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Ассоциации полиморфизмов генов NOD2/CARD15, CRP и FTO с распространённостью сердечно-сосудистых факторов риска, поражений органов-мишеней и кардиоваскулярных заболеваний у пациентов с язвенным колитом и болезнью Крона

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АННОТАЦИЯ

Актуальность. Роль генетических факторов в развитии сердечно-сосудистых заболеваний (ССЗ) у пациентов с воспалительными заболеваниями кишечника (ВЗК) практически не известна.

Цель. Изучить частоту носительства аллельных полиморфных вариантов генов NOD2/CARD15 (3020insC rs5743293, Gly908Arg rs2066845), CRP (+1444C>T rs1130864), FTO (A23525Trs9939609) и провести комплексную оценку наличия ассоциаций генетических факторов с распространённостью сердечно-сосудистых факторов риска (ССФР), субклинических поражений органов-мишеней, диастолической дисфункции миокарда левого желудочка и ССЗ у больных язвенным колитоми болезнью Крона, проживающих в Рязанской области.

Материалы и методы. В исследование включено 62 пациента (41 (66%) женщина, 40,5 [33,5; 52,25] лет), страдающих ВЗК (51 пациент с язвенным колитом, 11 пациентов с болезнью Крона), у которых методом аллель-специфической полимеразной цепной реакции определяли полиморфизмы 3020insC и Gly908Arg в гене CARD15 (NOD2), C1444T в гене CRP и A23525T в гене FTO, а затем оценивали частоту носительства их аллельных вариантов и связь с ССФР, поражениями органов-мишеней (оценка артериальной жёсткости, пульсового давления, гипертрофии миокарда левого желудочка, лодыжечно-плечевого индекса) и ССЗ.

Результаты. В соответствии с целями исследования частота носительства аллельных полиморфных вариантов генов: 3020insC в гене CARD15 (NOD2) rs5743293 85,5% — гомозигота по аллели 1, 14,5% — гетерозигота; Gly908Arg в гене CARD15 (NOD2) rs2066845 93,5% — гомозигота по аллели 1, 6,5% — гетерозигота; C1444T в гене CRP rs1130864 50% — гомозигота по аллели 1, 41,9% — гетерозигота, 8,1% — гомозигота по аллели 2; A23525T в гене FTO rs9939609 38,7% — гомозигота по аллели 1, 38,7% — гетерозигота, 22,6% — гомозигота по аллели 2. Распространённость артериальной гипертензии — 31%, ожирениядо по индексу массы тела — 18%, по окружности талии — 29%, и дислипидемии — 53%. Обнаружены статистически значимые ассоциации: 1) гиперхолестеринемии с полиморфизмом Gly908Arg в гене CARD15 (NOD2) rs2066845 ($\chi 2 = 6{,}005$; $p = 0{,}014$), 2) семейного анамнеза ранних ССЗ с полиморфизмом 3020insC в гене CARD15 (NOD2) rs5743293 ($\chi 2 = 5{,}561$; $p = 0{,}018$), а также с полиморфизмом Gly908Arg в гене CARD15 (NOD2) rs2066845 ($\chi 2 = 4{,}561$; $p = 0{,}033$), 3) артериальной гипертензии с полиморфизмом 3020insC в гене CARD15 (NOD2) rs5743293 ($\chi 2 = 4{,}65$; $p = 0{,}031$).

Заключение. Показатели распространённости артериальной гипертензии, ожирения и дислипидемии у пациентов с ВЗК в настоящем исследовании существенно ниже, чем у лиц сопоставимого возраста по данным эпидемиологического исследования МЕРИДИАН-РО в Рязанском регионе. Обнаружены статистически значимые ассоциации изучаемых полиморфизмов с гиперхолестеринемией, семейным анамнезом ранних сердечно-сосудистых заболеваний и артериальной гипертензией.

Ключевые слова: *3020insC; Gly908Arg; C1444T; A23525T; воспалительные заболевания кишечника; артериальная жесткость; сердечно-лодыжечный сосудистый индекс*

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Associations of Polymorphisms of NOD2/CARD15, CRP and FTO Genes with Cardiovascular Risk Factors, Damages to Target Organs and Cardiovascular Diseases in Patients with Ulcerative Colitis and Crohn's Disease

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ABSTRACT

INTRODUCTION: The role of genetic factors in the development of cardiovascular diseases (CVD) in patients with inflammatory bowel diseases (IBD) is practically unknown.

AIM: To study the frequency of carriage of allelic polymorphous variants of NOD2/CARD15 (3020insC rs5743293, Gly908Arg rs2066845), CRP (+1444C>T rs1130864), FTO (A23525T rs9939609) genes and to perform a comprehensive assessment of damage to the target organs, diastolic myocardial dysfunction of the left ventricle and CVD in patients with ulcerative colitis and Crohn's disease living in the Ryazan District.

MATERIALS AND METHODS: The study involved 62 patients (41 (66%) women, 40.5 [33.5; 52.25] years old) with IBD (51 patients with ulcerative colitis, 11 patients with Crohn's disease) in whom 3020insC and Gly908Arg polymorphisms in CARD15 (NOD2) gene, C1444T in CRP gene and A23525T in FTO gene were determined using allele-specific polymerase chain reaction. After that, the frequency of carriage of their allelic variants and association with CVRFs, damage to the target organs (through evaluation of the arterial stiffness, pulse pressure, hypertrophy of the left ventricular myocardium, ankle-brachial index) and CVDs was evaluated.

RESULTS: According to the aims of the study, the frequency of carriage of allelic polymorphic variants of genes was determined: 3020insC in gene CARD15 (NOD2) rs5743293 85.5% — homozygote for allele 1, 14,5% — heterozygote; Gly908Arg in gene CARD15 (NOD2) rs2066845 93.5% — homozygote for allele 1, 6.5% — heterozygote; C1444T in gene CRP rs1130864 50% — homozygote for allele 1, 41.9% — heterozygote, 8.1% — homozygote for allele 2; A23525T in gene FTO rs9939609 38.7% — homozygote for allele 1, 38.7% — heterozygote, 22.6% — homozygote for allele 2. The prevalence of arterial hypertension was 31%, of obesity by body mass index — 18%, by waist circumference — 29%, and of dyslipidemia — 53%. Statistically significant associations were found between: 1) hypercholesterolemia and Gly908Arg polymorphism in gene CARD15 (NOD2) rs2066845 ($\chi 2 = 6.005$; $p = 0.014$), 2) family history of early CVD and 3020insC polymorphism in gene CARD15 (NOD2) rs2066845 ($\chi 2 = 4.561$; $p = 0.033$), 3) arterial hypertension and 3020insC polymorphism in geneCARD15 (NOD2) rs5743293 ($\chi 2 = 4.65$; $p = 0.031$).

CONCLUSION: The prevalence of arterial hypertension, obesity and dyslipidemia in patients with IBD is considerably lower in the given study than in the individuals of comparable age in the epidemiological MERIDIAN-RO study conducted in the Ryazan region. Statistically significant associations were found between the studied polymorphisms and hypercholesterolemia, family history of early cardiovascular diseases and arterial hypertension.

Keywords: *3020insC; Gly908Arg; C1444T; A23525T; inflammatory bowel diseases; arterial stiffness; cardio-ankle vascular index*

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ECO•VECTOR

LIST OF ABBREVIATIONS

AH — arterial hypertension
BMI — body mass index
CARD15 — caspase recruitment domain-containing protein 15
CAVI — cardio-ankle vascular index
CD — Crohn's disease
CI — confidence interval
CRP — C-reactive protein
CVD — cardiovascular disease
DNA — deoxyribonucleic acid
FTO — fat mass- and obesity-associated gene
IBD — inflammatory bowel diseases
IQR — interquartile range
MERIDIAN-RO — epidemiological study of health state and behavioral risk factors in population of the Ryazan region
NF — nuclear factor
NOD2 — nucleotide-binding oligomerization domain-containing protein 2
OR — odds ratio
UC — ulcerative colitis

INTRODUCTION

Individuals with certain chronic immune-mediated inflammatory diseases (e.g., psoriasis, systemic lupus erythematosus and ankylosing spondylitis) are known to have a higher risk of developing cardiovascular pathology than individuals with similar social and demographic characteristics in the population, therefore the presented target group is actively considered from positions of early and timely cardiovascular prevention measures [1]. As for the inflammatory bowel diseases (IBDs) including Crohn's disease (CD), ulcerative colitis (UC) and unclassifiable (undifferentiated) colitis, no ideas have been generally accepted up to date concerning their role in the development and progression of cardiovascular diseases (CVD).

IBDs are a consequence of complex interactions between environmental factors, the intestinal microbiota and the immune system in genetically predisposed individuals, leading to development of a multifaceted chronic systemic inflammatory process associated with the effects of proinflammatory cytokines. In realization of subclinical phenotypes of lesions of the cardiovascular system, of the formation and destabilization of atherosclerotic plaques, special attention is given to the regulatory role of inflammation both in the concept of traditional cardiovascular risk factors, and irrespective of them. The cardiologic community analyzes successively emerging information for patients with IBDs about the elevated burden of subclinical lesions of target organs, which are components and predictors of the increased cardiovascular risk and are inextricably linked to the 'hard clinical endpoint' — mortality [2]. In the updated Clinical Guidelines for prevention of CVDs in clinical practice (2021), the experts of the European Society of Cardiology concluded: '...there is evidence indicating an approximately twenty percent increase in the risk of CVDs in patients with IBD during relapse' [3]. The statement has been placed immediately after a widely debated topic of cardiovascular risks in patients with rheumatoid arthritis, and is probably a way to express special hopes for creating research field about the role of IBDs in the development of CVDs, and demonstrates the prospect for its further study.

This research field is located at the interface of many specialties, in particular, one should note genetic and epigenetic constituent of IBD, which determines the diversity of its extra-intestinal manifestations [4]. It should also be noted that IBDs are associated with more than 200 genetic loci, many of which are also linked with other immune-inflammatory diseases including the process of atherogenesis [5, 6].

More than 20 years ago, the first gene associated with susceptibility to Crohn's disease — NOD2/CARD15 (nucleotide-binding oligomerization domain-containing protein 2/caspase recruitment domain-containing protein 15) was identified, which is responsible for activation of kB nuclear factor (NF) — one of the key regulators of inflammation, immune homeostasis, production of free radicals (the data on which were presented in detail in investigation of the pathogenesis of coronavirus infection [7]), playing a significant role in the pathogenesis of both IBDs and atherosclerotic lesion of vessels [8].

According to an Italian prospective study (n = 218), common genetic variations (polymorphisms) in the NOD2/CARD15 gene (irrespective of traditional cardiovascular risk factors) were associated with an increased risk of clinically diagnosed and angiographically confirmed coronary atherosclerosis and clinical destabilization of coronary heart disease, and rs9939609 polymorphism in the FTO gene associated with fat mass and obesity, according to a number of studies, was independently associated with the risk of developing both CD and CVD, which may point to a common genetic basis for IBD and atherosclerosis; however, currently there is very little reliable evidence in this aspect [9,10].

The **aim** of this study to evaluate the frequency of carriage of allelic polymorphisms in NOD2/CARD15 (3020insC rs5743293, Gly908Arg rs2066845), CRP (+1444C>T rs1130864), FTO (A23525Trs9939609) genes and to analyze the existence of associations of genetic

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factors with the prevalence of cardiovascular risk factors, subclinical lesions of target organs (through assessment of arterial stiffness, pulse pressure, left ventricular hypertrophy, ankle-brachial index), diastolic dysfunction of the left ventricular myocardium and cardiovascular diseases in patients with ulcerative colitis and Crohn's disease living in the Ryazan region.

MATERIALS AND METHODS

The clinical basis of the study was Regional Clinical Cardiologic Dispensary (Ryazan). The laboratory basis was the Central Scientific and Research laboratory of Pavlov Ryazan State Medical University. The study was approved by the Local Ethics Committee of Pavlov Ryazan State Medical University (Protocol No. 9 of April 05, 2021).

In the period from June 22, 2021 to July 8, 2022, the pilot single center clinical study included 62 patients with IBD of any duration, extent of lesion and any basic therapy (51 patients with UC (82.3%) and 11 patients with CD (17.7%), Table 1)). The median and interquartile age for the age 40.5 [33.5; 52.25] years (41 women (66.1%) and 21 men (33.9%)).

Table 1. General Clinical Characteristics of Patients Included in Study

Groups of Patient		n	%
Gender	Men	21	34
	Women	41	66
Age at the time of blood sampling, years	18–19	1	2
	20–29	10	16
	30–39	16	26
	40–49	16	26
	50–59	10	16
	60–69	5	8
	70 and above	4	6
Diagnosis	Ulcerative colitis	51	82
	Crohn's disease	11	18
Phase of disease	Clinical remission (partial Mayo index for ulcerative colitis 0-2 or Best index < 150)	51	82
	Recurrence (attack, active phase; partial Mayo index > 2 or Best index 150 and more)	11	18
Course	Chronic continuous	4	6,5
	Chronic recurrent	58	94

Inclusion criteria:

- signing of a voluntary informed consent to participation in the study and publication of the results obtained;
- established diagnosis of UC or CD;
- age 18 years and more.

Exclusion criteria:

- the patient's decision to withdraw from the study at any time (notifying the researcher by phone or email);
- known mental illnesses;
- known alcohol and drug abuse.

On the initial examination, the history was taken, medical documentation was studied, anthropometric data were studied with the calculation of body mass index (BMI) according to the Quetelet formula, waist and hip circumference. The 'office' blood pressure on both arms was taken (three times) with a CS Medica CS-109 Pro mechanical tonometer (Shenzhen Complect service Industrial & Trade Co., Ltd., China), and of the heart rate (auscultation of the heart). Using the method of volume sphygmography on a VaSera VS-1500N device (Fukuda Denshi, Japan), the cardio-ankle vascular index (CAVI) (arterial stiffness index) and ankle-brachial index were

determined. Patients filled out an EQ-5D-5L (EuroQol group) questionnaire to assess the quality of life. Instrumental examinations of the cardiovascular system were performed, including 12-channel electrocardiography, daily blood pressure monitoring, echocardiography, and a treadmill test. All patients included in the study, were taken blood from a vein in a volume of 5 ml into vacuum tubes with the anticoagulant ethylenediaminetetraacetic acid with subsequent isolation of genomic deoxyribonucleic acid (DNA) from whole blood leukocytes.

Allele-specific polymerase chain reaction was used to determine 3020insC and Gly908Arg polymorphisms in the CARD15 (NOD2) gene, C1444T in the CRP gene and A23525T in the FTO gene (SNP-EXPRESS-RV product detection scheme; Litech Research and production company, Russia) in the central research laboratory of Pavlov Ryazan State Medical University. Based on the results of the analysis, three types of conclusions were made: homozygote for allele 1, heterozygote, homozygote for allele 2 (allele 1 is indicated before the designation of the polymorphism position, allele 2, respectively, after the designation of the polymorphism position).

The choice of candidate genes in the study was based on the analysis of the literature data on the association of their polymorphisms with risks of IBD and CVD [9–13]. The associations of the studied polymorphisms with the prevalence of the traditional cardiovascular risk factors, subclinical lesions of the target organs (by evaluation of arterial stiffness, pulse pressure, ankle-brachial index), hypertrophy of the left ventricular myocardium, diastolic dysfunction of the left ventricle (by echocardiography data) and cardiovascular diseases were analyzed.

The results of the study were analyzed using Microsoft Office Excel 2007 (Microsoft Corporation, USA) and Stat Soft Statistica 13.0 (Stat Soft Inc., USA) programs. The fundamental methods were descriptive and nonparametric statistics. The qualitative data are presented in the form of absolute and relative (%) values, quantitative data in the form of Me (IQR), where Me is the median, IQR is the interquartile range. The significance of differences in the frequencies of genotypes and alleles was determined by methods of nonparametric statistics (Pearson's chi-squared test) and linear regression analysis. The correspondence of genotype frequencies in the samples to Hardy–Weinberg equilibrium was assessed. The differences were considered statistically significant at $p < 0.05$. The strength of the identified associations was assessed by the odds ratio (OR) and its 95% confidence interval (CI). A CI that did not include unity, i. e. in which both values of its boundaries were above or below unity at significance level of $p < 0.05$, was considered statistically significant.

Based on the Montreal classification of UC by the extent of lesion, 11.8% (n = 6) of patients were diagnosed with proctitis, 52.9% (n = 27) with left-sided colitis, 27.5% (n = 14) with total colitis, and in 7.8% (n = 4) of cases it was impossible to reliably determine the extent of the lesion based on the provided documentation. In all patients with CD, an extended CD (Montreal classification of CD, 2005) was recorded; based on localization of the lesion, 72.7% (n = 8) of patients were diagnosed with ileocolitis (in two patients with anorectal lesions), 18.2% (n = 2) with terminal ileitis, and one patient had colitis with lesion of the anorectal zone. Genetic engineering biological therapy was conducted in 21% (n = 13) of patients. At the time of inclusion in the study, 4.8% (n = 3) of patients received treatment with infliximab, 6.5% (n = 4) used the drug earlier (withdrawal of the drug or replacement with another one), 6.5% (n = 4) of patients received adalimumab, 3.2% (n = 2) used the drug earlier, 4.8% (n = 3) received vedolizumab, 3.2% (n = 2) — golimumab, 1.6% (n = 1) — certolizumab paegol.

The age of patients with arterial hypertension (AH) in the study was 55 [48.5; 66.5] years. Seventeen patients (27.4%) were smoking at the time of inclusion in the study or stopped smoking less than 5 years before inclusion, 21% (n = 13) had smoking in history and stopped smoking more than 5 years before inclusion in the study. Six patients had no smoking in history but were subjected to passive smoking (18.8% of never-smokers in the group). The age of obese patients in the study was 45 [37.5; 50] years old, of overweight patients — 58 [46.75; 65.75] years, of patients with abdominal obesity — 51.5 [42; 60.75] years. The ratio of waist circumference to hip circumference > 0.9 in men and > 0.85 in women was recorded in 62.5% of overweight patients (n = 10), in all obese patients and in 20% of patients (n = 7) with normal body weight (Table 2).

Higher education — in 66.1% (n = 41) of patients.

An obvious excess salt intake was detected in 11.3% (n = 7).

In patients without cardiovascular diseases, diabetes mellitus, chronic kidney disease, and having not very high levels of separate risk factors, the relative (for people under 40 years; n = 29) and absolute (for people of 40 years and older; n = 22) total cardiovascular risks were evaluated on the SCORE scale (Systematic Coronary Risk Assessment, a scale of absolute risk of fatal cardiovascular complications in the next 10 years of life). High and very high-risk category (≥ 5%) was determined on the scale in 5 patients (9.8%), each patient was over 40. In 16 patients (25.8%) the category of high and very high cardiovascular risk was determined using the traditional stratification model of the total risk of cardiovascular complications. The average CAVI value was 7.2 ± 1.2 (Table 3). Pulse pressure of 60 mmHg or more was recorded in 50% of persons (4 out of 8 patients) aged 60 years or older. According to echocardiography, the values of the left ventricular ejection fraction were 66 [64; 68]%.

Table 2. Anthropometric Data of Examined Patients

Parameter	Value
Body mass index, Me (IQR), kg/m^2	24.1 [21.13; 28.15]
Waist circumference, Me (IQR), cm	84 [73.5; 99.5]
Obesity by BMI, %	17.7
Overweight by BMI, %	25.8
Obesity in waist circumference, %	29

Notes: Me — median, IQR — interquartile range, BMI — body mass index

Table 3. Data of Prevalence of Cardiovascular Risk Factors, Lesions of Target Organs and Cardiovascular Diseases among Patients Included in the Study

Disease/Condition	n	%
Arterial hypertension	19	31
Hypercholesterolemia (total cholesterol above 4.9 mmol/l (190 mg/dl))	33	53
Active smoking or less than 5 years after giving up smoking before inclusion in the study	17	27
Passive smoking	22	35
Obesity by body mass index	11	18
Obesity by waist circumference (waist circumference ≥ 102 cm in men, ≥ 88 cm in women)	18	29
Overweight	16	26
Type 2 diabetes mellitus / disorder in glucose tolerance / disorder in fasting glycemia (by medical documentation data)	5	8
Family history of early cardiovascular diseases (< 55 years in men, < 65 years in women)	24	39
Insufficient physical activity (moderate physical activity < 150 minutes a week, intensive physical activity < 75 minutes a week)	13	21
Consumption of fruit and vegetables less than once a day	20	32
Anxiety/depression (based on EQ-5D-5L questionnaire)	35	56
Cardio-ankle vascular index above threshold values for the corresponding age group in Japanese population	9	15
Cardio-ankle vascular index above threshold values for the corresponding age group in Russian population	3	5
Ankle-brachial index < 0.91 or > 1.29	4	6
Myocardial hypertrophy of the left ventricle (of 52 examined patients)	3	6
Diastolic dysfunction of the myocardium of the left ventricle (of 52 examined patients)	10	19
Coronary heart disease	5	8
IIA stage chronic heart failure	1	2
Atrial fibrillation	1	2
Transient ischemic attack	1	2

RESULTS

The main results of the study are given in Tables 4–6.

Table 4. Frequency of Carriage of Allelic Polymorphisms and Correspondence of Genotype Frequency to Hardy–Weinberg Equilibrium (with use of $\chi 2$)

Polymorphism	Frequency of Carriage of Allelic Polymorphisms, %			$\chi 2$	p
	Homozygote for allele 1	Heterozygote	Homozygote for allele 2		
3020insC in gene CARD15 (NOD2) rs5743293	85.5	14.5	0	0.38	*0.54*
Gly908Arg in gene CARD15 (NOD2) rs2066845	93.5	6.5	0	0.07	*0.79*
C1444T in gene CRP rs1130864	50.0	41.9	8.1	0.02	*0.89*
A23525T in gene FTO rs9939609	38.7	38.7	22.6	2,61	*0.11*

Table 5. Associations between Genetic Factors and Cardiovascular Risk Factors, Lesions of Target Organs

Allelic Polymorphisms	Parameter							
	Cardio-Ankle Vascular Index		Total Cholesterol		Body Mass Index		Waist Circumference	
	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
3020insC in gene CARD15 (NOD2) rs5743293 homozygote for allele 1	7.29	7.05–7.53	5.21	4.95–5.49	25.15	24.04–26.26	86.12	82.87–89.37
3020insC in gene CARD15 (NOD2) rs5743293 heterozygote	6.68	6.13–7.23 (*p = 0.051*)	4.74	4.08–5.41 (*p = 0.161*)	24.07	22.00–26.13 (*p = 0.445*)	85.33	79.95–90.72 (*p = 0.845*)
Regression coefficient B	-0.611 (-1.22: 0.003), *p = 0.051*		5.218 (4.952: 5.483), *p = 0.161*		-1.08 (-3.89: 1.72), *p = 0.445*		-0.79 (-8.75: 7.18), *p = 0.845*	
Gly908Arg в гене CARD15 (NOD2) rs2066845 гомозигота по аллели 1	7.22	6.88–7.55	5.14	4.87–5.41	24.95	23.93–25.97	85.70	82.76–88.64
Gly908Arg в гене CARD15 (NOD2) rs2066845 гетерозигота	6.98	6.31–7.64 (*p = 0,595*)	5.16	5.02–5.30 (*p = 0.961*)	25.60	20.88–30.32 (*p = 0.751*)	91.67	76.41–106.93 (*p = 0.364*)
Regression coefficient B	-0.241 (-1.13: 0.65), *p = 0.595*		5.141 (4.883: 5.398), *p = 0.961*		0.65 (-3.38: 4.68), *p = 0.751*		5.97 (-7.02: 18.96), *p = 0.364*	
C1444T in gene CRP rs1130864 homozygote for allele 1	7.20	6.95–7.44	5.33	4.96–5.70	24.20	22.93–25.47	84.59	80.53–88.64
C1444T in gene CRPrs1130864 heterozygote	7.05	6.65–7.45	5.02	4.67–5.38	25.59	23.80–27.38	86.60	81.86–91.34
C1444T in gene CRP rs1130864 homozygote for allele 2	7.98	7.00–8.96 (*p = 0.092*)	4.40	3.30–5.50 (*p = 0.182*)	26.82	24.92–28.72 (*p = 0.288*)	91.20	83.58–98.82 (*p = 0.441*)
Regression coefficient B	0.159 (-0.18: 0.50), *p = 0.361*		5.347 (5.017: 5.678), *p = 0.074*		1.35 (-0.19: 2.89), *p = 0.09*		2.76 (-1.67: 7.20), *p = 0.220*	
A23525T in gene FTOrs9939609 homozygote for allele 1	6.99	6.67–7.32	4.94	4.54: 5.34	25.30	23.70: 26.90	86.22	81.49–90.94
A23525T in gene FTOrs9939609 heterozygote	7.42	7.00–7.84	5.29	4.94: 5.65	25.16	23.55–26.76	88.09	83.33–92.85
A23525T in gene FTOrs9939609 homozygote for allele 2	7.18	6.82–7.54 (*p = 0.232*)	5.40	4.88: 5.91 (*p = 0.257*)	24.19	21.95–26.43 (*p = 0.684*)	82.36	76.53–88.19 (*p = 0.317*)
Regression coefficient B	0.129 (-0.16: 0.42), *p = 0.372*		4.965 (4.638: 5.292), *p = 0.111*		-0.51 (-1.80: 0.78), *p = 0.437*		-1.57 (-5.25: 2.10), *p = 0.398*	

Notes: CI — confidence interval, OR — odds ratio

Table 6. Calculation of $\chi 2$ Criteria for a Number of Parameters of Traditional Cardiovascular Risk Factors in Terms of Single Nucleotide Replacements

Аллельные полиморфные варианты	Polymorphisms							
	3020insC in gene CARD15 (NOD2) rs5743293		Gly908Arg in gene CARD15 (NOD2) rs2066845		C1444T in gene CRP rs1130864		A23525T in gene FTO rs9939609	
	$\chi 2$	p	$\chi 2$	p	$\chi 2$	p	$\chi 2$	p
Arterial hypertension	**4.65**	***0.031***	0.064	*0.8*	0.413	*0.813*	2.291	*0.318*
Hypercholesterolemia	1.859	*0.173*	**6.005**	***0.014***	1.957	*0.376*	0.129	*0.938*
Family history of early cardiovascular diseases	**5.561**	***0.018***	**4.561**	***0.033***	2.033	*0.362*	2.268	*0.322*
Obesity	0.634	*0.426*	0.309	*0.578*	0.224	*0.894*	0.337	*0.845*
Abdominal obesity	0.474	*0.491*	0.67	*0.795*	5.080	*0.079*	0.004	*0.998*

Note: statistically significant differences are highlighted in bold and background

DISCUSSION

The associations obtained in our study were previously considered only in isolated publications. Thus, in an Italian prospective case-control study, the incidence of the NOD2/CARD15 gene Gly908Arg polymorphism of interest to us was significantly lower in the group of patients with coronary heart disease than in the control group (1.8% vs. 6.4% in the control group; odds ratio (OR) 0.05; 95% CI 0.01–0.69; p = 0.031, multivariate analysis)), which is a source of discussion about a probable protective effect of this polymorphism in the context of coronary atherogenesis, while the prevalence of Leu1007fsinsC polymorphisms in the NOD2/CARD15 gene (significantly — 11.9% vs. 1.8% in the control group; OR 7.2; 95% CI 1.5–32.9; p = 0.01), and of Arg702Trp (not significantly — 10.1% vs. 3.7% in the control group; OR 2.9; 95% CI 0.91–9.6; p = 0.07) was higher in patients with coronary heart disease compared with the control group. In patients with acute coronary syndrome, the prevalence of Leu1007fsinsC polymorphism was significantly higher than in patients with stable exertion angina (OR 5.7; 95% CI 1.1–39.7; p = 0.034) [11].

Different results were obtained in a large Danish study: polymorphic variants in the NOD2/CARD15 genes (Gly908Arg, Arg702Trp, Leu1007fsinsC) were not associated with increased risk of CVDs [12].

In a German study, no statistically significant difference in the incidence of the same three gene NOD2/CARD15 polymorphisms between patients with coronary heart disease and the control group was either found [13].

To note, in the Ryazan region, a study of polymorphic variants in the NOD2/CARD15 gene associated with the risk of IBDs (2 of them were the same as in our study — Gly908Arg, Leu3020insC), have already been conducted, but no association between the studied polymorphisms with the development of UC and features of its course was found.

We emphasize the lack of reliable international evidence-based data even in the aspect of the prevalence of traditional cardiovascular risk factors, in patients with IBD [15]. The need to create tools for an objective assessment of the risk of CVD in patients with UC and CD, taking into account a steady growth of the morbidity in industrialized countries, motivates us to further explore this unexplored area. Obviously, the data on the increased risks of cardiovascular diseases in IBD cannot be attributed to the existence of traditional cardiovascular risk factors in this group of patients alone. A number of studies provide information on a lower prevalence of classic cardiovascular risk factors among patients with IBD compared to the general population, in particular, lower parameters of blood lipid spectrum and BMI, as well as lower prevalence of diabetes mellitus and obesity, while other studies, on the contrary, point to an increased prevalence or frequency of several traditional risk factors, including hypertension and diabetes mellitus, in patients with IBD compared with healthy individuals [1, 15].

A dissonance has also been identified in the parameters of the blood lipid spectrum in patients with IBD and the control group. Tobacco smoking is known to have a damaging effect in CD, including a higher risk of development and progression of the disease, a poor response to medicinal and surgical treatment, while in UC smoking can have a protective effect, however, no

convincing results have been demonstrated in the studies of nicotine substitution therapy in UC. The prevalence of obesity by BMI in the Ryazan region based on a prospective MERIDIAN-RO study among patients who have not reached the combined endpoint (the mean age 42.9 ± 0.3 years), made 41%. The prevalence of this cardiovascular risk factor in our study (mean age 43.72 ± 14.87 years) was 17.7%, the risk factor for hypertension: 44.5% in the MERIDIAN-RO study versus 31% in our study; for dyslipidemia: 83.3% versus 53% in our study, which may indirectly evidence a lower prevalence of these cardiovascular risk factors in patients with IBD in the region [16].

The data on the prevalence of smoking are consistent: 24.4% in MERIDIAN–RO study in patients without combined endpoint versus 27% in our study, the prevalence of anxiety / depression in patients without a combined endpoint in the population study was 62.1% versus 56% in our study, insufficient physical activity was 23.3% against 21%, respectively. The results of the study of vascular stiffness in patients with IBDs appeared rather unexpected: in only 5% of patients, CAVI was above the threshold values for the respective age group in the Russian population (besides, all patients in this group had AH). To note, the use of this method claimed an 'indicator of true arterial stiffness' mathematically purified of the effect of the blood pressure [17] in IBD, is not described in the available literature. Limitations of the study were a short follow-up period (a bit more than one year), high proportion of young patients (53% under 44 years), absence of a control group or comparison group.

The frequency of carriage of polymorphisms in the genes NOD2/CARD15 (3020insC rs5743293, Gly908Arg rs2066845), CRP (+1444C>T rs1130864), FTO (A23525Trs9939609) and the revealed associations with cardiovascular risk factors require additional verification in large-scale studies. The results obtained contribute to prioritization in further search for common genetic determinants of inflammatory bowel diseases and cardiovascular pathology and can be considered as the 'necessary first step' towards the formation of a concept of high-quality management of cardiovascular risks in patients with inflammatory bowel diseases in the Russian Federation.

CONCLUSION

In accordance with the aims of the study, the frequency of carriage of allelic polymorphism variants: 3020insC in the CARD15 (NOD2) rs5743293 gene 85.5% — homozygote for allele 1, 14.5% — heterozygote; Gly908Arg in the CARD15 (NOD2) rs2066845 gene 93.5% — homozygote for allele 1, 6.5% — heterozygote; C1444T in the CRP rs1130864 gene 50% — homozygote for allele 1, 41.9% — heterozygote, 8.1% — homozygote for allele 2; A23525T in the FTOrs9939609 gene 38.7% — homozygote for allele 1, 38.7% — heterozygote, 22.6% — homozygote for allele 2, was determined.

Statistically significant associations were found of:

1) hypercholesterolemia with Gly908Arg polymorphism in the CARD15 (NOD2) rs2066845 gene ($\chi 2 = 6.005$; $p = 0.014$);

2) family history of early cardiovascular diseases with 3020insC polymorphism in the gene CARD15 (NOD2) rs5743293 ($\chi 2 = 5.561$; $p = 0.018$), as well as with Gly908Arg polymorphism in the gene CARD15 (NOD2) rs2066845 ($\chi 2 = 4.561$; $p = 0.033$);

3) arterial hypertension with 3020insC polymorphism in the gene CARD15 (NOD2) rs5743293 ($\chi 2 = 4.65$; $p = 0.031$).

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