EXTENDED REPORT

Implication of *IL-2/IL-21* region in systemic sclerosis genetic susceptibility

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▶ Additional supplementary data are published online only. To view these files please visit the journal online (http://dx.doi.org/10.1136/annrheumdis-2012-202357).

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Accepted 21 October 2012
Published Online First 21 November 2012

ABSTRACT

Objective The interleukin 2 (*IL-2*) and interleukin 21 (*IL-21*) locus at chromosome 4q27 has been associated with several autoimmune diseases, and both genes are related to immune system functions. The aim of this study was to evaluate the role of the *IL-2/IL-21* locus in systemic sclerosis (SSc).

Patients and methods The case control study included 4493 SSc Caucasian patients and 5856 healthy controls from eight Caucasian populations (Spain, Germany, The Netherlands, USA, Italy, Sweden, UK and Norway). Four single nucleotide polymorphisms (rs2069762, rs6822844, rs6835457 and rs907715) were genotyped using TaqMan allelic discrimination assays.

Results We observed evidence of association of the rs6822844 and rs907715 variants with global SSc (p_c=6.6E-4 and p_c=7.2E-3, respectively). Similar statistically significant associations were observed for the limited cutaneous form of the disease. The conditional regression analysis suggested that the most likely genetic variation responsible for the association was the rs6822844 polymorphism. Consistently, the rs2069762A-rs6822844T-rs6835457G-rs907715T allelic combination showed evidence of association with SSc and limited cutaneous SSc subtype (p_c=1.7E-03 and p_c=8E-4, respectively).

Conclusions These results suggested that the *IL-2/IL-21* locus influences the genetic susceptibility to SSc. Moreover, this study provided further support for the *IL-2/IL-21* locus as a common genetic factor in autoimmune diseases.

INTRODUCTION

Interleukin 2 (*IL-2*) and interleukin 21 (*IL-21*) are equally attractive biological candidates that may influence the pathogenesis of autoimmune diseases. Both are cytokines involved in the proliferation of T and B lymphocytes and different immunological activation pathways.[1] Moreover, the *IL-2* and *IL-21* genes cover a region of approximately 200 kb that maps in the 4q27 locus. *IL-2* has an important role in the maintenance of immune system homeostasis and self-tolerance. This cytokine has two paradoxical roles: promoting T cell proliferation and terminating T cell responses. Moreover, *IL-2* facilitates the production of immunoglobulins through B cells and induces the differentiation and proliferation of natural killer cells.[1 2] *IL-21* is a potent immunomodulatory cytokine with pleiotropic effects on both innate and adaptive immune responses. These actions include the following positive effects: enhanced proliferation of lymphoid cells, increased cytotoxicity of CD8 T cells and natural killer cells, and differentiation of B cells into plasma cells. *IL-21* is also produced by T helper 17 (Th17) cells and is a critical regulator of Th17 development.[1 3] Genetic association studies have demonstrated that several *IL-2/IL-21* polymorphisms influence the risk for autoimmune diseases (AIDs). The first evidence of this association was found in type 1 diabetes, Graves' disease, coeliac diseases and rheumatoid arthritis.[4–7] These results have been confirmed through replication studies in different populations and extended to other autoimmune diseases, such as inflammatory bowel diseases, giant cell arthritis, psoriasis and systemic lupus erythematosus (SLE).[8–17]

Systemic sclerosis (SSc) is a chronic fibrotic autoimmune disease in which patients are commonly classified into the following two major subgroups that are related to the specific autoantibodies against several nuclear and/or nucleolar antigens: (i) limited cutaneous SSc (lcSSc), which is related to the positive status of anticentromere autoantibodies (ACA) and (ii) diffuse cutaneous (dcSSc), which is related to the positive status of antitopoisomerase

autoantibodies (ATA).[18–22] More than 40 susceptibility loci to SSc have been identified during the last 10 years. Half of these variants need to be replicated in different populations and many of these variants are shared among different AIDs, especially SLE.[22–25] In this regard, one single nucleotide polymorphism (SNP) of the *IL-2* gene was proposed as risk factor to lcSSc subtype,[26] but this association has not been confirmed by other studies. Moreover the *IL-21* gene has been implicated as a potential driver of AIDs and recently a fine-mapping in SLE demonstrated that variants of the *IL-2/IL-21* region are implicated in the genetic susceptibility to SLE.[12 16] Thus, the aim of this study was to evaluate the influence of the *IL-2/IL-21* region in SSc genetic susceptibility.

PATIENTS AND METHODS

Subjects

This case-control association study was comprised of 4493 SSc patients and 5896 controls of Caucasian ancestry. The discover cohort included the Spanish group, which consisted of 1176 SSc patients and 1721 healthy controls. The follow-up phase consisted of the following subjects: 609 SSc cases and 426 controls from Germany, 365 SSc cases and 734 controls from the Netherlands, 916 SSc cases and 884 controls from USA, 595 SSc cases and 1107 controls from Italy, 225 SSc cases and 273 controls from Sweden, 374 SSc cases and 436 controls from the UK and 102 SSc cases and 278 controls from Norway. There was an overlapping of 1726 SSc and 2578 controls with the previous GWAS in SSc.[25] The patients fulfilled the 1980 American College of Rheumatology classification criteria for SSc[27] or the criteria proposed for early SSc.[21] In addition, the patients were classified as having lcSSc or dcSSc as described by LeRoy *et al*.[21] The following clinical data were collected for the ascertainment of the clinical phenotype of the SSc patients: age, gender and presence of SSc-specific autoantibodies (Ab; ACA and ATA). The control population consisted of unrelated healthy individuals recruited in the same geographical regions as the SSc patients, and they were matched by age, sex and ethnicity with the SSc patient groups. The study was approved by local ethical committees from all the participating centres. Both patients and controls were included in the study after written informed consent was obtained.

SNP Selection and genotyping

Four SNPs of the *IL-2/IL-21* region were selected for this study. The rs2069762 SNP was selected because it has been suggested to be a genetic factor of lcSSc subtype susceptibility by a study in a small Italian cohort.[26] SSc and SLE share some immunogenetic pathways; thus, the rs6822844, rs6835457 and rs907715 *IL-2/IL-21* polymorphisms were studied because they are the most associated variants in a recent fine-mapping of the region in SLE.[12]

DNA from the patients and the controls were extracted from peripheral white blood cells following standard procedures. The samples were genotyped for the rs2069762, rs6822844, rs6835457 and rs907715 *IL-2/IL-21* region polymorphisms using predesigned SNP genotyping assays from Applied Biosystems (Assay IDs: C__15859930_10, C__28983601_10, C___1597475_10 and C___8949748_10, respectively). TaqMan SNP genotyping was performed using a 7900HT Real-Time PCR system from Applied Biosystems following the manufacturer's suggestions (Foster City, California, USA). In all the cohorts, the genotyping success rate was greater than 95%, and randomly selected samples were genotyped twice to verify the genotyping accuracy. Ninety-nine per cent of the genotypes were identical.

Statistical analysis

The Hardy-Weinberg equilibrium was tested for all the SNPs in all the studied populations. Significance was calculated using 2×2 contingency tables and Fisher's exact test or the χ^2 test when necessary to obtain p values, ORs and 95% CIs using PLINK (V.1.07) software (http://pngu.mgh.harvard.edu/purcell/plink/).[28] The p values less than 0.05 were considered to be statistically significant. The Bonferroni correction was applied to the significant p values and referred in the text as p_c ($p_{corrected}$). Cochran-Mantel-Haenszel meta-analysis was performed to control the differences among populations as implemented by the PLINK software. In addition, the Breslow-Day test (BD test) and the Higgins' test (I^2) were performed using the PLINK software in each meta-analysis. The random-effects model was checked in the significant BD P_{values} analysis. The dependency of the association between each SNP and every studied genetic variant was determined by a conditional logistic regression analysis (considering the different cohorts as covariates) using the PLINK software. Linkage disequilibrium (LD) patterns between the four studied SNPs were estimated by the expectation-maximisation algorithm using HAPLOVIEW (V.4.2; Broad Institute of MIT and Harvard) and PLINK software. To evaluate the allelic combination difference between cases and controls, the conditional haplotype-based associations test was applied using the PLINK software.[29] The statistical power of the combined analysis was between 91% and 99% for all the SNPs, allowing for the detection of associations with an OR equal to 1.2 at a 5% significance level and the lowest minor allelic frequency, according to the Power Calculator for Genetic Studies 2006 software, which uses the methods described by Skol *et al*.[30]

RESULTS

The cases and controls of the eight Caucasian populations were in Hardy-Weinberg equilibrium at a 5% significance level. Additionally, the minor allelic frequencies of the four studied SNPs were similar to those reported by the HapMap project for the Utah residents with ancestry from northern and western Europe (CEU) population (http://hapmap.ncbi.nlm.nih.gov/). The LD structure of the eight cohorts is shown in the supplemental material (see online supplementary figure S1).

First, an association study was conducted in a Spanish case-control set, and a significant association was observed between the rs907715 SNPs minor allele and the global SSc (p_c=0.03, OR=0.85 95% CI 0.8 to 0.9) and the lcSSc subtype (p_c=0.04, OR=0.83 95% CI 0.7 to 0.9). A trend of association was observed between the minor allele of the rs6822844 SNP and the global SSc (p_{value}=0.04, OR=0.84 95% CI 0.7 to 1) and lcSSc subtype (p_{value}=0.04, OR=0.79 95% CI 0.7 to 0.9). Also a trend of association was detected between the minor allele of rs6835457 and lcSSc subtype in this population (p_{value}=0.03, OR=0.87 95% CI 0.8 to 1). In contrast, no association was observed with the rs2069762 SNP (p_{value}=0.8 for both SSc and lcSSc) (see online supplementary tables S1–S3). Based on these observations, we decided to evaluate other Caucasian cohorts and to perform a meta-analysis.

Table 1 shows the meta-analysis results for the *IL-2/IL-21* SNPs, the global SSc, the main SSc subtypes, the ACA and the ATA antibodies positive status. The combined analysis showed that the minor allele frequencies of the rs6822844 and rs907715 SNPs were significantly higher in controls than in SSc (p_c=6.6E-04 OR=0.86 95% CI 0.79 to 0.93 and p_c=7.2E-3 OR=0.91 95% CI 0.85 to 0.96, respectively)

Table 1 Genotype and minor allele frequencies of meta-analysis of four *IL-2/IL-21* SNPs located in SSc patients and healthy controls from European and US populations

			Genotype, N (%)				Allele test		
SNP	**1/2**	**Subgroup (N)**	**1/1**	**1/2**	**2/2**	**MAF (%)**	**p Value***	**p_c†**	**OR (CI 95%)‡**
rs2069762	C/A	Controls (n=5482)	510 (9.30)	2266 (41.34)	2706 (49.36)	29.97			
		SSc (n=4281)	429 (10.02)	1778 (41.53)	2074 (48.45)	30.79	0.08	NA	1.06 (0.99 to 1.13)
		lcSSc (n=2897)	295 (10.18)	1203 (41.53)	1399 (48.29)	30.95	0.09	NA	1.06 (0.99 to 1.14)
		dcSSc (n=1384)	134 (9.68)	575 (41.55)	675 (48.77)	30.46	0.31	NA	1.05 (0.96 to 1.15)
		ACA+ (n=1736)	170 (9.79)	730 (42.05)	836 (48.16)	30.82	0.25	NA	1.05 (0.97 to 1.14)
		ATA+ (n=1031)	94 (9.12)	428 (41.51)	509 (49.37)	29.87	0.98	NA	1.00 (0.90 to 1.11)
rs6822844	T/G	Controls (n=5792)	149 (2.57)	1475 (25.47)	4168 (71.96)	15.31			
		SSc (n=4407)**	98 (2.22)	996 (22.60)	3313 (75.18)	13.52	1.7E-04	6.6E-04	0.86 (0.79 to 0.93)
		lcSSc (n=2977)***	67 (2.25)	659 (22.14)	2251 (75.61)	13.32	1.5E-04	6.0E-04	0.84 (0.76 to 0.92)
		dcSSc (n=1430)	31 (2.17)	337 (23.57)	1062 (74.27)	13.95	0.06	NA	0.89 (0.79 to 1)
		ACA+ (n=1763)	38 (2.16)	395 (22.40)	1330 (75.44)	13.36	0.01	0.06	0.87 (0.78 to 0.97)
		ATA+ (n=1074)	29 (2.70)	257 (23.93)	788 (73.37)	14.66	0.67	NA	0.97 (0.85 to 1.11)
rs6835457	G/A	Controls (n=5720)	668 (11.68)	2507 (43.83)	2545 (44.49)	33.59			
		SSc (n=4392)****	445 (10.13)	1908 (43.44)	2039 (46.43)	31.85	0.013	0.05	0.93 (0.87 to 0.98)
		lcSSc (n=2965)*****	312 (10.52)	1255 (42.33)	1398 (47.15)	31.69	0.014	0.06	0.92 (0.86 to 0.98)
		dcSSc (n=1427)	133 (9.32)	653 (45.76)	641 (44.92)	32.20	0.28	NA	0.95 (0.87 to 1.04)
		ACA+ (n=1765)	186 (10.54)	756 (42.83)	823 (46.63)	31.95	0.12	NA	0.94 (0.86 to 1.02)
		ATA+ (n=1064)	113 (10.62)	481 (45.21)	470 (44.17)	33.22	0.99	NA	1.00 (0.90 to 1.10)
rs907715	T/C	Controls (n=5644)	670 (11.87)	2491 (44.14)	2483 (43.99)	33.94			
		SSc (n=4341)******	437 (10.07)	1883 (43.38)	2021 (46.56)	31.76	1.8E-03	7.2E-03	0.91 (0.85 to 0.96)
		lcSSc (n=2929)*******	307 (10.48)	1236 (42.20)	1386 (47.32)	31.58	2.7E-03	0.01	0.90 (0.84 to 0.96)
		dcSSc (n=1412)	130 (9.21)	647 (45.82)	635 (44.97)	32.12	0.14	NA	0.93 (0.85 to 1.02)
		ACA+ (n=1744)	180 (10.32)	754 (43.23)	810 (46.44)	31.94	0.05	NA	0.92 (0.85 to 1)
		ATA+ (n=1056)	109 (10.32)	475 (44.98)	472 (44.70)	32.81	0.48	NA	0.96 (0.87 to 1.07)

*All p values have been calculated for the allelic model.
**Breslow-Day p_{value}=0.29. Higgins' test (I^2)=17.3%. Random-effects model p_{value}=8.8E-04 p_c=3.5E-3 Random-effects OR=0.86.
***Breslow-Day p_{value}=0.16. I^2=33.9%. Random-effects model p_{value}=4.1E-03. Random-effects OR=0.84.
****Breslow-Day p_{value}=0.06. I^2=48.6%. Random-effects model p_{value}=0.1. Random-effects OR estimate=0.93.
*****Breslow-Day p_{value}=0.09. I^2=43.4%. Random-effects model p_{value}=0.11. Random-effects OR estimate=0.92.
******Breslow-Day p_{value}=0.02. I^2=58%. Random-effects model p_{value}=0.08. Random-effects OR estimate=0.91.
*******Breslow-Day p_{value}=0.09. I^2=43.7%. Random-effects model p_{value}=0.05. Random-effects OR estimate=0.91.
†If it is applicable, Bonferroni correction is shown.
‡OR for the minor allele.
ACA, anticentromere autoantibodies; ATA, antitopoisomerase autoantibodies; dcSSc, diffuse cutaneous SSc; NA, not applicable; SNP, single nucleotide polymorphisms; SSc, systemic sclerosis.

and lcSSc patients (p_c=6E-4 OR=0.84 95% CI 0.76 to 0.92 and p_c=0.01 OR=0.9 95% CI 0.84 to 0.96, respectively). A trend of association was observed in the meta-analysis for the rs6822844 and rs6835457 variants and ACA positive status (p_{value}=0.01 OR=0.87 95% CI 0.78 to 0.97 and p_{value}=0.05 OR=0.92 95% CI 0.85 to 1, respectively). The rs6835457 SNP also had a trend of association with global SSc and lcSSc (p_{value}=0.01 OR=0.93 95% CI 0.87 to 0.98 and

Table 2 Conditional logistic regression analysis for the *IL-2/IL-21* SNPs located in SSc considering the eight European and US populations as covariate

						r2 with rs6822844							
Group of analysis	**SNP**	**MAF Cases**	**MAF Controls**	**p value of each SNP conditioned by rs6822844**	**p value of rs6822844 conditioned by each SNP**	**Spain**	**Germany**	**The Netherlands**	**USA**	**Italy**	**Sweden**	**UK**	**Norway**
SSc													
	rs2069762	0.31	0.30	0.69	1.30E-03	0.06	0.06	0.09	0.07	0.07	0.11	0.06	0.09
	rs6835457	0.32	0.34	0.43	0.024	0.25	0.37	0.38	0.36	0.28	0.48	0.37	0.49
	rs907715	0.32	0.34	0.19	0.026	0.26	0.37	0.39	0.36	0.29	0.39	0.37	0.49
lcSSc													
	rs2069762	0.31	0.30	0.69	9.07E-04	–	–	–	–	–	–	–	–
	rs6835457	0.32	0.34	0.53	0.014	–	–	–	–	–	–	–	–
	rs907715	0.32	0.34	0.3	0.015	–	–	–	–	–	–	–	–
ACA+													
	rs2069762	0.31	0.30	0.64	0.015	–	–	–	–	–	–	–	–
	rs6835457	0.32	0.34	0.81	0.061	–	–	–	–	–	–	–	–
	rs907715	0.32	0.34	0.56	0.063	–	–	–	–	–	–	–	–

ACA, anticentromere autoantibodies; lcSSC, limited cutaneous SSc; MAF, minor allelic frequencies; SNP, single nucleotide polymorphisms; SSc, systemic sclerosis.

p_{value}=0.01 OR=0.92 95% CI 0.86 to 0.98, respectively). We did not detect any significant association between the rs6835457 or rs2069762 SNPs and the global SSc diseases or its different phenotypes (for detailed information see supplementary tables S1 through S3). It is worth noting that the minor I^2 percentage was observed in the meta-analysis for the rs6822844 SNP with SSc (17.3%) and lcSSc (33.9%), suggesting that the variation between the populations is moderate. Moreover, these analyses were the only ones that remained significant in the random-effect model (rs6822844 and SSc p_c=3.5E-3, rs6822844 and lcSSc p_c=0.016).

A conditional logistic regression analysis was used to identify which SNP could be the causal SNP for the observed associations between the studied polymorphisms. The association of each SNP was evaluated using the populations as covariates, and the association was conditioned to the rs6822844 SNP because the lowest p_{value} and strongest effect (OR) were observed in this locus. Pairwise conditional analysis showed that the association of the rs907715 SNP was explained by the rs6822844 effect, because only the latter SNP remained significant after conditioned to each other (rs907715 conditioned p_{value}=0.19; rs6822844 conditioned p_{value}=0.026). Moreover, the rs2069762 and rs6835457 SNPs exhibited significance only when conditioned to the rs6822844 SNP. These results suggested that the rs6822844 signal could explain the association observed in the *IL-2/IL-21* locus (table 2).

Finally, the results of the conditional haplotype-based association testing are shown in table 3. The allelic combination formed by the rs2069762 major allele and the rs6822844, rs6835457 and rs907715 minor alleles was significantly increased in the controls compared with the global SSc (p_c=1.7E-3, OR=0.89 95% CI 0.81 to 0.98), the lcSSc subtype (p_c=8E-4, OR=0.86 95% CI 0.77 to 0.96) and the ACA positive status (p_c=2.7E-2, OR=0.86 95% CI 0.75 to 0.98). Interestingly, the OR observed for this analysis was not different from the one observed in the allelic test. Moreover, the significant effect of the omnibus analyses for SSc, lcSSc and ACA positive status disappeared when they were controlled by the rs6822844 SNP (p_{values} of the likelihood ratio test were: p_{value}=0.66 for global SSc, p_{value}=0.74 for lcSSc and p_{value}=0.93 for ACA+).

DISCUSSION

Our study suggests for the first time the influence of the rs6822844 polymorphism of the *IL-2/IL-21* region in susceptibility to SSc. This variant also influences the lcSSc subtype of the diseases and probably the ACA positive status due to the trend of association observed between the rs6822844 polymorphism and this phenotype. Although, our study had sufficient statistical power for both dcSSc and ATA analysis (95% and 91%, respectively), we observed that there were not significant associations between the four *IL-2/IL-21* SNPs and dcSSc or ATA positive status. The ORs exhibited the same direction as the significant associations with SSc and lcSSc, suggesting that an increment in the sample size with future studies could show a significant relation between the *IL-2/IL-21* SNPs and dcSSc or ATA. Interestingly, the rs6822844 variant was associated in the same OR direction as that observed in SLE. The minor allele of this variant is more frequent in healthy donors than in SSc patients, lcSSc subtype subjects and SLE patients.[12 16] The logistic regression and the allelic combination analyses support that the rs6822844 SNP association was responsible for the observed associations. The rs2069762A-rs6822844T-rs6835457G-rs907715T allelic combination was associated as a protective factor to SSc, lcSSc subtype and ACA positive status, which is the same effect observed for the T allele of the rs6822844. Importantly, the ORs observed for this allelic combination were not different from the ORs observed for the rs6822844 SNP analysis. These observations were slightly different from the results of the SLE study performed by Hughes *et al*[12] where the observed association between *IL-2/IL-21* region and SLE could be explained by the rs6835457 and rs907715 SNPs. Together, these results support the idea that the common genetic factors in autoimmune diseases may be associated at a regional level but differ in the specific SNPs associated with each disease, including the magnitude and direction of the association.[31 32] Although, the logistic regression test and the allelic combination analyses conditioned by the rs6822844 SNP suggest that this variant is responsible for the association observed in the region; we cannot totally discard a slight role of the rs6835457 and rs907715 polymorphisms in SSc due to the moderate LD between them and the r6822844 SNP.

The rs6822844 and rs6835457 SNPs are located in the flanking 3′-untranslated region of *IL-21*, and the rs907715 polymorphism is located in intron 3 of the *IL-21* gene. In contrast, the rs2069762 SNP is located in the flanking 5′-untranslated region of *IL-2*, which did not exhibit significant association with SSc, the subtypes of the disease or the antibodies' status. The rs2069762 minor allele has been previously associated with the lcSSc subtype.[26] Our study has a considerably larger sample size than the previous study; therefore the previously reported

Table 3 Conditional haplotype-based association analysis of four *IL-2/IL-21* SNPs located according to diseases, lcSSc diseases subtype and ACA status and considering the eight European and US populations as covariate

	Frequency					Frequency				Frequency			
Allelic combination†	Controls	SSc	OR (CI 95%)	P*	Pc‡	lcSSc	OR (CI 95%)	P*	Pc‡	ACA+	OR (CI 95%)	P*	Pc‡
AGGT	0.182	0.181	-ref-	–**	–	0.181	-ref-	–***	–	0.183	-ref-	–****	–
ATGT	0.152	0.133	0.89 (0.81 to 0.98)	4.12E-04	1.65E-03	0.131	0.86 (0.77 to 0.96)	2.01E-04	8.04E-04	0.132	0.86 (0.75 to 0.98)	6.81E-03	2.72E-02
CGAC	0.298	0.306	1.04 (0.96 to 1.12)	0.18	NA	0.308	1.03 (0.94 to 1.13)	0.2	NA	0.308	1.02 (0.92 to 1.14)	0.27	NA
AGAC	0.369	0.379	1.04 (0.96 to 1.13)	0.1	NA	0.38	1.03 (0.94 to 1.13)	0.12	NA	0.377	1.02 (0.91 to 1.13)	0.4	NA

†The order of the SNPs is rs2069762, rs6822844, rs6835457, rs907715.
*p_{value} of the likelihood ratio test. Based on WHAP method.[29]
‡If it is applicable, Bonferroni correction is shown. Not applicable (NA).
**Omnibus test X^2=14.5 (df=3); p value=2.35E-03; Pc=9.4E-03.
***Omnibus test X^2=14.5 (df=3); p value=2.32E-03; Pc=9.28E-03.
****Omnibus test X^2=8.92 (df=3); p value=0.03; Pc=0.12.
ACA, anticentromere autoantibodies; lcSSc, limited cutaneous SSc; SNP, single nucleotide polymorphisms; SSc, systemic sclerosis.

significant association for rs2069762 might stem from type 1 statistical error. This fact together with the location of the associated SNP suggests a highlighted role of the *IL-21* cytokine. By examining the expression and regulation of *IL-21* and the *IL-21* receptor (*IL-21R*) in patients with SSc, a previous study demonstrated an upregulation of *IL-21R* in epidermis samples.[33] However, a recent study has demonstrated that the scleroderma burden in allogeneic haemopoietic stem cell transplantations is driven by Th17 induction via *IL-21* and *IL-23* signalling.[34] Together, these results suggest that *IL-21/IL-21R* signalling has a pathogenic function in SSc.

The role of *IL-2* and *IL-21* in the immune system makes these genes plausible candidates for the genetic component of autoimmune diseases.[1 35 36] Our results increase the evidence that have showed that the rs6822844 is significantly associated with multiple autoimmune diseases.[10–13] According to the HapMap project for the CEU population (http://hapmap.ncbi.nlm.nih.gov/), the rs682284 polymorphism tags seven other variants located along the *IL-2/IL-21* region (rs13132245, rs13122573, rs4459999, rs13151961, rs13140464, rs6814280 and rs2069778), but clear evidence that connects any of these variants with the *IL-2* and/or *IL-21* regulation is lacking. Together, all point out these genetic variants as good candidates for functional studies in SSc pathogenesis and in other autoimmune diseases.

Although, the combined analyses of the rs6822844 polymorphism did not show heterogeneity (BD p_{value}=0.29. I^2=17.3%) between the eight European populations, a weak point of our study is that we did not have enough data available to control the association by principal component. Furthermore, as we mentioned before, an increment in the sample size for the stratified analysis could define in an accurate way the role of the studied variants in different clinical manifestations of SSc as their influence in the presence of coautoimmunity. Consequently, it is necessary to replicate the actual observation.

To conclude, **c**onsistent with previous studies on autoimmune diseases, the *IL-2/IL-21* region is a susceptibility genetic factor for SSc and its lcSSc subtype. The rs6822844 polymorphism confers the best association signal for SSc. It is also worth mentioning that this study shows the importance of the study of different populations and broad collaboration to find the missing heritability for relatively rare diseases like SSc.

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Acknowledgements We thank Sonia García and Gema Robledo for their excellent technical assistance and all the patients and control donors for their essential collaboration. We thank Banco Nacional de ADN (University of Salamanca, Spain). We are also thankful to EUSTAR (The EULAR Scleroderma Trials and Research group) and the German Network of Systemic Sclerosis for the facilitation of this project.

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Contributors All the authors listed participated in all or at list one of these activities: Conception and design, acquisition of data or analysis and interpretation of data. Drafting the article or revising it critically for important intellectual content. Final approval of the version published.

Funding This work was supported by the following grants: LMDG was funded by the 'Ayudas Predoctorales de Formación en Investigación en Salud (PFIS-FI09/00544)' from the Instituto de Salud Carlos III. JM was funded by GEN-FER from the Spanish

Society of Rheumatology, SAF2009-11110 from the Spanish Ministry of Science, CTS-4977 from Junta de Andalucía, Spain, in part by Redes Temáticas de Investigación Cooperativa Sanitaria Program, RD08/0075 (RIER) from Instituto de Salud Carlos III (ISCIII), Spain and by Fondo Europeo de Desarrollo Regional (FEDER). TRDJR was funded by the VIDI laureate from the Dutch Association of Research (NWO) and Dutch Arthritis Foundation (National Reumafonds). JM and TRDJR were sponsored by the Orphan Disease Program grant from the European League Against Rheumatism (EULAR). BPCK is supported by the Dutch Diabetes Research Foundation (grant 2008.40.001) and the Dutch Arthritis Foundation (Reumafonds, grant NR 09-1-408). TW was granted by DFG WI 1031/6.1 and DFG KFO 250 TP03. NOC was funded by PI-0590-2010, Consejería de Salud, Junta de Andalucía, Spain.US National Institutes of Health and National Institute of Arthritis and Musculoskeletal Diseases (NIH-NIAMS) R01-AR-055258, Two-Stage Genome Wide Association Study in Systemic Sclerosis and by the NIH-NIAMS Center of Research Translation (CORT) in SSc (P50AR054144), K23AR061436, and the Department of Defense Congressionally Directed Medical Research Programs (W81XWH-07-01-0111), (NIH/NIAID) 1U01AI09090.

Competing interests None.

Ethics approval The local ethical committees in accordance with the tenets of the Declaration of Helsinki.

Provenance and peer review Not commissioned; externally peer reviewed.

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