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Insulin resistance changes in obese hypertensive patients under the influence of treatment depending on ace (i/d), ppar-γ2 (pro12ala) genes polymorphism

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Abstract
The dynamics of glycemic profile and functional activity of pancreatic β-cells data in patients with essential arterial hypertension (EAH) and abdominal obesity (AO) in association with insertion-deletion (I/D) polymorphism of ACE gene (rs4646994) and Pro12Ala polymorphism of PPAR-γ2 gene (rs1801282) under the influence of treatment were evaluated. Complex treatment improved the carbohydrate balance but only in I-allele carriers of ACE gene significantly: reducing plasma fasting glucose and C-peptide (ID-genotype) by 16.5% (p=0.035) and 33.0% (p=0.018), Insulin and insulin resistance HOMA-IR index (II-genotype) by 21.1% (p=0.011) and 23.7% (p=0.008) respectively, contrary functional activity of pancreatic β-cells HOMA-Fβ index increased (ID-genotype) by 53.9% (p=0.007). Depending on PPAR-γ2 gene genotypes C-peptide decreased by 26.2% (p=0.05) but only in ProPro-genotype carriers. The glycemic profile improved more effective under the treatment in EAH and AO patients with I-allele and ProPro-genotype in haplotype (II/Pro12, ID/Pro12 combinations).

Keywords: ACE (I/D), PPAR-γ2 (Pro12Ala) genes, hypertension, obesity, insulin-resistance, treatment

1. Introduction
According to the prognostic data of the WHO experts till 2025 half of the world population will suffer from alimentary obesity. Even today 2/3 inhabitants of the American continent "pay off" for their life style by abdominal obesity (AO). In the countries of Western Europe there is 30% of adult population with AO, in Eastern Europe – up to 38-42% [1]. Important that adipose tissue possesses pronounced endocrine activity, and adipokines and cytokines produced by it, are involved in all the processes forming the components of metabolic syndrome (MS), insulin resistance (IR), chronic systemic inflammatory response, lipoperoxidation, oxidative stress, activation of blood sedimentation processes etc. [2, 3]. Obesity is a binding component in pathogenetic chain between arterial hypertension (AH) and IR at the presence of MS. IR and compensatory hyperinsulinemia are found to be combined with AO only under condition of persistent chronic systemic inflammation and increased level of C-reactive protein (CRP) [4].
In addition, hypertrophy of the visceral adipose tissue is accompanied by macrophage infiltration and intensified synthesis of about 40 kinds of proinflammatory cytokines. The control of these processes is conducted due to peroxisome proliferator-activated receptor gamma-γ (PPAR-γ) in the normal condition. PPAR-γ is mostly expressed in the adipose tissue and regulates proliferation and differentiation of adipocytes. Also PPAR-γ is the primary factor of adipocytes hyperplasia and hypertrophy [5]. Out of three types of PPARs' receptors (α, β/σ- i 3 γ types) it is PPAR-γ activation (pharmacological agonists - thiazolidinedione) increases reliably tissue sensitivity to insulin, decrease IR, affecting the central mechanism of AH and AO development [6].
PPAR receptors modulate the activity of rennin-angiotensin-aldosterone system (RAAS) through the transcription control of synthesis of renin, angiotensin (AGT), angiotensin-converting enzyme (ACE) and angiotensin II of the 1st type receptor (AT-R1) [7], 3 binding biological effects of RAAS with PPARs. Some studies indicate the RAAS involvment in pathophysiology of obesity and AO-associated hypertension [8]. ACE, as a key component of RAAS, via AGT II enhances platelet aggregation and blood clotting, intensifying vessels susceptibility to inflammation, potentiating hyperplasia of the arterial wall. Also ACE via bradykinin degradation decrease the content of nitrogen monoxide (NO), promote the endothelial dysfunction development and accumulation of cholesterol of very low density

lipoproteids, formation of atherosclerotic changes or destabilizing available atherosclerotic plaque [9-12].

In the first step, 2-amino-6-methylpyridine (60 g, 0.55 mol) was treated with HBr (1.0L, 48% in water) and liquid bromine (80 mL) at 0-10 °C followed by $NaNO_2$ (100 g, 1.45 mol) addition in water to give 2-bromo-6-picoline (82 g, 0.47 mol). This was then treated with NBS (167 g, 0.94 mol) using CCl_4 (750 mL) as a solvent and column purification of crude mass over silica gel using EtOAc/Hexane =1:9 as an eluent gave 2-bromo-6-dibromomethyl-pyridine (135 g, 0.41 mol) as a white solid which was hydrolyzed with $CaCO_3$ (92 g, 0.92 mol) in water at reflux temperature. Column purification over silica gel afforded compound **1** (63 g, 0.34 mol) as a pure white solid However, the questions of association of certain and combined genetic mutations of ACE (I/D) and PPAR-γ2 (Pro12Ala) genes on IR and functional activity of pancreatic β-cells in patients with essential AH (EAH) and AO remain unsolved and require further examination with the aim of pharmacogenetic therapy correction.

Consequently, the aim of our study was to analyze the dynamics of glycemic profile and functional activity of pancreatic β-cells data in patients with EAH and AO in association with of insertion-deletion (I/D) polymorphism of angiotensin converting enzyme (ACE) gene (dbSNP id:rs4646994) and Pro12Ala polymorphism of PPAR-γ2 gene (dbSNP id: rs1801282) under the influence of treatment.

2. Materials and Methods

2.1 Compliance with bioethics

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. Patients' Examination Cards and Patients' Informed Consent Forms were approved by the Biomedical Ethics Commission of Bukovina State Medical University, Ministry of Health of Ukraine (Chernivtsi, Ukraine). All enrolled patients were treated in the Municipal Policlinics No.1 and No.3 (Chernivtsi, Ukraine) during 2010-2014 y.y. Genetic bench study performed in the laboratory of Medical Biology and Genetics Department of Bukovina State Medical University. After screening (matching inclusion/exclusion criteria) 110 hypertensive overweight / obese patients were selected for further examination. The control group included 50 practically healthy individuals who were not relatives with the patients, without reliable differences of sex and age.

2.2 Inclusion / Exclusion criteria.

Inclusion criteria. EAH patients were included in current Study with or without target-organs damage (I-III severity stages), in which after discontinuation of antihypertensive drugs mean office BP level was higher than 140/90 mmHg, several times a month, as national and ESH / ESC (2013) recommendation require [13, 14]; age above 20 y.o.; voluntary sign consent to participate in the study.

Exclusion criteria. We excluded patients with chronic heart failure (CHF) higher than II functional class (NYHA III-IV), severe sub and decompensated complication of EAH, type I diabetes mellitus (DM 1), sub- and decompensated type 2 DM, malignant, uncontrolled arterial hypertension, sub- and decompensated diseases of the liver (three times over the norm level of aspartate aminotransferase, alanine aminotransferase) and kidneys (blood serum creatinine 200 mkmol/l and higher), bronchial asthma, chronic obstructive pulmonary disease of III-IV stage (GOLD 2011), exacerbated oncologic and infectious diseases or during unstable remission, psychological disorders; after oral corticosteroids or contraceptives; pregnancy or lactation period.

2.3 Diagnosis of Arterial Hypertension and Abdominal Obesity.

Screening of patients and distribution into groups depending on target organs damaging, cardio-vascular risks and AO degrees was performed according to the Recommendations of Ukrainian and European Societies of Cardiology and Hypertension ESC/ESH (2013), American Heart Association (AHA), as well as current Orders of the Ministry of Public Health of Ukraine [13-15]. The diagnosis of AH and AO was made on the basis of the acting national and international recommendations criteria [13-15]. All the patients underwent a complex of examinations: waist and thighs circumferences, body mass index (BMI, kg/m^2), according to which the degree of AO was determined [15], ECG in 12 leads, ultrasound examination of the kidneys, general clinical and biochemical analyses, consultations by ophthalmologist and neurologist. Office systolic and diastolic blood pressure (SBP, DBP), heart rate (HR), Daily Holter BP monitoring were measured according to Ukrainian and European recommendations ESC/ESH, 2013 [13, 14].

2.4 Functional activity of pancreatic β-cells and insulin resistance investigation

Immunoreactive insulin (IRI, mkU/ml) and C-peptide (CP, ng/ml) were detected on empty stomach in the venous blood plasma by Immuno-enzyme method (ELISA) with the set of reagents of DRG (USA). Insulin resistance (IR) was defined by HOMA-IR index (Homeostasis Assessment of Insulin Resistance), (U): (plasma glucose x insulin) / 22.5. The function of the Langerhans β-cells pancreatic island was estimated by CP and integral HOMA-Fβ index (Homeostasis Model Assessment of β-Cell Function), (%): (20 x insulin) / (glucose – 3.5) [16]. IRI, CP, HOMA-IR and HOMA-Fβ were detected before and after the treatment.

2.5 Principles of Arterial Hypertension with Obesity Management

Basic pharmacotherapy of EAH patients included into the study during the whole period of examination was conducted according to the current legal national and international recommendations and protocols [13-15]. Patients with EAH and AO II and III stages receive additionally Orlistat® (120 mg three times daily before meals), or herbal medication based on Garcinia Combination (Stifimol®, UA) 1 capsule three times daily before meals for body weight correction. 25 patients with EAH III degree, AO and concomitant compensated DM type 2 take additionally Metformin 1000 mg/daily. The period of observation was 6 months ± 2 weeks.

2.6 Genotyping of the ACE (I/D) and PPAR-γ2 (Pro12Ala) polymorphisms

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′–gaaactgatgtcttgactcatg ggtg–3′ and reverse 5′-caacctggaagacaaactacaagagc-3′), for ACE gene (forward 5′–Gccggggactctgtaagccactgc–3′ and

reverse 5′-ccttgtctcgccagccctccca-3′). Amplified polymorphic locus was detected by polymerase chain reaction (PCR) on "Amply-4L" amplificator according to the manufacturer's protocol [17]. Alleles' discrimination of PPAR-γ2 gene was performed by restriction endonuclease Cse I (HgaI) ("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 minutes). The obtained PCR 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).

2.7 Statistical analysis

Statistical analysis was performed using Statistica 7.0 (StatSoft Inc, USA) software. Reliability of the data for independent quantitative sampling was calculated using 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). Risk ratio (RR) were estimated by odds ratio (OR) with 95% confidence interval (CI) using a chi-square test (df=1). P values <0.05 were considered statistically significant.

3. Results and Discussions

Prospective Study included 56.4% (62) women and 43.6% (48) men. The age of patients was from 25 to 79 (on the average 53.3±6.05 y.o.). There were 22.7% (25) subjects with EAH I stage, 45.45% (50) EAH II stage patients, and 31.8% (35) EAH III stage. Among them there were 8.18% (9) individuals with normal body weight, overweight – 38.2% (42), with AO – 53.6% (59): with I degree AO – 27.3% individuals (30), with II degree AO – 17.3% (19), with III degree AO – 9.09% (10) patients.

The content of fasting plasma glucose, IRI, HOMA-IR index were higher before treatment in EAH individuals with AO II and III degrees than in those with 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 (Table 1). The IRI, HOMA-IR index in EAH patients with AO II and III degrees were also higher than 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), with lower HOMA-Fβ – by 32.25% (p=0.003) and 25.1% (p=0.008), respectively. After treatment a reliable decrease of HOMA-IR in patients with AO II and III degrees was found by 41.7% (p=0.004) and 47.1% (p=0.007), and in overweight persons – by 43.7% (p=0.043), with considerable increase of functional activity of pancreatic β-cells by HOMA-Fβ index, but only in patients with AO III and I degrees by 31.4% (p=0,026) and 35.5% (p=0.012) respectively (Table 1).

Table 1: Influence of treatment on dynamics of carbohydrate metabolism indices in patients with arterial hypertension depending on the body weight and degrees of abdominal obesity, M±S.D.

Indices	Before/ after	Normal weight, n=9	Overweight, n=42	AO I, n=30	AO II, n=19	AO III, n=10
Glucose, mmol/L	before	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
	after	4.50±0.25	4.67±0.24	4.65±0.92	5.67±0.76	5.10±0.56
Insulin, mkU/ml	before	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
	after	7.50±0.69 p_4=0.011	12.8±1.11 p=0.005	17.5±4.38 p=0.031	19.0±3.89 p=0.007	17.8±4.11 p=0.025
CP, ng/ml	before	1.88±0.23	2.31±0.30	2.41±0.37	2.55±0.34	2.15±0.28
	after	1.80±0.11	1.77±0.18	1.98±0.50	1.89±0.22	1.86±0.39
HOMA-IR Index	before	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
	after	1.47±0.14 p_4=0.005	2.06±0.56 p_4=0.043	3.62±1.01 p=0.042	4.65±0.26 p=0.0002 p_1=0.004 p_4=0.004	4.03±0.32 p=0.0008 p_1=0.003 p_4=0.007
HOMA-Fβ Index	before	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
	after	152.8±15.6 p_4=0.005	218.8±21.2 p=0.016	304.0±34.2 p=0.005 p_1=0.038 p_4=0.012	175.1±16.3 p_2=0.009	222.5±27.4 p=0.041 p_2=0.047 p_4=0.026

Notes: Before / after – treatment; AO I-III – Abdominal obesity I-III degree of severity; CP – C-peptide; HOMA-IR – Homeostasis Model Assessment Insulin Resistance Index; HOMA-Fβ – Index of pancreas β-cells functional activity. 2. p – reliability of differences concerning individuals with normal body weight; p_1 – reliability of differences concerning overweight individuals; p_2 – reliability of differences concerning individuals with I degree AO; p_3 – reliability of differences concerning individuals with II degree AO; p_4 – reliability of differences concerning the condition before treatment

Fasting glycaemia and CP reduced significantly under the influence of treatment in I-allele of ACE gene carriers (Table 2) by 16.5% (p=0.035) and 33.0% (p=0.018), IR and HOMA-IR indices (II-genotype carriers) – by 21.1% (p=0.011) and 23.7% (p=0.008) respectively, with increasing of HOMA-Fβ index in ID-genotype carriers by 53.9% (p=0.007).

Table 2: Glycemic profile depending on ACE I/D gene's polymorphism in hypertensive patients, M±S.D.

Parameters	Before/after	Control group	II genotype, n=17	ID genotype, n=50	DD genotype, n=43
Glucose, mmol/L	before	4.43±0.32	4.67±0.22	6.20±0.28 p.p_1=0.004	5.66±0.34 p=0.011 p_1=0.018
	after		4.38±0.13	5.18±0.38 p_1=0.05 p_3=0.035	5.20±0.45
Insulin, mkU/ml	before	11.4±1.95	15.6±0.80 p=0.05	18.3±3.14 p=0.054	15.9±1.18 p=0.051
	after		12.3±0.65 p_3=0.011	17.7±2.58 p=0.053 p_1=0.047	16.0±0.78 p=0.032 p_1=0.007
CP, ng/ml	before	1.52±0.14	2.07±0.17 p=0.016	2.70±0.30 p=0.007	1.99±0.38
	after		1.85±0.10	1.81±0.21 p_3=0.018	1.65±0.13
HOMA-IR Index	before	2.24±0.30	3.28±0.23 p=0.008	5.09±0.49 p=0.002 p_1<0.001	4.0±0.33 p=0.005 p_1<0.001
	after		2.50±0.15 p_3=0.008	4.15±1.07 p=0.058	3.59±0.35 p=0.004 p_1=0.005
HOMA-Fβ Index	before	245.2±23.0	268.0±40.5	136.9±12.8 p=0.0045 p_1=0.003	146.8±43.5 p=0.048 p_1=0.046
	after		279.5±38.6	210.7±16.5 p_3=0.007	188.2±25.1 p_1=0.052

Notes: Before / after – treatment; CP – C-peptide; HOMA-IR – Homeostasis Model Assessment Insulin Resistance Index; HOMA-Fβ – Index of pancreas β-cells functional activity. 2. p – reliability of differences concerning control group; p_1 – reliability of differences concerning patients with II-genotype; p_2 – reliability of differences concerning patients with ID-genotype; p_3 – reliability of differences concerning the condition before treatment

The dynamics of glycemic profile under the influence of treatment depending on PPAR-γ2 Pro12Ala gene's polymorphism in hypertensive patients is presented in Table 3. The Pro12-genotype carriers demonstrated higher levels of fasting glycemia, IR and HOMA-IR than in the control group and AlaAla-genotypes carriers: after glycemia – by 36.6% (p=0.016) and 24,7% (p=0.046), IRI – in 2.0 (p=0.045) and 1.49 times (p=0.049), HOMA-IR index – in 2.73 times (p=0.002) and 1.84 times (p=0.006). After treatment boundary reliable decrease of CP was found only in ProPro-genotype carriers by 26.2% (p=0.05).

Table 3: Glycemic profile depending on PPAR-γ2 Pro12Ala gene's polymorphism in hypertensive patients, M±S.D.

Parameters	Before/after	Control group	12Ala, ProAla, n=40	ProPro, n=70
Glucose, mmol/L	before	4.43±0.32	4.85±0.46	6.05±0.57 p=0.016 p_1=0.046
	after		4.81±0.42	5.19±0.45
Insulin, mkU/ml	before	11.4±1.95	15.3±1.58	22.8±3.42 p=0.045 p_1=0.049
	after		14.7±1.68	18.5±1.98 p=0.013
CP, ng/ml	before	1.52±0.14	2.21±0.22 p=0.01	2.44±0.36 p=0.02
	after		1.81±0.30	1.80±0.19 P_{treat}=0.05
HOMA-IR Index	before	2.24±0.30	3.32±0.40 p=0.034	6.12±0.65 p=0.002 p_1=0.0065
	after		3.19±0.72	4.27±0.58 p=0.002
HOMA-Fβ Index	before	245.2±23.0	224.7±18.1	178.8±34.7 p=0.056
	after		243.0±13.2	218.9±28.1

Notes: Before/after – treatment; CP – C-peptide; HOMA-IR – Homeostasis Model Assessment Insulin Resistance Index; HOMA-Fβ – Index of pancreas β-cells functional activity. 2.p – reliability of differences concerning control group; p_1 – reliability of differences concerning patients with 12Ala, ProAla-genotypes; p_2 – reliability of differences concerning patients with ID-genotype; p_{treat} – reliability of differences concerning the condition before treatment

According to haplotypes of analyzed genes it was found (Table 4) that fasting glycemia and IRI were reduced under the influence of therapy effectively in I-allele and ProPro-genotype carriers in haplotype (II/Pro12, ID/Pro12) by 15.4% and 20.3% (p<0.05) respectively. HOMA-IR was reliably reduced in patients with unfavourable homozygous Pro12-allele in haplotype regardless of ACE gene allele's condition (II/Pro12, ID/Pro12, DD /Pro12 variants) - by 28.1%, 24.8% and 22.3% (p<0.05), respectively. HOMA-Fβ index increased significantly after treatment, but only in ID/12Ala, ID/ProAla haplotypes carriers by 33.3% (p=0.016).

Table 4: Glycemic profile depending on haplotypes of ACE (I/D) and PPAR-γ2 (Pro12Ala) genes in patients with arterial hypertension, M±S.D.

Haplotypes of ACE and PPAR-γ2 genes		Glycemia, mmol/L	Insulin, mkU/ml	C-peptide, ng/ml	HOMA-IR Index	HOMA-Fβ Index
II/12Ala, II/ProAla, n=7	before	4.73±0.30	15.5±1.06	2.12±0.19	3.29±0.28	253.6±33.0
	after	4.52±0.23	13.1±0.99	1.84±0.13	2.73±0.34	267.3±30.1
II/Pro12, n=10	before	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
	after	4.79±0.29	15.3±1.17 $p_{treat}<0.05$	1.83±0.15	3.38±0.37 $p_{treat}<0.05$	250.3±33.1
ID/12Ala, ID/ProAla, n=24	before	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**
	after	5.06±0.39	16.7±2.28	1.81±0.24	3.83±0.75	221.5±15.4 $p_{treat}<0.05$
ID/Pro12, n=26	before	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/ProAla DD/12Ala, DD/ProAla**	157.8±23.7 **II/12Ala,II/ProAla**
	after	5.18±0.35 **II/12Ala, II/ProAla** $p_{treat}<0.05$	18.0±2.12 **II/12Ala, II/ProAla**	1.81±0.20	4.21±0.62 **II/12Ala, II/ProAla** $p_{treat}<0.05$	215.4±22.3
DD/12Ala, DD/ProAla, n=9	before	5.40±0.35	15.7±1.31	2.06±0.32	3.77±0.35	172.8±35.0 **II/12Ala,II/ProAla**
	after	5.04±0.44	15.2±1.04	1.70±0.19	3.46±0.47	206.5±21.0
DD/Pro12, n=34	before	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/ProAla DD/12Ala, DD/ProAla**	162.9±39.2 **II/12Ala,II/ProAla**
	after	5.19±0.40 **II/12Ala, II/ProAla**	17.1±1.35 **II/12Ala, II/ProAla**	1.73±0.16	3.93±0.45 **II/12Ala, II/ProAla** $p_{treat}<0.05$	205.4±24.8

Note. Before / after – treatment; reliability of index differences concerning a certain haplotype is raised to the power/degree ($p<0,05$); p_{treat} – reliability of differences concerning the condition before treatment.

Epidemiological analysis found a reliably high probability of hyperglycemia and IR development in case of ProPro-genotype and D-allele presence in haplotype: ID/Pro12 variant – OR=4.09 (p=0.026) and DD/Pro12 combinations – OR=3.38 (p=0.045), respectively. In normal weight and overweight EAH patients the haplotypes of analyzed genes are not the risk factors of hyperglycemia and IR. However, AO in EAH patients with presence of ProPro-genotype and D-allele (ID/Pro12, DD/Pro12) in haplotype increases a relative risk of hyperglycemia and IR for ID/Pro12 haplotype – in 2.46 times [OR=4.80; p=0,036], for DD/Pro12 – in 2.44 times [OR=4.71; p=0.024].

The evidence concerning the association of PPAR-γ2 (Pro12Ala) gene polymorphism with IR remains disputable: according to some authors [18] the presence of AlaAla-genotype is accompanied by increased sensitivity to insulin and reduced lipid oxidation in response to insulin stimulation, but only in men as compared to homozygous Pro-allele carriers (p=0,03); the "Atherosclerosis Risk in Communities" Study (middle age African-Americans of general population) [19] has found that among the AlaAla-genotype carriers without obesity there is less number of patients with DM 2 type, lower level of IRI (p=0,001), HOMA-IR (p=0,002), higher ratio of fasting glucose to insulin (p=0,005), lower DBP (p=0,02), than in those with ProPro-genotype. These findings corresponds to our results. And among individuals with increased BMI (up to 29,9 kg/m^2) similar dependence on the PPAR-γ2 gene polymorphism of was not found [19].

During the last decade a lot of evidence of ACE (I/D) gene polymorphism association with AH, left ventricle hypertrophy, myocardial infarction, chronic heart failure, kidney diseases, IR and vascular complications of DM 2 type has been accumulated [9, 12, 20-25]. Our results corresponds partially to Tanno-Sobetsu Study (Japan), where the relations of IR by HOMA-IR index with ACE I/D gene polymorphism were not found in 550 individuals of general population [26]. D. Conen *et al*. in Prospective cohort "Women's Genome Health Study" during 10 years did not find any relation of 2 type DM occurrence or MS signs with polymorphism of ACE, AGTR1, AGT та eNOS genes. These findings partially corresponds to our results but only for the practically healthy subjects of control group [27]. In spite of numerous studies, the evidence concerning relations of ACE (I/D) gene polymorphism with the AO and IR development, as well as its combined mutations with PPAR-γ2 (Pro12Ala) gene polymorphism is limited, and the available data are debatable [22, 28,29].

4. Conclusion

After treatment in normal body weight and overweight EAH patients the level of IRI and HOMA-IR index reliably decreased and did not depend on the haplotypes of ACE (ACE) and PPAR-γ2 (Pro12Ala) genes. On the contrary, HOMA-Fβ index increased, but only in obese patients, and did not depend on haplotypes either. Complex treatment promoted reliable improvement of glycemic profile, but only in I-allele of ACE gene carriers: decrease of fasting glycemia and CP (ID-genotype), IRI and HOMA-IR index (II-genotype), with increase of HOMA-Fβ index in ID-genotype carriers. Depending on the PPAR-γ2 gene genotypes after treatment CP reduced only in ProPro-genotype carriers. Depending on haplotypes the fasting glycemia and IRI decreased more effective under the treatment influence in case of ProPro-genotype and I-allele presence in haplotype of EAH and obese patients (II/Pro12, ID/Pro12 combinations).

In perspective we plan to analyze the association of haplotypes of ACE (rs 4646994) and PPAR-γ2 (rs1801282) genes with lipids profile, leptin and adiponectin in EAH patients with / without obesity, inc. under the treatment influence.

5. Limitations of the Study. 93 of 110 consecutive patients were previously pharmacologically treated for essential hypertension.

6. Conflict of Interest: None declared.

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8. References

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