PPARγ2 GENE ALLELES EXPRESSION IN ELDERLY PATIENTS WITH OBESITY AND CORONARY ARTERY DISEASE

L.I. Folomeeva[1*], *E.V. Filippov*[2], *N.Y. Grigorieva*[3]

[1] State budgetary institution of health care of the city of Moscow "City clinical hospital No. 29 Of the Department of health of the city of Moscow", Moscow, Russia.
[2] Federal state budgetary educational institution of higher education "Ryazan state medical University named after the academician I. P. Pavlov" of the Ministry of Health of the Russian Federation, Ryazan, Russia.
[3] National Research Lobachevsky State University of Nizhny Novgorod.

* Corresponding author: larisaigorevna.kudryavtseva@mail.ru

Abstract. We have explored the features of PPARγ2 gene alleles expression in elderly patients with comorbid conditions such as obesity and coronary artery disease. 140 patients of both sexes (54.3% men and 45.7% women) aged 60-89 were examined. The main group included 70 pts with CHD; the control group included 70 pts without CHD. Pro12 allele carrier state in the main group was 85%, and Ala12 allele carrier state – 15%. Pro12Ala and Ala12Ala genotypes, Ala12 allele were detected more often in patients with coronary artery disease than in the control group ($p = 0.0008$ and $p = 0.0003$, respectively). Pro12Ala genotypes (OR = 2.02, CI = 1.28–3.19, $p = 0.003$) and Ala12Ala (OR = 2.002, CI = 1.32–3.04, $p = 0.0006$) of Ala12 gene PPARγ2 carrier state increases the risk of CHD developing by 2 times. It was confirmed that nuclear PPARs are capable of controlling development, differentiation, metabolic homeostasis and reproduction. The role of various genotypes of the PPARγ2 gene in the regulation of lipid metabolism and angiogenesis in comorbid conditions in elderly patients may help to identify new methods of their treatment.

Keywords: peroxisome activation receptors, γ2 promoter, obesity, PPARγ2 gene, coronary artery disease, elderly people.

List of Abbreviations

BMI – body mass index
CAD – coronary artery disease
CHD – coronary heart disease
CI – confidence interval
CVD – coronary vascular diseases
PPARs – peroxisome proliferator-activated receptors
WHO – World Health Organization

Introduction

According to WHO, obesity is a «global non-infectious epidemics of the 21st century» (Soboleva et al., 2014; Hales et al., 2017). Eating disorders, accompanied by body weight increase, caused the widespread occurrence of obesity in the world – approximately 30%. Overweight patients are exposed to a greater risk of developing of vessel wall atherosclerotic lesion, coronary artery disease (CAD) and other cofactor diseases such as diabetes and dyslipidemia, especially in elderly patients.

Peroxisome proliferator-activated receptors (PPARs), being a family of ligand-inducible transcription factors, which is a part of nuclear hormone receptors PPARs superfamily, interact with their lipophile genuine ligands and are included in the composition of desaturated fatty acids, vitamins, steroid hormones and prostaglandins. By this means, nuclear receptors regulate gene expression programs controlling development, differentiation, metabolic homeostasis and reproduction both in time and selective tissue mode (Ivanova et al.,2015; McEwan, 2016).

It is a known fact that a human has 3 isoforms PPARs, coded by different genes: PPARα, PPARß/δ и PPARγ, whose activity profiles only partly overlap and are differentially expressed in different organs and tissues (Lamas & Ferreira, 2019).

PPARγ is abundantly expressed in adipose tissue and to a lesser extent – in macrophages and other cell types, regulating adipogenesis,

lipid accumulation and glucose homeostasis. PPARγ gene has several promoters: γ1, γ2, γ3. Among the above only PPARγ2 has an additional N-terminal area which consists of 30 amino acids. Being expressed only in adipose tissue, PPARγ2 is a powerful activator of transcription and has a protective effect on other types of tissue, protecting them from lipid overexertion by means of maintaining of adequate adipocytokines, adiponectin and leptin expression, which mediate insulin signalization in peripheral tissues (Janani & Kumari, 2015).

At present time PPARγ ligands (pioglitazone and rosiglitazone) are anti-diabetic and insulin sensitizers. Preliminary data on PPAR-γ dual agonists has shown a positive effect on lipid profile, arterial tension, atherosclerosis, inflammation and anti-coagulative effects (Derosa et al., 2018).

A wide range of PPARγ2-effects activation can be applied to the treatment of various coronary vascular diseases (CVD), such as atherosclerosis, arterial hypertension, hypertrophic cardiomyopathy and aortic aneurism (Ivanova et al., 2017). However, their potential capability in the treatment of high coronary vascular risk-related conditions, including coronary artery disease, is not understood.

Taking into account prolongation of life expectancy and the age of retirement raising in Russia, researching of PPARγ2 effect on lipid metabolism in patients with its various genotypes is becoming especially relevant.

The aim of the paper is to research the features of the expression of PPARγ2 gene alleles in elderly patients with obesity and coronary artery disease.

Materials and methods

In the period of 2017-2019 in Moscow «Clinical Hospital № 29 named after N.E. Bauman» 140 patients of both sexes (54.3% men and 45.7% women) aged 60–89 were examined on their own free will. The patients were divided into 2 groups, 70 people in each. The main group included 70 pts with CHD, and the control group included 70 pts without CHD. The examination was performed according to the clinical examination protocols, including anthropometry as well as an in-depth examination of the participants' gerontological and cardiological characteristics. For molecular genetic analysis, the DNA samples obtained by oral cavity epithelial cells withdrawal with a universal sensitive element were used. DNA was extracted from buccal epithelium with the set of reagents Diatom TM DNA Prep (Biokom). Gene PPARγ2 polymorphism was defined through the method of polymerase chain reaction (PCR) by means of amplification with the direct 5'-GCC AAT TCA AGC CCA GTC-3' and reverse 5'-GAT ATG TTT GCA GAC AGT GTA TCA GTG AAG GAA TCG CTT TCC G-3' primers («Metabion», Germany), followed by restriction with endonuclease Bsh1236I («Fermentas», Lithuania). Assessment of Pro12 and Ala12 alleles and their genotypes: Pro12Pro, Pro12Ala and Ala12Ala incidence has been performed. Hardy-Weinberg analysis has been done.

Conducting of the research was based on ethical principles of scientific research with the involvement of people (Declaration of Helsinki) and regulations of GCP. The design of the research has been approved by the ethical issues committee of RyazSMU named after I.P. Pavlov (№ 141617).

Statistical processing of the data has been done with a standard set of Non-parametric tests and statistical programs (Statistica 10.0), with calculating of the average value (M), error of mean (m) and occurrence (%). For prognostic assessment of experimental criteria chances evaluation method with calculating of the confidence interval (C.I.) has been applied. Evaluation of statistical importance of the differences in dependent samples at normal distribution has been performed with the use of Student t-criterium. The differences were thought significant at $p < 0,05$.

Results

The body mass index (BMI) average value indicator in the main group was 32,7 ± ± 3,5 kg/m^2, and 31,5 ± 2,4 kg/m^2 in the patients without CHD, which aligns with obesity.

Table 1

Gene PPARγ2 allele Pro12 and Ala12 genotypes abundance (%) in the patients of the main and control groups

Examined groups	Gene PPARγ2 allele Pro12 and Ala12 genotypes		
	Pro12Pro	Pro12Ala	Ala12Ala
Patients with CAD	74,0%	23,0%*	3,0%*
Control group	85,0%	14,0%	1,0%

Note: * – the values are significantly different ($p < 0,05$)

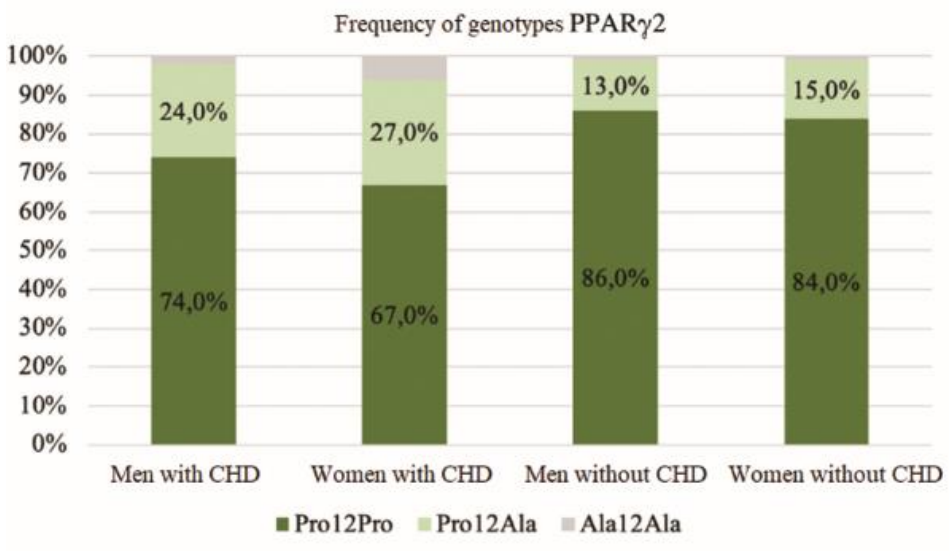

Fig. 1. Carrier state occurrence (%) of gene PPARγ2 alleles Pro12 and Ala12 genotypes Pro12Pro, Pro12Ala and Ala12Ala in patients of the main and control groups depending on the sex

It was found that cumulative obesity risk increased with age.

Departure from Hardy-Weinberg population equilibrium has not been observed.

Carrier state occurrence of gene PPARγ2 Pro12 allele in the main group was 85,0%, aand 92,0% in the control group. It has been documented that 15,0% of the examined with CAD have gene PPARγ2 allele Ala12, which was definitely ($p < 0,05$) higher in comparison with control indicator (8,0%).

The gene PPARγ2 alleles Pro12 and Ala12 genotyping showed that genotypes Pro12Ala (23,0%, $p = 0,0008$) and Ala12Ala (3,0%, $p = 0,0003$) significantly predominated in the main group compared with the control group results, as shown in Table 1.

It has been discovered that gene PPARγ2 alleles Pro12Ala (OR = 2,02, CI = 1,28–3,19, $p = 0,003$) and Ala12Ala (OR = 2,002, CI = = 1,32–3,04, $p = 0,0006$) carrier state increases the risk of CAD developing by 2 times.

The undertaken analysis of various gene PPAR-γ2 genotypes distribution in patients with CAD and the control group, of both sexes has not exposed gender differences of Pro12Pro, Pro 12Ala and Ala12Ala genotypes carrier state. Allele Pro12 occurrence in the main group was 85,0% in both the men and the women, and in the control group – 92,0% in the women and 93,0% in the men. Gene PPARγ2 allele Ala12 occurrence was 15,0% both in the men and the women in the main group, and 7,0% and 8,0% in the men and the women respectively in the control group.

Picture 1 represents carrier state occurrence (%) of gene PPARγ2 alleles Pro12 and Ala12 genotypes Pro12Pro, Pro12Ala and Ala12Ala

in patients of the main and control group depending on the sex.

The analysis results of risk factors for coronary vascular diseases (hypertensive disease, aggravated CAD heredity, smoking, dyslipidemia, obesity and overweight) have not exposed statistically significant differences in gene PPAR-γ2 Pro12Pro, Pro12Ala and Ala12Ala genotypes carriers.

In the process of examining blood lipids in patients with CAD, gene PPAR-γ2 Pro12Pro, Pro12Ala and Ala12Ala genotypes carriers, statistically significant differences have not been documented.

Discussion

Angiogenesis plays an important role in the development of coronary vascular diseases, including coronary artery disease (McEwan, 2016). Angiogenesis regulation is done by means of the set of growth factors and cytokines which are produced in response to hypoxic and inflammatory signals (McEwan, 2016). PPAR participate in angiogenesis under various conditions, although the role of this gene is still discussible. It was proved that the effect of PPARγ agonists on endothelial cells depended on the specimen dosage: angiogenic effect occurred only with low concentrations of the specimen (Fukunaga et al.,2001). M. Fujii and co-authors' research results (2012) indicate angiogenesis positive regulation by gene PPAR-γ2, by means of receptor-2 up-regulation vascular endothelium growth factor (VEGF) (Fujii et al.,2012). On the other hand, according to Z. Wu and co-authors (2012) and A. Qu and co-authors (2012), PPARγ2 is capable of preventing atherosclerosis developing (Wu et al.,2012; Qu et al., 2012).

Having performed a meta-analysis of 23375 pieces, Z. Wu and co-authors. (2012) found that the gene PPARγ2 allele Ala12 homozygosity may have a potential increased risk of CAD development, which is also confirmed by the results of our research (Wu et al., 2012).

Of interest are the results of T. Pischon and co-authors research (2005). They examined 249 women and 266 men with coronary artery disease and with a history of an acute coronary syndrome (Pischon et al., 2005). The authors established that relative risk for non-fatal infarction or fatal CAD in gene PPARγ2 allele Ala12 carriers compared with non-carriers is 1,17 in women (95%, CI = 0,82–1,68) and 1,44 (95%, CI = 1,00–2,07) in men. Also, the above paper reveals the discovered significantly increased risk related to allele Ala12, in people with body mass index > or = 25 kg/m2 (women: RR = 1,88; 95%, CI = 1,01–3,50; men: RR = 1,55; 95%, CI = 0,92–2,60) (Pischon et al., 2005). The received data correspond with the results of our research as the BMI of the examined cohort with CAD was 32,7 kg/m2. It is proven that the gender in the presence of overweight aggravates CVD development: 44% and 37% of women with obesity have an increased risk for myocardial infarction and stenocardia, respectively (Neifeld et al., 2012).

It was proved that gene PPAR-γ allele Ala12 carrier state affects the underlying risk for CAD and related to myocardial infarction risk in people at the age of 45 and under among the population Northern-Western region of the Russian Federation, which emphasizes the relevance of the undertaken research results (Sergeeva et al., 2017).

However, the contrary data also exist. It was established that gene PPARγ2 genotype Ala12 can decrease the number of damaged vessels and the severity of CAD, which may be related to the direct antiatherogenic effect of this polymorphism as well as to the indirect effect, connected with a lower inflammatory parameters level and occurrence of insulin resistance (Youssef et al., 2013).

The results of our research represent lower allele Pro12 carrier state occurrence (85,0%) in patients with CAD, compared to the control group (95,0%). It can be assumed that the protection of organs and tissues from the hyperlipidemic load is done through increased expression of this particular allele of gene PPARγ2. Further studying of this theory may be interesting for defining the role of gene PPARγ2 in lipid exchange regulation and angiogenesis in the presence of such comorbid conditions as obesity and coronary artery disease. In addition, in animal models, PPAR activation leads to an improvement in the lipid profile and regulates glucose homeostasis, which in the

long term may determine new therapies (Ivanova et al.,2015; McEwan, 2016).

Conclusions

Expression of PPARγ2 ligands, which are produced by adipose cells, has a multidirectional impact on patients' organisms, both young and elderly. Overweight is of critical pathogenetic importance to the development of cardiovascular diseases, including coronary artery disease. However, the features of gene PPARγ2 alleles expression in elderly patients with obesity and coronary artery disease identified in this research confirm the capability of specifically PPARs nuclear receptors to control development, differentiation, metabolical homeostasis and reproduction both in time and selective tissue mode. The perspective goal is to define the role of various gene PPARγ2 genotypes in lipid metabolism and angiogenesis more precisely, in the presence of the discussed comorbid conditions with the aim of discovering new treatment methods.

Conflict of interests does not exist.

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