Influence of the *IL6* Gene in Susceptibility to Systemic Sclerosis

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ABSTRACT. ***Objective.*** Systemic sclerosis (SSc) is a genetically complex autoimmune disease; the genetic component has not been fully defined. Interleukin 6 (IL-6) plays a crucial role in immunity and fibrosis, both key aspects of SSc. We investigated the influence of *IL6* gene in the susceptibility and phenotype expression of SSc.

Methods. We performed a large metaanalysis including a total of 2749 cases and 3189 controls from 6 white populations (Germany, The Netherlands, Norway, Spain, Sweden, and United Kingdom). Three *IL6* single-nucleotide polymorphisms (SNP; rs2069827, rs1800795, and rs2069840) were selected by SNP tagging and genotyped using TaqMan® allele discrimination technology.

Results. Individual SNP metaanalysis showed no evidence of association of the 3 *IL6* genetic variants with the global disease. Phenotype analyses revealed a significant association between the minor allele of rs2069840 and the limited cutaneous SSc clinical form (Bonferroni p = 0.036, OR 1.14, 95% CI 1.04–1.25). A trend of association between the minor allele of the rs1800795 and the diffuse cutaneous SSc clinical form was also evident (Bonferroni p = 0.072, OR 0.86, 95% CI 0.77–0.96). In the *IL6* allelic combination analyses, the GGC allelic combination rs2069827-rs1800795-rs2069840 showed an association with overall SSc (Bonferroni p = 0.016, OR 1.13, 95% CI 1.04–1.23).

Conclusion. Our results suggest that the *IL6* gene may influence the development of SSc and its progression. (J Rheumatol First Release Oct 1 2012; doi:10.3899/jrheum.120506)

Key Indexing Terms:
SYSTEMIC SCLEROSIS | SCLERODERMA | *IL6* GENE
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Systemic sclerosis (SSc) is a genetically complex disease clinically characterized by progressive fibrosis of the skin and internal organs, vascular damage, and autoimmune events. Two major subgroups in the commonly accepted classification of SSc are determined in the prognostic differentiation: limited cutaneous scleroderma (lcSSc) and diffuse cutaneous scleroderma (dcSSc). Anticentromere antibodies (ACA) and antitopoisomerase antibodies (ATA) are 2 almost mutually exclusive SSc-associated autoantibodies that correlate with these distinct clinical subsets[1].

Familial clustering and ethnic influences support the genetic component of this disease[2]. Initially, only MHC genes were firmly associated with SSc[3]. Current genetic studies, including genome-wide association studies (GWAS), have shown that several genes located within non-MHC chromosomal regions also contribute to SSc susceptibility and clinical features[4,5,6]. However, the genetic component of SSc and its clinical subsets remain to be elucidated[3].

Interleukin 6 (IL-6) is a pleiotropic cytokine that plays a crucial role in both adaptive and innate immunity and in the link between them. It is produced by a variety of cell types, including lymphocytes, monocytes, and fibroblasts, in response to inflammatory stimuli; and it exerts proinflammatory and antiinflammatory functions[7]. IL-6 induces the production of collagen by dermal fibroblasts, and investigators have demonstrated higher production of IL-6 by fibroblasts in SSc[8,9] contributing to skin fibrosis[10]. High IL-6 levels have been reported in both skin and serum of patients with SSc, apparently correlating with the presence of lung involvement[11,12,13]. Further, IL-6 induces development of Th17 cells and inhibits development of regulatory T cells (T_{reg})[14], modulating the Th17/T_{reg} balance that is involved in SSc pathogenesis[15]. Current studies reveal a possible beneficial effect of anti-IL-6 receptor antibody therapy (tocilizumab) for patients with SSc, supporting the crucial role of IL-6 in SSc pathophysiology[16].

It is well known that the *IL6* gene is mainly regulated at the transcriptional level, and several polymorphisms affecting transcription have been reported to influence final IL-6 levels[17,18]. A promoter *IL6* –174*G allele has been associated with risk for systemic-onset juvenile chronic arthritis[19] and systemic lupus erythematosus[20], diseases that share a substantial genetic component with SSc[3].

Considering all these findings, we investigated the potential contribution of *IL6* gene variants to susceptibility to SSc and its major clinical phenotypes.

MATERIALS AND METHODS

Study population. A large multicenter case-control study was carried out including a total of 3189 controls and 2749 cases from 6 European populations of white ancestry (Germany, The Netherlands, Norway, Spain, Sweden, and United Kingdom). The control population consisted of unrelated healthy individuals recruited in the same geographical regions as patients and matched for ethnicity. Approval from the local ethical committees and written informed consent from all participants were obtained.

Patients with SSc were diagnosed according to the 1980 American College of Rheumatology classification criteria[21], and were subdivided into those with lcSSc and dcSSc as defined by LeRoy, *et al*[22], and by autoantibody status based on the presence/absence of ACA or ATA. The presence of the SSc-specific autoantibodies antitopoisomerase I (anti-Scl70) and ACA was assessed by passive immunodiffusion against calf thymus extract (Inova Diagnostics) and indirect immunofluorescence of HEp-2 cells (Antibodies Inc.), respectively. Additionally, fibrotic lung was assessed in patients with SSc, defined as the presence of typical features on high-resolution computed tomography of the chest, as described[23].

Single-nucleotide polymorphism selection and genotyping. We analyzed 3 single-nucleotide polymorphisms (SNP) located within the *IL6* gene, on

chromosome 7, that tag over 80% of the variability of this locus: rs2069827, rs1800795, and rs2069840. Tagging was performed using the "aggressive tagging" option in the Haploview software v4.2 (website: http://broad.mit.edu/mpg/haploview), with the r^2 threshold set at 0.8 and minimum minor allele frequency at 0.1. The inclusion of the rs1800795 polymorphism (–174 G/C) located in the promoter of this gene was forced in our study.

Genomic DNA was extracted from peripheral white blood cells following standard procedures. Samples were genotyped for the *IL6* genetic variants using TaqMan® 5' allele discrimination assays (rs2069827 and rs2069840 were predesigned assays with identification C__15860047_10 and C__15804104_10; and rs1800795 was designed as a custom assay) in a 7900HT Fast Real-Time PCR System (Applied Biosystems).

The 3 selected SNP are in a linkage disequilibrium block of *D*' = 1 and their correlation values measured by the r^2 coefficient were 0.097 between rs2069827 and rs1800795, 0.051 between rs2069827 and rs2069840, and 0.476 between rs1800795 and rs2069840 (data from the International HapMap Project, Website: http://www.hapmap. org).

Statistical analysis. Hardy–Weinberg equilibrium was tested for all SNP at significance level = 0.01. Genotyping success was greater than 95% for all polymorphisms.

Association was calculated by 2 × 2 contingency tables and/or Fisher's exact test when necessary, obtaining p values, OR and 95% CI using PLINK (v1.07; http://pngu. mgh.harvard.edu/purcell/plink/). Allelic combinations were constructed using PLINK and Haploview v4.2 using the expectation–maximization algorithm implemented in the program for all samples included in the case–control study (excluding individuals with > 40% missing genotypes). Allelic combinations represented in over 5% of the healthy controls in any of the 6 study populations were selected for pooled analysis. Multiple testing corrections were applied to the resulting p values with Bonferroni correction. Bonferroni correction was applied in the haplotype-based association analysis by dividing the detected significance level by the number of major haplotypes detected (frequency > 5.0%). After correction, p values < 0.05 were considered statistically significant.

The metaanalysis was carried out using PLINK (v1.07) and Review Manager (RevMan 5.0). Homogeneity among cohorts was calculated using the Breslow–Day test, and overall OR calculations were performed under a fixed-effects Mantel–Haenszel model.

RESULTS

Genotypic and allelic frequencies for the pooled analysis of the 3 selected *IL6* SNP are shown in Table 1; and genotype and allelic frequencies for 6 national subpopulations are shown in Appendixes 2 to 7. In the different metaanalyses performed, the Breslow-Day test revealed no significant heterogeneity among OR of the different populations in the SNP separately or in their allelic combinations (p > 0.05). Independent metaanalysis of the 3 SNP showed no evidence of association with the global disease (Table 1). However, the Mantel-Haenszel test of the different allelic combinations of the SNP, including sex as a covariate, showed a statistically significant association between the rs2069827-rs1800795-rs2069840 GGC allelic combination and global disease risk after Bonferroni correction for multiple testing (p = 0.014, OR 1.14, 95% CI 1.04–1.23). On the other hand, the TCC combination showed a protective effect for SSc (Bonferroni p = 0.020, OR 0.82, 95% CI 0.72–0.94), but statistical significance was lost when sex was included as a covariate (p = 0.117; Table 2). In addition, metaanalysis for the different *IL6* allelic combinations in the different subtypes of patients with SSc showed that the associated *IL6**GGC allelic combination with global disease seemed to confer a similar effect (size and direction) in both

Table 1. Genotype and minor allele frequencies of SNP located within *IL6* gene in patients with SSc and healthy controls of the different study cohorts.

SNP	1/2	Subgroup (n)	Genotype, n (%)			MAF, %	Allele Test		
			1/1	1/2	2/2		p*	p_{BFN}**	OR (95% CI)***
rs2069827	G/T	Controls (3189)	2687 (84.26)	473 (14.83)	29 (0.91)	8.33			
		SSc (2739)	2339 (85.40)	379 (13.84)	21 (0.77)	7.69	0.018	0.162	0.85 (0.74–0.97)
		lcSSc (1848)	1574 (85.17)	257 (13.91)	17 (0.92)	7.87	0.083		0.87 (0.75–1.02)
		dcSSc (891)	765 (85.86)	122 (13.69)	4 (0.45)	7.30	0.024	0.216	0.79 (0.64–0.97)
		ACA+ (1049)	902 (85.99)	137 (13.06)	10 (0.95)	7.48	0.051		0.83 (0.69–1.00)
		ATA+ (611)	521 (85.27)	86 (14.08)	4 (0.65)	7.69	0.141		0.84 (0.66–1.06)
		FLA+ (715)	604 (84.48)	106 (14.83)	5 (0.70)	8.11	0.297		0.89 (0.72–1.11)
rs1800795	G/C	Controls (3179)	1215 (38.22)	1492 (46.93)	472 (14.85)	38.31			
		SSc (2749)	1077 (39.18)	1250 (45.47)	422 (15.35)	38.09	0.252		0.96 (0.89–1.03)
		lcSSc (1850)	705 (38.11)	843 (45.57)	302 (16.32)	39.11	0.985		1.00 (0.92–1.09)
		dcSSc (899)	372 (41.38)	407 (45.27)	120 (13.35)	35.98	0.008	0.072	0.86 (0.77–0.96)
		ACA+ (1059)	401 (37.87)	494 (46.65)	164 (15.49)	38.81	0.829		0.99 (0.89–1.10)
		ATA+ (611)	244 (39.93)	281 (45.99)	86 (14.08)	37.07	0.141		0.91 (0.80–1.03)
		FLA+ (716)	251 (35.06)	352 (49.16)	113 (15.78)	38.09	0.575		1.00 (0.91–1.17)
rs2069840	C/G	Controls (3162)	1300 (41.11)	1459 (46.14)	403 (12.75)	35.82			
		SSc (2725)	1176 (43.16)	1252 (45.94)	297 (10.90)	33.87	0.060		0.93 (0.86–1.00)
		lcSSc (1840)	819 (44.51)	835 (45.38)	186 (10.11)	32.80	0.004	0.036	0.88 (0.81–0.96)
		dcSSc (885)	357 (40.34)	417 (47.12)	111 (12.54)	36.10	0.497		1.04 (0.93–1.16)
		ACA+ (1044)	444 (42.53)	500 (47.89)	100 (9.58)	33.52	0.125		0.92 (0.83–1.02)
		ATA+ (612)	260 (42.48)	281 (45.92)	71 (11.60)	34.56	0.499		0.96 (0.84–1.09)
		FLA+ (705)	310 (43.97)	325 (46.10)	70 (9.93)	32.98	0.124		0.91 (0.80–1.03)

* Values calculated for the allelic model and Mantel-Haenszel test under fixed effect. ** Multiple testing Bonferroni (BFN) correction. *** For the minor allele. SSc: systemic sclerosis; lcSSc: limited cutaneous SSc; dcSSc: diffuse cutaneous SSc; ACA: anticentromere antibodies; ATA: antitopoisomerase antibodies; FLA: fibrotic lung; MAF: minor allele frequency; SNP: single-nucleotide polymorphism.

Table 2. Pooled analysis of rs2069827-rs1800795-rs2069840 allelic combinations in white patients with SSc and healthy controls.

Allelic Combination	SSc, n (%)	Controls, n (%)	p_{MH}	p (covariates sex and population)	OR (95% CI)	p_{BD}
GGC	1598 (28.4)	1710 (26.5)	0.016*	0.014	1.13 (1.04–1.23)	0.16
GCC	1693 (30.1)	1915 (29.7)	0.980	0.455	1.00 (0.93–1.09)	0.70
GGG	1920 (34.1)	2289 (35.5)	0.280	0.014	0.95 (0.88–1.03)	0.32
TCC	419 (7.4)	534 (8.3)	0.020*	0.117	0.82 (0.72–0.94)	0.68

p_{MH}: allelic Mantel-Haenszel fixed-effects model. p_{BD}: Breslow-Day value. * Multiple testing Bonferroni correction (× 4 analyzed haplotypes).

subgroups of patients (lcSSc and dcSSc) although, probably because of statistical power, in the comparison of dcSSc against controls, statistical significance was lost (Appendix 8, Appendix 9).

An association was evident when the minor allele frequencies of the rs2069840 genetic variant were tested in patients with lcSSc versus controls (Bonferroni p = 0.036, OR 0.88, 95% CI 0.81–0.96), whereas the rs2069840 minor allele frequencies were similar between the dcSSc subgroup and controls ($p_{uncorrected}$ = 0.497, OR 1.04, 95% CI 0.93–1.16). For this reason, in the allelic combination analysis the *IL6**GGG combination showed a risk effect for global disease, although haplotype analysis in the different subtypes of patients revealed that this allelic combination, which is marked for the rs2069840*G allele, confers risk for only the limited clinical disease form (Appendix 8, Appendix 9).

A trend for association was also evident when the minor allele frequencies of the rs1800795 variant were compared between cases with dcSSc and controls (Bonferroni p = 0.072, OR 0.86, 95% CI 0.77–0.96), while the rs1800795 minor allele frequencies were similar between lcSSc and controls ($p_{uncorrected}$ = 0.985, OR 1.00, 95% CI 0.92–1.09).

Finally, the analysis according to autoantibody status (ACA/ATA) or development of pulmonary fibrosis achieved no statistical significance in either the independent analyses (Table 1) or the allelic combination tests (data not shown).

DISCUSSION

The cytokine IL-6 plays an important role in immunity and fibrosis, both key aspects of SSc[9]. To date, several GWAS have led to the discovery of many common genetic variants associated with SSc. However, SNP located within *IL6* locus have not been associated with SSc in these studies[4,5,6]. Even though genome-wide studies of SSc susceptibility did not highlight the association with this locus, poor coverage of the specific chromosomal region in these studies might hamper identification of genetic risk factors. It is important to note that the SNP analyzed in our study were not included in previous GWAS data and, as well, the *IL6* region has not been properly covered in GWAS[4]. In addition, it should be noted that the allelic combination analyses often help to uncover hidden signals. Thus, the allelic combinations may also tag other regional polymorphic sites, and therefore they are considered to provide more robust results than the study of single SNP in unraveling the genetic background of complex diseases. Indeed, our data showed that the *IL6**GGC allelic combination influences predisposition to SSc, conferring risk to the global disease. This associated allelic combination may contain the causal genetic variant or may act as a marker of another nearby causal polymorphism in tight linkage disequilibrium with it. However, further comprehensive studies are needed to elucidate causal polymorphism/s of the detected associations.

To date few and relatively underpowered studies have been conducted to analyze the influence of the *IL6* gene in susceptibility to SSc, and results have been controversial[24,25]. Moreover, these studies assessed only the rs1800795 polymorphism (–174G/C) and this may provide a limited view of the role of *IL6* in SSc pathophysiology, because several polymorphisms are likely to modify levels of IL-6[24,25,26]. Our investigation is the first tagging study to explore the influence of most of the *IL6* gene variability in the susceptibility to SSc and/or its major clinical features in a large and well-defined white cohort.

Interestingly, the *IL6* rs1800795 (–174G) variant, reported to increase the levels of IL-6[19], is carried in 2 of the 4 commonly conformed *IL6* allelic combinations (GG*C and GG*G). We investigated the effects of these 2 combinations in the development of SSc. Our results showed that only the GG*C combination confers risk for the global disease, suggesting that *IL6* rs1800795 may not confer disease susceptibility by itself, and other variant/s carried in the GG*C combination could be necessary to confer the described effect (Table 2). Thus, our results are in agreement with studies showing that several polymorphisms influence final IL-6 levels[18].

Additionally, we observed that the *IL6* rs2069840*G seems to play a protective role in the lcSSc clinical form, whereas the rs1800795*G allele seems to confer risk for the dcSSc clinical form. A recent study showed overexpression of IL-6 in patients with dcSSc and supports the potential of IL-6 as a surrogate marker for clinical outcome in SSc[27]. Other studies have suggested that blocking Th17-inducing cytokines such as IL-6 and IL-23 may intervene in the progression of SSc[13]. Our results would be in agreement with

these functional studies, and genetic variants located within the *IL6* locus affecting the final IL-6 levels probably influence specific subgroups of patients with SSc.

Because the pathogenesis of SSc is still unclear, an improved treatment for SSc is a major challenge. Our results might open new fields of study for treatment of SSc, and further investigations are needed to determine the role of the *IL6* locus in this genetically complex autoimmune disorder.

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APPENDIX 1

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APPENDIX 2

Genotype and minor allele frequencies of SNP within *IL6* gene in patients with SSc and healthy controls from Germany.

CHR	SNP	Locus	1/2	Subgroup (n)	Genotype, n (%)			MAF, %	Allele Test		OR (95% CI)***
					1/1	1/2	2/2		p*	p_{FDR}**	
7	rs2069827	*IL6*	G/T	Controls (289)	226 (78.20)	57 (19.72)	6 (2.08)	11.94			
				SSc (579)	466 (80.48)	107 (18.48)	6 (1.04)	10.28	0.29	0.44	0.84 (0.62–1.16)
				lcSSc (338)	267 (78.99)	67 (19.82)	4 (1.18)	11.09	0.64	0.90	0.92 (0.65–1.30)
				dcSSc (241)	199 (82.57)	40 (16.60)	2 (0.83)	9.13	0.14	0.21	0.74 (0.50–1.11)
				ACA+ (224)	182 (81.25)	39 (17.41)	3 (1.34)	10.04	0.34	0.51	0.82 (0.55–1.23)
				ATA+ (190)	154 (81.05)	35 (18.42)	1 (0.53)	9.74	0.29	0.43	0.80 (0.52–1.21)
7	rs1800795	*IL6*	G/C	Controls (290)	83 (28.62)	148 (51.03)	59 (20.34)	45.86			
				SSc (568)	205 (36.09)	262 (46.13)	101 (17.78)	40.85	0.05	0.14	0.82 (0.67–1.00)
				lcSSc (331)	112 (33.84)	158 (47.73)	61 (18.43)	42.30	0.21	0.62	0.87 (0.69–1.08)
				dcSSc (237)	93 (39.24)	104 (43.88)	40 (16.88)	38.82	0.02	0.06	0.75 (0.59–0.96)
				ACA+ (219)	79 (36.07)	105 (47.95)	35 (15.98)	39.95	0.06	0.18	0.79 (0.61–1.01)
				ATA+ (189)	75 (39.68)	81 (42.86)	33 (17.46)	38.89	0.03	0.10	0.75 (0.58–0.98)
7	rs2069840	*IL6*	C/G	Controls (287)	125 (43.55)	136 (47.39)	26 (9.06)	32.75			
				SSc (572)	254 (44.41)	251 (43.88)	67 (11.71)	33.65	0.71	0.71	1.04 (0.84–1.29)
				lcSSc (334)	149 (44.61)	149 (44.61)	36 (10.78)	33.08	0.90	0.90	1.02 (0.80–1.29)
				dcSSc (238)	105 (44.12)	102 (42.86)	31 (13.03)	34.45	0.56	0.56	1.08 (0.83–1.40)
				ACA+ (220)	90 (40.91)	109 (49.55)	21 (9.55)	34.32	0.60	0.60	1.07 (0.82–1.40)
				ATA+ (189)	84 (44.44)	78 (41.27)	27 (14.29)	34.92	0.49	0.49	1.10 (0.84–1.45)

* Values calculated for the allelic model. ** Multiple testing false discovery rate correction. *** For the minor allele. SNP: single-nucleotide polymorphism; IL: interleukin; SSc: systemic sclerosis; MAF: minor allele frequency.

APPENDIX 3

Genotype and minor allele frequencies of SNP within *IL6* gene in patients with SSc and healthy controls from Sweden.

CHR	SNP	Locus	1/2	Subgroup (n)	Genotype, n (%)				Allele Test		
					1/1	1/2	2/2	MAF, %	p*	p_{FDR}**	OR (95% CI)***
7	rs2069827	*IL6*	G/T	Controls (268)	206 (76.87)	59 (22.01)	3 (1.12)	12.13			
				SSc (254)	208 (81.89)	38 (14.96)	8 (3.15)	10.63	0.45	0.67	0.86 (0.59–1.26)
				lcSSc (184)	150 (81.52)	28 (15.22)	6 (3.26)	10.87	0.56	0.83	0.88 (0.58–1.34)
				dcSSc (70)	58 (82.86)	10 (14.29)	2 (2.86)	10.00	0.49	0.73	0.81 (0.44–1.48)
				ACA+ (71)	60 (84.51)	10 (14.08)	1 (1.41)	8.45	0.22	0.66	0.67 (0.35–1.38)
				ATA+ (44)	35 (79.55)	6 (13.64)	3 (6.82)	13.64	0.69	0.69	1.14 (0.59–2.22)
7	rs1800795	*IL6*	G/C	Controls (273)	83 (30.40)	138 (50.55)	52 (19.05)	44.32			
				SSc (252)	81 (32.14)	117 (46.43)	54 (21.43)	44.64	0.92	0.92	1.01 (0.79–1.29)
				lcSSc (181)	56 (30.94)	87 (48.07)	38 (20.99)	45.03	0.83	0.83	1.03 (0.79–1.34)
				dcSSc (71)	25 (35.21)	30 (42.25)	16 (22.54)	43.66	0.89	0.89	0.97 (0.67–1.41)
				ACA+ (69)	25 (36.23)	31 (44.93)	13 (18.84)	41.30	0.52	0.74	0.88 (0.61–1.29)
				ATA+ (44)	11 (25.00)	21 (47.73)	12 (27.27)	51.14	0.23	0.54	1.32 (0.84–2.06)
7	rs2069840	*IL6*	C/G	Controls (270)	128 (47.41)	114 (42.22)	28 (10.37)	31.48			
				SSc (255)	138 (54.12)	95 (37.25)	22 (8.63)	27.25	0.13	0.40	0.82 (0.62–1.06)
				lcSSc (184)	98 (53.26)	70 (38.04)	16 (8.70)	27.72	0.22	0.67	0.83 (0.62–1.12)
				dcSSc (71)	40 (56.34)	25 (35.21)	6 (8.45)	26.06	0.21	0.63	0.77 (0.51–1.16)
				ACA+ (70)	34 (48.57)	30 (42.86)	6 (8.57)	30.00	0.74	0.74	0.93 (0.62–1.40)
				ATA+ (45)	25 (55.56)	16 (35.56)	4 (8.89)	26.67	0.36	0.54	0.79 (0.48–1.31)

* Values calculated for the allelic model. ** Multiple testing false discovery rate correction. *** For the minor allele. SNP: single-nucleotide polymorphism; IL: interleukin; SSc: systemic sclerosis; MAF: minor allele frequency.

APPENDIX 4

Genotype and minor allele frequencies of SNP within *IL6* gene in patients with SSc and healthy controls from The Netherlands.

CHR	SNP	Locus	1/2	Subgroup (n)	Genotype, n (%)				Allele Test		
					1/1	1/2	2/2	MAF, %	p*	p_{FDR}**	OR (95% CI)***
7	rs2069827	*IL6*	G/T	Controls (596)	529 (88.76)	63 (10.57)	4 (0.67)	5.96			
				SSc (374)	329 (87.97)	45 (12.03)	0 (0.00)	6.02	0.96	0.96	1.01 (0.69–1.49)
				lcSSc (254)	229 (90.16)	25 (9.84)	0 (0.00)	4.92	0.40	0.60	0.82 (0.51–1.31)
				dcSSc (120)	100 (83.33)	20 (16.67)	0 (0.00)	8.33	0.17	0.51	1.44 (0.86–2.41)
				ACA+ (90)	79 (87.78)	11 (12.22)	0 (0.00)	6.11	0.93	0.93	1.03 (0.53–1.98)
				ATA+ (102)	89 (87.25)	13 (12.75)	0 (0.00)	6.37	0.82	0.45	1.08 (0.58–1.98)
7	rs1800795	*IL6*	G/C	Controls (585)	246 (42.05)	258 (44.10)	81 (13.85)	35.90			
				SSc (375)	152 (40.53)	179 (47.73)	44 (11.73)	35.60	0.89	0.96	0.99 (0.82–1.20)
				lcSSc (254)	102 (40.16)	125 (49.21)	27 (10.63)	35.24	0.80	0.80	0.97 (0.78–1.21)
				dcSSc (121)	50 (41.32)	54 (44.63)	17 (14.05)	36.36	0.89	0.89	1.02 (0.77–1.36)
				ACA+ (92)	36 (39.13)	49 (53.26)	7 (7.61)	34.24	0.66	0.93	0.93 (0.67–1.29)
				ATA+ (102)	48 (47.06)	43 (42.16)	11 (10.78)	31.86	0.27	0.45	0.84 (0.61–1.15)
7	rs2069840	*IL6*	C/G	Controls (578)	204 (35.29)	277 (47.92)	97 (16.78)	40.74			
				SSc (367)	149 (40.60)	181 (49.32)	37 (10.08)	34.74	0.01	0.03	0.77 (0.64–0.94)
				lcSSc (248)	106 (42.74)	118 (47.58)	24 (9.68)	33.47	0.01	0.02	0.73 (0.59–0.91)
				dcSSc (119)	43 (36.13)	63 (52.94)	13 (10.92)	37.39	0.34	0.51	0.87 (0.65–1.16)
				ACA+ (88)	35 (39.77)	46 (52.27)	7 (7.95)	34.09	0.09	0.28	0.75 (0.54–1.05)
				ATA+ (103)	40 (38.83)	50 (48.54)	13 (12.62)	36.89	0.30	0.82	0.85 (0.63–1.16)

* Values calculated for the allelic model. ** Multiple testing false discovery rate correction. *** For the minor allele. SNP: single-nucleotide polymorphism; IL: interleukin; SSc: systemic sclerosis; MAF: minor allele frequency.

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APPENDIX 5

Genotype and minor allele frequencies of SNP within *IL6* gene in patients with SSc and healthy controls from Spain.

CHR	SNP	Locus	1/2	Subgroup (n)	Genotype, n (%) 1/1	1/2	2/2	MAF, %	Allele Test p*	p_{FDR}**	OR (95% CI)***
7	rs2069827	*IL6*	G/T	Controls (1400)	1208 (86.29)	180 (12.86)	12 (0.86)	7.29			
				SSc (1012)	890 (87.94)	119 (11.76)	3 (0.30)	6.18	0.13	0.39	0.84 (0.67–1.06)
				lcSSc (689)	603 (87.52)	83 (12.05)	3 (0.44)	6.46	0.33	0.33	0.88 (0.68–1.14)
				dcSSc (323)	287 (88.85)	36 (11.15)	0 (0.00)	5.57	0.12	0.18	0.75 (0.52–1.08)
				ACA+ (502)	434 (86.45)	65 (12.95)	3 (0.60)	7.07	0.82	0.82	0.97 (0.73–1.28)
				ATA+ (234)	206 (88.03)	28 (11.97)	0 (0.00)	5.98	0.31	0.93	0.81 (0.54–1.22)
7	rs1800795	*IL6*	G/C	Controls (1412)	611 (43.27)	638 (45.18)	163 (11.54)	34.14			
				SSc (1021)	444 (43.49)	448 (43.88)	129 (12.63)	34.57	0.75	0.75	1.02 (0.90–1.15)
				lcSSc (694)	298 (42.94)	293 (42.22)	103 (14.84)	35.95	0.24	0.33	1.08 (0.95–1.24)
				dcSSc (327)	146 (44.65)	155 (47.40)	26 (7.95)	31.65	0.23	0.23	0.89 (0.74–1.07)
				ACA+ (508)	212 (41.73)	221 (43.50)	75 (14.76)	36.52	0.17	0.32	1.11 (0.96–1.29)
				ATA+ (234)	94 (40.17)	121 (51.71)	19 (8.12)	33.97	0.95	0.95	0.99 (0.81–1.22)
7	rs2069840	*IL6*	C/G	Controls (1391)	568 (40.83)	648 (46.59)	175 (12.58)	35.87			
				SSc (998)	411 (41.18)	475 (47.60)	112 (11.22)	35.02	0.54	0.76	0.96 (0.85–1.09)
				lcSSc (684)	302 (44.15)	313 (45.76)	69 (10.09)	32.97	0.06	0.19	0.88 (0.77–1.01)
				dcSSc (314)	109 (34.71)	162 (51.59)	43 (13.69)	39.49	0.09	0.18	1.17 (0.98–1.39)
				ACA+ (499)	213 (42.69)	236 (47.29)	50 (10.02)	33.67	0.21	0.32	0.91 (0.78–1.06)
				ATA+ (228)	87 (38.16)	117 (51.32)	24 (10.53)	36.18	0.90	0.95	1.01 (0.82–1.25)

* Values calculated for the allelic model. ** Multiple testing false discovery rate correction. *** For the minor allele. SNP: single-nucleotide polymorphism; IL: interleukin; SSc: systemic sclerosis; MAF: minor allele frequency.

APPENDIX 6

Genotype and minor allele frequencies of SNP within *IL6* gene in patients with SSc and healthy controls from the United Kingdom.

CHR	SNP	Locus	1/2	Subgroup (n)	Genotype, n (%) 1/1	1/2	2/2	MAF, %	Allele Test p*	p_{FDR}**	OR (95% CI)***
7	rs2069827	*IL6*	G/T	Controls (376)	305 (81.12)	68 (18.09)	3 (0.80)	9.84			
				SSc (420)	363 (86.43)	54 (12.86)	3 (0.71)	7.14	0.05	0.16	0.70 (0.49–1.01)
				lcSSc (316)	273 (86.39)	40 (12.66)	3 (0.95)	7.28	0.09	0.27	0.72 (0.49–1.06)
				dcSSc (104)	90 (86.54)	14 (13.46)	0 (0.00)	6.73	0.17	0.25	0.66 (0.37–1.20)
				ACA+ (160)	144 (90.00)	14 (8.75)	2 (1.25)	5.63	0.02	0.07	0.55 (0.32–0.93)
				ATA+ (58)	51 (87.93)	7 (12.07)	0 (0.00)	6.03	0.19	0.28	0.59 (0.26–1.31)
7	rs1800795	*IL6*	G/C	Controls (373)	120 (32.17)	184 (49.33)	69 (18.50)	43.16			
				SSc (434)	168 (38.71)	189 (43.55)	77 (17.74)	39.52	0.14	0.21	0.86 (0.71–1.05)
				lcSSc (323)	122 (37.77)	143 (44.27)	58 (17.96)	40.09	0.25	0.37	0.88 (0.71–1.09)
				dcSSc (111)	46 (41.44)	46 (41.44)	19 (17.12)	37.84	0.16	0.25	0.80 (0.59–1.09)
				ACA+ (169)	55 (32.54)	86 (50.89)	28 (16.57)	42.01	0.72	0.91	0.95 (0.74–1.24)
				ATA+ (59)	23 (38.98)	25 (42.37)	11 (18.64)	39.83	0.50	0.50	0.87 (0.59–1.30)
7	rs2069840	*IL6*	C/G	Controls (376)	157 (41.76)	171 (45.48)	48 (12.77)	35.51			
				SSc (435)	179 (41.15)	207 (47.59)	49 (11.26)	35.06	0.85	0.85	0.98 (0.80–1.20)
				lcSSc (325)	129 (39.69)	161 (49.54)	35 (10.77)	35.54	0.99	0.99	1.00 (0.80–1.25)
				dcSSc (110)	50 (45.45)	46 (41.82)	14 (12.73)	33.64	0.61	0.61	0.92 (0.67–1.26)
				ACA+ (166)	61 (36.75)	91 (54.82)	14 (8.43)	35.84	0.91	0.91	1.02 (0.78–1.33)
				ATA+ (62)	33 (53.23)	23 (37.10)	6 (9.68)	28.23	0.11	0.28	0.71 (0.47–1.09)

* Values calculated for the allelic model. ** Multiple testing false discovery rate correction. *** For the minor allele. SNP: single-nucleotide polymorphism; IL: interleukin; SSc: systemic sclerosis; MAF: minor allele frequency.

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APPENDIX 7

Genotype and minor allele frequencies of SNP within *IL6* gene in patients with SSc and healthy controls from Norway.

CHR	SNP	Locus	1/2	Subgroup (n)	Genotype, n (%)				Allele Test		
					1/1	1/2	2/2	MAF, %	p*	p_{FDR}**	OR (95% CI)***
7	rs2069827	*IL6*	G/T	Controls (260)	213 (81.92)	46 (17.69)	1 (0.38)	9.23			
				SSc (100)	83 (83.00)	16 (16.00)	1 (1.00)	9.00	0.92	0.97	0.97 (0.55–1.72)
				lcSSc (67)	52 (77.61)	14 (20.90)	1 (1.49)	11.94	0.35	0.35	1.33 (0.73–2.43)
				dcSSc (33)	31 (93.94)	2 (6.06)	0 (0.00)	3.03	0.09	0.15	0.31 (0.07–1.30)
				ACA+ (54)	44 (81.48)	9 (16.67)	1 (1.85)	10.19	0.76	0.82	1.12 (0.56–2.23)
				ATA+ (14)	13 (92.86)	1 (7.14)	0 (0.00)	3.57	0.31	0.31	0.36 (0.05–2.74)
7	rs1800795	*IL6*	G/C	Controls (246)	72 (29.27)	126 (51.22)	48 (19.51)	45.12			
				SSc (99)	27 (27.27)	55 (55.56)	17 (17.17)	44.95	0.97	0.97	0.99 (0.71–1.38)
				lcSSc (67)	15 (22.39)	37 (55.22)	15 (22.39)	50.00	0.32	0.35	1.22 (0.83–1.78)
				dcSSc (32)	12 (37.50)	18 (56.25)	2 (6.25)	34.38	0.10	0.15	0.64 (0.37–1.10)
				ACA+ (54)	16 (29.63)	26 (48.15)	12 (22.22)	46.30	0.82	0.82	1.05 (0.69–1.59)
				ATA+ (14)	7 (50.00)	5 (35.71)	2 (14.29)	32.14	0.18	0.31	0.58 (0.26–1.30)
7	rs2069840	*IL6*	C/G	Controls (260)	118 (45.38)	113 (43.46)	29 (11.15)	32.88			
				SSc (98)	45 (45.92)	43 (43.88)	10 (10.20)	32.14	0.85	0.97	0.97 (0.68–1.37)
				lcSSc (65)	35 (53.85)	24 (36.92)	6 (9.23)	27.69	0.26	0.35	0.78 (0.51–1.20)
				dcSSc (33)	10 (30.30)	19 (57.58)	4 (12.12)	40.91	0.19	0.19	1.41 (0.84–2.39)
				ACA+ (53)	27 (50.94)	20 (37.74)	6 (11.32)	30.19	0.59	0.82	0.88 (0.56–1.39)
				ATA+ (14)	4 (28.57)	8 (57.14)	2 (14.29)	42.86	0.28	0.31	1.53 (0.71–3.31)

* Values calculated for the allelic model. ** Multiple testing false discovery rate correction. *** For the minor allele. SNP: single-nucleotide polymorphism; IL: interleukin; SSc: systemic sclerosis; MAF: minor allele frequency.

APPENDIX 8

Pooled analysis of rs2069827-rs1800795-rs2069840 allelic combinations in white patients with dcSSc and healthy controls.

Haplotype	dcSSc, n (%), n = 1822	Controls, n (%), n = 6448	p_{MH}	p (covariates sex and population)	OR (95% CI)	p_{BD}
GGC	525 (28.8)	1710 (26.5)	0.080*	0.064	1.16 (1.03–1.30)	0.09
GCC	518 (28.4)	1915 (29.7)	0.171	0.494	0.92 (0.82–1.04)	0.9
GGG	648 (35.6)	2289 (35.5)	0.642	0.806	1.03 (0.92–1.15)	0.14
TCC	131 (7.2)	534 (8.3)	0.080*	0.089	0.78 (0.64–0.96)	0.24

p_{MH}: allelic Mantel-Haenszel fixed-effects model. p_{BD}: Breslow-Day value. * Multiple testing Bonferroni correction (× 4 analyzed haplotypes). dcSSc: diffuse cutaneous systemic sclerosis.

APPENDIX 9

Pooled analysis of rs2069827-rs1800795-rs2069840 allelic combinations in white patients with lcSSc and healthy controls.

Haplotype	lcSSc, n (%), n = 3765	Controls, n (%), n = 6448	p_{MH}	p (covariates sex and population)	OR (95% CI)	p_{BD}
GGC	1073 (28.5)	1710 (26.5)	0.016*	0.014	1.14 (1.04–1.25)	0.36
GCC	1176 (30.2)	1915 (29.7)	0.180	0.149	1.06 (0.97–1.17)	0.69
GGG	1228 (32.6)	2289 (35.5)	0.024*	0.001	0.89 (0.81–0.97)	0.40
TCC	288 (7.6)	534 (8.3)	0.160*	0.185	0.85 (0.73–0.99)	0.60

p_{MH}: allelic Mantel-Haenszel fixed-effects model. p_{BD}: Breslow-Day value. * Multiple testing Bonferroni correction (× 4 analyzed haplotypes). lcSSc: limited cutaneous systemic sclerosis.

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