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COMBINED INFLUENCE OF POLYMORPHIC VARIANTS OF PPAR-γ2 (rs1801282) AND ACE (rs4646994) GENES ON THE ONSET OF NON-ALCOHOLIC FATTY LIVER DISEASE IN PATIENTS WITH ESSENTIAL ARTERIAL HYPERTENSION AND OBESITY

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Abstract

Objective: to study a combined influence of polymorphic variants of PPAR-γ2 (rs1801282) and ACE (rs4646994) genes on the onset of non-alcoholic fatty liver disease (NAFLD) in patients with essential arterial hypertension (EAH) and obesity.

Materials and methods: The study involved 96 patients with NAFLD, stage II EAH of the 1-2 degrees, with high and very high risk with associated AO including males - 41.67% (40), women - 58.33% (56), the average age was 53.70 ± 5.34 years. The function of the liver was studied by the activity of organ-specific enzymes. The polymorphism of PPAR-γ2 (Pro12Ala) and ACE (I / D) genes was studied by the PCR method. The control group consisted of 50 virtually healthy individuals.

Results. A third of patients with NAFLD (31.25%) are carriers of an unfavorable combination of homozygous deletion of the ACE gene (rs4646994) and the ProPro genotype of the PPAR-γ2 gene (rs1801282) (DD / Pro12), and one in five (22.92%) is a carrier of a heterozygote combination for two genes (ID / ProAla variant), which is by 2.6 and 2.9 times more frequent than in the control group (p = 0.01 and p = 0.025, respectively). The controls are dominated by individuals with a combination of the ID-polymorphic variant of the ACE gene and the ProPro genotype of the PPAR-γ2 gene by 1.84 times: 44.0% versus 23.96%

(χ^2=6,19, p=0,013). The risk of NAFLD in the surveyed population of the inhabitants of Northern Bukovyna increases with a combination of the Ala allele of the PPAR-γ2 gene and the ID-genotype of the gene ACE (ID / ProAla, ID / 12Ala variants) by 2.3 times (OR = 3.17, 95% CI OR = 1.13-8.88, p = 0.023), due to NASH – by 4.4 times (OR = 7.0, 95% CI OR = 1.81-27.07, p = 0.006). While the dominant effect of the homozygous deletion of the ACE gene, without the significant effect of the Pro12-genotype of the PPAR-γ2 gene, on the owners of the DD / Pro12 variant, increases the risk of NAFLD by 2.6 times (OR = 3.33, 95% CI OR = 1.28 -8.68, p = 0.01), due to NALS – by 2.7 times (OR = 3.53, 95% CI OR = 1.33-9.34, p = 0.008), respectively. Instead, the combination of the I-allele of the ACE gene and the Pro-allele of the PPAR-γ2 gene is protective with the lowest chance of NAFLD (OR = 0.40, 95% CI OR = 0.19-0.83, p = 0.013) and its subtypes, especially steatohepatitis (OR = 0.08, 95% CI OR = 0.01-0.69, p = 0.006) than liver steatosis (OR = 0.48, 95% CI OR = 0.23-1, 01, p = 0,051).

Conclusions: Having the DD genotype of the ACE gene in combination with the Pro12 genotype of the PPAR-γ2 gene increases the risk of NAFLD by 2.6 times (due to non-alcoholic liver steatosis). The combination of the I-allele of the ACE gene and the Pro-allele of the PPAR-γ2 gene is protective with the lowest chance of NAFLD in the population (especially of non-alcoholic steatohepatitis).

Key words: non-alcoholic fatty liver disease, *PPAR-γ2* і *ACE* genes, arterial hypertension, obesity.

Introduction. At the present stage non-alcoholic fatty liver disease (NAFLD) attracts the attention of a wide range of specialists both in our country and abroad. According to the NHANES III (Third National Health and Nutritional Examination Survey) study, the prevalence of fatty liver dystrophy is up to 16% in patients with normal body weight and up to 76% in obese patients. The prevalence of NAFLD in Western Europe is 20-30%, in Asia - 15% [1].

NAFLD can act both as a single manifestation of lipid metabolism disorders, and as an integral part of the metabolic syndrome (MS). The course of NAFLD can be in the form of fatty dystrophy, non-alcoholic steatohepatitis or fibrosis with the possibility of developing cirrhosis, or even hepatocellular carcinoma [2].

Over the past decade, there has been a large accumulation of data on the association of I / D polymorphism of the ACE gene and Pro12Ala polymorphism of the PPAR-γ2 gene

with the development of myocardial infarction (MI), chronic heart failure (CHF), atherosclerosis, hypertension, left ventricular hypertrophy (LVH), insulin resistance, kidney diseases and micro / macroangiopathies in type 2 diabetes [3, 4, 5, 6]. However, the findings on their combined interaction in the pathogenesis of NAFLD development against the background of hypertension and obesity, as well as their indirect effect on clinical and laboratory manifestations of steatosis and steatohepatitis are controversial, and the available data are quite limited [7, 8, 9].

And since the *PPAR-γ2* and ACE gene polymorphism may be one of the important components of the circulus vitiosus of genetically induced hepatocyte dysfunction, the aim of our work was to investigate the combined effect of *PPAR-γ2* (rs1801282) and ACE (rs4646994) polymorphic variants on the onset of NAFLD in patients with EAH and obesity.

Material and methods

The prospective study was conducted in compliance with the main provisions of the GCP (1996), the Council of Europe Convention on Human Rights and Biomedicine, the Helsinki Declaration of the World Medical Association on the ethical principles for the implementation of human medical research, with the informed consent of the patient to participate in the research. The clinical material was collected from September 2013 to April 2015 on the basis of communal medical institutions of the city clinics №1 and №3 in Chernivtsi, as well as the outpatient department of general practice - family medicine of Vyzhnytsya district of Chernivtsi region. The study involved 96 patients with stage II EAH of the 1-2 degrees, with high and very high cardiovascular risk, with accompanying AO and NAFLD including 41,67% (40) of men, 58,33% (56) of women; the average age was 53.70 ± 5.34 years. 45,83% (44) of patients had compensated type 2 diabetes, lasting from 2 to 7 years. The control group consisted of 50 virtually healthy individuals, comparable by age (47.99 ± 8.46 years) and sex distribution (60% - women, 40% men) who were not in family relationships with the patients.

The clinical diagnosis of EAH and NAFLD was made in accordance with Order No. 384 of the Ministry of Health of Ukraine of May 24, 2012 and Order No. 826 of the Ministry of Health of Ukraine of November 6, 2014 [10,11] AO was diagnosed by the waist circumference (WC) for men> 94 cm, for women > 80 cm [10,12]. The abdominal type of obesity was confirmed by the waist / hip ratio: in men> 1.0, in women> 0.85. The body mass index (BMI) (the ratio of the body mass to the height squared) ≥ 30 kg / m^2, served as an indicator to diagnose obesity (OB) [10,12]. The surveyed denied alcohol abuse: for men -> 50 g of ethanol / a week, for women -> 30 g of ethanol / a week during the last year. Non-

inclusion criteria were: EAH I and III stages; chronic viral hepatitis (HBV, HCV, HDV); autoimmune and drug-induced hepatitis, idiopathic hemochromatosis; congenital a1-antitrypsin deficiency, Konovalov-Wilson's disease; chronic kidney disease with glomerular filtration rate <89 ml / min / 1.73 m2 (stage II-V); acute, or exacerbation of chronic inflammation of any localization during the last 3 months; active phase of the course of autoimmune diseases; hypothyroidism; oncopathology; mental disorders that made impossible a contact with the patient. Polymorphisms of PPAR-γ2 (Pro12Ala) (rs1801282) and ACE (I / D) (rs4646994) genes were studied using polymerase chain reaction (PCR). DNA was isolated from the peripheral venous blood lymphocytes using a DNA-sorb-V reagent set (RU). The PCR reaction was performed using Taq-DNA polymerase and specific primers for the PPAR-γ2 gene (direct 5'-GAAACTGATGTCTTGACTCATGGGTG-3 'and reverse 5'-CAACCTGGAAGA CAAACTACAAGAGC -3') and ACE (direct 5'-GCCGGGGACTCTGTAAGCCA CTGC- 3 'and reverse 5'-CCTTGTCTCGCCAGCCCTCCCA -3'). [13]. The discrimination of PPAR-γ2 gene alleles was performed using the restriction endonuclease Cse I (HgaI) ("Fermentas®", Lithuania). The products of the amplification of I/D polymorphism of the ACE gene and the restriction products of PCR of Pro12Ala of the PPAR-γ2 gene polymorphism were separated in a horizontal electrophoresis in a 3% agarose gel, concentrated with 4 μl of ethidium bromide for 45-60 min, and was visualized with a transluminator in the presence of a marker of molecular weights of 100- 1000 Mon ("SibEnzim", RU).

The statistical processing was carried out using the applications MS® Exxel® 2003 ™, Primer of Biostatistics® 6.05 and Statistica® 7.0 (Statsoft Inc., USA). The difference was considered to be reliable at $p < 0.05$.

Results and discussion

Among the patients with NAFLD, 27.08% (26) had the I degree OB, 58.33% (56) people – the II degree OB, 14.58% (14) patients - the III degree OB. 83,33% (80) persons were diagnosed with steatohepatosis and the remaining patients 16,67% (16) had steatohepatitis with the minimum activity of the mesenchymal and inflammatory process.

The distribution of combinations of polymorphic variants of the *PPAR-γ2* (Pro12Ala) and ACE (I / D) genes among the examined individuals is presented in Table 1.

Table 1

Combination of the polymorphic variants of the *PPAR-γ2 (Pro12Ala)* and *ACE (I/D)* genes in the examined people

Combination of the genotypes of the *PPAR-γ2* and *ACE* genes, n (%)	Groups of observations, n (%)			Total of patients, n=96 (%)	χ^2 P
	Control, n=50 (%)	Steatohepatitis, n=16 (%)	Steatohepatosis, n=80 (%)		
II/Pro12, n=17 (%)	9 (18,0)	2 (12,5)	6 (7,50)	8 (8,33)	χ^2=2,99; p>0,05
II/ProAla, n=13 (%)	5 (10,0)	2 (12,5)	6 (7,50)	8 (8,33)	p>0,05
II/12Ala, n=1 (%)	0	0	1 (1,25)	1 (1,04)	-
ID/Pro12, n=45 (%)	22 (44,0)	1 (6,25)	22 (27,50)	23 (23,96)	χ^2=6,19; p=0,013
ID/ProAla, n=26 (%)	4 (8,0)	6 (37,5)	16 (20,0)	22 (22,92)	χ^2=5,0; p=0,025
ID/12Ala, n=4 (%)	1 (2,0)	1 (6,25)	2 (2,50)	3 (3,12)	p>0,05
DD/Pro12, n=36 (%)	6 (12,0)	4 (25,0)	26 (32,50)	30 (31,25)	χ^2=6,56; p=0,01
DD/ProAla, n=3 (%)	3 (6,0)	0	0	0	-
DD/12Ala, n=1 (%)	0	0	1 (1,25)	1 (1,04)	-
Total, n=146 (%)	50 (34,25)	16 (10,96)	80 (54,79)	96 (65,75)	χ^2=28,55; p<0,001

Note. p – the reliability of differences between the total of patients and the control group.

Almost every third patient with NAFLD had an unfavorable combination of the homozygous deletion (DD) of the ACE gene and the *ProPro*-genotype of the PPAR-γ2 gene (31.25%) (DD / Pro12), which was 2.6 times more frequent than in the control group (12.0%) (p = 0.01). In each fifth patient, a combination of heterozygotes for two genes (ID / ProAla variant) was detected, which was 2.9 times more frequent than in the control group: 22.92% vs. 8.0% (χ^2 = 5.0, p = 0.025), respectively. Instead, the people with a combination of the ID-polymorphic variant of the ACE gene and the ProPro genotype of the *PPAR-γ2* gene were dominant by1.84 times: 44.0% versus 23.96% (χ^2 = 6.19, p = 0.013.

Potential genetic risk factors for NAFLD and its subtypes (NASH, NALS) in the population based on six combinations of *PPAR-γ2 (Pro12Ala*) and ACE (I / D) gene genotypes are presented in Table 2.

Table 2

Combinations of polymorphic variants of *PPAR-γ2 (Pro12Ala)*and ACE (I / D) genes as risk factors of non-alcoholic fatty liver disease in people.

Combination of genotypes of the *PPAR-γ2* and *ACE* genes		*RelR*	*OR*	*95%CI RR*	*95%CI OR*	P
II/Pro12	NAFLD	0,46	0,41	0,19-1,13	0,15-1,15	0,08
	NASH	0,69	0,65	0,17-2,89	0,12-3,38	>0,05
	NALS	0,42	0,37	0,16-1,10	0,12-1,11	0,07
II/ProAla, II/12Ala	NAFLD	0,94	0,93	0,33-2,65	0,29-2,94	>0,05
	NASH	1,25	1,29	0,27-5,83	0,22-7,37	>0,05
	NALS	0,87	0,86	0,29-2,61	0,26-2,88	>0,05
ID/Pro12	NAFLD	0,54	0,40	0,34-0,87	0,19-0,83	0,013
	NASH	0,14	0,08	0,02-0,97	0,01-0,69	0,006
	NALS	0,62	0,48	0,39-1,0	0,23-1,01	0,051
ID/ProAla, ID/12Ala	NAFLD	2,30	3,17	1,06-6,39	1,13-8,88	0,023
	NASH	4,37	7,0	1,61-11,89	1,81-27,07	0,006
	NALS	2,25	2,61	0,89-5,68	0,90-7,56	0,07
DD/Pro12	NAFLD	2,60	3,33	1,16-5,84	1,28-8,67	0,01
	NASH	2,08	2,44	0,67-6,47	0,59-10,08	>0,05
	NALS	2,71	3,53	1,20-6,11	1,33-9,34	0,008
DD/ProAla, DD/12Ala	NAFLD	0,17	0,16	0,02-1,63	0,02-1,63	>0,05
	NASH	No patients with this genotype combination				
	NALS	0,21	0,20	0,02-1,95	0,02-1,96	>0,05

Note. NAFLD – non-alcoholic fatty liver disease; NALS– non-alcoholic liver steatosis; NASH – non-alcoholic steatohepatitis; *RelR* -relative risk; *OR* - Odds Ratio; *95%CI RR, OR* - confidence interval of risks (*RR*) and odds (*OR*).

The combination of the I-allele of the ACE gene and the Pro-allele of the PPAR-γ2 gene (Table 2) is protective and makes the chances of the occurrence of NAFLD and its subtypes (especially steatohepatitis) lowest in the surveyed population (OR = 0.40, 95% CI OR = 0.19-0.83, p = 0.013, OR = 0.08, 95% CI OR = 0.01-0.69, p = 0.006 and OR = 0.48, 95% CI OR = 0.23 -1.01, p = 0.051, respectively). Instead, the presence of the *Ala*-allele of the *PPAR-γ2* gene and the ID-genotype of the ACE gene in the combination (*ID / ProAla, ID / 12Ala* variants) increases the risk of NAFLD by 2.3 times (OR = 3.17, 95% CI OR = 1, 13-8,88, p = 0,023), at the expense of NASH – by 4,4 times (OR = 7,0, 95% CI OR = 1,81-27,07, p = 0,006). At the same time in the barriers of the *DD / Pro12* variant the dominant effect of the homozygous deletion of the ACE gene without a significant impact of the *ProPro*-genotype of the *PPAR-γ2* gene increases the risk of NAFLD by 2.6 times (OR = 3.33, 95% CI

OR = 1.28- 8.68, p = 0.01), mainly due to NALS - by 2.7 times (OR = 3.53, 95% CI OR = 1.33-9.34, p = 0.008), respectively.

Conclusions: Bearing the DD genotype of the ACE gene in combination with the Pro12 genotype of the PPAR-γ2 gene increases the risk of NAFLD by 2.6 times (due to non-alcoholic liver steatosis). The combination of the I-allele of the ACE gene and the Pro-allele of the PPAR-γ2 gene is protective with the lowest chance of NAFLD in the population (especially non-alcoholic steatohepatitis).

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