

Distribution of polymorphic markers of genes encoding the renin-angiotensin system (*ACE*, *AGT*, *AGTR1*), *ITGB3*, *PPARG* in patients with essential hypertension depending on the age

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Abstract—Essential hypertension (EH) is a multifactorial disease with a hereditary predisposition. Genes encoding the renin-angiotensin system (RAS) play a leading role in the stabilization of elevated BP in the course of EH, which determines the relevance of our clinical and genetic study. The study was based on the determination of the frequencies of polymorphic markers of the *AGT*, *AGTR1*, *ACE*, *ITGB3*, *PPARG* genes in 2 groups, depending on the age of patients (up to 60 years - group1, n=18, and after 60 years - group2, n=31), for the intergroup frequencies comparison and comparison of group frequencies with population data. Genotyping by gene polymorphisms was performed by real-time polymerase chain reaction. 24-hour ambulatory BP monitoring (ABPM) and Holter HR monitoring were conducted. Phenotypic features of the course of EH were accompanied by changes in the frequency characteristics of the studied genotypes. In patients of group 1, an increase in the frequency of protective genotypes of the *ACE(II)* and *ITGB3(TT)* genes was observed ($p=0.004;0.015$), which confirms the hypothesis of a possible favorable course of EH in patients under 60 years.

Keywords—Essential hypertension, renin-angiotensin system, gene polymorphism, *AGT*, *AGTR1*, *ACE*, *ITGB3*, *PPARG*, age, ABPM.

I. INTRODUCTION

ESSENTIAL hypertension (EH) is a multifactorial disease with a hereditary predisposition. Among the genes that affect the activity of the cardiovascular system with the development of this disease, a special place belongs to the genes encoding the components of the renin-angiotensin system (RAS), as a different degree of activation of this

system proteins depending on the gene polymorphism of the *ACE*, *AGTR*, *AGTR1* genes [1, 2, 3, 4, 5, 6] may lead to the formation of varying degrees of hypervolemia. Which is one of the pathogenetic mechanisms underlying the stabilization of elevated BP values. The inclusion of *ACE* (angiotensin converting enzyme gene) I/D polymer-phism in the study is traditional in the exploration of EH. The *ACE* gene, which is 22 kb in size, is mapped on chromosome 17 (17q23) and consists of 26 exons and 25 introns. In the 16th intron, insertion-deletion polymorphism was detected. The Alu Ins/Del I>D polymorphism of the *ACE* gene has two variants, differing in the presence (insertion, I) or absence (deletion, D) of the Alu sequence in the *ACE* gene intron. Different degrees of *ACE* gene expression are associated with this polymorphism. The overall activity of the enzyme in carriers of the D/D allele combination of *ACE* gene is 30% higher than in individuals with variant I/I. The presence of the D allele in the genotypes of *ACE* gene is considered as a predisposition factor for the development of EH and increases the frequency of kidney damage with the formation of chronic kidney failure, in particular, against the background of antihypertensive therapy in patients with EH [7, 8]. We also studied the A1166C polymorphism of the *AGTR1*(angiotensin type 1 receptor angiotensin 2 gene), which leads to the replacement of adenine (A) with cytosine (C) at the 1166 position of the *AGTR1* gene, in connection with the available literature data on the effect of mutation in this position of the nucleotide sequence on the functional activity of the angiotensin II receptor and its association with the risk of developing EH and DM type 2 [2]. The main function of this receptor is to bind angiotensin II, vasoconstriction, signal proliferation, and antagonism to nitric oxide (NO). The location of the *AGTR1* gene is the third chromosome.

The inclusion of the T/C polymorphism of the *AGT* (angiotensinogen gene), which leads to the amino acid replacement of M 235T, is explained by the fact that an

increase in the frequency of the C allele even in the heterozygous state leads to a change in the activity of the angiotensin protein [3]. The *AGT* gene is located at locus 1q42 of the short arm of the first chromosome. When studying the *AGT* gene, about 30 single-nucleotide polymorphisms were identified, most of them lead to aminoacid changes. The most studied allelic variants of mutations associated with the exchange of methionine (Met) for threonine (Thr) in codon 235 (Met → Thr or Met235Thr; M235T). There is also an evidence in the literature indicating the presence of a different hypotensive effect of *ACE* inhibitors depend on the M235T genotype of the *AGT* gene [9].

The receptor activated by the peroxisome proliferator, synthesized on the basis of the *PPARG* gene, is of considerable interest. Being a transcription factor, it is involved in the regulation of carbohydrate and lipid metabolism [10]. β -3 integrin, synthesized on the basis of the *ITGB3* gene is also of great interest. Being a component of the platelet fibrinogen receptor, TC polymorphism of which, traditionally denoted A1/A2, promotes irreversible platelet aggregation. The accretion of the frequency of allele C (A2) is associated with cardiovascular accidents [11]. This gene is located in the long arm of the seventh chromosome. The mutation (T1565C) leads to the substitution of the proline of aminoacid leucine in the 59th position and is represented in the form of two allelic variants, namely PLA1 and PLA2. We'd mark that in the European population, the frequency of the A2 allele is approximately 13-14%.

The *PPARG* gene encodes a protein-receptor activated by peroxisome proliferators, a gamma-nuclear receptor that regulates the expression of genes involved in cell differentiation, muscle tissue metabolism, determining the metabolism of fats and carbohydrates. Its activation and binding to the retinoid receptor *X* forms a heterodimer that interacts with specific DNA sequences that encode proteins involved in the metabolism of lipids and glucose. For the first time, the relationship of the polymorphic marker rs1801282 of the *PPARG* Pro12Ala gene located on chromosome 3p25 with an increased risk of developing DM type 2 was described in 1997. It was later shown that individuals homozygous for 12Pro have more expressed insulin resistance, obesity, dyslipidemia, and hypertension, and have 20% higher risk of developing DM type 2 compared to carriers of the 12Ala allele [12].

The need to study the clinical and genetic aspects of the formation of EH without metabolic syndrome, taking into account the age of patients, is due to its rare occurrence. At a young age, this form of hypertension often occurs covertly. There are conflicting literature data on the frequency characteristics of genes-candidates of EH depending on the patient's age [13, 14]. Thus, the aim of our study was to compare the phenotype of the course of EH depending on the age of patients with the frequency of occurrence of polymorphic variants of the RAS genes (*AGT*, *AGTR1*, *ACE*), *ITGB3*, *PPARG* to determine the possible impact of the selected genetic polymorphisms on the mechanisms of the formation of EH without metabolic syndrome (RAS genes) and on the possibility of complications in the form of kidney damage (*ACE* gene), an increase in the frequency of cardiovascular accidents (*ITGB3* gene), metabolic disorders (*PPARG* gene).

II. MATERIALS AND METHODS

The clinical and genetic study involved 49 patients who underwent examinations and treatment with their written informed consent at the Out-patient department of the City Clinical Hospital No 13 (Moscow). The size of the sample group 1 is conditioned by both the rare occurrence of EH without metabolic syndrome, the frequent latent course of this form of the disease in young patients, and the strict criteria used when including patients in the study. The most common criteria for exclusion from the study were the presence of concomitant pathology in the form of coronary heart disease and diabetes mellitus, secondary hypertension (Table 1) capable to change the course of EH. The exclusion of metabolic syndrome was carried out on the basis of the determination of waist circumference (WC), body mass index (BMI), glycated hemoglobin and the level of immune-reactive insulin (IRI). Patients were subdivided into 2 groups according to their age: Group 1 – patients with EH aged 60 years or less (n=18), Group 2 - patients with EH aged over 60 years (n=31). The justification of the stratification of the entire group by using 60 years as the cut-off is based on our own analysis and literature data, which indicate the presence of gender differences in patients before and after 60 years of age [15].

All the patients received adequate drug therapy aimed at correcting BP to reach the target values. Basically, a combination of two drugs was used (significantly more often in group 1). The gender and age structure and clinical characteristics of the study groups are presented in Table 1. To analyze hemodynamic parameters a control group (n=15) aged from 25 to 69 years was introduced into the study.

Genotyping gene polymorphisms of angiotensin converting enzyme (*ACE* rs 4646994, I/D), angiotensinogen (*AGT* rs699, M235T), angiotensin II type 1 receptor (*AGTR1* rs5186, A1166C), integrin β -3 (*ITGB3* rs5918, Leu33Pro), peroxisome proliferator-activated receptor (*PPARG* rs1801282, Pro12Ala) was performed by using the Real time-PCR method with utilizing commercially available kits (Syntol, Russia). The material for the study was DNA isolated from venous blood samples. Venous blood was collected from each participant into sterile EDTA tubes. The blood samples were stored at -20°C until DNA extraction. Genomic DNA was extracted from the peripheral blood by standard procedures using a commercially available blood DNA extraction kit ("DNA-extran-1", Syntol, Russia). The kit uses a three-step procedure: Blood Lysis, DNA precipitation, Washing and dissolving of DNA. Extracted DNA was stored at -20°C .

All the patients underwent 24-hour ambulatory BP monitoring (ABPM) and Holter HR monitoring from 8.00 to 8.00 next day. BP measurements were carried out from 8.00 to 22.00 with intervals of every 15 minutes and from 22.00 to 8.00 every 30 minutes. The obtained data was processed using the program EZDoctor 2.7. (AND, Japan) and the program of the company Schiller. The time of night sleep was compared with the records of the patient's diaries. The daily dynamics of BP and HR indicators were analyzed and the coefficient of variation (standard deviation/mean) was calculated to assess the homogeneity of groups according to hemodynamic parameters. In addition, the index time (% of the time during the day with changed BP indicators, and the index area (% of the area under the daily curve with changed BP parameters) were analyzed. According to the Holter

monitoring data, the presence or absence of autonomic dysfunction was determined. The % of patients with altered autonomic regulation of heart rhythm was evaluated. The changes based on analysis of the histogram of RR intervals were determined. In the absence of autonomic dysfunction, the normal distribution of the histogram was recorded (Fig. 1), with the shift of the vertex to the right – sympatheticotonia, to the left – there was an increase in the tone of the parasympathetic part of the autonomic nervous system.

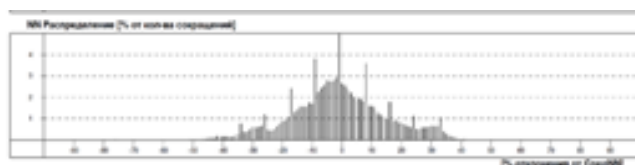


Figure 1. Histogram of the daily distribution of RR intervals without autonomic dysfunction

The double-humped distribution curve indicates simultaneous dysfunction of both parts of the vegetative nervous system (Fig.2). The observed change of vegetative heart rate control from sympathetic (day) to parasympathetic (night) is not accompanied by a change in the normal distribution of RR intervals on the daily histogram. Therefore, the shift of the hump to the right or to the left, or the appearance of 2 humps on the daily histogram of RR intervals may be regarded as a manifestation of autonomic dysfunction in the regulation of heart rhythm. This method of analyzing autonomic dysfunction from our point of view has an advantage over spectral analysis. It preserves the discreteness of the studied parameter value, and does not transform it into the category of continuous quantities which may lead to the loss of some information.

For an adequate description of the statistical methods we used, the following explanations are required. For assessing significance of differences in the studied quantitative indicators authors used the concept of the error of representativeness of sample indicators, which monotonically decreases with increasing sample size and therefore depends on its size. However, the error of representativeness depends not only on the size of the sample, but mostly on the method of selecting its elements, which should be random. In our case, the selection of patients was carried out in accordance with a very strict deterministic clinical criterion, excluding the presence of concomitant pathology in the form of secondary EH, EH with metabolic syndrome, coronary heart disease and diabetes mellitus, which may change the course of EH. At the same time, the genetic component of each selected patient remained unknown *a priori*, until the genetic analysis was performed *a posteriori*. For this reason, the selection of the genetic component of this study was random, and therefore the sample groups of our study were representative samples from the point of view of the genetic component, despite the small size. Therefore, to conduct a reliable statistical analysis, one can use criteria for assessing significance not depending on the small sample size. According to the theory of statistical inference, if the obtained results are clearly significant with such criteria, checking by a more powerful criteria for large samples is not required. Therefore, our statistical data processing was

carried out taking into account the small size of sample group 1, n=18. The significance of differences in the studied quantitative indicators (Table 1, 2) were evaluated by the nonparametric Mann-Whitney U-test in the Biostat® program (Praktika Publishing House 1998, version 3.03). The significance of differences of frequency shares (%) in Table 3 were evaluated by the Fisher ϕ^* criterion at the level of $p \leq 0.05$ when comparing the data of groups 1 and 2

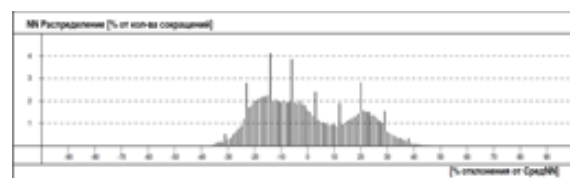


Figure 2. Histogram of the daily distribution of RR intervals with autonomic dysfunction

with each other (2 groups) and at the level of $p \leq 0.025$ when comparing groups 1 and 2 with population data (3 groups) to exclude false positive results when conducting multiple comparisons. Both methods (Mann-Whitney and Fisher ϕ^*) have no restrictions on the small size of the representative samples. In order to eliminate errors during multiple comparisons, the reliable results obtained in Table 3 were verified by using the Holm-Bonferroni correction. In addition, the verification of homogeneity of the studied groups for hemodynamic parameters, based on the use of coefficient of variation (standard deviation/mean), was made.

A. Clinical and Laboratory Findings

First of all, it is necessary to justify the stratification of the entire group by using 60 years as the cut-off. The fact is that there is a clinical concept that the early development of hypertension is associated with more severe clinical variants of its course. However, our own clinical analysis does not support this concept – the disease flowed more favorably, which was reflected in the absence of beta-blockers and calcium antagonists in the list of drug's antihypertension therapy. In addition, our own analysis and literature data [15, 16, 17] indicate the presence of gender differences in patients under and over 60 years of age.

Analysis of the data in Table 1 shows a significant difference between Groups 1 and 2 in the duration of the disease and in the levels of the main biochemical parameters such as creatinine, urea, the level of microalbuminuria (MAU) and IRI.

Table 1. Clinical and laboratory features of the analyzed groups of patients.

Indicator	Group 1 (n=18)	Group 2 (n=31)
Age, years	43.2±2.8	73.9±1.27*
Men, %	61	32*
Women, %	39	68
BMI, kg/m ²	25.05±0.48	28.1±0.16
WC, cm	87.9±1.04	92.4±1.03
Duration of hypertension, years	8.9	15.4*
Dipper, %	58	45
Non-dipper, %	23	30
Over-dipper, %	6	9

Night peaker, %	13	16
ACE and receptor ACE block, % of patients	94	81
β-blockers, % of patients	0	19*
Diuretics, % of patients	22	71*
Calcium channel blockers, % of patients	0	19*
HbA1c,(6) %	5.5	6
Creatinine,(64-104) mmol/l	71.2±2.76	93.66±2.21*
Urea,(3-9) mmol/l	3.02±0.22	7.97±0.11*
UIA,(0.3) mg/l	0.4±0.001	0.7±0.003*
IRI(2-25)mkED/ml	6.5±3.09	19.4±1.86*

* $p < 0.05$ in comparison with Group 1;

BMI – body mass index;

WC – waist circumference;

HbA1 – glycated hemoglobin;

MAU – microalbuminuria;

IRI – immune-reactive insulin;

UIA – microalbuminuria.

But the values of the parameters were within the normal range, even without taking into account the age.

The main significant difference that could affect the frequency characteristics of the genotypes of the studied genes was the patients' gender: in Group 1 men and in Group 2 women were significantly more common. This gender distribution of patients who were older and younger 60 years of age was noted earlier in the literature [18]. The analysis of the applied treatment allows us to indirectly consider the differences in pathogenetic mechanisms of EH formation. In particular, for Group 1, considering the use of only ACE inhibitors and receptors to angiotensin II (94%) in combination with diuretics (22%), we can discuss activating RAS proteins as the leading mechanism of the pathogenesis of hypertension in this group. The use of beta-blockers (19%) and calcium channel blockers (19%) in Group 2 suggests the involvement of the sympathoadrenal system in the realization of pathogenetic mechanisms of aggravated hypertension together with the activation of RAS proteins (ACE inhibitors and ARBs – 81%, diuretics – 71%). Analysis of histograms of RR intervals obtained by Holter monitoring confirmed that the patients of group 1 significantly more likely had signs of increased tone of the parasympathetic part of the vegetative nervous system (50% in group 1 vs. 29% in the second group).

B. Comparative Analysis of Daily Values of Hemodynamic Parameters

Since the analysis of hemodynamic parameter indicators (Table 2) of this study was carried out under antihypertensive therapy, the sensitivity to which depends on the activity of RAS proteins, it is important to prove the homogeneity of both groups in terms of the studied parameters. The data on the coefficient of variation of BP and HR hemodynamic parameters is presented in Table 2 to prove the homogeneity of the analyzed groups. After determining the homogeneity, the actual analysis of hemodynamic parameters was carried out.

Table 2. Parameters of daily hemodynamics.

Indicator	Control (n=15)	Group 1 (n=18)	Group 2 (n=31)
Office SBP, mmHg	123.8±1.2	153.9±1.03*	152.6±1.1*

Indicator	Control (n=15)	Group 1 (n=18)	Group 2 (n=31)
Office DBP, mmHg	75.6±1.74	88.3±1.85*	85.6±1.13*
Daytime SBP, mmHg	121.3±1.85	142.4± 2.7*	137.9±1.08*
Nighttime SBP, mmHg	104.6±2.05	133.4±3.91*	126.3±2.31*▼
Daytime DBP, mmHg	76.8±2.01	82.7± 2.83*	77.4 ±1.08▼
Nighttime DBP, mmHg	63.6±1.01	72.5± 2.65*	64.7± 1.85▼
Average daytime HR	75.9±1.24	77.1± 1.79	70.8 ±1.29*▼
Average nighttime HR	64.3±3.14	64.8± 1.41	60.5±0.86▼
Daily CV for SBP, %	1	9	6
Daily CV for DBP, %	1.2	12	10
Daily CV for HR, %	10	10	10
Time index of the SBP, %	22.9±3.21	53.7 ±5.18*	59.7± 3.67*
Time index of the DBP, %	18.4±2.78	41.05± 5.4*	32.2± 2.88*
SBP area index, %	4.9±3.42	10.5± 2.06*	14.5±1.73*
DBP area index%	4.7±0.08	4.4±0.94	7.9± 0.97*

* $p < 0.05$ in comparison with the Control;

▼ – $p < 0.05$ in comparison Group 1.

The patients of Groups 1 and 2 did not have any significant differences in mean BP, but differed in the level of nighttime systolic blood pressure (SBP), diastolic blood pressure (DBP) and HR. Moreover, in Group 2, DBP area index% was significantly higher compared to Group 1. The groups did not differ in the profile of nighttime BP. Groups 1 and 2 had significant differences in the hemodynamic parameters compared to the control group. The analysis of the coefficient variation (CV) for BP and HR is given in Table 2 to prove the homogeneity of the analyzed groups.

C. Results of Genetic Analysis

Taking into account the obtained differences in the features of the clinical course of EH in groups 1 and 2, the presence of significant gender differences in the groups and the nature of the therapy used, one can expect that the genetic component may be involved in the basis of phenotypic differences in the course of the disease. For its determination, a comparison analysis of the frequencies of alleles and genotypes of the studied genes was performed. Mapping of the frequency characteristics of genotypes and alleles of the studied genes was carried out both with population data (from these studies, the frequencies of genotypes of genes in healthy individuals who made up the control group were taken) and between groups themselves (Table 3) In order to eliminate errors during multiple comparisons, the reliable results obtained in Table 3 were verified by using the Holm-Bonferroni correction.

Comparative analysis of the frequency characteristics of the genotypes of two studied groups (Table3) revealed a decrease in the frequency of the genotype D of ACE gene with a probability of 89%, a significant increase ($p < 0.017$) in the in the frequency of the TT genotype and a decrease ($p < 0.003$) in the frequency of TS genotype of the ITGB3 gene in patients of group 1. Also in this group, a decrease in the frequency of CC genotype of the PPARG gene was

detected with a probability of 92%. The obtained data confirmed the validity of the subdivision of patients with hypertension, using the 60-year as a cut-off. Taking into account the size of the samples, this results were obtained with help of the Fisher criterion ϕ^* . This method has no restrictions on the size of the samples and evaluates the significance of differences between frequency shares (%) of two studied samples.

Table 3. Frequencies of genotypes and alleles of genes *AGT*, *AGTR1*, *ACE*, *ITGB3*, *PPARG*.

Genotypes (replacement of amino acids), Alleles	Group 1 (n=18)	Group 2 (n=31)	$p \leq 0.05$	Population frequencies	$p \leq 0.025$
Gene <i>ACE</i> rs4646994					
<i>II</i>	0.588 ^x	0.388	0.09	0.261 (n=117) ¹	0.004 (1) 0.098 (2)
<i>ID</i>	0.25	0.29	ID	0.443	0.053 (1) 0.057 (2)
<i>DD</i>	0.162	0.322	0.097	0.296	0.097 (1)
Allele <i>D</i>	0.287	0.467	0.11	0.517	0.031 (1)
Gene <i>AGT</i> rs699					
<i>TT (M235M)</i>	0.205	0.188	ID	0.314 (n=115) ²	0.071 (2)
<i>TC (M235T)</i>	0.54	0.577 ^x	ID	0.40	0.02 (2)
<i>CC (T235T)</i>	0.255	0.255	ID	0.21	ID
Allele <i>C</i>	0.53	0.54	ID	0.41	0.097 (2)
Gene <i>AGTR1</i> rs5186					
<i>AA</i>	0.54	0.48	ID	0.66 (n=115) ²	0.03 (2)
<i>AC</i>	0.375	0.48	ID	0.294	0.027 (2)
<i>CC</i>	0.085	0.04	ID	0.046	ID
Allele <i>C</i>	0.27	0.28	ID	0.193	ID
Gene <i>ITGB3</i> rs5918					
<i>TT (A1/A1)</i>	0.915 ^{x*}	0.67	0.017	0.724 (n=858) ³	0.015 (1)
<i>TC (A1/A2)</i>	0.0425 ^{x*}	0.33	0.003	0.257	0.003 (1)
<i>CC (A2/A2)</i>	0.0425	0	0.081	0.019	0.055
Allele <i>C (A2)</i>	0.0638 ^x	0.165	ID	0.15	ID
Gene <i>PPARG</i> rs1801282					
<i>CC (Pro12Pro)</i>	0.4965	0.70	0.08	0.69 (n=556) ⁴	0.042 (1)
<i>CG (Pro12Ala)</i>	0.42	0.27	ID	0.28	ID
<i>GG (Ala12Ala)</i>	0.0835	0.03	ID	0.03	ID
Allele <i>G</i>	0.2935	0.17	ID	0.17	ID

* $p \leq 0.05$ for differences in the genotypes and alleles between Groups 1 and 2;

^x $p \leq 0.05$ for differences of the studied groups in comparison with the population data: 1 – [4]; 2 – [20]; 3 – [21]; 4 – [22];

ID – invalid difference.

Analysis of the frequency characteristics of the genotypes of the studied genes revealed unidirectional changes in the frequency of genotypes in the analyzed groups compared to the population data for the genotypes of the *AGT* and *ATRI* genes. Thus, for the *AGT* genotypes, there was a decrease in the frequency of the *TT* genotype and an increase in the frequency of the *TC* genotype. The frequency of the latter had a significant difference from the frequency of this genotype in the population ($p \leq 0.02$).

III. DISCUSSION

Since earlier studies showed an increase in the activity of angiotensin II by 5% in the presence of a heterozygous *TC* genotype of the *AGT* gene, this genotype may increase the risk of hypertension and is attributed to genetic markers of hypertension [3, 23]. For the *ATRI* gene, there was a decrease in the *AA* genotype for both groups compared to the population data (for group 2 probability 97%) and an increase in the frequency of the *AC* genotype in group 2

compared to the population data (probability 98.3%). Considering the fact that the replacement of adenine in the 1166 position by cytosine leads to a change in the functional activity of *AT II* receptors, an increase in the frequency of this genotype is also considered as one of genetic markers of EH [24].

Changes in the frequency characteristics of the *ACE*, *ITGB3*, and *PPARG* genotypes have already been considered from the point of view of the development of possible complications of EH. It should be noted that the patients of Group 1 were characterized by the dominance of protective alleles of all the three genes.

Thus, for Group 1, there was an increase in the frequency of genotype II of the *ACE* gene and a significant decrease in the *D* allele ($p \leq 0.031$) compared to the population $p \leq 0.004$. A significant increase in the frequency of the *TT* (*A1/A1*) genotype of the *ITGB3* gene ($p \leq 0.015$) compared to the population data can be considered as a protection against further development of cardiovascular accidents in patients of group 1. When comparing the frequencies of the studied genotypes in the groups up to 60 (Group 1) with population frequencies, the tendency towards decrease ($p \leq 0.042$) for the allele *C* of the *PPARG* gene was revealed, which may be associated with a reduced risk of developing insulin resistance and DM type 2 in patients of this group [5]. For patients after 60, according to the polymorphisms of these three genes, their frequencies corresponded to the population data. It should be noted that the implementation of increased BP in group 2 occurs without the interest from the *ID* genotype of the *ACE* gene with the participation of the *TC* genotype of the *AGT* gene and the *AC* of the *AGTR1* gene. The frequency characteristics of the allele *C* of the *ITGB3* gene responsible for the development of cardiovascular accidents, the allele *C* of the *PPARG* gene responsible for the formation of metabolic syndrome, and the allele *D* of the *ACE* gene responsible for kidney damage were at the general population level, therefore, the risks of developing these complications in EH also corresponded to the population risks.

IV. CONCLUSION

The features of the selection process of patients for the analysis of the role in the study of the genetic component of the essential hypertension consisted in eliminating the combination of essential hypertension and coronary heart disease, as well as in excluding from the analysis patients with altered insulin sensitivity (the level of immunoreactive insulin in the blood serum was determined). This approach made it possible to identify a group of patients up to 60 years old, whose genome was dominated by protective ones in terms of changes in the activity of *RAS* proteins (*ACE* gene), cardiovascular catastrophes (*ITGB3* gene) and the possibility of the formation of a metabolic syndrome (*PPARG*) in the future. These results were obtained for the first time and suggest that in the presence of these protective polymorphisms in patients under 60 years, a favorable course of essential hypertension may be expected. The revealed clinical differences between the patients of group 1 and 2 in the course of EH without metabolic syndrome corresponded to the differences in the distribution of alleles and genotypes according to the studied gene polymorphisms

in the intergroup comparison and group comparison with population data. This allows us to discuss the possibility of the existence of phenotypic differences in the course of EH in patients of group 1 and 2, which are genetically realized in different frequencies of the studied genotypes of the *ACE*, *ITGB3* and *PPARG* genes. It should be noted that the protective genotypes of these genes dominated in the patients of group 1, which also corresponds to the clinical data on the absence of renal complications in this group (creatinine level 71.2 ± 2.76 mmol/l, UREA level 3.02 ± 0.22 mmol/l, MAU 0.4 ± 0.001 mg/l) associated with the protective genotype *II* of the *ACE* gene. The absence of cardiovascular pathology in the form of CHD, on the one hand, is a selection criterion, but it is genetically ensured by the dominance of the protective *TT* genotype of the *ITGB3* gene. It should also be noted that for group 1 the revealed trend towards a decrease in the occurrence of the *CC* genotype of the *PPARG* gene and, accordingly, an increase in the occurrence of the *G* allele, from our point of view, can be used to objectify exactly the EH form without a metabolic syndrome, since with the dominance of the *G* allele, the risk of developing a metabolic syndrome is decreased. This is due to the fact that the altered properties of the protein synthesized with the participation of the *G* allele of the *PPARG* gene lead to the fact that the – receptor activated by peroxisome proliferates ceases to respond to the altered activity of RAS proteins, as a result, there is no realization of the metabolic effects of RAS proteins. Actually, by results of the study, the *AC* genotype of the *AGTR1* gene and the *TC* genotype of the *AGT* gene are involved in the formation of EH in both groups.

Thus, it can be concluded that in the current study we succeeded to reveal the domination of the protective genotypes of the *ACE*, *ITGB3* and *PPARG* genes in the group of patients under 60 years of age (men predominated). This result confirms the concept that early onset of hypertension is not always associated with a severe course of this disease.

REFERENCES

- [1] M. Singh, A. K. Singh; P. Pandey, S. Chandra, K. A. Singh, I. S. Gambhir, “Molecular genetics of essential hypertension”. *Clin. Exp. Hypertens*, 38(3), pp. 268-277, 2016, doi: 10.3109/10641963.2015.1116543.
- [2] T. Yu. Zotova, A. P. Kubanova, M. M. Azova, Amira Aut Aissa, “Peculiarities of the clinical picture of arterial hypertension depending on polymorphic variant of *AGTR1* gene”. *Clinical Medicine*, 95(5), pp. 404-407, 2017, doi: <http://dx.doi.org/10.18821/0023-2149-2017-95-4-404-407>.
- [3] M. E. Dickson, C. D. Sigmund, “Genetic basis of hypertension: revisiting angiotensinogen”. *Hypertension*, 48(1), pp. 14-20, 2006, doi: 10.1161/01.HYP.0000227932.13687.60.
- [4] O. S. Glotov, A. S. Glotov, O. A. Tarasenko, T. E. Ivaschenko, V. S. Baranov, “Analysis of *ACE*, *AGTR1*, *eNOS*, *MTHFR*, *MTRR* and *APOE* genes polymorphisms in the population of North-West of Russia”. *Ecological genetics*, 2, pp. 32-35, 2004, doi: 10.17816/ecogen2332-35.
- [5] O. S. Pavlova, S. E. Ogurtsova, M. M. Liventseva, T. H. Lakotko, I. Y. Korobko, V. I. Shyshko, A. G. Mrochek, “Association of the I/D polymorphism of angiotensin converting enzyme gene with the development of essential hypertension”. *The Siberian Journal of Clinical and Experimental Medicine*, 34(3), pp. 87-96, 2019, <https://doi.org/10.29001/2073-8552-2019-34-3-87-96>.
- [6] A. Jones, S. S. Dhamrait, J. R. Payne, E. Hawe, P. Li, I. S. Toor et al., “Genetic variants of angiotensin II receptors and cardiovascular risk in hypertension”. *Hypertension*, 42(4), pp. 500-506, 2003, <https://doi.org/10.1161/01.hyp.0000088853.27673.d0>.
- [7] A. V. Baranova, O. Yu. Fadeva, R. R. Shilyaev, I. V. Bedina, E. G. Kuznetsova, “The role of polymorphism of I/D angiotensin converting enzyme in the development of tubulointerstitial kidney lesions”. *Bulletin of the Ivanovo Medical Academy*, 17, pp. 47-50, 2012.
- [8] Amal Al-Eisa, Mohammad Z. Haider, “B Angiotensin converting enzyme gene insertion/deletion polymorphism in idiopathic nephrotic syndrome in Kuwaiti Arab children Strivastva Scand”. *J. Urol. Nephrol*, 35, pp. 239–242, 2001, <https://doi.org/10.1080/003655901750292033>.
- [9] N. V. Drobotya, L. V. Arutyunyan, A. A. Pirozhenko, V. V. Kaltykova, “Prospects of using genetic testing to increase the effectiveness of antihypertensive therapy”. *Russian Journal of Cardiology*, 25(5), p. 3911, 2020, <https://doi.org/10.15829/1560-4071-2020-3911>.
- [10] S. Azhar, “Peroxisome proliferator-activated receptors, metabolic syndrome and cardiovascular disease”. *Future Cardiol*, 6(5), pp. 657-691, 2010, doi: 10.2217/fca.10.86.
- [11] M. Khatami, M. M. Heidari, S. Soheilyfar, “Common rs5918 (PLA1/A2) polymorphism in the *ITGB3* gene and risk of coronary artery disease”. *Arch. Med. Sci. Atheroscler. Dis.*, 1(1), pp. e9-e15, 2016, doi: 10.5114/amsad.2016.59587.
- [12] D. K. Sanghera, P. R. Blackett, “Type 2 Diabetes Genetics: Beyond GWAS *J. Diabetes Metab*”, 3(05), pp. 2–17, 2012, <https://doi.org/10.4172/2155-6156.1000198>.
- [13] E. G. Lakatta, “The reality of getting old”. *Nature Reviews Cardiology*, 15(9), pp. 499–500, 2018, DOI: 10.1038/s41569-018-0068-y.
- [14] Z. Sun, “Aging, arterial stiffness, and hypertension”. *Hypertension*, 65(2), pp. 252-256, 2015, doi: 10.1161/HYPERTENSIONAHA.114.03617.
- [15] Y. V. Kotovskaya, J. D. Kobalava, “Features of arterial hypertension in old age *Consilium medicus*”, 6(12), 2004, [old.consilium medicum.com](http://old.consilium-medicum.com).
- [16] K. I. Ibragimova, S. N. Mammaev, J. A. Omarova, “Gender-specific regulation of blood pressure and antihypertensive treatment”. *Arterial Hypertension*, 24(3), pp. 303-308, 2018, <https://doi.org/10.18705/1607-419X-2018-24-3-303-308>.
- [17] M. E. Statsenko, T. G. Shcherbakova, O. E. Sporova, S. V. Belenkova, Yu. V. Kalashnikova, “Arterial hypertension treatment effectiveness in elderly patients”. *Russian Journal of Cardiology*, 6, 27-32, 2008.
- [18] Y. A. Balanova, S. A. Shalnova, A. D. Deev, V. V. Konstantinov, A. V. Kapustina, “Arterial hypertension prevalence in Moscow in different time intervals”.

Arterial Hypertension, 19(2), 102-108, 2013, doi: <https://doi.org/10.18705/1607-419X-2013-19-2-102-108>.

- [19] Z. V. Maksimova, D. M. Maksimov, "Hypertension in working age population: influence of gender and education". *Kardiologiya*, 60(2), pp. 24-32, 2020, <https://doi.org/10.18087/cardio.2020.2.n441>.
- [20] A. R. Tuguz, N. A. Agadzhanian, P. P. Lysenko, T. M. Ashkanova, E. N. Anokhina, "Frequencies of Met235Thr, Thr174Met polymorphisms of the angiotensinogen gene (AGT) and A1166C allele of the angiotensinogen - 2 receptor type 1 (AGT2R1) in ethnic groups of the population of Maykop". *Modern problems of science and education*, 3, p. 15, 2011, <https://science-education.ru/ru/article/view?id=4693>.
- [21] I. A. Goncharova, N. P. Babushkina, L. I. Minaïcheva, V. V. Markova, E. V. Kulish, R. R. Salakhov, O. A. Makeeva, V. P. Puzyrev, "Prevalence of alleles of polymorphic variants Leu33Pro and Leu66Arg gene ITGB3 among inhabitants of Siberia". *Genetika*, 49(8), pp. 1008-1012, 2013, PMID: 25474888.
- [22] I. A. Bondar, M. L. Filipenko, O. Y. Shabelnikova, E. A. Sokolova, "Association between gene PPARG RS1801282 PRO12ALA and type 2 diabetes in Novosibirsk region and other populations". *The Siberian Journal of Clinical and Experimental Medicine*, 29(2), pp. 75-78, 2014, <https://doi.org/10.29001/2073-8552-2014-29-2-75-78>.
- [23] T. Yu. Zotova, M. M. Azova, A. A. Lukanina, A. Ait Aissa, M. L. Blagonravov, "Distribution of polymorphic marker of genes of the renin-angiotensin system RAS (AGT, AGTR1, ACE), ITGB3, PPARG) in patients with essential arterial hypertension depending on the nature of the nocturnal decrease of BP". *International journal of biology and biomedical engineering*, 15, pp. 212-217, 2021, DOI: 10.46300/91011.2021.15.24.
- [24] A. Bonnardeaux, E. Davies, X. Jeunemaitre, I. Féry, A. Charru, E. Clauser, L. Tiret, F. Cambien, P. Corvol, F. Soubrier, "Angiotensin II type 1 receptor gene polymorphisms in human essential hypertension". *Hypertension*, 24(1), pp. 63-69 1994, doi: 10.1161/01.hyp.24.1.63.

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