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.

Materials and Methods: The study included 109 Uzbek men (mean age 48 ± 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.

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 polymorph 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 ± 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'-ATCCACATGGCTGCCCAGTGTCA-3'. Reverse primer was R-5'-CACATGAGCTAACAACCAGACTCA-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 BsmI 181 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’-TGACGGAGTGCCAGGAGTAC-3’. Reverse primer was R-5’-ACAAACATTGCTGGGAATGAGA-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’- CACTGCCTTCAATTTTCACT-3’. Reverse primer was R-5’- AAATACAACCAGCTTGAG-3. Reverse primer was R-5’-ACAAACATTGCTGGGAATGAGA-3’. The conditions of amplification reaction were as follows: 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). Reaction condition: a warm bath (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/*1- genotype showed a band of 120 bp, *1/*2- genotype showed a bands of 120, 49 bp, and *2/*2- genotype showed a band of 149 bp.

CYP2C9*2/ PCR-RFLP

Sense primer was F-5’- ATATTGAGGGTGAGGCTTG-3. Reverse primer was R-5’- GGTTCCAAACAGGGAAGAGGT-3’. The conditions of amplification reaction were as follow: 94°C for 5 min (initial denaturation); 94°C for 30 s; 53°C for 40 s; 72°C for 30 s; 35 cycles; 72°C for 7 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 then conducted to a horizontal electrophoresis in 3% agarose gel and then conducted to a horizontal electrophoresis in 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, 349 bp, and *2/*2- genotype showed a band of 349 bp.

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].

CYP2C9*2: 16 individuals (16-patients: 0-healthy) carried two mutated allele being homozygous for CYP2C9-2, thus could be classified as poor metabolizers, while 22 subjects (12-pations: 10-healthy) classified as poor metabolizes, while 22 subjects (12-pations: 10-healthy) carried one mutated allele.
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].

<table>
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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 0.32 in healthy men, and 0.27, 0.41, and 0.32 in healthy women, 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 and CYP3A5 genes). Distribution of allele frequency of, CYP2C9*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