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Independent Replication and Metaanalysis of Association Studies Establish TNFSF4 as a Susceptibility Gene Preferentially Associated with the Subset of Anticentromere-positive Patients with Systemic Sclerosis

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Abstract

Objective—Independent replication with large cohorts and metaanalysis of genetic associations are necessary to validate genetic susceptibility factors. The known tumor necrosis factor (ligand) superfamily, member 4 gene (*TNFSF4*) systemic lupus erythematosus (SLE) risk locus has been found to be associated with systemic sclerosis (SSc) in 2 studies, but with discrepancies between them for genotype-phenotype correlation. Our objective was to validate *TNFSF4* association with SSc and determine the subset with the higher risk.

Methods—Known SLE and SSc *TNFSF4* susceptibility variants (rs2205960, rs1234317, rs12039904, rs10912580, and rs844648) were genotyped in 1031 patients with SSc and 1014 controls of French white ancestry. Genotype-phenotype association analysis and metaanalysis of available data were performed, providing a population study of 4989 patients with SSc and 4661 controls, all of European white ancestry.

Results—Allelic and genotypic associations were observed for the 5 single-nucleotide polymorphisms (SNP) with the subset of patients with SSc who are positive for anticentromere antibodies (ACA) and only a trend for association with SSc and limited cutaneous SSc. Rs2205960 exhibited the strongest allelic association in ACA+ patients with SSc [p = 0.0015; OR 1.37 (1.12–1.66)], with significant intracohort association when compared to patients with SSc positive for ACA. Metaanalysis confirmed overall association with SSc but also raised preferential association with the ACA+ subset and strongest effect with rs2205960 [T allele p = 0.00013; OR 1.33 (1.15–1.54) and TT genotype p = 0.00046; OR 2.02 (1.36–2.98)].

Conclusion—We confirm *TNFSF4* as an SSc susceptibility gene and rs2205960 as a putative causal variant with preferential association in the ACA+ SSc subphenotype. (First Release March 15 2012; *J Rheumatol* 2012;39:997–1003; doi:10.3899/jrheum.111270)

Key Indexing Terms

SYSTEMIC SCLEROSIS; *TNFSF4*; AUTOIMMUNITY; AUTOANTIBODIES

Systemic sclerosis (SSc) is a chronic autoimmune disease with a complex pathogenesis involving a combination of genetic risk factors and environmental events[1]. There is now strong evidence that various autoimmune diseases share genetic susceptibility factors. The particular role of T cells in this setting was recently underscored by the involvement of members of tumor necrosis ligand factor superfamily in various autoimmune diseases such as systemic lupus erythematosus (SLE). Among them, tumor necrosis factor (ligand) superfamily, member 4 gene (*TNFSF4*) encodes the costimulatory molecule OX40 ligand

(OX40L), involved in T cell regulatory functions. Interestingly, in SSc a lack of regulatory T cells was recently found in the fibrotic skin[2]. In addition, the serum soluble OX40 were found to be increased in SSc, compared to patients with SLE and controls[3]. This finding is in agreement with the concept of the implication of inflammation and autoimmunity, in particular at the early stages of SSc pathogenesis, with a potential role of the OX40 axis. Genetic studies have shown that the *TNFSF4* locus, on the chromosome 1q25, is associated with autoimmune diseases, notably SLE[4]. On these bases, 4 single nucleotide polymorphisms (SNP; rs10912580, rs12039904, rs2205960, and rs1234317) in the upstream region of *TNFSF4*, tagging a unique risk haplotype, and 1 SNP (rs844644), tagging a protective haplotype, were highlighted in both family-based and case control studies[5] and later replicated in independent cohorts of different ancestries[6,7,8,9] in SLE.

By a cross-disease approach, an association study performed in the North American European populations identified 3 SSc risk variants (rs1234314, rs2205960, and rs844648) involved in the overall disease susceptibility[10]. A first replication study performed in European white populations observed a weak association between SSc and 2 *TNFSF4* variants (rs1234314 and rs12039904). Interestingly, a higher magnitude of the association signals was found in both limited cutaneous SSc (lcSSc) and anticentromere antibody-positive (ACA+) subsets[11]. More recently, in a genome-wide association study of white individuals, association signals for *TNFSF4* variants were found in both lcSSc and ACA+ subsets and differed from previous reports by also finding association with the antitopoisomerase I antibody (ATA+) subset[12]. Putative genetic associations require independent replications using large cohorts to confirm a previously detected association, especially in the case of small OR, for which strong power is needed. Metaanalysis is a powerful tool to resolve discrepancies among genetic studies and notably to estimate the influence of a genetic factor on the phenotype[13]. This tool is particularly critical regarding the SSc phenotypic heterogeneity, which mirrors genetic heterogeneity.

Therefore, conforming to current guidelines about replication studies[14], our objectives were (1) to replicate the association between *TNFSF4* and SSc; (2) to perform a metaanalysis; and (3) to perform a genotype-phenotype association analysis.

MATERIALS AND METHODS

We performed a large case-control association study using a French white population consisting of 1031 patients with SSc and 1014 healthy controls, as reported[15]. For all patients with SSc, we determined LeRoy's cutaneous subtype[16] and carried out a phenotypic assessment, as recommended[17]. Taking into account the reported association of *TNFSF4* with both SLE and rheumatoid arthritis (RA) and in order to avoid this bias, we excluded prior to the analysis all overlap syndromes consisting of associated SLE or RA in the French SSc sample. Demographic data and disease characteristics of patients with SSc and controls are summarized in Table 1. All patients with SSc were tested for antinuclear antibodies by indirect immunofluorescence (IIF), with human epithelial cell line 2 cells as the antigen substrate (Antibodies Inc., Davis, CA, USA). We systematically checked for antibodies specific to SSc. ACA were identified on the basis of their distinctive IIF pattern. ATA were determined by counter immunoelectrophoresis. Our study was approved by the local institutional review board, and written informed consent was obtained from all subjects.

Genotyping

We selected the *TNFSF4* SNP, for which convincing associations with SLE and SSc have been reported[5,6,7,8,9,10,11]. These SNP were genotyped using a competitive allele-specific polymerase chain reaction system (Kaspar genotyping, KBioscience, Hoddesdon, UK) as described[15]. The average genotype completeness for these SNP was 99% for both the SSc

and the control samples. The accuracy was > 99%, according to duplicate genotyping of 10% of all samples using the Taqman SNP genotyping assay-allelic discrimination method (Applied Biosystems, Foster City, CA, USA). *Statistical analyses and metaanalysis*. The statistical analyses were performed using the R computer package software (version 2.13.0). The level of significance for all the tests corresponds to a type-I error-rate $\alpha = 5\%$. OR and their 95% CI are reported. Tests for conformity with Hardy-Weinberg equilibrium (HWE) were performed using a standard chi-squared test (1° of freedom). Individual association analyses of the *TNFSF4* SNP with SSc were performed by comparing cases and controls with both an additive association test providing OR and p values for alleles and a genotypic test providing OR and p values for genotypes. The OR and p values of these tests were computed by the mean of logistic regression models. The same procedure was applied to subgroups stratified according to SSc phenotype. The Bonferroni's correction was applied for all the tests performed, by multiplying the p values by 5 for testing SNP association with the disease and by 10 when comparing SSc subgroups and controls (10 phenotypic subsets).

A metaanalysis was conducted by logistic regression, adjusted for each study population, as described, using data from the studies by Gourh, *et al*[10] and Bossini-Castillo, *et al*[11], taking into account that these latter respected HWE. This method is equivalent to the Mantel–Haenszel metaanalysis method. Statistical power was assessed as described[15,18] and determined for the previously reported OR. As an example, in the French sample, taking into account the expected frequency of the rare allele of rs2205960, the set has a power of 72.3% and 98.0% for detecting an association of magnitude 1.3 and 1.5 (OR), respectively, at the 5% significance level. In the combined populations, the set has a power of 93.5% and 99.9% for detecting an association of magnitude 1.3 and 1.5 (OR), respectively.

Haplotype analysis

An analysis of haplotype diversity was performed for the 5 contiguous SNP using the expectation-maximization algorithm as implemented in the Haplo.Stats R library. The most frequent haplotype is then considered as a reference and the other haplotypes are compared to it using Fisher's exact test. A global p value comparing all haplotypes is also computed by applying a chi-squared test on the corresponding contingency table.

RESULTS

The *TNFSF4* SNP were at HWE in the control population. Allelic frequencies were found to be in good agreement with those reported for the European population[10] (Table 2).

No variant showed allelic association with the overall SSc; however, the rs2205960 TT genotype was found to be associated ($p_{corr} = 0.0035$, OR 2.16, 95% CI 1.37–3.4). However, the 5 SNP showed association or trend for association with lcSSc, but the level of association was much higher for the subgroup of ACA+ patients with SSc. In this latter subset, rs2205960 had the highest magnitude of association (Table 2). The T risk allele was found on 26.8% of SSc chromosomes compared to 21.1% in controls ($p_{corr} = 0.015$, OR 1.37, 95% CI 1.12–1.66) and the TT homozygous risk genotype in 7.6% compared to 2.9% in controls ($p_{corr} = 0.00042$, OR 2.98, 95% CI 1.73–5.1). The data fit well with a recessive model of inheritance for the SSc susceptibility rs2205960 T allele (p-value for recessive model: 0.00017; dominant model: 0.02; additive: 0.00089; Table 2).

Intracohort analyses revealed significant allelic association between the 5 TNFSF4 SNP and the ACA+ subset when compared to the ATA+ subset. No trend for association was observed for the subset of patients with fibrosing alveolitis, and pulmonary arterial hypertension (data not shown). Linkage disequilibrium (LD) analyses revealed that the 5

SNP belong to the same LD block (Figure 1), in good agreement with the above results and published data[5].

Haplotype analyses led to the identification of 3 common haplotypes (frequency > 5%). All of them were found to act with a neutral effect regarding the overall SSc susceptibility. One risk haplotype and 1 protective were significantly associated with ACA positivity, and a trend of association was found with lcSSc (Table 3).

Metaanalysis was performed of the 3 most strongly associated SNP, according to the previous reports[10,11], providing a study population of 4989 patients with SSc and 4661 controls. The metaanalysis strengthened association with SSc disease *per se* but confirmed stronger association in the ACA+ SSc subsets (Table 4). For rs220590, the T risk allele was found associated with the overall SSc (p = 0.0021, OR 1.19, 95% CI 1.06–1.32), lcSSc (p = 0.0003, OR 1.25, 95% CI 1.11–1.42), and ACA+ subsets (p = 0.00013, OR 1.33, 95% CI 1.15–1.54). Intracohort analysis revealed significant allelic association of rs12039904 and rs844648 with ACA+ subset when compared to ATA+ SSc individuals: p = 0.011, OR 1.21, 95% CI 1.04–1.41 and p = 0.0025, OR 1.14, 95% CI 1.05–1.23, respectively.

DISCUSSION

Results from genetics association studies can show discrepancies and a lack of reproducibility that can be due to several factors: population stratification, phenotype differences, selection biases, genotyping errors, variations in LD blocks among different populations, and other factors[19]. Thus, independent replication with large sample sizes represents the best way to definitely identify genetic risk loci[19]. Our study aimed at the replication of the putative association of *TNFSF4* variants with the genetic susceptibility to SSc. Our supportive results establish *TNFSF4* as an SSc susceptibility gene, but the association signal appears to be preferential in the subset of patients with SSc who are ACA +.

The 5 *TNFSF4* SNP investigated show association with the ACA+ SSc subset with the greatest association signal for the rs2205960 SNP. These results are congruent with previous studies concerning SLE and SSc[5,11]. In SLE, the strongest associated variants were rs844648 and rs12039904 in white European patients[5,6]. Another replication study in a Chinese population found the 2 *TNFSF4* rs844648 and rs2205960 SNP independently associated with the overall SLE[7]. Genotype-phenotype association analysis revealed an influence of *TNFSF4* SLE susceptibility variants on autoantibody subphenotypes (anti-Ro), supporting a link between *TNFSF4* and autoantibody production. Anti-Ro status, as the presence of secondary Sjögren's syndrome (SS), was only available in the French sample and we found no association of *TNFSF4* variants with these subsets (data not shown). However, the sample does not allow firm conclusions and therefore investigations regarding these subsets remain to be done in a very large sample.

Regarding SSc, the 2 previously reported association studies concerning *TNFSF4* found an overall association with SSc but discrepancies regarding both cutaneous and autoantibody subsets[10,11]. In contrast, our replication study performed in a French white population found an association between *TNFSF4* and SSc restricted to both ACA+ and lcSSc phenotypes. This led us to perform a metaanalysis on available data. The results reinforce our initial findings and further clarify the putative role of *TNFSF4* in modifying the SSc phenotype, specifically for the ACA+ subset, the antibody mostly associated with the limited cutaneous phenotype. Secondary analyses of a genome-wide association study recently showed an association between rs2205960 and lcSSc that partly supports our findings. However, when SSc autoantibody subgroups were analyzed, the authors observed the strongest association

in the ATA+ subset, without association with dcSSc. This will need further investigation because ATA+ are strongly correlated with dcSSc[20].

In our population, the 5 *TNFSF4* SNP are located in the same LD block and explained the trend for association of all tested SNP (Table 2).

The rs2205960 SNP is located in the 5′ upstream region and is believed to affect *TNFSF4* expression[5]. Indeed, functional analyses were performed by Cunninghame Graham, *et al*[5] using lymphoblastoid cell lines and peripheral blood lymphocytes from patients with SLE. The authors have reported that homozygous cells, for either the overtransmitted haplotype or the undertransmitted haplotype, showed, after activation, differential upregulation of the OX40L messenger RNA (mRNA) levels and cell-surface expression of OX40L. Although not being in a coding region, rs2205960 variant could therefore affect mRNA levels through regulatory functions. However, this remains to be directly demonstrated, as only correlations have been reported so far. A first step could be the investigation of mRNA/protein expression using various cellular models coming from patients with SSc and controls with stratification according to genotypes. We could not do this because cellular material had not been collected. Moreover, it may be that a deep regional sequencing is now required in order to definitively identify the causal variant. Moreover, taking into account the sera results[3] together with the herein genetic data, we only have only incomplete evidence of a specific role of *TNFSF4* in SSc. Indeed, the relationships with ACA production need to be further investigated. Even more importantly, the effect of *TNFSF4* on the specific tissular and vascular fibrotic component that characterizes SSc, even in its limited cutaneous subset, must be studied in order to clarify whether *TNFSF4* only reflects shared autoimmunity or is a critical actor in the pathogenesis of SSc.

Other haplotypes were shown associated with other autoimmune diseases such as SS[21]. Interestingly, autoimmune disease coexisting with SSc is predominantly observed in patients with lcSSc, suggesting a specific involvement of autoimmunity in this feature[22]. In the same way, another risk locus of SSc and other autoimmune diseases influencing T cell activation, *PTPN22*, has been shown to be restricted to the lcSSc subtype in a recent metaanalysis[23].

Phenotypic heterogeneity is another concern regarding genetic studies. Because of the critical role of shared autoimmunity in systemic autoimmune disease, it is important to investigate patients with SSc for other candidate gene-associated diseases. That is why we excluded from our replication cohort SLE and RA SSc overlap. Gourh, *et al*[10], excluded SLE from their cohort. Unfortunately this exclusion criterion was not applied in the report from Bossini-Castillo, *et al*[11]. Nevertheless, in the French cohort, SLE or RA SSc overlap represent < 1% of the cohort (data not shown) and together with the very large sample made available by the metaanalysis, we regard this bias as weak.

Our data helped to clarify the role of *TNFSF4* in modifying the SSc phenotype. *TNFSF4* is preferentially involved in ACA+ SSc susceptibility, a subset that seems to have a high frequency of multiple autoimmune diseases. This suggests that future therapy targeting the *TNFSF4* pathway and T cell modulation should be preferentially investigated in this subgroup.

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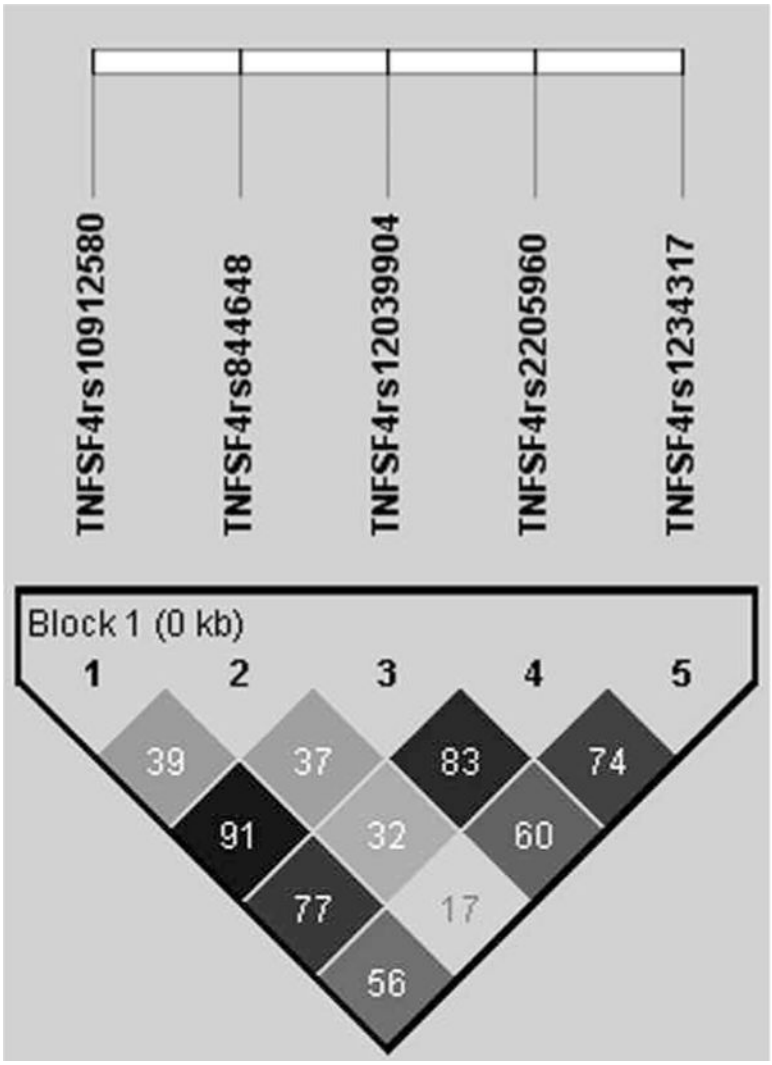

Figure 1.
Linkage disequilibrium (LD) and haplotype block structure of *TNFSF4* gene within healthy controls. Measure of strength of LD, r^2 values are given as numerical values within each box.

Table 1

Characteristics of the patients with systemic sclerosis in the white French population. Data are n (%) unless otherwise indicated.

Characteristics	SSc Cohort, n = 1031
Age, yrs ± SD	46.2 ± 14.8
Female, %	85.7
Disease duration, yrs ± SD	10.6 ± 8.1
Diffuse cutaneous subtype	321/971 (33)
Limited cutaneous subtype	650/971 (67)
Antinuclear antibodies (≥ 1/180)	739/928 (80)
Positive antitopoisomerase I antibodies	253/928 (27)
Positive anticentromere I antibodies	377/928 (41)
Fibrosing alveolitis on CT scan	359/920 (39)
Pulmonary arterial hypertension	68/965 (7)
Digital ulcers (past or present)	333/918 (36)

CT: computed tomography.

Table 2

Genotype and allele frequencies of *TNFSF4* single-nucleotide polymorphisms in patients with systemic sclerosis (SSc) and controls from the French population. Numbers in boldface are significant values.

	MAF	Allelic p	Corrected Allelic p	OR (95% CI)	Genotype, n (%)	Genotypic p	Corrected Genotypic p	OR (95% CI)		
rs2205960	T				TT	TG	GG			
SSc, n = 1011	23.49	0.06	0.3	1.15 (0.99–1.33)	61 (6.0)	353 (34.9)	597 (59.1)	**0.0007**	**0.0035**	**2.16 (1.37–3.4)**
dcSSc, n = 315	21.11	0.98	NA	1.0 (0.8–1.2)	18 (5.7)	97 (30.8)	200 (63.5)	**0.035**	0.35	1.9 (1.04–3.5)
SSc ATA+, n = 248	21.17	0.96	NA	1.0 (0.8–1.3)	16 (6.5)	73 (29.4)	159 (64.1)	**0.02**	0.2	2.13 (1.1–4.0)
lcSSc, n = 636	25	**0.0086**	0.086	1.25 (1.06–1.47)	41 (6.4)	236 (37.1)	359 (56.5)	**0.0003**	**0.003**	**2.4 (1.48–3.96)**
SSc ACA+, n = 368	26.77	**0.0015**	**0.015**	**1.37 (1.12–1.66)**	28 (7.6)	141 (38.3)	199 (54.1)	**0.000042**	**0.00042**	**2.98 (1.73–5.1)**
Controls, n = 1011	21.07	NA	NA	NA	29 (2.9)	368 (36.4)	614 (60.7)	NA	NA	NA
Expected frequencies in controls (Hapmap)	20.0									
rs1234317	T				TT	TC	CC			
SSc, n = 1010	26.24	0.33	NA	1.07 (0.9–1.2)	75 (7.4)	380 (37.6)	555 (55.0)	**0.027**	0.135	1.53 (1.05–2.2)
dcSSc, n = 314	24.36	0.79	NA	0.97 (0.8–1.2)	21 (6.7)	111 (35.3)	182 (58.0)	0.33	NA	1.3 (0.76–2.2)
SSc ATA+, n = 249	24.9	0.99	NA	1.0 (0.8–1.3)	19 (7.6)	86 (34.6)	144 (57.8)	0.16	NA	1.5 (0.85–2.6)
lcSSc, n = 637	27.63	0.08	0.8	1.15 (0.98–1.35)	52 (8.2)	248 (38.9)	337 (52.9)	**0.0075**	0.075	1.75 (1.16–2.7)
SSc ACA+, n = 371	28.98	**0.031**	0.31	1.2 (1.02–1.49)	34 (9.2)	147 (39.6)	190 (51.2)	**0.0026**	**0.026**	**2.03 (1.27–3.2)**
Controls, n = 990	24.9	NA	NA	NA	48 (4.9)	397 (40.1)	545 (55.0)	NA	NA	NA
Expected frequencies in controls (Hapmap)	23.3									
rs12039904	T				TT	TG	GG			
SSc, n = 1011	23.99	0.29	NA	1.08 (0.9–1.3)	57 (5.6)	371 (36.7)	583 (57.7)	0.19	NA	1.31 (0.9–2.0)
dcSSc, n = 313	20.61	0.3	NA	0.89 (0.7–1.1)	15 (4.8)	99 (31.6)	199 (63.6)	0.97	NA	1.01 (0.6–1.9)
SSc ATA+, n = 246	20.73	0.38	NA	0.9 (0.7–1.1)	13 (5.3)	76 (30.9)	157 (63.8)	0.75	NA	1.1 (0.6–2.1)
lcSSc, n = 639	25.9	**0.029**	0.29	1.20 (1.02–1.4)	39 (6.1)	253 (39.6)	347 (54.3)	**0.07**	0.7	1.51 (0.96–2.4)
SSc ACA+, n = 368	27.99	**0.003**	**0.03**	**1.33 (1.1–1.6)**	27 (7.3)	152 (41.3)	189 (51.4)	**0.01**	0.1	1.92 (1.16–3.2)
Controls, n = 997	22.57	NA	NA	NA	44 (4.4)	362 (36.3)	591 (59.3)	NA	NA	NA
Expected frequencies in controls (Hapmap)	21.9									

	MAF	Allelic p	Corrected Allelic p	OR (95% CI)	Genotype, n (%)	Genotypic p	Corrected Genotypic p	OR (95% CI)		
rs10912580	G				GG	GA	AA			
SSc, n = 1007	24.53	0.18	NA	1.11 (0.95–1.3)	64 (6.4)	366 (36.3)	577 (57.3)	0.13	NA	1.35 (0.9–2.0)
dcSSc, n = 314	21.66	0.58	NA	0.94 (0.8–1.2)	18 (5.7)	100 (31.9)	196 (62.4)	0.71	NA	1.1 (0.6–2.0)
SSc ATA+, n = 249	21.69	0.62	NA	0.9 (0.7–1.2)	14 (5.6)	80 (32.1)	155 (62.3)	0.77	NA	1.1 (0.6–2.0)
lcSSc, n = 634	26.34	**0.019**	0.19	1.22 (1.03–1.4)	43 (6.8)	248 (39.1)	343 (54.1)	0.055	0.55	1.5 (0.99–2.3)
SSc ACA+, n = 368	28.8	**0.001**	**0.01**	**1.38 (1.14–1.7)**	32 (8.7)	148 (40.2)	188 (51.1)	**0.0024**	**0.024**	**2.06 (1.28–3.3)**
Controls, n = 999	22.72	NA	NA	NA	29 (2.9)	368 (36.4)	614 (60.7)	NA	NA	NA
Expected frequencies in controls (Hapmap)	20.8									
rs844648	A				AA	AG	GG			
SSc, n = 1009	43.61	0.086	0.43	1.12 (0.99–1.3)	214 (21.2)	452 (44.8)	343 (34.0)	**0.047**	0.235	1.29 (1.003–1.7)
dcSSc, n = 313	41.05	0.96	NA	1.0 (0.8–1.2)	64 (20.5)	129 (41.2)	120 (38.3)	0.59	NA	1.1 (0.8–1.6)
SSc ATA+, n = 249	39.55	0.94	NA	0.9 (0.8–1.2)	51 (20.9)	91 (37.3)	102 (41.8)	0.87	NA	1.0 (0.7–1.5)
lcSSc, n = 637	44.98	**0.022**	0.22	1.18 (1.02–1.4)	136 (21.4)	301 (47.2)	200 (31.4)	**0.019**	0.19	1.4 (1.06–1.9)
SSc ACA+, n = 367	46.87	**0.0054**	0.054	1.27 (1.07–1.5)	77 (21.0)	190 (51.8)	100 (27.3)	**0.0089**	0.089	1.6 (1.12–2.3)
Controls, n = 1004	40.94	NA	NA	NA	171 (17.0)	480 (47.8)	353 (35.2)	NA	NA	NA
Expected frequencies in controls (Hapmap)	40.8									

dcSSc: diffuse cutaneous SSc; ATA+: antitopoisomerase I antibodies; lcSSc: limited cutaneous SSc; ACA+: anticentromere antibodies; NA: not applicable.

Table 3

Predicted *TNFSF4* haplotypes in systemic sclerosis (SSc), SSc subsets, and healthy controls. Numbers in boldface are significant values.

***TNFSF4* Haplotypes**	**rs12039904**	**rs10912580**	**rs844648**	**rs1234317**	**rs2205960**	**Frequency in Comparator**	**Frequency in Patients**	**p**	**OR (95% CI)**
SSc vs controls									
1	C	A	G	C	G	0.53	0.51	0.037	1.10 (0.97–1.24)
2	C	A	A	C	G	0.18	0.19	0.22	0.93 (0.79–1.09)
3	T	G	A	T	T	0.19	0.21	0.048	0.89 (0.76–1.04)
lcSSc vs controls									
1	C	A	G	C	G	0.53	0.50	0.0022	0.87 (0.76–1.00)
2	C	A	A	C	G	0.18	0.19	0.86	1.01 (0.84–1.22)
3	T	G	A	T	T	0.19	0.23	**0.00046**	**1.21 (1.02–1.44)**
dcSSc vs controls									
1	C	A	G	C	G	0.53	0.53	1	1.00 (0.83–1.19)
2	C	A	A	C	G	0.18	0.20	0.03	1.13 (0.90–1.42)
3	T	G	A	T	T	0.19	0.19	0.42	0.95 (0.75–1.20)
lcSSc vs dcSSc									
1	C	A	G	C	G	0.53	0.50	**0.012**	0.87 (0.72–1.06)
2	C	A	A	C	G	0.20	0.19	0.13	0.90 (0.70–1.15)
3	T	G	A	T	T	0.19	0.23	**0.0004**	**1.27 (1.00–1.63)**
ACA+ vs controls									
1	C	A	G	C	G	0.53	0.47	**5.53×10^{-7}**	**0.80 (0.67–0.95)**
2	C	A	A	C	G	0.18	0.18	1	1.00 (0.80–1.24)
3	T	G	A	T	T	0.19	0.24	**7.71×10^{-7}**	**1.31 (1.07–1.61)**
ATA+ vs controls									
1	C	A	G	C	G	0.53	0.53	0.88	1.00 (0.82–1.23)
2	C	A	A	C	G	0.19	0.18	0.50	1.04 (0.80–1.34)
3	T	G	A	T	T	0.19	0.19	0.29	0.95 (0.73–1.22)
ACA+ vs ATA+									
1	C	A	G	C	G	0.47	0.53	**0.0013**	**1.26 (1.00–1.59)**
2	C	A	A	C	G	0.19	0.19	0.78	1.03 (0.76–1.39)
3	T	G	A	T	T	0.24	0.18	**0.00011**	**0.72 (0.53–0.96)**

dcSSc: diffuse cutaneous SSc; ATA+: antitopoisomerase I antibodies; lcSSc: limited cutaneous SSc; ACA+: anticentromere antibodies.

Table 4

Metaanalysis of *TNFSF4* minor allele frequencies, heterozygous and homozygous genotype of rs2205960, rs844648, rs12039904 in French, North American, and other European combined white populations. Numbers in boldface are significant values.

	Allele Genotype	p	OR (95% CI)
rs2205960 (French and North American)			
SSc, n = 2043	T	**0.0021**	**1.19 (1.06–1.32)**
	TG	0.070	1.14 (0.99–1.30)
	TT	**0.0029**	**1.60 (1.18–2.19)**
dcSSc, n = 700	T	0.12	1.13 (0.97–1.31)
	TG	0.33	1.10 (0.91–1.33)
	TT	0.14	1.37 (0.90–2.08)
SSc ATA+, n = 422	T	0.082	1.17 (0.98–1.40)
	TG	0.85	0.98 (0.78–1.23)
	TT	**0.0024**	**1.99 (1.27–3.10)**
lcSSc, n = 1231	T	**0.0003**	**1.25 (1.11–1.42)**
	TG	**0.021**	**1.20 (1.03–1.40)**
	TT	**0.00093**	**1.78 (1.26–2.49)**
SSc ACA+, n = 664	T	**0.00013**	**1.33 (1.15–1.54)**
	TG	**0.013**	**1.27 (1.05–1.54)**
	TT	**0.00046**	**2.02 (1.36–2.98)**
Controls, n = 1703		NA	NA
rs844648 (French, North American, and other European)			
SSc, n = 4989	A	**0.0014**	**1.10 (1.04–1.16)**
	AG	0.058	1.11 (0.99–1.24)
	AA	**0.0016**	**1.20 (1.07–1.35)**
dcSSc, n = 1447	A	0.14	1.07 (0.98–1.16)
	AG	0.90	1.01 (0.86–1.19)
	AA	0.19	1.12 (0.94–1.33)
SSc, ATA+, n = 949	A	0.75	1.02 (0.92–1.12)
	AG	0.063	1.20 (0.99–1.45)
	AA	0.48	1.07 (0.88–1.31)
lcSSc, n = 2916	A	**0.0018**	**1.11 (1.04–1.19)**
	AG	**0.041**	**1.14 (1.01–1.29)**
	AA	**0.0017**	**1.24 (1.08–1.41)**
SSc ACA+, n = 1526	A	**0.0025**	**1.14 (1.05–1.23)**
	AG	0.35	1.08 (0.92–1.26)
	AA	**0.0047**	**1.27 (1.08–1.50)**
Controls, n = 4661		NA	NA
rs12039904 (French and other European)			
SSc, n = 3905	T	**0.00027**	**1.15 (1.06–1.23)**
	CT	**0.037**	**1.10 (1.01–1.21)**
	TT	**0.00038**	**1.42 (1.17–1.72)**

	Allele Genotype	p	OR (95% CI)
dcSSc, n = 1050	T	0.76	1.02 (0.91–1.14)
	CT	0.39	0.94 (0.81–1.09)
	TT	0.15	1.24 (0.92–1.66)
SSc, ATA+, n = 769	T	0.76	1.02 (0.90–1.16)
	CT	0.76	0.97 (0.83–1.15)
	TT	0.40	1.16 (0.82–1.63)
lcSSc, n = 2278	T	**0.000057**	**1.19 (1.09–1.29)**
	CT	**0.0019**	**1.19 (1.07–1.32)**
	TT	**0.0016**	**1.43 (1.15–1.79)**
SSc ACA+, n = 1208	T	**0.000089**	**1.23 (1.11–1.37)**
	CT	**0.0056**	**1.21 (1.06–1.39)**
	TT	**0.00076**	**1.58 (1.21–2.07)**
Controls, n = 3988		NA	NA

dcSSc: diffuse cutaneous SSc; ATA+: antitopoisomerase I antibodies; lcSSc: limited cutaneous SSc; ACA+: anticentromere antibodies. NA: not applicable.