ORIGINAL INVESTIGATIONS

Association of ankylosing spondylitis activity indicators in a Russian population of patients with *STAT4* rs7574865 gene polymorphism

Krylov M.Yu., Starkova A.S., Samarkina E.Yu., Dubinina T.V., Erdes Sh.F.
V.A. Nasonova Research Institute of Rheumatology, Moscow, Russia
34A, Kashirskoe Shosse, Moscow 115522

Family and twin studies have shown that ankylosing spondylitis (AS) has a hereditary nature that is based on a strong association with the leukocyte antigen HLA-B27. However, only 1–5% of HLA-B27 carriers develop AS, which indicates that there are other genetic markers involved in the formation of a predisposition to this disease. A number of genome-wide association studies have convincingly confirmed the role of the STAT4 gene. This gene encodes the protein – the signal transducer and activator of transcription (STAT) protein, which is a predisposing factor for the development of many autoimmune diseases. There are not so many studies of the relationship of STAT4 polymorphisms to the predisposition to AS, and there are no these studies regarding the Russian population.

Objective: *to study whether there is a possible association of STAT4 rs7574865 gene polymorphism with the predisposition to AS and to assess the activity of this disease using BASDAI and ASDAS scores in the Russian patient population.*

Patients and methods. *A cohort of 203 individuals, including 100 patients (79 men and 21 women) with AS, and 103 healthy volunteers (a control group) was surveyed. Age, gender, duration, and specific features of AS onset, ESR, and CRP levels were assessed. BASDAI and ASDAS scores were calculated to evaluate disease activity.*

Results and discussion. *There was a significant relationship between STAT4 polymorphism and C-reactive protein (CRP) levels and BASDAI and ASDAS-CRP scores. The TT genotype carriers had significantly higher mean activity indices compared to the GG (p=0.001) and GT (p=0.005) genotype carriers for CRP, BASDAI (p=0.0001 and p=0.009, respectively) and ASDAS-CRP (p=0.009 and p=0.001, respectively). High disease activity (BASDAI >4 and ASDAS-CRP >3.5) was also associated with the high frequency of the T allele (p=0.046 and p=0.004, respectively). The value of STAT4 rs7574865 gene polymorphism in the pathogenesis of autoimmune diseases is confirmed by a study in which the T allele in STAT4 rs7574865 enhances mRNA transcription and protein expression. Italian authors have shown that there is a relationship between the minor T allele of rs7574865 and the high risk of arthritis. We have previously established a relationship between the T allele and the predisposition to diffuse systemic scleroderma, interstitial lung damage, and elevated anti-topoisomerase I antibody levels.*

Conclusion. *The present study has shown for the first time a significant association of STAT4 rs7574865 polymorphism with the main AS activity indicators: CRP levels, BASDAI and ASDAS-CRP scores. The studied polymorphism may be a new genetic marker for predicting the severity of AS.*

Keywords: *ankylosing spondylitis; STAT4 gene; rs7574865 polymorphism; C-reactive protein; activity indices BASDAI, ASDAS.*
Contact: *Mikhail Yuryevich Krylov;* ***mekry@yandex.ru***
For reference: *Krylov MYu, Starkova AS, Samarkina EYu, et al. Association of ankylosing spondylitis activity indicators in a Russian population of patients with STAT4 rs7574865 gene polymorphism. Sovremennaya Revmatologiya=Modern Rheumatology Journal. 2019;13(2):55–60.*
DOI*: 10.14412/1996-7012-2019-2-55-60*

Key points:

1. The STAT4 gene is involved in the formation of susceptibility to ankylosing spondylitis (AS).
2. The association of the rs7574865 T allele of the STAT4 gene polymorphism with the AS activity: the level of CRP, BASDAI and ASDAScrp indices has been established.
3. Allele rs7574865 T is a risk factor for activity and severity of the disease.

1. Introduction

Ankylosing spondylitis (AS) is a chronic inflammatory disease from the group of spondyloarthritides, characterized by damage to the sacroiliac joints and / or the spine which potentially results in ankylosis, with frequent involvement of the entheses and peripheral joints in the pathological process [1]. The disease is characterized by inflammatory back pain (IBP) and stiffness, which can lead to pronounced functional impairment and reduced quality of life. The frequency of AS in European population is 0.55%, in Russian population – 0.1%. AS is rarely found in African and Japanese populations. The disease is more common in men, the ratio of men and women is about 3: 1 [2, 3], but, according to the latest data, it can approach 2:1 or even 1:1 in the early forms of the disease [4]. Family and twin studies have shown a clear hereditary nature of AS and its strong association with the leukocyte antigen HLA-B27 [5]. At the same time, only 1–5% of HLA-B27 carriers develop AS [6,7], which indicates the existence of other genetic markers involved in the formation of susceptibility to this disease [8].

Signal converters and transcription activators (STAT proteins) are potential factors that induce transcription of their target genes by recognizing specific consensus DNA sequences. Among the 6 described STAT proteins, STAT-4 is the most interesting. The STAT4 gene is mapped on chromosome 2q33 and is expressed in activated peripheral blood monocytes, macrophages and dendritic cells at inflammation sites [9]. STAT4 transmits signals induced by interleukin (IL) -12, IL-23, and interferon-c (IFN-c), which are key cytokines in the development of autoimmune diseases [10,11]. In addition, STAT4 plays a major role in the differentiation and proliferation of T-helper cells (Th) 1 and Th17 [11]. A number of associative and genome-wide studies convincingly confirmed the role of the STAT4 gene as a predisposing factor for the development of autoimmune diseases, such as systemic lupus erythematosus, rheumatoid arthritis (RA), Sjogren's syndrome, systemic scleroderma [12–16]. All the above said indicates that the STAT4 gene may also play an important role in the pathogenesis of AS. Previously published data related to the rs7574865 polymorphism of the STAT4 gene showed an increased risk of developing a variety of complex autoimmune diseases, such as RA in different ethnic populations [17–19]. The rs7574865 polymorphism of the STAT4 gene is a single nucleotide polymorphism (SNP) associated with the replacement of the guanine base by thymine (G→T), the functional significance of which remains unclear. In the 3rd intron of the STAT4 gene, several SNPs in non-equilibrium adhesion with rs7574865 were found, for which participation in regulatory processes was confirmed [20]. There are a rather limited number of studies that analyze the association of STAT4 polymorphisms with susceptibility to AS and phenotypes that determine the activity of the disease [21,22], and such studies have not been carried out in the Russian population.

The purpose of this study was to analyze the association of rs7574865 (G→T) polymorphism of the STAT4 gene with susceptibility to AS and disease activity, determined by the serum level of C-reactive protein (CRP), BASDAI (Ankylosing Spondylitis Disease Activity Index) and ASDAS (Ankylosing Spondylitis Disease Activity Score) indices in the Russian population of patients.

2. Materials and methods

2.1 Patients

A multiethnic cohort of 100 patients with AS (68 Slavs, 23 Caucasians, 9 from other ethnic groups) was investigated; of them 79 were men and 21 women (ratio 4: 1); all patients were treated at V.A. Nasonova Institute of Rheumatology in the period from 2016 to 2017. All patients were positive for HLA-B27, the mean age was 39.6 ± 10.9 years, the average disease duration was 241.7 ± 113.7 weeks. The control group included 103 healthy non-related subjects (the Institute staff) of comparable sex and age without signs of spondyloarthritis and other chronic inflammatory diseases. The diagnosis of AS was made according to the modified New York criteria [23]. Age, gender, AS duration, specific features of the onset of the disease, erythrocyte sedimentation rate (ESR), and CRP levels were estimated. To determine the disease activity BASDAI and ASDAS were used. ESR was determined by Westergren method, CRP level – by a highly sensitive immunonephelometric method.

The study was approved by the Ethical Committee of V.A. Nasonova Institute, and informed consent was requested from all patients.

2.2 Genotyping of rs7574865 STAT4 gene polymorphism

Venous blood samples were taken from all the participants on admission to the clinic. DNA from 100 patients and 103 unrelated healthy individuals was isolated from fresh or frozen blood samples using the commercial kit "GS genetics" manufactured by DNA-Technology (Moscow). The rs7574865 polymorphism of the STAT4 gene was studied using real-time allele-specific polymerase chain reaction. The design of primers and labeled probes, their synthesis and amplification conditions were developed in the company "SINTOL" (Moscow). 5% of the DNA samples of patients who were randomly selected and re-genotyped showed 100% concordance of the results.

2.3 Statistical analysis

Clinical phenotypes were presented as dichotomous variability. Normal distributions were compared using an ANOVA post hoc variant assay or Student's t-test, and presented as mean ± standard deviation (M ± δ). The correlation between the level of CRP and categorical clinical variability was studied using the parametric Pearson method. A level of $p < 0.05$ was considered statistically significant. For small values of variability, the 2-sided Fisher criterion was used. Differences in the distribution of genotype frequencies between patients and controls were evaluated by means of a 2×2 pairing table using the χ^2 criterion. All data were analyzed using the Statistica 6.1 software package (StatSoft Inc, Tulsa, USA).

3. Results

The results of the examination of patients with AS are presented in Table 1.

The ratio of males and females in our sample was 4: 1 and differed from the corresponding indicator in European populations of AS patients. The age and duration of the disease were within the average values observed in Europe.

Table 1. Characteristics of patients

Characteristic	Values
Gender: Men/Women, n	79/21
Age (mean), years (M ± δ)	39.6 ± 10.9
Disease duration, weeks (M ± δ)	241.7 ± 113.7
Onset of the disease: n	
- with inflammatory back pain	53 (53%)
- with extra-axial arthritis	47 (47%)
Sacroiliitis (SI), n	
- stage 3	39 (39%)
- stage 4	61 (61%)
BASDAI (M ± δ)	5.2 ± 2.2
ASDAScrp (M ± δ)	3.6 ± 1.6
ESR mm / hour (M ± δ)	25.2 ± 25.5
CRP mg / l (M ± δ)	34.9 ± 47.7

Table 2. The frequency distribution of genotypes and alleles of the STAT4 gene, n (%)

Group	Genotype			Allele		p
	GG	GT	TT	G	T	
Controls, n (%)	65 (63.1)	34 (33.0)	4 (3.9)	164 (79.6)	42 (20.4)	
Patients	51 (51.0)	42 (42.0)	7 (7.0)	144 (72.0)	56 (28.0)	0.073

Table 3. Association of STAT4 G / T polymorphism with specific AS onset, n (%)

Clinical phenotype of the onset	Genotype GG	GT	TT	P
Group A (n = 53)	30 (56.6)	17 (**32.1**)	6 (11.3)	
Group B (n = 47)	21 (44.7)	25 (**53.2**)	1 (2.1)	**0.033**

Group A - inflammatory back pain (IBP) as the first symptom of AS; group B – IBP as the second and subsequent symptom of AS.

Table 4. Association of STAT4 G/T polymorphism with the disease activity indicators (M ± δ)

Clinical phenotype	Genotype GG[1]	GT[2]	TT[3]	p
ESR, mm/h	20.2±23.7	29.4±27.6	36.8±18.5	NS
CRP, mg/l	20.5±30.8	45.8±52.1	74.7±81.8	P^{3-1}= 0.001, P^{3-2}= 0.005
BASDAI	4.5±2.0	5.5±2.2	7.8±0.9	P^{3-1}= 0.0001, P^{3-2}=0.009
ASDAScrp	3.2±1.4	3.7±1.5	5.5±1.7	P^{3-1}= 0.009, P^{3-2}=0.0001

Note. NS – non-significant

3.1 The frequency of genotypes and alleles of rs7574865 polymorphism of the STAT4 gene

The frequency distribution of genotypes and alleles of the STAT4 gene in the controls and in the group of patients with AS is presented in Table 2.

The distribution of genotype frequencies in the control and patient groups was in agreement with the Hardy–Weinberg law.

The frequency of the T-allele in patients with AS was increased compared with the controls, but the differences did not reach statistical significance (28.0% and 20.4%, respectively, p = 0.073).

3.2. Correlation analysis

Pearson's correlation analysis showed the negative correlation of STAT4 G/T polymorphism with inflammatory back pain (IBP) at the onset of the disease (r = -0.270, p = 0.007).

3.3. Frequency distribution of genotypes of polymorphism STAT4G / T depending on the onset of AS

A significant association between the STAT4 polymorphism and the specific features of the onset of AS was revealed (Table 3). Patients whose first symptom of AS was IBP (group A) had a significantly lower incidence of the GT genotype compared with patients in group B in whom IBP was not the first symptom of the disease (p = 0.033).

There was no association between STAT4 polymorphism and the onset of the disease, when the first symptom of AS was non-axial arthritis.

3.4. The frequency distribution of genotypes of polymorphism STAT4G/T depending on the level of laboratory and clinical indicators of the activity of AS

The frequency of the TT genotype was related to the BASDAI and ASDAS values. BASDAI in the carriers of the TT genotype (see Table 4) was significantly higher than in those with genotypes GG and GT (7.8 ± 0.9; 4.5 ± 2.0 and 5.5 ± 2.2; p = 0.0001 and p = 0.009, respectively). The TT genotype carriers also had a significantly higher ASDAScrp index (5.5 ± 1.7; 3.2 ± 1.4 and 3.7 ± 1.5; p = 0.009 and p = 0.0001, respectively).

Table 5. Distribution of the frequency of the STAT4 G/T polymorphism in patients with AS depending on the degree of disease activity according to BASDAI and ASDAScrp, n (%)

Activity	Genotype			T-allele	p
	GG	GT	TT		
Activity (BASDAI):					
moderate ≤4	18 (64.3)	10 (35.7)	0	10 (17.8)	
high >4 (78%)	33 (45.8)	32 (44.4)	7 (9.7)	46 (31.9)	0.046
Activity (ASDAScrp):					
moderate ≤3.5	32 (61.1)	20 (38.9)	0	20 (19.2)	
high >3.5 (48%)	19 (39.6)	22 (45.8)	7 (14.6)	36 (37.5)	0.004

3.5 Frequency distribution of genotypes of STAT4 polymorphism in groups of patients with high and low BASDAI activity

In order to assess the effect of the studied polymorphism on the clinical activity of AS according to BASDAI index, all patients were divided into two groups: low disease activity (BASDAI ⩽ 4, n = 28) and high disease activity (BASDAI > 4, n = 72) (Table 5).

The frequency of the minor T-allele in the group with high AS activity was significantly higher than in the group with low activity (31.9 and 17.8%, respectively; p = 0.046). A similar distribution of patients according to ASDAScrp parameters into groups with moderate (ASDAScrp ⩽ 3.5 index; n = 52) and high (ASDAScrp > 3.5 index; n = 48) disease activity also showed a significantly increased frequency of the minor allele in the group with high activity (37.5 and 19.2%, respectively; p = 0.004).

There were no significant age differences between the carriersof the studied STAT4 polymorphism genotypes. No connection of this polymorphism with the duration of the disease, stage of sacroiliitis and ESR was established.

Thus, the present study showed an increased frequency of mutant allele T in patients with AS; an association between the polymorphism of the STAT4 gene and the onset of the disease; an association of the frequency of the allele T rs7574865 of the STAT4 gene with a high AS activity.

4. Discussion.

Genetic, environmental and demographic factors are involved in the pathogenesis of AS, but the genetic background of an individual remains the main determinant of the disease. These data were confirmed in experimental and clinical studies, which proved the involvement of the STAT4 gene in the development of many autoimmune diseases [15–19]. STAT4 is an important signaling molecule for IL12, IL23 and IFN-γ, which play a major role in the pathogenesis of these diseases [11]. STAT4 is essential for the functioning of cells of the innate and adaptive immunity.

Our data did not confirm the presence of a significant association of the rs7574865 polymorphism of the STAT4 gene with a predisposition to AS (p= 0.073). The studies conducted in the Chinese population in order to identify an association of the rs7574865 polymorphism of the STAT4 gene with a predisposition to AS, showed ambiguous results [21, 22]. One group of authors found an association of the rs7574865 polymorphism of the STAT4 gene with a predisposition to AS [21] and showed that the T-allele is associated with an increased risk of AS (allele T compared with allele G: odds ratio, OR 1.48; 95% confidence interval, [CI] 1.22–1.79; p <0.0001) in the dominant model (GT+TT genotypes were also associated with the risk of AS development; OR 1.56), but in the recessive model TT-genotype was not associated with a predisposition to AS. Another group of Chinese researchers, when studying four polymorphisms of the STAT4 gene (rs3077; rs9277535; rs7453920 and rs7574865) in 400 patients with AS and 379 control subjects, found no significant differences in the frequency of alleles of the studied polymorphisms between patients with AS and healthy controls [22]. The significance of rs7574865 polymorphism of the STAT4 gene in the pathogenesis of autoimmune diseases is confirmed by another study [25]. The authors showed that the T-allele rs7574865 of the STAT4 gene enhances mRNA transcription and protein expression. The presence of one copy of the T-allele is associated with elevated mRNA levels. In patients with the TT genotype, a higher level of STAT4 protein was detected compared with patients having the GG genotype. Carriers of at least one T-allele rs7574865 are characterized by a reduced level of IL6 compared with GG homozygotes. These data suggest that the presence of the rs7574865 T-allele enhances STAT4 mRNA transcription and protein expression, which can lead to enhanced signals of STAT4 family molecules.

In the available literature, we did not find studies on the relationship between the rs7574865 polymorphism of the STAT4 gene and the activity of AS. In the work of Italian colleagues, such connection has been established for early arthritis [26]. The authors showed an association of the minor rs7574865 T-allele with a high risk of developing arthritis. The TT rs7574865 polymorphism genotype correlated with increased disease activity (according to DAS28 parameters) compared with the GG genotype (p = 0.044). We have previously shown that this polymorphism of the STAT4 gene is associated with clinical and serological phenotypes of systemic scleroderma [16]. An association of rs7574865 T-allele with a predisposition to the diffuse form of systemic scleroderma, interstitial lung damage and an increased level of antibodies to topoisomerase I was established.

Conclusions. In our study, a reliable association of the rs7574865 polymorphism of the STAT4 gene with the main signs of AC activity: the values of CRP, BASDAI, and ASDAScrp indices, was first revealed. The polymorphism studied may be a new genetic marker for AS severity.

An important limitation of this study is a relatively small group of patients, which makes it difficult to interpret the relationship between STAT4 polymorphism and predisposition to AS. Further research is needed in different ethnic populations and with larger samples of patients.

REFERENCES

1. Эрдес ШФ, Бадокин ВВ, Бочкова АГ и др. О терминологии спондилоартритов. Научно-практическая ревматология. 2015;53(6):657-60. [Erdes ShF, Badokin VV, Bochkova AG, et al. On the terminology of spondyloarthritis. *Nauchno-prakticheskaya revmatologiya = Rheumatology Science and Practice*. 2015;53(6):657-60. (In Russ.)]. doi: 10.14412/1995-4484-2015-657-660.
2. Australo-Anglo-American Spondyloarthritis Consortium (TASC), Reveille JD, Sims AM, Danoy P, et al. Genome-wide association study of ankylosing spondylitis identifies non-MHC susceptibility loci. *Nat Genet.* 2010 Feb;42(2):123-7. doi: 10.1038/ng.513. Epub 2010 Jan 10.
3. Эрдес ШФ, Дубинина ТВ, Абдулганиева ДЭ и др. Клиническая характеристика анкилозирующего спондилита в реальной практике в России: результаты одномоментного многоцентрового неинтервенционного исследования ЭПИКА2. Научно-практическая ревматология. 2016;54(Прил 1):10-4. [Erdes ShF, Dubinina TV, Abdulganieva DE, et al. Clinical characteristics of ankylosing spondylitis in real practice in Russia: results of the cross-sectional non-interventional trial EPICA2. *Nauchno-prakticheskaya revmatologiya = Rheumatology Science and Practice*. 2016;54(1S):10-4. (In Russ.)]. doi: 10.14412/1995-4484-2016-1S-10-14.
4. Эрдес ШФ, Румянцева ДГ, Смирнов АВ. Оценка прогрессирования аксиального спондилоартрита на ранних стадиях болезни в реальной клинической практике: возможности использования суммарного счета рентгенологического сакроилиита. Научно-практическая ревматология. 2018;56(4):461-5. [Erdes ShF, Rumyantseva DG, Smirnov AV. Evaluation of the progression of axial spondyloarthritis in the early stages of the disease in real clinical practice: the possibilities of using the summary score of radiographic sacroiliitis. *Nauchno-prakticheskaya revmatologiya = Rheumatology Science and Practice*. 2018;56(4):461-5. (In Russ.)]. doi: 10.14412/1995-4484-2018-461-465
5. Brown MA, Kennedy LG, MacGregor AJ, et al. Susceptibility to ankylosing spondylitis in twins: the role of genes, HLA, and the environment. *Arthritis Rheum.* 1997 Oct; 40(10):1823-8. doi: 10.1002/1529-0131(199710)40:10<1823::AID-ART15>3.0.CO;2-1
6. Brewerton DA, Hart FD, Nicholls A, et al. Ankylosing spondylitis and HL-A 27. *Lancet*. 1973 Apr 28;1(7809):904-7.
7. Khan MA, Ball EJ. Genetic aspects of ankylosing spondylitis. *Best Pract Res Clin Rheumatol.* 2002 Sep;16(4):675-90.
8. Brown MA, Laval SH, Brophy S, Calin A. Recurrence risk modelling of the genetic susceptibility to ankylosing spondylitis. *Ann Rheum Dis.* 2000 Nov;59(11):883-6. doi: 10.1136/ard.59.11.883
9. Frucht DM, Aringer M, Galon J, et al. Stat4 is expressed in activated peripheral blood monocytes, dendritic cells, and macrophages at sites of Th1-mediated inflammation. *J Immunol.* 2000 May 1; 164(9):4659-64. doi: 10.4049/jimmunol.164.9.4659
10. Mathur AN, Chang HC, Zisoulis DG, et al. Stat3 and Stat4 direct development of IL-17-secreting Th cells. *J Immunol.* 2007 Apr 15;178(8):4901-7. doi: 10.4049/jimmunol.178.8.4901
11. Watford WT, Hissong BD, Bream JH, et al. Signaling by IL-12 and IL-23 and the immunoregulatory roles of STAT4. *Immunol Rev.* 2004 Dec;202:139-56. doi: 10.1111/j.0105-2896.2004.00211.x
12. Harley JB, Alarcon-Riquelme ME, Criswell LA, et al. Genome-wide association scan in women with systemic lupus erythematosus identifies susceptibility variants in ITGAM, PXK, KIAA1542 and other loci. *Nat Genet.* 2008 Feb;40(2):204-10. doi: 10.1038/ng.81. Epub 2008 Jan 20.
13. Kobayashi S, Ikari K, Kaneko H, et al. Association of STAT4 with susceptibility to rheumatoid arthritis and systemic lupus erythematosus in the Japanese population. *Arthritis Rheum.* 2008 Jul;58(7):1940-6. doi: 10.1002/art.23494.
14. Korman BD, Alba MI, Le JM, et al. Variant form of STAT4 is associated with primary Sjoþgren's syndrome. *Genes Immun.* 2008 Apr;9(3):267-70. doi: 10.1038/gene.2008.1. Epub 2008 Feb 14.
15. Zhao Y, Liu X, Liu X, et al. Association of STAT4 gene polymorphism with increased susceptibility of rheumatoid arthritis in a northern Chinese Han subpopulation. *Int J Rheum Dis.* 2013 Apr;16(2):178-84. doi: 10.1111/1756-185X.12093.
16. Крылов МЮ, Ананьева ЛП, Конева ОА и др. Влияние полиморфизма rs7574865 (G/T) гена STAT4 на риск развития клинических и иммунологических фенотипов системной склеродермии в русской популяции больных: результаты пилотного исследования. Терапевтический архив. 2017;89(5):20-5. [Krylov MYu, Anan'eva LP, Koneva OA, et al. Effect of rs7574865 (G/T) gene STAT4 polymorphism on the risk of clinical and immunological phenotypes of systemic scleroderma in the Russian patient population: results of a pilot study. *Terapevticheskii arkhiv.* 2017;89(5): 20-5. (In Russ.)].
17. Mohamed RH, Pasha HF, El-Shahawy EE. Influence of TRAF1/C5 and STAT4 genes polymorphisms on susceptibility and severity of rheumatoid arthritis in Egyptian population. *Cell Immunol.* 2012;273(1):67-72. doi: 10.1016/j.cellimm.2011.11.005. Epub 2011 Dec 4.
18. Zervou MI, Sidiropoulos P, Petraki E, et al. Association of a TRAF1 and a STAT4 gene polymorphism with increased risk for rheumatoid arthritis in a genetically homogeneous population. *Hum Immunol.* 2008 Sep; 69(9):567-71. doi: 10.1016/j.humimm.2008.06.006. Epub 2008 Jul 14.
19. Orozco G, Alizadeh BZ, Delgado-Vega AM, et al. Association of STAT4 with rheumatoid arthritis: a replication study in three European populations. *Arthritis Rheum.* 2008 Jul;58(7):1974-80. doi: 10.1002/art.23549.
20. Sigurdsson S, Nordmark G, Garnier S, et al. A risk haplotype of STAT4 for systemic lupus erythematosus is over-expressed, correlates with anti-dsDNA and shows additive effects with two risk alleles of IRF5. *Hum Mol Genet.* 2008 Sep 15;17(18):2868-76. doi: 10.1093/hmg/ddn184. Epub 2008 Jun 25.
21. Liu Z, Zhang P, Dong J. Genetic variants of STAT4 are associated with ankylosing spondylitis susceptibility and severity in a Chinese Han population. *Int J Clin Exp Med.* 2014 Dec 15;7(12):5877-81. eCollection 2014.
22. Liu X, Yang B, Li L, et al. Association of HLA-DP/DQ and STAT4 polymorphisms with ankylosing spondylitis in Southwest China. *Int Immunopharmacol.* 2016 Oct; 39:10-15. doi: 10.1016/j.intimp.2016.06.033. Epub 2016 Jul 7.
23. Van der Linden S, Valkenburg HA, Cats A. Evaluation of diagnostic criteria for ankylosing spondylitis. A proposal for modification of the New York criteria. *Arthritis Rheum.* 1984 Apr;27(4):361-8.
24. Nguyen KB1, Watford WT, Salomon R, et al. Critical Role for STAT4 Activation by Type 1 Interferons in the Interferon-γ Response to Viral Infection. *Science*. 2002 Sep 20;297(5589):2063-6.
25. Lamana A, Lopez-Santalla M, Castillo-Gonzales R, et al. The Minor Allele of rs7574865 in the STAT4 Gene Is Associated with Increased mRNA and Protein Expression. *PLoS One.* 2015 Nov 16;10(11):e0142683. doi: 10.1371/journal.pone.0142683. eCollection 2015.
26. Lamana A, Balsa A, Rueda B, et al. The TT Genotype of the STAT4 rs7574865 Polymorphism is Associated with High Disease Activity and Disability in Patients with Early Arthritis. *PLoS One.* 2012;7(8): e43661. doi: 10.1371/journal.pone.0043661. Epub 2012 Aug 24.

Article received 8.04.2019