

## Investigation of Five Common Mutations on Phenylalanine Hydroxylase Gene of Phenylketonuria Patients from Two Provinces in North of Iran

### Abstract

**Background:** There are more than 500 different mutations on phenylalanine hydroxylase (PAH) gene that is responsible for phenylketonuria (PKU) diseases and the spectrum of these mutations is varied in different populations. The main clinical manifestation of untreated patients is severe mental retardation. The PAH gene, that is 90 kb long, is consisted of 13 exons and 12 introns. The aim of the present study was to identify the frequency of five common mutations on PAH gene among patients with PKU in Mazandaran and Golestan provinces including c.1066-11G>A, p. R261Q, p. R252W, p. R261X, and c.1200 + 1G>C. **Methods:** Forty unrelated PKU patients, that 22 of them, were from Mazandaran and 18 of them from Golestan provinces were enrolled in the study. Genomic DNA was extracted from leukocytes using Qiagen DNA extraction kit and polymerase chain reaction - restriction fragment length polymorphism method was applied to detect five common mutations. **Results:** Three out of the 5 investigate mutations were identified among the patients. The c.1066-11G>A mutation has the highest frequency (27.5%) among the patients and the frequency of p. R261Q and p. R261X mutations were 3.75 and 1.25%, respectively. In Golestan province, only c.1066-11G>A mutation was observed in investigated alleles. **Conclusions:** The high frequency of c.1066-11G>A mutation in Golestan province may be related to genetic drift, founder effect, and consanguinity.

**Keywords:** Mutation, phenylalanine hydroxylase, phenylketonuria

### Introduction

Phenylketonuria (PKU) is an autosomal recessive genetic disorder which is caused by lack or reduced activity of phenylalanine hydroxylase (PAH) enzyme that converts phenylalanine to tyrosine. Any defects on PAH activity leads to toxic accumulation of phenylalanine on patient's body fluids, and the severity of the disease depends on the level of enzyme activity.<sup>[1]</sup> In the most severe form of the disorder which is named classic PKU, the serum level of phenylalanine reaches more than 20 mg/dl.<sup>[2]</sup> Severe mental retardation is the most noticeable clinical manifestation of the disease in untreated subjects.<sup>[3]</sup> Early diagnosis of the PKU and adopting low-phenylalanine dietary treatment to control the serum level of phenylalanine is the main approach in preventing mental retardation.<sup>[3]</sup>

The frequency of PKU is 1 in 10,000 and 1 in 16,500 in Caucasians and Oriental live births, respectively.<sup>[3,4]</sup> Based on screening results, the frequency of PKU is 1 in 10,000 live births in Iran.<sup>[5]</sup>

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The gene responsible for the disease PAH is located on 12 q22-q24.1. The PAH gene, that is 90 kb long, is consisted of 13 exons and 12 introns. To date, more than 500 different mutations have been identified on PAH gene.<sup>[4,6]</sup> The spectrum of these mutations is varied in different populations, and it is necessary to identify the common mutations in each distinct area to facilitate prenatal diagnosis procedure and genetic counseling.

The aim of this study was to identify the frequency of c.1066-11G>A, p. R261Q, p. R252W, p. R261X, and c.1200 + 1G>C mutations on PAH gene among patients with PKU in Mazandaran and Golestan provinces.

### Methods

Forty unrelated PKU patients that 22 of them were from Mazandaran and 18 of them from Golestan provinces were enrolled the study. The parents of all patients signed an informed consent and agreed that their children can share participation in the study.

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Genomic DNA was extracted from 200 µl of peripheral blood using Qiagen DNA extraction kit.

Polymerase chain reaction (PCR) - restriction fragment length polymorphism method was used to detect five mutations including c.1066-11G>A, p. R261Q, p. R252W, p. R261X, and c.1200 + 1G>C.<sup>[6]</sup> The regions on PAH gene containing sites of mutations were amplified using PCR with specific primers derived from a published article.<sup>[6]</sup> The PCR mixture included 200 ng of DNA, 12.5 µ of a 2X PCR master mix containing Taq enzyme, buffer and dNTPs (Takara, Japan), and 10 pmol of reverse and forward primers in total of 25 µ. The amplification program was as follows 5 min at 95°C, 35 cycles of 1 min at 95°C, 1 min at an appropriate annealing temperature, and 1 min at 72°C, ended by a final extension step for 7 min at 72°C.

For the detection of the mentioned mutations, the amplified fragments were treated with specific restriction enzymes as published before (DdeI for c.1066-11G>A, c.1200 + 1G>C and p. R261X, HinfI for p. R261Q, and AvaI for p. R252W mutations) and subsequently were analyzed on 2% gel agarose [Figure 1].<sup>[6]</sup>

## Results

Of the investigated cases, 16 were male, and 24 were female. Three out of the five common investigated mutations were identified among the patients. In 26 alleles (39.9%), the mutation was detected, and 54 alleles did not carry the investigated mutations. The c.1066-11G>A mutation had the highest frequency in patients and p. R261X mutation was identified just in one allele [Table 1]. Twenty four patients did not have the five common mutations. In patients with Golestani origin only c.1066-11G>A mutation was found (homozygote state in six patients and heterozygote state in two cases) while in Mazandaran province three different mutations were identified. In Mazandaran province in 4 patients, the mutation was identified in both alleles (three cases homozygote for c.1066-11G>A and one subject homozygote for p. R261Q mutations) and in four cases, the mutation was detected just in one allele (two

cases with c.1066-11G>A, one with p. R261Q, and one with p. R261X mutations).

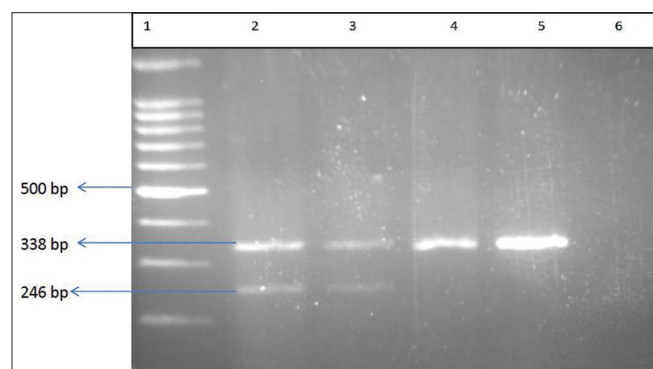
## Discussion

Mazandaran and Golestan are two neighboring provinces of Iran located on Southern coastlines of the Caspian Sea with a population of around 3 and 2 million, respectively. In Mazandaran, the majority of the population has Mazandarani origin while in Golestan province, Turkmens are one of the major ethnic groups. This is the first report investigating the mutations responsible for PKU disorder in Mazandaran and Golestan provinces.

Bonyadi *et al.* at 2010 showed that in Azeri Turkish patients of Iran and among 44 PKU patients, c.1066-11G>A and p. P261 L mutations had the highest incidence rate (19.3% and 19.3%) and the frequencies of p. R261Q, p. R261X, and c.1200 + 1G>C mutations were 5.7, 4.5, and 1.1%, respectively.<sup>[7]</sup> The results of our study showed that among the 80 investigated alleles, c.1066-11G>A was the most common mutation (27.5%) and the frequency of this mutation in Golestani patients was higher than patients with Mazandarani origin (43.75 vs. 18.3%). The p. R261Q and p. R261X mutations were only found in patients from Mazandaran with close frequencies to Azeri patients.

Zare-Karizi Sh *et al.* at 2011 investigated the mutation spectrum of PAH gene among 248 unrelated chromosomes of Iranian patients.<sup>[6]</sup> The results showed that c.1066-11G>A is the most frequent mutation in Iran with the frequency of 24.6% and p. R261Q and p. R261X were found in 12.1% and 6% of the alleles, respectively while in our study, the frequency of c.1066-11G>A in Golestani patients was much higher than other provinces and the frequency of p. R261Q mutation on Mazandarani patients were almost half of what was reported in the mentioned study.

Fifteen different mutations were observed on molecular analysis of PAH gene in 51 of the 54 alleles in Kurdish PKU patients in West of Iran.<sup>[8]</sup> The c.168 + 5G>C mutation was the most prevalent mutation with the frequency of 26%. In Kermanshah province – located at West of Iran with mainly Kurd population-p. R261Q was observed just in one chromosome of 25 PKU patients.<sup>[9]</sup> In another study in Qazvin and Zanjan provinces at 2015, Biglari *et al.* identified 74% of the mutations in 70 investigated chromosomes. P. R176X (10.25%) and p. P281 L (10.25%) were the most common mutations and c.1066-11G>A was not found in any of the patients.<sup>[10]</sup> PAH mutation analysis of PKU patients in Isfahan Province – Central Iran showed that p. R252W (15.38%) was the most frequent mutation among 26 cases<sup>[11]</sup> while in our study, none of the patients from Mazandaran and Golestan provinces had that mutation. Besides, in Isfahan province c.1066-11G>A was observed just in 5.7% of the alleles. In Khuzestan province-southeast of Iran-13 different mutations with mutation detection rate of 53.75% was observed on 40 PKU patients and



**Figure 1:** The results of polymerase chain reaction-restriction fragment length polymorphism test for the identification of IVS10-11G>A mutation. Lane 1: Ladder (100 bp), Lanes 2 and 3: heterozygote samples, Lanes 4 and 5: normal samples, Lane 6: Nontemplate control

**Table 1: The allelic frequency of investigated mutations in Mazandaran and Golestan provinces**

Trivial name (protein effect)	Systematic name	Exon/intron	Number of alleles	Frequency (%)		
				Total	Mazandaran	Golestan
IVS10-11G>A	c.1066-11G>A	I 10	22	27.5	18.2	38.9
p.R261Q	c.782G>A	E7	3	3.75	6.8	0
p.R261X	c.781C>T	E7	1	1.25	2.3	0
p.R252W	c.754C>T	E7	0	0	0	0
IVS11+1G>C	c.1200+1G>C	I 11	0	0	0	0
Total			26	32.5	27.3	39.9

**Table 2: Frequency of the most common mutations in different provinces of Iran and Middle East**

Region	Number of investigated cases	The most frequent mutation	Frequency (%)
Khuzestan	40	IVS10-11G>A	10
Azerbaijan	40	IVS10-11G>A	23.75
Azerbaijan	44	IVS10-11G>A	19.3
Zanjan and Ghazvin	39	p.P281L and p.R176X	10.25
Isfahan	26	p.R252W	15.38
Mazandaran	22	IVS10-11G>A	18.2
Golestan	18	IVS10-11G>A	38.9
NonAshkenazi Jews	78	IVS10-11G>A	18.8
Ashkenazi Jews	18	p.A403V	23.3
Arabs of Israel	45	IVS10-11G>A	16.1
Turkey	44	IVS10-11G>A	28.6

c.1066-11G>A splice site mutation had the highest relative frequency (10%).<sup>[12]</sup> These differences in the frequencies of mutations represent the variety of ethnic groups and high heterogeneity of the PAH locus in Iran.

The mutation analysis of 163 patients in Israel showed that like the results of our study c.1066-11G>A mutation is the most common mutations among non-Ashkenazi Jews (18.8%) and Arab (16.1%) patients with PKU while this mutation was not identified in Ashkenazi Jews and p. A403V mutation (23.3%) was the most frequent one.<sup>[13]</sup> In China, p. R243Q mutation is the most frequent mutation and the frequency of c.1066-11G>A mutation is 0.25%.<sup>[14]</sup>

The results of the present study showed that in comparison to other parts of Iran and the Middle East c.1066-11G>A has the highest frequency in Golestan province [Table 2] that may be related to genetic drift, founder effect, and consanguinity.

Although the present study have investigated the frequencies of 5 mutations on PAH gene, the mutations were found only on 32.5% of the chromosomes. Hence, different mutations are responsible for PKU disease in north of Iran, and further studies are recommended to identify all of the mutations on PAH gene in the region.

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### Conflicts of interest

There are no conflicts of interest.

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