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ESSENTIAL HYPERTENSION AND GENETIC POOL IN UZBEK POPULATION

Research

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ABSTRACT

Background and objectives: 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 13 polymorphic loci distinctive susceptibility to hypertension. There are 12 polymorphisms of genes in nuclear DNA and a pool of mitochondrial DNA in D-loop region (HVR1).

Materials and methods: All samples were extracted from human whole blood collected in EDTA or sodium citrate eppendorf tubes. The study included 100 healthy volunteers and 312 patients with I-II grade of essential hypertension EH, all of them were Uzbek males in the mean age of 48.3 ± 8.1 years.

Results: We have found that 17% of inheritance of hypertension in Uzbek population the most closely to Asian type. We have also found dominance of European contribution (83%) to the development of hypertension. Heritage of hypertension in Uzbek population has been evaluated as 17% Eastern, 33% Western, 25% Central Europe, 8% for Anatolia and 17% for South East Asia. The functional significance of all 12 genes in hypertensive patients with combination of atherosclerosis and metabolic syndrome is similar for the most part with European type of heritage. However, a distinctive sensitivity to the exchange of sodium in these individuals is due to 17% of the Asian contribution.

Conclusion: Based on these findings, we have assumed that monogenic Western and hybrid Eastern population of Uzbekistan have different ethnic origins of hypertension heritage. This is confirmed by high population density of the west and low population of the east of country. Since the western region was densely populated, respectively, the number of such settlements exceeded the number of conquerors, which eventually led to small genetic effects on hypertension heritage. The functional significance of 12 genes in Uzbek population with a combination of atherosclerosis and metabolic syndrome is similar 83% in most of European heritage type of hypertension. However, a distinctive sensitivity to the exchange of sodium in these individuals is due to 17% of the Asian contribution.

KEYWORDS: Essential hypertension (EH); Linkage disequlibrium (LD); Biomarkers; Central Asia; Genetic drift

INTRODUCTION

Each patient has a combination of the genes bound to cardiovascular illnesses [1]. About 20 years ago it was discovered that 60% of population variability of blood pressure (BP) - genetically determined (Ward R. et al

1995). However, most studies on have been performed in 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 2011).

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.

With this in mind, we would like to trace the heritage of population events associated with hypertension in modern Uzbekistan. 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 13 polymorphic loci distinctive susceptibility to hypertension. There are 12 polymorphisms of genes in nuclear DNA (B2BKR, eNOS, PPARγ2, ADRB3, AGTR1, AGTR2, ADRB2, ENDRA, ADD, GNB3, ACE, and CYP11B2) and a pool of mitochondrial DNA in D-loop region (HVR1). We have also analyzed of linkage disequilibrium in chromosome 17 where ACE gene is localized and had a strong association with blood pressure in previous studies.

MATERIALS AND METHODS:

Sample Collection and Study Design

All samples were extracted from human whole blood collected in EDTA or sodium citrate eppendorf tubes. The study included 100 healthy volunteers and 312 patients with I-II grade of essential hypertension EH, all of them were Uzbek males in the mean age of 48.3 ± 8.1 years. The study was approved by the medical ethical committee of the center of cardiology, Tashkent Uzbekistan. Informed consent was obtained from each individual recruited.

Genotyping

Genomic DNA was extracted from whole-blood leukocytes using a commercially available kit (Diatom DNA Prep 200). Genotyping of the SNP was performed by polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP).

Sequencing mtDNA HVR1

Sequencing was performed on the sequencer «ABI PRISM 3100 DNA Sequencer».

Linkage disequilibrium

For linkage disequilibrium research, have been taken microsatellite SSR-markers, specific to a chromosome-17 (D17S1866, D17S1798, D17S1840, D17S1529, D17S831, D17S1832, D17S1298, CHRNB1, D17S578, D17S938, D17S960, D17S516, D17S786, D17S1791, D17S1303, D17S1857).

Statistical analyses

Relativity risk (RR) with 95% confidence intervals (CI) were estimated for the effects of high risk alleles (Altman theorem). A level of $P < 0.05$ was considered statistically significant. Continuous variables were expressed as mean± standard deviation and categorical variables as percentages. Differences in continuous variables between cases and controls were examined using the unpaired Student's t-test, where the Mann-Whitney U -test was used in case of abnormal distribution. Deviations from the Hardy-Weinberg equilibrium and differences in allele distributions between the two groups were assessed by the chi-square test with 1 degree of freedom, whereas differences in genotype distributions were assessed by the chi-square test with 2 degrees of freedom. Associations between alleles or genotypes 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 (StatSoft, USA) software.

RESULTS: We have studied genotype of 20 gene of cardiovascular continuum and identified 12 diagnostically significant genes. Registration of the genotyping results has identified an association SNP genes (ET_A ENDRA T/T; ADDUCIN G/G; GNB3 C/C; ACE D/D; CYP11B2 T/T) with the risk of violations of water-salt metabolism, (PPR2Y P/P; ADRB3 T/T, AGTR1 A/A, AGTR2 G/G; β_2-AR G/G) with the risk of metabolic syndrome, ENDOTHEL system genes (B_2BKR +9-+9; eNOS; 4a/4b) with the risk of endothelial dysfunction.

Analysis has shown that +9/+9 genotype of B2BKR gene is associated with vascular remodeling (50%; p = 0.031). Distribution of allele frequency was the most closely with Caucasian (Brull D., et al. 2001). The damaging TT genotype of CYP11B2 gene was associated with higher SBP (160,43 ± 13,16mm Hg; p= 0.044) and left ventricular hypertrophy (LVH) (77.72%; p<0.05). A similar pattern was observed in Italian and French populations (Brand E., et al. 1998). The negative role of carrier of 4a-allele and 4a/4b-genotype of eNOS gene was characterized by high values of LV mass index (LVMI) (181,4 ± 50,1 g/m2; p<0,05), intima media thickness (0,93±0,13mm; p < 0.02). The distribution of allele frequency of eNOS gene was the most closely with Turkish and European patients (Akar N. et al. 1999). The damaging Glu27Glu-genotype of ADRB2 gene was associated with high SBP variability (7,25±8,41mm Hg, p<0,05), and LVH. Allele's frequency of ADRB2 gene was closely with Kyrgyz population. High blood pressure was found in carriers of TT- genotype of ADRB3 gene and LVMI was significantly higher in patients with T-allele (326,8±80,5 g/m2; p= 0,009). Carriage of C-allele of GNB3 gene was associated with a higher threshold of salt sensitivity taste, regardless of serum electrolytes level. The frequency of GNB3 gene genotypes was closely with representatives of Italy (Sartori M et al. 2003). Results of study have shown T-allele carriers of EDNRA gene was associated with expressed LVH and vascular lesions (LVMI 140,54±6,85 g/m2; p=0,002). The frequency of T-allele of EDNRA gene was closely with Chinese population [3]. We have found that carriers of Pro/Pro-genotype of PPARγ gene have had increased risk of hypertension developing in metabolic syndrome (OR 2.06; 95% CI, 1.02-4.15). Distribution of genotype frequency was closely with Serbian population [5]. Aallele carriers of AGTR1 gene and the G-allele carriers of AGTR2 gene have had an increased risk of hypertension developing in metabolic syndrome (OR 1.79; 95% CI, 1,00-3.18, and OR 1.17; 95% CI, 0,63 -2.17, respectively). Distribution of allele frequency of AGTR1and AGTR2 genes was similar with Russian population (Fig.1).

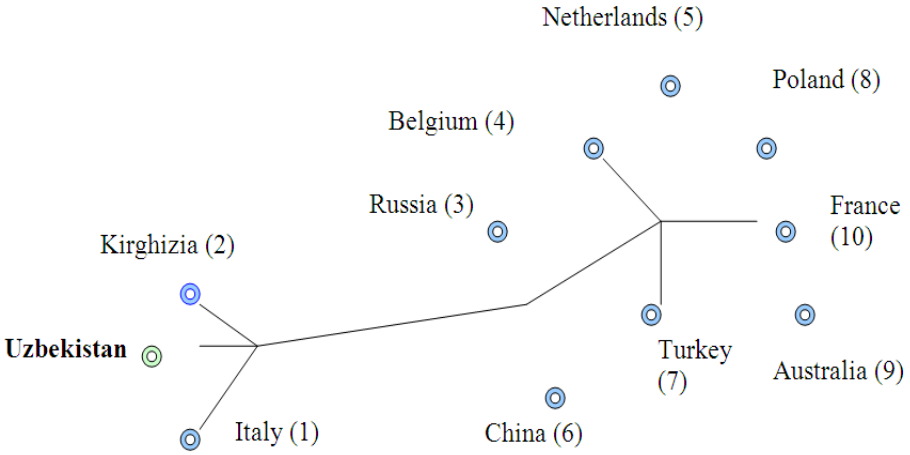


Fig. 1 : Distribution of negative genotype for 12 genes/ the close countries

The heritage of hypertension in Uzbek population is estimated as: 17% of east, 33% of the south, 25% from the central Europe, 8% for Anatolia and 17% for Southeast Asia (Fig.2).

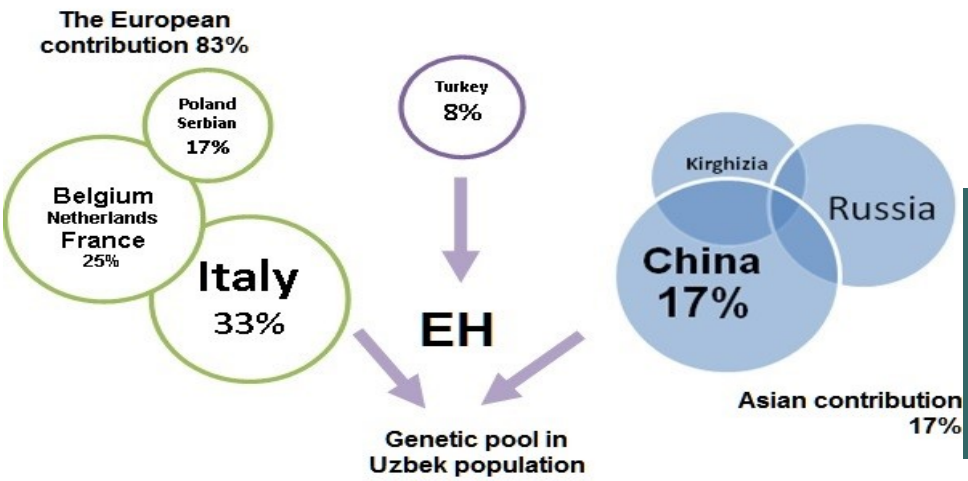


Fig. 2 : The heritage of hypertension in Uzbek population

mtDNA

The sequencing of the nucleotide sequences revealed the most common mutations: 16126 T/C, 16129 G/A, 16189 T/C, 16223 C/T, 16298 T/C, 16327 C/T. In the analysis of mtDNA HVI region in patients with EH, we have identified six common point of nucleotide substitutions, of which 5 are most common in European populations (H, T, X, I, V) and a common mutation with the highest frequency in African populations (L3). In the con-

trol group, the most frequently encountered mutation 16172 T/C which is prevalent in Southeast Asia. Comparative analysis of HVI region of mtDNA and SNP C344T in hypertension candidate gene CYP11B2, showed a definite connection with carriage of "negative" TT genotype of CYP11B2 gene with substitutions in 16129, 16298 and 16327 loci of HVI. Thus in patients with EH with the substitution in locus 16129 TT-genotype was significantly predominant in compare with CC and CT genotypes of the CYP11B2 gene: 66.7% vs 8.3% and 25% respectively (χ^2=9.7; p=0.008), TT-genotype carriage also was associated with substitution in 16298 locus: 66.7% vs 25% and 8.3% (χ^2=9.7; p=0.008) respectively, and 16327 locus: 69.2% versus 23.1% and 7.7% (χ^2=12; p=0.002). In healthy volunteers with the substitution in 16126 locus of HVI carriage of CT-genotype of the CYP11B2 gene was absolutely dominated (χ^2=15; p=0.000). For the rest of parameters both groups have no statistical differences. As a result, discovered N, R, J-super clusters in Uzbek population has denoted appearance from territories of South-East Asia and Western Europe (fig.3).

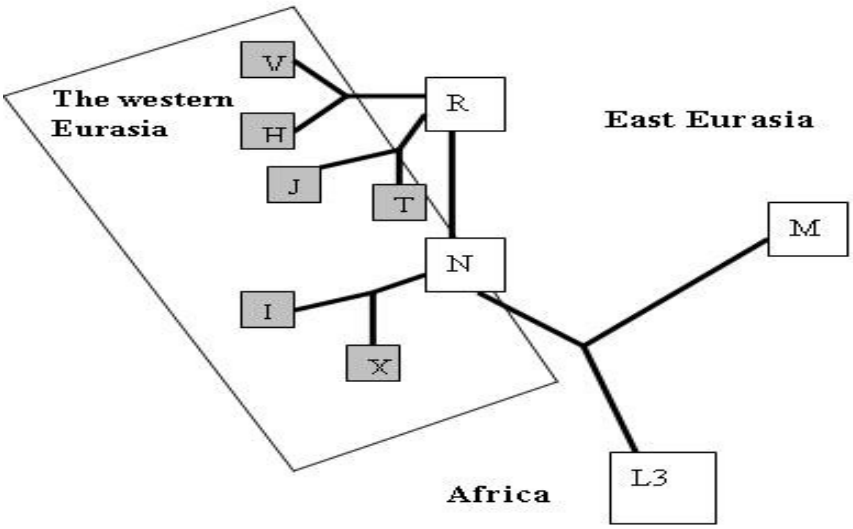
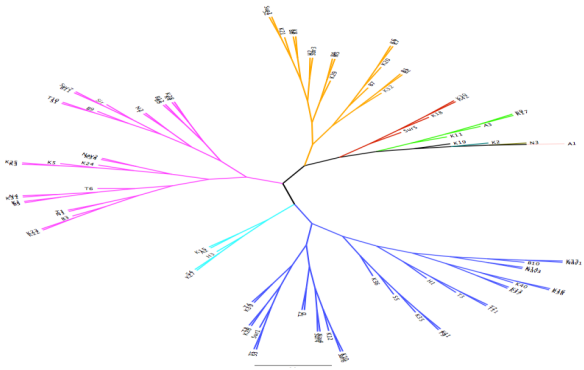


Fig. 3 : Median net of R, N, L3 super clusters of mtDNA in Uzbek population

A comparative analysis of the frequency of occurrence of mtDNA mutations in healthy subjects and patients with EH showed no statistically significant differences, however, observed an association with carriage of "negative" TT genotype of CYP11B2 gene and substitutions in 16129, 16298 and 16327 loci of HVI region of mtDNA. This fact suggests the possibility of the contribution of these mutations in development of the EH in Uzbek patients in continental specificity conditions.



Linkage disequilibrium

We have analyzed of level of linkage disequilibrium (LD) to investigate of population structure. Analysis of phylogenetic relationships on SSR-markers has shown that individuals were divided into 6 phylogenetic groups (Fig.4).

Fig. 4 : Molecular filogenesis of chromosome 17. SSR markers

Markers precisely distributed of population according to genomic groups for genome wide association mapping. As a result phylogenetic tree came from individuals from Andijan, Namangan and Kashkadarya regions (A1, N3, K2), which are located in the root of the tree, which indicates a high diversity of their genome. We calculated a genetic distance (GD) and determined that differences in the genome amounts 9-61%. We have also determined that monogenic Western and hybrid Eastern population of Uzbekistan are often carriers of damaging DD-genotype of ACE gene which localized in chromosome 17. Similar results have been described in Russian, Kyrgyz and Polish populations. D allele of ACE gene was also considered as damaging allele in these populations [4]. We have found that 17% of inheritance of hypertension in Uzbek population the most closely to Asian type. We have also found dominance of European contribution (83%) to the development of hypertension. Heritage of hypertension in Uzbek population has been evaluated as 17% Eastern, 33% southern, 25% western Europe, 8% for Anatolia and 17% for South East Asia. The functional significance of all 12 genes in hyperten-

sive patients with combination of atherosclerosis and metabolic syndrome is similar for the most part with European type of heritage. However, a distinctive sensitivity to the exchange of sodium in these individuals is due to 17% of the Asian contribution.

CONCLUSION: We have found only one association of combined carriage of HVR1 region of mtDNA and T-allele of CYP11B2 gene of nuclear DNA among 12 genes. We have identified two European, one African super cluster and J haplogroupe of Western Asia. Detected super clusters in the Uzbek population have indicated to occurrence from South-East Asia and Western Europe. Perhaps this similarity in hypertension heritage between Uzbek and European populations, due to the fact that the ancient settlements of the Paleolithic Age could migrate to Central Asia from the east and west, not from north and south. We have determined that differences in genome were between 9 and 61%. We have also established the highest diversity of genome in individuals from Andijan and Namangan region. Based on these findings, we have assumed that monogenic Western and hybrid Eastern population of Uzbekistan have different ethnic origins of hypertension heritage. This is confirmed by high population density of the west and low population of the east of country. Since the western region was densely populated, respectively, the number of such settlements exceeded the number of conquerors, which eventually led to small genetic effects on hypertension heritage. East region has a low population density, which leads to increase in genetic effects on variation symptoms of high blood pressure. Identified similarities of ADD, GNB3, ACE, CYP11B2 genes distribution between Uzbek and Italian populations is effect of genetic drift. The functional significance of 12 genes in Uzbek population with a combination of atherosclerosis and metabolic syndrome is similar 83% in most of European heritage type of hypertension. However, a distinctive sensitivity to the exchange of sodium in these individuals is due to 17% of the Asian contribution.

Thus, our study has demonstrated the distinctive susceptibility to hypertension among ethnic Uzbeks reflected in the imposition of the population of western and central Europe and East Asia, migration settlements through the Silk Road, which took place on the territory of modern Uzbekistan.

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