Value of Angiotensin-Converting Enzyme and Monoxide Nitrogen in Pathogenesis of Myocardium Remodeling Depending on Genes' Polymorphism of Ace (I/D) and eNOS (894T>G) in Patients with Arterial Hypertension

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Abstract

Introduction: Humans genes mutations in altered social conditions through interaction with environmental factors and harmful habits become an individual risk factor.

Objectives: To evaluate Angiotensin-Converting Enzyme (ACE) and nitrogen monoxide metabolites ($NO/NO_2^-/NO_3^-$) blood levels depending on I/D polymorphism of ACE gene (dbSNP id:rs4646994), 894T>G of Endothelial Nitric Oxide Synthase (eNOS, dbSNP id:rs1799983) in pathogenesis of left ventricular hypertrophy (LVH) in patients with Essential Arterial Hypertension (EAH).

Methods: 120 screened patients with EAH I-III stages (48.3% – women, 51.7% – men, average age 52.9±9.24 years) and 40 healthy persons participated in prospective study. Alleles of polymorphic locus were studied by PCR method. Serum ACE level was detected by ELISA. Plasma NO metabolites levels were determined by colorimetric method. Structural myocardium changes – by EchoCG, ECG. Results analyzed according to the European guidelines ESC/ESH (2009).

Results: D-allele presence (ACE) associated with high risk of LVH geometric patterns and higher level of ACE. Presence of TT-genotype of eNOS gene (ID/TT-haplotype) associated with lower levels of NO metabolites by 14.5% ($p<0.05$). Homozygous D-allele carriers (regardless of genotypes combination of eNOS gene – DD/TG, DD/GG haplotypes) have ACE concentration increased by 18.1% and 17.5%, accordingly ($p<0.05$). The absence of mutations in haplotypes (II/GG) is a protective factor against LVH (OR=0.13, p=0.047), with the lowest level of ACE, followed by higher concentration of NO metabolites ($p<0.05$). The combination of D+T allele in haplotypes (ID/TG, DD/TG) increases the relative risk of LVH and EAH II and III in 1.19-2.25 times (OR=4.75-13.5, $p\leq0.021$-0.001), which is confirmed by severity of clinical course, and increased ACE serum level (DD/TG carriers).

Conclusion: Risk groups for eccentric or concentric LVH models are D-allele carriers (ACE gene) with highest ACE level and T-allele of eNOS gene with lower concentration of NO metabolites.

Key words: arterial hypertension, genetics, left ventricular hypertrophy, ACE, NO.

Background

Single- or polynucleotide replacement in the chromosome loci of genes-candidates for Essential Arterial Hypertension (EAH) associates with activity of the renin-angiotensin-aldosterone (RAAS), or Nitric Oxide Synthase (NOS) systems. This may manifest phenotypically as their hemodynamic expression or early damage of corresponding target-organs.[1,2] Interesting in this respect is the role of polymorphic markers of arterial hypertension (AH) candidate genes, which cover different parts of the system and tissue circulation regulation and proliferation of vascular smooth muscle cells and cardiomyocytes. These include genes of renin (R14+17G), angiotensinogen (AGT), angiotensin receptors (AGTR1, AGTR2), ACE and endothelial NOS (eNOS), endothelin-1 (BsiY1) and kallikrein (S1386A, A1789G), aldosterone (SYP11B2), β1-adrenoceptor (ADRβ1), catecholamines metabolism enzymes genes, glucagon, II β-hydroxylase, genes associated with insulin resistance (PC-1 k121q, PPAR-g2), etc. The role of mutations in key genes encoding RAAS activity, NOS and sympathoadrenal systems, in pathogenesis of target-organ damaging in EAH patients, including left ventricle hypertrophy (LVH), is not fully explored, available results are contradictory and differ greatly between populations and ethnic groups, with significant racial differences.[1,3] LVH develops in 60% patients with hypertension, determining the risk level of cardiovascular events (CVE).[4,5] However, LVH is not only a result of the hemodynamic component of the RAAS, NO and the sympathetic-adrenal system activity. 60% of LVH appear regardless of blood pressure (BP).[6-9] Some molecular and cellular mechanisms of RAAS, NO-system participation in the progression of cardio-vascular diseases (CVD), EAH and LVH realization, mainly studied in experimental models in vitro, including the morphological level.[6,7,10]

The aim of our research is to evaluate the blood levels of angiotensin-converting enzyme (ACE) and nitrogen monoxide metabolites (NO/NO_2-/NO_3-) depending on the I/D polymorphism of the ACE gene (dbSNP id: rs4646994), 894T>G polymorphism of Endothelial Nitric Oxide Synthase gene (eNOS, dbSNP id: rs1799983) and their role in the pathogenesis of LVH in patients with EAH.

Methods

Study was performed in compliance with the Council of Europe Convention on Human Rights and Biomedicine and recommendations of the Committee on Bioethics of the Ministry of Health of Ukraine. Research list and Informed Consent patients' Form were approved by the Biomedical Ethics Commission of Bukovina State Medical University, Ministry of Health of Ukraine (Chernivtsi, Ukraine). All enrolled patients were under observation in the Regional Clinical Cardiology Dispensary or municipal ambulatory hospital №1, or under the supervision of family physicians in Chernivtsi city (Ukraine) from 2007 to 2012. Genetic research performed

in the laboratory of Medical Biology and Genetics Department of Bukovina State Medical University.

The prospective study included 150 patients with EAH I-III stages of severity (WHO/ISH, 2003), and moderate, high and very high CV risk (ESC/ESH 2009, 2010) with average office BP higher than 140/90 mm Hg measured according to the ESH and ESC requirements (2009). Duration of EAH varied from 2 to 28 years (mean 15.73 ± 8.02 years).

After screening (matching inclusion/exclusion criteria) 120 EAH patients were selected for further examination. Groups' stratification by target-organ damage, complications, and CV risk was performed according to the classifications of WHO, national and European Societies of Cardiology and Hypertension (ESC, ESH, 2009, 2010). 15 patients (12.5%) were with EAH I stage (st) and moderate CV risk, 72 (60.0%) – with EAH II st and high CV risk, 33 (27.5%) - with EAH III st, high and very high CV risk; 58 (48.3%) women and 62 (51.7%) men, mean age 52.91±9.24 years, mean body weight 72.2±12.05 kg, height – 169.7±9.63 cm, body mass index (BMI) – 25.58±3.15 kg/m^2, respectively. The control group included 40 practically healthy individuals of appropriate age and gender ($p>0.05$).

Exclusion criteria were: symptomatic (secondary) hypertension, sub- and decompensate liver and kidney diseases, chronic heart failure (CHF) II class (NYHA) and higher, ejection fraction (EF) less than 45%, an acute coronary syndrome or stroke prior of 3 months, acute heart failure, diabetes mellitus type 1 (DM1), sub- and decompensated type 2 diabetes (DM2), mental disorders, corticosteroids administration, oral contraceptives, pregnancy and lactation period, acute inflammatory processes or exacerbation of chronic inflammatory processes regardless location.

Office systolic and diastolic BP (SBP, DBP), heart rate (HR) were measured according to European[4,5] and National[11] guidelines. 24-hour blood pressure monitoring (ABPM) performed with "ABPE-02" ("SOLVAIG", Ukraine, France) and "ABPM" ("Meditech", Hungary) according to standard protocol (40-55 measures/day). Data analysis was performed with the devices' software. BP results we described in previously published data.[8]

LVH estimated using M- and B-modes of Echocardiography (SonoAce8000 SE, "Medison", Korea), analyzed standard linear indicators of structural and functional state of the left ventricle, including the LV geometry. Left ventricular mass (LVM) was evaluated according to the Penn Convention, LVM index (LVMI) was calculated as the LVM to body surface area (g/m^2) ratio. LVH was evaluated according to the European Guidelines (ESH, ESC, 2009) criteria: LVMI in men ≥125 g/m^2, in women ≥110 g/m^2. According to LVMI and left ventricular relative wall thickness (RWT) the following geometric models of LV (ESH, ESC, 2009) were identified: normal LV geometry (LV NG), LV concentric remodeling (LV CR), eccentric LVH (LV EH), concentric LVH (LV CH). In addition, patients' examination list included ECG in 12 standard leads, Ultrasonography of kidneys and abdominal organs, clinical and biochemical analysis, consultations by ophthalmologist, neurologist.

The ACE level serum in 88 EAH patients was studied in enzyme immunoassay (ELISA) with "Quantikine®" (R&D Systems, Inc., USA) set of reagents. The nitrogen monoxide metabolites (NO_2+NO_3) concentration was determined in 30 EAH patients' plasma, stabilized with EDTA (1 mg/ml), by colorimetric method with "Total NO/NO_2-/NO_3-" (RDS, UK) set of reagents after recovery of nitrate to nitrite by nitrate-reductase enzyme.

Genomic DNA was extracted from peripheral blood leukocytes using the "DNA-sorb-B" test system, with primers specific to the genes' alleles.[12] Amplified polymorphic loci were detected by polymerase chain reaction (PCR) on "Amply-4L" device according to the manufacturer's protocol. PCR restriction fragment visualization was performed according to previously described methods to determine the I/D polymorphism of the ACE gene[13] and T894G polymorphism of the NOS gene.[14] Alleles' discrimination of eNOS gene was performed by restriction endonuclease BanII (Eco241) ("Fermentas", USA), digested for 16 hours at 37°C. Amplified DNA fragments were separated by gel electrophoresis, stained with xylene zianol, visualized by transluminator in the presence of molecular mass ladder (100-1000 bp).[9]

Statistical processing was performed with MS® Excel® 2003™, Primer of Biostatistics® 6.05 and Statistica® 7.0 (StatSoft Inc., USA) software. Differences in continuous variables between cases and controls were analyzed using the unpaired Student's t-test (distribution in Kolmogorov-Smirnov and W-Shapiro-Wilk test were close to normal) or Wilcoxon-Mann-Whitney U-test; analysis of qualitative data (categorical variables) were assessed using the chi-square test (χ^2), incl. 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 LVH or EAH severity were tested using odds ratio (OR) with 95% confidence intervals (CI). The differences were considered significant at $p<0.05$.

Results

Level of nitric oxide metabolites (total NO/NO_2-/NO_3-) in plasma of patients decreased with increasing CV risk and severity of EAH, but significantly only in patients with EAH III st., high and very high CV risk versus (vs) EAH I st. and moderate CV risk by 21.9% (22.82±1.96 vs 29.23±2.70 μmol/l, $p<0.05$). ACE concetration in serum reliably was higher in patients with target-organ damage, high and very high CV risk (EAH II and III stages) than in patients with moderate CV risk (EAH I st.) by 32.5% (33.10±5.07 vs 22.34±3.58 ng/ml, $p=0.05$) and by 38.0% (36.03±4.90 vs 22.34±3.58 ng/ml, $p=0.039$), respectively.

The distributions of all alleles and genotypes of analyzed genes were within the Hardy-Weinberg equilibrium. No changes of monoxide nitrogen metabolites were observed in dependence on ACE genotypes (table 1). The NO metabolites concentration was reliably lower in homozygous carriers of the mutant T-allele of eNOS gene than in patients with the G-allele (GG-and TG-genotypes): by 20.6% ($p<0.05$) and 15.8% ($p=0.05$), respectively. ACE level was higher in D-allele carriers of ACE gene (ID-and DD-genotypes) than in patients with the II genotype by 21.6% ($p=0.049$) and 32.0% ($p=0.028$), respectively, and does not differ between genotypes of eNOS gene.

LV NG was revealed in 10 (8.3%) patients with EAH I st; LV CR – in 15 (12.8%) patients: 5 persons with EAG I st, 10 – with EAG II st; LV EH – in 38 (31.7%) EAH patients: 25 subjects with EAG II st, 13 – with EAH III st; LV CH - in 57 (47.5%) subjects: 37 individuals with EAH II st, 20 – with EAH III st, ($\chi_2<1.0$, $p>0.05$). The distribution of ventricular geometric patterns depending on alleles of the analyzed genes: LV EH and CH occurred more often in D-allele carriers (DD + ID-genotypes) of ACE gene (85.3% and 88.7% vs 58.3% cases of II-genotype carriers, $p<0.001$), in T-allele (TT + TG-genotypes) of eNOS gene (100.0% and 89.1% vs 63.8%

cases of GG-genotype carriers, p≤0.014-0.003). The D-allele of ACE gene and T-allele of eNOS gene were identified as "damaging, mutant" alleles.

Analysis of possible genotypes combinations and distribution depending on the LV geometrical model is shown in table 2. The most frequent heterozygous combinations were ID/TG haplotypes – in 25.8% cases, and ID/GG, DD/TG – in 20.0% and 16.7%, respectively. The combination of homozygous "mutant" alleles of ACE gene and eNOS gene (D- and T-) was not detected. Genotypes combinations of DD/GG, II/TG and II/TG were distributed almost equally – 11.7%, 10.8% and 7.5%, respectively (p>0.05). ID/TT, II/TT haplotypes were observed less often, in 5.8% and 1.7% cases, respectively. Comparative analysis of the LV geometric patterns frequencies showed that in II/GG haplotype carriers NG and LV CR were revealed more often than in patients with II/TG- (χ^2=12.84, p<0.001), ID/TT- (χ^2=7.65, p=0.006) and ID/GG-haplotype (χ^2=6.23, p=0.013). LV hypertrophic models (LV EH and LV CH) were observed more frequently in ID/TG-haplotype patients, than in ID/GG-haplotype carriers (χ^2=6.70, p=0.01); in ID/TT-haplotype patients, than in II/TT patients (χ^2=4.37, p=0.037) with borderline advantage in II/TG-haplotype EAH patients (χ^2=3.76, p=0.052) (tab. 2).

ACE and nitrogen monoxide metabolites content depending on genotypes combinations is shown in table 3. The level of NO_2-/NO_3- was lower by 14.5% in the ID/TT haplotype carriers than in those with II/GG combination (p<0.05). The ACE level in patients with II/GG-haplotype was significantly lower than in homozygous D-allele carriers of ACE gene (DD/TG and DD/GG-combinations) by 18.1% and 17.5%, respectively (p<0.05). Presence of the TT-genotype of eNOS gene (including conjunction with unfavorable D-allele of ACE gene) is accompanied by a reliable decrease of nitric oxide plasma level. However, homozygous D-allele of ACE gene presence in EAH patients (regardless of the eNOS gene genotypes combination) is characterized by significantly higher concentration of ACE serum.

The nitrogen monoxide metabolites content in plasma was significantly lower in patients with LV CH (mostly patients with EAH II and III stages, high and very high CV risk) than in patients with normal geometric model of left ventricle (EAH I st, moderate CV risk) by 23.5% (p<0.05). ACE level was significantly higher in LV CH patients than in LV NG and LV CR by 38.1% and 24.6% (p<0.05), respectively (table 4). ACE concentration in patients with LV EH also prevailed such in patients with normal left ventricular geometry by 35.0% (p<0.05).

Risk analysis of pathological changes in the geometric structure of the LV in patients with EAH based on haplotypes (table 5) showed significant increase in probability of hypertrophic left ventricle (LV EG and LV CH) in ID/TG and DD/TG haplotype carriers at 1.34 and 2.25 times (OR=5.98, and OR=13.5, respectively, χ^2≤ 6.01-13.6, p≤0.015-0.001) at the lowest chances for normal geometry or concentric LV remodelling (OR=0.17 and OR=0.07, respectively, χ^2≤4.68-11.6, p≤0.031-0.001). The probability of favourable patterns of LV NG and LV CR in EAH patient with II/GG haplotype increases 2.53 times (OR=7.87, χ^2=5.14, p=0.04), and the risk of LV EG and LV KG become the lowest in the population (OR=0.13, χ^2=4.60, p=0.047).

Discussion

A number of studies have been devoted to the search of genetic markers that predispose to unfavorable clinical course of EAH with development of severe organ damage. However, the results are contradictory and vary depending on population or study.[7,15-17]

The main findings of our study showed association between I/D polymorphism of the ACE gene and 894T>G polymorphism of the eNOS gene and severity of EAH, presence and types of LVH: high-risk groups of LVH in patients with EAH are male carriers of DD-genotype and female carriers of D-allele of the ACE gene, and men with TT-genotype of eNOS gene. High-risk groups of the eccentric or concentric LVH models are D-allele carriers of ACE gene and the T-allele carriers of eNOS gene. The combination of D and T "mutant" alleles in haplotypes (ID/TG and DD/TG options) increases the relative risk of LVH and EAH II and III stages 1.19-2.25 times (OR=4.75-13, $p \leq 0.021$-0.001), correspondes with severity of clinical course of the disease, with the lowest chance for a normal geometry or concentric LV remodelling or milder EAH I st course (OR=0.07-0.17, $p \leq 0.031$-0.001). Our results suggest that D-allele and T-allele may contribute to unfavourable hypertrophic patterns of LV in hypertensive patients. We hypothesized that possible mechanism of LVH (LV CH and EH) realization in relation to D-allele of ACE gene is associated with high ACE serum concentration, in TT-genotype carriers of eNOS gene – with low level of nitrogen monoxide metabolites. Our data is partly compatible with studies conducted in Japanese population (n=87)[18], Uzbek population (n=172)[19], Polish.[20] O. Hitoshi[18] found reliable association of DD-genotype of ACE gene with concentric LVH, but not eccentric; with RWT, but not with LVMI. In addition, no association was found between ACE (I/D) genotypes and BP level. A. Ganau and R.B. Devereux[21] suggested that concentric LVH is a result of pressure overload, whereas eccentric LVH is caused by concomitant hemodynamic volume and pressure overload, which associates partially with our findings. In other Japanese study[22] no relationship was found between I/D polymorphism of the ACE gene and increased LVM in women with hypertension, whereas such association was observed in Japanese hypertensive men who had concentric LVH.

At present, it is not clear whether there is a relationship between the 894T> allele or 894G allele of eNOS gene and LVH and EAH severity. Results of existing studies are controversial. Our findings of the mutant T-allele of eNOS gene relationship with the EAH severity are consistent with the Bogalusa Heart Study[23], and the frequency of LVH – with the data of O.I. Yakovleva[24]. However, no relation is found between the size of LVH and geometric patterns.[24,25] The relationship between plasma NO metabolites, serum ACE level and genotypes of the ACE I/D and eNOS 894T>G genes observed in our study are very similar to those reported for other Caucasian and non-Caucasion populations[26-28] with limited information devoted to the haplotypes variants of ACE and eNOS genes distribution and association with ACE and NO levels, but not with LVH.[29,30]

Limitations of the Study

The present study was limited by a number of enrolled subjects. Furthermore, 105 of 120 consecutive patients were previously pharmacologically treated for essential hypertension.

Conclusion

The results of our study provide evidence for associations between ACE (I/D), eNOS (894T>G) polymorphism and EAH and LVH patterns in North Bukovina (Western Ukraine) population. The presence of D-allele of ACE gene in hypertensive patients associates with higher frequency of left ventricular hypertrophic geometric patterns (LV EH and CH) and higher levels of serum ACE. Presence of TT-genotype of eNOS gene (including conjunction with unfavorable DD-

genotype of ACE gene – ID/TT haplotype) is characterized by lower levels of total plasma NO metabolites. Homozygous D-allele of ACE gene (regardless of the genotypes combination of the eNOS gene – DD/TG and DD/GG) is accompanied by a significantly higher concentration of serum ACE. The combination of "mutant" D and T allele in haplotypes (ID/TG and DD/TG options) 1.19-2.25 times increases the relative risk of LVH and EAH II and III stages. The absence of mutations in haplotypes (II/GG variant) is a protective factor against LVH.

Thus, the variations of ACE, I/D and eNOS 894T>G are additional individual predictive risk factors of target-organ damage (LVH) in hypertensive patients. In perspective, additional studies are needed to determine the pathogenetic mechanisms by which the ACE (I/D) and eNOS (894T>G) genotypes and haplotypes are linked to the LVH patterns, structural changes of the elastic type arteries in hypertensive patients, additional relations to RAAS and NO-system for early detection of target-organ damage.

Conflict of Interest: None declared.

References

1. Paynter NP, Chasman DI, Buring JE, et al. Cardiovascular disease risk prediction with and without knowledge of genetic variation at chromosome 9p21.3 *Ann Intern Med.* 2009;150:65-72.
2. Perez-Cadahia B, Laffon B, Valdiglesias V, et al. Cytogenetic effects induced by Prestige oil on human populations: the role of polymorphisms in genes involved in metabolism and DNA repair. *Mutat Res*. 2008;653(1-2):117-123.
3. Conen D, Cheng S, Steiner L, et al. 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.
4. ESC and ESH Committee for Practice Guidelines. *Compendium of abridged ESC Guidelines 2010*. London, UK: Springer Healthcare; 2010.
5. Mancia G, Laurent S, Agabiti-Rosei E, et al Working ESC/ESH Group. Guidelines for the Management of Arterial Hypertension 2009. Reaprisal of European Guidelines on hypertension management: European Society of Hypertension (ESH) Task Force Document. *J. Hypertens*. 2009;27:2121-2158.
6. Deschepper CF, Boutin-Ganache I, Zahabi A, Jiang Z. In search of cardiovascular genes. Interaction between phenotypes and genotypes. *J Hypertens*. 2002;39:332-336.
7. Fraser R. Studying genes and the development of cardiac hypertrophy: convenient intermediate phenotypes in man. *J Hypertens*. 2003;21:873-874.
8. Sydorchuk LP, Amosova KM. Influence of pharmacogenetically determined treatment on parameters of peripheral hemodynamics in patients with arterial hypertension. *The New Armenian Medical J.* 2011;5(2):35-43.
9. Sydorchuk LP. *Pharmacogenetics of hypertension*. Chernivtsi: BSMU; 2010.
10. Sydorchuk II, Ushenko OG, Sydorchuk RI, Levitska SA. Laser polarimetry of biotissues. *Proceedings of SPIE (Coherent Optics of Ordered and Random Media)*. 2000;4242:218-227.
11. Working group on hypertension of the Ukrainian Association of Cardiology. *Order of the Ministry of Health of Ukraine № 384 from 24.05.2012. (Guidelines) Recommendations and clinical protocol of care "Hypertension": updated, adapted, evidence based*. Kiev: Ministry of Health of Ukraine; 2012.
12. Entrez Gene. Sequence analysis. National Center for Biotechnology Information. http://www.ncbi.nlm.nih.gov

13. Gardemann A, Fink M, Stricker J, et al. ACE I/D gene polymorphism: presence of the ACE D allele increases the risk of coronary artery disease in younger individuals. *Atherosclerosis*. 1998;139(1):153-159.
14. Hingorani AD, Liang CF, Fatibene J, et al. A common variant of the endothelial nitric oxide synthase (Glu298Asp) is a major risk for coronary artery disease in the UK. *Circulation*. 1999;100:1515-1520.
15. Rai H, Fitt J, Sharma AK, et al. Lack of association between Glu298Asp polymorphism and coronary artery disease in North Indians. *Mol Biol Rep*. 2012;39(5):5995-6000.
16. 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.
17. 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.
18. Oeno H, Takata M, Yasumoto K, Chin T, Hiroshi I. Angiotensin converting enzyme Gene Polymorphism and Geometric Patterns of Hypertensive Left Ventricle Hypertrophy. *Jpn Heart J*. 1999;40(5):589-598.
19. Kurbanova D, Eliseyeva M. Genetic background of left ventricular hypertrophy in Uzbek hypertensive men. *Arch Turk Soc Cardiol*. 2010;38(7):466-472.
20. Buraczynska M, Pijanowski Z, Spasiewicz D, et al. Renin-angiotensin system gene polymorphisms: assessment of the risk of coronary heart disease. *Kardiol. Pol.* 2003;58(1):1-9.
21. Ganau A, Devereux RB, Roman MG, et al. Patterns of Left Ventricle Hypertrophy and geometric remodelling in essential hypertension. *J Am Coll Cardiol*. 1992;19:1550-1558.
22. Kimura M, Yokota M, Fujimura T, et al. Association of a deletion polymorphism of the angiotensin-converting enzyme gene with left-ventricular hypertrophy in Japanese women with essential hypertension; multicenter study of 1,919 subjects. *Cardiology*. 1997;88:309-14.
23. Chen W, Srinivasan SR, Elkasabany A, Ellsworth DL, Boerwinkle E, Berenson GS. 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.
24. Yakovleva OI, Vakhrameeva NV, Larionov V. Polymorphism of endothelial NO-synthase and the structural and functional state of the large vessels in hypertensive patients with left ventricular hypertrophy. *Arterial Hypertens*. 2005;11(3):78-85.
25. 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.
26. Eisenhardta A, Sperlingb H, Hauck E, et al. ACE gene I/D and NOS3 G894T polymorphisms and response to sildenafil in men with erectile dysfunction. *Urology*. 2003;62(1):152-157.
27. Rahimi Z. ACE insertion/deletion (I/D) polymorphism and diabetic nephropathy. *J Nephropathology*. 2012;1(3):143-151. doi: 10.5812/jnp.8109.
28. Yoon Y, Song J, Hong SH, Kim JQ. Plasma nitric oxide concentrations and nitric oxide synthase gene polymorphisms in coronary artery disease. *Clin Chem.* 2000;46:1626-1630.
29. Ahsana A, Ram R, Baig MA, Qadar PM. ACE I allele and eNOS G allele crosstalk may have a role in chronic obstructive pulmonary disease. *Clinical Biochemistry*. 2004;37(11):1037-1040.
30. Kumar R, Nejatizadeh A, Ehtesham A, et al. Multi-locus interactions of vascular homeostasis genes in essential hypertension: A gender-based study. *Clinica Chimica Acta*. 2009;405:87-93.

Table 1: Angiotensin-converting enzyme level and monoxide nitrogen metabolites blood content in the hypertensive patients depending on the genotypes of ACE and eNOS genes

Genes	Genotypes, n=120 (%)	#	NO_2^-/NO_3^-, µmol/l	ACE, ng/ml
Control, n=40			35.60±3.45	20.53±4.26
ACE (I/D)	II, n=24 (20.0)	1	29.0±3.50	24.95±3.11
	DD, n=34 (28.3)	2	24.58±2.91 p<0.03	36.70±4.20 p<0.01 p_1=0.028
	I/D, n=62 (51.7)	3	27.6±3.67 p<0.05	31.81±2.16 p=0.018 p_1=0.049
eNOS (T894G)	GG, n=47 (39.2)	1	29.74±3.40	30.50±4.39 p<0.05
	TG, n=64 (53.3)	2	28.02±1.85	31.02±6.34 p<0.05
	TT, n=9 (7.5)	3	23.60±2.20 p<0.01 p_1<0.05 p_2=0.05	32.97±5.56 p<0.05

Notes:

– group supervision;
p – difference compared to the control group;
p_1 – difference compared to the group #1 (homozygous patients II ACE, GG eNOS, respectively);
p_2 – difference compared to the group #2 (heterozygous patients ID, TG, respectively).

Table 2: Geometric patterns of the left ventricle in patients with arterial hypertension depending on haplotypes of analyzed genes

Left ventricle geometric pattern, n (%)	Genotypes combination of ACE (I/D) and eNOS (894T>G) genes		
	II/TT, n=2 (%)	**II/TG, n=13 (%)**	**II/GG, n=9 (%)**
LV NG, n=3	0	0	3 (33.3)
LV CR, n=7	0	3 (23.1)	4 (44.4)
LV EH, n=7	1 (50.0)	5 (38.5)	1 (11.1)
LV CH, n=7	1 (50.0)	5 (38.5)	1 (11.1)
	ID/TT, n=7 (%)	**ID/TG, n=31 (%)**	**ID/GG, n=24 (%)**
LV NG, n=5	0	0	5 (20.8)
LV CR, n=5	1 (14.3)	2 (6.5)	2 (8.3)
LV EH, n=19	2 (28.6)	13 (41.9)	4 (16.7)
LV CH, n=33	4 (57.1)	16 (51.6)	13 (54.2)
	DD/TT, n=0 (%)	**DD/TG, n=20 (%)**	**DD/GG, n=14(%)**
LV NG, n=2	–	1 (5.0)	1 (7.1)
LV CR, n=3	–	1 (5.0)	2 (14.3)
LV EH, n=12	–	7 (35.0)	5 (35.7)
LV CH, n=17	–	11 (55.0)	6 (42.9)

Notes:

LV NG – normal geometry of left ventricle;
LV CR – concentric remodeling of left ventricle;
LV EH – eccentric hypertrophy of left ventricle;
LV CH – concentric hypertrophy of left ventricle;
n (%) – number (%) of observed patients.

Table 3: Angiotensin-converting enzyme and nitrogen monoxide metabolites blood contents depending on haplotypes

Genotypes combination of ACE (I/D) and eNOS (894T>G) genes	ACE, ng/ml	NO_2^-/NO_3^-, µmol/l
II/TT, n=2	28.84±4.74	26.05±2.85
II/TG, n=13	27.90±3.72	28.61±2.67
II/GG, n=9	27.72±2.01	29.37±1.25
ID/TT, n=7	33.30±3.86	25.12±2.73 $^{II/GG}$
ID/TG, n=31	30.94±2.23	27.64±2.07
ID/GG, n=24	31.18±4.25	28.50±2.18
DD/TG, n=20	33.86±3.27 $^{II/GG}$	26.30±4.38
DD/GG, n=14	33.60±3.29 $^{II/GG}$	27.16±3.15

Notes: The probability difference regarding certain haplotype elevated to degree ($p<0.05$).

Table 4: Angiotensin-converting enzyme and nitrogen monoxide metabolites blood contents depending on left ventricle geometric models

LV Geometric Model	NO_2^-/NO_3^-, µmol/l	ACE, ng/ml
LV NG, n=10 (8.3%)	30.12±2.08	22.08±2.46
LV CR, n=15 (12.8%)	27.09±2.60	26.89±3.40
LV EH, n=38 (31.7%)	23.95±4.33	33.97±5.03 $p<0.05$
LV CH, n=57 (47.5%)	23.04±3.71 $p<0.05$	35.66±4.17 $p,p_1<0.05$

Notes:

LV NG – normal geometry of left ventricle;
LV CR – concentric remodeling of left ventricle;
LV EH – eccentric hypertrophy of left ventricle;
LV CH – concentric hypertrophy of left ventricle;
p – difference compared to the patients with LV NG;
p_1 – difference compared to the patients with LV CR;
p_2 – difference compared to the patients with LV EH.

Table 5: Haplotypes of ACE and eNOS genes as risk factors for left ventricular geometry changes in patients with essential hypertension

Data		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
LV Normal Geometry or Concentric Remodeling	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;
95CI RR,OR– confidence interval of Risk Ratio (RR), Odds Ratio (OR);
LVH – left ventricle hypertrophy.