ARTICLE ORIGINAL

Interleukin-1 gene polymorphisms in axial spondyloarthritis Tunisian patients

Etude des polymorphismes du gène codant pour l'interleukine-1 au cours de la spondyloarthrite axiale

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RÉSUMÉ

Objectifs : Déterminer l'association entre les polymorphismes génétiques du gène codant pour l'interleukine-1 (IL-1) et l'antagoniste du récepteur de l'IL1 avec le phénotype de la spondyloarthrite axiale (SpA) chez des patients tunisiens ainsi que l'impact sur la sévérité de la maladie

Méthodes : 101 patients atteints de SpA et 100 patients témoins sains ont été inclus. Les caractéristiques démographiques, cliniques, radiologiques et thérapeutiques ont été recensées. Les fréquences alléliques et génotypiques d'IL1B (−511) et (+3953) et le nombre de variable de répétitions en tandem (VNTR) d'IL1RN (VNTR) et (-889) ont été comparées entre les 2 groupes.

Résultats : Il existait une différence significative dans la distribution allélique des quatre loci entre les patients atteints de SpA et les témoins. Les allèles C / T des polymophismes IL-1α (-889) et 1/1 de IL-1Ra étaient significativement augmentés chez les patients atteints de SpA, (p=0.0001; p=0.000004, respectivement). L'allèle T (3954) de IL-1β et l'allèle 1 de IL-1Ra étaient significativement associé à la forme articulaire périphérique (p=0.047, p=0.05 respectivement). De plus, le génotype 1/1 de IL-1Ra était significativement abaissé chez les patients SpA ayant une maladie active (BASDAI> 4) (p = 0.033).

Conclusion : Cette étude montre que les polymorphismes du cluster de gène de IL-1/IL-1Ra sont associés au développement de la SpA chez les Tunisiens. Ils étaient aussi corrélés à la sévérité de la maladie.

Mots clés : Interleukine-1, Polymorphisme, Spondyloarthrite axiale

SUMMARY

Introduction: Axial spondyloarthritis (SpA) is a common inflammatory arthritis characterized by axial skeletal inflammation, enthesitis, and association with HLA-B27. Pro-inflammatory cytokines play important roles in the regulation of inflammatory response and seem to be good candidates involved in the development of this pathology.

Aim: To assess the influence of the functional polymorphisms of single nucleotide polymorphims (SNPs) of IL-1 and IL-1 Ra in SpA susceptibility Tunisian patients.

Methods and Results: One hundred and one patients and 100 ethnic-matched healthy controls were genotyped. Susceptibility to SpA was showed with SNP's: C/T of IL-1α (-889) (p=0.0001) and 1/1 of IL-1Ra (p<10-3). Analysis of SpA patients according to clinical behavior of the disease reveled the influence of these polymorphisms in SpA course. Indeed, individuals carrying the allele T (+3954) of IL-1β and allele 1 of IL-1Ra had an increased risk of peripheral arthritis (p=0.047, p=0.05, respectively). Also the 1/1 genotype of IL-1Ra was significantly decreased in SpA patients having an active disease (BASDAI>4) (p=0.033).

Conclusion: Genetic polymorphisms of pro-inflammatory IL-1/IL-1Ra cytokines seem to be involved in susceptibility and clinical course of SpA in Tunisian patients.

Key-Words: 'spondylitis, ankylosing'; 'spondylarthritis'; 'Polymorphism,Genetic' ; interleukin-1

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INTRODUCTION

Axial Spondyloarthritis (SpA) is a common cause of inflammatory arthritis after rheumatoid arthritis (1). Genetic factors have been strongly implicated in its etiology and heritability, as assessed by twin studies, has been estimated to be greater than 90% (2). Although HLA-B27 is the major gene associated with AS as it is present in almost 95% of white individuals with this disease, its contribution to the overall genetic predisposition has been estimated at only 20-30% (1). Moreover, the exact mechanism of the effect of HLA-B27 on SpA is still unclear.

There is evidence supporting the notion of additional genes in the HLA region with a total estimated contribution of 40-50% for this region, so it is apparent that non-major histocompatibility complex genes contribute a major portion of genetic susceptibility to SpA (3). Whole genome scan studies as well as case controls studies suggest other susceptible genes such as the interleukin-1 (IL-1) family gene (4).

The IL-1 gene family contains, within a 430-kb region, three related genes: IL-A, IL-B and IL-1RN, which respectively encode the pro-inflammatory cytokines: IL-1a, IL-1b and their endogenous receptor antagonist IL-1 Ra (5).

Several polymorphisms in these genes have been described and they are known to influence the production of these cytokines. Bi-allelic polymorphisms, at position -889 in the promoter region of the IL-1A gene (6), at position +3953 in exon 5 (7) and position -511 in the promoter region (8) of the IL-1B gene have been described. Concerning the IL-1RN gene, a penta-allelic polymorphism of variable number of tandem repeat (VNTR) in intron 2 has been identified with combined frequencies of alleles IL-1RN*1 and IL-1RN*2 exceeding 96% (9,10).

Association of members of the interleukin 1 (IL-1) gene cluster with AS have been previously reported by several studies (6,11,12). However, these studies have conflicting results in different populations, making it difficult to draw a conclusion about the relationship between IL-1 gene clusters and SpA. Furthermore, none of them provided detailed clinical data of reported populations. We intend in this present work to determine the association between genetic polymorphisms of IL-1 gene and SpA in Tunisian patients.

METHODS

This was a cross-sectional study at a rheumatology outpatient in Charles Nicolle Hospital. Informed consent was obtained from all subjects, and the study was approved by the Hospital local Ethics Committee. Diagnosis of SpA was based on the 1984 modified criteria of New York (13). Patients aged 16 years or older were recruited. Exclusion criteria were patients diagnosed with psoriatic arthritis, enteropathic spondyloarthritis, reactive arthritis and undifferentiated spondyloarthritis.

Clinical information was collected systematically and included gender, age of disease onset, peripheral arthritis, hip involvement, extra-articular symptoms (anterior uveitis) and treatment received by patients. The disease activity was evaluated using the Tunisian version of the Bath Ankylosing Spondylitis Activity Index (BASDAI) and the functional impairment was determined by the Tunisian version of the Bath Ankylosing Spondylitis Functional Index (BASFI) (14). The carriage of the antigen HLA-B27 and its subtype were also determined. A control group of 100 unrelated, healthy Tunisian blood donors age- and sex-matched were enrolled.

DNA extraction and genotyping analysis

Samples of peripheral blood were obtained from all participants and the genomic DNA was extracted from EDTA-treated whole blood by a standard salting out procedure (15). HLA-B27 typing and sub-typing was performed by polymerase chain reaction with sequence-specific primers (PCR-SSP) using a commercial kit manufactured by One Lambda. The bi-allelic transition polymorphisms C/T at position -511 in the promoter region and position +3953 in exon 5 on the IL-1B gene were analyzed by restriction fragment length polymorphism (RFLP).

The -889 C/T polymorphism in the promoter region of IL-1A gene and the penta-allelic variable numbers of tandem repeat polymorphism in intron 2 of the IL-1RN gene were genotyped according to PCR-SSP.

Statistical analysis

For the statistical analysis we used the software Statistical Package for Social Sciences (SPSS) 11.5 for Windows. Both genotypes and alleles frequencies were calculated in

patients and controls and compared by the Chi2-test. A p value ≤ 0,05 was considered significant. The magnitude of associations was expressed as an odds ratio (OR) with a 95% confidence interval (CI). To determine the correlation between alleles and genotypes of each polymorphism and clinical disease features, we used the test of Chi2 to study the liaison of two qualitative variables and the t-test of Student to compare quantitative variables.

RESULTS

IL-1 polymorphisms and SpA susceptibility: 101 patients were enrolled, the cohort was mostly male (sex-ratio: 3.2). Table1 illustrates the patients' baseline demographic characteristics. The mean age of the sample was 39.8 [19-72] years; the mean disease duration at study baseline was 12.27 years [6 months - 41years]. Respectively, 62.4% and 32.7% of the cohort have peripheral arthritis and extra-articular symptoms. A hip osteoarthritis was present in 37.6%.

Table 1. Demographic and clinical characteristics of SpA patients

Parameters	Value
Mean age, years (range)	39.8 (19-72)
Male (%) / Female (%)	76.2 / 23.8
Mean age at disease onset, year (SD)	27.47 (±10.91)
Relatives with SpA (%)	16.8
Peripheral arthritis (%)	64.4
Hip involvement (%)	37.6
Anterior uveitis (%)	16
Mean BASDAI (SD)	38.26 (±15.6)
Mean BASFI (SD)	37.86 (±9.2)
HLA-B27 positive (%)	72.3
TNFi (%)	19.8

SpA: axial spondyloarthritis, BASDAI: Bath Ankylosing Spondylitis Disease Activity Index, BASFI Bath ankylosing spondylitis Functional Index, TNFi: Tumor necrosis Factor inhibitors, SD: Standard deviation

At baseline, the mean BASDAI score was 38.26; 50% have BASDAI greater than or equal to 4 and the mean BASFI score was 37.86. More than half of the patients included in the study were taking non-steroidal anti-inflammatory drugs (91.1%); up to 49.5% of the participants were taking conventional synthetic disease-modifying antirheumatic drugs and 19.8% received tumor necrosis factor inhibitors.

The HLA-B27 antigen was present in 72.3% of patients and 3 subtypes were identified. The two alleles B2705 and B2702 were predominant with a frequency of 49% and 47% respectively. The allele B2707 was found in 3 patients.

The distributions of (-889) IL-1a, (-511) and (+3953) IL-1b polymorphisms genotypes and alleles frequencies in SpA patients and controls are shown in Table 2.

The CT genotype of C/T (-889) IL-1a polymorphism was significantly increased in SpA patients in comparison with controls (60.4% and 37% respectively; p=0.0001; OR=3.4; 95% CI=1.85-6.32). The allele T was also significantly increased in SpA patients in comparison with blood-donors (p=0.002; OR=2.2; 95% CI= 1.44-3.73). The association of the T allele of (-889) IL-1a did not depend on HLA-B27. Indeed, the distribution of the two alleles was similar in B27-negative and B27-positive individuals.

Concerning the two SNPs C/T (-511) and (+3953) of the IL-1b, no significant differences were observed between SpA patients and controls in alleles or genotypes frequencies.

For the VNTR of IL-1 Ra polymorphism, the rare alleles IL-1RN*3 or IL-1RN*4 were present only in six SpA patients and three controls. None of the subjects was homozygous for allele IL-1 RN*3 or IL1-RN*4. For this raison, and in order to have more valid statistic results, we divided cases and controls into 2 groups: subjects carrying the 1 allele and subjects not carrying this allele.

Table 2. IL-1a and IL-1b genotype and alleles frequencies in cases and controls

		Genotypes (%)					Alleles			
Marker		**CC**	**CT**	**TT**	**p**	**OR (95% CI)**	**C**	**T**	**p**	**OR (95% CI)**
IL-1 A (-889)	Cases (101)	26.7	60.4	12.9			0.569	0.431		
	Controls (100)	56	37	7	**0,0001**	**3.4 (1.85-6.32)**	0.745	0.255	**0.002**	**2.2 (1.44-3.73)**
IL-1 B (+3953)	Cases (101)	37.6	45.5	16.8			0.604	0.396		
	Controls (100)	40	42	18	0.88		0.61	0.39	0.82	
IL-1 B (-511)	Cases (101)	55.4	33.7	10.9			0.723	0.277		
	Controls (101)	49	32	19	0.27		0.65	0.35	0.12	

IL-1: Interleukin-1

Our results show that the frequency of allele IL-1Ra*1 and the genotype 1/1 was significantly increased in SpA patients in comparison with controls (Table 3). The association of the 1 allele of IL-1 Ra did not depend on HLA-B27. Indeed, the distribution of this allele was similar in B27-negative and B27-positive individuals.

Genotypes and alleles frequencies of studied polymorphisms were found to be similar in SpA patients carrying HLA-B2705 or HLA-B2702 subtypes.

Table3. IL-1 Ra genotypes and alleles distribution in cases and controls

		Genotypes (%)				Alleles			
		1/1	Others	p	OR (95% CI)	1	Others	p	OR (95% CI)
IL-1Ra	Cases	84.2	15.8	$p<10^{-3}$	**3.47**	0.91	0.75	$p<10^{-3}$	**3.35**
	Controls	54	46		**(1.89-6.37)**	0.09	0.25		**(1.57-6.24)**

IL-1Ra: Interleukin-1 receptor antagonist; OR: Odds ratio; CI: confidence interval

IL-1 polymorphisms and clinical manifestations

Among SpA patients, individuals carrying the C allele of (+3953) IL-1β had an increased risk of peripheral arthritis (*p*=0.047, OR : 0.5, IC à 95‰ [0.28-0.99]). Patients carrying the 1 allele of IL-1Ra had a lower BASDAI score in comparison with patients carrying other alleles (37.3% versus 53,2% p=0.04), and had more frequently peripheral arthritis (p=0.05). No significant association were found between these alleles and gender, age at disease onset, peripheral arthritis, hip involvement, anterior uveitis, BASDAI and BASFI scores.

No significant associations were found between carriage of each of the alleles IL-1B -511, IL-1A -889 and different disease clinical features.

DISCUSSION

We demonstrated that the IL-1 gene cluster significantly influences susceptibility to SpA in Tunisian population. Indeed, the allele T of -889 IL-1a and the allele 1 of IL-1 Ra were significantly increased in SpA patients in comparison with controls. The role of this proinflammatory cytokine in SpA inflammation is well known. IL-1 is secreted by activated macrophages in the inflamed synovium and initiates the recruitment of immune cells and the progression of inflammation (16). Production of such mediators in various rates may influence the susceptibility to many inflammatory diseases since the cytokine IL-1 is a major factor in the control of inflammatory response. Production phenotype is likely to be determined by transcriptional and/or post transcriptional mechanisms. In either case, the presence of polymorphism within non-structural parts of the gene (such as the promoter region or other regulatory elements) might determine the different producer phenotypes (17).

In our study, the allele T of -889 IL-1a was found to be a genetic factor of susceptibility to SpA in Tunisian population. Only few studies have focused in the polymorphism C/T of IL-1 a(-889) and its relationship with SpA genetic susceptibility. For instance, no association was found in English and Iranian patients (6,18); this discordance can be explained by ethnically differences. In a recent meta-analysis, the OR of the T allele of IL-1A-889 was found to be significantly increased in Europeans with SpA (OR=1.357, 95% CI=1.085-1.697, P=0.007) but not in Asiatic SpA. This meta-analysis included a total of nine studies of 20 separate comparisons of association between IL-1 polymorphisms and SpA susceptibility. These were performed on European, Asian, and Latin American population samples (19).

Concerning the IL-1 Ra polymorphism, our study showed a significant association between the allele 1 and genetic susceptibility to SpA in Tunisian population. Our results are quite different from reported studies. Indeed, some studies found that the allele 2 of IL-1 Ra, rather than the 1 allele is associated with SpA in English, German and Taiwanese populations (11,12,20). However, no association was found between SpA and IL-1 Ra alleles in Canadian, north-American, Iranian and Chinese population (18, 21-23). Some of these differences can be due to ethnic variations. Moreover, the studies include a small number of patients and are then probably underpowered. Therefore, larger studies in different ethnic populations are required so that more reliable conclusions can be obtained.

IL-1RN encodes the IL-1Ra protein. This protein inhibits IL-1a and IL-1β activities and modulates a variety of IL-1-related immune and inflammatory responses. The findings of a recent meta-analysis revealed 3 single-nucleotide polymorphisms in IL-1RN having strong evidence of association with SpA risk. Authors reported significant positive associations between the rs30735*C

allele/carrier and the rs31017*G allele and susceptibility to AS in both Caucasian and Asian populations, while the positive association between the rs315952*T carrier and SpA susceptibility was significant only in Asian populations (24).

While IL-1R1 contributes to IL-1 functional signaling, IL-1R2 inhibits the activity of the ligands by binding IL-1a, IL-1b due to its lack of the intracellular Toll-IL-1 receptor domain (25). Through a large cohort, Yu Xia et al showed that genetic variation rs2302589 in IL-1R2 gene was significantly associated with SpA susceptibility in Northern Han Chinese (26).

In this study, no significant association between the two polymorphisms at (-511) and (+3953) C/T of the IL-1 b and the susceptibility to SpA was found. Our results are similar to the findings of studies from Iran (18), Canada (21), Taiwan (20), China (23) and Germany (12). In contrast, the C allele of the (-511) IL-1B polymorphism was significantly associated with SpA in an English study including 930 AS patients (6).

In the meta-analysis of the IL-1B-511, IL-1B+3953 polymorphisms and of the IL-1RN VNTR the variable numbers of tandem repeats revealed no association between SpA and these polymorphisms (19).

The majority of the studies focusing in IL-1 genes polymorphisms and SpA have analyzed their possible contribution to susceptibility to the disease, while repercussions on disease prognosis and phenotype have been less thoroughly investigated. Predicting the prognosis of SpA patients has been difficult due to disease heterogeneity, its slow progression, and the absence of adequate outcome measures. Several factors are considered to be associated with a worse prognosis, including male gender, early age at disease onset, peripheral arthritis particularly with hip involvement, the presence of extra-articular symptoms, and high BASDAI and BASFI scores (27). It is known that SpA severity is largely genetically determined, and the outcome of a family member can be predicted by the disease pattern of previously affected members (28). Our results suggest that polymorphisms in IL-1 gene could influence the disease course and, therefore, be a predictor of SpA long-term prognosis. Indeed, the C allele of (+3953) and the 1 allele of IL-1 Ra were associated with an increased risk of peripheral arthritis. In accordance with our results, Cubino N et al found that the G allele polymorphism of IL1B (-511 A/C) was associated with higher peripheral joint disease activity in patients with psoriatic arthritis (29). In another hand, the allele 1 of IL-1 Ra was associated with less active disease. The study of Van der Praadt et al didn't found any association between alleles of the studied polymorphisms and peripheral arthritis, anterior uveitis and age at disease onset (12).

CONCLUSION

Results from this study suggest that IL-1a gene promoter polymorphisms at positions −889 and IL-1 Ra are additional genetic factors of SpA in Tunisian population besides the HLA-B27 gene. The polymorphism (+3593) of IL-1b and IL-1Ra have an influence on SpA phenotype and prognosis, although these data need further confirmation in a larger population and in family-based study.

Conflicts of interest :

We wish to confirm that there are no known conflicts of interest associated with this publication.

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