Association analysis of interleukin-1 gene polymorphisms in autoimmune thyroid diseases in the Tunisian population

Maha Kammoun-Krichen[1], Noura Bougacha-Elleuch[1], Kaouthar Makni[1], Maha Rebai[3], Sylvie Peraldi-Roux[2], Ahmed Rebai[3], Mouna Mnif[4], Mohamed Abid[4], Joumaa Jouida[5], Hammadi Ayadi[1]

[1] Unité cibles diagnostiques et thérapeutiques en pathologies humaines, Centre de biotechnologie de Sfax, Tunisie
[2] UMR CNRS 5232 CPID, Faculté de Pharmacie, Montpellier, France
[3] Unité de bioinformatique, Centre de biotechnologie de Sfax, Tunisie
[4] Service d'endocrinologie, CHU Hedi Chaker, Sfax, Tunisie
[5] Dispensaire Bir El-Hfai-Sidi Bouzid, Tunisie

Correspondence : H. Ayadi, Unité cibles diagnostiques et thérapeutiques en pathologie humaine, Centre de biotechnologie de Sfax, BP "K", 3038 Sfax, Tunisie.
<directeur.general@cbs.rnrt.tn>

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ABSTRACT. Autoimmune thyroid diseases (AITDs), including Graves' disease (GD) and autoimmune hypothyroidism (AH), are inherited as complex traits. Among the genes contributing to AITDs susceptibility are genes of the IL-1 family. IL-1 regulates T and B lymphocyte maturation, including the induction of several cytokines and cytokine receptors. Therefore, disturbances of this balance may not only play a role in inflammation but also in the pathogenesis of autoimmunity. In order to investigate genetic association of IL-1 gene polymorphisms with AITDs, we performed both a familial study in a large Tunisian pedigree with high prevalence of AITDs (64 patients and 176 controls), and a case-control study (131 GD unrelated patients and 225 healthy controls). PCR and PCR-RFLP methods were used to analyse respectively a VNTR in the IL-1RN gene and three SNPs in both IL-1B genes (-511 C/T and +3954 C/T) and IL-1A (-889 C/T). The family-based association study showed an association of the IL-1B+3954 C/T polymorphism (p = 0.02) and two haplotypes IL-1RN*3/C/T/T and IL-1RN*1/C/T/T (p = 0.009 and p = 0.047 respectively) with AITDs. The case-control study is the first study revealing a significant association of the IL-1A-889 C/T polymorphism (chi2 = 10.23; p = 0.0014) with susceptibility to GD. Our data suggest that the IL-1 gene cluster may harbour susceptibility genes for AITDs and GD pathogenesis in the Tunisian population.

Keywords: interleukin-1, polymorphisms, genetic association, autoimmune thyroid diseases

Cytokines, a large group of non-enzymatic protein hormones, are involved in the induction and effector phases of all inflammatory and immune responses, and are therefore likely to play a critical role in the development of autoimmune diseases (AIDs) [1]. The proinflammatory cytokines interleukin (IL)-1α and β (OMIM 147760 and OMIM 147720), and their receptor antagonist (IL-1Ra) (OMIM 147679) play major roles in initiating and modulating immune responses [2]. IL-1 is a family of three proteins IL-1α, IL-1β and IL-1Ra, which are encoded by different genes (IL-1A for IL-1α, IL-1B for IL-1β and IL-1RN for IL-1Ra), spanning a 430 kb region on chromosome 2q13-21 [3]. These genes are highly polymorphic, encompassing both single nucleotide polymorphisms "SNPs", and length variants (microsatellites and variable number tandem repeats "VNTR"). IL-1 genes have been reported to be involved in the development of various inflammatory and immune diseases, including Graves' disease (GD), a major, autoimmune thyroid disease (AITD) [4, 5]. AITDs also include autoimmune hypothyroidism (AH): primary idiopathic myxoedema (PIM) and Hashimoto's thyroiditis (HT). It is known that IL-1 influences the function of the thyroid cells [6]. In fact, IL-1 downregulates the expression of thyroid-specific proteins such as thyroglobulin [7] and thyroperoxidase [8], inhibits iodide organification [9] and the Na+/I- symporter NIS [10], and reduces the delivery of thyroid hormone to the circulation [11]. These inhibitory effects are demonstrated both in apparently normal human thyrocytes adjacent to adenomas or cancers, in thyrotoxic cells, and, to some extent, in FRTL5 cells.

In the present study, we examined the genetic association of four polymorphic loci in the IL-1 gene cluster with AITDs in a large Tunisian family (Akr family), and with GD in unrelated Tunisian patients. Two of these polymorphisms were found to be associated with AITDs in family and case-control studies.

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METHODS AND PATIENTS

Patients and controls

Patients were recruited from a large family in South Tunisia which has a high prevalence of AITDs (Akr family) [12]. This family consists of more than 400 members spanning 10 generations and including 176 controls and 65 patients. The latter are subdivided into 34 patients affected with GD and 31 patients with AH (9 HT + 21 PIM). The case-control study included 131 GD patients and 225 controls (healthy subjects with no history of AITDs). The diagnosis of GD and AH was performed as previously described [13].

Genotype analysis

DNA was extracted from peripheral blood as previously described [14]. PCR was used to identify the genotypic pattern of the different IL-1 genes *(table 1)*. The four polymorphisms studied are located on chromosome 2q14. The IL-1B and IL-1A gene polymorphisms : IL-1B-511 C/T, IL-1B+3954 C/T and IL-1A-889 C/T were analysed by PCR followed by restriction fragment length polymorphism (PCR-RFLP) as previously described [15-17]. The VNTR located in intron 2 of IL-1RN was amplified as previously described [18]. Alleles are conventionally defined as follows: allele 1 (412 bp, representing 4 repeats); allele 2 (240 bp, 2 repeats); allele 3 (498 bp, 5 repeats); allele 4 (326 bp, 3 repeats) [19].

Statistical analysis

In the familial study, we used the FBAT program (Family-Based Association Test) [20] to test for association in the presence of linkage [21]. We used three diagnostic models; *(i)* the AITDs model: all AITDs patients were considered as affected, *(ii)* the GD model: only GD patients were considered to be affected and AH patients were considered as unaffected, and *(iii)* the AH model: only AH patients were considered to be affected. We used Version 1.5 of FBAT, which provides a haplotypic test of association [22].

The distribution of alleles in unrelated patients affected with GD *versus* controls was compared by a standard, chi-square in a 2×2 contingency table. A corrected p-value (p_c) < 0.05 was considered significant, where corrected p-values were calculated according to the Bonferroni correction. Odds ratios (OR) were calculated according to Woolf's formula, with 95% confidence intervals (95% CI). IL-1 haplotypes were estimated from population genotype data by PHASE version 2.02 software [23, 24].

The power of the association study was evaluated using functions from the Genetics R package (available on the URL http://cran.r-project.org) based on the method of Long and Langley [25].

RESULTS

Family study

The investigation of the four polymorphisms in the Akr family showed only an association of the IL-1B+3954 polymorphism with AITDs model (multi-allelic mode, recessive model; p = 0.02). No association was found with IL-1RN VNTR, IL-1B-511 C/T SNP or IL-1A-889 C/T SNP with the three models of the FBAT package (p > 0.05). In order to search for a haplotype associated with AITDs, the haplotype-based association test (hbat) was used. The haplotypes were given as follows: IL-1RN/IL-1B-511/IL-1B+3954/IL-1A-889. This analysis showed that the IL-1RN*3/C/T/T and IL-1RN*1/C/T/T haplotypes were found to be associated with AITDs ($\chi^2 = 3.95$, p = 0.047; $\chi^2 = 6.8$, p = 0.009) respectively, but not associated with neither GD nor AH models.

Case-control study

The genotype and allele frequencies of the IL-1RN VNTR, IL-1B-511 C/T, IL-1B+3954 C/T and IL-1A-889 C/T polymorphisms in unrelated GD patients and healthy controls are shown in *table 2*. Departure from Hardy-Weinberg equilibrium (HWE) was tested for each polymorphism investigated using a chi-square test. Only IL-1A-889 C/T SNP was found not to be in HWE in the control population *(table 2)*. The two SNPs in HWE show very high heterozygosity as compared to expected value.

Four alleles were observed for the IL-1RN VNTR, both in case-control samples and the Akr family. IL-1RN*1 and IL-1RN*2 alleles were the most frequent *(table 2)*. There was no significant difference in IL-1RN allele frequencies between GD and the control group ($\chi^2 = 3.16$; df = 3; p = 0.367) *(table 3)*. Also, allele frequencies of the IL-1B-511 and IL-1B+3954 showed no significant difference between GD and the control group ($\chi^2 = 2.18$, p = 0.14; $\chi^2 = 0.01$; p = 0.98 respectively). However, we found a significant increase in the allele and genotype frequencies of the IL-1A-889 C/T polymorphism, in GD patients as compared to controls (χ^2=9.46; p = 0.0021). As this SNP was not in HWE in controls, we calculated a chi-square test of association that corrected for deviation from HWE [26].

Table 1
Characteristics of the four polymorphisms investigated

Set	Position	Id SNP (rs)	PCR product (bp)	Restriction site	Reference
IL-1A promoter	-889 C/T	rs1800587	116	*NcoI*	[17]
IL-1B promoter	-511 C/T	rs16944	304	*AvaI*	[15]
IL-1B Exon 5	+3954 C/T	rs1143634	249	*TaqI*	[16]
IL-1RN	Intron 2	-	240, 325, 410 and 500	-	[18]

Table 2
Allele and genotype frequencies of the polymorphisms investigated in both controls and patients, and the corresponding Hardy-Weinberg equilibrium test

Polymorphism	Controls[a] n = 225 (%)	GD[a] patients n = 131 (%)	H_{obs}[b]	H_{th}[c]	HWE[d]
Allele frequencies					
IL-1RN*1	356 (79.1)	200 (76.3)			
IL-1RN*2	72 (16)	43 (16.4)			
IL-1RN*3	10 (2.2)	12 (4.6)			
IL-1RN*4	12 (2.7)	7 (2.7)			
IL-1B-511 C	216 (48)	110 (42)			
IL-1B-511 T	234 (52)	152 (58)			
IL-1B+3954 C	249 (55.3)	144 (55)			
IL-1B+3954 T	201 (44.7)	118 (45)			
IL-1A-889 T	413 (91.8)	220 (84)			
IL-1A-889 C	37 (8.2)	42 (16)			
Genotype frequencies					
(IL-1RN*1/ IL-1RN*1)	140 (62.2)	79 (60.3)			
(IL-1RN*1/ IL-1RN*2)	62 (27.5)	33 (25.2)			
(IL-1RN*1/ IL-1RN*3)	8 (3.5)	4 (3.0)			
(IL-1RN*1/ IL-1RN*4)	7 (3.1)	5 (3.8)			
(IL-1RN*2/ IL-1RN*2)	4 (1.8)	4 (3.1)			
(IL-1RN*2/ IL-1RN*3)	0	2 (1.5)	0.346	0.348	25.06 ($1.5\ 10^{-5}$)
(IL-1RN*2/ IL-1RN*4)	1 (0.5)	0			
(IL-1RN*3/ IL-1RN*3)	1 (0.5)	3 (2.3)			
(IL-1RN*3/ IL-1RN*4)	0	0			
(IL-1RN*4/ IL-1RN*4)	2 (0.9)	1 (0.8)			
IL-1B-511C/C	26 (11.6)	17 (13)			
IL-1B-511T/C	165 (73.3)	86 (65.7)	0.733	0.500	49.39 ($< 10^{-6}$)
IL-1B-511T/T	34 (15.1)	28 (21.3)			
IL-1B+3954 T/T	23 (10.2)	13 (9.9)			
IL-1B+3954 T/C	155 (68.9)	92 (70.2)	0.689	0.495	34.86 ($< 10^{-6}$)
IL-1B+3954 C/C	47 (20.9)	26 (19.9)			
IL-1A-889 T/T	188 (83.6)	89 (67.9)			
IL-1A-889 T/C	37 (16.4)	42 (32.1)	0.164	0.151	1.80 (0.18)
IL-1A -889 C/C	0	0			

[a] Number (frequency in %) of the alleles or genotypes.
[b] H_{obs}: observed heterozygosity.
[c] H_{th}: expected heterozygosity under HWE ($1-\Sigma p_i^2$ where p_i are allele frequencies).
[d] Chi-square (p-value) for the Hardy-Weinberg equilibrium test.

We found that the association of IL-1A-889 C/T is highly significant after correction ($\chi^2 = 20.63$; $p = 0.000005$). Haplotype analysis of the four IL-1 polymorphisms showed no haplotype associated with GD ($p > 0.05$) in the case-control study.

Linkage disequilibrium analysis between the four markers showed a linkage only between two SNPs: IL-1B-511 and IL-1B+3954 ($\chi 2 = 8.9$; $p = 0.0028$).

Following the recommendations of Kharrat *et al*. [27], we calculated the power of our study at a significance level of $5\ 10^{-5}$ for values of genetic risk ranging from 1.5 to 3, and a risk-allele frequency ranging from 0.1 to 0.5 under the multiplicative model. The power of our sample for a gene with a 1.5 risk ranged from 83.1% to 98.7% depending on allele frequency. For a gene having a relative risk of 2 or more, power is close to 1 for all allele frequencies.

Table 3
Statistical results for the case-control association study

Polymorphism	χ^2		Corrected p-value		OR CI 95%
	Allelic	Genotypic	Allelic	Genotypic	
IL-1RN	3.16	7.55	0.367	0.48	---
IL-1B-511 C/T	2.18	2.70	0.14	0.26	---
IL-1B+3954 C/T	0.01	0.07	0.985	0.964	---
IL-1A-889 C/T	9.46	10.81	0.0021	0.0014	0.47 0.29-0.7

DISCUSSION

The etiology of AITDs is complex and involves multiple genetic and environmental influences. Genetic susceptibility to AITDs is controlled both by genes implicated in thyroid physiology and in the immune reaction [28]. Of the interleukins, the TNF-α gene was found to be implicated in AITDs pathogenesis in the Akr family [29]. The IL-1RN gene has been reported to be associated with GD [30]. However, to our knowledge, to date only few studies have evaluated the relationship between the IL-1B or IL-1A gene polymorphisms and AITDs and they yielded contradictory results [30-32]. We have reported a novel finding that demonstrates a significant association between the IL-1A-889 polymorphism and susceptibility to GD ($p = 0.0021$) in a case-control study. The C/C genotype was absent in both GD patients and controls *(table 2)* even though the effector allele was C. The IL-1A-889 C/C genotype has been associated with significantly lower transcriptional activity of the IL-1A gene and lower levels of IL-1A in plasma compared with the T/T genotype [33]. The mechanism of interaction between IL-1A-889 polymorphism and the level of protein synthesis has not yet been elucidated. It is still unclear whether the IL-1A-889 polymorphism has a direct influence on protein expression or is in linkage disequilibrium with the effector gene in the IL-1 cluster. As regards the IL-1B gene, we focused on two C/T SNPs: the first located at position -511 and the second at position +3954. Investigation of these polymorphisms revealed an association of the IL-1B+3954 C/T with AITDs (multi-allelic mode, recessive model: $p = 0.02$) in the Akr family. No IL-1B polymorphisms was found to be associated with GD in the case-control study ($p > 0.05$). However, Chen *et al*. [34] showed that the IL-1B-511 C/T polymorphism was associated with susceptibility to GD ($p = 0.038$) rather than the IL-1B+3954 C/T polymorphism. The discrepancy between our results and those of others may reflect the different genetic pools represented in the different ethnicities. On the other hand, we were also interested in a VNTR in intron 2 of the IL-1RN gene. Our study showed no association between the IL-1RN polymorphism and AITDs in either familial or the case-control study. Studies that examined the association of this gene polymorphism with susceptibility to GD are limited and contradictory [30-32].

In conclusion, in the Tunisian population, IL-1 gene polymorphisms seem to be associated with AITDs pathogenesis in both familial and case-control cohorts, with different polymorphisms involved (IL-1B+3954 and IL-1A-889 SNPs respectively). In the family studied, two major haplotypes seem to predispose to AITDs. The discrepancy between results in the case-control and familial studies may be explained by the genetic heterogeneity of AITDs and by the fact that the Akr family is an isolated and consanguine population. Whether these polymorphisms have a direct, functional effect on gene expression or this association was due to linkage disequilibrium with another disease-causing polymorphism within or close to the IL-1 gene cluster, remains to be investigated.

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