PHD THESIS

ROLE OF INTERLEUKIN-23 RECEPTOR GENE POLYMORPHISMS IN AUTOIMMUNE AND IMMUNE MEDIATED DISEASES

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LIST OF ABBREVIATIONS

bp	basepair
CRP	C-reactive protein
DNA	deoxyribonucleic acid
dNTP	deoxyribonucleotide triphosphate
EDTA	ethylenediaminetetraacetic acid
HLA	human leukocyte antigene
IgAN	immunglobuline-A nephropathy
IL	interleukin
IL23R	interleukin-23 receptor
IL-12Rβ1	interleukin-12 receptor β1 subunit
INF-γ	interferon gamma
LD	linkage disequilibrium
MAF	minor allele frequency
OR	odds ratio
PCR	polymerase chain reaction
RFLP	restriction fragment length polymorphism
SLE	systemic lupus erythematosus
SNP	single nucleotide polymorphism
SPA	ankylosing spondylitis, Bechterew syndrome
SS	Sjögren syndrome
STAT	signal trancducer and activator of transcription
Th17/Th_{IL-17}	T helper 17 cells
TNF-α	tumor necrosis factor-α
3'-UTR	3'-untranslated region
95% CI	95% confidence interval

1. INTRODUCTION

1.1 The interleukin-23/interleukin-17 axis

Cytokines are small molecular weight glykoproteins that play an important role in information transmission and regulation during immune responses. The interleukin (IL) name used as a synonym refers to the connections made between leucocytes. They play a role in the cooperation between accessory cells and lymphocytes, help the maturation, activation, differentiation of cells, participate in effector functions also. The interleukin-23 (IL.23) is a heterodimeric cytokine composed of two disulfide-bridged subunits, p19 which is unique to IL-23 and p40 which is shared with IL-12. This complex is expressed by activated dendritic cells and has biological activities that are similar but not equal to those of IL-12. The receptor complex of IL-23 is also similar to that of IL-12: the common p40 subunit is recognized by interleukin-12 receptor beta-1 subunit (IL-12Rβ1) and the p19 subunit associates to the specific interleukin-23 receptor (IL-23R). Binding of IL-23 to its receptor leads to the activation of Janus Kinases (Jak2 and Tyk2), which can phosphorylate IL-23R at discrete locations and thus form docking sites for signal transducers and activators of transcription (STATs). The STATs are then phosphorylated by the Jaks, and are thus capable of dimerising and translocating to the nucleus where they influence the transcription of key proinflammatory genes such as IL-17.

Interleukin-17 is a pro-inflammatory cytokine that is produced by activated T cells. In chronic inflammation, the antigen-stimulated dendritic cells and macrophages produce IL-23 which acts on naïve $CD4^+$ T cells and induces their differentiation towards becoming helper T 17 cells (Th17/$Th_{IL\text{-}17}$). These cells produce IL-17, which enhances T-cell priming and causes inflammatory responses by inducing the production of several inflammatory mediators. IL-23 also acts on macrophages and dendritic cells in an autocrine/paracrine manner to stimulate the generation of pro-inflammatory.

In case of bacterial infection IL-23 is rapidly produced by the macrophages and dendritic cells at the site of infection, where it then activates the local resident Th17 cells. Generation of IL-17 by these cells then induces granulocyte-colony stimulating factor production from stromal cells and subsequently recruitment of neutrophils to the infection site, contributing to extracellular bacterial clearance. The neutrophils transmigrate into tissues where the apoptotic neutrophils are then phagocytosed by the macrophages and dendritic cells. This reduces IL-23 production and the resulting IL-17 production of Th17 cells.

The human *IL23R* gene is located on chromosome 1 (1p31.3). The standard form of IL-23R is encoded by 11 exons. Through alternative splicing at least six spliced isoforms can be generated. These variants result in either premature termination to generate a diverse form of receptor ectodomain, or a frameshift to produce various lengths of IL-23R intracellular domain. A genome-wide association found strong association with Crohn's disease and polymorphisms of the IL23R gene. In total 10 single nucleotide polymorphisms (SNPs) were reported, which showed highly significant association with inflammatory bowel disease. Theoretical considerations also suggested association of these SNPs with other autoimmune diseases.

The biological impact of the investigated polymorphisms on the expression and functionality of IL23R is currently unknown but it is obvious that these SNPs can represent an important link in the development of autoimmune and inflammatory diseases. Several mechanisms can be suggested by which polymorphisms can change the function of the receptor. SNPs located in the 3'-UTR can possibly cause over expression of the receptor (e.g. by increasing mRNA stability) driving differentiation of T-cells towards a Th17 sub-population. In

case of the rs11209026 Arg381Gln SNP the arginine allele has a side chain with positive charge while the rare glutamine an uncharged polar one and thus the substitution can have considerable effect on the protein structure. The intronic polymorphisms could perhaps exert their influence via regulating the differential splicing.

1.2 Studied diseases

1.2.1 Psoriasis

Psoriasis is a chronic inflammatory disease that affects skin and joints. It is a chronic immune mediated disease, so unlike in autoimmune disease, the target antigene is not one of the organism's but rather an intolerance of joint bacterial populations. Its prevalence is about 2% in Hungary, it occurs in both sexes equally. . It begins with red, scaly skin patches that later start to peel off and drop off. Although psoriasis can have a massive effect on life-quality, life-span does not seem to be shortened due to the disease.

Psoriasis was considered an epidermal disease but after realizing that immunosupressive agents can improve the patient's status, this conception has radically changed. The role of many genes and loci is presumed in its development beside environmental factors. The association of several human leucocyte antigens (HLAs) was confirmed with the disease. There is a relation with the 17q25 region, TNF-α gene and with the 3q21, 19p13 and 20q13 loci.

1.2.2 Systemic lupus erythematosus

Systemic lupus erythematosus (SLE) is a chronic, remitting, relapsing, inflammatory autoimmune connective tissue disease. The word 'lupus' originating from latin, means wolf, the lupus erythematosus (red wolf) name denotes to the red skin accompanying the disease. Its prevalence rate is about 12-64 in 100,000 people and is ten times more common in women than men. Both external and internal factors are involved in its development, amongst the triggering factors are for example UV radiation, infections and some drugs. The result is a complex disorder of the immune system, characterized by the loss of tolerance against the organism's own antigenes. The inflammation can affect any organ.

SLE is a multifactorial disease with genetic factors enhancing its development, however one single gene is not enough for its development. This association was confirmed first with MHC I-, II-, III-polymorphisms and inherited complement-deficiency. Beside these, the role of numerous genes can be presumed. Amongst the loci associated with SLE three are found on chromosome 1, and within these loci several genes have been identified. Most of them are polymorphic and one of the alleles is associated with the disease.

1.2.3 Ankylosing spondylitis

Ankylosing spondylitis (AS) usually starts in sacroiliac joints with axial skeleton involvement as the disease progresses with inflammation of the joints and entheses eventually leading to new bone formation with syndesmophytes and ankylosis. Men are more often affected than women; most patients develop the first symptoms at an age younger than 30 years. Az the surface of the joints degenerates, the vertebrea begin to coalesce. A quarter of the patients develop symptoms that are not associated with movement organs.

The exact cause of AS is unknown however its occurence shows a familial aggregation. The HLA B27 allele can be found int he majority of patients but it is presumable that it is responsible only for 20-30% of disease risk. The role of other MHC genes like HLA B60 and

HLA DR1 is presumed also. Several investigations have shown association with non-MHC genes also.

1.2.4 Sjögren-syndrome

Sjögren-syndrome (SS) is one of the most common problems in ophthalmology. It is clinically characterized by keratoconjunctivitis sicca and xerostomia. It is an autoimmune disease that mainly effects the exocrine glands. Due to the inflammation the number of cells forming the glands decreases constantly. The symptoms can vary from mild irritation to pain. An itching, burning sensation in the eye is the most common, accompanied with xerostomia, pain and disturbance in tasting. More than half of patients develop extraglandular manifestations, like myalgia, arthralgia, and involvement of the pulmonary system. More, than 90% of the patients are women, with a mean age of 50-60 years.

Primary SS occurs when there are no other immune diseases in the background while secondary SS accompanies diseases of joint and connective tissue most often. Autoantibodies can be found against two antigenes, namely SS-A and SS-B. The pathogenesis of the disease is not completely known, however it is a multifactorial disease with a long process leading to the selective lesion of exocrine glands. Studies have shown disease association with HLA DRB1*03 and DQB1*02 alleles, but only in the patients in whom anti-SSA and/or anti-SSB antibodies were present.

1.2.5 Immunoglobulin A nephropathy

Immunoglobuline A nephropathy (IgAN) is a slowly progressing immunmediated disease of the kidneys that effects the glomeruli. IgA1 deposits can be detected in the mesangium with immunehistology; it can cause macroscopic or microscopic haematuria. It is the most common form of glomerulonephritis. Nearly half of the patients have decreased renal functions, 20-50% needs renal replacement treatment 20 years after the diagnosis. Besides primary IgAN of unknown origin we can distinguish forms that are observed together with the disease of another organ. Although cause and effect are not always obvious, these forms are named secunder IgAN.

Eventhough IgAN occurs sporadicaly and it can't be considered an inherited disease, some facts seem to allude to its genetic background, like the ethnical and geographic variability in its prevalence and the familial aggregation observed in several cases. The association of IgSN with the 6q22-23 region was shown in a linkage analysis while another study identified two other loci (4q26-31 and 17q12-22). IgAN was correlated also with polymorphisms in E-selectin and L-selectin genes and several genes in the renin-angiotensin system.

1.2.6 Systemic sclerosis

The word scleroderma originates from Greek, it means 'stiff, hard skin'. The disease can be characterized by the fibrosis and late atrophy of skin and some internal organs and generalised obliterative vasculopathy. Tissue structures degenerate gradually, tissue atrophy develops and internal organs suffer irreversible lesions.

The disease affects women mainly, it begins usaully in the fifth decade. Its two forms, diffuse cutan systemic sclerosis and limited cutan systemic sclerosis differ from each other in terms of clinical symptoms and prognosis. In the first case, the skin of the limbs and torso is effected and this is a form with severe internal symptoms and a bad prognosis, while in the later case only the skin of the face and limbs is affected and prognosis is more favorable also.

Although there are some genetic associations known in systemic sclerosis, its occurrence in homo- and heterozygotic twin studies is low (4.2-5.9%) denoting the important effect of environmental factors. Significant association from a clinical point of view can be found between the HLA-DR52a and lung fibrosis. Beside HLA region, the fibrillin and SPARC genes can be considered as susceptibility loci. The role of other factors arose also, like cytokine- and cytokinereceptor genes and other genes responsible for collagen synthesis. Several environmental factors like some solvents and drugs can cause scleroderma or scleroderma-like diseases.

2. AIMS OF THE STUDY

The objective of our work was to study polymorphisms of the IL23R gene in autoimmune diseases in Hungarian population. We aimed to study the rs1004819, rs11805303, rs7517847, rs7530511 (Pro310Leu), rs10489629, rs2201841, rs11209026 (Arg381Gln), rs10889677 and rs11209032 genetic variant of the IL23R gene and the rs2201841, rs10889677 and rs1884444 genetic variants in case of systemic sclerosis in the following diseases:

a) Psoriasis
b) Systemic lupus erythematosus
c) Ankylosing spondylitis
d) Sjögren-syndrome
e) Immunoglobuline A nephropathy
f) Systemic sclerosis

We also aimed to identify the occurring IL23R haplotypes in the studied diseases and to investigate the role of the frequent haplotypes in the development of the diseases.

3. MATERIALS AND METHODS

3.1 Studied populations

The DNA samples of the patients with psoriasis were collected at the Department of Dermatology and Allergology, University of Szeged. The group consisted of 214 patients (mean age 47.5±12.3 years). A total of 189 Caucasian subjects served as controls (mean age 45.3±11.0 years). The DNA samples of the patients with SLE were collected at the Department of Immunology and Rheumatology, University of Pecs, at the 3rd Department of Internal Medicine, Semmelweis University, Budapest and at the 3rd Department of Internal Medicine, University of Debrecen, Hungary. The SLE population represented a total of 383 clinically well-characterized SLE-patients (mean age: 46.5±13.6 years). A total of 253 Caucasian subjects (mean age: 44.0±11.8 years) served as controls. The DNA samples of the patients with AS were collected at the National Institute of Rheumatology and Physiotherapy in Budapest, Hungary; the group consisted of 206 patients (mean age: 40.7±15.4 years). Data for B27 status was available for 191 patients; 153 patients were B27 positive (80.1%), 38 were tested negative (19.9%). A total of 235 Caucasian subjects (mean age: 45.0±10.9 years) served as controls just like in case of Sjögren-syndrome (156 patients, mean age 59.5±12.3 years). Primary Sjögren syndrome was present in 141 patients (90.4%), secondary Sjögren syndrome was present only in 15 patients (9.62%). In the secondary forms the disease was associated with rheumatoid arthritis. Positive anti-SSA was

measured in 25% of the patients (mean: 100.3 ±49.9U/ml), positive SSB in 12.2% of the patients (mean: 50.8±25.1U/ml). The samples of the SS patients were collected at the University of Debrecen. The IgAN population represented a total of 143 clinically well-characterized subjects (mean age 49.9±13.4 years), all originating from the 2nd Department of Internal Medicine and Nephrology Center, University of Pécs. A total of 189 Caucasian subjects served as controls (mean age 45.3±11.0 years). We also examined 224 patients with systemic sclerosis (27 males and 197 females, mean age: 56.8 ± 12.0 years). The DNA samples were collected at the Department of Rheumatology and Immunology, University of Pécs and were compared to 220 healthy individuals (mean age: 43.6±12.5 years). The patients and controls gave their informed consent previous to the study in all cases. During the collection and use of DNA samples and clinical data guidelines and regulations of the Ethics Committee (ETT TUKEB) and the Helsinki Declaration in 1975 were followed.

3.2 Molecular biology methods

The molecular analyses were performed using DNA extracted from peripheral blood leukocytes with a routine salting out procedure. PCR-RFLP methods were applied to test the alleles of the IL23R gene which consisted specific, synthetic oligonucleotide primers, Taq polymerase, dNTP, buffer and genomic DNA template. For detection of the PCR product gelelectrophoresis, ethidium-bromide staining and UV light was used. For genotype determination, restriction fragment length polymorphism (RFLP) was used. The primers were designed to create obligatory cleavage sites of the proper restriction enzymes in the amplicons to control the accuracy of the digestion. The following parameters were applied for amplification: predenaturation on 95ºC for 2 min, 35 cycles of: denaturation on 95ºC 30 sec, annealing on 55ºC 45 sec (in case of rs188444 58^{o}C 45 sec, rs11805303 59^{o}C 30 sec, rs7530511 and rs10889677 60^{o}C 45 sec), polymerization on 72ºC 45 sec, and a final annealing on 72ºC for 5 min. The restriction endonucleases, PCR product lengths and the restriction patterns are also shown in Table 1. In case ofrs2201841 we had to introduce mismatch bases to generate artificial cleavage sites (underlined in the sequence).

3.3 Statistical evaluation

Differences between the patient and control groups were examined by χ^2-test. Associations of the studied diseases and the examined genetic variants were tested using binary logistic regression analysis using SPSS 11.5 for Windows. Odds ratios refer to the patient population vs. controls and are calculated with 95% confidence intervals (95% CI), indicated in brackets.

Haploview 4.1 was used to study linkage disequilibrium (LD) patterns. We required the minor allele frequency at each locus to be >0.05, with an R^2 value of <0.8 between pairs of loci, based on the default settings in Haploview. After applying these criteria, 6 of the 9 SNPs were retained for haplotype analysis. Haplotype frequencies were estimated using PHASE version 2.1.

4. RESULTS

4.1 Psoriasis

We examined nine polymorphisms of the IL23R gene in 214 psoriatic patients and 189 healthy controls. All the genotype and allele distributions were in Hardy-Weinberg-equilibrium

both groups. The results are shown in Table 2. The presence of the mutant T allele of rs11805303 was significantly increased in patients with psoriasis compared to the healthy controls (p=0.019). The logistic regression analysis revealed that carrying the minor allele confers a 1.55-fold risk for the development of psoriasis (OR=1.60; 95% CI: 1.08-2.38). We performed a χ^2-test to investigate the dissimilarity between the allele frequencies of the patient and control group and found a statistically significant difference (p=0.037; OR=1.39; 95% CI: 1.02-1.89).

For the rs10889677 variant the prevalence of the AA genotype showed a more than 3-fold increase in the psoriasis group compared to the controls (p=0.005; OR=3.44; 95% CI: 1.45-8.15). The homozygous form of the minor allele of rs2201841 was also significantly increased in psoriasis patients, conferring a 2.4-fold risk for the disease (p=0.016; OR=2.64; 95% CI: 1.20-5.81). The allele frequencies showed also significant differences between the psoriasis patients and controls (rs10889677: p=0.040; OR=1.38; 95% CI: 1.01-1.87 and rs2201841: p=0.048; OR=1.36; 95% CI: 1.01-1.86).

The highest linkage disequilibrium (LD) values (R^2) were observed between rs1004819 and rs11805303, as well as between rs2201841 and rs10889677 variations (R^2=0.80).

Association of IL23R haplotypes with psoriasis is shown in Table 3. SNP rs11209026 had a minor allele frequency less than 0.05 so it was not taken into account in the haplotype analysis. The R^2 values between rs11805303and rs1004819 furthermore between rs10889677 and rs2201841 were 0.8 so rs1004819 and rs2201841 were removed from the haplotype analysis. The TTCAAA haplotype of rs11805303, rs7517847, rs7530511, rs10489629, rs10889677, rs11209032 variants was associated with risk of psoriasis (p=0.003; OR=1.76). The CGCACG haplotype showed the strongest protective effect (p=0.001; OR=0.13), while the TTCACA and CTCACG haplotypes proved to be somewhat less protective.

4.2 Systemic lupus erythematosus

All the genotype and allele distributions were in Hardy-Weinberg-equilibrium both in the patient and control groups. The genotypes and minor allele frequencies of the nine examined IL23R variants are summarized in Table 4. We could not detect any significant difference in the genotype distributions or allele frequencies between the two groups. The R^2 values between rs11805303and rs1004819 furthermore between rs10889677 and rs2201841 were >0.8 so rs1004819 and rs2201841 were removed from the haplotype analysis since they had lower minor allele frequencies than rs11805303 and rs10889677, respectively. The distribution of haplotypes did not differ either between the examined groups.

4.3 Ankylosing spondylitis

All the genotype and allele distributions were in Hardy-Weinberg-equilibrium both in the patient and control groups. The obtained results are shown in Table 5. The minor allele frequency in case of rs11805303 was significantly increased in patients compared to controls (p=0.009; OR=1.47; 95% CI: 1.10-1.95) what is true in presence of the minor T allele also as 59.2% of the patient population carries the mutant allele while only 47.2% of the controls. The logistic regression analysis revealed that carrying the minor allele confers a 1.6-fold risk for the development of AS (p=0.017; OR=1.60; 95% CI: 1.09-2.36).

Similarly to the rs11805303 allele, carrying the rs1004819 A allele was more frequent in patient than in controls (64.1% vs. 48.5%), moreover, the allele frequencies showed also significant difference between the AS patients and controls (p=0.004; OR=1.51; 95% CI: 1.14-2.01). In case of rs10889677 and rs2201841 carriage of the mutant homozygotic genotype was

elevated in patients compared to controls.

We weren't able to find a homozygous form of the rs11209026 polymorphism in any of our examined groups, however, the GA heterozygous genotype showed a significant decrease in AS patients compared to the controls (5.34% vs. 11.5%, p=0.031) and both logistic regression analysis and the χ^2-test for the difference of the allele frequencies (p=0.025; OR=0.45; 95% CI: 0.22-0.92) revealed a protective effect of this variant for the development of the disease.

When only the B27 positive AS patients were included in the statistical analysis, the same genotypes were found significantly different compared to the controls as in the whole AS group, except for rs11209026 where we could not detect significant difference. As for the allele frequencies, for rs11805303 the difference between the B27 positive patients and the controls was statistically significant however only marginally (p=0.042; OR=1.38; 95% CI: 1.01-1.89). The same was true for rs10889677 (p=0.046; OR=1.37; 95% CI: 1.01-1.86). In case of rs1004819 the minor allele was associated with risk of AS (p=0.015; OR=1.46; 95% CI: 1.08-1.99). In all cases the level of significance was always less than was in the total group, probably due to the smaller number of samples. The number of B27 negative AS patients was too few for a statistically valid analysis.

The highest LD values (R^2) were observed between rs1004819 and rs11805303 (R^2=0.84).

Association of IL23R haplotypes with AS is shown in Table 6. SNP rs11209026 had a minor allele frequency less than 0.05 so it was not taken into account in the haplotype analysis. The R^2 value between rs1004819 and rs11805303 was >0.8 so the latter was also removed from the haplotype analysis since it had lower minor allele frequency than rs1004819. The ATCACAG and ATCACAA haplotypes consisting of rs1004819, rs7517847, rs7530511, rs10489629, rs2201841, rs10889677, rs11209032 variants proved to be susceptibility factors for the development of the disease in Hungarian population, while GGCATCG and AGCACAA haplotypes showed a protective effect. In case of B27 positive patients only the GGCATCG and ATCACAA haplotypes showed association with AS, the previous being a protective, the later a susceptibility factor.

4.4 Sjögren syndrome

All the genotype and allele distributions were in Hardy-Weinberg-equilibrium both in the patient (n=156) and control (n=235) groups. The genotype- and allele frequencies are shown in Table 7. There was no difference in the distribution of any of the examined IL23R variants between the controls and the Sjögren syndrome patients. No association was detected in the subgroup of primary form of SS either (Table 7, colored in grey). The number of secondary SS patients was too few for a statistically valid analysis.

We could not detect any association between IL23R polymorphisms and autoantibody secretion either. The highest LD values were observed between rs1004819 and rs11805303 (R^2=0.83). Rs1004819 was not taken into account in the haplotype analysis due to the strong linkage detected previously. None of the IL23R haplotypes showed association with Sjögren syndrome.

4.5 Immunoglobuline A nephropathy

All the genotype and allele distributions were in Hardy-Weinberg-equilibrium both in the IgAN patient (n=143) and control (n=189) groups. The genotype- and allele frequencies are shown in Table 8. We could not detect any significant difference between the two groups neither in case of genotypes nor in case of allele frequencies. The LD value between rs11805303 and

rs1004819 was over 0.8 so rs1004819 was not taken into account in the haplotype analysis since it had a lower MAF than rs11805303. None of the IL23R haplotypes showed association with IgAN.

4.6 Systemic sclerosis

All the genotype and allele distributions were in Hardy-Weinberg-equilibrium both in the systemic sclerosis patients (n=233) and in the control (n=220) groups. The genotype- and allele frequencies are shown in Table 9. We could not detect any significant difference between the two groups neither in case of genotypes nor in case of allele frequencies. None of the LD values exceeded 0.8 so all SNPs were taken into account in the haplotype analysis however none of the haplotypes showed association with the disease.

5. DISCUSSION

The IL23R gene is located on chromosome 1p31.3, the encoded protein forms a receptor for the IL23 molecule together with the β1 subunit of IL12R. IL23 plays a role in numerous inflammatory processes, so changes occurring in its gene can have an effect on the development of autoimmune processes and diseases. Our aim was to analyse IL23R mutations in Hungarian population in so far not studied autoimmune diseases and also its possible effects on the development of these diseases.

According to studies the haplotype composed of the C allele of rs7530511 and the G allele of rs11209026 had a significantly higher occurrence in patients suffering from psoriasis than in healthy controls. Several studies have confirmed the association of the later SNP and of this haplotype with psoriasis later on. Association was also found between rs2201841 and psoriasis. Our results somewhat contradict those of other investigators since we could not detect an association between the Arg381Gln mutation and psoriasis in Hungarian population. The possible explanation for this could be that we weren't able to find a homozygous mutant genotype in our sample population. However this fact isn't unmached either in literature since similar phenomenon was detected in Thai population in similar number of samples. It should be noted that those studies that were able to detect an association used much larger populations. This calls attention to the importance of the fact that it is quite necessary to analyse large number of patients since factors with lower odds ratios can be detected only this way. The rs2201841 variant proved to be a significant risk factor in Hungarian population also and the rs11805303 and rs10889677 polymorphisms were described first in literature as factors associated with psoriasis.

A previous study on a group of Spanish SLE patients analysed eight polymorphisms of IL23R, and could not find any association between the variants and the disease. Also, in other study Korean SLE patients no association between the disease and IL23R could be detected either. Our analyses confirm these results since we could not find aggregation of any of the IL23R genotypes or haplotypes in Hungarian SLE patients compared to controls. Our results suggest that IL23R gene variants do not pose a risk for SLE; the Hungarian population does not differ from other populations in this respect.

The association of the IL23R gene and ankylosing spondylitis was first demonstrated by the Wellcome Trust Case Control Consortium in a genome wide association study. They analysed eight IL23R SNPs in total; all of them showed an association with the disease with rs11209032 being the most significant one. These results are confirmed by a Spanish study in which the Arg381Gln and rs1343151 variants were found to have a protective effect. Similar results were obtained in British population where the rs11209032 showed the strongest association with AS also, while in Portugese patients rs1004809 was the most significant risk factor. The mentioned studies were all conducted on Caucasian patients, with similar results. The data in Asian population is not so unified: a Korean study involving ten IL23R polymorphisms could not detect an association with the disease. Among Chinese patients the genotype frequency of rs11209032 and the genotype- and allele frequencies of rs6677188 showed a significant difference compared to controls, however another Chinese study did not detect any association. Our results confirm those obtained in other Caucasian populations. We could show the association of five IL23R polymorphisms with AS. The AA genotype of rs10889677 polymorphism and the CC genotype of rs2201841 showed an elevated prevalence in the AS group compared to controls. In the patients carrying either the AA or the CC genotype, so possessing two of either mutant allele, disease risk was elevated more than 2-folds compared to those with a normal genotype. This data seems to imply a gene-dose effect in the role the minor alleles play in disease susceptibility. The

obtained results were similar when only the B27 positive AS patients were included in the statistical analysis except for the rs11209026 variant in which case we did not detect difference between cases and controls. This results suggest that B27 status has no effect on the IL23R gene.

Until now there were no studies conducted to identify the possible association between Sjögren syndrome and IL23R gene polymorphisms, our study on Hungarian population is the first in literature. None of the IL23R variant showed a significant difference of genotype- or allele distribution in patients compared to controls. We could not detect significant difference between the two groups when only primary Sjögren syndrome patients were included in the statistical analysis and we obtained similar negative results in regard of autoantibody secretion also. According to our analyses we can conclude that polymorphisms of the IL23R gene do not play a role in the development of Sjögren syndrome neither alone nor combined into haplotypes.

Similar to Sjögren syndrome, there is no previous data in literature on the association of the examined polymorphisms and immunoglobuline A nephropathy. The fact that the IL23R gene showed association rather with immune mediated diseases like psoriasis or ankylosing spondylitis than with autoimmune diseases like SLE lead us to study the possible association of these variations with this immune mediated nephropathy. We could not detect any association between the analysed variants and IgAN however. This does not exclude the possibility that the IL-23/IL-17 pathway plays some kind of a role in the development of IgAN however these studies would require many populations and a great number of patients.

In regard of systemic sclerosis the results obtained so far are somewhat contradictory. A study conducted on Dutch population demonstrated an association with two polymorphisms (rs11209032, rs1495965) out of the studied seven, however the same investigation failed to find association between the gene and a Spanish patient population. When taking together the two populations, no association was found between IL23R variants and systemic sclerosis either. A similar negative result was obtained in another investigation, however in case of anti-topoisomerase I antibody positive patients the rs11209026 (Arg381Gln) and rs11465804 SNP-s showed an association with the disease. We studied the possible effects of ten IL23R gene variants in Hungarian patient population but could not find any association with the disease. Our results confirm the assumption that IL23R gene variants do not play an important role in the development of systemic sclerosis, however to clarify whether this is true in all subgroups of the disease more studies will be needed in the future.

According to data in international literature it seems likely that IL23R gene plays a more important role in the development of immune mediated diseases than in autoimmune ones. In both cases inflammatory processes cause organic lesions, but their immune biology is different since in case of immune mediated diseases the target antigene is not one of the organism's own ones but the key factor is rather the loss of tolerance against the associated bacteria (mainly in the gastrointestinal tract and those of the skin). The fact that the role of IL23R polymorphisms arose in Crohn's disease first coincides with this observation. Our investigations support this approach. This is presumably in concordance with the assumed evolutionary role of the IL-23/IL-17 axis: IL-23 is produced by macrophages and dendritic cells hours after contact with lipopolysaccharides and other microbial products. This generates a rapid IL-17 response of the local tissue T-cells. Inflammatory cytokines are produced in response to IL-17, which recruit neutrophil granulocytes to the infection site, so it is obvious that IL-23 molecule has a important role in the early response to pathogens. Later on during the process common Th17 cells appear in the tissues and produce more IL-17 in favour of the continuous neutrophil presence. Without the rapid IL-23/IL-17 response animals appear to be more susceptible to sepsis-type diseases. One can assume that the IL-23 molecule plays an important role in surviving severe local damage like

large aseptic wounds or other serious trauma, when an immediate immune response is needed to prevent sepsis and/or tissue necrosis. The immediate neutrophil granulocyte response buys time for the development of an efficient antimicrobial Th1 response which may take days to develop. This effective immune response seems to have a high price however, since if the regulation of signal transduction pathways is damaged, the process can turn against the organism's own tissues and antigenes.

Eventually, the results of our investigations contribute to cognition and understanding of the genetic background of several autoimmune and immune mediated diseases, what is crucial to the early recognition of these diseases and can also help in identifying new therapeutic targets. It is of course necessary to identify additional genetic variants and conduct thorough genotype-phenotype analyses, since the increasing amount of knowledge can lead to effective prevention and treatment of autoimmune diseases this way.

6. SUMMARY

I. In the presence of the rs11805303 minor allele and in case of rs2201841 or rs10889677 homozygosity the chance to develop psoriasis was increased significantly. The TTCAAA haplotype comprised ofrs11805303, rs7517847, rs7530511, rs10489629, rs10889677, rs11209032 variants associates with disease susceptibility. The CGCACG, TTCACA and CTCACG haplotypes showed a protective effect.

II. Our results suggest that in Hungarian systemic lupus erythematosus patients none of the IL23R polymorphisms or haplotypes associate with the disease.

III. In ankylosing spondylitis the carriage of the minor allele of rs1004819 or rs11805303 was a susceptibility factor for disease development both in whole patient population and in the B27 positive subgroup also. The Arg381Gln variant showed a protective effect against the disease. The homozygotic forms of rs10889677 and rs2201841 have proven to be predisposing factors for ankylosing spondylitis.

IV. None of the IL23R variants or haplotypes associated with Sjögren syndrome. We obtained the same result in case of the primary Sjögren syndrome subgroup.

V. We investigated first in literature the association of immunoglobuline A nephropathy and IL23R polymorphisms. According to our studies, IL23R variants do not play a role in the development of the disease.

VI. We did not detect difference in the distribution of the examined ten IL23R gene polymorphisms or their haplotypes in systemic sclerosis in Hungarian population.

7. THE THESIS IS BASED ON THE FOLLOWING PUBLICATIONS

1. Faragó B, Magyari L, **Sáfrány E**, Csöngei V, Járomi L, Horvatovich K, Sipeky C, Maász A, Radics J, Gyetvai A, Szekanecz Z, Czirják L, Melegh B. Functional variants of interleukin-23 receptor gene confer risk for rheumatoid arthritis but not for systemic sclerosis. Ann Rheum Dis. 2008 Feb;67(2):248-50. IF=7.188
2. **Sáfrány E**, Pazár B, Csöngei V, Járomi L, Polgár N, Sipeky C, Horváth IF, Zeher M, Poór G, Melegh B. Variants of the IL23R gene are associated with ankylosing spondylitis but not with Sjögren syndrome in Hungarian population samples. Scand J Immunol. 2009 Jul;70(1):68-74. IF=2.108
3. **Safrany E**, Melegh B. Functional Variants of the Interleukin-23 Receptor Gene in Non-Gastrointestinal Autoimmune Diseases. Curr Med Chem. 2009;16(28):3766-74. IF=4.708
4. **Safrany E**, Hobor R, Jakab L, Tarr T, Csongei V, Jaromi L, Sipeky C, Valasek A, Zeher M, Fust G, Czirjak L, Melegh B. Interleukin-23 receptor gene variants in Hungarian systemic lupus erythematosus patients. Inflamm Res. 2010 Feb;59(2):159-64. IF=1.589 (2009)
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8. OTHER PUBLICATIONS

1. Magyari L, Bene J, Komlósi K, Talián G, Faragó B, Csöngei V, Járomi L, **Sáfrány E**, Sipeky C, Lakner L, Varga M, Gasztonyi B, Melegh B. Prevalence of SLC22A4 1672T and SLC22A5 -207C combination defined TC haplotype in Hungarian ulcerative colitis patients. Pathol Oncol Res. 2007;13(1):53-6. IF=1.272
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3. Maász A, Kisfali P, Horvatovich K, Mohás M, Markó L, Csöngei V, Faragó B, Járomi L, Magyari L, **Sáfrány E**, Sipeky C, Wittmann I, Melegh B. Apolipoprotein A5 T-1131C variant confers risk for metabolic syndrome. Pathol Oncol Res. 2007;13(3):243-7. IF=1.272
4. **Sáfrány E**, Balikó L, Guseo A, Faragó B, Melegh B. The autosomal dominant cerebellar ataxias are hereditary neurodegenerative diseases. Orv Hetil. 2007 Nov 11;148(45):2125-32.
5. Illes Z, **Safrany E**, Peterfalvi A, Magyari L, Farago B, Pozsonyi E, Rozsa C, Komoly S, Melegh B. 3'UTR C2370A allele of the IL-23 receptor gene is associated with relapsing-remitting multiple sclerosis. Neurosci Lett. 2008 Jan 24;431(1):36-8. IF=2.200
6. Horvatovich K, Orkényi M, Bíró E, Pongrácz K, Kisfali P, Talián G, Csöngei V, Járomi L, **Sáfrány E**, Harangi F, Sulyok E, Melegh B. Pseudo-Bartter syndrome in a case of

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15. Kisfali P, Polgár N, **Sáfrány E**, Sümegi K, Melegh BI, Bene J, Wéber A, Hetyésy K, Melegh B. Triglyceride level affecting shared susceptibility genes in metabolic syndrome and coronary artery disease. Curr Med Chem. 2010;17(30):3533-41. IF=4.708 (2009)

Impact factor of publications which have served as a base for the thesis:	17.235
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Table 1. **Primer sequences, PCR product lengths, restriction endonucleases and restriction patterns for the analysed genetic variants**

	Forward primer	Reverse primer	Product length (bp)	Restriction endonuclease	Fragments of frequent allele genotype (bp)	Fragments of heterozygous genotype (bp)	Fragments of rare allele genotype (bp)
rs1884444	CAGTCTTTTCCTGCTTCCAGACAT	AATAAAATCATACTCTTGCCAATGGCCC	509	PscI	191+318	28+191+290+318	28+191+290
rs1004819	GCATTCTAGGACCGTTTTGG	ATCTGGTGGAAATATGTGAAACCTA	270	TaaI	13+71+185	13+71+185+257	13+257
rs11805303	TCTTCCCAGTCTCCAGTGTG	CCGAACAATTTTTGTTTCCC	373	MnlI	39+136+198	39+136+198+237	136+237
rs7517847	AAACATTGACATTCCCTTCATAC	GAAATGAGTCACCAATAATCCAC	530	BseMII	29+91+410	29+91+410+501	29+501
rs7530511	TACCCATCCATTTTAGGTTAAAGAA	GTCTTGAAGTCCTGACCTAAGGTAATC	614	HphI	51+134+429	51+134+185+429	185+429
rs10489629	CCACACCTCGCCAAGACTTT	TATAAGCTTGTTTGATTATGATGTCAGCAA	348	SspI	31+119+198	31+119+150+198	150+198
rs2201841	GGCAAAAGGGAATTGAGAGG	GGCCTATGATTATGCTTTTTCCTG	420	HpyF3I	163+257	25+163+232+257	25+163+232
rs11209026	AGTCACTCTGTGGCCTAAAGTAAAG	AGATTTTTCTAGTAAACAACTGAAATGA	350	Hpy188I	35+65+250	35+65+250+287	65+287
rs10889677	ATCGTGAATGAGGAGTTGCC	TGTGCCTGTATGTGTGACCA	470	MnlI	61+185+224	61+185+224+285	185+285
rs11209032	TTGTTACTGGAGTTAAACCTCTTGC	AGGAATAATTGCTGAGATGCAATG	265	BseMI	24+67+174	24+67+174+242	24+242

Table 2. **Genotype distribution and minor allele frequency (MAF) of the examined IL23R variants in the groups of patients with psoriasis and healthy controls.**

			Psoriasis patients (n=214)					Controls (n=189)						
	Alleles		Genotypes				MAF	Genotypes				MAF	p	OR (95% CI)
IL23R SNPs	1	2	11	12	22	12+22		11	12	22	12+22			
rs1004819	G	A	107 (50.0)	81 (37.9)	26 (12.1)	107 (50.0)	0.31	103 (54.5)	73 (38.6)	13 (6.88)	86 (45.5)	0.26	0.367 [a]	1.20 (0.81-1.77)
rs11805303	C	T	96 (44.9)	100 (46.7)	18 (8.41)	118 (55.1)*	0.32*	107 (56.6)	69 (36.5)	13 (6.88)	82 (43. 4)	0.25	0.019 [a]	1.60 (1.08-2.38)
rs7517847	T	G	70 (32.7)	111 (51.9)	33 (15.4)	144 (67.3)	0.41	55 (29.1)	101 (53.4)	33 (17.5)	134 (70.9)	0.44	0.435 [a]	0.84 (0.55-1.29)
rs7530511	C	T	167 (78.0)	46 (21.5)	1 (0.47)	47 (22.0)	0.11	140 (74.1)	45 (23.8)	4 (2.12)	49 (25.9)	0.14	0.352 [a]	0.80 (0.51-1.27)
rs10489629	A	G	55 (25.7)	119 (55.6)	40 (18.7)	159 (74.3)	0.47	54 (28.6)	96 (50.8)	39 (20.6)	135 (71.4)	0.46	0.518 [a]	1.16 (0.75-1.80)
rs2201841	T	C	102 (47.7)	87 (40.7)	25 (11.7)*	112 (52.3)	0.32*	101 (53.4)	79 (41.8)	9 (4.76)	88 (46.6)	0.26	0.016 [b]	2.64 (1.20-5.81)
rs11209026	G	A	201 (94.7)	13 (6.07)	0	13 (6.07)	0.03	167 (88.4)	22 (11.6)	0	22 (11.6)	0.06	0.051 [a]	0.49 (0.24-1.00)
rs10889677	C	A	101 (47.2)	88 (41.1)	25 (11.7)*	113 (52.8)	0.32*	99 (52.4)	83 (43.9)	7 (3.70)	90 (47.6)	0.26	0.005 [b]	3.44 (1.45-8.15)
rs11209032	G	A	94 (43.9)	91 (42.5)	29 (13.6)	120 (56.1)	0.35	90 (47.6)	84 (44.4)	15 (7.94)	99 (52.4)	0.30	0.458 [a]	1.16 (0.78-1.72)

[*] $p<0.05$; [a] *P* value for presence of minor allele (heterozygous plus homozygous subjects together in both groups); [b] *P* value for homozygousity of minor allele

Table 3. **IL23R haplotypes associated with psoriasis.**

	CTCACG			CGCACG			TTCACA			TTCAAA		
	p value	OR	95% CI	p value	OR	95% CI	p value	OR	95% CI	p value	OR	95% CI
psoriasis patients	0.021*	0.54	0.32-0.91	0.001*	0.13	0.04-0.43	0.042*	0.26	0.07-0.95	0.003*	1.76	1.22-2.54

* $p < 0.05$

Table 4. **Genotype distribution and minor allele frequency (MAF) of the examined IL23R variants in the groups of patients with SLE and healthy controls.**

			SLE patients (n=383)					Controls (n=253)						
	Alleles		Genotypes				MAF	Genotypes				MAF	p	OR (95% CI)
IL23R SNPs	1	2	11	12	22	12+22		11	12	22	12+22			
rs1004819	G	A	179 (46.7)	168 (43.9)	36 (9.39)	204 (53.3)	0.31	124 (49.0)	105 (41.5)	24 (9.49)	129 (51.0)	0.30	0.437	1.14 (0.82-1.57)
rs11805303	C	T	167 (43.6)	180 (47.0)	36 (9.39)	216 (56.4)	0.33	127 (50.2)	103 (40.7)	23 (9.09)	126 (49.8)	0.29	0.067	1.35 (0.98-1.87)
rs7517847	T	G	125 (32.6)	176 (46.0)	82 (21.4)	258 (67.4)	0.44	73 (28.9)	139 (54.9)	41 (16.2)	180 (71.1)	0.44	0.260	0.82 (0.58-1.16)
rs7530511	C	T	291 (76.0)	89 (23.2)	3 (0.78)	92 (24.0)	0.12	183 (72.3)	65 (25.7)	5 (1.98)	75 (27.7)	0.15	0.231	0.80 (0.56-1.15)
rs10489629	A	G	104 (27.2)	193 (50.4)	86 (22.5)	279 (72.1)	0.48	80 (31.6)	128 (50.6)	45 (17.8)	173 (68.4)	0.43	0.276	1.22 (0.86-1.72)
rs2201841	T	C	171 (44.6)	179 (46.7)	33 (8.62)	212 (55.4)	0.32	123 (48.6)	114 (45.1)	16 (6.32)	140 (51.4)	0.29	0.225	1.22 (0.89-1.68)
rs11209026	G	A	346 (90.3)	36 (9.40)	1 (0.26)	37 (9.43)	0.05	226 (89.3)	27 (10.7)	0	27 (10.7)	0.05	0.606	0.87 (0.51-1.47)
rs10889677	C	A	168 (43.9)	183 (47.8)	32 (8.36)	215 (56.1)	0.32	117 (46.2)	121 (47.8)	15 (5.93)	136 (53.8)	0.30	0.423	1.14 (0.83-1.57)
rs11209032	G	A	144 (37.6)	198 (51.7)	41 (10.7)	239 (62.4)	0.37	109 (43.1)	120 (47.4)	24 (9.49)	144 (56.9)	0.33	0.154	1.27 (0.92-1.75)

P value for presence of minor allele (heterozygous plus homozygous subjects together in both groups)

Table 5. **Genotype distribution and minor allele frequency (MAF) of the examined IL23R variants in the groups of patients with ankylosing spondylitis and healthy controls.**

			AS patients (n=206) B27+ AS patients (n=153)					Controls (n=235)						
	Alleles		Genotypes				MAF	Genotypes				MAF	p	OR (95% CI)
IL23R SNPs	1	2	11	12	22	12+22		11	12	22	12+22			
rs1004819	G	A	74 (35.9) 57 (37.2)	110 (53.4) 80 (52.3)	22 (10.7) 16 (10.5)	132 (64.1)* 96 (62.8)*	0.37* 0.37*	121 (51.5)	95 (40.4)	19 (8.09)	114 (48.5)	0.28	0.003 [a] 0.011 [a]	1.80 (1.22-2.67) 1.77 (1.14-2.66)
rs11805303	C	T	84 (40.8) 65 (42.5)	97 (47.1) 71 (46.4)	25 (12.1) 17 (11.1)	122 (59.2)* 88 (57.5)*	0.36* 0.34*	124 (52.8)	93 (39.6)	18 (7.66)	111 (47.2)	0.27	0.017 [a] 0.040 [a]	1.60 (1.09-2.36) 1.55 (1.02-2.36)
rs7517847	T	G	67 (32.5) 48 (31.3)	115 (55.8) 85 (55.6)	24 (11.7) 20 (13.1)	139 (67.5) 105 (68.7)	0.40 0.41	69 (29.4)	126 (53.6)	40 (17.0)	166 (70.6)	0.44	0.556 [a] 0.692 [a]	0.88 (0.58-1.34) 0.91 (0.58-1.43)
rs7530511	C	T	160 (77.7) 117 (76.5)	42 (20.4) 32 (20.9)	4 (1.94) 4 (2.61)	46 (22.3) 36 (23.5)	0.12 0.13	166 (70.6)	64 (27.2)	5 (2.13)	69 (29.4)	0.16	0.095 [a] 0.259 [a]	0.69 (0.44-1.07) 0.76 (0.47-1.22)
rs10489629	A	G	61 (29.6) 49 (32.0)	105 (51.0) 75 (49.0)	40 (19.4) 29 (19.0)	145 (70.4) 104 (68.0)	0.45 0.43	76 (32.3)	117 (49.8)	42 (17.9)	159 (67.7)	0.43	0.568 [a] 0.997 [a]	1.13 (0.75-1.71) 1.00 (0.64-1.56)
rs2201841	T	C	91 (44.2) 68 (44.4)	89 (43.2) 65 (42.5)	26 (12.6)* 20 (13.1)*	115 (55.8) 85 (55.6)	0.34 0.34	118 (50.2)	102 (43.4)	15 (6.38)	117 (49.8)	0.28	0.030 [b] 0.040 [b]	2.12 (1.08-4.15) 2.12 (1.4-4.32)
rs11209026	G	A	195 (94.7) 143 (93.5)	11 (5.34) 10 (6.54)	0 0	11 (5.34)* 10 (6.54)	0.03* 0.03	208 (88.5)	27 (11.5)	0	27 (11.5)	0.06	0.031 [a] 0.113 [a]	0.44 (0.21-0.93) 0.54 (0.25-1.16)
rs10889677	C	A	88 (42.7) 65 (42.5)	94 (45.6) 68 (44.4)	24 (11.7)* 20 (13.1)*	118 (57.3) 88 (57.5)	0.34 0.35*	114 (48.5)	108 (46.0)	13 (5.53)	121 (51.5)	0.29	0.016 [b] 0.009 [b]	2.42 (1.18-4.96) 2.70 (1.28-5.69)
rs11209032	G	A	79 (38.3) 60 (39.2)	108 (52.4) 79 (51.6)	19 (9.22) 14 (9.15)	127 (61.7) 93 (60.8)	0.35 0.35	106 (45.1)	110 (46.8)	19 (8.09)	129 (56.9)	0.31	0.224 [a] 0.300 [a]	1.27 (0.86-1.88) 1.25 (0.82-1.91)

[*] $p<0.05$; [a] *P* value for presence of minor allele (heterozygous plus homozygous subjects together in both groups) , [b] *P* value for homozygousity of minor allele

Table 6. **IL23R haplotypes associated with AS.**

	GGCATCG			AGCACAA			ATCACAG			ATCACAA		
	p value	OR	95% CI	p value	OR	95% CI	p value	OR	95% CI	p value	OR	95% CI
AS patients	0.033*	0.33	0.12-0.92	0.023*	0.09	0.01-0.72	0.027*	2.51	1.11-5.69	0.039*	1.43	1.02-2.00
$B27^+$ AS patients	0.042*	0.21	0.05-0.95	0.063	0.14	0.02-1.11	0.082	1.39	0.96-2.02	0.011*	2.96	1.28-6.83

* $p < 0.05$

Table 7. **Genotype distribution and minor allele frequency (MAF) of the examined IL23R variants in the groups of patients with Sjögren syndrome and healthy controls**

			Sjögren syndrome patients (n=156) **primary Sjögren syndrome patients (n=141)**					**Controls (n=235)**						
	Alleles		Genotypes				MAF	Genotypes				MAF	p	OR (95% CI)
IL23R SNPs	1	2	11	12	22	12+22		11	12	22	12+22			
rs1004819	G	A	75 (48.1) 66 (46.8)	68 (43.6) 62 (44.0)	13 (8.33) 13 (9.22)	81 (51.9) 75 (53.2)	0.30 0.31	121 (51.5)	95 (40.4)	19 (8.09)	114 (48.5)	0.28	0.344 0.470	1.26 (0.78-2.06) 1.20 (0.73-1.99)
rs11805303	C	T	71 (45.5) 62 (44.0)	73 (46.8) 67 (47.5)	12 (7.69) 12 (8.51)	85 (54.5) 79 (56.0)	0.31 0.32	124 (52.8)	93 (39.6)	18 (7.66)	111 (47.2)	0.27	0.087 0.127	1.53 (0.94-2.50) 1.48 (0.89-2.46)
rs7517847	T	G	46 (29.5) 44 (31.2)	86 (55.1) 74 (52.5)	24 (15.4) 23 (16.3)	110 (70.5) 97 (68.8)	0.43 0.43	69 (29.4)	126 (53.6)	40 (17.0)	166 (70.6)	0.44	0.663 0.321	0.89 (0.53-1.51) 0.76 (0.44-1.31)
rs7530511	C	T	115 (73.7) 104 (73.8)	35 (22.4) 32 (22.7)	6 (3.85) 5 (3.55)	41 (26.3) 37 (26.2)	0.15 0.15	166 (70.6)	64 (27.2)	5 (2.13)	69 (29.4)	0.16	0.166 0.274	0.67 (0.39-1.18) 0.73 (0.41-1.29)
rs10489629	A	G	51 (32.7) 48 (34.0)	78 (50.0) 68 (48.2)	27 (17.3) 25 (17.7)	105 (67.3) 93 (65.9)	0.42 0.42	76 (32.3)	117 (49.8)	42 (17.9)	159 (67.7)	0.43	0.638 0.533	0.88 (0.53-1.48) 0.85 (0.50-1.44)
rs2201841	T	C	71 (45.5) 62 (44.0)	71 (45.5) 66 (46.8)	14 (8.97) 13 (9.22)	85 (54.5) 79 (56.0)	0.32 0.33	118 (50.2)	102 (43.4)	15 (6.38)	117 (49.8)	0.28	0.193 0.258	1.38 (0.85-2.25) 1.34 (0.81-2.22)
rs11209026	G	A	137 (87.8) 123 (87.2)	19 (12.2) 18 (12.8)	0 0	19 (12.2) 18 (12.8)	0.06 0.06	208 (88.5)	27 (11.5)	0	27 (11.5)	0.06	0.634 0.666	0.83 (0.39-1.78) 0.84 (0.39-1.84)
rs10889677	C	A	71 (45.5) 62 (44.0)	68 (43.6) 64 (45.4)	17 (10.9) 15 (10.6)	85 (54.5) 79 (56.0)	0.33 0.33	114 (48.5)	108 (46.0)	13 (5.53)	121 (51.5)	0.29	0.526 0.645	1.17 (0.72-1.90) 1.13 (0.68-1.86)
rs11209032	G	A	74 (47.4) 65 (46.1)	69 (44.2) 63 (44.7)	13 (8.33) 13 (9.22)	82 (52.6) 76 (53.9)	0.30 0.32	106 (45.1)	110 (46.8)	19 (8.09)	129 (56.9)	0.31	0.870 0.991	1.04 (0.64-1.69) 1.00 (0.60-1.65)

P value for presence of minor allele (heterozygous plus homozygous subjects together in both groups)

Table 8. **Genotype distribution and minor allele frequency (MAF) of the examined IL23R variants in the groups of patients with IgAN and healthy controls**

			IgAN patients (n=143)					Controls (n=189)						
	Alleles		Genotypes				MAF	Genotypes				MAF	p	OR (95% CI)
IL23R SNPs	1	2	11	12	22	12+22		11	12	22	12+22			
rs1004819	G	A	83 (58.0)	46 (32.2)	14 (9.79)	60 (42.0)	0.26	103 (54.5)	73 (38.6)	13 (6.88)	86 (45.5)	0.26	0.554	0.88 (0.56-1.36)
rs11805303	C	T	80 (55.9)	49 (34.3)	14 (9.79)	63 (44.1)	0.27	107 (56.6)	69 (36.5)	13 (6.88)	82 (43.4)	0.25	0.924	1.02 (0.66-1.59)
rs7517847	T	G	53 (37.0)	66 (46.2)	24 (16.8)	90 (63.0)	0.40	55 (29.1)	101 (53.4)	33 (17.5)	134 (70.9)	0.44	0.102	0.68 (0.42-1.08)
rs7530511	C	T	112 (78.3)	29 (20.3)	2 (1.40)	31 (21.7)	0.12	140 (74.1)	45 (23.8)	4 (2.12)	47 (25.9)	0.13	0.474	0.83 (0.49-1.39)
rs10489629	A	G	43 (30.0)	70 (49.0)	30 (21.0)	100 (70.0)	0.45	54 (28.6)	96 (50.8)	39 (20.6)	135 (71.4)	0.43	0.601	0.88 (0.54-1.43)
rs2201841	T	C	78 (54.5)	53 (37.1)	12 (8.39)	65 (45.5)	0.27	101 (53.4)	79 (41.8)	9 (4.76)	88 (46.6)	0.26	0.839	0.96 (0.62-1.49)
rs11209026	G	A	124 (86.7)	19 (13.3)	0	19 (13.3)	0.07	167 (88.4)	22 (11.6)	0	22 (11.6)	0.06	0.866	1.10 (0.54-2.07)
rs10889677	C	A	79 (55.2)	51 (35.7)	13 (9.09)	64 (44.8)	0.27	99 (52.4)	83 (43.9)	7 (3.70)	90 (47.6)	0.26	0.551	0.87 (0.56-1.36)
rs11209032	G	A	80 (55.9)	50 (35.0)	13 (9.09)	63 (44.1)	0.27	90 (47.6)	84 (44.4)	15 (7.94)	99 (52.3)	0.30	0.134	0.71 (0.46-1.11)

P value for presence of minor allele (heterozygous plus homozygous subjects together in both groups)

Table 9. **Genotype distribution and minor allele frequency (MAF) of the examined IL23R variants in the groups of patients with systemic sclerosis and healthy controls.**

			Systemic sclerosis patients (n=233)					Controls (n=220)						
	Alleles		Genotypes				MAF	Genotypes				MAF	p	OR (95% CI)
IL23R SNPs	1	2	11	12	22	12+22		11	12	22	12+22			
rs1884444	G	T	56 (24.0)	170 (73.0)	7 (3.00)	177 (76.0)	0.39	55 (25.0)	162 (73.6)	3 (1.36)	165 (75.0)	0.38	0.895	1.04 (0.60-1.79)
rs1004819	G	A	119 (51.1)	99 (42.5)	15 (6.44)	114 (48.9)	0.28	108 (49.1)	90 (40.9)	22 (10.0)	112 (50.9)	0.30	0.698	1.10 (0.68-1.76)
rs11805303	C	T	112 (48.1)	107 (45.9)	14 (6.01)	121 (51.9)	0.29	110 (50.0)	88 (40.0)	22 (10.0)	110 (50.0)	0.30	0.298	1.29 (0.80-2.07)
rs7517847	T	G	68 (29.2)	115 (49.3)	50 (21.5)	165 (70.8)	0.46	70 (31.8)	118 (53.6)	32 (14.5)	150 (68.1)	0.41	0.598	1.15 (0.69-191)
rs7530511	C	T	167 (71.7)	63 (27.0)	3 (1.29)	66 (28.3)	0.15	157 (71.4)	58 (26.4)	5 (2.27)	63 (28.6)	0.15	0.798	1.07 (0.64.1.80)
rs10489629	A	G	68 (29.2)	106 (45.5)	59 (25.3)	165 (70.8)	0.48	71 (32.3)	111 (50.4)	38 (17.3)	149 (67.7)	0.43	0.973	1.00 (0.61-1.68)
rs2201841	T	C	120 (51.5)	101 (43.3)	12 (5.15)	113 (48.5)	0.27	108 (49.1)	98 (44.5)	14 (6.36)	112 (50.9)	0.29	0.942	0.98 (0.61-1.58)
rs11209026	G	A	196 (84.1)	35 (15.0)	2 (0.86)	37 (15.9)	0.08	194 (88.2)	26 (11.8)	0	26 (11.8)	0.06	0.774	0.90 (0.45-1.81)
rs10889677	C	A	116 (49.8)	109 (46.8)	8 (3.43)	117 (50.2)	0.27	101 (45.9)	106 (48.2)	13 (5.91)	119 (54.1)	0.30	0.472	0.84 (0.52-1.35)
rs11209032	G	A	121 (51.9)	94 (40.3)	18 (7.73)	112 (48.1)	0.29	94 (42.7)	105 (47.7)	21 (9.55)	126 (57.3)	0.33	0.160	0.71 (0.44-1.14)

P value for presence of minor allele (heterozygous plus homozygous subjects together in both groups)