Influence of STAT4 Polymorphism in Primary Sjögren's Syndrome

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ABSTRACT. ***Objective.*** To examine the influence of *STAT4* rs7574865 gene polymorphism on patients with primary Sjögren's syndrome (SS).

Methods. Two different cohorts were studied: 69 patients with primary SS and 296 controls from Colombia and 108 patients with primary SS and 227 controls from Germany. Samples were genotyped for the *STAT4* rs7574865 single-nucleotide polymorphism with a predesigned TaqMan single-nucleotide polymorphism genotyping assay. We carried out a metaanalysis of our results combined with data published to date.

Results. Although no significant differences were observed in the allele frequencies of *STAT4* rs7574865 gene polymorphism between patients and controls in Colombians ($p = 0.28$, OR 1.24, 95% CI 0.82–1.87) and Germans ($p = 0.08$, OR 1.40, 95% CI 0.96–2.02), the metaanalysis disclosed a significant effect of the T allele on disease ($p = 4.7 \times 10^{-6}$, OR 1.40, 95% CI 1.21–1.62).

Conclusion. These data reinforce the influence of *STAT4* gene on primary SS and as a general autoimmune gene. (First Release April 1 2010; J Rheumatol 2010;37:1016–9; doi:10.3899/jrheum.091007)

Key Indexing Terms:
STAT4 SJÖGREN'S SYNDROME AUTOIMMUNITY GENETIC STUDIES

Sjögren's syndrome (SS) is a chronic autoimmune disease characterized by progressive lymphocytic and plasma cell infiltration of the salivary and lachrymal glands, accompanied by the production of autoantibodies leading to xerostomia and keratoconjunctivitis sicca (sicca symptoms). SS may occur alone (primary SS; pSS) or in association with other autoimmune diseases (secondary SS; sSS), of which the most frequent are Hashimoto's thyroiditis and rheumatoid arthritis (RA). The powerful influence of genetic predisposition on susceptibility is usually based on disease concordance rates in monozygotic twins. Although genetic studies in pSS twins have not been performed, the observed aggregation of autoimmune diseases in families of patients with pSS supports a genetic component in SS etiology[1-4]. The efforts to identify the genetic component of SS have relied on association studies for disease gene identification. However, robust analyses of candidate gene variants have not been undertaken and no linkage study has been reported to date. In the absence of chromosomal regions identified by linkage studies, research has focused on candidate gene approaches (by biological plausibility) rather than on positional approaches. Association with *HLA* genes has been largely described and confirmed[5-7]. Genes outside the *HLA* region have also been associated with pSS; nevertheless these positive associations, including *PTPN22*[8,9] and *CTLA*, have proven difficult to replicate. Recently, an association of *STAT4* gene polymorphism (rs7574865) at 2q32 with SS has been reported[12,13]. Our objective was to assess the influence of *STAT4* rs7574865 polymorphism on pSS in German and Colombian populations, and to evaluate the global influence of this variant in disease susceptibility with a metaanalysis.

MATERIALS AND METHODS

Study population. Peripheral blood samples were obtained from 2 unrelated cohorts of pSS patients and controls. The Colombian cohort consisted of 69 pSS patients and 296 healthy controls and the German cohort 108 pSS patients and 227 healthy controls. All patients fulfilled the international classification criteria[14] for pSS. Patients and controls were all Caucasian and were matched for age (by mean age) and for sex (by frequency matching). Written informed consent was obtained from all subjects, and the study was approved by the local ethics committee of each center.

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Supported by the Junta de Andalucía, grant CTS-1880, and by Universidad del Rosario, Medellin, Colombia.

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Accepted for publication November 12, 2009.

Demographic characteristics of the patients and controls in each population have been described[15-17].

For the metaanalysis we combined data from North American (case-control ratio 120/1112), Norwegian (case-control ratio 228/669), and Swedish (case-control ratio 140/148) cohorts[12,13] with our findings. It is important to note that Nordmark, *et al*[13] analyzed rs7582694 *STAT4* gene polymorphism, which is a perfect proxy of rs7574865.

Genotyping for STAT4 polymorphism. Samples were genotyped for *STAT4* rs7574865 using a polymerase chain reaction system with a predeveloped TaqMan allelic discrimination assay (Applied Biosystems, Foster City, CA, USA)[18]. Duplicate samples and negative controls were included to check the accuracy of genotyping. The genotyping success rate was ≥ 96%.

Statistical analysis. We used the chi-square test for Hardy-Weinberg equilibrium and statistical analysis to compare allelic and genotypic distributions. Odds ratios (OR) and 95% confidence intervals (95% CI) were calculated according to Woolf's method[19] using the Statcalc program (EpiInfo 2002, Centers for Disease Control and Prevention, Atlanta, GA, USA). P values < 0.05 were considered statistically significant.

To estimate the common effect size of the T allele on disease, a metaanalysis of previous results together with our own was performed. Characteristics of studies included in the metaanalysis are shown in Figure 1. The metaanalysis was conducted using StatsDirect software (StatsDirect, Cheshire, UK). In all the studies, allele and genotyping frequencies were consistent with Hardy-Weinberg equilibrium in patients and controls. Since the number of studies and the average sample size were small, to calculate the inconsistency of the metaanalysis with a power > 80%, pooled calculations of OR were obtained using random effect models[20]. Publication bias was investigated by funnel plotting. The significance of the intercept was determined by the t test suggested by Egger.

The power of the study to detect an effect of a polymorphism on disease susceptibility was estimated for an OR of 1.4 (first described effect size), a type I error rate of 0.05, dominant inheritance mode, and 0.005% of population risk. The power was calculated using Quanto version 0.5 software (Department of Preventive Medicine, University of Southern California, Los Angeles, CA, USA).

RESULTS

No deviation from Hardy-Weinberg equilibrium was observed in the study cohorts for the rs7574865 single-nucleotide polymorphism (SNP). Table 1 shows the *STAT4* rs7574865 genotype and allele distributions for Colombian and German populations. The T allele increased the risk of developing pSS by 24% in Colombian subjects. The same trend was found in the German cohort, in which the frequencies of the T allele showed a clear trend of association (p = 0.08, OR 1.40, 95% CI 0.96–2.02). Since the observed effect of the T allele was not significant due to low sample size (40% power for the Colombian and 45% for the German cohort), we combined data from previous studies in a metaanalysis in order to improve statistical power (> 99% in the metaanalysis). Interestingly, *STAT4* rs7574865 T allele frequencies were quite similar in patients and controls in all the populations, except for the Colombian population, where the T allele frequency in pSS patients was similar to the frequencies in patients with RA and systemic lupus erythematosus (SLE) reported before[18]. Figure 1 shows results of the metaanalysis of all studies with published allele frequencies for *STAT4* rs7574865 in pSS, including the present study. There was no statistically significant evidence for heterogeneity of OR among the studies for this polymorphism in pSS, and the fixed-effects model was chosen (I^2 = 0%). Metaanalysis of all 5 cohorts using the Mantel-Haenszel test revealed a highly significant association of this variant with pSS (pooled OR 1.40, 95% CI 1.21–1.62, p = 4.7×10^{-6}). No publication bias was found in these studies; the Egger test was not statistically significant (bias = 1.09, 95% CI = –1.35651 to 3.53, p = 0.19), with symmetrical funnel plots (data not shown).

DISCUSSION

Our study adds further evidence indicating an influence of

Population	Study	Year	Patients, n	T Allele, %	Controls, n	T Allele, %
American	Korman[12]	2008	120	29.2	1112	22.3
Colombian	Current study	—	69	34.1	296	29.4
German	Current study	—	108	28.2	227	22.0
Norwegian*	Nordmark[13]	2009	228	29.0	669	22.0
Swedish*	Nordmark[13]	2009	140	29.0	148	23.0

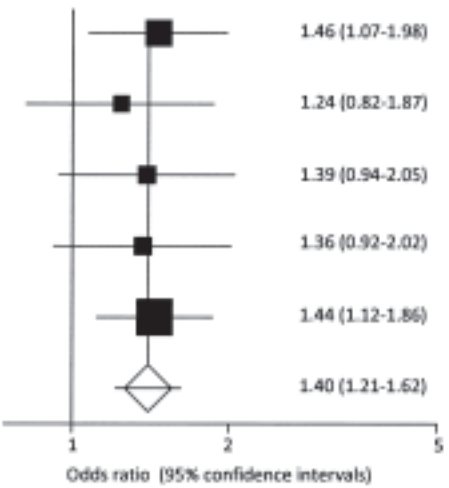

Figure 1. Pooled data for the *STAT4* rs7574865 T allele frequency of North American, Colombian, German, Norwegian, and Swedish populations. OR estimate of each study is marked with a square; the size of the square represents the weight that study exerts in the metaanalysis. Confidence intervals of pooled estimates are shown as a horizontal line. No asymmetry was found, as indicated by the p value of Egger's t test: bias = –1.46 (95% CI –3.44 to 1.14), p = 0.20. *Data correspond to rs7582694 *STAT4* polymorphism that is a perfect proxy of rs7574865.

Table 1. Genotype and allele frequencies of the rs7574865 *STAT4* polymorphism in healthy controls and patients with primary SS.

STAT4 rs7574865	pSS	Controls	p	OR (95% CI)
Colombians	n = 69 (%)	n = 296 (%)		
GG	28 (40.6)	152 (51.4)	0.11	0.50 (0.37–1.14)
GT	35 (50.7)	114 (38.5)	0.06	1.64 (0.94–2.88)
TT	6 (8.7)	30 (10.1)	0.71	0.84 (0.30–2.25)
G	91 (65.9)	418 (70.6)	0.28	0.81 (0.53–1.22)
T	47 (34.1)	174 (29.4)	0.28	1.24 (0.82–1.87)
Germans	n = 108 (%)	n = 227 (%)		
GG	55 (50.9)	138 (60.8)	0.09	0.67 (0.41–1.09)
GT	45 (41.7)	78 (34.4)	0.19	1.36 (0.83–2.24)
TT	8 (7.4)	11 (4.8)	0.34	1.57 (0.56–4.37)
G	155 (71.8)	354 (78)	0.08	0.72 (0.49–1.04)
T	61 (28.2)	100 (22)	0.08	1.40 (0.96–2.02)

STAT4 rs7574865 T allele in pSS, and confirms *STAT4* as one of the most important and replicable genes influencing susceptibility to pSS apart from the *HLA* region.

The association of rs7574865 variant in the *STAT4* gene was initially reported in Korean patients with RA[20a], and almost at the same time in Caucasian patients with RA and SLE[21]. These associations were replicated later in different populations[18,22-25] and in autoimmune diseases such as type I diabetes[26,27] and systemic sclerosis[28]. Together, these data suggest that *STAT4* could be a useful genetic marker of autoimmunity, and they add further evidence for a common genetic origin of diverse autoimmune diseases[29]. Nevertheless, there are other autoimmune diseases in which *STAT4* rs7465865 showed no influence, such as giant cell arteritis[30] and multiple sclerosis[26]; the reason *STAT4* has different effects in different autoimmune diseases remains unknown.

STAT4 genes encode a transcription factor that transmits signals induced by interleukin 12 (IL-12), IL-23, and type 1 interferon[31]. A major action of IL-12 through *STAT4* signaling is to promote the differentiation of naive CD4+ T cells into T-helper 1 (Th1) cells, which produce interferon-γ. These Th1 cells are thought to drive the chronic autoimmune response. *STAT4* is also important for the development of IL-17-secreting Th cells in response to IL-23[32]. As both lineages are master regulators of the etiopathology of pSS in humans[33,34], *STAT4* may exert its influence through defective signaling in these pathways.

Although it seems clear that *STAT4* plays a key role in several pathways in autoimmunity, the functional role(s) of the associated polymorphism(s) remains to be elucidated. The rs7574865 *STAT4* variant is located at the third intron of the gene, suggesting a role in *STAT4* splicing. Nevertheless, Abelson, *et al*[25] observed no differences in splicing of the gene in peripheral blood mononuclear cells (PBMC). Instead, they reported a correlation between expression levels of *STAT4* in PBMC and the risk allele of the *STAT4* rs7574865-associated SNP. Additional studies are needed to clarify the role of *STAT4* as a novel marker for autoimmune diseases.

ACKNOWLEDGMENT

We thank all the participants in this study.

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