

COMBINED EFFECTS OF ACE (I/D) AND ENOS (894T>G) GENES POLYMORPHISM IN PATIENTS WITH ARTERIAL HYPERTENSION IN THE REALIZATION OF MOLECULAR MECHANISMS OF LEFT VENTRICULAR HYPERTROPHY

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Received 1/27/2013; accepted in final form 4/25/2013

ABSTRACT

Aim: To determine the frequency of alleles and genotypes of insertion-deletion (I/D) polymorphism gene of the angiotensin-converting enzyme (ACE) and a missense mutation of the endothelial NO-synthase (eNOS) gene among residents of Western Ukraine (Bukovina) with essential arterial hypertension (EAH), depending on EAH severity stages and their association with the frequency and patterns of left ventricular hypertrophy (LVH).

Material and Methods: The observation involved 120 patients with EAH I-III stages (female: 48.3%, male: 51.7%) and 40 practically healthy persons. Alleles of polymorphic loci were studied by polymerase chain reaction. Structural and functional changes of the myocardium and LVH models were investigated using echocardiography and electrocardiography methods.

Results and Discussion: Among EAH patients 35.8% had a mutation in coding regions of ACE gene or eNOS. Carriers of a pathological DD-genotype of ACE gene in haplotypes made 28.3% patients, whereas the combination of homozygous mutations of eNOS was observed in 7.5% cases. A combination of two abnormal genotypes of both genes (DD/TT) was not observed at all. Haplotypes ID/TG and ID/GG were the most common both in patients with EAH (25.8% and 20.0%) and in the control group (32.5% and 22.5%), respectively. High-risk groups for LVH among EAH patients were presented by male carriers of DD-genotype and female carriers of D-allele of the ACE gene, as well as men with TT-genotype of eNOS gene. D-allele carriers of ACE gene and T-allele carriers of eNOS gene made high-risk groups by frequency of left ventricle eccentric or concentric hypertrophic model. The combination of D and T "mutant" alleles in haplotypes (ID/TG and DD/TG options) increased the relative risk of LVH variants and EAH II and III stages 1.19-2.25 times, a finding consistent with severity of the disease clinical course and the lowest chance for a normal geometry or concentric left ventricular remodeling and onset of EAH I stage. The absence of mutations in haplotypes (II/GG variant) was a protective factor against LVH, target organ damage and EAH complications; it increased chances for mild EAH I stage 1.44 times and favourable patterns of left ventricular geometric structure 2.53 times.

Conclusion: The ID/TG and DD/TG combinations of genotypes of ACE (I/D) and eNOS (893T>G) genes are the additional independent predictors of target-organ damage, in particular for appearance of LVH, and the severity of EAH.

Keywords: genes ACE (I/D) and eNOS (893T>G), arterial hypertension, left ventricular hypertrophy.

INTRODUCTION

The use of molecular genetics methods to identify and assess genetic risk factors for early detection of cardiovascular disease (CVD) before the onset of clinical symptoms is an actual problem of

medicine today. The basis of the modern strategy for medical management of specified patients is to achieve adequate control of blood pressure (BP), limit the formation and progression of target organ damage and prevent the occurrence of comorbid conditions and metabolic atherothrombotic events [Fagard R. et al., 2009; Sydoruchuk L., Amosova K., 2011]. Among the CVDs one of the most common is arterial hypertension (AH) that makes from

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5.1% in Asia to 70.7% in Western Europe [Kearney P. et al., 2005; Jaddou H. et al., 2011]; from 25.2% to 75.0% of patients are aware of the presence of hypertension, while controlled BP is recorded only in 5.4-58.0% of cases. In Ukraine, hypertension is diagnosed in 32.2% of adults; 80.8% of patients in the urban population and 67.8% in rural area are aware of it, 48.4% and 38.3% are treated, and the effectiveness of treatment is only 18.7% and 8.1%, respectively [Working group on Hypertension, 2012]. Left ventricular hypertrophy (LVH) develops in 60% patients with AH and thus determines the risk magnitude of cardiovascular events (CVE) [Mancia G. et al., 2009; ESC Guidelines, 2010]. However, LVH is not only a result of the implementation of the hemodynamic component of the renin-angiotensin-aldosterone (RAAS), NO and the sympathetic-adrenal system, 60.0% of LVH appears regardless of BP [Deschepper C. et al., 2002; Fraser R., 2003; Sydorчук L., 2010]. Angiotensin-2 (AT2) plays an important role in the manifestation of the clinical phenotype of CVD, including the essential arterial hypertension (EAH) [Tikellis C., Thomas M., 2012]. Some authors have found that AT2 promotes expression of “immediate-early” fetal genes such as *jun B*, *erg-1*, *c-myc*, *c-fos*, *c-jun*, which are responsible for the intensity of myocardial intracellular protein synthesis, signal transduction and activate fetal type of metabolism [Dias-Peixoto M. et al., 2012].

Increased expression of genes of the heavy β -myosin chain, α -actin, atrial natriuretic peptide is accompanied by an increase in fetal isoforms of contractile proteins and the formation of LVH, respectively [Verdecchia P. et al., 2012] with a subsequent decrease of the relaxation first, and then the pumping function of the heart [Fournier D. et al., 2012]. The participation of the majority of molecular and cellular mechanisms of RAAS NO-system in the progression of CVD, EAH and LVH realization were mainly studied in experimental models *in vitro*, including the morphological level [Sydorчук I. et al., 2000]. Part of the pathogenetic mechanisms is not yet fully established, the role of key genes mutations are not fully understood, some data are contradictory and significantly different in separate populations, ethnic groups and races [Conen D. et al., 2009; Paynter N. et al., 2009; Rai H. et al., 2012]. Separate clinical and

prognostic values of many genetic factors are generally unknown and require further study.

The aim of the present study was to evaluate the frequency of alleles and genotypes of insertion-deletion (I/D) polymorphism of the angiotensin-converting enzyme (ACE) (dbSNP id: rs4646994) and a missense mutation (894T<G polymorphism) of the endothelial NO-synthase (eNOS) gene (dbSNP id: rs1799983) among residents of Western Ukraine (Bukovina), patients with EAH depending on the severity of EAH stages and their association with the frequency and patterns of LVH.

MATERIAL AND METHODS

In prospective study we included 150 patients with EAH I-III stages of severity with average office BP greater than 140/90 mm Hg measured according to the requirements of ESC/ESH(2009) [Mancia G. et al., 2009] and disease duration from 2 to 28 years (mean: 15.73±8.02 years). All the patients were treated in the Regional Clinical Cardiology Dispensary and the Municipal Ambulatory Hospital No.1 in Chernivtsi city (Ukraine). The genetic research was performed in the Laboratory of Medical Biology and Genetics Department at Bukovina State Medical University (BSMU). The research list and patients' Prior Informed Consent Form were approved by the Biomedical Ethics Commission of BSMU, Ministry of Health of Ukraine.

After screening (matching inclusion/exclusion criteria) 120 EAH patients were selected for further examination. Group distribution by target-organ damage and complications appearance was performed according to WHO classifications, guidelines of national and European Societies of Cardiology and Hypertension [Mancia G. et al., 2009; ESC Guidelines, 2010]. Among the selected cohort 12.5% (n=15) of patients were with EAH I stage, 60.0% (n=72) – with EAH II stage; 27.5% (n=33) – with EAH III stage. Female patients made 48.3% (n=58) and male –51.7% (n=62); mean age made 52.91±9.24 years. The control group involved 40 practically healthy individuals of appropriate age and gender ($p>0.05$).

Exclusion criteria were as follows: symptomatic hypertension, sub- and decompensated liver diseases (AST, ALT levels three times above the normal upper limit), decompensated renal diseases (serum creatinine 200 $\mu\text{mol/L}$ and above), chronic

heart failure (CHF) higher than II class (NYHA), ejection fraction (EF) less than 45%, acute coronary syndrome 3 months prior to survey, acute heart failure, acute stroke prescription 3 months prior, diabetes mellitus type 1 (DM1), sub- and decompensated type 2 diabetes (DM2), mental disorders. Subjects taking corticosteroids, oral contraceptives, as well as those with exacerbation of chronic inflammatory processes or background endocrine and acute inflammatory processes, wherever located, women in pregnancy and lactation period were also excluded from the survey.

Office systolic and diastolic BP, heart rate (HR) were measured according to European guidelines ESC/ESH (2009) [Mancia G. et al., 2009]. The 24-hour blood pressure monitoring (ABPM) was performed on "ABPE-02" ("SOLVAIG", Ukraine-France) and "ABPM" ("Meditech", Hungary) according to the standard protocol (40-55 measurements/day). Data analysis was performed with the software of mentioned devices.

Genomic DNA was extracted from peripheral blood leukocytes using the test system "DNAsorb-B" (Russia), with primers specific to the alleles' genes [Entrez Gene, 2011]. Amplified polymorphic locus was detected by polymerase chain reaction on "Amply-4L" amplificator according to the manufacturer's protocol. Alleles' discrimination of eNOS gene was performed by restriction endonuclease BanII (Eco241) ("Fermentas", USA). Amplified DNA fragments were separated by gel electrophoresis, stained with xylene zianol, visualized by transluminator in the presence of molecular mass ladder/marker (100-1000 bp).

The LVH was estimated using M- and B-modes of echocardiography (Echo-CG) on automated diagnostic complex "SonoAce8000 SE" ("Medison", Korea), standard linear indicators of structural and functional state of the left ventricle (LV) were analyzed, including the LV geometry. The LV mass (LVM) was evaluated according to the Penn Convention, LVM index (LVMI) was calculated by the ratio of LVM to body surface area (g/m^2). The criteria of LVH, according to the European Guidelines (ESC/ESH, 2009) were as follows: LVMI in men $\geq 125 g/m^2$, in women $\geq 110 g/m^2$. According to LVMI and LV relative wall thickness (RWT) we identified the following geometric models of LV (ESC/ESH, 2009): LV normal geometry (LV NG),

LV concentric remodeling (LV CR), eccentric LVH (LV EH), concentric LVH (LV CH). In addition, patients' examination list included electrocardiogram in 12 standard leads, ultrasound of kidneys and abdominal organs, clinical and biochemical analysis, consulting by ophthalmologist and neurologist.

Statistical processing was performed with MS® Excel® 2003™, Primer of Biostatistics® 6.05 and Statistica® 7.0 (StatSoft Inc., USA) programs software. Differences in continuous variables between cases and controls were analyzed using the unpaired Student's *t*-test (distribution by Kolmogorov-Smirnov and W-Shapiro-Wilk test was near to normal) or U-test Wilcoxon-Mann-Whitney; analysis of qualitative data (categorical variables) was done using the chi-square test (χ^2), including deviation from the Hardy-Weinberg equilibrium, comparison of allele frequencies with 1 degree of freedom (df), haplotypes and genotypes between the groups and the control with 2 df. The associations between alleles or genotypes, and EAH and LVH severity were analyzed using odds ratio (OR) with 95% confidence intervals (CI). The differences were considered significant at $p < 0.05$.

RESULTS

Among EAH patients mutation in intron 16 of ACE gene was observed in 28.3%, that was 1.4 times more frequent than in the control group ($\chi^2 = 4.34$; $p = 0.037$). In healthy subjects the "wild" I allele frequency of ACE gene exceeded that in hypertensive patients by 6.7% ($p < 0.001$), respectively (Table 1). Carriers of I allele insignificantly dominated (52.5 versus 47.5% D allele, $\chi^2 < 1.0$; $p > 0.05$, respectively).

The distribution obtained in observed groups reflected the general picture in the studied population; this latter was confirmed by the calculation of inbreeding coefficient (F) with unreliable heterozygote excess ($F = -0.04-0.10$; $p > 0.05$), spread to the whole sample ($F = -0.05$; $p < 0.05$), and indicated a normal population distribution corresponding to the expected Hardy-Weinberg equilibrium (Table 2).

"Wild" G allele of eNOS gene was observed in 214 (66.9%) of the 320 cases of selected alleles, where in a 7-chromosome 7 exon q35-36 of eNOS gene no missense mutation occurred with structural nucleotide substitution of thymine for guanine (T@G) and subsequent synthesis of modified

TABLE 1.

The allele frequencies and genotype of ACE (I/D), eNOS (894T>G) genes polymorphism in association with arterial hypertension

Groups	Control group, n=40		Hypertension, n=120		χ^2	OR (95% CI)	p	
	%	n	%	n				
Alleles								
ACE gene	I	42	52.5	110	45.8	16.02	3.02 (1.77-5.13)	<0.001
	D	38	47.5	130	54.2	16.36	3.04 (1.79-5.17)	<0.001
eNOS gene	T	24	30.0	82	34.2	0.30	1.21 (0.70-2.09)	0.58
	G	56	70.0	158	65.8	0.47	0.82 (0.48-1.43)	0.49
Genotypes								
ACE gene	II	10	25.0	24	20.0	4.36	2.98 (1.17-7.71)	0.037
	ID	22	55.0	62	51.7	0.79	0.66 (0.27-1.65)	0.51
	DD	8	20.0	34	28.3	5.35	3.04 (1.16-3.93)	0.021
eNOS gene	GG	16	40.0	47	39.2	0.01	0.97 (0.46-2.01)	0.92
	TG	24	60.0	64	53.3	12.52	3.02 (1.66-5.48)	0.004
	TT	0	0	9	7.5	–	–	–

NOTES: OR=odds ratio; CI=confidence interval.

protein G [Glu]@A[Asp]. Unfavourable “minor” T-894 (Asp) variant was recorded 2 times less frequent than the G allele: 106 cases (33.1%) ($\chi^2=7.15$; $p=0.011$). Similar frequency distribution was observed in both experimental and control groups (Table 1), where “wild” G allele significantly prevailed over mutant allele: 65.8% versus 34.2% in the experimental group ($\chi^2=6.37$; $p=0.012$) and 70.0% versus 30.0% ($\chi^2=7.41$; $p=0.008$) in the control, respectively. However, the distribution of “minor” and “mutant” alleles of EAH patients and healthy control groups were not significantly different ($\chi^2<1.0$; $p>0.05$). Allelic distribution among the observed cohort was within the expected Hardy-Weinberg equilibrium (Table 3) with

the insignificant tendency to heterozygosity increase in all observed groups ($F=-0.18-0.43$) and overall ($F=-0.24$), with no statistically significant difference ($p>0.05$).

In patients with EAH II and III stages the “mutant” D allele was found 4.5 and 7.3 times more frequent, respectively, than the II-genotype (81.9-87.9% against 12.1-18.1% ($\chi^2=7.35-14.28$; $p\leq 0.007-0.001$). Among patients with EAH I stage the favorable I allele was recorded 6.5 times more frequent than the DD-genotype ($\chi^2=5.53$; $p=0.048$). In general, DD-genotype was by 8.3% more common in patients with EAH (Table 1) than in healthy subjects ($\chi^2=5.35$, OR=3.04, 95%CI=1.16-3.9; $p=0.021$), with no significant differences in the frequency of ID-genotype.

TABLE 2.

Analysis of heterozygosity and allelic state of the ACE I/D gene polymorphism

Groups	Alleles				P_I	P_D	H_O	H_E	F	χ^2	p
	I		D								
	n	%	n	%							
EAH patients	110	45.8	130	54.2	0.45	0.54	0.52	0.49	-0.04	<1.0	>0.05
Control	42	52.5	38	47.5	0.53	0.47	0.55	0.50	-0.10	<1.0	>0.05
Total	152	47.5	168	52.5	0.48	0.52	0.52	0.50	-0.05	<1.0	>0.05

NOTES: P_I – relative I allele frequency; P_D – relative D allele frequency. H_O – heterozygosity observed; H_E – heterozygosity expected; F – inbreeding coefficient (deviation of genotypic frequencies from panmictic frequencies in terms of heterozygous deficiency or excess); $\chi^2(p)$ – criterion of the “zero” hypothesis validity between observed and expected heterozygosity.

TABLE 3.

Analysis of heterozygosity and allelic state of the eNOS 894G>T gene polymorphism

Groups	Alleles				P _G	P _T	H _O	H _E	F	χ ²	P
	G		T								
	n	%	n	%							
EAH patients	158	65.8	82	34.2	0.66	0.34	0.53	0.45	-0.18	2.46	>0.05
Control	56	70.0	24	30.0	0.70	0.30	0.60	0.42	-0.43	2.28	>0.05
Total	214	66.9	106	33.1	0.67	0.33	0.55	0.44	-0.24	2.29	>0.05

NOTES: P_G – relative G frequency; P_T – relative T allele frequency. H_O – heterozygosity observed; H_E – hetero-zygosity expected; F – inbreeding coefficient (deviation of genotypic frequencies from panmictic frequencies in terms of heterozygous deficiency or excess); χ²(p) – criterion of the “zero” hypothesis validity between observed and expected heterozygosity.

Among II-genotype carriers the number of EAH II patients were significantly, 1.85 times, greater than those with EAH I stage (χ²=15.38; p<0.001). The total frequency of II-genotype by 5.0% (p=0.037) prevailed in control group *versus* the experimental one.

Homozygous mutation in the eNOS gene (TT genotype) was found only in patients with severe target-organ damage and complications (EAH II and III) with the same relative frequency (55.5% and 44.5%; p>0.05). EAH III stage was associated with the dominance of “mutant” TT genotype 1.5-2.1 times compared to the G allele (χ²=6.06-11.13; p≤0.014-0.001). Among the favorable GG-genotype carriers of eNOS gene EAH I stage patients dominated 1.54-1.98 times (60.0% *versus* 38.9% and 30.3% of EAH II and III patients, respectively (χ²=4.36-22.3; p≤0.037-0.001). EAH I and II stages were associated with G allele higher frequency (1.27-1.4 times) as compared to TT genotype (χ²= 11.12-17.26; p<0.001). Overall, in patients with EAH the TG-genotype of eNOS gene was detected 6.7% more frequent than in the control group (χ²=12.52, OR=3.02, 95% CI=1.66-5.48; p=0.004), with no significant differences in the frequency of GG-genotype; TT genotype was absent among control subjects (Table 1).

According to Echo-CG the size of the left atrium (LA), end-diastolic and end-systolic sizes and volumes (EDS, ESS, EDV, ESV), EF between genotypes of ACE gene were not significantly different, but the LV posterior wall thickness in diastole (PWTd) and interventricular septum in diastole (IVSd) substantially prevailed in DD-genotype carriers by 20.4% and 21.7% (p<0.05) than in II-genotype carriers, and by 11.7% and 17.3%

(p<0.05) than in I/D-genotype carriers, respectively. LVM and LVMI in men was also greater in DD-genotype carriers of ACE gene (p≤0.05-0.009), but the LVMI in women was associated with D-allele and prevailed the index in homozygous I-allele carriers by 32.5% (p<0.03) and 20.3% (p<0.05), respectively. The RWT did not differ between genotypes of ACE gene in hypertensive subjects, but was significantly higher in EAH patients with DD-genotype, than in controls. The T894G polymorphism analysis of eNOS gene showed that the sizes of the LA in T-allele carriers exceeded those in patients with GG-genotype by 19.7% (p<0.05) and 21.5% (p<0.03), with no difference after EF, EDS, ESS, EDV, ESV and PWTd, IVSd between genotypes in EAH patients. LVM was not significantly higher in T-allele carriers, with more reliable difference of LVMI also in T-allele carriers, but only in men, by 18.0% (p<0.04) and 16.9% (p=0.05), respectively.

The haplotypes distribution of examined genes is shown as Table 4. Mutations in two genes were not found in 8.1% (n=13) patients: 5.6% (n=9) – in the experimental group, 2.5% (n=4) – in control. Pathological DD variant of ACE gene was recorded almost in one fourth of surveyed (26.3%; n=42): 21.3% (n=34) – in the experimental group, 5.0% (n=8) – in control (χ²=4.34; p=0.037). The eNOS gene mutation was observed 4.7 times less frequent than ACE gene mutation: 5.6% (n=9) cases among patients and none in the control group. Nearly one third patients with EAH (35.8%) were the homozygous “minor” D allele carriers of ACE gene or the T allele of eNOS gene. The combination of two abnormal genotypes of both genes (DD/TT) was not

TABLE 4.

Distribution of haplotypes of ACE and eNOS genes in observed population

Combination of genotypes of ACE and eNOS genes, n=160 (100%)	Groups				χ^2	p	OR (95% CI)
	EAH patients, n=120		Control, n=40				
	n	%	n	%			
II/TT, n=2 (1.25)	2	1.7	0			–	–
II/TG, n=19 (11.9)	13	10.8	6	15.0	2.05	0.152	0.34 (0.09-1.17)
II/GG, n=13 (8.13)	9	7.5	4	10.0	1.05	0.316	0.36 (0.08-1.39)
ID/TT, n=7 (4.37)	7	5.8	0	0		–	–
ID/TG, n=44 (27.5)	31	25.8	13	32.5	6.07	0.014	0.39 (0.14-0.76)
ID/GG, n=33 (20.6)	24	20.0	9	22.5	4.24	0.039	0.53 (0.12-0.86)
DD/TT, n=0 (0)	0	0	0	0		–	–
DD/TG, n=25 (15.6)	20	16.7	5	12.5	2.40	0.122	0.32 (0.10-1.10)
DD/GG, n=17(10.6)	14	11.7	3	7.5	1.26	0.262	0.32 (0.07-1.39)

NOTES: OR=odds ratio; CI=confidence interval.

observed (Table 4). Heterozygous presence of both genes in the haplotypes or in combination with favourable homozygous variant (II/TG, ID/TG, ID/GG) was revealed in the overwhelming majority, viz., 60.0% (n=96) of surveyed subjects: 42.5% (n=68) patients in the experimental group, 17.5% (n=28) – in the control ($\chi^2=13.91$; $p<0.001$). The

most common combinations were ID/TG and ID/GG haplotypes (n=77), both among the EAH patients (25.8% and 20.0%) and in control group (32.5% and 22.5%), appropriately ($\chi^2=6.07$; $p=0.014$ and $\chi^2=4.24$; $p=0.039$) (Table 4).

The analysis of geometric models of the LV distribution, depending on the haplotypes of the ana-

TABLE 5.

Geometric model of the left ventricle in patients with hypertension, depending on the ACE (I/D) and eNOS (894T>G) genes haplotypes

Geometric model of the left ventricle, n=120 (100%)	Genotypes combinations of ACE (I/D) and eNOS (T894G) genes, n=120 (100%)					
	n	%	n	%	n	%
	II/TT, n=2 (1.67)		II/TG, n=13 (10.8)		II/GG, n=9 (7.5)	
LV NG. n=3 (2.5)	0	0	0	0	3	33.3
LV CR. n=7 (5.8)	0	0	3	23.1	4	44.4
LV EH. n=7 (5.8)	1	50.0	5	38.5	1	11.1
LV CH. n=7 (5.8)	1	50.0	5	38.5	1	11.1
	ID/TT, n=7 (5.83)		ID/TG, n=31 (25.8)		ID/GG, n=24 (20.0)	
LV NG. n=5 (4.2)	0	0	0	0	5	20.8
LV CR. n=5 (4.2)	1	14.3	2	6.5	2	8.3
LV EH. n=19 (15.8)	2	28.6	13	41.9	4	16.7
LV CH. n=33 (27.5)	4	57.1	16	51.6	13	54.2
	DD/TT, n=0 (%)		DD/TG, n=20 (16.7)		DD/GG, n=14(11.7)	
LV NG. n=2 (1.7)	–	–	1	5.0	1	7.1
LV CR. n=3 (2.5)	–	–	1	5.0	2	14.3
LV EH. n=12 (10.0)	–	–	7	35.0	5	35.7
LV CH. n=17 (14.2)	–	–	11	55.0	6	42.9

NOTES: LV NG – normal geometry of left ventricle; LV CR – concentric remodelling of left ventricle; LV EH – eccentric left ventricle hypertrophy; LV CH – concentric left ventricle hypertrophy. n (%) – number (%) of observations.

lyzed genes is presented as Table 5. In II/GG-haplotype carriers NG and CR LV were more common than in patients with II/TG ($\chi^2=12.84$; $p<0.001$), ID/TT ($\chi^2=7.65$; $p=0.006$) and ID/GG haplotypes ($\chi^2=6.23$; $p=0.013$). Hypertrophic models (EG and CG LV) were significantly more frequent in patients with ID/TG-haplotype than in ID/GG-haplotype carriers ($\chi^2=6.70$; $p=0.01$) and in patients with ID/TT-haplotype than in II/TT patients ($\chi^2=4.37$; $p=0.037$) with the borderline advantage for those with II/TG-haplotype ($\chi^2=3.76$; $p=0.052$).

Potential genetic risk factors for complications or severe EAH course in six haplotypes combinations are shown in Table 6. The presence of ID/TG and DD/TG haplotypes increases the relative risk of EAH II and III stages 1.19 and 1.33 times, while odds increase 6.08 and 4.75 times ($\chi^2=6.06$; $p=0.014$ and $\chi^2=5.13$; $p=0.021$, respectively), which is confirmed by severity of EAH clinical course. However, the presence of the above mentioned genotypes combination makes the likelihood of EAH I stage appearance the lowest in the studied patient

population (OR=0.16; $\chi^2=4.70$; $p=0.03$ and OR=0.21; $\chi^2=4.98$; $p=0.027$, respectively). The absence of mutations in haplotypes (II/GG variant) is a protective factor against target-organ damages and EAH complications appearance (OR=0.56, $p>0.05$) and 1.44 times (OR=1.80; $p>0.05$) increases the chances for EAH I stage (Table 6).

Risk analysis of pathological changes in the geometric structure of the LV in patients with EAH based on haplotypes (Table 7) showed a significant 1.34 and 2.25 times (OR=5.98 and OR=13.5; $\chi^2\leq 6.01-13.6$; $p\leq 0.015-0.001$) increase in the probability of hypertrophic LV options (LV EH and LV CH) in ID/TG and DD/TG haplotype carriers, respectively, at the lowest chances for LV NG or CR (OR=0.17 and OR=0.07, respectively, $\chi^2\leq 4.68-11.6$; $p\leq 0.031-0.001$). In the EAH patient with II/GG haplotype the probability of favourable patterns of LV NG and LV CR increases 2.53 times (OR=7.87; $\chi^2=5.14$; $p=0.04$), and the risk of LV EG and LV CG becomes the lowest in the population (OR=0.13; $\chi^2=4.60$; $p=0.047$).

TABLE 6.

Haplotypes of ACE and eNOS genes as risk factors for the severity of essential hypertension

Severity of EAH	Indices	Potential risk factor					
		II/TG	II/GG	ID/TG	ID/GG	DD/TG	DD/GG
EAH II and III stages	ARI / ARR	-0.08	0.14	-0.23	-0.11	-0.15	-0.10
	RRI / RRR	-0.12	0.20	-0.33	-0.15	-0.19	-0.13
	RelR	1.12	0.80	1.33	1.15	1.19	1.13
	RR	1.30	0.71	3.62	1.48	1.92	1.93
	OR	1.54	0.56	6.08	1.87	4.75	2.79
	95% CI RR	0.46-3.67	0.27-1.92	0.97-13.52	0.61-3.55	0.47-18.0	0.34-10.99
	95% CI OR	0.31-7.72	0.09-3.25	1.26-29.3	0.50-7.01	0.51-44.5	0.26-30.27
	χ^2	<1.0	<1.0	6.06	<1.0	5.13	<1.0
	p	>0.05	>0.05	0.014	>0.05	0.021	>0.05
EAH I stage	ARI / ARR	0.08	-0.14	0.23	0.11	0.15	0.11
	RRI / RRR	0.27	-0.44	0.78	0.39	0.75	0.59
	RelR	0.73	1.44	0.22	0.61	0.25	0.40
	RR	0.77	1.40	0.28	0.68	0.34	0.52
	OR	0.65	1.8	0.16	0.63	0.21	0.36
	95% CI RR	0.27-2.17	0.52-3.76	0.07-1.03	0.28-1.63	0.06-2.11	0.09-2.96
	95% CI OR	0.13-3.26	0.31-10.52	0.03-0.79	0.14-1.99	0.02-1.97	0.03-3.90
	χ^2	<1.0	<1.0	4.70	<1.0	4.98	<1.0
	p	>0.05	>0.05	0.03	>0.05	0.027	>0.05

NOTES: ARI/ARR – absolute risk increase / absolute risk reduction; RRI/RRR – relative risk increase / relative risk reduction; RelR – relative risk; RR – Risk Ratio; OR – Odds Ratio; 95 CI RR, OR – confidence interval of RR, OR; EAH – essential arterial hypertension.

DISCUSSION

A set of EAH genes-candidates was studied in different ethnic populations, but with contradictory results. Conducted population, ethnic and racial analysis shows that the frequency of unfavorable D allele of ACE gene (0.48-0.52) among EAH patients in our survey corresponds to a majority of Caucasian populations (0.43-0.56), exceeding the corresponding averaged index in Mongoloid race (0.32-0.40; $p < 0.05$), and is slightly lower than in Equatorial race (0.62-0.66; $p < 0.05$) [Oeno Hitoshi et al., 1999; Zhang Ying-Min et al., 2001; Sekerli E. et al., 2008].

The frequency of unfavorable T allele of eNOS gene (30.0% – in control, 34.2% – in the experimental group) corresponded to that of Caucasian individuals ($P_T = 26.5-31.2\%$ – in control, $P_T = 26.5-37.7\%$ – in patients of the experimental group; $p > 0.05$), being significantly higher than in the most of Mongoloid individuals ($P_{Tcontrol} = 5.8-16.9\%$, $P_{Tresearch\ group} = 7.5-21.0\%$; $p < 0.05$) and Equatorial race ($P_T = 8.6\%$; $p < 0.05$) [Luzon M. et al., 2009; Serrano N. et al., 2010; Li J. et al., 2011; Ali A. et al., 2012; Metzger I. et al., 2013].

Population analysis of the haplotypes frequency in our study shows the presence of unfavourable D allele of ACE gene and the T allele of eNOS gene (DD/TG, ID/TT haplotypes) in 20.0% cases totally (22.5% – in EAH patients, 12.5% – in the controls) and that matches to such frequency in Caucasian population (20.0-27.0%) and somewhat exceeds the figure in Non-caucasian (Asians and African Americans) (12.6-15.4%) [Ali A. et al., 2012; Iri-janto Fredie Nurrohmah et al., 2012; Jun-Ge Han et al., 2012; Rahimi Z. et al., 2012]. “Mutant” combination DD/TT was not observed in our patients.

The relationship between severity of EAH and I/D polymorphism of ACE gene is ambiguous: our results are consistent with the statements that the presence of D-allele of ACE gene in patients with previously untreated EAH is a marker of LVH [Kuznetsova T. et al., 2000], an independent indication of target-organ damage and additional cardiovascular risk factor [Buraczynska M. et al., 2003], with 2-fold burden of hypertension anamnesis, frequent crisis trend, associated with DM2

TABLE 7.

Haplotypes of ACE and eNOS genes as risk factors for left ventricular geometry changes in patients with essential hypertension

	Indices	Potential risk factor					
		II/TG	II/GG	ID/TG	ID/GG	DD/TG	DD/GG
Eccentric and concentric LVH	ARI / ARR	-0.09	0.47	-0.24	0.02	-0.50	-0.19
	RRI / RRR	-0.14	0.68	-0.34	0.03	-1.25	-0.31
	RelR	1.14	0.32	1.34	0.97	2.25	1.31
	RR	1.31	0.29	3.65	0.95	6.88	1.99
	OR	1.56	0.13	5.98	0.91	13.5	2.44
	95% CI RR	0.48-3.69	0.07-1.08	0.92-13.6	0.49-1.84	1.75-27.1	0.63-6.27
	95% CI OR	0.35-7.80	0.02-0.91	1.49-25.1	0.28-2.92	2.75-66.3	0.59-10.2
	χ^2	<1.0	4.60	6.01	<1.0	13.6	<1.0
	p	>0.05	0.047	0.015	>0.05	<0.001	>0.05
Normal Geometry, Concentric Remodeling of left ventricle	ARI / ARR	0.09	-0.47	0.24	-0.02	0.50	0.19
	RRI / RRR	0.28	-1.53	0.79	-0.07	0.83	0.46
	RelR	0.75	2.53	0.23	1.07	0.17	0.54
	RR	0.79	3.50	0.29	1.06	0.15	0.50
	OR	0.63	7.87	0.17	1.10	0.07	0.41
	95% CI RR	0.27-2.19	0.90-13.24	0.06-1.09	0.54-2.05	0.04-0.57	0.16-1.58
	95% CI OR	0.18-3.30	1.10-56.1	0.05-0.88	0.34-3.53	0.01-0.36	0.10-1.70
	χ^2	<1.0	5.14	4.68	<1.0	11.6	<1.0
	p	>0.05	0.04	0.031	>0.05	<0.001	>0.05

NOTES: ARI/ARR – absolute risk increase / absolute risk reduction; RRI/RRR – relative risk increase / relative risk reduction; RelR – relative risk; RR – Risk Ratio; OR – Odds Ratio; 95 CI RR, OR – confidence interval of RR, OR; LVH – left ventricle hypertrophy.

and CHF, higher levels of BP, LVM and concentric pattern of LVH [Oeno Hitoshi et al., 1999]. However, several authors found no relationship of ACE gene polymorphism with more severe EAH course; these studies were conducted in patients receiving long-term antihypertensive therapy [Gomez-Angelats E. et al., 2000; Lopez-Contreras J. et al., 2000; Headley A. et al., 2007].

Our findings of the mutant T-allele of eNOS gene relationship with the EAH severity and BP levels are consistent with the Bogalusa Heart Study [Chen W. et al., 2001], while according the frequency of LVH appearance – with data of O.I. Yakovleva and co-workers [Yakovleva O. et al, 2005]. However, we found no relation with the intensity of LVH and geometric models [Yakovleva O. et al, 2005; Lapu-Bula R. et al., 2005].

CONCLUSION

One third of patients with EAH (35.8%) had a mutation in the coding regions of the ACE gene (I/D, intron 16, 17q23, dbSNP id: rs4646994) or eNOS (894T<G, codon 298 of exon 7, 7q35-36, dbSNP id: rs1799983). Every fourth patient (28.3%) was the carrier of a pathological DD-genotype of ACE gene in a haplotype, whereas the combination of homozygous mutations of eNOS gene was observed 4.7 times less frequent (7.5%). A combination of two abnormal genotypes of both genes (DD/

TT) was not observed. The combined mutation of genes ACE (I/D) and eNOS (894T<G) in 8.1% (n=13) cases was missing. The most common in the observed population group were ID/TG and ID/GG haplotypes (48.1%), both among EAH patients (25.8% and 20.0%; $p=0.014$) and in the control group (32.5% and 22.5%; $p=0.039$), respectively.

Risk-groups of LVH in patients with EAH were male DD-genotype carriers and female D-allele carriers of ACE gene, and men with TT genotype of eNOS gene. Risk groups of the eccentric or concentric LV hypertrophy models frequency were D-allele carriers of ACE gene and the T-allele of eNOS gene. The combination of “mutant” D and T allele in haplotypes (ID/TG and DD/TG options) 1.19-2.25 times (OR=4.75-13.5; $p\leq 0.021-0.001$) increased the relative risk of LVH and EAH II and III stages, which was also confirmed by the severity of the clinical course of the disease, with the lowest chance for a normal geometry or concentric LV remodeling and the emergence of EAH I stage (OR=0.07-0.17; $p\leq 0.031-0.001$).

The absence of mutations in haplotypes (II/GG variant) is a protective factor against LVH (OR=0.13; $p=0.047$), emergence of target-organ damage and EAH complications (OR=0.56; $p> 0.05$); furthermore, it increases both the chances for mild EAH I stage course (1.44 times; OR=1.80; $p>0.05$) and the number of favourable patterns of LV geometric structure (2.53 times; OR=7.87; $p=0.04$).

REFERENCES

1. Ali A., Alghasham A., Ismail H., Dowaidar M., Settin A. ACE I/D and eNOS E298D gene polymorphisms in Saudi subjects with hypertension. J. of Renin-Angiotensin-Aldosterone System. 2012; DOI: 10.1177/1470320312459976.
2. Buraczynska M., Pijanowski Z., Spasiewicz D., Nowicka T., Sodolski T., Widomska - Czekajska T., Ksiazek A. Renin-angiotensin system gene polymorphisms: assessment of the risk of coronary heart disease. Kardiolog. Pol. 2003; 58(1): 1-9.
3. Chen W., Srinivasan S.R., Elkasabany A., Ellsworth D.L., Boerwinkle E., Berenson G.S. Combined effects of endothelial nitric oxide synthase gene polymorphism (G894T) and insulin resistance status on blood pressure and familial risk of hypertension in young adults: the Bogalusa Heart Study. Am. J. Hypertens. 2001; 14(10): 1046-1052.
4. Conen D., Cheng S., Steiner L.L., Buring J.E., Ridker P.M., Zee R.Y. Association of 77 polymorphisms in 52 candidate genes with blood pressure progression and incident hypertension: the Women’s Genome Health Study. J. Hypertens. 2009; 27(3): 476-483.
5. Deschepper C.F., Boutin-Ganache I., Zahabi A., Jiang Z. In search of cardiovascular genes. Interaction between phenotypes and genotypes. J. Hypertens. 2002; 39: 332-336.
6. Dias-Peixoto M.F., Ferreira A.J., Almeida P.W., Braga V.B., Coutinho D.C. The cardiac expression of Mas receptor is responsive to different physiological and pathological stimuli. Peptides. 2012; 35(2): 196-201.
7. Entrez Gene. Sequence analysis. National Center for Biotechnology Information. U.S. National Library of Medicine. 2011; Online ver-

- sion <http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene>
8. *ESC Guidelines Desk Reference. Compendium of abridged ESC Guidelines 2010. ESC and ESH Committee for Practice Guidelines. London. UK. Springer Healthcare. 2010. 392p.*
 9. *Fagard R.H., Celis H., Thijs L., Wouters S. Regression of left ventricular mass by antihypertensive treatment: a meta-analysis of randomized comparative studies. J. Hypertens. 2009; 54(5): 1084-1091.*
 10. *Fournier D., Luft F.C., Bader M., Ganten D., Andrade-Navarro M.A. Emergence and evolution of the renin-angiotensin-aldosterone system. J. Mol. Med. 2012; 90(5): 495-508.*
 11. *Fraser R. Studying genes and the development of cardiac hypertrophy: convenient intermediate phenotypes in man. J. Hypertens. 2003; 21: 873-874.*
 12. *Gomez-Angelats E., de la Sierra A., Enjuto M., Sierra C., Oriola J., Francina A., Pare J.C., Poch E., Coca A. Lack of association between ACE gene polymorphism and left ventricular hypertrophy in essential hypertension. J. Hum. Hypertens. 2000; 14(1): 47-49.*
 13. *Headley A.P., Li Y., Li L.H. Left ventricular hypertrophy in relation to systolic blood pressure and the angiotensin converting enzyme I/D polymorphism in Chinese. J. Hypertens. 2007; 25(Suppl. 2): 39-253.*
 14. *Irijanto Fredie Nurrohmah, Oom Presanto, Heru Aziza Bawazier, Lucky Hamim Sadewa, Ahmad Sjabani, Mochammad Tomino, Yasuhiko. Angiotensin II type 1 receptor A1166C (AT-1R), high D allele frequency of ACE I/D gene, endothelial nitric oxide synthase G894T (eNOS), IL-6, polymorphism in familial hypertension in Javanese Indonesian. J. of Clinical Hypertension. 2012; 14(Suppl. 1): 215.*
 15. *Jaddou H.Y., Batieha A.M., Khader Y.S., Kanaan A.H., El-Khateeb M.S., Ajlouni K.M. Hypertension Prevalence, Awareness, Treatment and Control, and Associated Factors: Results from a National Survey. Jordan. Intern. J. of Hypertension. 2011; 2011. Article ID 828797. doi:10.4061/2011/828797.*
 16. *Jun-Ge Han, Hong Jin, Lin-Bo Gao, Jian Zhang, Xue-Ke Deng, Li-Juan Li, Chang-Ping Song, Tao Wang, Lin Zhang. Relationship between Polymorphisms of Angiotensin-converting Enzyme Gene Insertion/Deletion, Endothelial Nitric Oxide Synthase Gene Intron 4 VNTR and Risk for Cervical Cancer. Life Science J. 2012; 9(2): 100-104.*
 17. *Kearney P.M., Whelton M., Reynolds K., Muntner P., Whelton P.K., He J. Global burden of hypertension: analysis of worldwide data. Lancet. 2005; 365: 217-223.*
 18. *Kuznetsova T., Staessen J.A., Wang J. Antihypertensive treatment modulates the association between the D/I ACE gene polymorphism and left ventricular hypertrophy: a meta analysis. J. Hum. Hypertens. 2000; 14(7): 447-454.*
 19. *Lapu-Bula R., Quarshie A., Lyn D., Oduwole A., Pack C., Morgan J. The 894T allele of endothelial nitric oxide synthase gene is related to left ventricular mass in African Americans with high-normal blood pressure. J. Natl. Med. Assoc. 2005; 97(2): 197-205.*
 20. *Li J., Cun Y., Tang W.R., Wang Y., Li S.N., Ouyang H.R., Wu Y.R., Yu H.J. Association of eNOS gene polymorphisms with essential hypertension in the Han population in south western China. Genet. Mol. Res. 2011; 10(3): 2202-2212. DOI <http://dx.doi.org/10.4238/vol10-3gmr1160>.*
 21. *Lopez-Contreras J., Blanco-Vaca F., Borrás X., Carreras F., Pons-Llado G., Gallego F., Sole M.J., Cirera S., Benet M.T., Negro E., Roca-Cusachs A. Usefulness of the I/D angiotensin-converting enzyme genotype for detecting the risk of left ventricular hypertrophy in pharmacologically treated hypertensive men. J. Hum. Hypertens. 2000; 14(5): 327-331.*
 22. *Luizon M.R., Izidoro-Toledo T.C., Simoes A.L., Tanus-Santos J.E. Endothelial Nitric Oxide Synthase Polymorphisms and Haplotypes in Amerindians. DNA and Cell Biology. 2009; 28(7): 329-334.*
 23. *Mancia G., Laurent S., Agabiti-Rosei E. et al. Working ESC/ESH Group. Guidelines for the Management of Arterial Hypertension 2009.*

- Reappraisal of European Guidelines on hypertension management: European Society of Hypertension (ESH) Task Force Document. *J. Hypertens.* 2009; 27: 2121-2158.
24. Metzger I.F., Luizon M.R., Lacchini R., Ishizawa M.H., Tanus-Santos J.E. Effects of endothelial nitric oxide synthase tag SNPs haplotypes on nitrite levels in black subjects. *Nitric Oxide.* 2013; 28: 33-38.
 25. Oeno Hitoshi, Takata Masanobu, Yasumoto Kotaro, Chin Tomita, Hiroshi Inoue. Angiotensin converting enzyme Gene Polymorphism and Geometric Patterns of Hypertensive Left Ventricle Hypertrophy. *Jpn. Heart J.* 1999; 40(5): 589-598.
 26. Paynter N.P., Chasman D.I., Buring J.E., Shiffman D., Cook N.R., Ridker P.M. Cardiovascular disease risk prediction with and without knowledge of genetic variation at chromosome 9p 21.3. *Ann. Intern. Med.* 2009; 150: 65-72.
 27. Rahimi Z., Vaisi-Raygani A., Rahimi Z., Parsian A. Concomitant presence of endothelial nitric oxide 894T and angiotensin II-converting enzyme D alleles are associated with diabetic nephropathy in a Kurdish population from Western Iran. *Nephrology (Carlton).* 2012; 17(2): 175-181.
 28. Rai H., Fitt J., Sharma A.K., Sinha N., Kumar S., Pandey C.M., Agrawal S., Mastana S. Lack of association between Glu298Asp polymorphism and coronary artery disease in North Indians. *Mol. Biol. Rep.* 2012; 39(5): 5995-6000.
 29. Sekerli E., Katsanidis D., Papadopoulou V., Makedou A., Vavatsi N., Gatzola M. Angiotensin-I converting enzyme gene and I/D polymorphism distribution in the Greek population and a comparison with other European populations. *J. of Genetics.* 2008; 87(1): 91-93.
 30. Serrano N.C., Diaz L.A., Casas J.P., Hingorani A.D., Moreno-De-Luca D., Paez M.C. Frequency of eNOS polymorphisms in the Colombian general population. *BMC Genetics.* 2010; 11: 54 doi: 10.1186/1471-2156-11-54. <http://www.biomedcentral.com/1471-2156/11/54>.
 31. Sydorчук I.I., Ushenko O.G., Sydorчук R.I., Levitska S.A. [Laser polarimetry of biotissues] [Article in Russian]. *Proceedings of SPIE (Coherent Optics of Ordered and Random Media).* 2000; 4242: 218-227.
 32. Sydorчук L.P. [Pharmacogenetics of hypertension] [published in Ukrainian]. Chernivtsi BSMU. 2010. 532p.
 33. Sydorчук L.P., Amosova K.M. [Influence of pharmacogenetically determined treatment on parameters of peripheral hemodynamics in patients with arterial hypertension] [Article in Russian]. *The New Armenian Medical Journal.* 2011; 5(2): 35-43.
 34. Tikellis C., Thomas M.C. Angiotensin-converting Enzyme 2 (ACE2) is a key Modulator of the Renin Angiotensin System in Health and Disease. *Int. J. Pept.* 2012; 2012: 256-294.
 35. Verdecchia P., Gentile G., Angeli F., Reboldi G. Beyond blood pressure: evidence for cardiovascular, cerebrovascular, and renal protective effects of renin-angiotensin system blockers. *Ther. Adv. Cardiovasc. Dis.* 2012; 6(2): 81-91.
 36. Working group on hypertension of the Ukrainian Association of Cardiologists. Order of the Ministry of Health of Ukraine No.384 of 24.05.2012. Guidelines. Recommendations and clinical protocol of care "Hypertension": updated, adapted, evidence based. Kiev. Ministry of Health of Ukraine. 2012. 108p.
 37. Yakovleva O.I., Vakhrameeva N.V., Larionov V. [Polymorphism of endothelial NO-synthase and the structural and functional state of the large vessels in hypertensive patients with left ventricular hypertrophy] [Article in Russian; abstract in English]. *Arterial Hypertension.* 2005. 11(3): 78-85.
 38. Zhang Ying-Min, Zhang Li-Ying, Wang Ke-Qiang, CE Jun-Bo. Distribution of Angiotensin converting enzyme Gene Polymorphism among Northern Hans, Daurs, and Ewenkis. *Acta Pharmacol. Sin.* 2001; 22(8): 747-750.