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INSULIN RESISTANCE IN PATIENTS WITH ARTERIAL HYPERTENSION AND ABDOMINAL OBESITY DEPENDING ON ACE AND PPAR-γ2 GENES POLYMORPHISM: A NEW OPINION CONCERNING AN OLD PROBLEM

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Abstract

The objective of this study was to determine an association of insertion-deletion (I/D) polymorphism of ACE (ACE) gene (dbSNP ID: rs4646994) and Pro12Ala polymorphism of peroxisome proliferator-activated receptor (PPAR) γ2 gene (dbSNP ID: rs1801282) with functional activity of pancreatic β-cells and signs of insulin resistance in patients with essential arterial hypertension depending on its severity, body weight and haplotype of the analyzed genes in the population of Western Ukrain (Bukovina).

III degree essential arterial hypertension patients with high and very high cardiovascular risk, and II and III degree abdominal obesity fasting plasma glucose levels, immunoreactive insulin, and HOMA-IR index were higher, while HOMA-Fβ index was lower, than that of other patients. Homozygous presence of PPAR-γ2 gene Pro12-allele in the haplotype of EAH patients, regardless of ACE gene allele condition (II/Pro12, ID/Pro12, DD/Pro12), is accompanied by higher levels of immunoreactive insulin and glycemia for ID/Pro12, DD/Pro12 carriers. The presence of II-genotype and Ala-allele in the haplotype (II/12Ala, II ProAla variants) is favorable and associated with lower HOMA-IR, as well as higher pancreatic β-cells functional activity of HOMA Fβ index. The haplotypes of the analyzed genes are not the risk factors of hyperglycemia and insulin resistance in normal body weight or overweight EAH patients. However, the presence of abdominal obesity and the presence of homozygous Pro-allele of PPAR-γ2 gene and D-allele of ACE gene in the haplotype (ID/Pro12, DD/Pro12 combinations) in EAH patients increases a relative risk of hyperglycemia and insulin resistance: for ID/Pro12 haplotype – 2.46 times, for DD/Pro12 – 2.44 times, respectively.

The presence of ProPro-genotype of PPAR-γ2 gene and D-allele of ACE gene in the haplotype is an independent additional risk factor of hyperglycemia and insulin resistance development in patients with EAH accompanied by abdominal obesity.

Keywords: *ACE (I/D) genes polymorphism, peroxisome proliferator-activated receptor γ2 (Pro12Ala), arterial hypertension, abdominal obesity, insulin resistance.*

Introduction

The risk factors that substantially determine the prognosis for patients with arterial hypertension are: dyslipidemia, abdominal obesity, disorders of glucose metabolism and development of insulin resistance, or diabetes mellitus (DM) [*Working group on hypertension of the Ukrainian Associa-*

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tion of Cardiologists, 2012; Mancia G et al., 2013]. Many researchers concluded, that arterial hypertension in 35-50% cases is accompanied by the formation of metabolic syndrome [*Girman CJ et al., 2005*]. Susceptibility of patients with arterial hypertension to insulin resistance is a distinctive adaptation of the body to environmental changes to maintain "energy balance" and normal functioning of all organs and systems, which in its turn is realized in connection with the genotype of a certain individual. Adipocytes of the visceral fat are known to secrete small amounts of about 40 kinds of cytokines, adipokines (adiponectin, leptin), resistin, grelin, PPAR- γ transcription factor, plasminogen-1 activator inhibitor, and the macrophages, which accumulate in the adipose tissue as it enlarges in size, additionally synthesize tumor necrosis factor-a [*Fasshauer M, 2003*]. Even so, anti-inflammatory cytokines of the adipose tissue disturb insulin signal transmission into the cell [*Ritchie SA et al., 2004*]. Adipocytes synthesize free fatty acids as well, which enter the systemic blood flow through the portal vein. In the liver free fatty acids are utilized through the activation of gluconeogenesis increasing glucose production and decreasing activity of phosphatidilinozitol-3-kinase of the insulin receptors, which in its turn disturbs glucose transport inside the cell, resulting in hyperglycemia (lipotoxic effect) and insulin resistance development; the second way is the synthesis of triacylglycerols which are highly atherogenic. Another aspect of the problem of insulin resistance formation is reduced gene expression of the nuclear family γ (γ1-γ3) of PPARs, especially PPAR-γ2, under conditions of "chronic diet saturated with lipids", which control differentiation and proliferation of adipocytes and free fatty acids deposition in the adipose tissue, and not in the skeletal muscles or liver [*Tsuchida A et al., 2005*].

However, a true role of metabolic syndrome, insulin resistance and leptin resistance in continuous cardio-vascular diseases is still being studied. In recent decade an active scientific research is conducted to find molecular-genetic mechanisms of insulin resistance formation in patients with arterial hypertension and abdominal obesity, regulation of carbohydrate metabolism activity, their place in the development of cardio-vascular complications or more severe course of the underlying disease with the aim of early diagnostics, prognosis and timely therapeutic correction of metabolic disorders [*Sydorchuk LP, Amosova KM, 2011; Sydorchuk LP et al., 2013*]. This direction of medical investigations is rather promising and topical on the current period of modern medicine development.

The objective of the present study is to determine association of insertion-deletion (I/D) polymorphism of ACE gene (dbSNP ID: rs4646994) and Pro12Ala polymorphism of PPAR-γ2 gene (dbSNP ID: rs1801282) with functional activity of pancreatic β-cells and signs of insulin resistance in patients with essential arterial hypertension (EAH) among residents of West Ukraine (Bukovina) depending on EAH severity, body weight and haplotypes of the analyzed genes.

Materials and Methods

Clinical study was performed at the No.1 Municipal Clinic in the city of Chernivtsy, Ukraine, from September 2010 to April 2014. Genetic examination was conducted at the Laboratory of Medical Biology and Genetics Department at Bukovinian State Medical University (BSMU). Patients' Examination Card and Patient's Informed Consent Form were approved by the Biomedical Ethics Board of BSMU, the Ministry of Public Health of Ukraine. Prospective study was performed in compliance with the Council of Europe Convention on Human Rights and Biomedicine, Helsinki Declaration of the World Medical Association on ethical principles to conduct scientific medical research with human participation and the Recommendations of the Committee on Bioethics of the Ministry of Public Health of Ukraine.

Screening, diagnosing, distribution of patients by groups depending on target organs damage and risks, abdominal obesity degrees, was conducted in accordance with the recommendations of the Ukrainian Society of Cardiology, European Society of Cardiology and European Society of Hypertension (ESC/ESH 2013), American Heart Association, as well as current orders of the Ministry of Public Health of Ukraine [*Jensen MD et al., 2013; Mancia G et al., 2013; Working group on arterial hypertension of the Ukrainian Association of Cardiologists, 2012*]. Screening was performed on 110 patients with EAH, overweight, or abdominal obesity, who signed an Informed Consent to participate in the

study with further venous blood work for genetic analysis. The patients were 25 to 79 years old (average age = 53.3±6.05 years). The patients included 56.4% (62) women and 43.6% (48) men. There were 22.7% (25) individuals with stage I EAH, 45.45% (50) patients with stage II EAH, and 31.8% (35) patients with stage III EAH. Among them were 8.18% (9) individuals with normal body weight, 38.2% (42) overweight, 53.6% (59) with abdominal obesity: 27.3% (30) of them with I degree abdominal obesity, 17.3% (19) – with II degree abdominal obesity, and 9.09% (10) – with III degree abdominal obesity. The control group was comprised of 50 practically healthy individuals who were not relatives to the patients under study and without reliable differences of sex and age.

Office systolic and diastolic blood pressure and heart rate were measured. All the patients underwent a complex of examinations: waist and thigh circumferences, body mass index (BMI, kg/m^2), according to which the degree of abdominal obesity was determined [*Jensen MD et al., 2013*], ECG in 12 leads, ultrasound examination of the kidneys, general clinical and biochemical analyses, consultations by ophthalmologist and neurologist. Immunoreactive insulin (*mkU/ml*) and C-peptide (*ng/ml*) were detected on an empty stomach in the venous blood plasma by an Immunoenzyme test (ELISA, DRG, USA). Insulin resistance was defined by the formula HOMA-IR index = Fasting glucose (μ*mol/L*) x Fasting insulin (μU/*ml*) / 22.5. The function of the Langerhans β-cells pancreatic island was estimated by C-peptide and integral HOMA-Fβ index (Homeostasis Model Assessment of β-Cell Function), (%): (20 x insulin) / (glucose – 3.5) [*Hill NR et al., 2013*].

Alleles of the polymorphic areas of ACE (I/D) and PPAR-γ2 (Pro12Ala) genes were studied by means of Genomic DNA extraction from the peripheral blood leukocytes using the DNA-sorb-B test system, with primers specific to the genes' alleles: for PPAR-γ2 gene (forward 5′–GAAACTGATGTCTTGACTCATGGGTG–3′ and reverse 5′-CAACCTGGAAGACAAACTACA AGAGC-3′), for ACE gene (forward 5′–GCCGGGGACTCTGTAAGCCACTGC–3′ and reverse 5′-CCTTGTCTCGCCAGCCCTCCCA-3′). Amplified polymorphic locus was detected by polymerase chain reaction on "Amply-4L" amplifier according to the manufacturer's protocol [*Entrez Gene. Sequence analysis, 2014*]. Alleles' discrimination of PPAR-γ2 gene was performed by Cse I (HgaI) endonuclease restriction (Fermentas®, Lithuania). I/D polymorphism amplification products of ACE gene and restriction products of PPAR-γ2 gene Pro12Ala polymorphism were separated by the horizontal electrophoresis in 3% agarose gel stained with 4 μl of ethidium bromide (45-60 *min*). The obtained polymerase chain reaction fragments of ACE I/D gene polymorphism (I-allele – 553 base pairs (*bp*), D-allele – 263 *bp*) and restriction fragments of PPAR-γ2 Pro12Ala gene polymorphism (Pro-allele – 305 *bp*, Ala-allele – 140 and 165 *bp*) were visualized by UV transluminator (Nyxtechnic, USA) in the presence of molecular mass ladder (100-1000 *bp*) (SibEnzyme, Russia).

Statistical processing was performed using Statistica® 7.0 (StatSoft Inc, USA) software. Reliability of the data for independent sampling was calculated using unpaired Student's t-test (distribution by Kolmogorov-Smirnov and W-Shapiro-Wilk test was close to the normal) and U-test *Wilcoxon-Mann-Whitney* (in case of irregular distribution), analysis of qualitative data (categorical variables) – by chi-square test (χ^2) (with the frequency less than 5 – Fisher Exact Probability Test). The influence of haplotypes as risk factors on insulin resistance development and hyperglycemia was estimated by the value of relative risk (RelR), risk ratio (RR) and odds ratio (OR) with 95% confidential interval [95% CI] considering χ^2 criterion (df=1), using logistic regression model. The differences were considered significant at $p<0.05$.

Results

Among the patients of high and very high cardiovascular risk with stage III EAH and abdominal obesity were 25 individuals with compensated DM type 2: 7 patients with III degree abdominal obesity, 16 patients with II degree abdominal obesity, 2 patients with I degree abdominal obesity. Average parameters of the plasma fasting glucose level and HOMA-IR index in EAH III stage patients were higher than in EAH I and II stages patients and the control group (Table 1): glucose level by 29.5% (p=0.012), 23.8% (p=0.043) and 32.9% (p=0.008), HOMA-IR - by 49.8% (p=0.007), 45.0% (p=0.002) and 67.6% (p=0.002), respectively. Immunoreactive

insulin and C-peptide were also higher in EAH III stage patients than in the control by 54.2% (p=0.019) and 48.5% (p=0.031) respectively. But the pancreatic Functional Activity of β-cells (HOMA-Fβ) in these patients was 33.3% lower (p=0.021), than in EAH I stage subjects.

Dynamics of carbohydrate metabolism data in patients with EAH depending on the body weight and abdominal obesity degree (Table 2) showed reliably bigger content of fasting plasma glucose, immunoreactive insulin, HOMA-IR index in individuals with EAH and II and III degree abdominal obesity than those with the normal body weight, by 32.5% and 30.1%, 46.7% and 46.0%, 68.4% and 63.5% (p≤0.052-0.001), and on the contrary HOMA-Fβ index was 39.25% lower (p=0.029) and 32.9% (p=0.003), respectively. The indices of immunoreactive insulin, HOMA-IR index in patients with EAH and II and III degrees abdominal obesity were also higher than those in overweight individuals by 36.9% (p=0.045) and 36.1% (p=0.019), 54.1% (p=0.003) and 52.0% (p=0.0025), in case of lower HOMA-Fβ – by 32.25% (p=0.003) and 25.1% (p=0.008), respectively (Table 2).

Homozygous presence of Pro12-allele in the haplotype (Table 3) (II/Pro12, ID/Pro12, DD/Pro12) regardless of ACE gene allele condition, is accompanied by 23.9-32.6% (p<0.05) higher level of immunoreactive insulin and 29.4% (p=0.012) and 23.7% (p=0.006) higher level of glycemia for ID/Pro12, DD/Pro12 carriers, respectively, than in homozygotic carriers of I-allele of ACE gene and Ala-allele of PPAR-γ2 gene (II/12Ala, II/ProAla), whose levels are 14.6% and 14.8% (p<0.05) higher, respectively. The presence of II-genotype and Ala-allele in haplotype (II/12Ala, II/ProAla) is favorable and is associated with lower index of HOMA-IR by 26.9-41.25% (p≤0.03-0.003), than in Pro12-allele homozygote subjects in haplotype (II/Pro12, ID/Pro12, DD/Pro12); it is also associated with a high index of functional activity of pancreatic β-cells HOMA-Fβ by 46.8-55.7% (p≤0.024-0.048) (Table 3).

Haplotypes as potential risk factors of insulin resistance development and hyperglycemia in patients with EAH are presented in Table 4. Hyperglycemia condition was detected with fasting glucose concentration more than 6.1 μ*mol/L* (ESH, ESC 2013), and insulin resistance condition with HOMA-IR index >3.0 U. Epidemiological analysis found a reliably high probability of hyperglycemia and insulin resistance development in case of ProPro-genotype and D-allele presence in haplotype: ID/Pro12 and DD/Pro12 combinations [OR=4.09; p=0.026 and OR=3.38; p=0.045, respectively] (Table 4).

In patients with EAH with normal weight and overweight the haplotypes of analyzed genes are not risk factors to provoke hyperglycemia and insulin resistance. Although abdominal obesity in patients with EAH with presence of ProPro-genotype and D-allele (ID/Pro12, DD/Pro12) in haplotype increases a relative risk of hyperglycemia and insulin resistance: for ID/Pro12 haplotype –2.46 times [OR=4.80; p=0.036], for DD/Pro12 –2.44 times [OR=4.71; p=0.024].

Discussion

The results of numerous population studies indicate clear correlation link between the MS risk and obesity: with the increase of BMI to 35 kg/m^2 the risk increases 42 times in men and 92 times in women [*Girman CJ et al., 2005; Tomlinson JW et al., 2008*]. The cause of MS development is the ability of inflammatory mediators such as TNF-a, interleukins (IL-1β, IL-6), C-reactive protein, plasminogen-1 activator inhibitor to provoke insulin resistance with a cascade of metabolic disorders [*Vardeny O et al., 2013*]. These processes are controlled within the norm condition due to peroxisome receptors γ activation (PPAR-γ), which are expressed mostly in the adipose tissue and regulate hyperplasia and hypertrophy of adipocytes [*Tsuchida A et al., 2005*]. Although, the question of genetic predisposition to insulin resistance development, carbohydrate homeostasis changes remains unsolved, and the results of the former studies are contradictory.

PPAR-γ receptors, being transcriptional mediators of adipogenesis and susceptibility to insulin, play a crucial role in inflammation of the cells in case of arterial hypertension, cardiac hypertrophy, congestive chronic heart failure and atherosclerosis [*Ostgren CJ et al., 2003*]. According to the data of some researche, "loss-of-function" mutation of PPAR-γ2 gene (loss of Pro-allele) results in insulin resistance reduction and signs of DM type 2 [*Trombetta M et al., 2013*], decrease the frequency of myocardial infarctions [*Ridker PM et al., 2003*], reduction in diastolic blood pressure [*Ostgren CJ*

Table 1.

Dynamics of carbohydrate metabolism indices depending on arterial hypertension severity.

Indices	Control	Essential arterial hypertension		
		I	II	III
Fasting glucose, μ*mol/L*	4.43±0.32	4.65±0.18	5.03±0.30	6.60±0.72 p=0.008 p_1=0.012 p_2=0.043
Insulin, *mkU/ml*	11.4±1.95	16.3±3.01	17.0±3.67	24.9±5.20 p=0.019
C-peptide, *ng/ml*	1.52±0.14	1.91±0.23	2.23±0.30 p=0.036	2.95±0.63, p=0.031
HOMA-IR Index	2.24±0.30	3.47±0.31	3.80±0.44 p=0.004	6.91±0.89 p.p_2=0.002 p_1=0.007
HOMA-Fβ Index	245.2±23.0	259.7±19.8	232.2±22.7	173.2±30.4 p_1=0.021

Notes: p – *reliability of index differences concerning the control;* p_1 *– reliability of index differences comparing to EAH I patients;* p_2 *– reliability of index differences comparing to EAH II patients.*

Table 2.

Dynamics of carbohydrate metabolism indices in patients with arterial hypertension depending on the body weight and degrees of abdominal obesity

Indices	Normal weight (n=9)	Overweight (n=42)	Abdominal obesity, I-III stages of severity		
			I (n=30)	II (n=19)	III (n=10)
Glucose, μ*mol/L*	4.75±0.19	5.08±0.52	5.38±0.58	7.04±0.88 p=0.018	6.80±1.01 p=0.052
Insulin, *mkU/ml*	13.6±1.67	16.1±2.02	18.5±2.60	25.5±4.12 p=0.013 p_1=0.045	25.2±3.13 p=0.012 p_1=0.019
C-peptide, *ng/ml*	1.88±0.23	2.31±0.30	2.41±0.37	2.55±0.34	2.15±0.28
HOMA-IR Index	2.88±0.31	3.66±0.54	4.46±0.51 p=0.012	7.98±0.74 p=0.001 p_1=0.003 p_2=0.005	7.62±1.14 p=0.006 p_1=0.002 p_2=0.016
HOMA-Fβ Index	227.5±11.8	204.0±16.3	196.2±22.9	138.2±36.6 p=0.029 p_1=0.003	152.7±8.49 p=0.003 p_1=0.008 p_2=0.024

Notes: *p – reliability of index differences comparing to individuals with normal body weight;* p_1 *– reliability of index differences comparing to overweight individuals;* p_2 *– reliability of index differences comparing to individuals with I degree abdominal obesity;* p_3 *– reliability of index differences comparing to individuals with II degree abdominal obesity.*

Table 3.

Carbohydrate metabolism indices in patients with arterial hypertension depending on haplotypes of ACE (I/D) and PPAR-γ2 (Pro12Ala) genes

Haplotypes of ACE and PPAR-γ2 genes	Glucose, *mmol/L*	Insulin, *mkU/ml*	C-peptide, *ng/ml*	HOMA-IR Index	HOMA-Fβ Index
II/12Ala, II/ProAla n=7	4.73±0.30	15.5±1.06	2.12±0.19	3.29±0.28	253.6±33.0
II/Pro12 n=10	5.38±0.40	19.2±2.11 II/12Ala II/ProAla	2.26±0.25	4.70±0.44 II/12Ala, II/ProAla	223.4±37.6
ID/12Ala, ID/ProAla n=24	5.75±0.34 II/12Ala II/ProAla	17.3±2.62	2.54±0.27	4.50±0.45 II/12Ala II/ProAla	166.2±15.5 II/12Ala II/ProAla
ID/Pro12 n=26	6.12±0.42 II/12Ala II/ProAla	20.55±3.05 II/12Ala II/ProAla	2.57±0.33	5.60±0.40 II/12Ala II/ProAlaDD/12Ala DD/ProAla	157.8±23.7 II/12Ala II/ProAla
DD/12Ala, DD/ProAla n=9	5.40±0.35	15.7±1.31	2.06±0.32	3.77±0.35	172.8±35.0 II/12Ala II/ProAla
DD/Pro12 n=34	5.85±0.24 II/12Ala II/ProAla	19.4±2.13 II/12Ala II/ProAla	2.21±0.35	5.06±0.49 II/12Ala II/ProAlaDD/12Ala DD/ProAla	162.9±39.2 II/12Ala II/ProAla

Note: *Reliability of index differences compared to a certain haplotype is raised to the power ($p<0.05$).*

Table 4.

Haplotypes of ACE (I/D) and PPAR-γ2 (Pro12Ala) genes as risk factors of hyperglycemia and insulin resistance in patients with essential arterial hypertension

Potential risk factor	RelR	RR	OR	95% CI RR	95% CI OR	p
II/12Ala, II/ProAla	0.57	0.58	0.5	0.09-3.95	0.05-5.22	>0.05
II/Pro12	1.60	1.55	2.0	0.57-4.21	0.40-10.1	>0.05
ID/12Ala, ID/ProAla	1.67	1.38	2.14	0.82-2.32	0.59-7.84	>0.05
ID/Pro12	2.31	1.77	4.09	1.06-2.97	1.14-14.7	0.026
DD/12Ala, DD/ProAla	1.33	1.31	1.5	0.43-4.03	0.27-8.34	>0.05
DD/Pro12	2.12	1.52	3.38	1.01-2.27	1.0-11.4	0.045

Notes*: RelR - (relative risk); RR - (Risk Ratio); OR - (Odds Ratio); 95% CI RR, OR – 95% confidential intervals of risk ratio (RR) and odds ratio (OR).*

et al., 2003]. The cardio-vascular effects did not depend on metabolic ones, which were determined by this mutation [*Trombetta M et al., 2013*]. On the contrary, Zhang R. et al (2014) did not prove the association of Pro12Ala polymorphism of PPAR-γ2 gene with MS signs. Temelkova-Kurktschiev T. et al (2004) proved that "intima-media" thickness in Ala-allele homozygotes with atherosclerosis (aged 40-70 y.o.) is less than in the ProPro-genotype carriers. PPAR-γ2 receptors were found in atherosclerotic plaques and macrophages. In the authors' opinion, it indicated of predisposition of ProPro-

genotype carriers to earlier development of atherosclerosis. The European project Genes in Hypertension, conducted in 6 European populations (n=2553), has found the involvement of ProPro-genotype of PPAR-γ2 gene in the development of metabolic syndrome and insulin resistance in patients with arterial hypertension [*Tikhonoff V et al., 2006*], and its association with diastolic blood pressure and DM type 2 risk [*Ostgren CJ et al., 2003; Motavallian A et al., 2013*], which corresponds to the results of our research. However, the data concerning a combined influence of Pro12Ala polymorphism of PPAR-γ2 gene and I/D polymorphism of ACE gene upon carbohydrate-lipid metabolism, metabolic syndrome and insulin resistance development, or leptin resistance in patients with EAH are limited, and this problem today is being actively studied [*Passaro A et al., 2011*].

The relationship between EAH, abdominal obesity severity, insulin resistance and I/D polymorphism of ACE gene is conflicting: our results correspond to the statements that the presence of D-allele of ACE gene in EAH patients not treated earlier is an independent sign of the target-organs damage and additional factor of cardio-vascular risk [*Buraczynska M et al., 2003*] with double frequent complicated heredity of arterial hypertension, frequent crisis course, comorbid DM type 2, MS and chronic heart failure, much higher blood pressure levels, myocardial mass of the left ventricle as well as its concentric remodeling [*Headley AP et al., 2007; Sydorchuk LP et al., 2013*]. Although, a number of researchers did not find connection of ACE I/D gene polymorphism with more severe course of EAH, signs of insulin resistance, metabolic syndrome, but some studies were conducted with participation of patients getting anti-hypertensive therapy for a long time [*Gomez-Angelats E et al., 2000; Lopez-Contreras J et al., 2000; Danková Z et al., 2009*]. Conen D. et al in prospective cohort 10-years study, called Women's Health, did not find any correlations of the MS or DM type 2 signs development with polymorphism of ACE, AGTR1, AGT and eNOS genes, but they were subjects of the general population. That corresponds partially to our results for the control group [*Conen D et al., 2009*].

Conclusion

The onset of insulin resistance, hyperglycemia and decrease of the integral index of pancreatic β-cells functional activity in EAH patients is associated with disease severity, abdominal obesity degrees and the magnitude of cardiovascular risk: in patients with stage III EAH, high and very high cardiovascular risk, II and III degree abdominal obesity fasting plasma glucose levels rise by 23.8-32.9% ($p \leq 0.043$-0.008), immunoreactive insulin rises by 36.1-46.7% ($p \leq 0.045$-0.012), HOMA-IR index – by 49.8-68.4% ($p \leq 0.007$-0.0012), whereas HOMA-Fβ index decreased by 25.1-33.3% ($p \leq 0.021$-0.003).

Homozygous presence of Pro12-allele of PPAR-γ2 gene in haplotype of EAH patients regardless of ACE gene allele condition (II/Pro12, ID/Pro12, DD/Pro12), is accompanied by 23.9-32.6% ($p<0.05$) increase of immunoreactive insulin levels and 29.4% ($p=0.012$) and 23.7% ($p=0.006$) increase of glycemia indices for ID/Pro12, DD/Pro12 carriers, respectively. The presence of II-genotype and Ala-allele in haplotype (II/12Ala, II ProAla variants) is favorable and is associated with 26.9-41.5% lower HOMA-IR index ($p \leq 0.03$-0.003) and with 46.8-55.7% higher HOMA-Fβ index ($p \leq 0.024$-0.048).

The risk of hyperglycemia and insulin resistance in EAH patients increases with the presence of ProPro-genotype of PPAR-γ2 gene and D-allele of ACE gene in the haplotype: for ID/Pro12 variant – 2.31 times [OR=4.09; $p=0.026$], for DD/Pro12 combination – 2.12 times [OR=3.38; $p=0.045$], respectively. In normal weight and overweight EAH patients, haplotypes of the analyzed genes are not risk factors of hyperglycemia and insulin resistance. Though, abdominal obesity in EAH patients with presence of ProPro-genotype and D-allele (ID/Pro12, DD/Pro12) in haplotype increases a relative risk of hyperglycemia and insulin resistance: for ID/Pro12 haplotype - 2.46 times [OR=4.80; $p=0.036$], for DD/Pro12 – in 2.44 times [OR=4.71; $p=0.024$].

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