

Association of an IRF-5 gene functional polymorphism with Sjögren's syndrome

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ABSTRACT

Objective.

Interferon (IFN) regulatory factor 5 (IRF-5) is a transcription factor involved in the regulation of host defense. Previous reports have demonstrated a significant association of various *IRF-5* polymorphisms with systemic lupus erythematosus (SLE), among Caucasians. This case-control study aimed to investigate whether IRF-5 polymorphisms were involved in the genetic predisposition to primary Sjögren Syndrome (pSS), an autoimmune disease closely related to SLE.

Methods.

We analyzed *IRF-5* rs2004640, rs2070197, rs10954213, and rs2280714 polymorphisms in a cohort of 212 pSS patients and 162 controls, all of Caucasian origin. The four studied polymorphisms were genotyped by competitive allele specific polymerase chain reaction (PCR) using FRET technology.

Results.

The IRF-5 rs2004640 GT or TT genotypes (T allele carriers) were found among 87% of pSS patients compared with 77% in controls ($P=0.01$; OR1.93, 95%IC [1.15-3.42]). Likewise, IRF-5 rs2004640 T allele was found on 59% of chromosomes in pSS patients compared with 52% in controls ($P=0.04$; OR 1.36, 95% CI [1.01-1.83]). No significant association was evidenced with rs2070197, rs10954213, and rs2280714 when analyzed independently. Nevertheless, haplotype reconstructions based on the four studied polymorphisms suggest that various allele combinations of rs2004640 and rs2070197 could define susceptibility or protective haplotypes.

Conclusion:

We demonstrated for the first time a significant association of IRF-5 rs2004640 T allele with pSS. These results, which require further replication on larger sample sized populations suggest that, beside association with identical major histocompatibility complex (MHC) gene polymorphisms, pSS and SLE also share IRF-5 polymorphism as a common genetic susceptibility factor.

Primary Sjögren's syndrome (pSS) is a complex auto-immune disease, involving both genetic and environmental factors. Recent data on pathogenic mechanisms involved in pSS have supported the role of the interferon (IFN) pathway through the demonstration of an IFN signature, both in peripheral blood mononuclear cells (PBMC) (1) and in salivary glands (2). Moreover, plasmacytoid dendritic cells, the professional cells secreting IFN α , are present in the salivary glands, the targeted organ of the autoimmune process.

Systemic lupus erythematosus (SLE) shares numerous pathogenic features with pSS: secretion of anti-SSA/SSB, activation of IFN pathways, and recruitment of plasmacytoid dendritic cells (3-6). SLE and pSS also share genetic predisposition factors; indeed, in both diseases, the same HLA alleles are associated with the anti-SSA/SSB secretion pattern: secretion of anti-SSA alone associated with HLA DRB1*15, and secretion of both anti-SSA and anti-SSB associated with DRB1*03 (7, 8).

Recently, polymorphisms of genes involved in interferon signaling have been reported to be significantly associated with SLE, including Tyrosine Kinase 2 (TYK2) and IFN regulatory factor 5 (IRF-5) (9, 10). The rs2004640 polymorphism is located in the first IRF-5 exon. Three different IRF-5 promoters lead to various transcripts containing exon 1A, exon 1B, or exon 1C (9). The rs2004640 polymorphism is located 2 bp downstream of the exon-intron border of exon 1B at a consensus GT donor splice site on exon 1B transcripts. The functional consequences of this polymorphism are important since only individuals with the rs2004640 T allele express transcripts containing exon 1B, beside the transcripts containing exon 1A and 1C. Individuals homozygous for the G allele express IRF5 isoforms containing only exon 1A and 1C (9). In SLE, a significant association of the IRF-5 rs2004640 T allele has been reported among Caucasians by two independent groups, through case-control and intrafamilial association studies (9, 10). A second IRF-5 polymorphism (rs2280714), located about 5 kb downstream of IRF-5, modulates IRF-5 expression and has also been associated with SLE genetic susceptibility (9). SLE is associated with the rs2280714 T allele which leads to an over expression of IRF5 (9). The rs2280714 SNP is in high linkage disequilibrium (LD) with rs2004640, therefore allowing haplotypic association studies. About 14% of IRF5

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haplotypes carrying the rs2280714 T allele, but lacking the rs2004640 T allele, are associated with IRF5 over expression, but are not associated with SLE both in family-based analyses and case-controls studies (9). Thus these results suggest that the genetic susceptibility to SLE is mediated rather by the presence of exon 1B (supported by the rs2004640 T allele), than by an over expression of IRF5 (supported by the rs2280714 A allele).

More recently, an extended analysis of IRF5 locus has evidenced that two additional IRF5 polymorphisms were of primary importance in order to define susceptibility or protective haplotypes for SLE (11). The first one (rs2070197) is located in the 3'UTR of IRF5 and was highly associated to SLE susceptibility; the second one (rs10954213) determines the length of the 3'-UTR of IRF5 mRNAs. The A allele at this SNP is located in the polyadenylation signal sequence that results in a shorter 3'-UTR of *IRF5* mRNA.

The aim of the present study was to search for an association of rs2004640, rs2070197, rs10954213 and rs2280714 IRF-5 polymorphisms with pSS, an auto-immune disorder closely related to SLE.

MATERIALS AND METHODS

Patients

Two hundred and twelve unrelated pSS patients diagnosed according to the European American consensus group criteria (12) (36% without autoantibodies, 30% with anti-SSA only, 33% with both anti-SSA and anti-SSB) and 162 healthy blood donors were genotyped for IRF-5 rs2004640 and rs2280714 polymorphisms. None of the patients had anti-dsDNA. All patients and controls were Caucasians and provided an informed consent. The study was approved by the local ethic committee.

Genotyping

After the isolation of genomic DNA from peripheral blood mononuclear cells, *IRF-5* rs2004640, rs2070197, rs10954213 and rs2280714 polymorphisms were genotyped by competitive allele specific polymerase chain reaction (PCR) using FRET technology.

Statistical analysis

Allelic and genotypic frequencies of *IRF-5* rs2004640, rs2070197, rs10954213, and rs2280714 polymorphisms were compared between patients and controls using 2-sided chi-square test. All genotyped SNPs were in Hardy-Weinberg equilibrium. *IRF-5* haplotypes based on the 4 studied SNPS were also examined for association with pSS. We used the software PHASE (version 2.1) to perform haplotype reconstructions. Alleles included in each haplotype are signaled following their order in the sequence throughout the manuscript: rs2004640, rs2070197, rs10954213 and rs2280714. The average probability of PHASE certainty in haplotype inference was 99% for *IRF-5* haplotypes. *P* values less than 0.05 were considered significant.

Logistic regression was performed to test for an association between the four genetic polymorphisms and the clinical status (patient or control). Adjustment for multiple tests was not considered in this exploratory analysis. If a significant relationship was found, conditional logistic regression was then performed for the other polymorphisms conditioning on this relationship.

RESULTS

Independent SNP analysis

We evidenced a significant difference between pSS patients and controls in phenotype and allele frequencies of IRF-5 rs2004640. Indeed, the IRF-5 rs2004640 GT or TT genotypes (T allele carriers) were found among 87% of pSS patients compared with 77% in controls ($P=0.01$; OR1.93, 95%IC [1.15-3.42]). Likewise, IRF-5 rs2004640 T allele was found on 59% of chromosomes in pSS patients compared with 52% in controls ($P=0.04$; OR 1.36, 95% CI [1.01-1.83]) (Table 1). No significant association was found when testing the TT genotype versus the two other allele combinations (GT and GG) (Table 1). As previous genetic associations in pSS were reported to be rather related to the profile of autoantibody secretion, we searched for a specific association of IRF-5 rs2004640 T allele with the auto-antibody status. The IRF-5 rs2004640 GT or TT genotype frequencies ranged from 84% to 89% in the subgroups of patients without autoantibody, with anti-SSA and with anti-SSA and anti-SSB, without significant difference between groups ($P=0.77$). Thus, the association of IRF-5 rs2004640 T allele was independent from the auto-antibody profile of secretion.

A trend of association was evidenced with rs2280714 polymorphism with the A allele carriers in phenotype studies (Table 1). In fact, IRF-5 rs2280714 A allele was carried by 94% of pSS patients compared with 88% of controls ($P=0.06$; OR 2.04, 95% CI [0.97-4.29]). The same trend concerns allele and genotype frequencies with P values ranging from 0.06 to 0.07. As observed for IRF-5 rs2004640, we did not find any significant association of IRF-5 rs2280714 with the auto-antibody status ($P=0.85$).

No significant association was evidenced with SSp for IRF-5 rs10954213 and IRF-5 rs2070197 when analyzed independently, neither at allelic nor at genotypic or phenotypic levels (Table 1)

Genotype association conditional in the other SNPs

Univariate logistic regression showed a relationship between the genotype for rs2004640 and the presence of pSS, which was attributable to a difference between the carriers of a T allele versus homozygous GG ($p=0.02$ using a χ^2 test). Conditional logistic regression stratifying on rs2004640 (homozygous GG versus other genotypes) showed that there was no significant effect of the three latter genetic polymorphisms once rs2004640 had been taken into account, with p-values of 0.81, 0.74 and 0.91 respectively for rs2280714, rs2070197 and rs10954213.

Haplotype analyses

Haplotype reconstruction with the 4 studied SNPs resulted in 4 main haplotypes among our French Caucasian population (TTAA 35%; GTGG 32%; GTAA 16%; TCAA 13%) and 2 rare haplotypes, TTGA and TTGG (Table 5). These haplotype frequencies among controls were in perfect accordance with those previously reported among 284 European DNA samples from the Human Genome Diversity Project (11). The insertion/deletion polymorphism leading to a 30bp in-frame deletion in exon 6 (exon 6 indel) was not genotyped since it can be unambiguously inferred from haplotype reconstructions due to the high LD with the studied SNPs (Table 5). Thus, 56% of pSS patients and 52% of controls are expected to carry exon 6 deletion, leading to a non significant association of exon 6 indel with pSS ($P=0.21$; OR 0.83, 95% CI [0.61-1.11]).

TTAA haplotype was observed with an increased frequency among pSS patients in comparison with controls ($P=0.03$; OR 1.39, 95% CI [1.02-1.88]) (Table 2). Conversely, two other haplotypes were observed with a slightly decreased frequency among pSS patients in comparison with controls: GTGG and GTAA ($P=0.03$; OR 0.72, 95% CI [0.54-0.97] when pooled together) (Figure 1). The TCAA haplotype was equally represented between patients and controls and thus had a neutral effect on genetic susceptibility to pSS ($P=0.84$; OR 0.96, 95% CI [0.62-1.48] (table 2 and Figure 1). Subsequent haplotype analyses suggest that

rs2004640 and rs2070197 allelic combination could define susceptibility or protective haplotypes (Figure 1).

DISCUSSION

The IRF-5 rs2004640 polymorphism has been previously associated to lupus susceptibility (9-11). We demonstrated for the first time a significant association of IRF-5 rs2004640 T allele with pSS. In our study, patients carrying one or two copies of IRF-5 rs2004640 T allele were at increased risk for pSS. An individual risk for patients homozygous for IRF-5 rs2004640 T allele was not evidenced in this study, probably due to the somehow limited sample size population of patients and controls. In fact, in a Swedish population previously studied, with similar number of lupus patients and controls, an homozygous effect was not evidenced neither (9). The association of IRF-5 rs2004640 T allele with pSS was independent from the auto-antibody status, but the study was underpowered to detect such a difference between autoantibody positive and negative patients. On that point, this result differs with previous positive studies in pSS demonstrating association restricted to the subgroup of patients with anti-SSB and/or anti-SSA antibodies (13). Nevertheless, the two previous significant associations of IRF-5 rs2004640 T allele with SLE did not conditionally involve patients with specific auto-antibody profiles either.

We also found a trend in favor of an association of rs2280714 T allele with pSS. Such association was mainly due to a linkage disequilibrium with rs2004640 T and did not persist after logistic regression conditional on rs2004640. Thus, these results demonstrated that what conferred risk of pSS was mainly the presence of the exon 1B (supported by the rs2004640 T allele) and not the over expression of IRF5 (supported by the rs2280714 A allele). These results are in accordance with those reported in SLE in which conditional analyses on the most associated IRF-5 SNP (rs2070197) led to a non significant association of rs2280714 and exon 6 indel with SLE (11).

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Interestingly, rs2070197 and rs10954213 were not associated with pSS when tested independently, suggesting that none of those two polymorphisms contributes to pSS susceptibility on their own. Nevertheless, subsequent haplotype analyses suggest that rs2004640 and rs2070197 allelic combination could define susceptibility or protective haplotypes. The TTAA haplotype, associated with pSS, has been previously, associated with SLE in the metaanalysis of both case-controls and intrafamilial association studies reported by Graham et.al. (11). Conversely, the TCAA haplotype, associated with SLE, had a neutral effect on pSS susceptibility in our study. It is possibly due to an under power of our study since this haplotype is present in only 13% of the patients versus 35 to 43% for the TTAA haplotype. On the other hand, the protective haplotypes for pSS (GTGG and GTAA) were the same protective haplotypes reported in SLE (11). The functional consequences of IRF-5 susceptibility haplotypes are not yet fully understood and need complementary investigations.

In pSS, there is not only an interferon signature both in PBMCs and in salivary glands, but also the presence of the professional interferon secreting cells, the plasmacytoid dendritic cells, within the salivary glands, the targeted organ of the autoimmune process. Thus, a more efficient function of IRF5 which acts both by promoting the secretion of type I IFN after stimulation of TLRs by microbes or immune complexes, and by amplifying the effects of IFN after fixation to its receptor (14), could be a key event in the pathophysiology of the disease. Moreover, our group recently suggested that in patients with pSS, responding cells could be more sensitive to IFN α effects. Indeed, B-cell activating factor (BAFF), which is an interferon-induced cytokine, is more increased in patients than in controls after stimulation with interferon α of salivary epithelial cells (15) and peripheral blood monocytes (submitted manuscript). Interestingly, epithelial cells secrete more IFN- β than IFN- α and the IRF5 exon 1B transcript, which is on the dependence of the IRF-5 rs2004640 T allele, stimulates production of IFN- β but not IFN- α 1 or IFN- α 2 (11).

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In conclusion, although preliminary, this study provides the first evidence of a significant association of IRF-5 rs2004640 T allele with pSS. Haplotype analyses also suggest an interaction between rs2004640 and rs2070197 alleles in determining protective or susceptibility haplotype for pSS. Nevertheless, further replication studies are necessary, on larger sample sized populations, as well as functional studies. This study is a novel piece of the puzzle suggesting the responsibility of type 1 interferon in the pathogenesis of pSS. A genetic susceptibility favoring a higher interferon response to different stimuli (infections or immune complexes) could be a key event in the onset or the perpetuation of the disease. Last, our results demonstrate new links between lupus and pSS suggesting that the later could be considered as the lupus of mucosa.

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Contribution of authors

CMR and XM performed the study design and wrote the manuscript. EC performed statistical analysis. PL, XP and EH contributed to DNA collection. All authors read and approved the final version.

Legend of Figure

Figure 1: Haplotype analysis restricted to rs2004640 and rs2070197 to define pSS susceptibility or protective haplotypes. OR and 95% CI are represented. In this representation, X appears in the place of any rs10954213 and rs2280714 genotype combination. TCXX haplotype: OR 0.96, 95% CI [0.62-1.48], $P=0.84$; TTXX haplotype: OR 1.44, 95% CI [1.07-1.95], $P=0.015$; GTXX haplotype : OR 0.72, 95% CI [0.54-0.97], $P=0.03$.

Table 1: Phenotype, genotype and allele distribution between patients and controls for IRF-5 rs2004640, rs2070197, rs10954213, and rs2280714. *The allelic frequencies are calculated on 420 chromosomes for patients and 308 chromosomes for controls for IRF-5 rs2004640, on 408 chromosomes for patients and 302 chromosomes for controls for IRF-5 rs2070197, on 380 chromosomes for patients and 262 chromosomes for controls for IRF-5 rs10954213 and on 426 chromosomes for patients and 308 chromosomes for controls for IRF-5 rs2280714. NS= non significant; CI= confidence interval; pSS = primary Sjögren's syndrome.

	Phenotype frequencies		Allele frequencies*		Genotype frequencies		
rs2004640	T positive	G positive	T	G	GG	GT	TT
SSp (n=210)	182 (87)	143 (68)	249 (59)	171 (41)	28 (13)	115 (55)	67 (32)
Controls (n=154)	118 (77)	113 (73)	159 (52)	149 (48)	36 (23)	77 (50)	41 (27)
Odds ratio (95% CI)	1.93 (1.15; 3.42)	0.77 (0.49;1.23)	1.36 (1.01;1.83)	0.73 (0.54;0.98)	1.93 (1.15; 3.42)		1.29 (0.81; 2.04)
P value	0,01	NS	0.04	0.04	(GG vs GT&TT) NS		(TT vs GT&GG) NS
rs2070197	T positive	C positive	T	C	TT	TC	CC
SSp (n=204)	201 (98)	52 (25)	353 (87)	55 (13)	152 (75)	49 (24)	3 (1)
Controls (n=151)	148 (98)	39 (26)	260 (86)	42 (14)	112 (74)	36 (24)	3 (2)
Odds ratio (95% CI)	1.02 (0.64; 1.62)	0.98 (0.61;1.56)	1.04 (0.67;1.60)	0.96 (0.62;1.48)	1.02 (0.62; 1.65)		0.98 (0.60; 1.59)
P value	NS	NS	NS	NS	(TT vs TC&CC) NS		(CC vs TT&TC) NS
rs10954213	A positive	G positive	A	G	AA	AG	GG
SSp (n=190)	175 (92)	104 (55)	261 (69)	119 (31)	86 (45)	89 (47)	15 (8)
Controls (n=131)	116 (98)	73 (56)	174 (66)	88 (34)	58 (44)	58 (44)	15 (11)
Odds ratio (95% CI)	1.06 (0.72; 1.55)	0.94 (0.64;1.38)	1.11 (0.79;1.55)	0.90 (0.64;1.26)	1.04 (0.66; 1.62)		0.96 (0.61; 1.50)
P value	NS	NS	NS	NS	(AA vs AG&GG) NS		(GG vs AG&AA) NS
rs2280714	A positive	G positive	A	G	GG	GA	AA
SSp (n=213)	200 (94)	102 (48)	311 (73)	115 (27)	13 (6)	89 (42)	111 (52)
Controls (n=154)	136 (88)	84 (55)	206 (67)	102 (33)	18 (12)	66 (43)	70 (45)
Odds ratio (95% CI)	2.04 (0.97; 4.29)	0.77 (0.51; 1.16)	1.34 (0.97;1.84)	0.75 (0.54;1.02)	2.04 (0.97; 4.29)		1.31 (0.86; 1.98)
P value	0.06	NS	0.07	0.07	(GG vs GA&AA) 0.06		(AA vs GA&GG) NS

Table 2: Haplotype frequencies of the 4 studied IRF5 SNPs. Only haplotypes with frequency over than 2% are shown. Alleles included in the haplotypes are signalled according to their order in the sequence: rs2004640, rs2070197, rs10954213 and rs2280714. *The exon 6 insertion (Ins) or deletion (Del) variants have been unambiguously inferred from previously reported linkage disequilibrium with the four studied SNPs (11).

No	Haplotypes (*)	% in controls (n)	% in pSS (n)	OR (95% IC)	P value
1	TCAA (Ins)	13 (41)	13 (54)	0.96 (0.62-1.48)	0.84
2	TTAA (Del)	35 (109)	43 (182)	1.39 (1.02-1.88)	0.03
3	TTGA (Ins)	2.6 (8)	4 (16)	1.48 (0.63-3.5)	0.37
4	GTGG (Ins)	32 (100)	27 (115)	0.78 (0.57-1.07)	0.13
5	GTAA (Del)	16 (50)	13 (56)	0.79 (0.52-1.20)	0.26

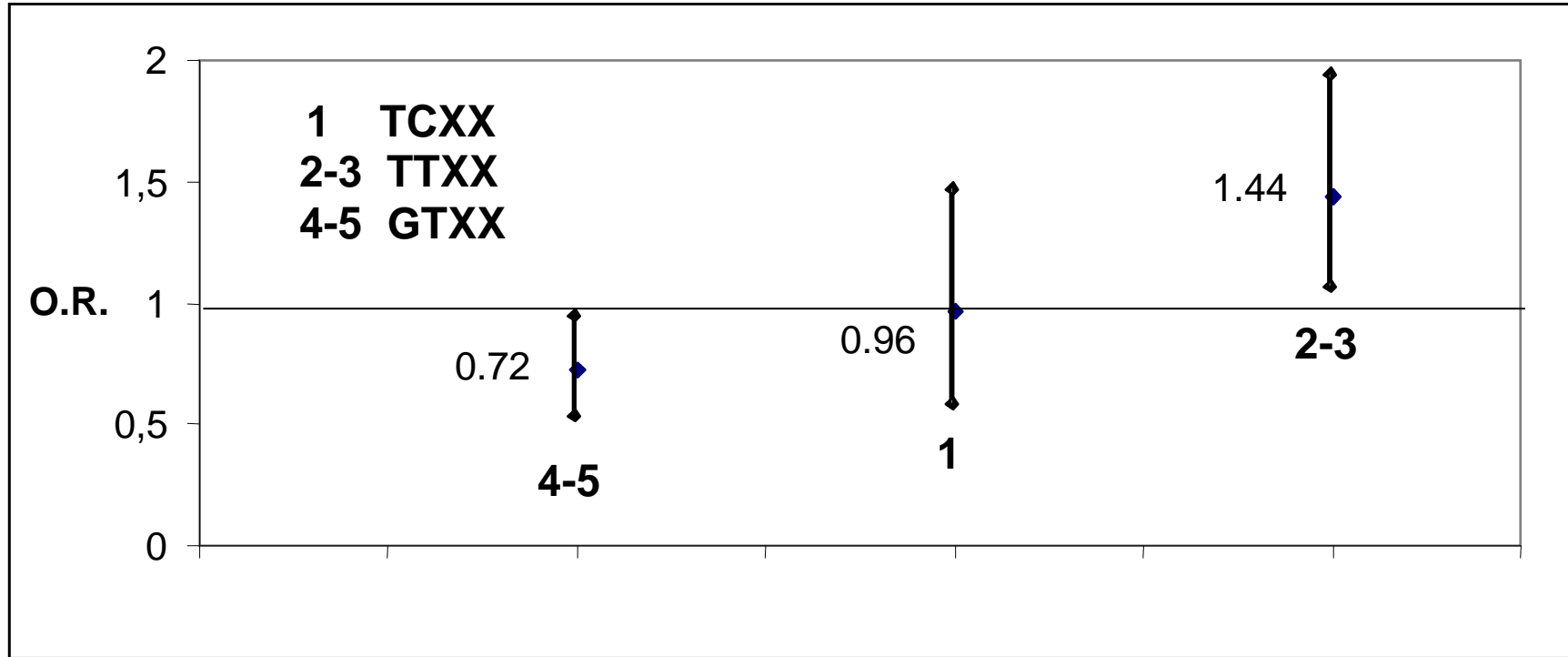


Figure 1

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