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Frequency of the VNTR-Polymorphisms at the PAH Gene in the Iranian Azeri Turkish Patients with Phenylketonuria

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ABSTRACT

Introduction: *This study was carried out to determine the frequency of the VNTR-polymorphisms at the PAH gene in the Iranian Azeri Turkish patients with phenylketonuria (PKU) and normal controls.*

Material and methods: *The VNTR-polymorphisms were determined by PCR in 43 PKU patients as well as 43 controls.*

Outcomes: *The frequencies of VNTR-alleles were 13(15.1%), 3(3.49%), 64(74.4%), 5(5.81%), and 1(1.16%) in the patients and 43(50%), 0(0%), 42(48.8%), 0(0%), and 1(1.16%) in the controls regarding 3, 7, 8, 9, and 11 repeat copies, respectively. The VNTR alleles with 12 and 13 repeats were not found in our samples. The frequencies of VNTR-genotypes were 25(58.1%), 1(2.33%), 1(2.33%), 10(23.3%), 2(4.65%), 2(4.65%), 1(2.33%), 1(2.33%), and 0(0%) in the patients and 13(30.2%), 13(30.2%), 0(0%), 16(37.2%), 0(0%), 0(0%), 0(0%), 0(0%) and 1(2.33%) in the controls regarding VNTR8/VNTR8, VNTR3/VNTR3, VNTR3/VNTR9, VNTR8/VNTR3, VNTR8/VNTR9, VNTR7/VNTR9, VNTR7/VNTR8, VNTR8/VNTR11, and VNTR3/VNTR11 genotypes, respectively. The comparisons of VNTR-polymorphisms imply that there are statistically significant differences between the patients and controls regarding VNTR3, VNTR8, and VNTR9 alleles as well as VNTR8/VNTR8 and VNTR3/VNTR3 genotypes (all P-Value <0.05). The frequency of "risk-associated genotype of VNTR8/VNTR8" was significantly higher in the cases.*

Conclusions: *It is concluded that this position is heterozygous and there were statistically significant differences between patients and controls concerning the VNTR8/VNTR8 genotype. We found higher frequencies of disease-associated genotype in our samples than controls. This report is the first in its own type in the west Azerbaijani population. Further studies require assessing how this genotype predicts adverse outcomes in tested population.*

Keywords: VNTR, polymorphism, PKU

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INTRODUCTION

Phenylalanine hydroxylase (PAH) insufficiency results in elevated levels of the essential amino acid phenylalanine (Phe) in blood and produces a variety of diseases including phenylketonuria (PKU) and non-PKU hyperphenylalaninemia (non-PKU HPA) (1). Deficiency of PAH in pregnancy is teratogenic and related to psychomotor impairment, microcephaly, spontaneous miscarriage, congenital heart disease, major bowel anomalies, craniofacial dysmorphic features, seizures, disruption of normal development and fetal growth, and irreversible intellectual impairment (2-7). The human PAH gene is mapped on chromosome 12q23.2 (8). To date, more than 530 mutations have been recognized in the patients with PKU or HPA in the PAH gene (PAHdb; http://www.mcgill.ca/pahdb). The human PAH gene contains allelic variations regarding the variable-numbers of tandem-repeat (VNTR) within 3′ un-translated region of the PAH gene that is a 30 bp AT-rich repeat (9). Polymorphism Information Content (PIC) value for multi-allelic VNTR at the PAH gene was 66%, 70%, and 32% in Iranian (10), European Caucasians (11), and Chinese (11) PKU families, respectively. PIC value shows importance of a marker locus that may be informative if randomly chosen person is likely to be heterozygous for that marker in a linkage study (11). Regarding the high rate of heterogeneity of the PAH gene as well as PKU incidence (1 in 3627 live births) in Iranian groups (12), this study was carried out to evaluate the frequency of the VNTR-polymorphisms at the PAH gene in the Iranian Azeri Turkish patients with PKU and normal controls. ❑

METHODS AND MATERIALS

This project was reviewed and approved for ethics by the Institutional Review Board (Urmia University of Medical Sciences). 43 unrelated PKU patients (mean age of 5.54 ± 4.98 years), that is, 86 alleles, as well as 43 normal matched controls from unrelated families have been analyzed. All families are living in the West Azerbaijan Province of Iran. Present investigation was carried out on PKU patients who were admitted to department of genetics and had been followed up in the department of pediatrics at the Motahary University Hospital (Urmia, Iran) for three years. The diagnosis of PAH deficiency is coined based on the elevated plasma Phe concentrations, that is, higher than 120 μmol/L (2 mg/dL) (8,13,14). Furthermore, patients with any other cause of PKU or HPA, such as dihydrobiopterin reductase deficiency were excluded from the study. All the patients underwent evaluation by the same pediatrician who made the clinical diagnosis and reviewed the medical history. Normal controls were randomly elected from the matched ethnic group among participants in genetic counseling sessions in the Genetic Center at Urmia University of Medical Sciences. Normal controls were selected regarding to their past medical history and exclusion of any specific disorders such as genetic and congenital diseases. Analyses of VNTR-polymorphisms were performed in the department of genetics, Urmia University of Medical Sciences. Every family was informed about the study and a written consent was obtained from the families. Genomic DNA was extracted from 3-4 ml peripheral blood leukocytes by salting out method (15). 43 unrelated PKU patients as well as 43 normal controls were genotyped for the VNTR marker located at the PAH gene. PAH-VNTR markers were genotyped using PCR amplification by a set of primers including 5′-TTTTAATGTTCTCACCCGCC-3′ and 5′-AAGAATCCCATCTCTCAGAG-3′(16). Condition of PCR amplification was as follow: denaturation at 95°C for 5 min; 35 cycles of 95°C for 1 min, 55°C for 1 min, and 72°C for 1 min; final extension at 72°C for 5 min. Length of PCR products were 325, 445, 475, 505, 565, 595 and 625 bp that corresponding to the presence of alleles with 3, 7, 8, 9, 11, 12, and 13 copies of the repeated unit, respectively. PCR products were separa-

VNTR-polymorphisms	Cases F(%) N=43	Controls F(%) N=43	P-Value
VNTR3	**13(15.12)**	**43(50)**	**0**
VNTR7	3(3.49)	0(0)	0.08
VNTR8	**64(74.42)**	**42(48.84)**	**0.0006**
VNTR9	**5(5.81)**	**0(0)**	**0.02**
VNTR11	1(1.16)	1(1.16)	1
VNTR8/VNTR8	**25(58.14)**	**13(30.23)**	**0.009**
VNTR3/VNTR3	**1(2.33)**	**13(30.23)**	**0.0005**
VNTR3/VNTR9	1(2.33)	0(0)	0.31
VNTR8/VNTR3	10(23.26)	16(37.21)	0.15
VNTR8/VNTR9	2(4.65)	0(0)	0.15
VNTR7/VNTR9	2(4.65)	0(0)	0.15
VNTR7/VNTR8	1(2.33)	0(0)	0.31
VNTR8/VNTR11	1(2.33)	0(0)	0.31
VNTR3/VNTR11	0(0)	1(2.33)	0.31

TABLE 1. Frequency of the VNTR-polymorphisms at the PAH gene in the Iranian Azeri Turkish patients with PKU and normal controls and a comparison between studied groups.

ted via electrophoresis in a 2.5% agarose gel.

Statistical Analysis

The alleles and genotypes frequencies were determined by direct counting as well as the number of chromosomes. Microsoft Office Excel 2010 was used for statistical analysis such as χ^2 and p- values. Significance level was set at p- value < 0.05. ❑

OUTCOMES

Results of this study are summarized in Table 1. The frequencies of VNTR-alleles were 13(15.1%), 3(3.49%), 64(74.4%), 5(5.81%), and 1(1.16%) in the patients and 43(50%), 0(0%), 42(48.8%), 0(0%), and 1(1.16%) in the controls regarding 3, 7, 8, 9, and 11 repeat copies, respectively. The VNTR alleles with 12 and 13 repeats were not found in our samples. The frequencies of VNTR-genotypes were 25(58.1%), 1(2.33%), 1(2.33%), 10(23.3%), 2(4.65%), 2(4.65%), 1(2.33%), 1(2.33%), and 0(0%) in the patients and 13(30.2%), 13(30.2%), 0(0%), 16(37.2%), 0(0%), 0(0%), 0(0%),0(0%) and 1(2.33%) in the controls regarding VNTR8/VNTR8, VNTR3/VNTR3, VNTR3/VNTR9, VNTR8/VNTR3, VNTR8/VNTR9, VNTR7/VNTR9, VNTR7/VNTR8, VNTR8/VNTR11, and VNTR3/VNTR11 genotypes, respectively. The comparisons of VNTR-polymorphisms imply that there are statistically significant differences between the patients and controls regarding VNTR3, VNTR8, and VNTR9 alleles as well as VNTR8/VNTR8 and VNTR3/VNTR3 genotypes (all P-Value < 0.05). The frequency of "risk-associated genotype of VNTR8/VNTR8" was significantly higher in our cases (58.1% vs. 30.2%). Figures 1 and 2 show the frequencies of the VNTR alleles/genotypes at the PAH gene in cases and controls. ❑

DISCUSSION

PAH-VNTR alleles at the PAH gene have been described previously by Eisensmith et al

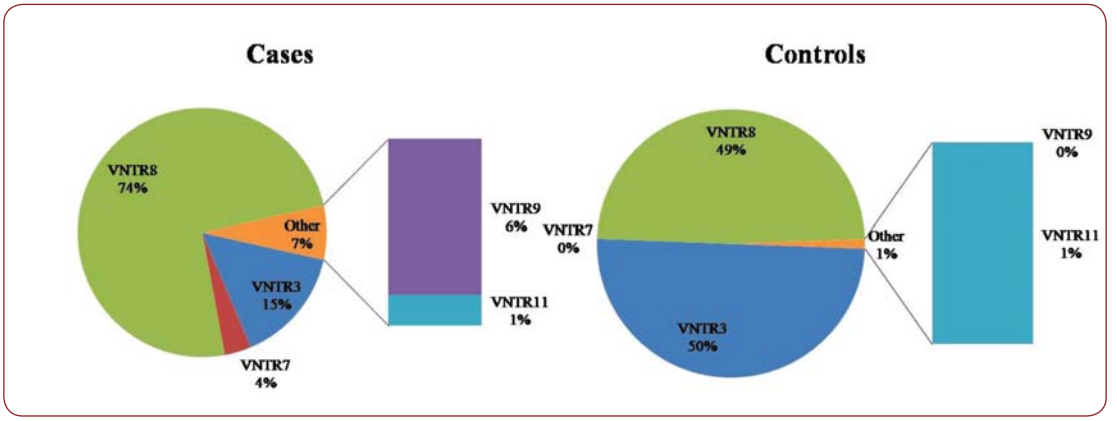


FIGURE 1. Frequency of the VNTR alleles at the PAH gene in the Iranian Azeri Turkish patients with PKU and normal controls.

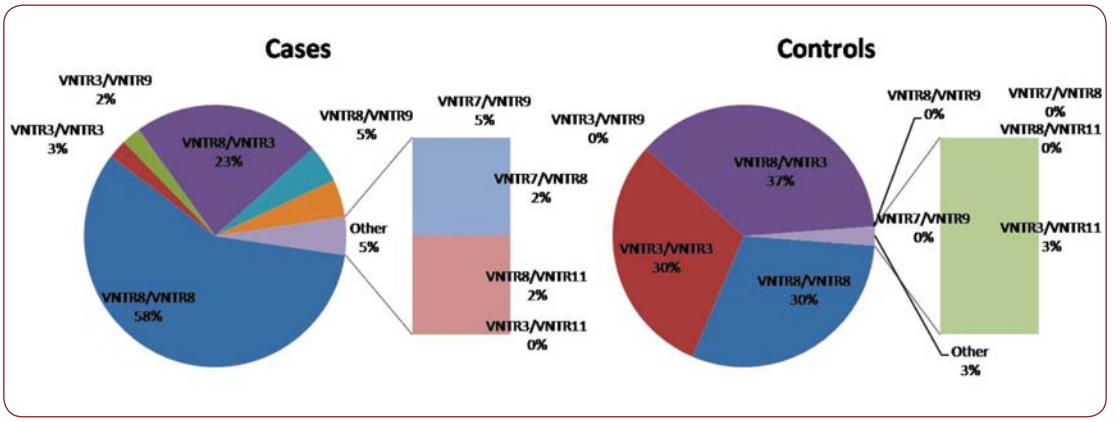
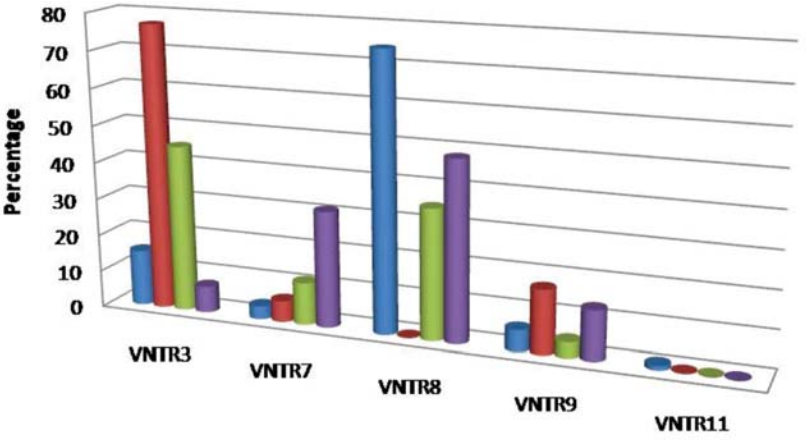


FIGURE 2. Frequency of the VNTR genotypes at the PAH gene in the Iranian Azeri Turkish patients with PKU and normal controls.



	VNTR3	VNTR7	VNTR8	VNTR9	VNTR11
This study	15.12	3.49	74.42	5.81	1.16
Chinese	77.1	5.7	0	17.1	0
European	45	11.4	35	4.3	0
Iranian	7.1	31.3	48.3	13.3	0

FIGURE 3. The frequency of the PAH-VNTR alleles at the PAH gene in the west Azerbaijani PKU patients versus Chinese, European and other Iranian groups.

(11). As the first study, our investigation was carried out in the PKU patients in the west Azerbaijan regarding PAH-VNTR alleles at the PAH gene. Totally, 5 PAH-VNTR alleles containing 3, 7, 8, 9, and 11 repeat copies were identified in this study. The PAH-VNTR alleles with 12 and 13 repeats were not identified in our tested group. This investigation demonstrates high rate of the PAH-VNTR variations in the west Azerbaijani PKU patients. The heterozygosity of the PAH-VNTR alleles in this ethnic group suggests that patients should be analyzed for other highly polymorphic and effective markers as well as mutations in association with studied VNTR alleles (20). Results of the recent studies indicated that the VNTR8 as disease-associated allele is linked to IVS10nt546 mutation in Iranian PKU Patients from Fars and West Azerbaijan Provinces (21,22). In our investigation, it is demonstrated that the most common PAH-VNTR alleles were VNTR8 in our samples. Figure 3 shows the frequency of the PAH-VNTR alleles at the PAH gene in this study versus Chinese, European and other Iranian groups (10). PAH-VNTR alleles at the PAH gene are informative in carrier detection of PKU in the Iranian population and European Caucasians but they are not informative in carrier detection of PKU in Chinese (10,11). Several studies have been conducted on carrier detection of PKU by many polymorphic and informative mini haplotypes of STR and VNTR systems including BglII/EcoRI/ VNTR markers at the PAH gene in different ethnic groups (20,23-25). Iran has one of the most heterogeneous populations in the world and the Iranian Azeri Turkish population with about 3 million is related to the Georgians (26). Analysis of the PAH gene mutations and its related inter-genic polymorphic markers such as PAH-VNTR alleles in combination with BglII and EcoRI markers could be understood as informative haplotypes in carrier screening and prenatal diagnosis of PKU disease (20). Our investigation had some limitations including low sample size and should be verified by further analysis regarding other polymorphic mini-haplotypes of STR system and RFLP/VNTR markers at the PAH gene. ❑

CONCLUSION

Based on our findings it can be concluded that PAHVNTR-polymorphisms are heterozygous and there were statistically significant differences between studied groups concerning the VNTR8/VNTR8 genotype. Also, higher frequencies of disease-associated genotype were found in our samples than controls. A further revision is needed to be carried out regarding more details.

Conflict of interests: none declared.

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