

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.

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Abstract — A clinical-genetic study using ABPM (24-hour BP monitoring) and Holter's ECG methods in 49 patients with essential arterial hypertension (group 1: 17 patients without sufficient nocturnal BP decrease $CI \leq 10\%$, and group 2: 32 patients with sufficient nocturnal BP decrease $CI \geq 10\%$.) was performed for comparative analysis of the genotype frequencies of *ACE*, *AGT*, *AGTR1*, *ITGB3*, and *PPARG*. The study was conducted in order to clarify the pathogenetic mechanisms of the implementation of different dynamics of nocturnal blood pressure in patients with hypertension without metabolic syndrome. It was found that in group 1, protective genotype *II* of the *ACE* gene was more common ($p \leq 0.025$) than in the population data. A significant increase ($p \leq 0.025$) in the frequency of the *CC* genotype of the *AGTR1* gene responsible for the formation of insulin resistance compared to the population data was combined with a significant increase in the frequency of autonomic dysfunction in patients of group 1 - 83.4% vs. 64.5% group 2 respectively. The results obtained indicate the possible pathogenetic links between genetically determined insulin resistance and autonomic nervous system dysfunction and allows us to determine therapeutic approaches for correcting the nocturnal blood pressure profile.

Key words: ABPM, essential arterial hypertension (EAH), RAS gene polymorphism (*AGT*, *AGTR1*, *ACE*), *ITGB3*, *PPARG*, nocturnal decrease of BP.

I. INTRODUCTION

Essential arterial hypertension (EAH), being a multifactorial disease, also has a genetic component in the form of polymorphism of candidate genes implemented in single-nucleotide DNA substitutions. These substitutions, by changing the properties of proteins, provide a change in the activity of, for example, proteins of the renin-angiotensin system

(RAS) [1.2.3]. It is the increased activity of RAS proteins in carriers of polymorphic markers of RAS genes that ensures the stabilization of elevated blood pressure at hypertension and provides a pathogenetic link with tissue effects. In this regard, the study of the frequency characteristics of genotypes in the presence of polymorphic gene variants is aimed at identifying candidate genes that increase the risk of developing EAH and its specific clinical forms-occurring with and without a nocturnal decrease of BP [4,5,6,7,8]. The selection of polymorphic markers of the *AGTR1* and *PPARG* genes was aimed at clarifying the role of the genetic component in the formation of nocturnal insulin resistance

The study of the diurnal chronostructure of BP in EAH led to the formation of the concept of a nocturnal BP, determined on the basis of the diurnal index (DI), according to which patients are subdivided in 4 different groups: dippers, non-dippers, over-dippers and night - pickers. According to the literature, the absence of a nocturnal decrease of BP is associated with the realization of vascular dysfunction against the background of a vegetative imbalance in the form of sympathicotonia and a decrease in the characteristic nocturnal period of increasing the tone of the parasympathetic nervous system [7]. Taking into account the pathogenetic links of increased activity of RAS proteins with activation of the sympathetic link of the nervous system, observed both at the central level (sodium-dependent mechanisms) and at the peripheral level (increased release of norepinephrine in sympathetic terminals) [8], the study of the frequency of occurrence of RAS gene polymorphisms (*ACE*, *AGT*, *AGTR1*) in a group of patients with hypertension without nocturnal decrease in BP (group 1) and with nocturnal decrease in BP (group 2), in combination with the study of the frequency of genotypes of the gene *ITGB3* (whose polymorphic marker, *A2*, increases the risk of vascular catastrophes [9]) and the *PPARG* gene responsible for the formation of insulin

resistance [5] (allele C (Pro12Pro)) in patients with metabolic syndrome.

Thus, the aim of the study was to clarify the pathogenetic mechanisms of the implementation of different dynamics of nocturnal blood pressure in patients with hypertension without metabolic syndrome.

II. MATERIALS AND METHODS.

In the study were included 49 patients, undergoing treatment and examination in Moscow polyclinic at the State Clinical Hospital No.13, with their written informed consent, without concomitant pathology that can change the nature of the course of EAH including metabolic syndrome. The volume of the sample is determined by the low frequency of occurrence of the isolated form of AH and the strictness of the selection criteria.

The study did not include patients with concomitant pathology that can change the nature of the course of arterial hypertension, patients with metabolic syndrome, ischemic heart disease.

To analyze the genotype frequencies for the studied genes, 2 groups of patients were formed: group 1 (n=17) patients with EAH with no nocturnal decrease in BP (non-dippers and night-pickers 100%, $CI \leq 10\%$) and group 2 (n=32) patients with EAH who had a nocturnal decrease in BP (dippers and over-dippers 100%, $CI \geq 10\%$).

All patients received adequate therapy that reached the target values. The gender and age composition and clinical characteristics of the studied groups are presented in Table 1. For the analysis of hemodynamic parameters, a control group (n=15) aged 25 to 69 years was included into the study. Genotyping by gene polymorphisms of angiotensin converting enzyme (ACE rs4646994, I/D), angiotensinogen (AGT rs699, M235T), angiotensin II type 1 receptor (AGTR1 rs5186, A1166C), integrin β -3 (ITGB3 rs5918, Leu33Pro), peroxisome proliferator-activated receptor (PGARG rs1801282, Pro12Ala)

was performed by a real-time polymerase chain reaction using the NPF Synthol and Litech reagent kits.

The material for the study was DNA isolated from a sample of venous blood. For comparison, we used the frequencies of the analyzed gene polymorphisms in healthy individuals in population studies [10,11,12,4] conducted on the territory of the Russian Federation, taking into account the race of patients, since the frequency characteristics of the studied genotypes depend on these parameters.

All patients underwent ABPM (24-hour arterial BP monitoring) and 24-hour Holter's ECG monitoring. The data obtained were processed by using the EZDoctor program (AND, Japan) and the MT-100 program (Schiller, Switzerland). The average analyzed indicators: Office BPS, mmHg, Office BPD, mmHg, DayBPS, mmHg, NightBPS, mmHg, DayBPD, mmHg, NightBPD, mmHg, DayHR, b/s, NightHR, b/s, Time load BPS - % of the time during the day with the changed values of BPS, Time load BPD - % of the time during the day with the changed values of d BPD, Area load BPS - % of the area under the curve of daily BP with the changed values of BPS, Area load BPD-% of the area under the curve of daily BP with the changed values of BPD are shown in Table 2.

Holter HR monitoring was performed to detect autonomic dysfunction: a qualitative analysis of the distribution form of RR intervals was performed. The normal form of distribution indicates the absence of autonomic dysfunction, skew to the

III.RESULTS AND DISCUSSION.

The data presented in Table 1 allow us to speak about the balance of both groups by gender, age, absence of metabolic syndrome and the level of reduction of the renal barrier (MAU). The patients of both groups did not differ in the duration of the disease and the nature of the therapy.

Despite the therapy, both groups differed significantly from the control group in terms of the main hemodynamic average indicators and the load index by time, as well as by area, except for the area index for BPD in group 1 (Table 2).

Table1. Clinical and laboratory average indicators (M±m) of the analyzed groups of patients

Indicator	Group 1 (n=18)	Group2 (n=31)
Age, years	60,9±4,22	63,7±3,17
Men, %	44	42
Women, %	56	58
BMI, kg/m ²	26,9±0,49	27,6±0,37
WC, cm	91,4±1,27	90,4±1,06
Duration of hypertension, years	12,6±1,19	13,3±1,27
HbA1c, %	6%	5,7%
Creatinine, mmol / l	84,44±3,73	85,97±2,99
Urea, mmol / l	6,44±0,6	5,99±0,45
MAU, mg / l	0,6±0,01	0,5±0,002

IRImkED/ml	12,7±1,58	15,3±1,1
Antihypertensive therapy, % number of patients		
ACE and rec.ACE block	88 (n=15)	78 (n=25)
β-block	11 (n=2)	13 (n=4)
Diuretics	59 (n=10)	47 (n=15)
Calcium antagonists	24 (n=4)	28 (n=9)

Where: MAU-microalbuminuria, IRI-immunoreactive insulin, ACE - angiotensin converting enzyme and receptor ACE blocker.

Table 2. Hemodynamic average indicators in the studied groups

Indicator	Control (n=15)	Group 1 (n=18)	Group 2 (n=31)
Office BPS, mmHg	123,8±1,2	155,3±1,1*	151,8±1,02*
Office BPD, mmHg	75,6±1,74	87,5±1,9*	86,1±1,1*
DayBPS, mmHg.	121,3±1,85	137,5±2,28*	140,7± 1,4*
NightBPS, mmHg.	104,6±2,05	142,8±2,29*•	120,8±1,77*
DayBPD, mmHg.	76,8±2,01	76,5± 1,8	81,1± 1,68
NightBPD, mmHg.	63,6±1,01	73,6±2,36*•	64,1± 1,87
DayHR, b/s	75,9±1,24	73,5 ±1,64	72,9 ±1,51
NightHR, b/s	64,3±3,14	63,4±1,52	61,3± 0,9
Time load BPS %	22,9±3,21	55,7±5,43*	58,2± 3,48*
Time load BPD %	18,4±2,78	33,6± 5,6*	36,2± 2,72*
Area load BPS %	4,9±3,42	12,6±3,1*	13,3±1,18*
Area load BPD%	4,7±0,08	5,5±1,28	7,3±0,9*

* p <0.05-the significance of differences between control and groups 1 and 2;

• p<0.05-the significance of the difference between groups

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

Genotypes, Alleles	Group 1 (n=17)	Group 2 (n=32)	p≤ 0,05*	Population frequencies	p≤ 0,05 ^x
Gene AGT rs699					
TT(M235M)	0,18	0,187	ID	0,314 (n=115) ¹	p2=0,07
TC(M235T)	0,47	0,593^x	ID	0,40	p2=0,02
CC(T235T)	0,35	0,22	ID	0,21	ID
Allele C	0,585	0,52	ID	0,41	p1=0,084
Gene AGTR1 rs5186					
AA	0,35^{*x}	0,593	0,045	0,66 (n=115) ¹	p1=0,008
AC	0,53^x	0,407	ID	0,294	p1=0,033
CC	0,12 [*]	0 ^x	0,009	0,046	p2=0,015
Allele C	0,385	0,204	0,091	0,193	p1=0,052
Gene ACE rs4646994					
II	0,71^{*x}	0,32	0,03	0,261(n =117) ²	p1=0,001
ID	0,29	0,25^x	ID	0,443	p1=0,067 p2=0,018
DD	0 ^x	0,41[*]	0,001	0,296	p1=0,001 p2=0,11

Allele D	0,145 ^{*x}	0,54	0,001	0,517	p1=0,001
Gene <i>ITGB3</i> rs5918					
<i>TT</i>	0,82	0,72	ID	0,724 (n=858) ³	ID
<i>TC</i>	0,18	0,25	ID	0,257	ID
<i>CC</i>	0	0,03	ID	0,019	ID
Allele <i>C</i>	0,09	0,15	ID	0,15	ID
Gene <i>PPARG</i> rs1801282					
<i>CC(Pro12Pro)</i>	0,65	0,593	ID	0,69 (n=556) ⁴	ID
<i>CG(Pro12Ala)</i>	0,29	0,343	ID	0,28	ID
<i>GG(Ala12Ala)</i>	0,06	0,064	ID	0,03	ID
Allele <i>G</i>	0,2	0,24	ID	0,17	ID

* p < 0.05-the significance of differences in genotypes and alleles between groups 1 and 2;

^xp ≤ 0.05-the significance of the difference between the studied groups in comparison with the comparison with the population data 1 – [10]; 2 - [11]; 3- [12]; 4 - [4]; ID – invalid difference

The analysis of the frequency characteristics of the genotypes of the studied genes we begin with the *ITGB3* gene that reflects platelet-vascular dysfunction. On the basis of this gene, the platelet receptor integrin-3 is synthesized, which is responsible for the irreversible aggregation of platelets. In this study, the frequency characteristic of the minor C (A2) allele responsible for the development of vascular pathology in patients with ACS and arterial hypertension developing within the framework of the metabolic syndrome [4,9] was not increased, and in group 1 there was a tendency to increase the protective TT genotype and decrease the C allele of this gene.

The analysis of the *AGT* rs699 gene genotype frequencies, which leads to the replacement of M235T, led to the following results: in both groups, there was a decrease in the frequency of the TT genotype in comparison with the population data (p ≤ 0.025). Thus, the increase in the frequency of the TC genotype of the *AGT* gene in comparison with the population data unites both groups within the general pathology in the form of EAH.

The analysis of changes in the frequency characteristics of the ACE gene I/D polymorphism led to the following results. For group 1 of patients with essential hypertension, there was a significant increase in genotype II and a decrease in the D allele both in the population and in the intergroup comparison. Group 2 patients showed a typical increase in the DD genotype of the ACE gene, which leads to an increase in the activity of the angiotensin protein in the blood plasma of patients with hypertension and is considered as a candidate gene that increases the frequency of EAH development [1].

Further, we analyzed the frequency of polymorphic markers of the *AGTR1* rs5186 and *PPARG* rs18001282 genes. The minor allele C of the *AGTR1* rs5186 gene and the major allele C (Pro12Pro) of the *PPARG* rs18001282 gene are considered in the literature as risk factors for the development of insulin resistance [4,5,6]. At the same time, the mechanisms of their implementation are different. Thus, the

PPARG rs18001282 gene, providing the synthesis of the gamma receptor activated by peroxisome proliferators, ensures the implementation of the tissue effects of RAS by an increase in the activity of proteins of this system. It should be noted that for both groups, there was no change in the frequency characteristics of the genotypes of this gene in comparison with the population data. However, the normal nocturnal decrease in BP, which develops against the background of an increase in the tone of the parasympathetic link of the innervation of the autonomic nervous system, was accompanied by the development of insulin resistance, which develops against the background of the action of contrinsular factors that provide endogenous nutrition of the body during sleep. The analysis of the *AGTR1* gene genotype frequencies suggests that a significant increase in the frequency in group 1 of patients without an adequate nocturnal decrease in BP of the AC, CC genotypes may be accompanied by a change in the nature of the autonomic regulation of the cardiovascular system. To confirm the presence of autonomic dysfunction, Holter HR monitoring was performed and it was found that in group 1, autonomic dysfunction was significantly more common: 83.4% versus 64.5% in group 2 patients.

Thus, our study provides the explanation of the specific mechanisms of the implementation of insulin resistance at night in patients with hypertension with the lack of adequate nocturnal blood pressure reduction, which occurs, among other things, due to the activation of the sympathetic link of the autonomic nervous system, which was observed in 64.5% of cases in group 1 versus 45.1% in the second group of patients.

IV. CONCLUSION

Currently EAH is considered from the standpoint of not only a multifactorial disease, but also a disease with different phenotypes. Thus, EAH and AH that develops within the framework of the metabolic syndrome are distinguished. The studied pathogenetic differences in the mechanisms of formation of these two clinical forms of the disease can be conditionally reduced to an assessment of the role of insulin resistance, including those that develop normally and at night, and the balance of local and central mechanisms for the implementation of insulin resistance, their relationship with the activity of RAS proteins.

This problem is most significant for patients with EAH in the absence of adequate BP reduction (group 1). The absence of pronounced differences in the frequency characteristics of the PPARG gene compared to the population data indicates the absence of the role of local tissue factors in the development of insulin resistance in patients with EAH of both groups. The dominance of the AC genotype of the AGTR1 gene in patients without nocturnal BP reduction (group 1), combined with the predominance of autonomic dysfunction in this group, indicates the presence of possible pathogenetic relationships of RAS proteins with autonomic regulation of HR in this group of patients. This makes it possible to predict the probability of developing an altered nocturnal BP profile and to develop therapeutic approaches for correcting insulin resistance in a specific group of patients based on the study of polymorphic markers of the AGTR1 gene[13].

In addition, the predominance of the protective genotype II of ACE gene allows to exclude kidney damage as a cause of fixing the nocturnal increase in BP. We believe that differences in the frequencies of polymorphic markers of RAS genes may be the leading pathogenetic mechanism that provides a combina-

tion of insulin resistance with the formation of autonomic dysfunction and form the basis for the creation of an altered nocturnal BP profile.

V. SUMMARY

The results obtained in this study suggest that the polymorphic marker of the AGTR1 gene and autonomic dysfunction are involved in the formation of an altered nocturnal blood pressure profile in group 1 patients. The role of the renal factor is unlikely due to the dominance of the genotype II of ACE gene in group 1 patients.

VI. LIST OF ABBRIVIATIONS

EAH- essential arterial hypertension
BP – blood pressure
ACE-angiotensin converting enzyme
BPD – diastolic blood pressure
BPS-systolic blood pressure
HR – heart rate
ARBs-angiotensin II receptor blockers
BMI – body mass index
MAU-microalbuminuria
WC-waist circumference
IRI- immune-reactive insulin
ABPM - 24-hour артериальное blood pressure monitoring
RAS-renin-angiotensin system
CI-circadian index
AGT-angiotensinogen
AGTR1-angiotensin I receptor
ITGB3-Integrin beta-3
HbA1c-glycosylated hemoglobin
PPARG-receptors activated by the peroxisome proliferator
IHD - ischemic heart disease

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Author's Contributions.

All authors contributed to this study:

Zotova T. Yu - conceptualization, methodology, data curation, writing—original draft preparation, supervision;

Azova M.M - methodology. data curation, visualization, investigation;

Lukanina A.A.- formal analysis, software, investigation, writing—original draft preparation;

Ait Aissa A. – formal analysis, investigation;

Blagonravov M.L – validation, data curation, writing—review and editing, supervision, project administration, funding acquisition.

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