Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env.Pharmacol. Life Sci., Vol 4 [4] March 2015: 101-106

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD
Global Impact Factor 0.533
Universal Impact Factor 0.9804

ORIGINAL ARTICLE

Check the status of Haplotypes VNTR, MspI and *Pvu*II (a) in the PAH gene in Tabriz population genotype data using Family Threesome

Shahin Asadi[*1], [•2]Elham Alizadeh Milani, Ali Nazirzadeh[#3], Saeed Gasemi Manesh[4], Negin Baegerpour[5], Rouya Pourjafar[6]
Young Researchers and Elite Club,Ahar Branch,Islamic Azad University,Ahar,Iran.
2#; Assistant Professor Azad Islamic University Ahar and Kaleybar

ABSTRACT
In this research the types Haplv VNTR, MspI and PvuII (a) non-related gene in 100 individuals and 20 families from the city of Tabriz and allele frequency determination, Degree and frequency Heterozygosity Haplotypes were estimated to be named. Among Haplotypes were determined using the FBAT, Eight haplotype referred to as Haplotypes represent the PAH gene in the population that can be used in prenatal diagnosis and identification of carriers of the disease phenylketonuria (PKU) can be used.
Keywords: haplotype, phenylketonuria, polymorphism markers; Tabriz, Iran.

Received 26.12.2014 Revised 13.01.2015 Accepted 21.03.2015

INTRODUCTION

In recent years, the prevalence of patients with phenylketonuria (PKU) in Iranian population, research in different regions of the gene for phenylalanine hydroxylase (PAH) has been [1,2]. PAH Mutation Analysis Consortium has more than 500 different mutations in the PAH gene has collected. About 60% of known mutations are mutations that alter amino acids in proteins other means are PAH [3]. Since mutation detection is very time-consuming and expensive, in the area now known markers or Determining haplotypes used for diagnosing infectious.

PAH gene polymorphism markers rich. Of these markers to identify normal chromosomes and chromosome is mutated [4]. This area includes 7 marker gene can be detected with variable length with restriction enzymes (RFLP), a short tandem repeat (STR), A variable length tandem repeat (VNTR) polymorphism and several off [4]. In a study on VNTR markers in 50 healthy subjects (100 alleles) in Shiraz population, The presence of the VNTR allele frequency was set to repeat 3,6,7,8,9 and 13 [5]. During a study in Tabriz, VNTR allele frequency marker and its application in identifying carriers of phenylketonuria by genotyping 150 healthy subjects and 10 patients with phenylketonuria were identified. In this study, 6 VNTR allele of the VNTR alleles with repeat repetitions Shdndkh known 3,7,8,9,12,13 3 VNTR allele frequency with Repeat 13 was the least abundant [6]. It can be used for diagnosis, but each marker independently analyzed haplotypes of markers as additional information is obtained.

Table 1: Sequences of primers used for amplification of these markers *Pvu*II (a), MspI and VNTR in the PAH gene.

PAH gene haplotypes in Tabriz:

Reference	Primer sequences	Marker
17	5′-GGCATGACTGGATACGATTAG-3′ 5′-CTAGACTCAGAATGCCTGGG-3′	*Pvu*II(a)
18	5′-TGAGCATATTGTATCTGCCC-3′ 5′-CACATGTCCCAACAGCTCAT-3′	MspI
19	5′-GCTTGAAACTTGAAAGTTG-3′ 5′-GCAAACTTAAGAATCCCATC-3′	VNTR

A study was conducted in Denmark, a significant correlation between the PAH gene and the mutant allele polymorphism was observed in the PAH gene, But when the polymorphic haplotypes were studied, mainly in patients with phenylketonuria haplotype 2 is more common than in controls. So it seems that the correlation between disease-causing mutations and haplotypes can be used in medical genetics studies [7,8]. In different populations around the world, research on PAH gene haplotypes and the haplotype associated with mutations causing phenylketonuria taken Ast [12-15], Yet research on PAH gene haplotypes in Iranian population is not accurate and complete. In this study, three marker haplotype frequencies of *Pvu*II (a), MspI and VNTR, using genotypic information of family of three (parents and one child) in the population was estimated at Tabriz. Haplotypes with a frequency greater than 5% can be referred to as haplotypes are illustrative of the population.

MATERIALS AND METHODS

Preparation of sample: In this study, three marker *Pvu*II (a), MspI, and intron 2 VNTR, respectively, introns 7 and the 3 'instead of the PAH gene were selected. Blood samples from 100 healthy subjects and 20 non-family household consists of three (parents and one child) that their parents were not relatives, were prepared. These individuals were selected from a population of Tabriz.

DNA extraction and PCR: Genomic DNA was prepared from blood samples using standard modified salt precipitation was recorded [16]. Then, the three markers of *Pvu*II (a), MspI and VNTR PAH gene were amplified using specific primers. Sequences of primers used in the PCR reaction are shown in Table 1. The polymerase chain reaction (PCR) in a volume of 25 l, containing 50 ng of genomic DNA 10 pico moles of each primer, 2.5 units of enzyme in a final concentration of 10 mM dNTP mix and Taq Polimeras, the buffer ox1, and $MgCl_2$ were performed with PCR. Then, the PCR products on agarose gel genotype 1/5% was determined.

Digestion product of PCR: After PCR, to determine the site of failures enzymes *Pvu*II (a) and MspI, digestion of the amplification products was the place. Digestion of PCR products with each of the enzymes in this case was a 10-mg vial of sample DNA (product PCR) With 5 units (0.5 microliter) buffer was added to the enzyme and 2.5 microliter and 25 microliter was brought to volume with distilled water. Enzymatic digestion vials containing reaction mixture overnight at 37 ° C in a water bath until digestion was complete. This product is a gel 1/5% agarose was investigated.

Allele frequency and degree Heterozygosity: Estimation of allele frequency and degree Heterozygosity website using GENEPOP [20]. The input data file that was used to estimate allele frequencies, genotype data of 100 subjects were non-relatives.

Haplotype frequency estimates: To determine haplotypes suggest VNTR-MspI - *Pvu*II (a) the population of Tabriz, Tuesday marker genotypes *Pvu*II (a), MspI, VNTR threesome in the PAH gene in 20 families were identified. The genotypic data by the software FBAT, version 2003, and analysis and haplotype frequencies were estimated [21].

RESULTS

Electrophoretic separation of PCR products for gene VNTR marker on PAH, 9 different sizes and lengths 380,440,470,500,530,560,590,620,680 bp was observed, indicating the presence 3,5,6,7,8,9,10,11,13 replication.

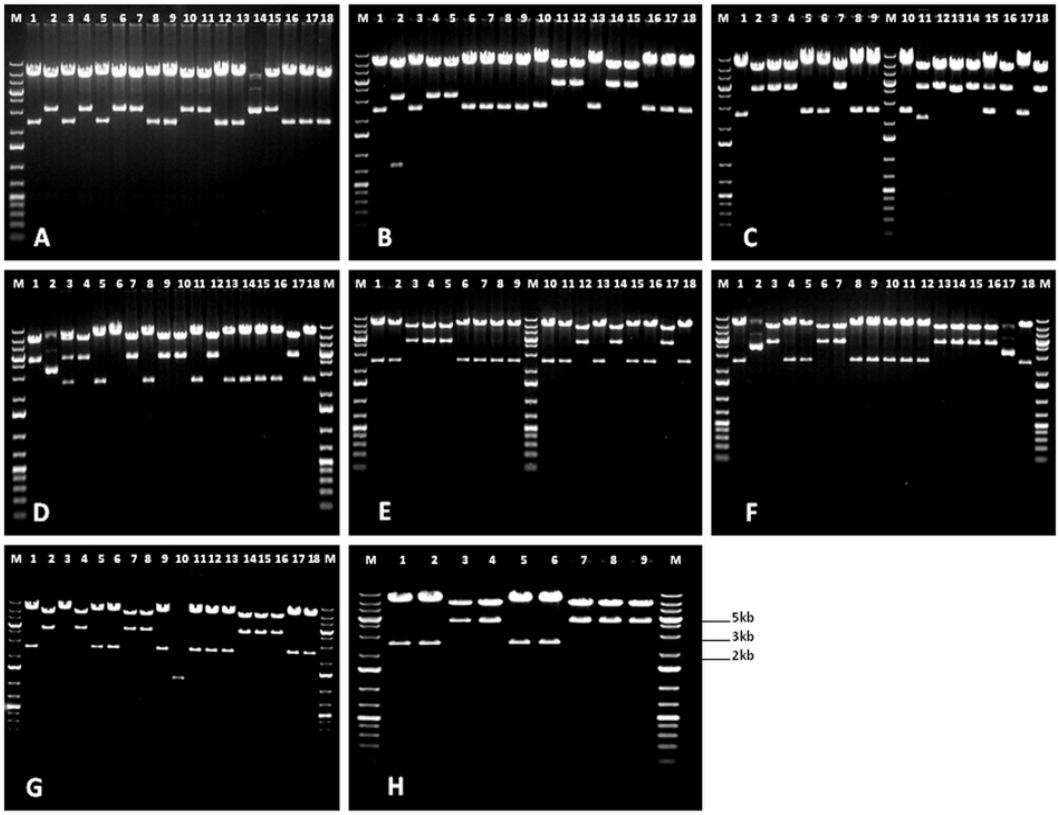

Figure1: VNTR marker alleles on agarose gel 1/5% (H represents the DNA marker is 5Kb).

Figure 1 shows a sample of the PCR product indicates the VNTR marker. The shape of the individual genotypes A and 6.11 B, D, F, G genotype 7/7, the 7/6 genotype C, genotype E of 3.7 is 8.13 and the H genotype. Related to the proliferation marker MspI and *Pvu*II (a), respectively, 379 and 445 bp fragments generated during the break in place of the enzyme *Pvu*II (a) and MspI, respectively, with 235 and 159 bp in length and over 400 pieces and 135 bp were broken.

After genotyping these markers *Pvu*II (a), MspI and VNTR in 100 non-related individuals in the population of Tabriz, the subjects with the genotype and allele frequency analysis GENEPOP website and Heterozygosity grade was calculated. Table 2 VNTR marker allele frequency and allele frequency and degree markers heterozygosity *Pvu*II (a) and MspI are shown in Table 3. In the population studied, with repeats VNTR alleles with repeat VNTR allele 3 highest and 5 the least abundant. Heterozygosity observed and expected VNTR markers in our study, 69% and 71% is estimated. As shown in Table 3, in the study population up Heterozygosity markers *Pvu*II (a) and MspI, respectively, 58% and 56%, respectively.

Table 2. VNTR marker on PAH gene allele frequency in a population

Percent	The number of VNTR repeats	Product size PCR (bp)
44	**3**	**380**
5	**5**	**440**
29	**6**	**470**
24	**7**	**500**
11	**8**	**530**
11	**9**	**560**
1	**10**	**590**
1	**11**	**620**
2	**13**	**680**

Allele frequencies +	Heterozygosity seen	Heterozygosity expected	Marker
48	%56	%50	**MspI**
63	%58	%46	***Pvu*II(a)**

Table 3: Heterozygosity marker allele frequency and degree of *Pvu*II (a) and MspI in the PAH gene in the population of Tabriz.

After genotyping these markers *Pvu*II (a), MspI and VNTR threesome in the PAH gene in 20 families, haplotype frequencies *Pvu*II (a) -MspI-VNTR was estimated using the software FBAT. The results of haplotype frequency estimates are shown in Table 4. Eight haplotypes in the population studied (121,214,115,221,111,215,226,114) had a higher frequency of 5%.

Haplotype	Haplotype frequencies of *Pvu*II (a) -MspI-VNTR
121	**./159986**
214	**./116667**
115	**./100000**
221	**./079167**
111	**./072091**
215	**./064152**
226	**./054167**
114	**./050000**
125	**./042924**
211	**./038757**
116	**./037500**
216	**./037502**
119	**./033333**
224	**./025000**
124	**./025000**
126	**./02083**
219	**./16667**
225	**./009591**
117	**./008333**
217	**./008333**

Table 4: Frequency of haplotype VNTR-*Pvu*II (a) -MspI of 20 families from three populations of. For each haplotype numbers from left to right, respectively show the marker allele VNTR-MspI-*Pvu*II (a) is. Markers for *Pvu*II (a) and MspI numbers 1 and 2 indicate the presence and absence of the restriction enzyme sites are failing. 9 VNTR marker alleles were numbered according to the number of repeats from 1 to 9.

DISCUSSION AND CONCLUSIONS

Set of haplotype may be the non-kin and Shjrnamh of three (parent and child) or larger did each of them benefits (ease of sampling from non-kin) and disadvantages (relative reduction in performance with increased number of people) there. Probably switch errors, incorrect connection to a piece of native haplotype block paternal haplotypes, in samples of three people rare hereditary pattern.

Most methods for determining haplotypes frequency estimates on the non-kin is concentrated. In cases where the pedigree information used by three to determine the haplotype, the haplotype phase can be determined with great accuracy unless all three at loci are heterozygous[10,11]. Genetic markers known in the PAH gene mutated genes are useful tools for the study of continuity and the high degree of Heterozygosity, they can be used to check for carriers of phenylketonuria [6]. For the study, nine VNTR alleles with repeats 3,5,6,7,8,9,10,11,13 in the PAH gene were detected in the population of Tabriz. VNTR allele with 13 repetitions for the first time in Shiraz population, were reported in 2003, the population was Tabriz. The study population in Tabriz, VNTR allele with 5 repeats that previously had not been reported in the Iranian population, first observed in a heterozygous individual. Marker allele frequency estimates based on VNTR, VNTR allele with 3 repeats, the Tabrizi had the highest frequency in the population (Table 2). Heterozygosity comparison of observed and expected for all three markers indicates that the observed Heterozygosity, Heterozygosity equal to or greater than expected.

Most of heterozygosity for VNTR markers have been reported. Thus it can be concluded that VNTR markers for genetic diagnosis is better than the other two markers. Comparing the results of the estimation of marker allele frequencies *Pvu*II (a) and MspI revealed that the frequency of failure status enzyme *Pvu*II (a) in the PAH gene in the population of Tabriz is higher than the position of the failure of the enzyme MspI (Table 3). So the probability of marker *Pvu*II (a) in prenatal diagnosis and detection of carriers of phenylketonuria is better than MspI marker. Marker allele frequencies and expected results Heterozygosity *Pvu*II (a) and MspI in 37 different countries crowd ALFRED shown on the website [22].

Marker *Pvu*II (a) Expected allele frequencies + Heterozygosity	MspI marker Expected allele frequencies + Heterozygosity	Population	Geographical area
./343 ./45	./654 ./45	Biaka	**Africa**
./321 ./44	./244 ./37	Mbuti	**Africa**
./375 ./47	./782 ./34	Yoruba	**Africa**
./708 ./41	./521 ./50	Druze	**Europe**
./725 ./40	./510 ./50	Danes	**Europe**
./667 ./44	./537 ./49	Russians	**Europe**
./896 ./19	./522 ./50	Melanesian	**Oceania**
./38 ./745	**./45 ./342**	**Yakut**	**Siberia**
./589 ./48	**./38 ./750**	**Cheyenne**	**North America**
./256 ./38	./811 ./31	Mexico	**North America**
./585 ./49	./636 ./46	Karitiana	**South America**
./261 ./39	./754 ./37	Ticuna	**South America**
./788 ./33	./113 ./20	Ami	**East Asia**
./881 ./21	./167 ./28	Atayal	**East Asia**
./724 ./40	./073 ./14	Japanese	**East Asia**
– –	./615 ./47	Keralite	**Asia**

Table 5: A comparison of allele frequencies and expected heterozygosity in 16 of the world's population with Iranian population studied.

Comparing these results with those reported for other populations ALFRED website indicates that the expected Heterozygosity marker *Pvu*II (a) in Iranian population greater than or equal Heterozygosity expect other people. Marker+ alleles *Pvu*II (a) the study populations in Africa, North America and South America are low frequency, but the frequency of the Iranian population, as well as European and East Asian populations, higher than 50% (Table 5). Thus marker *Pvu*II (a) in the PAH gene as a marker for prenatal diagnosis and detection of carriers of phenylketonuria proposed. As shown in Table 5, the frequency marker MspI + allele in most populations studied in Africa, Europe, North America and South America is higher than the frequency of this allele in Iranian population. But comparing MspI marker allele frequencies in the study population with East Asian populations suggests that the MspI markers with a frequency of 48% of the population, Awareness of the East Asian population and can identify carriers of phenylketonuria in prenatal diagnosis and helpful. To use prenatal diagnosis and detection of carriers of the disease haplotype in phenylketonuria is essential that the haplotype frequencies in the population are believed to be determined. Estimated haplotype frequencies of *Pvu*II (a) -MspI-VNTR using the software FBAT showed that eight haplotypes in 20 families threesome (121,214,115,221,111,215,226,114) in Tabriz population frequency greater than 5% (Table 4). Since Haplvtyphayy haplotypes with a frequency of ≥ 5% as if it were a defined population [15], The eight haplotypes haplotypes can be considered indicative of Tabriz and for prenatal diagnosis and detection of carriers of phenylketonuria used.

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CITATION OF THIS ARTICLE

Shahin A, Elham A M, Ali N, Saeed G M, Negin B, Rouya P. Check the status of Haplotypes VNTR, MspI and *Pvu*II (a) in the PAH gene in Tabriz population genotype data using Family Threesome. Bull. Env.Pharmacol. Life Sci., Vol 4 [4] March 2015: 101-106