INTERLEUKIN 1A GENE POLYMORPHISMS IN ROMANIAN PATIENTS WITH SERONEGATIVE SPONDYLOARTHROPATHIES

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Abstract

Objective. The aim of this study was to investigate whether two *IL1A* gene polymorphisms, rs17561 and rs1800587, influence disease susceptibility for seronegative spondyloarthropathies (SpA) in Romanians. Subsequently, we analysed separately ankylosing spondylitis (AS) and psoriatic arthritis (PsA) subgroups in relation with the two *IL1A* gene variants.

Methods. The study included 240 SpA patients (140 AS and 100 PsA patients) and 160 healthy controls. Real-time polymerase chain reaction (RT-PCR) was used to genotype the two single nucleotide polymorphisms (SNPs). Allele, genotype and haplotype frequencies of each SNP were compared between SpA patients and controls and also between AS and PsA cohorts and controls. The PLINK 1.9 software package was used to assess the potential associations; p values ≤ 0.05 were considered significant.

Results. The minor allele T frequency for rs17561 polymorphism was similar in general SpA cohort (31.4%) compared with controls (32.8%), the statistical analyses confirming the lack of association (p=0.67). Almost identical results were found for rs1800587: minor allele T frequency was 29.1% in SpA patients and 29.7% in controls (p=0.85). The same pattern persisted for the separate analysis of HLA-B27 positive SpA patients or AS and PsA cases against controls.

Conclusions. The *IL1A* gene polymorphisms (rs17561 and rs1800587) do not influence the disease predisposition for seronegative spondyloarthropathies in general, nor for ankylosing spondylitis and psoriatic arthritis in particular, in Romanians.

Keywords: *IL1A* gene, seronegative spondyloarthropathies (SpA), ankylosing spondylitis (AS), psoriatic arthritis (PsA), single nucleotide polymorphisms (SNPs)

INTRODUCTION

Ankylosing spondylitis (AS) and psoriatic arthritis (PsA) are the most prominent representatives of the seronegative spondyloarthropathies (SpA) group, which also include reactive arthritis, SpA related to inflammatory bowel disease (IBD-SpA), acute anterior uveitis (AAU), juvenile spondyloarthritis and undifferentiated spondyloarthritis (uSpA) (1). Axial and peripheral asymmetrical inflammatory arthritis is the clinical hallmark for these interrelated rheumatic diseases, while the intimate association with human leucocyte antigen (HLA) B27 represents the most recognisable genetic trait (2,3).

The prevalence and geographical distribution of HLA-B27 (4) influence the epidemiological profile of SpA. The lowest reported prevalence of SpA and

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AS was in Japan (less than 0.01%) (5), while the highest prevalence was registered in Nordic populations: 2.5% in Alaska (6) for SpA and 1.8% in Norwegian Samis (Lapps) for AS (7). After analysing 36 eligible studies, Dean *et al*. estimated the average AS prevalence to be 0.319% in North America, 0.238% in Europe, 0.167% in Asia, 0.102% in Latin America and 0.074% in Africa (8). The prevalence of psoriatic arthritis varies between <0.1 % (9) in Czech Republic and 0.4% in Italy (10), but this maximum value increases up to 42% in patients with existing psoriasis (1).

The association of HLA-B27 and AS has been documented for more than 40 years (11-14) and approximately 90% of AS and 60% of PsA patients are HLA-B27 positive (15). In the last 15 years (16) it has become clear that genes outside the major histocompatibility (MHC) system contribute to the genetic risk of AS and PsA. Several chromosomal regions (1p, 2q, 6p, 9q, 10q, 16q, and 19q) were identified in one of the first genome-wide scans ever performed, pointing to the presence of non-MHC genetic-susceptibility factors in AS (17). Since then, *endoplasmic reticulum aminopeptidase (ERAP1)* gene and *interleukin (IL) 23 receptor* gene have been identified as viable candidates by several genome-wide associations studies (GWAS) (18,19), while the involvement of *IL-1* family gene cluster in AS susceptibility has been less certain (20,21).

Initially, *IL-1* family gene cluster located on chromosome 2q.14.1-2 and extended across 360.1 kb, included *IL1A, IL1B* and *IL-1 receptor antagonist (IL-1RN)* genes (also called IL-1F1, IL-1F2, IL-1F3 genes). Later on, within the same 360.1 kb gene cluster, another 6 family members were identified (from *IL-1F5* to *IL-1F10*) (22), the gene order from centromere to telomere being: *IL1A-IL1B-IL1F7-IL1F9-IL1F6-IL1F8-IL1F5-IL1F10-IL1RN* (23). Along with another two interleukins, IL-18 (IL-1F4) and IL-33 (IL-1F11), located on different chromosomes (chr. 11 and chr. 9, respectively) they form the total 11 members of IL-1 family that we know today (24).

IL-1α and IL-1β are produced mainly, but not exclusively, by activated macrophages and they are responsible for a wide array of proinflammatory biological activities which comprise the host response to injury and/or infection: fever, acute phase protein synthesis, cytokines (especially TNF-α) production, up-regulation of adhesion molecules, fibroblast proliferation, vasodilatation, neutrophilia and lymphocytosis (22,25,26).

IL-1α and IL-1β bind to the same type I IL-1 receptor (IL-1RI) and accessory protein (AcP), also termed co-receptor IL-1RAcP, forming a complex responsible for the intracellular signalling secondary to the IL-1α/β recognition (24). The cytoplasmic Toll-IL-1 receptor (TIR) domains of IL-1RI and IL-1RAcP enlist the adapter protein MyD88 (myeloid differentiation primary response gene 88), determining the phosphorylation of IRAKs (IL-1R–associated kinases) and IKKβ (inhibitor of nuclear factor κB kinase β) (24). As a result NF-κB is transferred into nucleus, enhancing the expression of many inflammatory genes, such as *IL-6, IL-8, MCP-1, COX-2, IL1A, IL1B* and *MKP-1* (27).

IL-1 receptor antagonist (IL-1Ra) encoded by *IL-1RN* plays the role of a decoy molecule. Although it is recognized by the IL-1RI (like IL-1α and IL-1β), this recognition is not followed by the IL-1RAcP attachment and, thus, it fails to carry out the subsequent signalling interactions described above. Furthermore, blocking IL-1RI prevents the agonist molecules (IL-1α and IL-1β) from inducing the proinflammatory effect (22).

IL1A gene polymorphisms have been linked with disease predisposition in seronegative spondyloarthropathies as a whole and in AS in particular (28), while IL-1α serum levels were elevated in active IBD-SpA compared with inactive IBD-SpA (29).

The aim of this study was to investigate whether two *IL1A* gene polymorphisms, rs17561 and rs1800587, influence disease susceptibility for seronegative spondyloarthropathies in Romanians. Subsequently, we analysed separately AS and PsA subgroups in relation with the two *IL1A* gene variants.

PATIENTS AND METHODS

Case-control groups

The case-control cohorts selected for this study included a total of 400 unrelated Caucasians of Romanian ethnicity: 240 SpA patients (58.3% AS and 41.7% PsA patients) and 160 healthy controls. The AS patients were diagnosed according to the 1984 modified New York criteria (30), while Classification Criteria for Psoriatic Arthritis (CASPAR) (31) and Assessment of SpondyloArthritis international Society (ASAS) criteria (32, 33) were used to diagnose PsA patients in the Rheumatology Departments and Internal Medicine of "Dr. I. Cantacuzino" and "Sfânta Maria" Hospitals, Bucharest. General characteristics for the consecutively enrolled SpA (AS/

PsA) patients, as well as for the healthy controls are presented in Table 1.

TABLE 1. *Characteristics of the SpA (AS and PsA) patients and healthy controls*

Characteristics	SpA patients (n=240)	AS patients (n=140)	PsA patients (n=100)	Controls (n=160)
Average age (years)	45.3	38.9	53.2	38.1
Sex ratio (male:female)	162:78	118:22	44:56	84:76
HLA-B27+ %	56.5%	82.1%	20.2%	7.7%

HLA-B27+, human leucocyte antigen B27 positive; SpA, seronegative spondyloarthropathies; AS, ankylosing spondylitis; PsA, psoriatic arthritis.

The individuals included in the control group are healthy potential organ donors who do not present symptoms and/or history of SpA, screened by the National Institute of Blood Transfusion "Prof. Dr. CT Nicolau", Bucharest. The study had the approval of the local ethics committees and all the subjects recruited signed an informed consent.

DNA extraction was performed using mi-Blood Genomic DNA Isolation Kit - Metabion, Germany and QIAamp DNA Blood Mini Kit Qiagen, Germany, following the manufacturers' protocols. Polymerised chain reaction with specific primers was the principle used for genotyping HLA-B27 using HLA-B27-SSP low resolution kit (Olerup, Sweden) and AllSet+™ Gold SSP B27 Low-Resolution Kit (Invitrogen, Life Technologies, Thermo Fisher Scientific, USA).

IL1A genotyping

A single nucleotide polymorphism (SNP) represents a variation of a single nucleotide which occurs in certain specific position in the genome. Patients and controls were genotyped for two *IL1A* SNPs chosen based on previous association studies and meta-analysis (34, 35). The first *IL1A* polymorphism selected for this study is rs17561 [-340G/T] which is located in the coding sequence of IL1A and responsible for a non-synonymous (missense) mutation which changes the peptide structure of IL-1α. The residue change is located in the 114th amino acid position, alanine being replaced by serine in G to T allele change (Table 2). The second SNP investigated is rs1800587, also known as C-889T, which belongs to a non-coding sequence of the gene, the 5' untranslated region (5'UTR). Although the SNPs located here do not influence the quality of the resulting protein molecule, 5'UTR is an important region which regulates the translation of mRNA and therefore, changes made here may influence the final quantity of the synthetized polypeptide.

Real-Time PCR with TaqMan® Allelic Discrimination Assays (C___9546471_10 and C___9546481_20) were used to genotype the two SNPs in accordance with the protocols provided by the manufacturer (Thermo Fisher Scientific Inc, Applied Biosystems™, Foster City, MA, USA).

Statistical analysis

Hardy-Weinberg equilibrium (HWE) was tested for both SNPs in the control group (36). Allele frequencies and genotype frequencies of each SNP were compared between SpA patients and controls and also between AS and PsA cohorts and controls. The PLINK 1.9 software package (37) assessed the potential associations applying Fisher's Exact test and p values ≤ 0.05 were considered significant. The same software was used to calculate haplotypes frequencies and linkage disequilibrium (LD) estimation.

RESULTS

In the control group minor allele frequencies for both SNPs were higher than those reported for European descendants by HapMap (http://hapmap.ncbi.nlm.nih.gov), even reaching the statistical threshold for rs17561 (p=0.03), but closer to the values pre-

TABLE 2. *The genotyped IL1A gene single nucleotide polymorphisms.*

SNP	Chromosome position*	SNP location	Gene sequence Nucleotide change	Residue change
rs17561 [-340G/T]	112779646	1300	Coding: missense GCA – TCA	**A** [Ala] 114 **S** [Ser]
rs1800587 [C-889T]	112785383	12	Non-coding: 5'UTR C-T	–

**Genome Reference Consortium Human genome build 38; SNP, single-nucleotide polymorphisms; 5' UTR, 5' untranslated region; A [Ala], alanine; S [Ser], serine.*

sented by 1000 Genomes (http://www.1000genomes.org/data).

In the control group, HWE was observed for rs1800587. A minor deviation was obtained for rs17561 (p=0.03). The genotyping rate was 97.5% for rs17561 and 99.6% for rs1800587.

LD estimation for the two *IL1A* polymorphisms identified a high tendency to correlation between the two loci in our population: r^2=0.75.

For rs17561 [-340G/T] polymorphism, the minor allele T frequency was similar in general SpA cohort (31.4%) compared with controls (32.8%), the statistical analyses confirming the lack of association (p=0.67). Moreover, minor allele carriers, TT and GT genotypes, showed no difference in disease predisposition (p=0.42). Similar results were found for the second *IL1A* gene variant investigated: rs1800587 [C-889T]. Minor allele T frequency was 29.1% in SpA patients and 29.7% in controls (p=0.85) and minor allele T carriers presented the same risk of developing SpA as carriers of CC genotype (p=0.74) (Table 3).

The separate analysis of HLA-B27 positive SpA cases had the same outcome. For both *IL1A* polymorphisms, minor allele frequencies presented little variations between healthy controls (32.8% for rs17561 and 29.7% for rs1800587) and patients (32.3% for rs17561 and 31.8% for rs1800587), while minor allele carriers had a similar distribution among the two cohorts (Table 3).

A further examination of each subgroup of patients (AS and PsA) against the control cohort was performed. The minor allele frequencies for both polymorphisms investigated in AS patients (32.2% for rs17561 and 31.8% for rs1800587) were very close to those identified in controls (32.8% for rs17561 and 29.7% for rs1800587); no association with AS susceptibility was revealed (Table 4).

Furthermore, the minor allele frequencies calculated in PsA cohort for both SNPs presented minimal variations from the ones found in controls and these results were mirrored by the distribution of the genotypes in the two cohorts (Table 4).

Additional thorough analysis of HLA-B27 positive AS subgroup or separate male/female examination of SpA subgroups followed the same pattern of non-association.

Haplotype analysis showed almost identical distribution of the haplotypic combinations of rs17561|rs1800587 polymorphisms, resulting in similar haplotype frequencies, in all patients cohorts compared with controls (Table 5). No haplotypic combination, neither minor alleles TT haplotype, nor major alleles GC haplotype, influenced the risk of developing seronegative spondyloarthropathies as a whole, or ankylosing spondylitis and psoriatic arthritis in particular (p>0.05) (Table 5).

DISCUSSIONS AND CONCLUSIONS

This is the first Romanian based genetic association study which investigates the relation between *IL1A* gene variants and SpA. Our results show that the *IL1A gene* polymorphisms (rs17561 [-340G/T] and rs1800587 [C-889T]) do not influence the disease predisposition to seronegative spondyloarthropathies in general, nor for ankylosing spondylitis and psoriatic arthritis in particular, in Romanians.

TABLE 3. *Minor allele frequencies and genotypes frequencies for rs17561 and rs1800587 in SpA patients and HLA-B27 positive SpA patients versus controls*

SNP	Controls	SpA	Statistics	HLA-B27+ SpA	Statistics
rs17561	**n=160**	**n=234**		**n=130**	
Minor allele T	Number (frequency)	Number (frequency)	OR 0.938 C.I.=[0.69-1.27] p=0.67	Number (frequency)	OR 0.977 C.I.=[0.68-1.38] p=0.89
	105 (32.8%)	147 (31.4%)		84 (32.3%)	
Genotype TT+GT	11+83 (58.7%)	19+109 (54.7%)	OR 0.848 C.I.=[0.56-1.27] p=0.42	10+64 (56.9%)	OR 0.928 C.I.=[0.58-1.48] p=0.75
rs1800587	**n=160**	**n=239**		**n=132**	
Minor allele T	Number (frequency)	Number (frequency)	OR 0.971 C.I.=[0.71-1.32] p=0.85	Number (frequency)	OR 1.105 C.I.=[0.77-1.57] p=0.57
	95 (29.7%)	139 (29.1%)		84 (31.8%)	
Genotype TT+CT	14+67 (50.7%)	22+95 (49%)	OR 0.935 C.I.=[0.62-1.39] p=0.74	14+56 (53%)	OR 1.101 C.I.=[0.69-1.74] p=0.68

SpA, seronegative spondyloarthropathies; HLA-B27+, human leucocyte antigen B27 positive;
SNP, single-nucleotide polymorphisms, CI, 95% confidence interval; OR, odds ratio; p values <0.05 are considered significant.

TABLE 4. *Minor allele frequencies and genotypes frequencies for both SNPs investigated in AS patients and PsA patients versus controls*

SNP	Controls	AS	Statistics	PsA	Statistics
rs17561	**n=160**	**n=135**		**n=99**	
Minor allele T	Number (frequency)	Number (frequency)	OR 0.973 C.I.=[0.68-1.37] p=0.87	Number (frequency)	OR 0.890 C.I.=[0.60-1.30] p=0.55
	105 (32.8%)	87 (32.2%)		60 (30.3%)	
Genotype TT+GT	11+83 (58.7%)	11+65 (56.3%)	OR 0.904 C.I.=[0.56-1.43] p=0.67	8+44 (52.5%)	OR 0.777 C.I.=[0.46-1.28] p=0.32
rs1800587	**n=160**	**n=139**		**n=100**	
Minor allele T	Number (frequency)	Number (frequency)	OR 1.043 C.I.=[0.73-1.48] p=0.81	Number (frequency)	OR 0.876 C.I.=[0.59-1.29] p=0.50
	95 (29.7%)	85 (31.8%)		54 (27%)	
Genotype TT+CT	14+67 (50.7%)	14+57 (61.1%)	OR 1.018 C.I.=[0.64-1.60] p=0.93	8+38 (46%)	OR 0.831 C.I.=[0.50-1.37] p=0.46

AS, ankylosing spondylitis; PsA, psoriatic arthritis; SNP, single-nucleotide polymorphisms, CI, 95% confidence interval; OR, odds ratio; *p values <0.05 are considered significant.*

TABLE 5. *IL1A rs17561|rs1800587 haplotype frequencies for the investigated cohorts*

Haplotype	Controls (n=160)	SpA (n=233)	p	AS (n=134)	p	PsA (n=99)	p
TT	0.281	0.279	0.94	0.294	0.71	0.257	0.55
GT	0.015	0.012	0.74	0.011	0.64	0.015	0.96
TC	0.046	0.036	0.46	0.029	0.28	0.045	0.94
GC	0.656	0.671	0.65	0.664	0.83	0.681	0.54

SpA, seronegative spondyloarthropathies; AS, ankylosing spondylitis; PsA, psoriatic arthritis; SNP, single-nucleotide polymorphisms, p values <0.05 are considered significant.

The reports regarding *IL1* gene cluster located on chromosome 2q14 as a susceptibility locus for AS and PsA have been controversial since the early genetic studies (20, 21, 38). Genome wide association studies (18, 39), including the most recent International Genetics of Ankylosing Spondylitis Consortium GWAS (40) did not detect this locus among the ones being associated with AS, although loci located on chromosome 2q11 encoding *IL1R1* and *IL1R2* showed suggestive association with AS in Europeans (40). However, there are multiple association studies and meta-analyses which seem to implicate *IL1A, IL1B* or *IL1RN* gene polymorphisms as a risk factor for AS, PsA and SpA as a whole in various populations. One of the most cited studies examined three Canadian populations and identified several SNPs belonging to *IL-1* gene cluster as being associated with the risk of AS. Three *IL1A* gene polymorphisms were reported (rs3783550, rs3783543 and rs3783526), but, interestingly, the ones we investigated, rs17561 and rs1800794, showed no association with the disease (34). Similarly, *IL1A* polymorphism rs1800587 [-889C/T] was not associated with HLA-B27 positive AS in Iranians, although *IL1R* polymorphism rs2234650 did influence the risk of developing AS (41). A Korean study investigated 51 SNPs within *IL-1* gene cluster, including *IL1A* polymorphisms, and even though haplotype analysis revealed an association with AS, the study failed to identify any individual SNP association with AS susceptibility (42). Another study investigating an Asian population found that *IL-1* gene cluster is associated with AS in Chinese population (43). An early meta-analysis which included 6 studied and nine population samples, investigated *IL-1* gene complex and disease susceptibility for AS and reported among the relevant SNPs *IL1A* rs1800587 [C-889T] (44). A prospective meta-analyses aimed to determine the contribution of *IL-1* gene cluster to AS susceptibility in different populations identified three *IL1A* gene variants (rs17561, rs2856836, rs1894399) as having a strong association with AS (45). Another meta-analysis, comprising nine studies with population samples from Europe, Asia and Latin America, revealed that several SNPs belonging to *IL-1* gene cluster, including the ones we investigated (rs17561

[-340G/T], rs1800587 [C-889T]) present a significant association with AS, especially in Europeans (35). Similarly, *IL-1RN* polymorphisms were found to be implicated in AS pathogenesis (46).

The first report involving *IL1A* gene polymorphisms in PsA identified rs1800587 [C-889T] as a risk factor (47). Two years later, Rahman *et al*. reported two regions within *IL-1* gene complex contributing independently to the risk of disease in PsA: *IL1A* gene (rs3783547, rs3783543 and rs17561) and a region near the end of *IL1B*, through I*L-1F7*, *IL-1F8*, and into *IL-1F10* (48).

Monnet *et al*. (28) were the first to report that *IL1A* gene polymorphisms are associated with SpA as a whole and with AS susceptibility in particular. *IL1A* gene seems to influence also the AS phenotype, mainly sacroiliitis' severity (28). A significant variation of IL-1α and IL-1Ra serum levels in active enteropathic (IBD) SpA compared with inactive IBD-SpA was described (29), although a recent study found no statistical differences in *IL1A, IL1B* and *IL1RN* genotypes and allele distributions between IBD cases and healthy controls (49).

The results reported in the literature regarding the involvement of *IL-1* gene complex in SpA and AS susceptibility are conflicting. Furthermore, IL-1 blocking agent Anakinra, a recombinant IL1-Ra, approved for the treatment of rheumatoid arthritis patients, showed limited efficacy in AS (50) and PsA patients (51), while no large trials are currently evaluating its role in SpA (52). It is possible that some discrepancies between studies can be explained by study design or power limitations (53), but a more likely reason for the contrasting results may be disease heterogeneity itself. Spondyloarthropathies are multifactorial genetic disorders and the genetic factors implicated in disease susceptibility may differ from one population to another, which could explain why negative association studies, like ours, can coexist with positive ones. However, it has been hypothesized that *IL-1* gene cluster's contribution to SpA is closely related to other non-MHC gene susceptibility variants more firmly identified (*ERAP1*, *IL-23R*) (53), but only further studies can pinpoint the exact role of *IL-1* gene complex, particularly of *IL1A* gene, in SpA pathogenesis.

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