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GENETIC SUSCEPTIBILITY TO JUVENILE IDIOPATHIC ARTHRITIS IN THE BELARUSIAN POPULATION: GENE-GENE INTERACTIONS ANALYSIS

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❀ **Background.** GWASs revealed a huge amount of candidate genes for juvenile idiopathic arthritis (JIA) susceptibility. Individual SNP analysis has restrictions as an effect of each substitution may be too subtle to be detected but their interactions may significantly contribute to disease susceptibility. **Materials and methods.** 118 patients diagnosed with JIA and 202 controls were included into the study. The study was aimed to estimate interactions between SNPs of the immune and inflammatory responses genes: *RUNX3* (rs11249215), *RUNX1* (rs9979383), *STAT4* (rs7574865), *TRAF1/C5* (rs3761847), *MIF* (rs755622), *CTLA4* (rs5742909, rs231775), *PTPN2* (rs2542151) and to reveal their effects on the JIA susceptibility. SNPs were genotyped using PCR-RFLP and Real-time PCR. Multifactor dimensionality reduction analysis was performed using MDR 3.0.2 software. **Results.** *RUNX3*, *STAT4* and *PTPN2* polymorphisms were associated with systemic arthritis, RF- polyarthritis and oligoarthritis respectively. Interaction of *CTLA4* (rs5742909, rs231775), *TRAF1/C5* (rs3761847), *RUNX1* (rs9979383), *PTPN2* (rs2542151) SNPs is shown to be a risk factor for JIA ($p = 0.0099$). **Conclusion.** Some of the SNPs studied are associated with distinct JIA subtypes. MDR analysis identified a statistically significant high-order interaction of five polymorphisms which collectively may contribute to JIA genetic susceptibility in the Belarusian population.

❀ **Keywords:** juvenile idiopathic arthritis; genetic susceptibility; SNP; gene-gene interactions.

ГЕНЕТИЧЕСКАЯ ПРЕДРАСПОЛОЖЕННОСТЬ К ЮВЕНИЛЬНОМУ ИДИОПАТИЧЕСКОМУ АРТРИТУ В БЕЛОРУССКОЙ ПОПУЛЯЦИИ: АНАЛИЗ МЕЖГЕННЫХ ВЗАИМОДЕЙСТВИЙ

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❀ Определены частоты распространения аллельных вариантов и их комбинаций в полиморфных локусах генов *RUNX3*, *RUNX1*, *STAT4*, *TRAF1/C5*, *MIF*, *CTLA4*, *PTPN2* у 118 пациентов с ювенильным идиопатическим артритом (ЮИА). Выявлен неравнозначный вклад отдельных полиморфных вариантов в предрасположенность к различным подтипам ЮИА: с системным артритом ассоциированы минорный аллель A ($p = 0{,}0057$) и гомозиготы AA ($p = 0{,}042$) в локусе *RUNX3* (rs11249215); в группе серонегативного полиартрита чаще встречается минорный аллель T ($p = 0{,}03$) в локусе *STAT4* (rs7574865); у детей с олигоартритом выше частота гомозигот по минорному аллелю G ($p = 0{,}026$) в локусе *PTPN2* (rs2542151). Обнаружено 12 парных комбинаций генотипов, влияющих на вероятность развития ЮИА в общей выборке, при этом сочетания разных генотипов в пределах одной и той же пары локусов могут иметь противоположные эффекты. Несмотря на то что группа детей с ЮИА в целом и после стратификации по полу не отличалась от контрольных групп по результатам анализа отдельных локусов, изучение межгенных взаимодействий с помощью многофакторного сокращения размерности выявило сочетание генотипов по пяти локусам — CC (rs5742909) / AG (rs231775) / AG (rs3761847) / CT (rs9979383) / TT (rs2542151), оцениваемое как рисковое ($p = 0{,}0099$). Полученные результаты подтверждают целесообразность учета комбинаций генотипов при оценке рисковой значимости однонуклеотидных замен.

❀ **Ключевые слова:** ювенильный идиопатический артрит; генетическая предрасположенность; однонуклеотидный полиморфизм; межгенные взаимодействия.

INTRODUCTION

Juvenile idiopathic arthritis (JIA) is an autoimmune disease of unknown etiology and is the most common among pediatric rheumatic diseases. It manifests itself before the age of 16, with the incidence rate ranging from 0.83 to 23 new cases per 100,000 pediatric patients, depending on the population [1, 2].

It is believed that JIA is caused by dysfunction of the innate and adaptive immune systems, which is aggravated by adverse environmental factors [3]. To date, the role of certain variants of the genes encoding the major histocompatibility complex (human leukocyte antigen (HLA)) within the implementation of a genetic predisposition to JIA has been convincingly demonstrated; however, according to some estimates, this does not exceed 18% [4]. As a result of genome-wide analysis, the list of JIA-associated candidate genes that are not related to the HLA system has been significantly expanded and includes about 200 names [4], but the established associations have only been validated for single genes.

Thus, the question of JIA genetic architecture remains open. Causes of the so-called missing heritability include the presence of a large number of frequent variants with small individual effects along with the existence of functionally significant rare variants [5]. Based on this, the analysis of gene-gene interactions that exhibit an epistatic effect is of interest [6].

Existing clinical laboratory diagnostic methods are not specific enough nor informative for early detection of JIA; therefore, the detection of genetic variants that determine or modify the sensitivity or resistance to a given disease is necessary not only for understanding the molecular genetic basis of the disease but also primarily for identifying genetically predisposed individuals who are considered to be at risk. Since JIA has an autoimmune nature, close attention should be paid to gene variants of transcription factors and signaling molecules involved in immune system functioning.

This study aimed to analyze the contribution of genetic polymorphism and gene-gene interactions at the *RUNX3* (rs11249215), *RUNX1* (rs9979383), *STAT4* (rs7574865), *TRAF1/C5* (rs3761847), *MIF* (rs755622), *CTLA4* (rs5742909 and rs231775), and *PTPN2* (rs2542151) loci as related to the predisposition to JIA in the pediatric population within the Republic of Belarus.

MATERIALS AND METHODS

To conduct a case-control study in the city children's clinical hospital No.2 in Minsk, two groups were formed, which included 118 pediatric patients with clinically established JIA in accordance with the International League of Associations for Rheumatology (ILAR) criteria and 202 controls without joint pathology or autoimmune diseases. The average age of JIA patients was 8.08 ± 4.90, and that of the pediatric control group was 14.21 ± 2.56. Biological material was collected by employees of the medical institution after obtaining written informed consent and approval of the research by the ethics committee.

Genomic DNA from peripheral venous blood samples was isolated by phenol-chloroform extraction. The polymorphic variants of *RUNX3* (rs11249215), *CTLA4* (rs5742909), *STAT4* (rs7574865), and *TRAF1/C5* (rs3761847) loci were determined by the polymerase chain reaction restriction fragment length polymorphism products (PCR–RFLP). Genotyping of *RUNX1* (rs9979383), *CTLA4* (rs231775), and *PTPN2* (rs2542151) loci was performed using real-time PCR on a CFX96 thermocycler (BioRad); BioRad CFX Maestro 1.0 software was used for genotype identification. Table 1 shows the oligonucleotide sequences (Primetech) and enzymes used (Thermo Fisher).

Statistical data processing was performed using the programs MS Excel 2010 (Microsoft Corporation) and IBM SPSS V22.0. Statistically significant differences in the distribution of allele/genotype frequencies were determined using Fisher's exact test. Differences were considered statistically significant at $p \le 0.05$. Associations of each studied locus with the probability of JIA development were evaluated on the basis of the odds ratio (OR) indicator and 95% confidence interval values.

The analysis of gene-gene interactions was performed using the multifactor dimensionality reduction algorithm implemented in the multifactor dimensionality reduction (MDR) program V3.0.2 with the following query settings: attribute count range of 1–8 (where the maximum value coincided with the number of factors analyzed), model reproducibility (cross-validation count) of 100, analysis of top models (track top models) of 1000, search method configuration as exhaustive, comparison method (ambiguous cell analysis) as Fisher's exact test, and unclassified type related to cell classification (ambiguous cell assignment). Statistical significance of the obtained models was checked using the permutation test with the MDR permutation tool V1.0.2 beta 2.

RESULTS

The identification of the allelic variants of *RUNX3* (rs11249215), *RUNX1* (rs9979383), *STAT4* (rs7574865), *TRAF1/C5* (rs3761847), *CTLA4* (rs5742909 and rs231775), and *PTPN2* (rs2542151) loci showed that the frequencies of genotypes and minor alleles within the control group were in the frequency range characteristic of the European population [7].

Genotype distributions were checked for compliance with the Hardy-Weinberg equilibrium, and in two cases, namely, the control group for the *RUNX3* gene polymorphic variant ($p = 0.04$) and the group of JIA patients exhibiting the *TRAF1/C5* gene polymorphic variant ($p = 0.02$), a deviation from this equilibrium was

Table 1

Primers, probes and enzymes used for genotyping

Locus	Primer sequence 5' → 3'	Restriction enzyme / Probes sequences (5' → 3')
MIF rs755622	F: CTA-AGA-AAG-ACC-CGA-GGC R: GGG-GCA-CGT-TGG-TGT-TTA-C	Alu I
CTLA4 rs5742909	F: AGT-CTC-CAC-TTA-GTT-ATC-CAG-ATC-CT R: AAA-AGA-CAA-CCT-CAA-GCA-CTC-A	Tru1 I
STAT4 rs7574865	F: GCA-AAT-CTT-TGT-AAA-AAG-TCA-A R: TTA-TGG-AAA-ATT-ACA-TGA-GTG-TG	Tru1 I
TRAF1/C5 rs3761847	F: CCT-ACC-TGT-TCC-CTC-CTT-CC R: GGG-ATG-ATG-ATG-GCA-ATA-CC	Msp I
RUNX3 rs11249215	F: CAC-AGC-CAC-CTA-CGC-ACA R: CCA-ACT-CTA-TGG-CCT-CAG-CAC	Hpy99I
RUNX1 rs9979383	F: GGA-CAT-AAG-ATC-CTC-AGTT R: GAG-TGG-CAT-CTT-CTG-ATC	C: FAM-CCA-TCA-CAA-TAA-ACA-GGA-GTA-ATA-CTG-AT-BHQ1 T: HEX-TCA-CAA-TAA-ATA-GGT-GTA-ATA-CTG-ATA-CGA-BHQ1
CTLA4 rs231775	F: CCT-GAA-CAC-CGC-TCC-CAT R: GCT-CCA-AAA-GTC-TCA-CTC-ACC-T	A: FAM-AGC-TGA-ACC-TGG-CTA-CCA-GGA-CCT-BHQ1 G: HEX-AGC-TGA-ACC-TGG-CTA-CCA-GGA-CCT-BHQ1
PTPN2 rs2542151	F: TCC-TGT-CTC-CCA-AAC-TCT R: CAA-GAA-GGT-GTG-AAG-TTA-GTG	G: FAM-AGT-CTC-AGG-AAG-CGC-CCG-AA-BHQ1 T: HEX-AGT-CTC-AGG-AAG-AGC-CCG-AAC-CA-BHQ1

established. A comparative analysis of the frequencies of genotypes/alleles for all the studied loci did not reveal statistically significant differences between the JIA patient and control groups.

Taking into account the existing gender dimorphism of many autoimmune diseases, in particular rheumatoid arthritis within pediatric and adult patients [8], as well as the fact that our data in terms of gender ratio among pediatric patients with established JIA coincide with the global trend (69.5% female and 30.5% male), an attempt was made to evaluate the effect of the studied polymorphic gene variants on predisposition to the disease separately in male and female children. We were not able to identify any statistically significant gender-dependent effects of alleles/genotypes at the *RUNX3* (rs11249215), *RUNX1* (rs9979383), *STAT4* (rs7574865), *TRAF1/C5* (rs3761847), *MIF* (rs755622), *CTLA4* (rs5742909 and rs231775), or *PTPN2* (rs2542151) loci, as their frequency distributions within patients did not differ significantly from those within the corresponding control groups (data not shown).

There are seven subtypes of JIA according to current ILAR classifications [9]. An analysis of the patient cohort in the current study shows that, with the exception of undifferentiated arthritis, all these subtypes were diagnosed (Fig. 1).

Table 2 shows the analysis of possible associations of the studied polymorphic variants of the immune and inflammatory response genes within the most common forms of JIA, such as oligoarthritis, seronegative polyarthritis, and systemic arthritis. The percentage ratios of these disorders within this cohort were 67.8%, 16.1%, and 11.9%, respectively.

An analysis of the frequency distributions of genotypes and alleles at the *RUNX3* (rs11249215) locus for different JIA subtypes revealed that significant differences between patients with systemic arthritis and the control group, such as homozygous carriers of the A allele (OR = 4.29 [1.42–9.34], $p = 0.042$), as well as the allele itself (OR = 3.31 [1.38–7.97], $p = 0.0057$), were detected at a significantly higher frequency (50% and 75%, respectively) among patients exhibiting systemic disease. Other statistically significant differences should include an increase in the frequency of the T allele at the *STAT4* (rs7574865) locus (OR = 2.18 [1.09–4.35], $p = 0.03$) in cases of seronegative arthritis, a GG genotype at the *TRAF1/C5 (rs3761847)* locus (OR = 3.77 [1.15–12.36], $p = 0.057$)

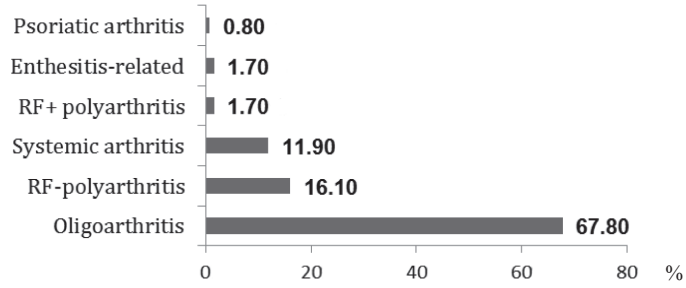

Fig. 1. The columns show the patients with different JIA subtypes percentagewise

Table 2

Frequency distribution of polymorphic variants in the genes studied in patients with different JIA subtypes

Polymorphism	Group tested							
Genotypes / alleles	Controls		Oligoarthritis		RF-polyarthritis		Systemic arthritis	
	n	%	*n*	%	*n*	%	*n*	%
RUNX3 rs11249215								
GG	48	23.9	15	19.0	1	5.3	0	0.0
GA	115	57.2	42	53.2	12	63.2	7	50.0
AA	38	18.9	22	27.8	6	31.5	7	50.0[1]
G	211	52.5	72	45.6	14	36.8	7	25.0
A	191	47.5	86	54.4	24	63.2	21	75.0[2]
RUNX1 rs9979383								
TT	87	43.9	31	38.8	4	21.1	8	57.2
CT	81	40.9	37	46.2	12	63.2	3	21.4
CC	30	15.2	12	15.0	3	15.7	3	21.4
T	255	64.4	99	61.9	20	52.6	19	67.9
C	141	35.6	61	38.1	18	47.4	9	32.1
MIF rs755622								
GG	138	68.7	50	62.5	11	57.9	12	85.7
GC	55	27.4	29	36.2	8	42.1	2	14.3
CC	8	3.9	1	1.3	0	0.0	0	0.0
G	331	82.3	129	80.6	30	78.9	26	92.9
C	71	17.7	31	19.4	8	21.1	2	7.1
CTLA4 rs5742909								
CC	162	80.2	66	82.5	16	84.2	11	87.6
CT	40	19.8	14	17.5	3	15.8	3	21.4
TT	0	0.0	0	0.0	0	0.0	0	0.0
C	364	90.1	146	91.3	35	92.1	25	89.3
T	40	9.9	14	8.7	3	7.9	3	10.7

Table 2 (continued)

Polymorphism	Group tested							
Genotypes / alleles	Controls		Oligoarthritis		RF-polyarthritis		Systemic arthritis	
	n	%	*n*	%	*n*	%	*n*	%
CTLA4 rs231775								
AA	62	30.7	23	28.8	4	21.1	5	35.7
AG	108	53.5	43	53.8	12	63.2	7	50.0
GG	32	15.8	14	17.4	3	15.7	2	14.3
A	232	57.4	89	55.6	20	52.6	17	60.7
G	172	42.6	71	44.4	18	47.4	11	39.3
STAT4 rs7574865								
GG	120	59.4	55	68.8	7	36.8	10	71.4
GT	71	35.1	23	28.8	9	47.4	4	28.6
TT	11	5.4	2	2.4	3	15.8	0	0.0
G	311	77.0	133	83.1	23	60.5	24	85.7
T	93	23.0	27	16.9	15	39.5[3]	4	14.3
TRAF1/C5 rs3761847								
AA	75	38.1	33	41.8	5	26.3	5	38.5
AG	94	47.7	29	36.7	11	57.9	3	23.0
GG	28	14.2	17	21.5	3	15.8	5	38.5[4]
A	244	61.9	95	60.1	21	55.3	13	50.0
G	150	38.1	63	39.9	17	44.7	13	50.0
PTPN2 rs2542151								
TT	152	75.2	62	77.5	14	73.7	12	85.7
TG	49	24.3	14	17.5	5	26.3	2	14.3
GG	1	0.5	4	5.0[5]	0	0.0	0	0.0
T	353	87.4	138	86.3	33	86.8	26	92.9
G	51	12.6	22	13.7	5	13.2	2	7.1

Note. [1]$p = 0.042$; [2]$p = 0.0057$; [3]$p = 0.03$; [4]$p = 0.057$; [5]$p = 0.026$.

in systemic JIA patients, and a homozygous GG genotype at the *PTPN2 (rs2542151)* locus (OR = 10.58 [1.16–96.16], $p = 0.026$) with oligoarthritis, when compared with controls. It should be emphasized that levels of statistical significance were relatively high, despite a decrease in the number of samples analyzed, which leads one to consider the aforementioned genetic variants as risk factors for the development of certain subtypes of the disease.

The minor C allele at the rs755622 locus of the *MIF* gene appeared less commonly in systemic disease patients compared to those with oligoarthritis and/or seronegative arthritis, but the revealed frequency differences (7% compared to 19% and 21%, respectively) were not statistically significant. Polymorphic variants of the *CTLA4* gene (rs5742909 and rs231775) did not affect the sensitivity of the current population to various JIA subtypes.

Table 3

Genotype combinations in loci of the immune and inflammatory response genes and their impact on JIA susceptibility in Belarusian population

Genotype combinations	JIA, *n* (%)	Control, *n* (%)	OR [95 % CI]	*p*
rs7574865 *STAT4* / rs11249215 *RUNX3*				
$STAT4_{GT}$ / $RUNX3_{GG}$	2 (1.7)	18 (9)	0.17 [0.04–0.77]	0.01
$STAT4_{GT}$ / $RUNX3_{AA}$	16 (13.6)	13 (6.5)	2.29 [1.05–4.95]	0.04
rs5742909 *CTLA4* / rs3761847 *TRAF1/C5*				
$CTLA4_{CC}$ / $TRAF1/C5_{GG}$	23 (20)	23 (11.6)	1.86 [1.01–3.55]	0.048
$CTLA4_{CT}$ / $TRAF1/C5_{AG}$	6 (5.2)	24 (12.2)	0.39 [1.01–3.55]	0.047
rs5742909 *CTLA4* / rs9979383 *RUNX1*				
$CTLA4_{CC}$ / $RUNX1_{TC}$	50 (42.4)	60 (30.3)	1.69 [1.05–2.7]	0.03
$CTLA4_{CT}$ / $RUNX1_{TC}$	5 (4.2)	21 (10.6)	0.37 [0.13–1.01]	0.056
rs755622 *MIF* / rs11249215 *RUNX3*				
MIF_{GC} / $RUNX3_{AA}$	13 (11.2)	10 (5)	2.4 [1.02–5.68]	0.045
rs755622 *MIF* / rs9979383 *RUNX1*				
MIF_{GC} / $RUNX1_{TC}$	22 (18.8)	19 (9.6)	2.18 [1.12–4.23]	0.02
rs3761847 *TRAF1/C5* / rs11249215 *RUNX3*				
$TRAF1/C5_{AG}$ / $RUNX3_{AG}$	20 (17.5)	55 (28)	0.5 [0.3–0.96]	0.03
rs3761847 *TRAF1/C5* / rs9979383 *RUNX1*				
$TRAF1/C5_{AA}$ / $RUNX1_{TT}$	14 (12.2)	45 (23.3)	0.45 [0.23–0.87]	0.016
$TRAF1/C5_{GG}$ / $RUNX1_{TT}$	10 (8.7)	6 (3.1)	2.96 [1.04–8.39]	0.037
rs3761847 *TRAF1/C5* / rs2542151 *PTPN2*				
$TRAF1/C5_{GG}$ / $PTPN2_{GT}$	7 (6)	3 (1.5)	4.19 [1.06–16.54]	0.04

Thus, unequal contribution of the polymorphic variants of the genes investigated within the inflammatory and immune response in terms of a predisposition to various subtypes of JIA was detected in pediatric and adolescent patients in Belarus.

An analysis of gene-gene interactions was performed based on the assumption that some gene variant combinations may result in a cumulative effect. Out of the 36 studied pair combinations, 12 showed a statistically significant effect (Table 3); 7 increased the likelihood of developing JIA, with OR values ranging from 1.86 to 4.19; and 5 exhibited some protective effect (see Table 3).

The multifactor dimensionality reduction algorithm was used for analysis of high-order gene-gene interactions and for querying the most informative predictive genotype combinations (Table 4).

The five-locus model was the most statistically significant and reproducible and included the *CTLA4* (rs5742909, rs231775), *TRAF1/C5* (rs3761847), *RUNX1* (rs9979383), and *PTPN2 (rs2542151)* polymorphic variants. The primary metrics of this model showed reproducibility of 100/100, balanced accuracy of 0.9239, sensitivity of 1.0, and specificity of 0.8478, χ^2:32.5141 ($p < 0.0001$). The risky combination (as defined within this model) is represented by the combination of the CC(rs5742909)/AG(rs231775)/AG(rs3761847)/CT(rs9979383)/TT(rs2542151) genotypes, OR = 3.38 [1.30–8.75], $p = 0.0099$.

The diagram constructed based on MDR analysis (Fig. 2) represents the contribution of the polymorphic variant of each gene to the probability of JIA development in the form of an entropy index in terms of percentage.

The maximum entropy indices for the pairs rs5742909 *CTLA4*/rs9979383 *RUNX1* (1.43%), rs3761847 *TRAF1/C5*/rs9979383 *RUNX1* (1.79%), and rs7574865 *STAT4*/rs11249215 *RUNX3* (1.95%) presented in the scheme confirm the data of pairwise interaction analysis, which showed the characteristic that their statistical significances serve as indicative risk factors for the development of the disease (Table 3).

Since the capabilities of MDR enable evaluation not only of paired combinations of polymorphic variants, a new

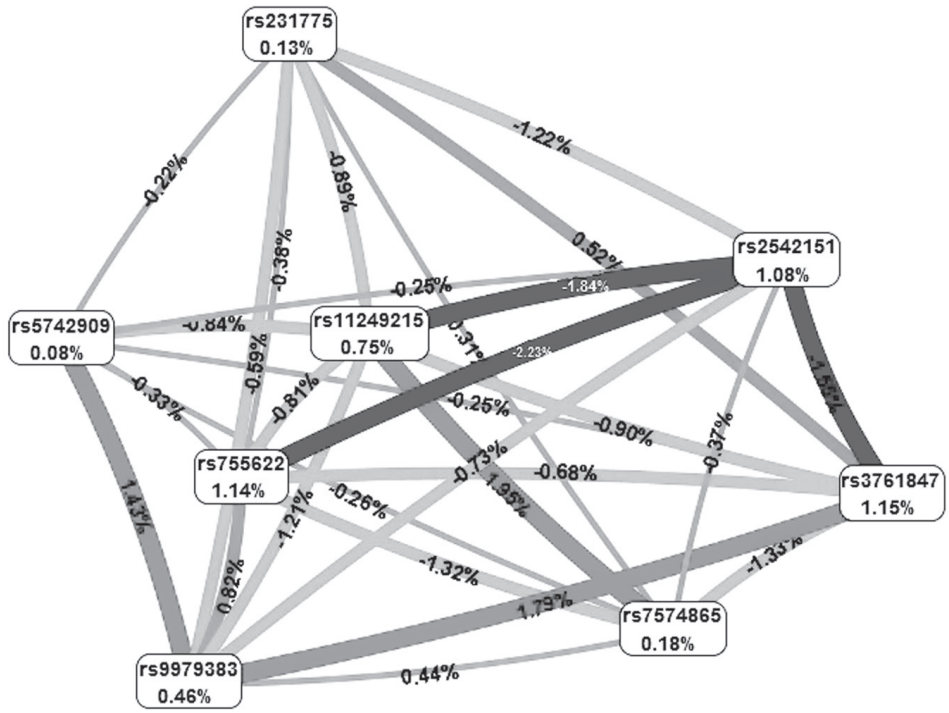

Fig. 2. Polymorphic loci and their contribution to disease susceptibility, measured as entropy in % are shown in the rectangles. Entropy values that characterize paired loci interactions are depicted on lines, connecting the respective rectangles

Table 4

Indices of balanced accuracy and cross-validation of the most significant models of different order

Model	Training ABA*	Tasting ABA*	Cross-validation consistency
rs755622	0.5442	0.4586	57/100
rs3761847, rs9979383	0.6098	0.5432	90/100
rs5742909, rs3761847, rs9979383	0.6362	0.4683	60/100
rs7574865, rs231775, rs3761847, rs11249215	0.6785	0.4687	92/100
rs5742909, rs231775, rs3761847, rs9979383, rs2542151	**0.6861**	**0.5934**	**100/100**
rs7574865, rs5742909, rs231775, rs3761847, rs11249215, rs2542151	0.6352	0.4339	69/100
rs5742909, rs231775, rs755622, rs3761847, rs11249215, rs9979383, rs2542151	0.6026	0.4494	86/100
rs7574865, rs5742909, rs231775, rs755622, rs3761847, rs11249215, rs9979383, rs2542151	0.5907	0.4427	100/100

Note. * Adjusted balanced accuracy. The most statistically significant and reproducible model (according to MDR) is written in bold type.

variable "rs5742909, rs231775, rs3761847, rs9979383, rs2542151" was created using attribute construction. Its contribution to the predictive potential of the model amounted to 10.44%, which is 3.6 times more than the simple arithmetic sum of the entropy indices for these loci.

DISCUSSION

Eight polymorphic variants of the genes implicated in the immune and inflammatory response *RUNX3* (rs11249215), *RUNX1* (rs9979383), *STAT4* (rs7574865), *TRAF1/C5* (rs3761847), *MIF* (rs755622), *CTLA4*

(rs5742909 and rs231775), and *PTPN2* (rs2542151), for which individual associations with JIA in the cohort studied were not detected, were analyzed as possible candidate genes for JIA diagnostic biomarkers.

Females were prevalent among JIA patients in the current study (i. e., 69.5%). It is assumed that such gender imbalance may be the result of differences in the implementation of the mechanisms of disease pathogenesis [10]. Within our JIA patient cohort, the frequencies of alleles and genotypes of the polymorphic variants studied were similar for both sexes and did not differ from controls.

Depending on the JIA subtype, inflammatory processes affect the joints and other organs to an unequal extent. The systemic variant, which is characterized by a high risk of disability and development of severe conditions, such as macrophage activation syndrome and lung and heart damage, is problematic for diagnostics and treatment. On the contrary, in patients with persistent oligoarthritis, in which polyarthritis has not developed, functional ability is preserved to a greater extent [11]. JIA clinical heterogeneity may be due to genetic factors specific to individual subtypes of the disease.

Genes of the *RUNX* family encode transcription factors containing an evolutionarily conserved runt domain providing interaction of protein with a specific DNA region [12]. It has been shown that RUNX1 and RUNX3 are expressed in all hematopoietic lines and play an important role in host immunity, namely, in that they control the expression of various cytokine genes, cell receptors, and cell cycle regulators and are involved in ensuring normal B-cell development, differentiation of T lymphocytes, and regulation of their immunological tolerance [13–15], in which malfunction can cause autoimmune diseases. In addition, it was found that RUNX1 is involved in chondrogenesis [16] and regulates the production of matrix metalloproteinases, of which increased levels are associated with radiographic changes in the joints [17]. The significant increase in the frequency of the minor A allele (OR = 3.31 [1.38–7.97], $p = 0.0057$) and the AA homozygotes (OR = 4.29 [1.42–9.34], $p = 0.042$) shown here at the *RUNX3* locus in systemic arthritis possibly implies an influence of this polymorphic variant on the implementation of the mechanisms of development of systemic lesions. It has been previously shown that by means of genome-wide association study (GWAS) associations of polymorphisms within the P1 *RUNX3* promoter were established for such systemic diseases as ankylosing spondylitis [18] and psoriasis [19]. Despite the fact that the polymorphic variant rs9979383 of the *RUNX1* gene is associated with a predisposition to rheumatoid arthritis (RA) [20], JIA [4, 20] and psoriatic arthritis [21], such an association was not detected in the cohort of Belarusian patients in this study.

Polymorphic variants of the *STAT4* gene have been verified as risk markers for JIA according to GWAS results [22], of which the encoded protein is a DNA-binding transcription factor expressed in activated monocytes, macrophages, and dendritic cells at sites of inflammation and regulates the transmission of cytokine signals necessary for differentiation of T-helper cells and production of interferon-γ (IFNγ) [23]. Thus, a change in the activity or expression of STAT4 can disrupt immune system function, potentially resulting in an autoimmune reaction. In accordance with these established data, we found that the T allele at the *STAT4* locus studied here is associated with the development of seronegative polyarthritis (OR = 2.18 [1.09–4.35], $p = 0.03$), while other groups have shown an association of this polymorphic variant with the degree of RA activity [24], which is probably the result of an increase in gene expression levels [25].

Based on the known functions of the *MIF* gene and its product, a pro-inflammatory cytokine and constitutive component of the immune system, as well as evidence concerning its effect of its polymorphism on the risk of autoimmune diseases, including RA in adults [26, 27], an association of the *MIF* polymorphic variant (rs755622) with JIA or its individual subtypes was predicted. However, the genotyping results of the *MIF* rs755622 gene locus in the existing patient cohort did not reveal statistically significant differences, and therefore, we were unable to draw unambiguous conclusions concerning the influence of the allelic state of this locus on the likelihood of JIA and its subtypes occurrence in the Belarusian population.

The effect of polymorphic variants of the *CTLA4* gene, which is involved in downstream regulation of the immune response [28], on the formation of a predisposition to autoimmune diseases has been investigated in different populations. Associations of a number of gene polymorphisms with Graves' disease, Hashimoto's hypothyroidism, insulin-dependent diabetes mellitus, multiple sclerosis, vitiligo, and RA have been previously established [29–34]. In the current study, genotyping of JIA patients at the rs231775 and rs5742909 loci did not reveal any differences in the distribution of genotypes/alleles either in the total group or after stratification by subtypes. In the case of rs5742909, this can be explained by a low minor allele frequency in populations (5%–9%).

It is known that the *TRAF1/C5* variant (rs3761847) affects corresponding mRNA levels [35], which can lead to a change in the expression of the TRAF1 protein involved in signal transmission through inflammatory cascades [36–38]. Some studies have shown the effect of *TRAF1/C5* polymorphic gene variants on susceptibility to autoimmune diseases, including RA and JIA [39, 40]. The data obtained in this cohort of Belarusian JIA pa-

tients indicate the likely contribution of the *TRAF1/C5* rs3761847 polymorphic variant to the pathogenesis of the systemic JIA ($p = 0.057$).

PTPN2 is another of the few genes for which associations with JIA have been confirmed in GWAS studies, which encodes T-cell tyrosine phosphatase, one of the negative regulators of the JAK-STAT signaling pathway that plays an important role in the implementation of immune responses [41]. Immune system cells express more PTP genes than those within other tissues, and knockout mice for these genes exhibit hyperreactivity of the immune system and impaired hematopoiesis [42]. It has been suggested that single nucleotide substitutions in the genes of tyrosine phosphatases, and *PTPN2* in particular, can reduce the effectiveness of inflammatory response suppression [41]. Our data concerning the association of homozygotes for the minor G allele at the *PTPN2* locus (rs2542151) with oligoarthritis (OR = 10.58 [1.16–96.16], $p = 0.026$) are consistent with the results of other studies [43].

Thus, a comparison of the allele/genotype frequencies in the studied gene loci within different JIA subtypes as compared to controls revealed certain characteristics of their potential risk implementation, indicating genetic heterogeneity of the disease and unequal contribution of individual polymorphic variants to predisposition to any of its subtypes.

Based on the hypothesis that enhancement of minor effects of single nucleotide substitutions impacts the risk of a disease with their "collective" exposure, we assessed the combined contribution of the studied variants to genetic predisposition to JIA. In this work, 12 paired combinations of genotypes in the studied loci affecting the susceptibility of Belarusian patients to JIA were identified.

Study of gene-gene interactions using the MDR program for identification of the most informative gene combinations for predicting the risk of JIA development in the Belarusian population confirmed the analysis of pair interactions for rs5742909 *CTLA4*/rs9979383 *RUNX1*, rs3761847 *TRAF1/C5*/rs9979383 *RUNX1*, and rs7574865 *STAT4*/rs11249215 *RUNX3*. In addition, the combination of CC(rs5742909)/AG(rs231775)/AG(rs3761847)/CT(rs9979383)/TT(rs2542151) genotypes with the highest risk significance (OR = 3.38 [1.30–8.75], $p = 0.0099$) was revealed using MDR. When previously studying gene-gene interactions within RA, it was established that the *MIF* gene is a key player in providing the maximum number of links with other components of the gene network, including *CTLA4* and *TRAF1/C5* [44], and ten paired gene interactions were discovered, among which there was a combination of *TRAF1/C5* and *RUNX1* [45]. In this study, polymorphic variants of these genes are involved in the formation of the risk potential of the five-locus model identified by MDR. Immune system function involves many signaling and receptor molecules, which provides a favorable environment for the formation of epistatic interactions. Understanding of this phenomenon is a key in revealing the mechanisms for predisposition to autoimmune diseases. Our data confirms the strengthening of minor effects (not reaching statistical significance in small samples) of individual polymorphic variants when combined, making it necessary to analyze genotype combinations when assessing the effect of polymorphic variants of the immune and inflammatory response genes on JIA susceptibility.

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