-1 g>c    | 6 (2.75)              | 3 (3.75)             |
| r408q             | 4 (1.83)              | 2 (2.5)              |
| q232q             | 2 (0.92)              | 1 (1.25)             |
| other             | 26 (11.9)             | 11 (13.75)           |

a: The allele frequency was based on 218 alleles including mutant (153/218) and normal (65/218) alleles in total (patients and parents) group; b: The allele frequency was based on 80 mutant alleles in case group; The mutation detection rate was 86.25% (69 out of 80 PKU chromosomes) and 83% (127 out of 153 PKU chromosomes) in case and total (patients and parents) groups in the West Azerbaijan population. F: Frequency
respectively. Deletion and nonsense mutations were not identified in this investigation. Figures 1 to 4 show PKU mutation analysis in this local population.

**Discussion**

The tested mutations were chosen based on similarity between population in the West Azerbaijan province and the Mediterranean groups. The IVS10nt-11g>a mutation with systematic name of c.1066-11g>a also known by the trivial name IVS10nt546 g>a, is the most common Mediterranean PKU mutation (1). Dworniczak et al (1991) reported transition of G to A at location 546 in intron 10 of the PAH gene (1). This mutation activates a splicing site and leads to insertion of nine nucleotides between exons 10 and 11 during splicing. IVS10nt546g>a is the major cause of PKU in parts of southern and southeastern Europe, mainly in Turkey. Several investigations have been conducted to study the spectrum of PKU causing mutations in various groups (8, 11, 13, 16-23). Interestingly, the frequency of IVS10nt-11 mutation in our cases is higher compared to other reports (8, 11, 13, 16-23). The high rate of consanguineous marriages (47.5%) is a contributing reason to this high prevalence in tested families. It has been suggested that the worldwide distribution of IVS10nt546 mutation has Turkish origin with expansion in different geographic regions (7). In this study, it was established that the IVS10-11 mutation has the highest frequency in the PKU patients among the Iranian Azerbaijanis, and it can be considered for molecular diagnosis in this population. The findings of the present study may be a sign of close familial link between West Azerbaijanis and Turks, which is consistent with the historical as well as geographical relations between West Azerbaijan and Turkey. The second most common mutation identified in our investigation, S67P (c.199T>C), is a missense mutation with low frequency in other populations (4). Unexpectedly, the mutation of S67P with 25% frequency seemed to be explained by the high rate of consanguineous marriages in the tested group (47.5%). The third most common mutation identified in our investigation, R261Q (c.782G>A), is a Mediterranean missense mutation and occurs on a CpG dinucleotide on exon 7 in the PAH gene. This mutation results in conversion of Arg→Gln at codon 261 and is the second most frequent mutation in Turks (19). The remaining mutations of R252W (c.754C>T), IVS11nt-1 G>C (c.1199+1G>C), R408Q (c.1223G>A), and Q232Q (c.696A/G) account for 12.47% of the identifiable mutations. This study indicated high level of heterogeneity of the PAH gene in PKU families in the West Azerbaijan, and thus further investigation should be carried out. Mutation analysis in the PAH gene can be used in carrier detection, prenatal diagnosis and prevent the incidence of PKU phenotypes in the West Azerbaijani population. The results of this study would be useful for biomedicine and molecular anthropology studies by tracing the anthropological characters of the population regarding autosomal chromosomes. This study had some limitations including the small sample size and poor quality of medical records.

**Conclusion**

Exon 11 and its intronic regions carry the most prevalent mutant alleles in PKU families in the West Azerbaijan. Screening of IVS10nt-11 g>a Mediterranean mutation should be tested for detection of possible mutations of PAH in the West Azerbaijan (Iran). This report reveals the genetic heterogeneity in the West Azerbaijani PKU population with a high frequency of IVS10nt-11 g>a mutation (35%) that shows similarity to Turks.
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