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Identification of *IL12RB1* as a novel systemic sclerosis susceptibility *locus*

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Genome-wide association studies (GWASs) have identified several immune-related *loci* associated with systemic sclerosis (SSc) that clearly support the important role of the immune system in the disease etiology (1–2). Using the Gene Set Enrichment Analysis (GSEA) and DAVID algorithms in the Biocarta pathway collection, we found that the most enriched pathways in SSc corresponded to the NO2-dependent IL-12 pathway in NK cells and the IL-12 and STAT4 dependent signaling pathway in Th1 development (Supp Methods and Supp Table 1). Moreover, several studies have implicated IL-12 in autoimmune inflammatory processes (3). Interestingly, in our recent large-scale fine mapping Immunochip study in SSc (4), we observed suggestive association signals in the *IL12RB1 locus*, which encodes the beta 1 subunit of the IL-12 receptor. Consequently, our aim was to evaluate for the first time the genetic contribution of the *IL12RB1* region in SSc through a follow-up strategy. Forty-six single-nucleotide polymorphisms (SNPs) within *IL12RB1* were screened in 1,871 SSc cases and 3,636 controls included in the SSc Immunochip discovery cohorts (4). In this first phase, eleven out of the 46 SNPs showed nominal association signals (Supp Table 2 and Supp Fig 1). After conditional logistic regression, we selected four SNPs (rs8109496, rs2305743, rs436857 and rs11668601) as the genetic variants which better explained the observed signals in the *IL12RB1* region (Supp Table 3 and Supp Methods). Therefore, these SNPs were selected for genotyping in six independent replication cohorts of European ancestry (Spain, Germany, Netherlands, Italy, Sweden and the United Kingdom) reaching 3,181 SSc patients and 5,076 controls. All SSc patients fulfilled previously described classification criteria for SSc (4). One SNP, rs2305743, achieved genome-wide significance level in the independent replication cohorts (P_{MH} = 3.936×10^{-8}, OR= 0.79) (Supp Table 4). Interestingly, the combined analysis (5,052 SSc patients and 8,712 controls) showed that the four selected SNPs were associated with SSc at the genome-wide significance level (Table 1 and Supp Table 5), providing robust evidence for the implication of *IL12RB1* in SSc development. Despite that dependence analysis could not discern among variants (Supp Table 6), our *in silico* functional analysis showed that minor alleles of both rs436857 promoter variant and rs2305743 were in *IL12RB1* cis-eQTLs that decreased *IL12RB1* expression (*P*-value = 2.4×10^{-81}, Z-score = −19.10; and *P*-value = 9.6×10^{-80}, Z-score = −18.91, respectively) (Supp Methods). Additionally, rs436857 showed evidence to affect the binding of several transcription factors in the ENCODE database (Supp Methods), such as POLR2A (the largest subunit of RNA polymerase II) and YY1 (a ubiquitously distributed transcription factor that interacts with POLR2A). Therefore, we hypothesize that this promoter SNP should be the best candidate for driving the reported association, narrowing down the signal to the promoter region. The protective OR and the decrease in *IL12RB1* expression related to these variants would be consistent with a reduced IL-12 response and a lower SSc susceptibility. Moreover, the coexpression of IL12Rβ1 and IL12Rβ2 is necessary to form the high-affinity IL-12 receptor and IL-12 binding leads to the activation of STAT4. Remarkably, coding genes for these proteins (*IL12RB2*, *IL12A*,

STAT4) are well-established risk factors for SSc (4–5,1). Therefore, we report for the first time the association of *IL12RB1* with SSc and add a novel IL-12 pathway related gene into the list of SSc susceptibility *loci.* These results highlight the special relevance of this pathway in SSc pathophysiology, its integration in the SSc genetic susceptibility context (Supp Fig 2), and suggest that blocking this pathway could be a possible new therapeutic target in an orphan disease such as scleroderma.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Analysis of *IL12RB1* SNPs minor allele frequencies in the overall combined analysis.

			Genotype, N (%)				Allele test		
SNP	**Minor/major**	**Subgroup (N)**	**1/1**	**1/2**	**2/2**	**MAF (%)**	P_{MH}	P_{BD}	**OR* [CI 95%]**
rs8109496	C/G	Controls (n=8697)	329 (3.78)	2736 (31.46)	5632 (64.76)	19.51			
		SSc (n=5036)	148 (2.94)	1343 (26.67)	3545 (70.39)	16.27	7.347E-09	0.32	0.82 [0.77–0.88]
rs2305743	A/G	Controls (n=8697)	353 (4.06)	2796 (32.15)	5548 (63.79)	20.13			
		SSc (n=5032)	166 (3.30)	1358 (26.99)	3508 (69.71)	16.79	4.294E-10	0.10	0.81 [0.76–0.87]
rs436857	A/G	Controls (n=8652)	297 (3.43)	2652 (30.65)	5703 (65.92)	18.76			
		SSc (n=4924)	142 (2.88)	1245 (25.28)	3537 (71.83)	15.53	3.938E-09	0.23	0.81 [0.76–0.87]
rs11668601	C/T	Controls (n=8682)	539 (6.21)	3302 (38.03)	4841 (55.76)	25.22			
		SSc (n=4962)	245 (4.94)	1690 (34.06)	3027 (61.00)	21.97	5.612E-09	0.06	0.84 [0.79–0.89]

* Odds ratio for the minor allele

CI, confidence interval; MAF, minor allele frequency; OR, odds ratio; P_{BD}, Breslow–Day test P-value; P_{MH}, allelic Mantel-Haenszel fixed effects model *P*-value; SNP, single nucleotide polymorphism; SSc, systemic sclerosis.