ORIGINAL INVESTIGATIONS

Relationship of genetic polymorphism of the acute phase marker of inflammation rs12218 of the *SAA1* gene with clinical phenotypes of juvenile idiopathic arthritis

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Objective. *To test the hypothesis of a possible relationship between the rs12218 polymorphism of the SAA1 gene and a predisposition to different clinical phenotypes of juvenile idiopathic arthritis (JIA).*

Patients and methods. *Genetic typing of rs12218 polymorphism was carried out in 142 children: 77 of them were diagnosed with JIA, including 30 patients with oligoarthritis (oJIA), 20 with polyarthritis (pJIA), and 27 with systemic onset (sJIA). Sixty five healthy volunteers were included in the control group. The rs12218 polymorphism of the SAA1 gene was investigated using real-time polymerase chain reaction.*

Results and discussion. *A high risk of developing the clinical phenotype of oJIA in carriers of the C mutant allele of the rs12218 T/C polymorphism of the SAA1 gene was established. Statistically significant differences between the clinical phenotypes of oJIA and sJIA in the frequency distribution of genotypes and alleles of rs12218 T/C polymorphism of the SAA1 gene are shown.*

Conclusion. *The results of the studies have confirmed the important role of the rs12218 T/C polymorphism of the SAA1 gene in the formation of susceptibility to clinical variants of JIA.*

Keywords: *juvenile idiopathic arthritis; predisposition to JIA; acute phase protein SAA; the SAA1 gene; SAA1 gene rs12218 T/C polymorphism; HLA-B27; anterior uveitis.*

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Introduction

Juvenile idiopathic arthritis (JIA) is one of the most common immune-inflammatory diseases of unknown etiology and occurs in children under the age of 16. The disease is a heterogeneous complex of chronic arthropathies [1]. The International League of Rheumatological Associations identifies 7 clinical phenotypes of JIA: systemic onset (sJIA), oligoarticular (oJIA), polyarticular (pJIA) rheumatoid factor positive or negative, enthesitis-associated arthritis, psoriatic arthritis, and undifferentiated arthritis [2]. In Europeans, oJIA is the most common variant of the disease, diagnosed in 50% of cases [3]. The second most common type is rheumatoid factor-negative pJIA, and it is diagnosed in 10–15% of JIA patients. It is characterized by damage to more than four joints at the time of the debut. The third most prevalent type is sJIA, which in European populations accounts for 5–15% of cases; in the Russian Federation its frequency reaches 22% [4]. The genetic component makes a significant contribution to the development of JIA. Relatives of children with JIA often have an increased predisposition to other autoimmune conditions. These observations support the view that different clinical autoimmune phenotypes have similar predisposing genetic factors. A number of genetic studies, including whole-genome ones, have confirmed non-random clustering of predisposing loci in different immune-inflammatory diseases [5]. It has been shown that genes encoding human leukocyte antigens (HLA) are located in the main histocompatibility complex on chromosome 6. It has been shown in numerous studies that antigens of the HLA-DR locus are associated with oJIA. However, the antigens at this locus determine only about 17% of th susceptibility to JIA. This confirms the involvement of non-HLA loci in susceptibility to JIA. Earlier testing of more than 100 genetic loci in order to identify associations with JIA showed that most of them did not demonstrate significant associations [6]. The most significant associations of non-HLA gene variants were shown for the PTPN22, MIF, STAT4 genes [7,8]. A relationship has been shown between JIA and some genetic variants of a number of other genes (TNFAIP3, C12orf30), for which associations with other autoimmune diseases, including rheumatoid arthritis (RA) and systemic lupus erythematosus were previously demonstrated [9].

Expression of serum amyloid A (SAA) protein, which is a hallmark of the acute phase inflammatory response, has been identified in many inflammatory conditions. It is a conservative response of vertebrate organisms to such external factors as tissue damage, infection, and surgery [10]. Human SAA protein is encoded by one of the four SAA1 genes and has well-studied characteristics. SAA protein is originally known as the main precursor of amyloid A (AA) and has been shown to play an important role in lipid metabolism, bacterial clearance, regulation of inflammation, and tumor pathogenesis. The SAA1 gene has five polymorphic coding alleles (SAA1.1-SAA1.5), which code for individual proteins with minor amino acid substitutions. SAA is an acute phase protein that can increase up to 1000 times in serum during inflammation and is proteolytically degraded to amyloid A (AA) protein, the main fibrillar protein in secondary amyloidosis. According to the materials of E. M. Tareev Clinic of I.M. Sechenov First Moscow Medical University, the most prevalent diseases predisposing to the development of secondary AA-amyloidosis include various forms of joint damage – rheumatoid

arthritis (RA), juvenile rheumatoid arthritis (JIA) and ankylosing spondylitis (Bekhterev's disease) [11, 12].

In the coding and non-coding regions of the human SAA1 gene, single nucleotide polymorphisms (SNPs) were identified, the presence of which was associated with predisposition to various diseases. It has been shown that rs12218 (-13T / C) polymorphism in the 5'-flanking region of the SAA1 gene is associated with increased transcriptional activity of this gene and in Japanese patients was associated with rheumatoid arthritis with susceptibility to type AA amyloidosis [13].

Aim: to test the hypothesis of possible relationship of the SAA1 gene rs12218 (T / C) polymorphism with a predisposition to different clinical phenotypes of JIA.

Materials and methods

Patients and control group

The study included 142 children, 77 of whom were diagnosed with JIA, and 65 healthy unrelated volunteers (college students) as a control group. The group of patients with JIA consisted of 48 girls and 29 boys, mean age 11.7 ± 4.2 years (range 3–17 years), and mean disease duration of 4.8 ± 3.8 years. All patients were treated in the pediatric department of V.A. Nasonova Institute of Rheumatology in 2016–2017. The diagnosis and classification of JIA were carried out according to the ILAR-2004 criteria [2]. The JIA group consisted of 30 (39%) patients with oJIA, of whom 20 patients (67%) were positive for HLA-B27 antigen (oJIA-B27 +) and 10 (33%) patients had anterior uveitis (oJIA- uveitis); 20 (26%) children were assigned to pJIA group, all were seronegative for rheumatoid factor; 27 (34%) patients were diagnosed with systemic onset JIA (sJIA).

Isolation of Genomic DNA and Genotyping of Samples

Venous blood samples were taken from all participants. Genomic DNA was isolated from fresh or frozen blood samples using the GS-genetics kit manufactured by DNA-Technology. The frequencies of rs12218 SAA1 gene polymorphism were estimated using allele-specific real-time polymerase chain reaction (RT-PCR). Labeled primers and probes were developed and synthesized at SINTOL (Moscow). The amplification conditions were in accordance with the manufacturer's recommendations. The study was carried out using DT-96 amplifier (NPO DNA-Tekhnologiya, Moscow).

Statistical analysis

Differences in the distribution of genotype frequencies between patients and controls were assessed using 2×2 conjugation tables and Fisher's exact test. The most frequent SAA1 TT genotype was used as a reference genotype. Clinical phenotypes are presented as dichotomous variability. Age and disease duration are presented as mean ± standard deviation (M±δ). ANOVA analysis of the relationship between dichotomous variability and the studied polymorphism was performed using the ANOVA post hoc test. Odds ratios (OR) and their 95% confidence intervals (95% CI) were calculated. Frequency distributions relative to the Hardy–Weinberg law in the control group were tested using the χ^2 test with one degree of freedom. Differences were considered statistically significant at $p<0.05$. For small values of variability, a 2-sided Fisher test was used. All calculations were performed using the Statistica 6.0 software package (StatSoft Inc, Tulsa, USA).

Written informed consent was obtained from the parents of all patients.

Table 1 Main basic characteristics of JIA patients

Characteristics	Patients with JIA n=77
Age, years (M±δ)	11.6 ± 4.1
Age of onset, years (M±δ)	6.8 ± 4.6
Duration, years (M±δ)	4.8 ± 3.8
Sex female/ male	48/29
Phenotypes n (%)	
JIA-oligoarthritis (oJIA)	30 (39.0)
JIA-polyarthritis (pJIA)	20 (26.0)
JIA-systemic (sJIA)	27 (35.0)

M±δ (mean ± standard deviation)

Results

Table 1 shows the main characteristics of patients with JIA.

Analysis of the frequency distribution of genotypes and alleles of the studied polymorphism in the group of children with JIA as a whole and in different clinical variants is presented in Table 2.

The distributions of genotype frequencies in the control group and in the JIA group (all patients) corresponded to the Hardy–Weinberg law. There were no significant differences in the distribution of genotype frequencies between patients with JIA in general and the control group ($p = 0.780$). In the group of patients with a clinical diagnosis of oJIA and its subtype oJIA + B27 (Table 2), the frequencies of the CC genotype were significantly higher compared with the controls (33.3% and 40.0% ver-

Table 2. Frequency distribution of genotypes and alleles of SAA1 rs12218 polymorphism in groups of patients with different clinical variants of JIA and in the control group.

Gene Genotypes alleles	Control group, n(%)	All patients, n(%)	oJIA, n(%)	oJIA + B27, n(%)	oJIA + uveitis, n(%)	pJIA, n(%)	sJIA n(%)
SAA1 (n)	65	**77**	30	20	10	20	27
TT	25(38.5)	27(35.1)	9(30.0)	6(30.0)	3(30.0)	7(35.0)	11(40.7)
TC	30(46.1)	35(45.4)	11(36.7)	6(30.0)	5 (50.0)	10(40.0)	14(51.8)
CC	10(**15.4**)	15(19.5)	10(**33.3**)*	8(**40.0**)*	2(20.0)	3(15.0)	2(7,.)
			P=0.046	p=0.018			
T	80(61.5)	89(57.8)	29(48.3)	18(45.0)	11(55.0)	24(60.0)	36(66.7)
C	50(38.5)	65(42.2)	31(51.7)	22(55.0)	9(45.0)	16(40.0)	18(33.3)

* p <0.05 compared with the control group.

sus 15.4%, p = 0.046 and p = 0.018, respectively). No significant differences were found in the frequencies of the C mutant allele between the clinical subtype of oJIA + uveitis, variants of pJIA, sJIA and the control group (Table 2). For the clinical variant of oJIA and both its clinical subtypes (oJIA + B27 and oJIA + uveitis) in the carriers of the CC genotype of the SAA1 gene, the odds ratio of various events (probabilities of susceptibility to JIA) was calculated (Table 3).

Table 3 Association between the SAA1 gene rs12218 polymorphism and the risk of predisposition to oJIA, oJIA + B27 and oJIA + uveitis phenotypes

SAA1 T/C	Genotypes			Alleles	
	TT	TC	CC	T	C
Control group n=65	25(38.5)	30(46.1)	10**(15.4)**	80(61.5)	50(38.5**)**
oJIA n=30	9(30.0)	11(36.7)	10**(33.3)**	29(48.3)	31(51.7)
OR 95% CI		2.75(0.87-8.55)		1.71(0.88-3.32)	
P		0.046		0.087	
oJIA+B27 n=20	6(30.0)	6(30.0)	8**(40.0)**	18(45.0)	22(5.,0)
OR 95% CI		3.67(1.01-12.82**)**		1.96(0.90-4/27)	
P		0.018		0.064	
oJIA+uveitis n=10	3(30.0)	5(50.0)	2(20.0)	11(55.0)	9(45.0)
OR 95% CI		1.38(0.12-8.45)		1.31(0.44-3.75)	
P		0.657		0.577	

OR-odds ratio, 95% CI - 95% confidence intervals

Logistic regression analysis of the distribution of genotype frequencies showed that carriage of the CC genotype was associated with a higher risk of predisposition to oJIA phenotype (OR 2.75, p = 0.046) and its variant oJIA + B27 phenotype (OR 3.67, p = 0.018) compared with the controls. Similar risk indicators were also observed for the frequencies of the C alleles, however, the differences did not reach statistical significance (OR = 1.71 and OR = 1.96, p = 0.087 and p = 0.064, respectively).

In this study, we identified for the first time significant differences in the frequency distribution of genotypes and alleles of rs12218 polymorphism of the SAA1 gene between different clinical JIA phenotypes and calculated the relative risk of probability (RR) of their development. A comparison of the frequencies of genotypes and alleles between the clinical variants of oJIA and sJIA showed significant differences. For oJIA variant, the frequency of the CC genotype and the C allele was 33.3% and 51.7%, while for sJIA variant – 7.4% and 33.3% (RR=4.33, p=0.020 and RR=1.58, p=0.043, respectively). The frequency of the CC genotype and the C allele in the clinical subtype oJIA+B27 was also significantly higher than the corresponding frequencies in the sJIA group (40% and 55% RR=5.40, p=0.011 and 7.4% and 33.3%, RR=1.65, p=0.036, respectively). Comparison of genotype and allele frequencies between the clinical variants of oJIA and pJIA and between pJIA and sJIA did not show any significant differences.

We also analyzed a possible association of SAA1 gene polymorphism with quantitative clinical characteristics among JIA phenotypes. All patients of the studied JIA groups were comparable in age and gender, except for the pJIA group, where girls predominated. In the group of children with pJIA, the carriers of the TT genotype had an older age at the disease onset compared with the carriers of the TS genotype (7.2 ±3.1 years and 3.6 ± 3.3 years, respectively, p=0.036). In this group, the gender ratio of females to males was 6:1. In the group of children with oJIA, the carriers of the TT genotype had a significantly shorter duration of the disease compared with the carriers of the TS genotype (2.4±2.7 years and 7.2±5.0 years, respectively, p=0.011). The female to male ratio in this group was 2:1. There was no correlation between the studied polymorphism and the clinical characteristics in the group of children with sJIA, where the gender ratio of patients was 1:1.

Thus, in this study, the following key points are established:

1. The existence of differences in the frequencies of genotypes and alleles of rs12218 T / C polymorphism of the SAA1 gene was shown between the oJIA phenotype, its subtype oJIA + B27, and controls.

2. Carriage of the CC rs12218 T / C mutant genotype of the SAA1 gene polymorphism was associated with a high risk of predisposition to oJIA phenotype and oJIA + B27 variant (OR 2.75 and OR 3.67, respectively) compared with the controls.

3. It was shown that the relative risk (RR) of carriage of the mutant CC genotype or C allele is 4.3 and 1.5 times higher in oJIA phenotype compared with sJIA phenotype (p = 0.020 and p = 0.043, respectively).

4. The association of rs12218 TT genotype of the SAA1 gene polymorphism with the age of debut in the pJIA group and the duration of the disease in the oJIA group was established.

Discussion

In this study, we for the first time examined the association of the SAA1 gene with JIA, since such studies in foreign and domestic literature are quite limited. Our interest in acute phase proteins was due, on the one hand, to the presence of chronic inflammation in JIA, and, on the other hand, to the possible formation of amyloidosis in this disease [11, 12].

In our study, rs12218 polymorphism of the SAA1 gene was investigated in 77 patients with JIA. The distribution of genotypes and alleles in the present study among all patients was similar to their distribution in controls. In the study of 3 main clinical phenotypes: oJIA, in which we included the subtypes oJIA + B27 and oJIA + uveitis, pJIA and sJIA, in patients with oJIA, a significantly higher frequency of the mutant CC genotype was revealed compared with the control group (p = 0.046).

The presence of the HLA-B27 antigen in this group of patients increased the frequency of this genotype (p = 0.018) and increased the risk of developing oJIA by 3.7 times. Mavragani et al. [14], studied 3 polymorphisms of the SAA1 gene: -13T / C, 2995C / T and 3010C / T in 88 patients with RA, 14 patients with familial Mediterranean fever (FMF) and 110 healthy controls. The authors found a similar distribution of genotypes and alleles in all studied groups and a rare detection of secondary AA amy-

loidosis in Greek population. Among Japanese patients with FMF, the opposite was true. It was shown that -13T / C polymorphism of the SAA1 gene is associated with a predisposition to FMF in Japanese population [15]. On the contrary, among Egyptian children, no association of this SAA1 gene polymorphism with a predisposition to FMF was found [16]. In the coding and non-coding regions of the human SAA1 gene, single nucleotide polymorphisms (SNPs) were identified, the presence of which was associated with a predisposition to various diseases. As shown above, the studied polymorphism rs12218 (-13T / C) located in the 5'-flanking region of the SAA1 gene is associated with increased transcriptional activity of the gene, and in Japanese patients it was associated with rheumatoid arthritis and susceptibility to type AA amyloidosis [13]. A recent study has confirmed an increased frequency of this polymorphism in patients with ankylosing spondylitis with amyloidosis [17]. Studies of rs12218 polymorphism in Han Chinese have shown that individuals with the CC genotype have lower HDL-C levels [18] and a higher risk of peripheral artery disease [19]. The CC rs12218 genotype was more frequent among patients with coronary artery disease [20]. This genotype was also more common in patients with cerebral infarction [21]. On the other hand, the TT rs12218 genotype was associated with an increased level of serum uric acid [22] in urolithiasis. Researchers from Ireland demonstrated a key role of A-SAA protein as a mediator of leukocytosis, angiogenesis, and matrix destruction, which eventually led to synovitis and joint damage in rheumatoid arthritis via the NF-kB signaling pathway [23]. A significant relationship has been established between obesity and predisposition to idiopathic AA amyloidosis [24]. The protein product of the SAA1 gene and a number of other cytokines (SEMA3G, TIMP 1, HEXB, ERN1) were found in the lacrimal fluid of children with JIA and uveitis.

Conclusion

This study was the first to establish that:

1. The SAA1 gene rs12218 polymorphism participates in the predisposition to the clinical phenotype oJIA and oJIA+B27.

2. A high risk of predisposition to these phenotypes is associated with the carriage of the mutant CC genotype and the C allele.

3. The mutant CC genotype of rs12218 polymorphism is 5.4 times more often detected in oJIA phenotype compared with sJIA phenotype.

4. There is an association of the mutant allele C of rs12218 polymorphism with the age of the debut in the pJIA group and the duration of the disease in the oJIA group.

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