UDC 616.728.3-007.248
DOI: 10.21668/health.risk/2022.4.13.eng

Read online

Research article

NFAT5 GENE POLYMORPHISM AS A RISK FACTOR OF KNEE OSTEOARTHROSIS

V.B. Novakov[1,2], O.N. Novakova[1], M.I. Churnosov[1]

[1]Belgorod National Research University, 85 Pobedy Str., Belgorod, 308015, Russian Federation
[2]City Hospital No. 2 of Belgorod, 46 Gubkina Str., Belgorod, 308036, Russian Federation

Knee osteoarthrosis (OA) is a multifactorial disease with genetic factors playing an important part in its development. Our research goal was to examine associations between polymorphic variants rs1060105 and rs56116847 of SBNO1 gene, rs6499244 of NFAT5 gene and rs34195470 of WWP2 gene and developing stage 4 knee osteoarthrosis in people living in the Central Chernozem Region in Russia.

Genotyping of polymorphic loci of candidate genes was accomplished in 95 patients with stage 4 knew osteoarthrosis and 500 people without the disease who were included into the reference group. We estimated associations between polymorphic loci of candidate genes and knee OA by using logistic linear regression within the allele, additive, recessive and dominant genetic models with gPLINK software.

As a result, we replicated an association between a GWAS-significant rs6499244 polymorphism of NFAT5 gene and knee OA in people living in the Central Chernozem Region in Russia. An allele variant A of rs6499244 of NFAT5 gene was established to be "a risk factor" regarding stage 4 knee OA within the additive (OR = 1.61, p_{perm} = 0.02) and recessive (OR = 2.07, p_{perm} = 0.02) genetic models. The rs6499244 locus of NFAT5 gene is located in an area of DNAse I hypersensitivity; it increases DNA affinity to four transcription factors (CDP_6, RFX5_known1, RORalpha1_2, TCF4_known1); it is localized in functionally active promoters and enhancers; it is associated with expression of nine genes (CLEC18A, COG4, EXOSC6, NFAT5, NOB1, NPIPB14P, NQO1, PDXDC2P, SMG1P7) and alternative mRNA splicing of three genes (NOB1, NPIPB14P, NQO1) in various organs and tissues in the body including those that are pathogenetically significant for OA (fat tissue, tibial nerves and arteries, and skeletal muscles).

Keywords: *knee osteoarthritis, NFAT5 gene, polymorphic locus, associations, candidate genes, risk factor, nuclear factor of activated T cells 5, evaluation of functional effects produced by polymorphism.*

Knee osteoarthrosis (OA) or gonarthrosis is a complex disease that involves degenerative, dystrophic and sometimes inflammatory changes in tissues forming the knee joint (cartilage, menisci, subchondral bone, synovial membrane, ligaments, and fibrous capsule) as well as in tissues beyond it, which are still an integral part of functions performed by the knee joint and are located close to it (tendons and muscles) [1]. According to data available in literature, OA prevalence is different in different populations across the world [2–5]. OA prevalence varies from 1.5 to 29.1 % in European countries [2, 3, 6–8] whereas this indicator is higher in Asia where it can reach 44 % [9–12]. According to official statistics, in Russia 13 % of people who are older than 18 have OA of the knee or hip joint [5, 13] and this corresponds to the disease prevalence in European populations. Gonarthrosis is also known to become more frequent among older people and its prevalence is higher among women [6, 14]. We should remember that knee OA develops in working age population thus deterio-

Vitaliy B. Novakov – postgraduate medical student; traumatologist-orthopedist (e-mail: v.novakov@bk.ru; tel.: +7 (472) 255-85-70; ORCID: https://orcid.org/0000-0001-5337-2156).

Olga N. Novakova – Candidate of Biological Sciences, Associate Professor (e-mail: litovkina@bsu.edu.ru; tel.: +7 (472) 230-13-83; ORCID: https://orcid.org/0000-0003-2700-1284).

Mikhail I. Churnosov – Doctor of Medical Sciences, Professor, Head of the Department of Biomedical Disciplines (e-mail: churnosov@bsu.edu.ru; tel.: +7 (472) 230-13-83; ORCID: https://orcid.org/0000-0003-1254-6134).

rating their health and resulting in untimely disability [15].

Osteoarthrosis, just like some other human diseases, is considered a multifactorial disease that occurs due to interactions between several environmental, epigenetic and genetic risk factors [16]. As it has been stated in research works, a contribution made by the genetic component to OA development may vary from 40 to 65 % [17, 18]. Genetic grounds for OA development are being actively studied by various research teams who perform whole-genome sequencings aimed at detecting relevant associations. According to the GWAS Catalog, over a period from 2008 up to now 24 whole-genome sequencings have been accomplished with their focus on the knee and hip joints as well as hand joints[1]. As a result, researchers have detected more than 250 GWAS-significant single-nucleotide polymorphisms (SNPs) associated with OA development. We should note that 13 out of 24 studies have concentrated on examining a role that belongs to genetic factors in knee OA development; they made it possible to establish more than 80 polymorphic loci associated with risks of knee OA development ($p \leq 5 \cdot 10^{-08}$). The majority of these loci that are GWAS-significant for knee OA (more than 95 %) were established by analyzing samplings made of Caucasian people. But we should also note that Russian people have never been included in samplings analyzed in the aforementioned whole-genome sequencings.

In addition to whole-genome sequencing of OA, we should mention replication studies that are also significant. They give an opportunity to establish a role played by specific polymorphic markers that are GWAS-significant for OA in development of the disease in a given population across the world, Russian ones included. According to data available in literature, by now there has been a rather limited number of replication studies with their focus on knee OA and OA with other localizations performed in various populations worldwide [19–26]. These replication studies have established associations with the disease for isolated GWAS-significant polymorphic loci: OA in general, 4 SNPs (rs2302061, rs7639618, rs4836732, rs3204689); knee OA, 7 SNPs (rs4867568, rs143383, rs3884606, rs10947262, rs7639618, rs6976, rs2302061). Three out of seven SNPs (rs4867568, rs143383, rs3884606) had a confirmed association with knee OA in Asian populations and four SNPs (rs10947262, rs7639618, rs6976, rs2302061) in European ones or in mixed samplings that included both Europeans and Asians.

It is important to point out that in Russian literature there has been only one replication study focusing on OA among women in Bashkortostan [26]. The authors analyzed associations between OA in women and nine GWAS-significant polymorphisms including rs4836732of *ASTN2* gene; rs1298744 and rs2302061 of *DOT1L* gene, rs3204689 of *ALDH1A2* gene, rs6976 of *GLT8D1* gene, rs11177 of *GNL3* gene, rs6094710 of *NCOA3* gene, rs11841874 of *MCF2L* gene, and rs7639618 of *DVWA* gene. The analysis was performed in two groups, the test one made of 256 patients and the reference one made of 161 women without the disease. The study confirmed associations with OA with different localizations only for five SNPs (rs2302061 *DOT1L*, rs7639618 *DVWA*, rs4836732 *ASTN2*, rs6976 *GLT8D1*, and rs3204689 *ALDH1A2*); an association with knee OA was confirmed only for three SNPs, namely rs2302061 *DOT1L*, rs7639618 *DVWA*, and rs6976 *GLT8D1*.

Therefore, there are more than 80 known polymorphic loci that are GWAS-significant for knee OA. Still, any associations with the disease among European populations are confirmed by replications studies only for four of them, namely rs10947262 of *BTNL2* gene, rs7639618 of *COL6A4P1* gene, rs2302061 of *DOT1L* gene, and rs6976 of *GLT8D1* gene and this is extremely insufficient. We should note that these replication studies performed

[1] GWAS Catalog. The NHGRI-EBI Catalog of human genome-wide association studies. *National Human Genome Research Institute*. Available at: https://www.ebi.ac.uk/gwas/search?query=osteoarthritis (May 20, 2022).

on European populations (or mixed samplings) have not confirmed any associations with knee OA for 10 SPNs. All the aforementioned facts call for conducting further replication studies of knee OA in various populations, Russian included.

Our research goal was to examine associations between polymorphic variants rs1060105 and rs56116847 of *SBNO1* gene, rs6499244 of *NFAT5* gene, and rs34195470 of *WWP2* gene and developing stage 4 knee osteoarthrosis among people living in the Central Chernozem Region in Russia.

Materials and methods. To conduct our study, we created two samples. The first one, or the test group, was made of 95 patients with knee OA (their average age was 52.69 ± 5.67 years); the second one was the reference group made of 500 people (their average age was 52.96 ± 6.72 years). All the patients had radiological stage 4 of the disease characterized with the most apparent destruction of various joint tissues, first of all, cartilage and bone [2]. Clinical and instrumental examinations of the patients were performed and their diagnosis was verified by certified physicians at the Traumatology Department No. 1 of the Belgorod City Hospital No. 2. We relied on the following criteria when including patients into our test group: a) a patient had primary knee OA diagnosed in accordance with clinical and radiological criteria developed by the American College of Rheumatology [27]; b) a patient had stage 4 knee OA as per Kellgren – Lawrence scale [28]; c) a patient suffered from knee joint pains (pain syndrome intensity was ≥ 40 mm as per the visual-analog scale when walking); d) movement was limited in the knee joint. The reference group included people without any pathology of the musculoskeletal system (its absence was identified during periodical medical examinations). All the participants were born in the Central Chernozem Region, were native Russians and were not blood relatives; they all gave their consent to participate in the study prior to it. People with severe hypertension, ischemic heart disease, diabetes mellitus, liver and kidney failure or oncological diseases were excluded from the analyzed samples.

We examined genome DNA in our study. It was isolated from leukocytes by phenol chloroform extraction. DNA samples were genotyped by performing the polymerase chain reaction with TaqMan probes.

We selected polymorphic loci of candidate genes to analyze them in our study using the following criteria: 1) an association with knee OA established in European population by previous whole-genome studies (GWAS); 2) apparent functional significance of a polymorphism (regulatory potential, an association with expression and alternative gene splicing) [29, 30]; 3) frequency of a polymorphism being not less than 5 %. Four SPNs of three genes were selected for the present study in accordance with these criteria. They were rs1060105 and rs56116847 of *SBNO1* gene, rs6499244 of *NFAT5* gene and rs34195470 of *WWP2* gene.

We determined empirical distribution of genotypes for the analyzed samplings and checked whether it corresponded to a theoretically expected one in accordance with the Hardy – Weinberg law (deviations were considered statistically significant at $p \leq 0.05$). When comparing frequency of alleles and genotypes in the test group (patients with OA) and the reference group, we applied χ^2 criterion with Yates's correction for continuity and contingency 2×2. Intensity of associations was estimated using odds ratio (*OR*) with 95 % confidence interval (95 % CI) with STATISTICA for Windows 6.0. Associations between OA and the analyzed polymorphic loci of candidate genes were estimated by using logistic linear regression (we considered four genetic models, namely, allele, additive, recessive and dominant one), such covariants as age and BMI taken into account. All the calculations were performed with gPLINK software [31]. We performed multiple comparison correction allowing for a permutation test ($p_{perm} < 0.05$ was considered statistically significant) [32].

We estimated functional effects produced by polymorphism of candidate genes that had significant associations with OA by using

HaploReg (v4.1)[2] to examine regulatory potential [29, 33] and GTExPortal[3] to examine influence exerted on gene expression and alternative splicing [30].

Results and discussion. We established that the actual distribution of genotypes corresponded to the expected one according to the Hardy – Weinberg equilibrium for all the analyzed polymorphic loci of candidate genes in the test and reference groups ($p > 0.05$) (Table 1).

We analyzed associations between alleles and genotypes of examined candidate genes polymorphism and developing stage 4 knee OA and established an association between rs6499244 locus of *NFAT5* gene and the disease (Table 1). The logistic regression analysis revealed that the minor allele A in rs6499244 of *NFAT5* gene had certain "risky" significance for OA within the additive (*OR* = 1.61, 95 % CI = 1.09–2.38, $p = 0.02$, $p_{perm} = 0.02$) and recessive (*OR* = 2.07, 95 % CI = 1.10–3.90, $p = 0.02$, $p_{perm} = 0.02$) genetic models.

Therefore, we identified a genetic risk factor of knee OA development in our study. This factor is the allele A in rs6499244 of *NFAT5* gene.

The next stage in our study involved analyzing regulatory effects produced by rs6499244 of *NFAT5* gene (using up-to-date bioinformatics resources). The analysis revealed that this locus had critical functional importance in the body.

Use of HaploReg (v4.1)[2] made it possible to establish that rs6499244 was located in the 3'-non-translated area in *NFAT5* gene, in an evolutionary conservative area of DNase I hypersensitivity in ovary tissues, in a region of regulatory motifs to four transcription factors (CDP_6, RFX5_known1, RORalpha1_2, TCF4_known1). We identified that rs6499244 of *NFAT5* gene was localized in a DNA area associated with histones (H3K27ac) that marked active enhancers in chondrocyte cell culture, brain, fat tissue, and peripheral blood cells. It was also localized in a DNA-area interacting with H3K9ac type histones that marked functionally active promoter sections in fat tissue cells and primary mononuclear cells in peripheral blood. The allele A, which is "risky" as regards OA development, increases affinity to four transcription factors: CDP_6 (ΔLOD = 3,3), RFX5_known1 (ΔLOD = 10,9), RORalpha1_2 (ΔLOD = 12), TCF4_known1 (ΔLOD = 5).

Use of GTExPortal[3] made it possible to establish that the locus rs6499244 had a significant association with expression of nine genes (*CLEC18A, COG4, EXOSC6, NFAT5, NOB1, NPIPB14P, NQO1, PDXDC2P, SMG1P7*) and alternative mRNA splicing of three genes (*NOB1, NPIPB14P, NQO1*) in cell cultures, tissues and organs that had pathogenetic significance for OA development (fat tissue, skeletal muscles, tibial arteries and nerves) (Table 2). We revealed that rs6499244 was associated with expression of *CLEC18A, EXOSC6, NPIPB14P, SMG1P7* genes and alternative mRNA splicing of three genes *NOB1, NPIPB14P, NQO1* in fat tissues. Fat tissue is known to produce adipokines that not only participate in metabolic processes but also make for chronic inflammatory reactions in the body. Adipokines and some other cytokines act together and these combined actions lead to cartilage tissue degradation [34]. T.N. Boer with colleagues (2012) showed that levels of adipokines were substantially higher in patients with radiological stage 4 osteoarthrosis than in the reference group ($p < 0.001$) [35]. We should note that *NFAT5* gene also participates in regulating inflammatory reactions in the body due to activation of immune cells, especially T-cells and macrophages [36].

We established that the allele A in rs6499244 of *NFAT5* gene was associated with low expression of *CLEC18A, NOB1, NPIPB14P* genes and alternative mRNA splicing of *NOB1* gene in skeletal muscles (Table 2). The condition of muscle tissues, muscle weakness included, is known to make a substantial contribution to OA development and progressing [37, 38]. Muscle weakness can be caused not only by absence of

[2] HaploReg v4.1. Available at: http://compbio.mit.edu/HaploReg (May 18, 2022).
[3] GTExPortal. Available at: http://www.gtexportal.org/ (May 18, 2022).

Table 1

Frequencies of alleles and genotypes established for polymorphic loci of candidate genes in the patients with stage 4 knee OA and the reference group

Polymorphism	Alleles, genotypes	Patients with stage 4 knee OA (n = 95), abs.(%)	Reference group (n = 500), abs.(%)	*OR* (95 % CI)	p
rs1060105	C	149 (78.42 %)	788 (78.80 %)	0.98 (0.66–1.95)	0.98
	T	41 (21.58 %)	212 (21.20 %)	1.02 (0.69–1.52)	
	C/C	58 (61.05 %)	315 (63.00 %)	0.92 (0.57–1.48)	0.81
	C/T	33 (34.74 %)	158 (31.60 %)	1.15 (0.71–1.87)	0.63
	T/T	4 (4.21 %)	27 (5.40 %)	0.77 (0.22–2.39)	0.82
	H_o/H_e (P_{HWE})	0.347/0.338 (1.000)	0.316/0.334 (0.229)		
	Minor allele T (allele model)			1.02 (0.70–1.49)	0.91
	C/Cvs. C/Tvs. T/T (additive model)			1.24 (0.77–1.98)	0.38
	C/Cvs. C/T + T/T (dominant model)			1.37 (0.78–2.42)	0.28
	C/C + C/Tvs. T/T (recessive model)			0.95 (0.25–3.65)	0.94
rs56116847	G	119 (62.63 %)	640 (64.26 %)	0.93 (0.67–1.30)	0.73
	A	71 (37.37 %)	356 (35.74 %)	1.07 (0.77–1.50)	
	G/G	37 (38.95 %)	213 (42.77 %)	0.85 (0.53–1.37)	0.56
	A/G	45 (47.37 %)	214 (42.97 %)	1.19 (0.75–1.90)	0.50
	A/A	13 (13.68 %)	71 (14.26 %)	0.95 (0.48–1.87)	1.00
	H_o/H_e (P_{HWE})	0.474/0.468 (1.000)	0.430/0.459 (0.145)		
	Minor allele A (allele model)			1.07 (0.78–1.48)	0.67
	G/G vs. A/G vs. A/A (additive model)			0.84 (0.56–1.28)	0.42
	G/Gvs. A/G + A/A (dominant model)			0.82 (0.47–1.45)	0.50
	G/G + A/Gvs. A/A (recessive model)			0.76 (0.33–1.77)	0.53
rs6499244	T	94 (49.47 %)	543 (54.30 %)	0.82 (0.60–1.14)	0.25
	A	96 (50.53 %)	457 (45.70 %)	1.21 (0.88–1.68)	
	T/T	26 (27.37 %)	157(31.40 %)	0.82 (0.49–1.38)	0.51
	A/T	42 (44.21 %)	229 (45.80 %)	0.94 (0.59–1.49)	0.86
	A/A	27 (28.42 %)	114 (22.80 %)	1.34 (0.80–2.26)	0.29
	H_o/H_e (P_{HWE})	0.442/0.500 (0.304)	0.458/0.496 (0.087)		
	Minor allele A (allele model)			1.21 (0.89–1.66)	0.22
	T/T vs. A/T vs. A/A (additive model)			**1.61 (1.09–2.38)**	**0.02**
	T/T vs. A/T + A/A (dominant model)			1.74 (0.91–3.32)	0.09
	T/T + A/Tvs. A/A (recessive model)			**2.07 (1.10–3.90)**	**0.02**
rs34195470	G	101 (53.72 %)	523 (52.40 %)	1.05 (0.76–1.46)	0.80
	A	87 (46.28 %)	475 (47.60 %)	0.95 (0.67–1.31)	
	G/G	24 (25.53 %)	136 (27.25 %)	0.92 (0.54–1.56)	0.83
	A/G	53 (56.38 %)	251 (50.30 %)	1.28 (0.80–2.04)	0.33
	A/A	17 (18.09 %)	112 (22.45 %)	0.76 (0.42–1.39)	0.42

End of the table 1

Polymorphism	Alleles, genotypes	Patients with stage 4 knee OA (n = 95), abs.(%)	Reference group (n = 500), abs.(%)	*OR* (95 % CI)	*p*
	H_o/H_e (P_{HWE})	0.564/0.497 (0.222)	0.503/0.499 (0.929)		
	Minor allele A (allele model)			0.95 (0.69–1.30)	0.74
	G/G vs. A/G vs. A/A (additive model)			0.97 (0.64–1.47)	0.89
	G/Gvs. A/G + A/A (dominant model)			1.05 (0.55–2.01)	0.88
	G/G + A/Gvs. A/A (recessive model)			0.87 (0.42–1.77)	0.69

Note: *OR* is odds ratio, 95 % CI is 95 % confidence interval of odds ratio, *p* is the significance level, H_o/H_e is observed / expected heterozygosis, P_{HWE} is the significance level of a deviation from the Hardy – Weinberg law.

Table 2

Data on associations between rs6499244 of *NFAT5* gene expression (eQTL) and alternative mRNA splicing (sQTL) of genes in cells, tissues and organs pathogenetically significant for OA

Gene	Tissue/organ/cell culture	The significance level *p*	Linear regression coefficient β
eQTL analysis			
CLEC18A	Thyroid gland	1.9e-9	-0.35
	Fat tissue	2.4e-7	-0.30
	Skeletal muscles	0.000057	-0.18
COG4	Fibroblast cell culture	0.000041	-0.10
EXOSC6	Fat tissue	0.0000038	-0.21
	Thyroid gland	0.00040	-0.14
NFAT5	Thyroid gland	9.1e-19	0.18
	Fibroblast cell culture	0.0000012	0.083
NOB1	Skeletal muscles	3.9e-10	-0.11
	Thyroid gland	3.3e-7	-0.14
	Brain	0.000020	-0.33
NPIPB14P	Tibial nerves	4.7e-15	-0.31
	Fibroblast cell culture	1.4e-13	-0.28
	Aorta	3.0e-10	-0.27
	Tibial artery	3.8e-10	-0.21
	Blood	1.0e-9	-0.17
	Brain	2.0e-9	-0.42
	Skeletal muscles	6.7e-9	-0.21
	Fat tissue	7.6e-7	-0.17
NQO1	Brain	0.000061	-0.21
PDXDC2P	Blood	0.000029	0.11
SMG1P7	Fat tissue	0.00013	-0.16
sQTL analysis			
NOB1	Skeletal muscles	3.8e-36	-0.61
	Fat tissue	8.1e-33	-0.66
	Tibial artery	2.0e-30	-0.62
	Thyroid gland	7.9e-30	-0.56
	Fibroblast cell culture	3.4e-29	-0.68
	Tibial nerves	3.6e-26	-0.63
	Blood	1.2e-16	0.42

End of the table 2

Gene	Tissue/organ/cell culture	The significance level *p*	Linear regression coefficient β
NPIPB14P	Blood	2.8e-9	-0.34
	Fat tissue	0.0000032	0.29
	Fibroblast cell culture	0.0000059	-0.28
NQO1	Fibroblast cell culture	2.9e-8	0.30
	Fat tissue	1.6e-7	0.31
	Tibial nerves	0.000023	0.25

Note: eQTL and sQTL are data taken from GTExPortal [30].

physical loads in muscles or their low levels but also by inflammation in them [38] which can be regulated, among other things, by *NFAT5* gene [36]. S. Muraki and others (2015) showed that weakness of the quadriceps muscle of thigh was associated with pains in patients with knee OA [37]. B.E. Oiestad with colleagues performed a systemic review and meta-analysis (2015) and obtained certain data on weakness of knee joint muscles being associated with elevated risks of knee OA both in men and women [38].

S.J. Rice and others (2019) provided data on associations between the locus rs7359336 that was not in equilibrium regarding coupling with rs6499244 ($r^2 = 0.91$), which we analyzed in our study, with elevated DNA methylation in *WWP2* gene in patients with OA [39]. The authors also established a substantial increase in *CLEC18A* gene expression in cartilage tissue in patients who had arthroplasty to treat OA ($p = 0.02$).

NFAT5 gene is known to code a transcription factor that regulates expression of genes participating in osmotic stress in mammals. The latter can become a basis for developing pathological processes in various hyperosmolar tissues and organs (the kidneys, intestinal epithelium, cornea and skin epidermis, and skeletal muscles)[4] [40, 41]. Some data confirm a role *NFAT5* gene plays in creating inborn immunity by activating gene expression in macrophages during TLR (Toll-like) receptor ligation [36, 42, 43]. We should note that immune disorders and inflammation of the synovial membranes (synovitis) are believed to contribute significantly to OA pathogenesis [2, 44, 45]. In future this may lead to "activation" of matrix metalloproteinases [46] that produce apparent proliferative and pro-inflammatory effects. *TLR2* and *TLR4* are known to be expressed intensely in macrophages of synovial fluid and to be responsible for macrophages activation [47]. *NFAT5* gene also plays an important role in proliferation of synoviocytes [48]. Inflammatory infiltrate of synovial fluid taken from patients with gonarthrosis contains large quantities of macrophages and T-lymphocytes [44]. According to data available in literature, macrophages mostly prevail in this infiltrate accounting for 65 %, followed by T-lymphocytes with their share being up to 22 % and B-lymphocytes up to 5 % [49].

There is a rather limited number of studies with their focus on how the locus rs6499244 of *NFAT5* gene (which is associated with knee OA as we have established in the present study) is involved into development of various human diseases. The GWAS Catalog provides data on the results produced by only two whole-genome sequencings that revealed significant associations between the analyzed locus and knee osteoarthrosis [50] and an age when menarche occurs [51]. Thus, I. Tachmazidou with colleagues performed a GWAS study (2019) on samples made of 77,052 patients who had OA with different localizations, knee OA included, and 378,169 people in the reference group. The study estab-

[4] GeneCards: The Human Gene Database. Available at: http://www.genecards.org/ (May 20, 2022).

lished an association between the allele A in rs6499244 of *NFAT5* gene and knee OA in an European population [50] and this is completely in line with our results.

Conclusion. Our study confirmed (replicated) an association between the GWAS-significant polymorphic variant rs6499244 of *NFAT5* gene and knee OA in people living in the Central Chernozem Region in Russia. We established that the allele A in rs6499244 *NFAT5* was a risk factor of stage 4 knee OA (*OR* = 1.61–2.07). rs6499244 polymorphism of *NFAT5* gene produces apparent functional effects (it is located in an area of DNase I hypersensitivity, increases DNA affinity to four transcription factors, is localized in functionally active promoters and enhancers and is associated with expression of nine genes (*CLEC18A, COG4, EXOSC6, NFAT5, NOB1, NPIPB14P, NQO1, PDXDC2P, SMG1P7*) and alternative mRNA splicing of three genes (*NOB1, NPIPB14P, NQO1*)) in various tissues and organs (tibial nerves and arteries, fat tissue, skeletal muscles and others) that are involved into pathophysiology of the disease.

Funding. The research was not granted any sponsor support.

Competing interests. The authors declare no competing interests.

References

1. Madry H., Kon E., Condello V., Peretti G.M., Steinwachs M., Seil R., Berruto M., Engebretsen L. [et al.]. Early osteoarthritis of the knee. *Knee Surg. Sports Traumatol. Arthrosc.*, 2016, vol. 24, no. 6, pp. 1753–1762. DOI: 10.1007/s00167-016-4068-3

2. Alekseeva L.I., Taskina E.A., Kashevarova N.G. Osteoarthritis: epidemiology, classification, risk factors, and progression, clinical presentation, diagnosis, and treatment. *Sovremennaya Revmatologiya*, 2019, vol. 13, no. 2, pp. 9–21. DOI: 10.14412/1996-7012-2019-2-9-21 (in Russian).

3. Branco J.C., Rodrigues A.M., Gouveia N., Eusébio M., Ramiro S., Machado P.M., da Costa L.P., Mourão A.F. [et al.]. Prevalence of rheumatic and musculoskeletal diseases and their impact on healthrelated quality of life, physical function and mental health in Portugal: results from EpiReumaPt – a national health survey. *RMD Open*, 2016, vol. 2, no. 1, pp. e000166. DOI: 10.1136/rmdopen-2015-000166

4. Wallace I.J., Worthington S., Felson D.T., Jurmain R.D., Wren K.T., Maijanen H., Woods R.J., Lieberman D.E. Knee osteoarthritis has doubled in prevalence since the mid-20th century. *Proc. Natl Acad. Sci. USA*, 2017, vol. 114, no. 35, pp. 9332–9336. DOI: 10.1073/pnas.1703856114

5. Balabanova R.M., Erdes S.F. Trends in the prevalence of rheumatic diseases in ICD-10 in the adult population of the Russian Federation over 2000–2010. *Nauchno-prakticheskaya revmatologiya*, 2012, vol. 50, no. 3, pp. 10–12. DOI: 10.14412/1995-4484-2012-702 (in Russian).

6. Comas M., Sala M., Roman R., Castells X. Impact of the distinct diagnostic criteria used in population-based studies on estimation of the prevalence of knee osteoarthritis. *Gac. Sanit*., 2010, vol. 24, no. 1, pp. 28–32. DOI: 10.1016/j.gaceta.2009.06.002 (in Spanish).

7. Bijlsma J.W.J., Berenbaum F., Lafeber F.P.J.G. Osteoarthritis: an update with relevance for clinical practice. *Lancet*, 2011, vol. 377, no. 9783, pp. 2115–2126. DOI: 10.1016/S0140-6736(11)60243-2

8. Kim C., Linsenmeyer K.D., Vlad S.C., Guermazi A., Clancy M.M., Niu J., Felson D.T. Prevalence of radiographic and symptomatic osteoarthritis in an urban United States community: the Framingham osteoarthritis study. *Arthritis Rheumatol*., 2014, vol. 66, no. 11, pp. 3013–3017. DOI: 10.1002/art.38795

9. Zhang Y., Xu L., Nevitt M.C., Aliabadi P., Yu W., Qin M., Lui L.-Y., Felson D.T. Comparison of the prevalence of knee osteoarthritis between the elderly Chinese population in Beijing and whites in the United States: The Beijing Osteoarthritis Study. *Arthritis Rheum*., 2011, vol. 44, no. 9, pp. 2065–2071. DOI: 10.1002/1529-0131(200109)44:9<2065::AID-ART356>3.0.CO;2-Z

10. Ho-Pham L.T., Lai T.Q., Mai L.D., Doan M.C., Pham H.N., Nguyen T.V. Prevalence of radiographic osteoarthritis of the knee and its relationship to self-reported pain. *PLoS One*, 2014, vol. 9, no. 4, pp. e94563. DOI: 10.1371/journal.pone.0094563

11. Nguyen T.V. Osteoarthritis in southeast Asia. *Int. J. Clin. Rheumatol*., 2014, vol. 9, no. 5, pp. 405–408. Available at: https://www.openaccessjournals.com/articles/osteoarthritis-in-southeast-asia.pdf (May 20, 2022).

12. Lee S., Kim S.-J. Prevalence of knee osteoarthritis, risk factors, and quality of life: The Fifth Korean National Health And Nutrition Examination Survey. *Int. J. Rheum. Dis*., 2017, vol. 20, no. 7, pp. 809–817. DOI: 10.1111/1756-185X.12795

13. Galushko E.A., Nasonov E.L. Prevalence of rheumatic diseases in Russia. *Al'manakh klinicheskoi meditsiny*, 2018, vol. 46, no. 1, pp. 32–39. DOI: 10.18786/2072-0505-2018-46-1-32-39 (in Russian).

14. Panikar V.I., Shcherban E.A., Pavlova I.A. Complex geriatric assessment of osteoarthrosis of knee joints in the senior age. *Nauchnye rezul'taty biomeditsinskikh issledovanii*, 2019, vol. 5, no. 1, pp. 131–139. DOI: 10.18413/2313-8955-2019-5-1-0-10 (in Russian).

15. Matveev R.P., Bragina S.V. Knee joint osteoarthrosis: problems and social significance. *Ekologiya cheloveka*, 2012, vol. 19, no. 9, pp. 53–62. DOI: 10.33396/1728-0869-2012-9-53-62 (in Russian).

16. Novakov V.B., Novakova O.N., Churnosov M.I. Genome-wide studies of knee osteoarthritis: Review. *Travmatologiya i ortopediya Rossii*, 2021, vol. 27, no. 4, pp. 131–144. DOI: 10.21823/2311-2905-2021-27-1580 (in Russian).

17. Zengini E., Finan C., Wilkinson J.M. The Genetic Epidemiological Landscape of Hip and Knee Osteoarthritis: Where Are We Now and Where Are We Going? *J. Rheumatol*., 2016, vol. 43, no. 2, pp. 260–266. DOI: 10.3899/jrheum.150710

18. Evangelou E., Kerkhof H.J., Styrkarsdottir U., Ntzani E.E., Bos S.D., Esko T., Evans D.S., Metrustry S. [et al.]. A meta-analysis of genome-wide association studies identifies novel variants associated with osteoarthritis of the hip. *Ann. Rheum. Dis*., 2014, vol. 73, no. 12, pp. 2130–2136. DOI: 10.1136/annrheumdis-2012-203114

19. Meulenbelt I., Chapman K., Dieguez-Gonzalez R., Shi D., Tsezou A., Dai J., Malizos K.N., Kloppenburg M. [et al.]. Large replication study and meta-analyses of DVWA as an osteoarthritis susceptibility locus in European and Asian populations. *Hum. Mol. Genet*., 2009, vol. 18, no. 8, pp. 1518–1523. DOI: 10.1093/hmg/ddp053

20. Shi D., Zheng Q., Chen D., Zhu L., Qin A., Fan J., Liao J., Xu Z. [et al.]. Association of single-nucleotide polymorphisms in HLA class II/III region with knee osteoarthritis. *Osteoarthritis Cartilage*, 2010, vol. 18, no. 11, pp. 1454–1457. DOI: 10.1016/j.joca.2010.07.009

21. Nakajima M., Shi D., Dai J., Tsezou A., Zheng M., Norman P.E., Chou C.-H., Lee M.T.M. [et al.]. A large-scale replication study for the association of rs17039192 in HIF-2α with knee osteoarthritis. *J. Orthop. Res*., 2012, vol. 30, no. 8, pp. 1244–1248. DOI: 10.1002/jor.22063

22. Valdes A.M., Styrkarsdottir U., Doherty M., Morris D.L., Mangino M., Tamm A., Doherty S.A., Kisand K. [et al.]. Large scale replication study of the association between HLA class II/BTNL2 variants and osteoarthritis of the knee in European-descent populations. *PLoS One*, 2011, vol. 6, no. 8, pp. e23371. DOI: 10.1371/journal.pone.0023371

23. Dai J., Ying P., Shi D., Hou H., Sun Y., Xu Z., Chen D., Zhang G. [et al.]. FTO variant is not associated with osteoarthritis in the Chinese Han population: replication study for a genome-wide association study identified risk loci. *J. Orthop. Surg. Res*., 2018, vol. 13, no. 1, pp. 65. DOI: 10.1186/s13018-018-0769-2

24. Zhao T., Zhao J., Ma C., Wei J., Wei B., Liu J. Evaluation of Relationship Between Common Variants in FGF18 Gene and Knee Osteoarthritis Susceptibility. *Arch. Med. Res*., 2020, vol. 51, no. 1, pp. 76–81. DOI: 10.1016/j.arcmed.2019.12.007

25. Li Y., Liu F., Xu X., Zhang H., Lu M., Gao W., Yin L., Yin Z. A novel variant near LSP1P3 is associated with knee osteoarthritis in the Chinese population. *Clin. Rheumatol*., 2020, vol. 39, no. 8, pp. 2393–2398. DOI: 10.1007/s10067-020-04995-8

26. Shapovalova D.A., Tyurin A.V., Khusainova R.I. Study of locuses associated with osteoarthritis on the basis of the results of full genomic investigations (GWAS) among women from Bashkortostan. *Terapiya*, 2019, vol. 5, no. 7 (33), pp. 91–96. DOI: 10.18565/therapy.2019.7.91-96 (in Russian).

27. Altman R.D. Criteria for classification of clinical osteoarthritis. *J. Rheumatol. Suppl.*, 1991, vol. 27, pp. 10–12.

28. Kellgren J.H., Lawrence J.S. Radiological assessment of osteo-arthrosis. *Ann. Rheum. Dis*., 1957, vol. 16, no. 4, pp. 494–502. DOI: 10.1136/ard.16.4.494

29. Ward L.D., Kellis M. HaploReg v4: systematic mining of putative causal variants, cell types, regulators and target genes for human complex traits and disease. *Nucleic Acids Res*., 2016, vol. 44, no. D1, pp. D877–D881. DOI: 10.1093/nar/gkv1340

30. GTEx Consortium. The GTEx Consortium atlas of genetic regulatory effects across human tissues. *Science*, 2020, vol. 369, no. 6509, pp. 1318–1330. DOI: 10.1126/science.aaz1776

31. Purcell S., Neale B., Todd-Brown K., Thomas L., Ferreira M.A.R., Bender D., Maller J., Sklar P. [et al.]. PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am. J. Hum. Genet*., 2007, vol. 81, no. 3, pp. 559–575. DOI: 10.1086/519795

32. Che R., Jack J.R., Motsinger-Reif A.A., Brown C.C. An adaptive permutation approach for genome-wide association study: evaluation and recommendations for use. *BioData Min.,* 2014, vol. 7, pp. 9. DOI: 10.1186/1756-0381-7-9

33. Polonikov A.V., Klyosova E.Yu., Azarova I.E. Bioinformatic tools and internet resources for functional annotation of polymorphic loci detected by genome wide association studies of multifactorial diseases (review). *Nauchnye rezul'taty biomeditsinskikh issledovanii*, 2021, vol. 7, no. 1, pp. 15–31. DOI: 10.18413/2658-6533-2020-7-1-0-2 (in Russian).

34. Strebkova E.A., Alekseeva L.I. Osteoarthritis and obesity. *Nauchno-prakticheskaya revmatologiya*, 2015, vol. 53, no. 5, pp. 542–552. DOI: 10.14412/1995-4484-2015-542-552 (in Russian).

35. De Boer T.N., van Spil W.E., Huisman A.M., Polak A.A., Bijlsma J.W.J., Lafeber F.P.J.G., Mastbergen S.C. Serum adipokines in osteoarthritis; comparison with controls and relationship with local parameters of synovial inflammation and cartilage damage. *Osteoarthritis Cartilage*, 2012, vol. 20, no. 8, pp. 846–853. DOI: 10.1016/j.joca.2012.05.002

36. Lee N., Kim D., Kim W.-U. Role of NFAT5 in the Immune System and Pathogenesis of Autoimmune Diseases. *Front. Immunol*., 2019, vol. 10, pp. 270. DOI: 10.3389/fimmu.2019.00270

37. Muraki S., Akune T., Teraguchi M., Kagotani R., Asai Y., Yoshida M., Tokimura F., Tanaka S. [et al.]. Quadriceps muscle strength, radiographic knee osteoarthritis and knee pain: the ROAD study. *BMC Musculoskelet. Dis*., 2015, vol. 16, pp. 305. DOI: 10.1186/s12891-015-0737-5

38. Oiestad B.E., Juhl C.B., Eitzen I., Thorlund J.B. Knee extensor muscle weakness is a risk factor for development of knee osteoarthritis. A systematic review and meta-analysis. *Osteoarthritis Cartilage*, 2015, vol. 23, no. 2, pp. 171–177. DOI: 10.1016/j.joca.2014.10.008

39. Rice S.J., Cheung K., Reynard L.N., Loughlin J. Discovery and analysis of methylation quantitative trait loci (mQTLs) mapping to novel osteoarthritis genetic risk signals. *Osteoarthritis Cartilage*, 2019, vol. 27, no. 10, pp. 1545–1556. DOI: 10.1016/j.joca.2019.05.017

40. Cen L., Xing F., Xu L., Cao Y. Potential Role of Gene Regulator NFAT5 in the Pathogenesis of Diabetes Mellitus. *J. Diabetes Res.*, 2020, vol. 2020, pp. 6927429. DOI: 10.1155/2020/6927429

41. Lopez-Rodríguez C., Aramburu J., Rakeman A.S., Rao A. NFAT5, a constitutively nuclear NFAT protein that does not cooperate with Fos and Jun. *Proc. Natl Acad. Sci. USA*, 1999, vol. 96, no. 13, pp. 7214–7219. DOI: 10.1073/pnas.96.13.7214

42. Buxadé M., Lunazzi G., Minguillón J., Iborra S., Berga-Bolaños R., Del Val M., Aramburu J., Lopez-Rodríguez C. Gene expression induced by Toll-like receptors in macrophages requires the transcription factor NFAT5. *J. Exp. Med.*, 2012, vol. 209, no. 2, pp. 379–393. DOI: 10.1084/jem.20111569

43. Kim N.-H., Choi S., Han E.-J., Hong B.-K., Choi S.Y., Moo Kwon H., Hwang S.-Y., Cho C.-S., Kim W.-U. The xanthine oxidase-NFAT5 pathway regulates macrophage activation and TLR-induced inflammatory arthritis. *Eur. J. Immunol.,* 2014, vol. 44, no. 9, pp. 2721–2736. DOI: 10.1002/eji.201343669

44. Shirinsky V.S., Kazygasheva E.V., Shirinsky I.V. Inflammation and immunity: a role in the pathogenesis of osteoarthritis. *Meditsinskaya immunologiya*, 2019, vol. 21, no. 1, pp. 39–48. DOI: 10.15789/1563-0625-2019-1-39-48 (in Russian).

45. Novakov V.B., Novakova O.N., Churnosov M.I. Risk factors and molecular entities of the etiopathogenesis of the knee osteoarthritis (literature review). *Genii Ortopedii*, 2021, vol. 27, no. 1, pp. 112–120. DOI: 10.18019/1028-4427-2021-27-1-112-120 (in Russian).

46. Rogova L.N., Lipov D.S. The role of matrix metalloproteinases in the pathogenesis of osteoarthrost. *Volgogradskii nauchno-meditsinskii zhurnal*, 2018, no. 1, pp. 12–16 (in Russian).

47. Huang Q.-Q., Pope R.M. The role of toll-like receptors in rheumatoid arthritis. *Curr. Rheumatol. Rep*., 2009, vol. 11, no. 5, pp. 357–364. DOI: 10.1007/s11926-009-0051-z

48. Yoon H.-J., You S., Yoo S.-A., Kim N.-H., Moo Kwon H., Yoon C.-H., Cho C.-S., Hwang D., Kim W.-U. NFAT5 is a critical regulator of inflammatory arthritis. *Arthritis Rheum*., 2011, vol. 63, no. 7, pp. 1843–1852. DOI: 10.1002/art.30229

49. Pessler F., Chen L.X., Dai L., Gomez-Vaquero C., Diaz-Torne C., Paessler M.E., Scanzello C., Cakir N. [et al.]. Schumacher A histomorphometric analysis of synovial biopsies from individuals with Gulf War Veterans Illness and joint pain compared to normal and osteoarthritis synovium. *Clin. Rheumatol.*, 2009, vol. 27, no. 9, pp. 1127–1134. DOI: 10.1007/s10067-008-0878-0

50. Tachmazidou I., Hatzikotoulas K., Southam L., Esparza-Gordillo J., Haberland V., Zheng J., Johnson T., Koprulu M. [et al.]. Identification of new therapeutic targets for osteoarthritis through genome-wide analyses of UK Biobank data. *Nat. Genet*., 2019, vol. 51, no. 2, pp. 230–236. DOI: 10.1038/s41588-018-0327-1

51. Pickrell J.K., Berisa T., Liu J.Z., Ségurel L., Tung J.Y., Hinds D.A. Detection and interpretation of shared genetic influences on 42 human traits. *Nat. Genet.*, 2016, vol. 48, no. 7, pp. 709–717. DOI: 10.1038/ng.3570

Novakov V.B., Novakova O.N., Churnosov M.I. NFAT5 gene polymorphism as a risk factor of knee osteoarthrosis. Health Risk Analysis, 2022, no. 4, pp. 137–147. DOI: 10.21668/health.risk/2022.4.13.eng

Received: 11.07.2022
Approved: 23.09.2022
Accepted for publication: 01.12.2022