



Research Article

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# CYPs Genotyping, Essential Hypertension and Frequencies of Mutant Alleles in an Uzbek Population

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## Abstract

**Background and Objectives:** Each patient has a combination of the genes bound to cardiovascular illnesses. The aim of our study was to determine the features of the distribution of genes associated with hypertension. In this paper we have described the 4 polymorphic loci distinctive susceptibility to hypertension. There are 4 polymorphisms of genes in nuclear DNA (*CYP2C19* \*2 *CYP3A5*, *CYP2C9-3* and *CYP2C9-2*).

**Materials and Methods:** The study included 109 Uzbek men (mean age  $48 \pm 11$  years) with untreated EH of stage 1-2 and 82 normotensive males.

**Results:** Distribution of allele frequency of, *CYP2C19* \*2 *CYP3A5*, *CYP2C9-3* and *CYP2C9-2* genes was similar with Saudi Arabian, Afro-American, Spain and European American population respectively. Registration of the genotyping results has identified an association CYPs gene (*CYP2C9-2*; *CYP3A5*) with the risk of EH. The *CYP2C9*-\*2 allele showed a higher association with hypertension in comparison to the \*1 allele (OR 3.89, 95% CI 0.48-10.20). In the analysis of cytochrome P450 in patients with EH, we have identified two common point of nucleotide substitutions (\*2, \*3). We have determined that monogenic Western and hybrid Eastern population of Uzbekistan are often carriers of damaging \*2/\*2 -genotype of *CYP2C9-2* and \*1/\*3-\*3/\*3 genotype of *CYP3A5* gene. Distribution of allele frequency was the most closely with American.

**Conclusion:** In the present study, we investigated the association between genotype variants of the CYPs genes and EH in Uzbek population. We found that the \*2/\*2 polymorphism of the *CYP2C9-2* gene was associated with EH in Uzbek hypertensive patients. A separate analysis of case-control studies (*CYP2C9-3*: *CYP2C19*\*2 genes) from Uzbekistan showed no significant differences in allele and genotype frequencies between hypertensive patients and normotensive control subjects. According to our opinion, the results are only preliminary and further large case-control studies in our and other Central Asia populations are needed to confirm this association.

**Keywords:** Essential hypertension EH; CYPs genes; Central Asia

## Introduction

Each patient has a combination of the genes bound to cardiovascular illnesses [1]. About 20 years ago it was discovered by ward et al that 60% of population variability of blood pressure can be genetically determined. However, most studies have been performed on European population [2]. It is still unknown to what extent the data in European populations may be applicable to other ethnic groups. Despite a number of major studies on genome-wide association and blood pressure (HyperGEN, PRIDEMED) it was found that these studies account for only 3% of the inter-individual variability in blood pressure (International Consortium for BP GWAS).

For the last 25 years, the population of Uzbekistan was enlarged twice. Today such tendency of population increase is followed by its aging. The positive aspect of this phenomenon is augmentation of average life expectancy. However, a negative side that the augmentation of life expectancy doesn't correspond to its quality. Deterioration first of all is bound to augmentation of prevalence of age chronic diseases, such as a hypertension. For example, today in Uzbekistan, one of three adults has raised by the ABP, and excess weight occurs at every second (WHO/STEPS 2014). As a result it caused double increasing of visits of the doctor and total number of patients in general.

Genetic polymorphisms of CYPs among different populations in different geographical regions could be different. *CYP2C9* is one of the major drug-metabolizing enzymes in humans. It has been shown to be polymorphic. The human *CYP2C9* gene is located on chromosome 10 with a length of approximately 55 kb [3]. *CYP2C9* is a major enzyme that belongs to the CYP2C subfamily. It constitutes about 20% of the hepatic cytochrome P450 enzyme expressed in humans and thus is responsible for the metabolism of a wide spectrum of clinically important drugs. The cytochrome P450 CYP3A5 subfamily plays a major role in the oxidative, peroxidative, and reductive biotransformation reactions of 50-60% of all currently used drugs exogenous carcinogens, and endogenous substrates such as steroids [4]. Genetic variation in a gene coding for a drug-metabolism enzyme can cause enzyme variants with high or no activity. Among these, *CYP2C19*\*2 exhibit genetic polymorphism. Thereby population can be divided into phenotypes of poor metabolizers, extensive metabolizers and ultra-rapid metabolizers.

## Materials and Methods

The study included 109 Uzbek men (mean age  $48 \pm 11$  years) with untreated EH of stage 1-2 and 82 normotensive males. Genomic DNA was extracted from whole-blood leukocytes using a commercially available kit (Diatom DNA Prep 200 RF).

### CYP2C9\*2/ PCR-RFLP

Genotyping of the SNP was performed by polymerase chain reaction and restriction fragment length polymorphism PCR-RFLP. Sense primer was F-5'-ATCCACATGGCTGCCAGTGTCA-3'. Reverse primer was R-5'-CACATGAGCTAACCAACCAGACTCA-3'.

The PCR primer was synthesized by EVROGEN Corporation. The conditions of amplification reaction were as follow: 95°C for 5 min (initial denaturation); 94°C for 30 s (denaturation); 56°C for 30 s (annealing); 72°C for 30 s (extension); 35 cycles; 72°C for 7 min (extension); 4°C (conservation). Five mL of PCR products were added in 3% agarose gel and then conducted to a horizontal electrophoresis for 40 min under a condition of 100 V constant voltages. Enzyme reaction system: a total volume of materials was 25 mL within 10 mL PCR products, 2.5 mL 10. buffer solution, 0.2 IU restriction endonuclease BmE 18I and 12.3 mL sterilization deionized water. Reaction condition: a warm bath (37°C) for 16 h was designed. The digests were then subjected to electrophoresis on a 3% agarose gel and visualized under ultraviolet illumination, where the undigested product \*1/\*1- genotype showed a band of 173 bp, \*1/\*2- genotype showed a bands of 173 and 349 bp, and \*2/\*2- genotype showed a band of 349 bp.

### CYP2C9\*3/ PCR-RFLP

Genotyping of the SNP was performed by polymerase chain reaction and restriction fragment length polymorphism PCR-RFLP. Sense primer was F-5'-TGCACGAGGTCCAGAGGTAC-3'. Reverse primer was R-5'-ACAACTTACCTTGGAATGAGA-3'. The conditions of amplification reaction were as follow: 95°C for 5 min (initial denaturation); 94°C for 30 s; 56°C for 30 s; 72°C for 30 s; 35 cycles; 72°C for 7 min (extension); 4°C (conservation). Enzyme reaction system: a total volume of materials was 25 mL within 10 mL PCR products, 2.5 mL 10. buffer solution, 0.2 IU restriction endonuclease KpnI and 12.3 mL sterilization deionized water. Reaction condition: a warm bath (37°C) for 16 h was designed (3.5% agarose).

### CYP3A5/ PCR-RFLP

Sense primer was F- 5'-CCTGCCTTCAATTTTCACT-3'. Reverse primer was R-5'-TATCACTTT CCATAAAGCAA G-3'. The conditions of amplification reaction were as follow: 95°C for 5 min (initial denaturation); 94°C for 1 min (denaturation); 61°C for 1min (annealing); 70°C for 1 min (extension); 35 cycles; 72°C for 7 min (extension); 4°C (conservation). Reaction condition: a warm bath (RsaI -37°C) for 16 h was designed. The digests were then subjected to electrophoresis on a 2.5% agarose gel and visualized under ultraviolet illumination, where the undigested product \*1/\*3- genotype showed a bands of 102, 94, 74, 20 bp, \*1/\*1- genotype showed a bands of 102, 74, 20 bp, and (\*3/\*3- genotype) showed a bands of 102, 94 bp.

### CYP2C19\*2/ PCR-RFLP

Sense primer was F-5'-AATTACAACCAGAGCTTGGC-3. Reverse primer was R-5'-GGTCCAAACAGGGAAGAGGT-3'. The conditions of amplification reaction were as follow: 94°C for 5 min (initial denaturation); 94°C for 45 s; 53°C for 40 s; 72°C for 30 s; 35 cycles; 72°C for 5 min (extension); 4°C (conservation). Reaction condition: a warm bath (SmaI -25°C) for 16 h was designed. The digests were then subjected to electrophoresis on a 3% agarose gel and visualized under ultraviolet illumination, where the undigested product \*1/\*1- genotype showed a bands of 120, 49 bp, \*1/\*2- genotype showed a bands of 169, 120, 49 bp, and (\*2/\*2- genotype) showed a band of 149 bp.

### Statistical analyses

Associations between alleles and EH were sought using odds ratios (OR) with 95% confidence intervals. The significance level for all the

analyses was set at  $p < 0.05$ . Statistical analyses were performed using GenePop and Statistica v6.0 software (StatSoft, USA).

### Results

CYP2C19 \*2: The frequencies of the \*1/\*1, \*1/\*2, and \*2/\*2 genotypes were 0.77, 0.17, and 0.05 in hypertensive men, and 0.68, 0.29, and 0.02 in healthy men, respectively ( $p < 0.05$ ). The frequencies of the \*1 and \*2 alleles were 0.86 and 0.14 in the hypertensive group, and 0.83 and 0.17 in the control group, respectively ( $p < 0.05$ ). A similar pattern was observed in Saudi Arabian and Iranian populations as shown in Figure 1 and Figure 2 [5].

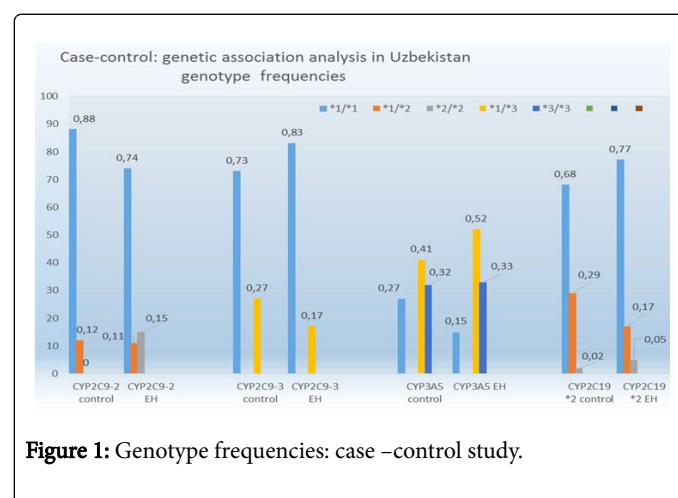


Figure 1: Genotype frequencies: case-control study.

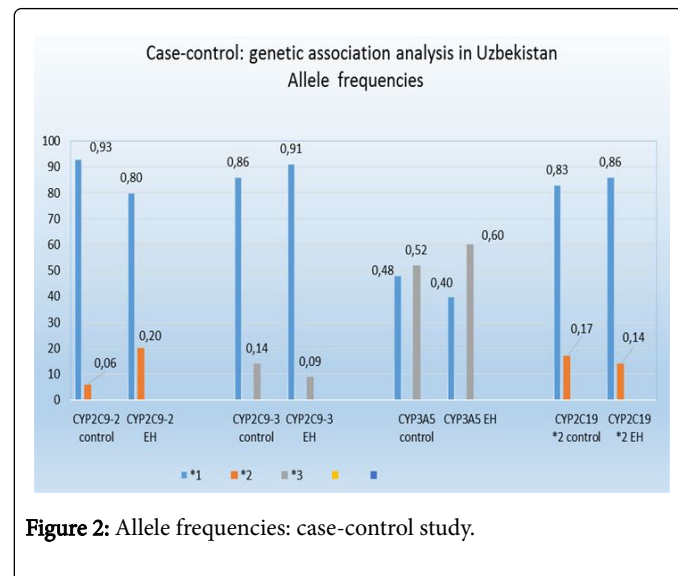


Figure 2: Allele frequencies: case-control study.

CYP2C9-2: 16 individuals (16-patients: 0-healthy) carried two mutated allele being homozygous for CYP2C9-2, thus could be classified as poor metabolizes, while 22 subjects (12-patients: 10-healthy) carried one mutated allele. The \*2 allele showed a higher association with hypertension in comparison to the \*1 allele (OR 3.89, 95% CI 0.48-10.20). The frequency of \*2-allele of CYP2C9-2 gene was closely with American population [6].

CYP2C9-3: The frequencies of the \*1/\*1, \*1/\*3, and \*3/\*3 genotypes were 0.83, 0.17, and 0 in hypertensive men, and 0.73, 0.27, and 0 in healthy men, respectively (Table 1). The frequencies of the \*1 and \*3

alleles were 0.91 and 0.09 in the hypertensive group, and 0.86 and 0.14 in the control group, respectively ( $p < 0.05$ ). Distribution of genotype frequency was closely with Spain population [7].

Uzbekistan CYP2C19*2 mt*2	Saudi Arabian	Irania n	Europea n America n	Japane se
0,17	0,15	0,14	0,13	0,23
Uzbekistan CYP3A5 mt*3	Afro- American s	India	Chinese	Turkish
0,52	0,47	0,59	0,75	0,07
Uzbekistan CYP2C9*3 mt*3	Spanish	India	Chinese	British
0,14	0,12	0,18	0,07	0,05
Uzbekistan CYP2C9*2 mt*2	European American	India	British	Spanish
0,06	0,08	0,04	0,1	0,22

**Table 1:** Distribution of CYPs variant among different ethnic groups.

CYP3A5: The frequencies of the \*1/\*1, \*1/\*3, and \*3/\*3 genotypes were 0.15, 0.52, and 0.33 in hypertensive men, and 0.27, 0.41, and 0.32 in healthy men, respectively ( $p < 0.05$ ). The frequencies of the \*1 and \*3 alleles were 0.40 and 0.60 in the hypertensive group, and 0.48 and 0.52 in the control group, respectively (OR 1.31, 95% CI 0.78-2.19). Distribution of allele frequency of *CYP3A5* gene was similar with Afro-American population and UK [8].

## Discussion

We have studied genotype of 4 gene of cardiovascular continuum and identified two diagnostically significant genes (*CYP2C9-2* and *CYP3A5* genes). Distribution of allele frequency of, *CYP2C19\*2* *CYP3A5*, *CYP2C9-3* and *CYP2C9-2* genes was similar with Saudi Arabian, Afro-American, Spain and European American population respectively [9]. Registration of the genotyping results has identified association of *CYPs* genes (*CYP2C9-2*; *CYP3A5*) with the risk of EH. In the analysis of cytochrome P450 in patients with EH, we have identified two common point of nucleotide substitutions (\*2, \*3). We have determined that monogenic Western and hybrid Eastern population of Uzbekistan are often carriers of damaging \*2/\*2 - genotype of *CYP2C9-2* and \*1/\*3-\*3/\*3 genotype of *CYP3A5* gene. Distribution of allele frequency was the most closely with American. Our study has several limitations. The number of patients and controls were limited for the research. Another limitation was that the healthy men were included as controls only for comparison of genotype and allele frequencies of the *CYPs* genes. Nonetheless, this is the first report examining the relationship between the \*2/\*2 polymorphism of the *CYP2C9-2* gene and EH in Uzbek population. According to our opinion, our results are only preliminary and further large case-control studies in our and other Central Asia populations are needed to confirm this association.

## Conclusion

In the present study, we investigated the association between genotype variants of the *CYPs* genes and EH in Uzbek population. We

found that the \*2/\*2 polymorphism of the *CYP2C9-2* gene was associated with EH in Uzbek hypertensive patients. A separate analysis of case-control studies (*CYP2C9-3*; *CYP2C19\*2* genes) from Uzbekistan showed no significant differences in allele and genotype frequencies between hypertensive patients and normotensive control subjects.

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**Protection of subjects:** The medical information gathered during the study was treated confidentially except as may be required by the law. The study was approved by the medical ethical committee of the center of cardiology, Tashkent Uzbekistan.

## References

- Levy D, Ehret G, Rice K, Verwoert GC, Launer LJ, et al. (2009) Genome-wide association study of blood pressure and hypertension. *Nat Genet* 41: 677-687.
- Walter S, Atzmon G, Demerath E, Garcia ME, Kaplan RC, et al. (2011) A genome-wide association study of aging. *J Neurobiol Aging* 32: 2109.e15-28.
- Lee C, Goldstein J, Pieper J (2002) Cytochrome P450 2C9 polymorphisms: a comprehensive review of the in-vitro and human data. *Pharmacogenetics* 12: 251-263.
- Gibson G, Plant N, Swales K, Ayrton A, Sankary W (2002) Receptor dependent transcriptional activation of cytochrome P4503A genes: induction mechanisms, species differences and interindividual variation in man. *Xenobiotica* 32: 165-206.
- Negin Z, Nader T, Mahmood H, Alireza S, Iraj M (2006) Allele frequency of *CYP2C19* gene polymorphisms in a healthy Iranian population. *IJPT* 4: 124-127.
- Goldstein JA, Ishizaki T, Chiba K, de Moraes SM, Bell D, et al. (1997) Frequencies of the defective alleles responsible for the mephenytoin poor metabolizer phenotype in various Oriental, Caucasian, Saudi Arabian and American black population. *Pharmacogenetics* 7: 59-64.
- Dorado P, Berecz R, Norbeto MJ, Yasar U, Dahl ML, et al. (2003) *CYP2C9* genotypes and diclofenac metabolism in Spanish healthy volunteers. *Eur J Clin Pharmacol* 59: 221-225.
- Barry K, Julian L, Elaine M, Faith M, Daly AK (2003) *CYP3A5* phenotype-genotype correlations in a British population. *Br J Clin Pharmacol* 55: 625-629.
- Xie HG, Prasad HC, Kim RB, Stein CM (2002) *CYP2C9* allelic variants: ethnic distribution and functional Significance. *Adv Drug Deliv Rev* 54: 1257-1270.