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Genetic variations in the IL-12B gene in association with IL-23 and IL-12p40 serum levels in ankylosing spondylitis

Mariana Ivanova[1] · Irena Manolova[2] · Lyuba Miteva[2] · Rada Gancheva[1] · Rumen Stoilov[1] · Spaska Stanilova[2]

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Abstract

In the present study, we evaluated the implication of *IL12Bpro* (rs17860508) and *IL12B* 3′ UTR A/C single nucleotide polymorphisms (SNPs) (rs3212227) for the ankylosing spondylitis (AS) development and the impact of *IL12B* genetic variations on IL-23 and IL-12p40 production and musculoskeletal disease characteristics. 80 patients with AS and 242 healthy controls were studied. Genotyping for the rs3212227 was performed by restriction fragment length polymorphisms–polymerase chain reaction (PCR) and for the rs17860508 by allele-specific PCR. Cytokines were measured by an enzyme-linked immunosorbent assay (ELISA). Clinical status was evaluated by calculation of the Ankylosing Spondylitis Disease Activity Score (ASDAS) using the C-reactive protein (CRP) level, the Bath Ankylosing Spondylitis Functional Index (BASFI) and the Bath Ankylosing Spondylitis Metrology Index (BASMI). An association was found for the rs17860508 polymorphism with AS under the allelic, the dominant, and the co-dominant models. Rs3212227 was not attributable to AS susceptibility by itself, but the carriage of C allele in the genotype amplifies the genetic risk for AS in the carriers of the high-risk *IL12Bpro* 2-allele, especially in homozygosity. Circulating IL-23 and IL-12p40 were raised among AS patients, as some of the genotypes of both *IL12B* polymorphisms positively regulate their expression. Carriage of the *IL12Bpro* genotype 2.2 has been linked to a worsened functional disability, while 3′ UTR CC genotype—with severe disease activity. *IL12B* polymorphisms can impact AS susceptibility and modulate IL-23 and IL-12p40 production levels, and have a contribution to the disease phenotype.

Keywords Ankylosing spondylitis · Genetic predisposition to disease · Cytokines

Introduction

Ankylosing spondylitis (AS) is an immune-mediated rheumatic disease belonging to the spectrum of axial spondyloarthritidis (axSpA), characterized mainly by chronic inflammatory back pain (axial inflammation) and radiographic sacroiliitis, but in which extra-spinal and extra-articular manifestations can also be presented. It has long been known that the disease is strongly associated with the specific genetic marker—human leukocyte antigen (HLA)-B27 [1, 2], as up to 94% of the affected individuals express this major histocompatibility complex (MHC) class I molecule [3]. Despite the undoubted contribution of HLA-B27 as a major susceptibility gene for AS, some published estimates indicate that it may only account for approximately 20–50% of overall genetic susceptibility to disease [4, 5]. Therefore, identification of other candidate genes related to AS is still a challenge. In recent years, genome-wide association studies identified polymorphisms in genes linked to interleukin-17 (IL-17)-mediated immune responses, including IL-1 receptor (IL-1R) and IL-23 receptor (IL-23R) with the condition [6–8], and also disease association with the endoplasmic reticulum aminopeptidase-1 (ERAP1) (an enzyme that trims peptides for antigen presentation by HLA class 1 molecules) genes [8, 9]. Genetic predisposition in close interaction with

Mariana Ivanova and Irena Manolova contributed equally to this work.

✉ Mariana Ivanova
mariana_ig@abv.bg

✉ Irena Manolova
imanolova@mf.uni-sz.bg

[1] Clinic of Rheumatology, Medical Faculty, University Hospital "St. Ivan Rilski", Medical University, 13, Urvich St, 1612 Sofia, Bulgaria

[2] Department of Molecular Biology, Immunology and Medical Genetics, Medical Faculty, Trakia University, 11, Armeiska St, 6003 Stara Zagora, Bulgaria

certain environmental factors (microbiomes, infections and drug and toxin exposure) seems to be the major factor playing a role in the disease pathogenesis [10].

Although the precise etiology of AS is not yet fully understood, the high prevalence of HLA-B27 carriage and predominant involvement of pro-inflammatory cytokines suggest a substantial role of the immunogenetics. Cytokine production and alterations in cytokine levels exert a pivotal role in the immune-mediated inflammatory disorders, including AS. Recent immunological studies revealed the substantial relative importance of the T-helper 17 (Th17) responses and the interleukin (IL)-23/IL-17 axis as key effectors in the autoimmunity, unlocking the pathogenesis of the disease [9]. Th17 responses induce IL-17 production, but for the Th17 cells pathogenicity IL-23 has a crucial role, acting on their maintenance, survival and stabilization of Th-17 cytokine signature expression [11]. IL-23 is a member of the IL-12 family, as both cytokines are heterodymeric proteins which share a common subunit p40 encoded by *IL12B*, localized in the long arm of the fifth chromosome (5q31.1-5q33.1). IL-12p70 is a central Th1 response cytokine, as it plays a key role in initial naïve T-cell (Th0) fate decision and thus skews the differentiation of Th0 into interferon-gamma-producing Th1 cells, recognized as responsible for the development of a variety of inflammatory diseases in earlier studies [12, 13].

Genetic variations within cytokine genes directly or indirectly involved in Th17/23 pathway are of interest, since genetic factors have been shown to be major determinants of susceptibility to the disease and contribute to amplification of immune responses. In this regard, the aim of this study was to determine whether genetic polymorphisms of the *IL-12B* gene are associated with AS in a Bulgarian population by genotyping of A/C single nucleotide polymorphisms (SNPs) in the 3′ untranslated region (UTR) (rs3212227) of the *IL12B* and *IL12Bpro* (rs17860508), a complex polymorphism in the promoter region of this gene, resulting from 4 bp microinsertion combined with AA/GC transition. Also, the variations of circulating IL-23 and IL-12p40, and musculoskeletal disease characteristics in relation to *IL-12B* polymorphisms were assessed.

Patients and methods

Study subjects

Study population consisted of 80 patients with definite AS fulfilling the modified New York criteria [14] and 242 age-, sex-, and ethnicity-matched (Caucasian race) healthy individuals. The verification of the diagnosis and the clinical evaluation of AS subject were accomplished in the inpatient and outpatient rheumatology clinics of University Hospital "St. Ivan Rilski", Sofia. A written informed consent has been obtained from each participant in accordance with the Declaration of Helsinki and the study has been approved by local Ethics Committee of the institution (University Hospital "St. Ivan Rilski", decision number 6, 29. Nov. 2016). Genetic and cytokine analyses were carried out in the Department of Molecular Biology, Immunology and Medical Genetics, Medical Faculty, Trakia University, Stara Zagora.

Clinical evaluation

Clinical assessment in AS patients included collecting demographic data and obtaining information regarding musculoskeletal manifestations, disease activity, functional ability and spinal mobility parameters, therapy for AS. Peripheral arthritis (40%) was defined as the presence of swelling in at least one peripheral joint. Since the enthesitis and dactylitis were manifested in a small number of patients, they were not included in the analyses. Disease activity state was evaluated by calculation of the index, showing better qualities—the Ankylosing Spondylitis Disease Activity Score (ASDAS) using the C-reactive protein (CRP), according to the standard formula [15, 16] via online calculator (https://www.asas-group.org) and was interpreted as inactive disease–low disease activity ASDAS-CRP < 2.1 or high–very high disease activity ASDAS-CRP ≥ 2.1 [17]. Physical function and axial status in AS were measured by the Bath Ankylosing Spondylitis Functional Index (BASFI) [18] and the Bath Ankylosing Spondylitis Metrology Index (BASMI) [19]. We defined two levels for the BASFI. A score below (<) 4 was assumed to mean mild functional impairment, while a score above (≥) 4, that there was a severe functional disability.

DNA extraction and genotyping

DNA extraction and genotyping for *IL12Bpro* (rs17860508) and *IL12B* 3′ UTR + 16974 A/C (rs3212227) were performed as described previously [20].

Serum IL-12p40 and IL-23 concentrations' measurement

Serum samples were stored at − 20 °C until assayed. The quantitative determination of both pro-inflammatory cytokines was performed by commercially available enzyme-linked immunosorbent assay (ELISA) kits (Invitrogen Corporation, Camarillo, CA 93012 for IL-12p40; Invitrogen Corporation, Frederick, MD 21704, USA for IL-23) according to the manufacturer's instructions. Serum concentration was determined using a standard curve constructed with kit's standards and expressed in picograms per ml (pg/ml). The minimum detectable concentration of IL-12p40 was less than 2.0 pg/ml and of the IL-23 ELISA kit—less

than 20 pg/ml. Serum samples of cases and controls were analyzed together in the same analytic batch.

Statistical analysis

Statistical analyses were performed using the Statistics Package for the Social Sciences (SPSS) version 16.0 (SPSS Inc., Chicago, IL). The differences in genotype distribution and allele frequency among cases and controls were analyzed using the Chi-squared test for contingency tables. When 2×2 tables contain small observed frequencies, Fisher's exact test p value was taken into account. StatPages.net website (http://statpages.org/index.html) was used to estimate odds ratios (OR), expressed with their 95% confidence intervals (95% CI) for disease susceptibility and severity in relation to *IL12B* polymorphisms. The goodness of fit to Hardy–Weinberg equilibrium, calculating the expected frequencies of each genotype and comparing them with the observed values for patients and healthy controls (HC), was performed using a χ^2 test. Comparison of mean concentrations of IL-12p40 and IL-23 with regard to *IL12B* gene polymorphisms between patients with AS and HC was done by the Kruskal–Wallis and the Mann–Whitney U tests. Two-tailed p values < 0.05 were considered statistically significant.

Results

Cohort description

In total, 80 AS patients (59 men and 21 women) aged 22–61 years with a mean (± SD) age of 38.1 ± 9.2 years were studied. 91% were HLA-B27 positive. The mean disease duration was 11.2 ± 7.4 years with a range from 0.6 to 36. The mean age at AS onset was 26.9 ± 8.0 years. 32 of all patients (40.0%) had a history of peripheral arthritis at the time point of the data collection. In terms of drug usage, 32 patients (40%) were only on first-line therapy, a nonsteroidal anti-inflammatory drug (NSAID), 14 (17.5%) were receiving a conventional synthetic disease-modifying antirheumatic drug (csDMARD) (sulfasalazine or methotrexate) and 34 (42.5%) were being treated with a tumor necrosis factor (TNF) inhibitor. The comparison was done with 242 healthy controls, 153 men and 89 women, age ranging from 18 to 74 years, mean age 39.0 ± 14.0 years.

IL12B polymorphisms and susceptibility to AS

Genotype distribution of the rs17860508 and rs3212227 was in agreement with Hardy–Weinberg equilibrium (HWE) among controls ($\chi^2 = 3.33$, $p = 0.188$; $\chi^2 = 1.025$; $p = 0.599$, respectively) and cases ($\chi^2 = 1.846$, $p = 0.397$; $\chi^2 = 0.197$; $p = 0.906$, respectively). Genotype and allele frequencies of *IL12Bpro* (rs17860508) and *IL12B* 3′ UTR (rs3212227) are displayed in Table 1.

We observed significant differences in the genotype distribution ($\chi^2 = 6.571$; $df = 2$; $p = 0.024$) and allele frequencies ($\chi^2 = 4.986$; $df = 2$; $p = 0.011$) of the rs17860508 polymorphism between the two sample cohorts. A statistically significant association was found for the rs17860508 polymorphism with AS under the allelic model (allele 2 vs. allele 1; OR = 1.595; 95% CI 1.095–2.232, $p = 0.011$), under the dominant model (1.2 + 2.2 vs. 1.1; OR = 2.695; 95% CI 1.349–5.448, $p = 0.002$), and the co-dominant model (2.2 vs. 1.1; OR = 2.601; 95% CI 1.041–6.01, $p = 0.013$; 1.2 vs. 1.1; OR = 2.736; 95% CI 1.324–5.736, $p = 0.003$). The heterozygous genotype 1.2 predominated in AS cases, occurring with frequency (56.3%), than in controls (43.4%), while homozygosity of the allele 1 (1.1) was less frequent in AS (16.3%) than in HC (34.3%). We also found that the homozygous genotype 2.2 was in a higher frequency in AS cases (27.5%) compared to healthy controls (22.3%), suggesting that the presence of allele 2 might increase the AS risk. With respect to genotype and allele frequencies of rs3212227 polymorphism of the *IL12B*, a similarity was observed in the patients and HC groups (Table 1).

Beyond the individual contribution, we also investigated the possible effect of combined genotypes of rs17860508 and rs3212227 on the susceptibility to AS in a Bulgarian population by calculating the ORs (Table 2). We choose for a reference group the individuals with the combination of the *IL12Bpro* 1.1 + *IL12B* 3′ UTR AA genotypes. The presence of the 2.2 genotype of *IL12Bpro* simultaneous with both AC and AC + CC genotypes of the *IL12B* 3′ UTR A/C polymorphism significantly increased the risk of developing AS, which was estimated to be 13.125 (95% CI 2.039–98.112; $p = 0.004$) for *IL12Bpro* 2.2 + *IL12B* 3′ UTR AC and 12.25 (95% CI 2.122–80.144; $p = 0.003$) for *IL12Bpro* 2.2 + *IL12B* 3′ UTR AC/CC compared to the reference genotype (*IL12Bpro* 1.1 + *IL12B* 3′ UTR AA). The heterozygosity of the allele 2 (1.2) of *IL12Bpro* in combination with AC and AC + CC genotypes of the *IL12B* 3′ UTR polymorphic variability conferred the AS susceptibility risk to be fourfold only (OR = 4.083, 95% CI 1.173–15.552, $p = 0.019$; OR = 3.946, 95% CI 1.152–14.833, $p = 0.021$, respectively).

Serum levels of IL-12p40 and IL-23 in AS patients and HC in relation to *IL12B* polymorphisms

We evaluated the possible influence of the genetic variants in these loci on the cytokine production. Serum levels of IL-12p40 and IL-23 in relation to various genotypes of rs17860508 and rs3212227 in 80 AS patients and 75 sex- and age- matched healthy controls are presented in Tables 3

Table 1 Allele and genotype frequencies of *IL12Bpro* (rs17860508) and *IL12B* A/C 3′ UTR (rs3212227) SNPs in AS patients and controls

Locus	Genotype	AS, $n=80$ (%)	Controls, $n=242$ (%)	OR (95% CI)	p value
IL12Bpro (rs17860508)					
Co-dominant model	1.1	13 (16.3)	83 (34.3)	Ref.	
	1.2	45 (56.3)	105 (43.4)	**2.736 (1.324–5.7361)**	**0.003**
	2.2	22 (27.5)	54 (22.3)	**2.601 (1.041–6.013)**	**0.013**
Dominant model	1.1	13 (16.3)	83 (34.3)	Ref.	
	1.2 + 2.2	67 (83.7)	159 (65.7)	**2.69 (1.349–5.448)**	**0.002**
Recessive model	1.2 + 1.1	58 (72.5)	188 (77.7)	Ref.	
	2.2	22 (27.5)	54 (22.3)	1.321 (0.712–2.439)	0.344
Over-dominant model	1.1 + 2.2	35 (43.7)	137 (56.6)	Ref.	
	1.2	45 (56.3)	105 (43.4)	**1.678 (0.977–2.884)**	**0.046**
Allelic model	Allele				
	1	71 (44.4)	271 (56)	Ref.	
	2	89 (55.6)	213 (44)	**1.595 (1.095–2.232)**	**0.011**
IL12B A/C SNP-3′ UTR (rs3212227)					
Co-dominant model	AA	41 (51.2)	138 (57.0)	Ref.	
	AC	32 (40.0)	85 (35.1)	1.267 (0.716–2.24)	0.386
	CC	7 (8.8)	19 (7.9)	1.24 (0.438–3.406)	0.651
Dominant model	AA	41 (51.2)	138 (57.0)	Ref.	
	AC + CC	39 (48.8)	104 (43.1)	1.262 (0.737–2.162)	0.436
Recessive model	AA + AC	73 (91.2)	223 (92.1)	Ref.	
	CC	7 (8.8)	19 (7.9)	1.125 (0.411–2.978)	0.798
Over-dominant model	AA + CC	48 (58.2)	157 (64.9)	Ref.	
	AC	32 (40.0)	85 (35.1)	1.231 (0.709–2.137)	0.432
Allelic model	Allele				
	A	114 (71.3)	361 (74.6)	Ref.	
	C	46 (28.8)	123 (25.4)	1.184 (0.779–1.798)	0.406

Bold values show the presence of statistical significance

AS ankylosing spondylitis, *OR* odds ratios, *CI* confidence intervals

Table 2 Combined effect of the *IL12B* rs17860508 and rs3212227genotypes on the AS susceptibility

IL12B pro	*IL12B* 3′ UTR A/C	Cases/controls	OR (CI 95%)	p value*
1.1	AA	4/35	**Ref**.	
1.1	AC	5/36	1.215 (0.254–5.983)	1.0
1.1	CC	4/12	2.917 (0.503–17.199)	0.322
1.1	AC + CC	9/48	1.641 (0.413–6.96)	0.645
1.2	AA	22/54	**3.565 (1.038–13.419)**	**0.036**
1.2	AC	21/45	**4.083 (1.173–15.552)**	**0.019**
1.2	CC	2/6	2.917 (0.29–26.707)	0.534
1.2	AC + CC	23/51	**3.946 (1.152–14.833)**	**0.021**
2.2	AA	15/49	2.679 (0.741–10.522)	0.153
2.2	AC	6/4	**13.125 (2.039–98.112)**	**0.004**
2.2	CC	1/1	8.75 (0.191–433.708)	0.463
2.2	AC + CC	7/5	**12.25 (2.122–80.144)**	**0.003**

Bold values show the presence of statistical significance

OR odds ratios, *CI* confidence intervals

*Fisher's exact test

Table 3 Serum levels of IL-12p40 in AS patients and healthy controls in relation to *IL12B* gene polymorphisms

	AS ($n=80$) Mean ± SD	Controls ($n=75$) Mean ± SD	p value*
Total IL-12p40 (pg/ml)	113.4 ± 81.5	74.0 ± 42.6	0.004
rs17860508 genotype			
1.1	153.4 ± 85.2	78.7 ± 40.1	0.01
1.2	114.4 ± 80.7	75.3 ± 41.0	0.02
2.2	83.3 ± 67.2	64.2 ± 26.2	n.s.
p value within the group**	0.012	n.s.	
rs3212227 genotype			
AA	103.8 ± 83.5	84.9 ± 48.6	n.s.
AC	119.6 ± 78.7	64.0 ± 27.3	0.002
CC	131.1 ± 73.2	92.3 ± 49.6	n.s.
p value within the group**	n.s.	n.s.	

*Mann–Whitney U test

**Kruskal–Wallis test

Table 4 Serum levels of IL-23 in AS patients and healthy controls in relation to *IL12B* gene polymorphisms

	AS ($n=80$) Mean ± SD	Controls ($n=75$) Mean ± SD	p value*
Total IL-23 (pg/ml)	23.6 ± 19.3	11.9 ± 10.2	< 0.001
rs17860508 genotype			
1.1	26.2 ± 15.2	8.4 ± 6.7	< 0.001
1.2	20.0 ± 17.0	10.9 ± 8.7	0.016
2.2	30.0 ± 25.5	21.1 ± 15.0	n.s.
p value within the group**	0.056	0.067	
rs3212227 genotype			
AA	26.2 ± 22.4	14.5 ± 12.2	0.032
AC	20.9 ± 16.6	10.9 ± 8.2	0.008
CC	23.2 ± 14.1	8.6 ± 7.4	0.06
p value within the group**	n.s	n.s.	

*Mann–Whitney U test

**Kruskal–Wallis test

and 4, respectively. The total (mean ± SD) levels of IL-12p40 and IL-23 were greatly increased in AS patients than in controls (113.4 ± 81.5 pg/ml vs. 74.02 ± 42.6 pg/ml, $p=0.004$; 23.6 ± 19.3 pg/ml vs. 11.9 ± 10.2, $p<0.001$; respectively).

After stratification of cytokine levels based on the genotypes, inter-group comparisons showed that the homozygous 1.1 and the heterozygous genotype 1.2 were associated with elevated serum concentrations of both IL-12p40 and IL-23 in AS patients as opposed to HC. With regard to homozygous genotype 2.2, no significant difference in serum levels of both cytokines was detected between patients and controls of the entire cohort (Tables 3, 4; Fig. 1a, c). Conversely, the male–female comparisons revealed that in males with AS the *IL12Bpro* 2.2 genotype was associated with raised IL-23 levels compared with healthy men (32.7 ± 28.9 pg/ml vs. 12.1 ± 4.3 pg/ml, $p=0.023$) in contrast to the women with the same genotype (24.5 ± 17.4 pg/ml vs. 21.1 ± 16.3 pg/ml; $p=0.651$).

Concerning polymorphic variability of *IL12B* 3′ UTR, only AC genotype in AS has been linked to a higher IL-12p40 production, while IL-23 was higher in AS than in HC regardless of the genotype as shown in Tables 3, 4 and Fig. 1b, d.

Overall, within each of both control and patient groups, no significant differences in the circulating cytokines regarding the genotypes were found, with the exception of serum concentrations of IL-12p40 in AS stratified on the basis of rs17860508 (Tables 3, 4; Fig. 1a–d).

Associations of *IL12B* polymorphisms with disease characteristics

As shown in Table 5, carriage of the *IL12Bpro* genotype 2.2 has been linked to a significantly higher BASFI and BASMI scores than 1.1 + 1.2 genotype, as the risk of having worsened functional capacity (BASFI > 4) in these patients was calculated to be at approximately 2.5-fold higher (OR = 2.637; 95% CI 0.842–8.479, $p=0.062$). AS patients carrying 3′ UTR CC genotype demonstrated a trend for higher mean values of ASDAS-CRP, indicating the most sever state of disease activity than those carrying allele A in the genotype (AA + AC) (3.9 ± 0.7 vs. 3.1 ± 1.3; $p=0.076$). Although not statistically significant, patients carrying 3′ UTR CC genotype were associated with earlier age at the onset of AS. No association of *IL12B* polymorphisms with the peripheral joints involvement was identified (data not shown).

Discussion

In this study, we examined the association between two *IL12B* polymorphisms, ankylosing spondylitis and serum levels of IL-23 and IL-12p40. One of them was the bi-allelic promoter polymorphism (*IL12Bpro*) located at -2703 bp of the transcription initiation site (rs17860508) and the second one, the A → C base pair substitution at position + 16974 in the 3′ UTR (rs3212227). The *IL12B* gene encodes the p40 polypeptide chain, which, together with p35 composes IL-12p70. *IL12B* polymorphisms are thought to influence also the production of the key Th17-promoting cytokine IL-23 which, in addition to the p40 subunit, also has a unique p19 chain [21]. By allowing differential production of cytokines, these polymorphisms may confer

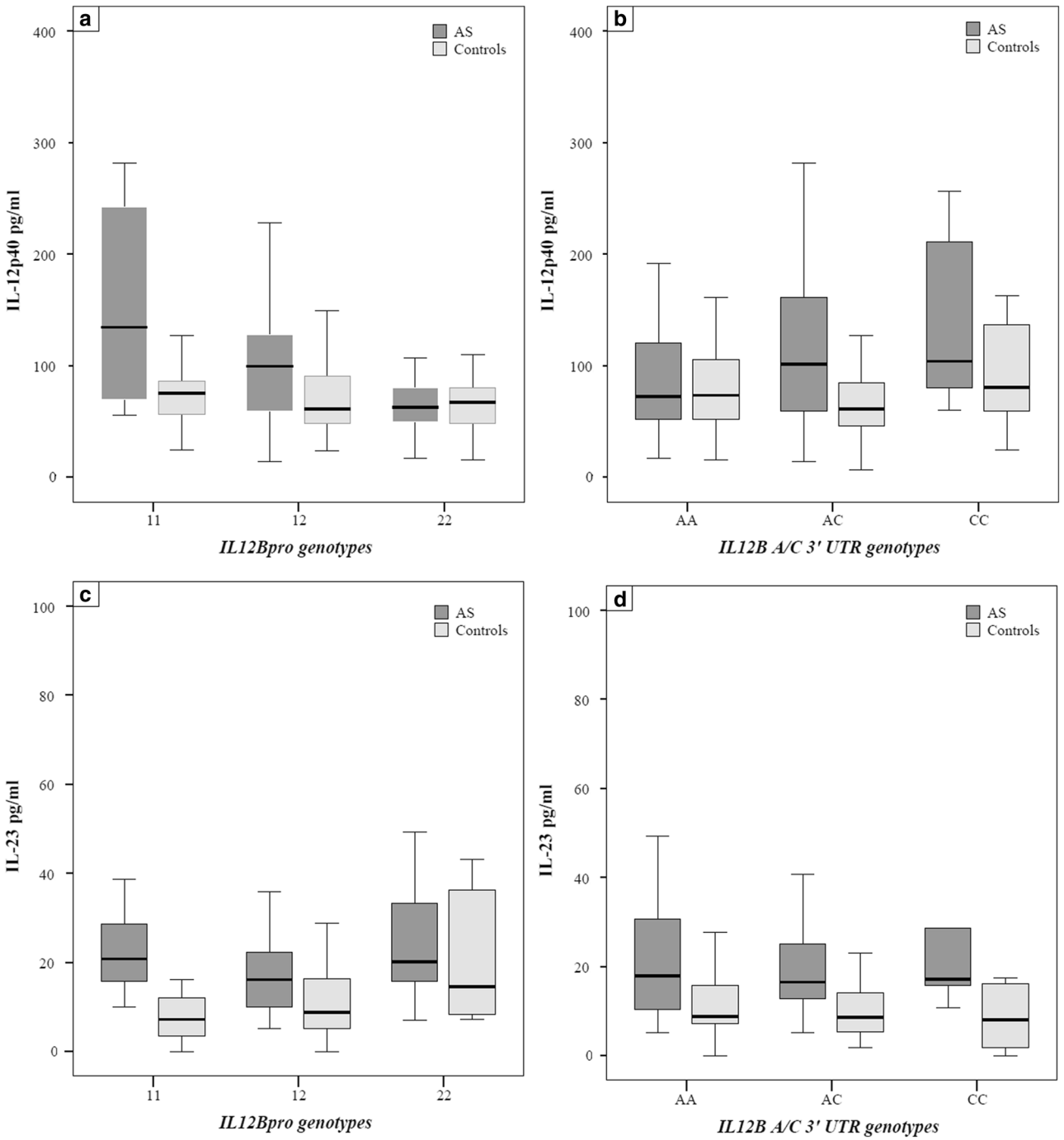

Fig. 1 Serum levels of IL-12p40 and IL-23 in AS patients and healthy controls regarding the genotypes of *IL12Bpro* (rs17860508) and *IL12B* A/C SNPs in the 3′ UTR (rs3212227). Boxplots show the median and interquartile range of the individual variables

variability in the immune response. Also, cytokine polymorphisms have been shown to be involved in susceptibility and resistance to autoimmune diseases [22]. Genetic variations and genomic research are at the forefront of personalized medicine as it provides information on disease predisposition and outcome, and can guide the choice of therapy.We characterized genetic variation in the *IL12B*, since their role in AS is largely unexplored. So far, there is very limited data concerning this topic, primarily for the association of the disease with the polymorphisms in the promoter region of the *IL12B* gene and for the joint effect of both gene loci.

The main findings gained from this study were that *IL12B* polymorphisms had linkage with increased genetic risk for AS in this Bulgarian population as well as with altered

Table 5 Clinical parameters in AS patients in relation to *IL12B* polymorphisms under the recessive model

Parameter	*IL12B rs17860508*		*p* value*
	1.1 + 1.2	2.2	
	Mean ± SD	Mean ± SD	
Age at onset (years)	26.8 ± 7.8	27.0 ± 8.8	0.893
ASDAS-CRP	3.1 ± 1.3	3.2 ± 1.1	0.726
BASFI	3.98 ± 2.61	5.26 ± 2.17	0.034
BASMI	3.48 ± 2.77	4.82 ± 2.15	0.037
Parameter	*IL12B rs3212227*		*p* value*
	AA + AC	CC	
	mean ± SD	mean ± SD	
Age at onset (years)	27.3 ± 8.1	22.9 ± 6.3	0.138
ASDAS-CRP	3.1 ± 1.3	3.9 ± 0.7	0.076
BASFI	4.22 ± 2.60	5.57 ± 1.68	0.128
BASMI	3.77 ± 2.7	4.71 ± 2.6	0.379

*Mann–Whitney *U* test

expression of the pro-inflammatory mediators of the immune response IL-12p40 and IL-23.

Numerous genetic studies on autoimmune diseases revealed that the differences in the frequencies of cytokine gene polymorphisms within geographically diverse populations depend on ethnicity and race [23, 24]. Hence, gene polymorhisms affecting major inflammatory pathways may help to explain racial discrepancies in disease risk. The rs3212227 has been linked to a genetic predisposition to AS in some populations, while in others no such relationship has been established. Wong et al. [25] published data that the rs3212227 CC/AC genotype is more frequent among the Taiwanese patients, whereas in mainland Han Chinese no significant differences of genotypic or allelic frequencies was found [26],as in our study. In contrast, *IL12B* rs3212227 has shown an association with psoriatic arthritis (PsA) [27]. Both AS and PsA belong to the group of the spondyloarthritides based on the overlapping clinical phenotype suggesting common pathogenic mechanisms [28]. In a meta-analysis, Li et al. [29] failed to find any association between rs3212227 polymorphism with AS. Our findings are consistent with this assertion, the rs3212227 and their genotypes did not influence on AS occurrence by itself. Apparently, the carriage of the *IL12Bpro* variant 2-allele in the genotype could increase susceptibility to AS regardless of homo- or heterozygosity (1.2 + 2.2), while its absence (1.1) might be protective. Its individual effect appears to be smaller, but AC and AC + CC genotypes of the rs3212227 multiply several times the genetic risk of AS development in subjects carrying the high-risk *IL12Bpro* 2-allele, especially in homozygosity.

The presence of certain alleles may influence a release of cytokines and thus the regulation of immune response [24]. Likewise,analyses of our dataset disclosed that some of the allelic variants in the *ILB12* have the potential to alter the expression of cytokines; carriage of the *IL12Bpro* variant 1-allele regardless of homo- or heterozygosity could enhance systemic IL-23 and IL-12p40 levels, as well as all the allelic variants in the 3′ UTR genes of the latter and only AC of the rs3212227 genotype could amplify IL-12p40 secretion. The elevation of both cytokines in our study is due to the fact that the result has been obtained from the entire AS cohort, but we cannot rule out the indirect realization through tight linkage with other nucleotide differences. It is difficult to draw firm conclusions based on the complexity of cytokine regulation. Clearly, further studies are warranted. In addition, serum levels of both cytokines were measured at a fixed point in time. Also, AS patients entered in the study were treated with various pharmacological agents including anti-TNF therapy at the time of evaluation. Therefore, we cannot rule out therapy influence on serum cytokines which was not detected by our assay. Moreover, conflicting results for the effect of anti-TNF treatment on certain cytokines were published [30].

According to our results, the *IL12Bpro* genotype 2.2 shares the determination of both more limited spinal mobility and corresponding severe functional disability than the carriage of allele 1 in the genotype. When stratifying the data according to the magnitude of ASDAS-CRP, we did not find a significant association of the investigated allelic variants with the disease activity state despite the tendency towards higher activity found in carriers *of IL12B* 3′ UTR CC genotype. These results might indicate that some of the genetic variants of *IL12B* influence disease activity while others influence chronicity.

The limitation of our study was the small sample size of AS patients. Further studies could strengthen the knowledge for the degree of linkage between the polymorphisms within the gene and AS. Taking this into account, this study may provide contribution in highlighting the ethnic genetic variation within *IL12B* gene in association with AS.

Conclusions

In summary, we propose the thesis that the combinatorial complexity of *IL12Bpro* (rs17860508) and 3′ UTR (rs17860508) polymorphisms of *IL12B* plays a role in AS genetic susceptibility in our population. Mostly, *IL12Bpro* variant 2-allele influences susceptibility to AS regardless of homo- or heterozygosity, especially when presented simultaneously with AC and AC + CC genotypes of the rs3212227. Also, allelic variants in the *IL12B* have the potential to alter the expression of both IL-12p40 and IL-23 cytokines in AS in a specific manner, thus influencing the immune response. Besides, the study demonstrates a contribution of IL-12p40 gene polymorphisms to disease severity in ankylosing spondylitis.

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Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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