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Evidence of the Contribution of the X Chromosome to Systemic Sclerosis Susceptibility

Association With the Functional *IRAK1* 196Phe/532Ser Haplotype

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Objective. **Several autoimmune disorders, including systemic sclerosis (SSc), are characterized by a strong sex bias. To date, it is not known whether genes on the sex chromosomes influence SSc susceptibility. Recently, an *IRAK1* haplotype that contains the 196Phe functional variant (rs1059702), located on Xq28, was found to confer susceptibility to systemic lupus erythematosus (SLE). This study was undertaken to test for an association between SSc and the *IRAK1* SLE risk haplotype.**

Methods. **We tested for an association with the *IRAK1* SLE risk haplotype in a discovery set of 849 SSc patients and 625 controls. *IRAK1* rs1059702 was further genotyped in a replication set, which included Caucasian women from Italy (493 SSc patients and 509 controls) and Germany (466 SSc patients and 1,083 controls).**

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Results. **An association between the *IRAK1* haplotype and SSc was detected in the discovery set. In both the discovery and replication sets, the rs1059702 TT genotype was found to be associated with specific SSc subsets, highlighting a potential contribution to disease severity. A meta-analysis provided evidence of an association of both the T allele and TT genotype with the overall disease, with an odds ratio (OR) of 1.20 and 95% confidence interval (95% CI) of 1.06–1.35 for the T allele ($P = 0.003$) and an OR of 1.49 and 95% CI of 1.06–2.10 for the TT genotype ($P = 0.023$). However, the most notable associations were observed with the diffuse cutaneous, anti–topoisomerase I antibody positive, and SSc-related fibrosing alveolitis subsets (OR 2.35 [95% CI 1.51–3.66], $P = 1.56 \times 10^{-4}$, OR 2.84 [95% CI 1.87–4.32], $P = 1.07 \times 10^{-6}$, and OR 2.09 [95% CI 1.35–3.24], $P = 9.05 \times 10^{-4}$, respectively).**

Conclusion. **Our study provides the first evidence of an association between *IRAK1* and SSc, demonstrating that a sex chromosome gene directly influences SSc susceptibility and its phenotypic heterogeneity.**

Systemic sclerosis (SSc) is a complex autoimmune disease that occurs in genetically predisposed individuals who have experienced certain environmental or stochastic stimuli (1). Despite phenotypic heterogeneity, the notion of a genetic contribution to the development of SSc is supported by the increased sibling recurrence risk ratio among patients with SSc (2) and a higher concordance rate for antinuclear autoantibodies in monozygotic than in dizygotic twins (3), suggesting that the genetic component may selectively contribute to susceptibility to SSc-related autoimmune processes. To date, most of the SSc-associated gene products have been shown to participate in key pathways involved in the pathogenesis of multiple autoimmune diseases, notably systemic lupus erythematosus (SLE) (1).

Recent studies have shown that the main genetic factors that contribute the most to SSc susceptibility involve the major histocompatibility complex (MHC) and non-MHC genes involved in antigen processing, T cell activation, and innate immunity (4–6). Regarding innate immunity, both NF-κB and interleukin-1 (IL-1) processing seem to play pivotal roles in SSc pathogenesis, as indicated by the contribution of *TNFAIP3* and *NLRP1* to SSc susceptibility (6,7). Among the NF-κB regulators, IL-1 receptor–associated kinase 1 (IRAK-1, encoded by the *IRAK1* gene), a serine/threonine protein kinase, was shown in mouse models to regulate NF-κB through T cell receptor signaling and Toll-like receptor activation as well as the induction of both interferon-α (IFNα) and IFNγ (8). Of interest, the type 1 IFN signature is a shared hallmark of various autoimmune diseases and connective tissue diseases, including SSc (9). Several autoimmune disorders, notably SSc, are characterized by a strong sex bias, with women being affected more frequently than men (10).

Research efforts over the past 3 decades have implicated sex hormones as being responsible for the sex difference in disease susceptibility. However, the effects of sex hormones do not rule out a more direct effect of the X chromosome. To date, nothing is known about whether genes on the sex chromosomes can directly influence SSc susceptibility. Interestingly, recent reports have indicated that various single-nucleotide polymorphisms (SNPs) of *IRAK1*, which are located on Xq28, confer susceptibility to SLE (8).

The SNPs that show the most significant associations (*IRAK1* rs2239673, rs763737, rs5945174, and rs7061789) are in a linkage disequilibrium block that extends from intron 10 to intron 13 of the *IRAK1* gene, encompassing exons 11–13, which corresponds to the C1 domain of IRAK-1 (8,11). It has been shown that this domain is at least partially responsible for the interaction with signal transduction factors such as TRAF6 (12). Additionally, 2 nonsynonymous *IRAK1* SNPs (196Phe/532Ser [rs1059702] and Leu532Ser [rs1059703]), which are in complete linkage disequilibrium and are also located within the SLE-associated haplotype block, were found to have functional consequences, such as increased NF-κB activity, in Caucasian women (13–16). Taken together, these findings make it tempting to speculate that the *IRAK1* SLE risk haplotype harboring the functional 196Phe/532Ser variant also contributes to SSc susceptibility.

PATIENTS AND METHODS

Study population and study design. We performed a large case–control association study that involved a total of 4,025 individuals and included a replication step. The *IRAK1* gene is located on Xp28, and the study included only women so that the contribution of *IRAK1* genotypes could be assessed. The discovery sample included 849 SSc patients and 625 controls, all of whom were from the French network as previously described (6). The replication step included women from Italy (493 SSc patients and 509 controls) and Germany (466 SSc patients and 1,083 controls). All of the SSc patients were classified by cutaneous subtype according to the criteria of LeRoy et al (17) and phenotypically assessed as recommended (18,19). The characteristics of the SSc patients are summarized in Table 1.

The control groups consisted of healthy unrelated women who were matched for ethnicity to the SSc cases (all individuals were of European Caucasian ancestry). All local institutional review boards approved the study, and written informed consent was obtained from all subjects. SSc patients

Table 1. Characteristics of the SSc patients in the 3 European populations genotyped for the *IRAK1* rs1059702 polymorphism*

	French population (n = 849)	German population (n = 466)	Italian population (n = 493)
Female	100	100	100
Age, mean ± SD years	57.0 ± 13.4	57.7 ± 14.4	56.9 ± 13.4
Disease duration, mean ± SD years	11.0 ± 8.3	18.3 ± 18.8	12.3 ± 9.1
Limited cutaneous subtype	70.2	69.1	78.3
Diffuse cutaneous subtype	29.8	30.9	21.7
Anti–topo I antibody positive	24.1	27.0	30.2
ACA positive	42.9	42.3	46.4
Fibrosing alveolitis on CT scan	39.3	31.4	33.9

* Except where indicated otherwise, values are the percent of patients. SSc = systemic sclerosis; anti–topo I = anti–topoisomerase I; ACA = anticentromere antibody; CT = computed tomography.

were tested for antinuclear antibodies using indirect immunofluorescence (IIF) and HEp-2 cells as antigen substrate (Antibodies Inc). Specific SSc antibodies were systematically assessed. The presence of anticentromere antibodies (ACAs) was determined by their distinctive IIF pattern on HEp-2 cells, and the presence of anti–topoisomerase I (anti–topo I) antibodies was determined by counterimmunoelectrophoresis. Regarding lung fibrosis, fibrosing alveolitis was defined as the presence of typical features (ground-glass opacity and/or a reticular or reticulonodular pattern in a peripheral distribution) on chest high-resolution computed tomography, which was performed on all of the SSc patients included in this study.

Genotyping. All of the subjects in the discovery set were genotyped for the *IRAK1* SNPs rs2239673, rs763737, rs5945174, rs7061789, and rs1059702 using a competitive allele-specific polymerase chain reaction system (KASPar genotyping; KBioscience) and the TaqMan SNP genotyping allele discrimination method (Applied Biosystems), as previously described (5). Given the complete linkage disequilibrium between rs1059702 and rs105973, only *IRAK1* rs1059702 was genotyped in the replication set. The average genotype completeness was 98% for SSc and control samples for all of the SNPs investigated except for rs5945174 (genotype completeness <85%), which was excluded from the study. The accuracy was >99%, according to duplicate genotyping of 10% of all samples. In the replication step, we used genome-wide data from 291 Italian and 993 female German controls from the HYPERGENES (www.hypergenes.eu) and KORA F3 (20,21) studies to enlarge our replication control groups, since the *IRAK1* rs1059702 marker was included in the chips used for these projects.

Statistical analysis. Statistical analyses were performed using the R software package, version 2.10.1. The level of significance for all tests corresponded to an alpha value of 5% for Type I error. Tests for conformity to Hardy-Weinberg equilibrium were performed using a standard chi-square test (1 df) to test for differences between observed and expected genotype distributions based on control population allele frequencies.

We first performed an analysis of haplotype diversity of the 4 contiguous *IRAK1* SNPs that determine the *IRAK1* SLE risk haplotype using the expectation-maximization algorithm as implemented in the Haplo.Stats R library. Haploview provided graphic representation of linkage disequilibrium. Bonferroni correction was applied for all tests performed for a "generating hypothesis step" when comparing SSc subgroups and controls (i.e., 6 tests since 6 phenotypic subsets were investigated, including the 2 cutaneous subtypes, ACA-positive and anti–topo I–positive subsets, and patients with and without fibrosing alveolitis). *P* values after this adjustment for multiple testing are indicated as P_{adj} in the text and tables.

Given our a priori hypothesis, if an association between the *IRAK1* SLE susceptibility haplotype and SSc was detected, we thereafter decided to test only the functional *IRAK1* 196Phe risk variant (rs1059702) for association in the replication sample. In the case of a defined hypothesis regarding any specific SSc subset detected in the discovery sample, we thereafter tested it in the replication samples, without correction for multiple testing.

Individual association analyses of the *IRAK1* rs1059702 polymorphism with SSc were performed by comparing cases and controls using Fisher's exact test on genotypes. The corresponding odds ratios (ORs) (and 95% confidence intervals [95% CIs]) were assessed using a standard logistic regression analysis with the most frequent homozygous genotype in the control population used as the reference. The same procedure was applied in subgroups stratified according to SSc phenotype and compared to controls. A genotype–phenotype correlation analysis was performed using intracohort tests comparing an SSc subset with a particular feature to the SSc subset without that feature. The main associations concerned the diffuse cutaneous SSc (dcSSc), anti–topo I–positive, and fibrosing alveolitis subsets. We performed multivariate logistic regression analysis including *IRAK1* rs1059702 and the 3 relevant SSc subsets in order to assess the respective independent effect of these 3 genetic and phenotypic factors on pulmonary fibrosis.

Meta-analysis of *IRAK1* rs1059702. The combined data including the 3 populations were analyzed by calculation of homogeneity of ORs among the cohorts using the Breslow-Day and Woolf Q methods, and by calculation of the pooled ORs under a fixed-effects model (Mantel-Haenszel meta-analysis) or random-effects model (DerSimonian-Laird) when necessary.

RESULTS

Discovery set. *Haplotype analysis.* Genotype frequencies of all 4 of the *IRAK1* SNPs investigated were in Hardy-Weinberg equilibrium in the study population.

Consistent with the results of the previously reported linkage disequilibrium analysis (8), the *IRAK1* SNPs rs2239673, rs763737, rs7061789, and rs1059702 belonged to the same linkage disequilibrium block (Figure 1). Two hundred women from the French Caucasian control population were also genotyped for *IRAK1* rs1059703 in order to assess linkage disequilibrium with rs1059702. Among the 400 X chromosomes investigated, rs1059702 and rs1059703 were found to be in complete linkage disequilibrium (data not shown).

Among the 16 *IRAK1* haplotypes defined by the 4 *IRAK1* SNPs rs2239673, rs763737, rs7061789, and rs1059702, 2 common haplotypes (frequency >5% in both controls and cases) were predicted from our sample. The TAAC haplotype (which carries the 196Ser protective variant) and its mirrored haplotype CGGT (which carries the 196Phe risk variant) were observed in 80% and 14% of the control chromosomes, respectively. The overall association between all of the *IRAK1* predicted haplotypes and disease status was not significant (global $P = 0.40$). Interestingly, when SSc subsets were analyzed, and after correction for multiple testing, association of the TAAC protective haplotype was detected in patients with dcSSc ($P_{adj} = 4.16 \times 10^{-4}$), anti–topo I–positive SSc patients ($P_{adj} = 4.07 \times 10^{-6}$), and SSc patients with fibrosing alveolitis ($P_{adj} = 0.0023$) (Table 2). The frequency of the mirrored haplotype of TAAC (CGGT) was found to be increased in chromosomes from patients with dcSSc, SSc patients who were anti–topo I positive, and SSc patients with fibrosing alveolitis compared to controls. This increase, however, did not reach statistical significance after correction for multiple testing (Table 2).

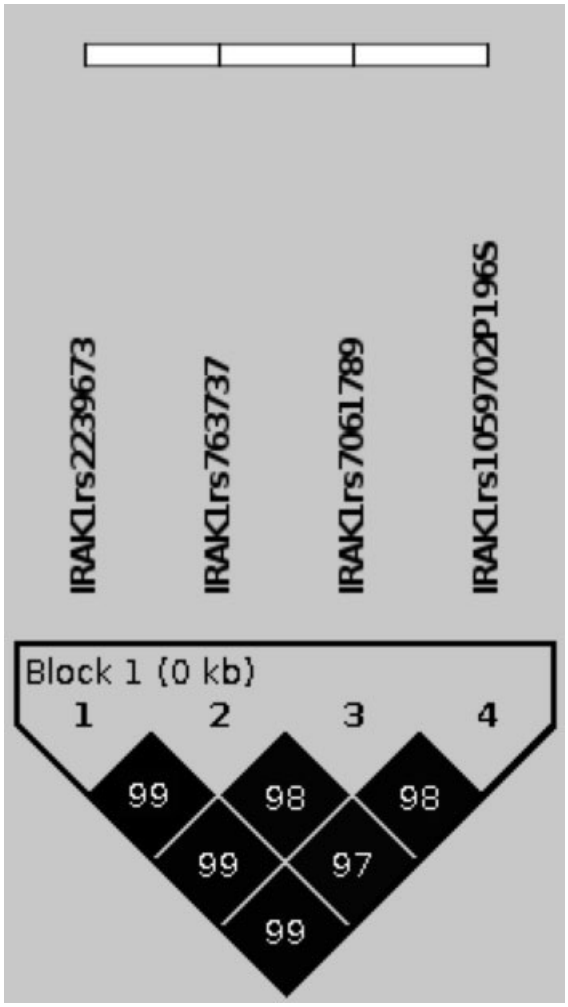

Figure 1. Schematic representation of the significant haplotype block structure in the *IRAK1* gene in the French Caucasian control population. Linkage disequilibrium was measured by D′. D′ values are shown within each box.

IRAK1 rs1059702 single marker analysis. In keeping with our a priori hypothesis, following the detection of an association between the IRAK-1 SLE susceptibility haplotype and SSc, we tested for an association with the functional IRAK-1 variant 196Phe (rs1059702). Consistent with the results of our haplotype analysis, we observed an increase in the frequency of the rs1059702 T risk allele in the SSc subsets that were previously found to be relevant (i.e., dcSSc, anti–topo I–positive SSc, and SSc with fibrosing alveolitis) in the French discovery population when compared to controls, but these increases did not reach statistical significance (Table 3). Genotype analysis revealed a strong increase in the frequency of the homozygous TT genotypes in dcSSc and anti–topo I–positive SSc compared to controls. The frequency in dcSSc was 6.2%, versus 2.9% in controls ($P = 0.022$, $P_{adj} = 0.13$, OR 2.24 [95% CI 1.11–4.54]) and 7.3% in anti–topo I–positive SSc, versus 2.9% in controls ($P = 0.0053$, $P_{adj} = 0.032$, OR 2.65 [1.31–5.40]). No statistically significant association was observed in the subset of SSc patients with fibrosing alveolitis ($P = 0.09$, OR 1.81 [95% CI 0.89–3.65]). (Additional data are available from the corresponding author upon request.) Taking into account the notable differences in the frequency of the *IRAK1* rs1059702 TT risk genotype when comparing SSc patients with fibrosing alveolitis to controls and the elevated OR (1.81) for a given complex disease, we decided to investigate this association in the replication sample despite the fact that the difference was not statistically significant in the discovery sample.

Replication set. In accordance with the haplotype and single marker results obtained in the discovery set, the *IRAK1* SNP rs1059702 was tested for associations with SSc, notably in the dcSSc, anti–topo I–positive SSc,

Table 2. Association of *IRAK1* haplotype with SSc in the French Caucasian population

Population	rs2239673	rs763737	rs7061789	rs1059702	Haplotype frequency	*P*	P_{adj}	Effect direction
Overall SSc (n = 849)								
	T	A	A	C	0.78	0.059	–	Neutral
	C	G	G	T	0.15	0.35	–	Neutral
dcSSc (n = 244)								
	T	A	A	C	0.75	6.94×10^{-5}	4.16×10^{-4}	Protective
	C	G	G	T	0.17	0.012	0.072	Risk
lcSSc (n = 573)								
	T	A	A	C	0.79	0.36	–	Neutral
	C	G	G	T	0.14	0.75	–	Neutral
Anti–topo I antibody positive (n = 205)								
	T	A	A	C	0.74	6.78×10^{-7}	4.07×10^{-6}	Protective
	C	G	G	T	0.17	0.0085	0.051	Risk
ACA positive (n = 365)								
	T	A	A	C	0.78	0.07	–	Neutral
	C	G	G	T	0.14	0.61	–	Neutral
SSc with fibrosing alveolitis (n = 301)								
	T	A	A	C	0.76	3.84×10^{-4}	0.0023	Protective
	C	G	G	T	0.16	0.050	0.30	Risk
SSc without fibrosing alveolitis (n = 464)								
	T	A	A	C	0.79	0.16	–	Neutral
	C	G	G	T	0.15	0.29	–	Neutral
Controls (n = 625)								
	T	A	A	C	0.80			
	C	G	G	T	0.14			

* dcSSc = diffuse cutaneous SSc; lcSSc = limited cutaneous SSc; $P_{adj} = P$ adjusted for multiple comparisons (see Table 1 for other definitions).

and SSc with fibrosing alveolitis subsets, in both the Italian and German populations. Genotype frequencies of rs1059702 were in Hardy-Weinberg equilibrium in the studied populations.

Italian population. Consistent with the association detected in the discovery set, the IRAK-1 rs1059702 TT genotype was found to be strongly associated with anti–topo I–positive SSc ($P = 0.0011$, OR 3.06 [95% CI 1.52–6.16]) in the Italian replication sample. Associations were also detected in both the dcSSc and SSc with fibrosing alveolitis subsets ($P = 0.030$, OR 2.45 [95% CI 1.06–5.62] for dcSSc and $P = 0.036$, OR 2.19 [95% CI 1.04–4.66] for SSc with fibrosing alveolitis). No association was observed for the remaining SSc subsets. (Additional data are available from the corresponding author upon request.)

German population. Similar results were found in the German population. The rs1059702 TT genotype was associated with both the anti–topo I–positive SSc and dcSSc subsets ($P = 0.025$, OR 2.45 [95% CI

Table 3. Meta-analysis of the *IRAK1* rs1059702 risk allele in the European Caucasian population*

	MAF					SSc		dcSSc		Topo+		FA+	
Population	SSc	dcSSc	Topo+	FA+	Controls	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)
French	15.3	17.4	17.8	16.6	14.0	0.34	1.11 (0.90–1.36)	0.07	1.30 (0.98–1.72)	0.06	1.33 (0.99–1.79)	0.14	1.22 (0.94–1.60)
Italian	22.0	25.2	25.8	25.8	18.9	0.08	1.21 (0.98–1.51)	0.034	1.45 (1.03–2.05)	0.008	1.50 (1.11–2.03)	0.007	2.19 (1.11–2.00)
German	18.1	19.6	19.4	19.9	15.3	0.047	1.23 (1.01–1.51)	0.06	1.36 (0.99–1.86)	0.08	1.34 (0.96–1.87)	0.047	1.38 (1.01–1.90)
Combined	–	–	–	–	–	0.003	1.20 (1.06–1.35)	0.001	1.35 (1.13–1.61)	9.39×10^{-5}	1.43 (1.19–1.71)	1.99×10^{-4}	1.37 (1.16–1.62)

* The *P* values and corresponding odds ratios (ORs) are for cases versus controls. MAF = minor allele frequency; SSc = systemic sclerosis; dcSSc = diffuse cutaneous SSc; Topo+ = anti–topoisomerase I antibody positive; FA+ = SSc-related fibrosing alveolitis; 95% CI = 95% confidence interval.

Table 4. Meta-analysis of the *IRAK1* rs1059702 genotype frequencies in the combined European Caucasian population*

	% of subjects	P†	OR (95% CI)	Genetic model	P‡	OR (95% CI)
SSc (n = 1,808)						
TT	4.3	0.023	1.49 (1.06–2.10)	A	0.0042	1.18 (1.05–1.33)
CT	27.1	0.041	1.16 (1.01–1.35)	D	0.010	1.20 (1.04–1.37)
CC	68.6	–	1 (reference)	R	0.039	1.43 (1.02–2.01)
dcSSc (n = 491)						
TT	6.7	1.56×10^{-4}	2.35 (1.51–3.66)	A	0.0018	1.32 (1.11–1.57)
CT	26.1	0.28	1.13 (0.90–1.43)	D	0.029	1.27 (1.02–1.57)
CC	67.2	–	1 (reference)	R	2.24×10^{-4}	2.27 (1.46–3.52)
lcSSc (n = 1,271)						
TT	3.2	0.65	–	–	–	–
CT	28.0	0.91	–	–	–	–
CC	68.8	–	–	–	–	–
Anti–topo I antibody positive (n = 480)						
TT	8.1	1.07×10^{-6}	2.84 (1.87–4.32)	A	1.97×10^{-4}	1.38 (1.17–1.64)
CT	25.2	0.41	1.10 (0.87–1.39)	D	0.018	1.29 (1.05–1.60)
CC	66.7	–	1 (reference)	R	1.48×10^{-6}	2.77 (1.82–4.19)
ACA positive (n = 789)						
TT	2.9	0.97	–	–	–	–
CT	28.5	0.89	–	–	–	–
CC	68.6	–	–	–	–	–
SSc with fibrosing alveolitis (n = 604)						
TT	5.8	9.05×10^{-4}	2.09 (1.35–3.24)	A	3.33×10^{-4}	1.34 (1.14–1.58)
CT	28.1	0.029	1.26 (1.02–1.56)	D	0.026	1.35 (1.11–1.65)
CC	66.1	–	1 (reference)	R	0.0022	1.95 (1.27–3.02)
SSc without fibrosing alveolitis (n = 1,088)						
TT	3.8	0.23	–	–	–	–
CT	27.0	0.54	–	–	–	–
CC	69.2	–	–	–	–	–
Controls (n = 2,217)						
TT	3.1	–	–	–	–	–
CT	25.3	–	–	–	–	–
CC	71.6	–	–	–	–	–

* In a genotype–phenotype association analysis comparing the frequency of the TT genotype among systemic sclerosis (SSc) subsets, $P = 0.0015$ for diffuse cutaneous SSc (dcSSc) versus limited cutaneous SSc (lcSSc), $P = 5.95 \times 10^{-5}$ for anti–topoisomerase I (anti–topo I) antibody–positive SSc versus anticentromere antibody (ACA)–positive SSc, and $P = 0.043$ for SSc with fibrosing alveolitis versus SSc without fibrosing alveolitis. OR = odds ratio; 95% CI = 95% confidence interval; A= additive; D = dominant; R = recessive.
† Mantel-Haenszel meta-analysis under a fixed-effects model.
‡ *P* value for each genetic model of inheritance.

1.09–5.52] for anti–topo I–positive SSc and $P = 0.017$, OR 2.49 [95% CI 1.15–5.42] for dcSSc). As observed in the French population, an increase in the frequency of the risk homozygous genotype was detected in the SSc with fibrosing alveolitis subset; however, this increase did not reach statistical significance. (Additional data are available from the corresponding author upon request).

Meta-analysis of *IRAK1* rs1059702 in the 3 populations. The Breslow-Day test of homogeneity showed no significant differences among the 3 populations (French, Italian, and German). Therefore, we performed a meta-analysis using the Mantel-Haenszel test under fixed effects. The meta-analysis of the 3 European Caucasian populations provided evidence of a strong association between the *IRAK1* rs1059702 T allele and overall SSc as well as the TT genotype and overall SSc ($P = 0.003$, OR 1.20 [95% CI 1.06–1.35] for the association between the T allele and SSc and $P = 0.023$, OR 1.49 [95% CI 1.06–2.10] for the association between the TT genotype and SSc).

In the subset analyses, we found a highly significant association of the *IRAK1* rs1059702 T allele and TT genotype with dcSSc, anti–topo I–positive SSc, and SSc with fibrosing alveolitis. Results of the meta-analysis are shown in Table 4 and summarized in Figure 2. The genotype–phenotype correlation analysis in the combined sample revealed that the *IRAK1* rs1059702 TT homozygous risk genotype discriminated patients with dcSSc from those with limited cutaneous SSc (lcSSc)

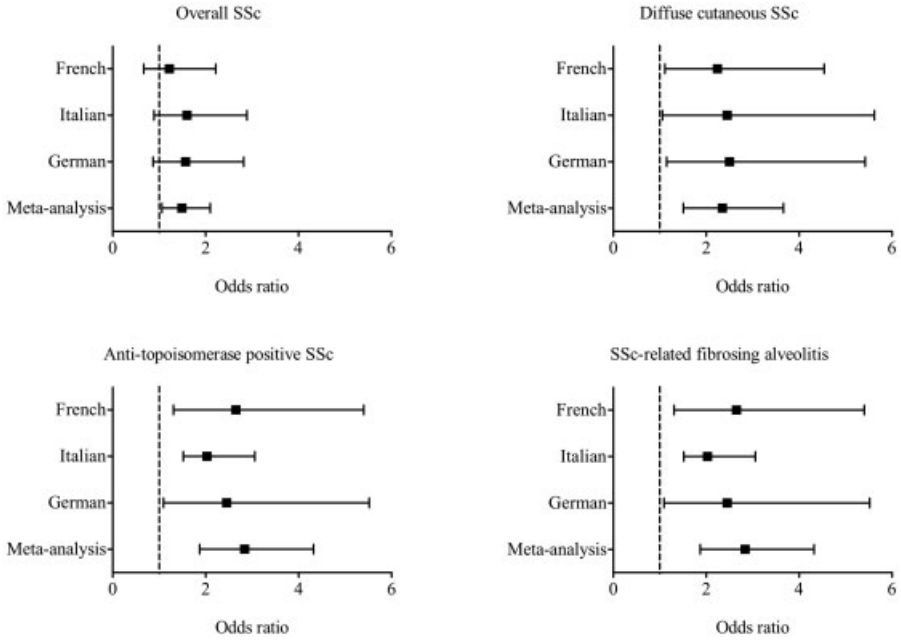

Figure 2. Meta-analysis of the *IRAK1* rs1059702 TT risk genotype in 3 European Caucasian populations (French, Italian, and German). Forest plots of the meta-analysis of the association of the *IRAK1* rs1059702 TT genotype with systemic sclerosis (SSc), diffuse cutaneous SSc, anti–topoisomerase I–positive SSc, and SSc-related fibrosing alveolitis in the 3 populations are shown. Bars represent the 95% confidence interval.

(6.7% versus 3.2%; $P = 0.0015$), anti–topo I–positive patients from ACA-positive patients (8.1% versus 2.9%; $P = 5.95 \times 10^{-5}$), and SSc patients with fibrosing alveolitis from those without (5.8% versus 3.8%; $P = 0.043$) (Table 4).

The recessive mode of inheritance was the best-fitting genetic model for the overall disease and the relevant SSc subsets (i.e., dcSSc, anti–topo I–positive SSc, and SSc with fibrosing alveolitis) (Table 4). A multivariate logistic regression analysis adjusted for dcSSc status had similar results (data not shown). Hence, *IRAK1* contributes to the subphenotypes dcSSc, anti–topo I–positive SSc, and SSc with fibrosing alveolitis, which are strongly linked.

DISCUSSION

In this study, our aim was to test for an association between *IRAK1* and SSc. In the discovery sample, we detected an association with the previously described *IRAK1* SLE risk haplotype (8). This association was restricted to dcSSc, anti–topo I–positive SSc, and SSc-related fibrosing alveolitis. Of the 4 *IRAK1* SNPs that define the SSc susceptibility haplotype block, we further investigated the functional variant *IRAK1* 196Phe (rs1059702). We found a strong association between the rs1059702 TT risk genotype and both the dcSSc and anti–topo I–positive SSc subsets; the association remained statistically significant after correction for multiple testing in the latter subgroup. We observed an increased frequency of the TT risk genotype in patients with fibrosing alveolitis; however, the increase did not reach statistical significance. Given the high correlation between anti–topo I positivity and SSc-related pulmonary fibrosis, we decided to further investigate the *IRAK1* rs1059702 SNP in 2 European Caucasian populations, with regard to the 3 relevant SSc subsets (i.e., dcSSc, anti–topo I–positive SSc, and SSc with fibrosing alveolitis).

The association was replicated in both the Italian and German populations for both dcSSc and anti–topo I–positive SSc. Interestingly, a significant association was also detected in the subgroup of SSc patients with fibrosing alveolitis. The meta-analysis that was corrected for population stratification further strengthened the evidence of this association and supported its soundness, indicating that the *IRAK1* rs1059702 TT genotype acts with a risk effect, under a recessive model on dcSSc, anti–topo I–positive SSc, and SSc with fibrosing alveoli-

tis. Multivariate analysis revealed that *IRAK1* was associated with those 3 specific SSc subgroups, illustrating the high correlation of those 3 SSc subsets. Of interest, genotype–phenotype correlation analyses revealed that the *IRAK1* rs1059702 TT genotype discriminated the cutaneous subtypes, the SSc-specific autoantibody status (i.e., anti–topo I positive versus ACA positive), and patients with pulmonary fibrosis from those without, suggesting that the risk genotype could contribute to a disease-specific phenotype, and may be regarded as a specific marker of a subgroup of SSc patients who are considered to have severe disease (22). However, one limitation of our study relates to the fact that the SSc phenotype can evolve during the course of the disease, and thus prospective followup of the SSc cohort is needed to improve subphenotype analyses.

In the present study, we focused on *IRAK1*, given the potential involvement of IRAK-1 in at least 2 immune cell functions that have been reported to be aberrant in SSc. First, IRAK-1 is involved in the induction of IFNα and IFNγ, the production of which has been shown to be aberrant in SSc (9,23). Second, IRAK-1 is a pivotal regulator of the NF-κB pathway. Of interest, we have recently reported that *TNFAIP3,* which encodes the A20 protein, a key regulator of the NF-κB pathway and *NLRP1*, which plays a pivotal role in IL-1β processing, are involved in the genetic background of SSc (6,7).

Although it is too early to determine the mechanism(s) by which IRAK-1 may contribute to SSc pathogenesis, the present study provides evidence of an association with 2 *IRAK1* variants in complete linkage disequilibrium (i.e., 196Phe/532Ser) that were found to have functional consequences. To our knowledge, the functional 196Phe/532Ser haplotype has not previously been investigated in autoimmune diseases, including SLE or SSc. Nonetheless, consistent with the hypothesis that a group of genes confers susceptibility to a broad spectrum of immune diseases, *IRAK1* should be considered as a genetic susceptibility factor shared by SLE and SSc. Our findings provide support for the evolving concept that common risk genes underlie SLE and SSc (5,7,24–26). The critical challenge will be to determine how shared autoimmune factors contribute to different phenotypes, such as SSc and SLE, and how they modulate the phenotypic heterogeneity in a given disease.

Both *IRAK1* risk variants are located in the haplotype block that corresponds to the C1 domain of IRAK-1 (11). There are several interpretations of the observed genetic association. First, the 2 nonsynonymous *IRAK1* variants could individually or together play the predominant mechanistic role that explains the association. Second, both amino acid changes together may be required to impart the functional changes that underlie the observed association (codominant effect). Third, these 2 markers may themselves be in linkage disequilibrium with other functional variant(s) located in the haplotype block that are the true mechanistic basis for the association. Consistent with this idea, it should be noted that a naturally occurring splice variant of IRAK-1, IRAK-1c, which lacks exon 11 and most of exon 12 (11), might suppress NF-κB activation and inhibits innate immune activation (27). Hence, it could be hypothesized that other *IRAK1* SNPs located in the SSc-associated haplotype block could have functional consequences.

IRAK1 is located on chromosome Xq28, juxtaposed to a second gene that has also been implicated in SLE susceptibility (28). Given the physical proximity of *IRAK1* and *MECP2* on Xq28 and their location in the same haplotype block, it is plausible that *MECP2* also contributes to SSc susceptibility (28). In addition, *MECP2* encodes for methyl-CpG–binding protein 2, which is critical in the transcriptional suppression of methylation-sensitive genes (29), underlying the putative role of epigenetics in autoimmunity (for review, see ref. 30). These findings make *MECP2* an attractive candidate gene for SSc susceptibility.

The present study provides the first evidence of an association between *IRAK1* and SSc, demonstrating that a sex chromosome gene directly influences SSc susceptibility and its phenotypic expression. Both deep resequencing of the *IRAK1 MECP2* haplotype block and functional studies are warranted to further establish the role of each gene in SSc susceptibility. Finally, the next challenge is to determine the exact contribution of X chromosome genes in both female and male SSc populations. However, this requires large samples, given the strong sex bias that characterizes SSc.

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AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Dieudé had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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