New insight on the Xq28 association with systemic sclerosis

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Abstract

Objective—To evaluate whether the systemic sclerosis (SSc)-associated *IRAK1* non-synonymous single-nucleotide polymorphism rs1059702 is responsible for the Xq28 association with SSc or whether there are other independent signals in the nearby methyl-CpG-binding protein 2 gene (*MECP2*).

Methods—We analysed a total of 3065 women with SSc and 2630 unaffected controls from five independent Caucasian cohorts. Four tag single-nucleotide polymorphisms of *MECP2* (rs3027935, rs17435, rs5987201 and rs5945175) and the *IRAK1* variant rs1059702 were genotyped using TaqMan predesigned assays. A meta-analysis including all cohorts was performed to test the overall effect of these Xq28 polymorphisms on SSc.

Results—*IRAK1* rs1059702 and *MECP2* rs17435 were associated specifically with diffuse cutaneous SSc (P_{FDR}=4.12×10^{-3}, OR=1.27, 95% CI 1.09 to 1.47, and P_{FDR}=5.26×10^{-4}, OR=1.30, 95% CI 1.14 to 1.48, respectively), but conditional logistic regression analysis showed that the association of *IRAK1* rs1059702 with this subtype was explained by that of *MECP2* rs17435. On the other hand, *IRAK1* rs1059702 was consistently associated with presence of pulmonary fibrosis (PF), because statistical significance was observed when comparing SSc patients PF+ versus controls (P_{FDR}=0.039, OR=1.30, 95% CI 1.07 to 1.58) and SSc patients PF+ versus SSc patients PF− (p=0.025, OR=1.26, 95% CI 1.03 to 1.55).

Conclusions—Our data clearly suggest the existence of two independent signals within the Xq28 region, one located in *IRAK1* related to PF and another in *MECP2* related to diffuse cutaneous SSc, indicating that both genes may have an impact on the clinical outcome of the disease.

INTRODUCTION

Autoimmune diseases are complex polygenic conditions in which multiple susceptibility genes interact with epigenetic and environmental factors for their predisposition and progression. In some cases, part of the genetic component is shared among different immune disorders, suggesting that these pathologies may be influenced by disease-specific and common molecular pathways.[1–4] For instance, most of the genetic associations described for systemic sclerosis (SSc), a fibrotic autoimmune disease of skin and internal organs, have also been reported to play a role in the susceptibility to systemic lupus erythematosus (SLE).[56]

Remarkably, a wide spectrum of autoimmune diseases shows a significant female preponderance. SSc represents a clear example of a sex biased immune disorder, with women reaching almost 90% of total affected individuals in some populations. Different factors have been proposed to explain this marked sexual dimorphism, including reproductive and sex hormones, fetal microchimerism, and gender differences in the immune system and lifestyle. Nevertheless, definitive evidences are still lacking and cumulative knowledge points to a major role of sex chromosomes in the immune system homeostasis. Due to the statistical complexity of testing for association between phenotype and genetic markers on the sex chromosomes, very few associations of sex-linked genes with the development of autoimmunity have been reported to date.[7–9]

One of the shared risk loci between SSc and SLE within the non-homologous region of the X chromosome is the interleukin-1 receptor-associated kinase 1 gene (*IRAK1*),[1011] that encodes a serine/threonine protein kinase with a special relevance in the signalling pathways of Toll-like receptors (TLRs)/IL-1R.[1213] However, *IRAK1* is in the same haplotypic block as the methyl-CpG-binding protein 2 gene (*MECP2*) on Xq28, which is also associated with

SLE,[1415] and functional genetic variants of the latter locus may explain the association signals with SLE observed in *IRAK1*.[16]

In this study we aimed to evaluate whether the SSc-associated *IRAK1* polymorphism rs1059702 (Phe196Ser) described by Dieudé *et al*[10] is the causal variant of the Xq28 association or whether it reflects another association signal from the nearby *MECP2*.

PATIENTS AND METHODS

Study population

Since the *IRAK1/MECP2* genes are located in a sex-linked region, only women were included in the study. Informed written consent from all participants and approval from the local ethical committees were obtained in accordance with the tenets of the Declaration of Helsinki.

We analysed a total of 3065 female SSc patients and 2630 female unaffected controls of Caucasian ancestry, from an initial discovery cohort of Spain (1016 SSc and 1520 controls) and four additional replication cohorts from USA (965 SSc and 489 controls), Germany (490 SSc and 180 controls), The Netherlands (235 SSc and 278 controls) and UK (359 SSc and 163 controls). Since most samples have not been subjected to genome-wide association study platforms, population substructure analysis could not been performed and this may represent a potential limitation. In all cases, SSc patients were classified based on their skin involvement into limited cutaneous SSc (lcSSc) or diffuse cutaneous SSc (dcSSc) according to the criteria by Leroy *et al*[17] A subset of the German cohort (43.73%) overlaps with that included in the study of *IRAK1* by Dieudé *et al*[10] To perform the subphenotype analyses, they were also phenotypically characterised accordingly with the presence or absence of anticentromere antibodies (ACA), antitopoisomerase antibodies (ATA) and pulmonary fibrosis (PF). ACA were determined by their characteristic distinctive pattern on HEP-2 cells, and ATAs were detected by passive immunodiffusion against calf thymus extract. PF was diagnosed by high resolution CT in all the European cohorts. However, the PF status of our US cohort was obtained by pulmonary function test (patients were considered to have PF if they showed a forced vital capacity (FVC) less than 70%). Since two different methodologies were used for the diagnosis, the meta-analyses on PF data were performed only in the European cohorts. The clinical characteristics of the analysed cohorts are summarised in table 1.

Single-nucleotide polymorphism selection

We followed a single-nucleotide polymorphism (SNP) tagging strategy using Haploview V. 4.2 (http://www.broad.mit.edu/mpg/haploview)[18] to identify taggers that may cover all the common genetic variation within *MECP2* (r2 0.8) in the Utah residents with ancestry from northern and western Europe (CEU) population of the HapMap database (http://hapmap.ncbi.nlm.nih.gov/). Four *MECP2* polymorphisms were selected with this method: rs3027935, rs17435, rs5987201 and rs5945175 (see online supplementary figure S1). Additionally, we also included in the study the non-synonymous *IRAK1* genetic variant rs1059702 (Phe196Ser), which was described as the SNP that best explains the SSc susceptibility *IRAK1* haplotype.[10] The location of the five genetic variants analysed within the *IRAK1/MECP2* region is shown in online supplementary figure S2.

Genotyping methods

DNA samples were obtained from peripheral white blood cells of participants following standard procedures. Predesigned TaqMan 5 SNP genotyping assays were used to genotype the selected Xq28 genetic variants (assay IDs: rs3027935: C__15765567_10, rs17435:

C___2597094_20, rs5987201: C__30089704_10, rs5945175: C__30485633_20, rs1059702: C___8966367_30) in a 7900HT Fast Real-Time PCR System (Applied Biosystems, Foster City, California, USA).

Statistical analyses

The overall statistical power of our study, according to Power Calculator for Genetic Studies 2006,[19] is shown in online supplementary table S1.

The Linux software Plink V.1.7 (http://pngu.mgh.harvard.edu/purcell/plink/)[20] was used to perform 2×2 contingency tables, χ^2 and/or Fisher's exact tests, when appropriate. p Values lower than 0.05 were considered as statistically significant. ORs, and 95% CI, were calculated according to Woolf's method. The Breslow-Day (BD) test was used to estimate the OR heterogeneity among the different cohorts. Combined data were analysed by Mantel-Haenszel tests under fixed effect, or random effect (DerSimonian–Laird) when the BD test reached statistical significance. Benjamini & Hochberg (1995) step-up false discovery rate (FDR) control correction[21] for multiple testing was applied to the p values in the independent analysis and the combined meta-analysis.

Plink V.1.7 was also used to carry out a conditional logistic regression analysis to test for dependence between the associated SNPs. Haplotype analyses of allelic combinations with a frequency higher than 5% in the control groups were performed with Plink V.1.7, Haploview V.4.2 and StatsDirect (V.2.6.6; StatsDirect, Altrincham, UK).

RESULTS

The genotyping success rate was higher than 95% in all analysed cohorts. No statistically significant deviation from Hardy-Weinberg equilibrium (p 0.01) was observed for every SNP in the controls sets, except for rs3027935 in the Spanish population (see online supplementary table S2). The *IRAK1* rs1059702 allele frequencies were similar to those described by Dieudé *et al*,[10] whereas the frequencies of the four analysed *MECP2* SNPs were in agreement with the data of the HapMap project.

Analysis of the discovery Spanish cohort

We first tested for association in a large Caucasian cohort of Spanish women (see online supplementary table S3). The statistical analysis of the allele frequencies indicated a significant association after FDR correction between *IRAK1* rs1059702 and dcSSc (P_{FDR}=0.028, OR=1.34, 95% CI 1.07 to 1.69). This genetic variant also showed trends towards association when the global SSc cohort and the PF+ patients were compared against the control set (P_{FDR}=0.096, OR=1.16, 95% CI 0.99 to 1.35, and P_{FDR}=0.058, OR=1.35, CI 95% 1.04 to 1.76, respectively). On the other hand, significant FDR-corrected p values were obtained for *MECP2* rs17435 in the analyses of global disease (P_{FDR}=0.014, OR=1.24, 95% CI 1.08 to 1.43), dcSSc (P_{FDR}=0.014, OR=1.38, 95% CI 1.12 to 1.70), and PF (P_{FDR}=0.047, OR=1.37, 95% CI 1.08 to 1.74). Other trends were suggested in the global SSc analysis for the *MECP2* polymorphisms rs5987201 (P_{FDR}=0.096, OR=1.32, 95% CI 0.97 to 1.79) and rs5945175 (P_{FDR}=0.053, OR=1.50, 95% CI 1.06 to 2.11).

Mantel-Haenszel test

Considering the above described associations, we decided to include in the study three additional Caucasian cohorts from Europe (Germany, The Netherlands and UK) and one from the USA. The results of the independent analyses of these populations are summarised in the online supplementary tables S4–S7.

A meta-analysis including all five cohorts was then performed to increase the statistical power thus allowing a better estimation of disease susceptibility (table 2). The overall analysis yielded significant p values only for *IRAK1* rs1059702 and MECP2 rs17435 (no heterogeneity of the ORs among the five populations was observed when applying the BD test in both cases). Regarding the *IRAK1* non-synonymous SNP rs1059702, the allele frequencies of the dcSSc subgroup differed significantly from those of the control set in the combined analysis (P_{FDR}=4.12×10^{-3}, OR=1.27, 95% CI 1.09 to 1.47), and trends towards association were evident in the global SSc/control (P_{FDR}= 0.070, OR=1.13, 95% CI 1.01 to 1.26) and ATA+/control (P_{FDR}=0.087, OR=1.23, 95% CI 1.04 to 1.46) comparisons, consistent with previously published data.[10] Similarly, the *MECP2* rs17435 variant reached statistical significant after comparing the global disease group and dcSSc subgroup with the control population (P_{FDR}=2.68×10^{-3}, OR=1.19, 95% CI 1.08 to 1.31, and P_{FDR}=5.26 ×10^{-4}, OR=1.30, 95% CI 1.14 to 1.48, respectively). The ATA +/control comparison also suggested a trend for this SNP (P_{FDR}=0.088, OR=1.18, 95% CI 1.01 to 1.38).

To confirm that the genetic association between these two SNPs relies on dcSSc rather than on the global disease, we performed another meta-analysis but comparing this time dcSSc patients against those with lcSSc instead of unaffected controls, thus avoiding a possible effect of having SSc as a confounding variable (table 3). This new combined analysis showed statistically significant differences between dcSSc and lcSSc patients for the allele frequencies of *IRAK1* rs1059702 (p=0.022, OR=1.19, 95% CI 1.02 to 1.37) and *MECP2* rs17435 (p=0.026, OR=1.16, 95% CI 1.02 to 1.32).

Dependence analysis

Taking into account that both SNPs seemed to be specifically associated with risk to develop dcSSc, a conditional logistic regression analysis considering the populations as covariate was carried out to determine whether one of them could be dependent on the other one (table 4). The pairwise conditioning showed that the association of *IRAK1* rs1059702 with dcSSc was explained by that of *MECP2* rs17435, because only the latter SNP remained significant in the conditional analysis (rs1059702 *conditioned* p=0.786; rs17435 *conditioned* p=0.049). The fact that both polymorphisms were in moderate linkage disequilibrium (r^2 0.60) may explain the dependence.

Analysis on PF data

Since the PF data of the SSc patients from the USA was estimated using a different methodology, we decided to include in the meta-analysis of this clinical feature only the European cohorts (Spain, Germany, The Netherlands and UK; table 5).

In a first step, we compared PF+ SSc patients with the unaffected control group, obtaining significant p values for *IRAK1* rs1059702 (P_{FDR}=0.039, OR=1.30, 95% CI 1.07 to 1.58) and *MECP2* rs17435 (P_{FDR}=0.039, OR=1.24, 95% CI 1.04 to 1.48). However, only *IRAK1* rs1059702 showed a consistent association with PF susceptibility, because the allele frequencies were significantly different between SSc patients *with* PF and SSc patients *without* PF (p=0.025, OR=1.26, 95% CI 1.03 to 1.55). On the contrary, in the case to case comparison, *MECP2* rs17435 was not associated with PF (p=0.346, OR=1.09, 95% CI 0.91 to 1.31).

Haplotype analysis

Finally, we further explore a possible haplotype effect within this genomic region by performing several allelic combinations. Although the combinations rs1059702*G-rs17435*A and rs1059702*A-rs17435*T showed clear association peaks (see online

supplementary table S8), the strength of these associations did not differ from those of *IRAK1* rs1059702 and *MECP2* rs17435 independently.

DISCUSSION

This study represents an important step forward towards a better understanding of the complex genetic association between the Xq28 genomic region and the predisposition to autoimmunity. Because of the linkage disequilibrium structure of this *locus*, there is controversy about whether one of the two juxtaposed genes *IRAK1* or *MECP2* may harbour the causal variant of the described association with SLE and other autoimmune diseases including SSc.[10][14–16][22] *IRAK1* was considered as the most likely candidate, mainly due to its crucial involvement in the TLR pathway (in which it modulates the IL1-induced activation of NF- B) and to the fact that this gene has been related with certain SLE manifestations in mouse models.[11–13]

On the other hand, *MECP2* is also emerging as an interesting putative susceptibility gene in autoimmunity. It encodes a nuclear protein that binds specifically to methylated DNA, playing a central role in the transcriptional regulation of methylation-sensitive genes. Interestingly, there is increasing evidence pointing to epigenetic alterations, including those in DNA methylation, as relevant players in the pathogenesis of autoimmune diseases.[23] For example, epigenetic dysregulation in T cells seems to be an important component of the SLE pathogenesis.[24–26] Additionally, SLE patients containing the *MECP2* SLE-risk haplotype show abnormal gene expression profiles of B cells, and this has been suggested to contribute to the interferon (IFN) signature observed in this pathology.[15] Moreover, it has also been described in mouse models that MeCP2 protein may regulate the transcriptional activation of *IRAK1* through microRNAs.[27–29] Strikingly, the risk allele of the *IRAK1* variant analysed here (rs1059702) has been recently associated with lower mRNA levels of *MECP2* in SLE cases and unaffected controls of multiple ancestral groups, which has led to propose that both genes contribute independently to SLE susceptibility.[30] Although no epistatic gene-gene interaction between *MECP2* and *IRAK1* was observed in our data, it could be possible that genetic variation within any of these two genes may be associated with the expression of the other one. In this regard, experimental or bioinformatic analysis on the association of *MECP2* rs17435 and *IRAK1* rs1059702 with expression levels of both genes would provide important clues that would help to completely unmask their inter-relation and its relevance in the genetic susceptibility to SSc and other related disorders.

Consistent with the latter idea, our data clearly indicate the existence of independent association signals with different SSc clinical conditions in each gene. One of them, rs17435, is located in the intron 2 of *MECP2* and tags the SLE-risk haplotype.[15][31] The same genetic variant has also been associated with Sjögren's syndrome (SS), one of the most frequent comorbidities in SSc patients.[22][32][33] Specifically, we found a firm association of *MECP2* rs17435 with the dcSSc subtype, which is the most severe form of the disease.[9] It should be noted that SLE, SS and SSc are related autoimmune disorders that share some of their immunological and genetic component.[1][2][34][35] For instance, a type I IFN signature similar to that described in SLE has also been identified in a subset of SSc patients.[36] Taking into account the above, it is likely that *MECP2* represents a real shared susceptibility factor for these systemic diseases probably affecting the epigenetic control of common pathogenic pathways (eg, the IFN signalling).

In addition, *MECP2* could also be one of the genes contributing to the gender bias in autoimmune diseases. X chromosome inactivation in women involves processes that include DNA methylation.[37] Considering that this chromosome contains a considerable number of important immune-related genes, such as inhibitor of light polypeptide gene enhancer in B

cells kinase (*IKBKG*),[38] interleukin 2 receptor gamma (*IL2RG*),[39] CD40 ligand (*CD40L*),[40] forkhead box P3 (*FOXP3*)[41] or *IRAK1*,[213] among others, some authors proposed that an abnormal gene dosage effect of X linked genes due to X chromosome defects may be involved in the female preponderance to develop autoimmunity.[8] Supporting this idea, it has been recently reported that demethylation of regulatory elements of *CD40L* on the inactive X chromosome may be directly involved in the overexpression of this gene observed in CD4 T cells from SSc patients.[4243]

On the other hand, we also found an independent association between the non-synonymous *IRAK1* SNP rs1059702 and susceptibility to develop PF in SSc patients. This association is in agreement with that described by Dieudé *et al*[10] However, these authors suggested that this *locus* is also associated with the dcSSc subtype and production of ATAs. In this regard, our data indicate that the association of *IRAK1* rs1059702 with dcSSc is dependant of that for *MECP2* rs17435, possibly due to the moderate linkage disequilibrium of both SNPs and the overlapping between this group of patients and those with pulmonary involvement (figure 1). The trend that we detected in the ATA analysis may also be a consequence of the latter cause. Since presence of ATAs and PF occur preferentially in dcSSc patients,[9] it is not easy to determine specific associations with these three phenotypes if only cases versus controls are compared (having one of these features may represent a confounding variable). We observed statistically significant differences between SSc patients with PF and those without PF only for *IRAK1* rs1059702 but not for *MECP2* rs17435, thus suggesting that only *IRAK1* rs1059702 is associated with PF predisposition.

Despite *MECP2* and *IRAK1* showing clear association signals with SSc, it should be noted that these associations are less robust than those described in SLE.[1114] It could be speculated that the risk variants in this genomic region may lead to a SLE-like phenotype, as it has been observed for other common susceptibility genes like *ITGAM*.[44]

In conclusion, the analysis of our data showed that *MECP2* and *IRAK1* are independent SSc susceptibility genes that influence distinct clinical phenotypes. *MECP2* is associated with the most severe subtype of the disease, consistent with the major role that epigenetics seem to play in this kind of diseases, and *IRAK1* may be a crucial gene underlying the pathogenic mechanisms leading to PF, probably by altering the TLR pathway.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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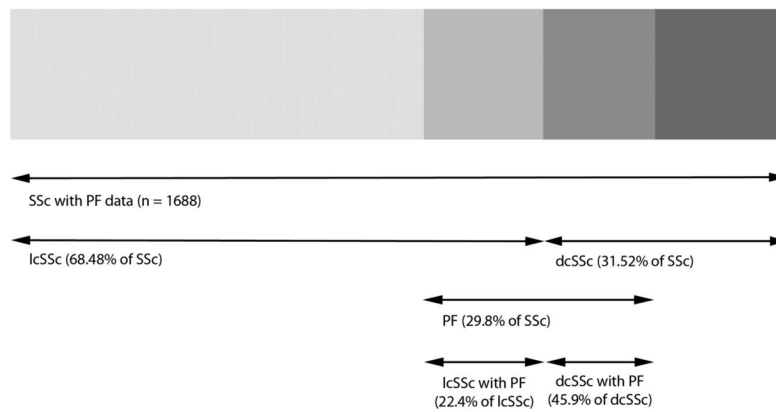
Figure 1.
Degree of overlapping between different case subsets from Spain, Germany, The Netherlands and UK. SSc, systemic sclerosis; lcSSc, limited cutaneous systemic sclerosis; dcSSc, diffuse cutaneous systemic sclerosis; PF, pulmonary fibrosis. Note: '*lcSSc with PF*' and '*dcSSc with PF*' percentages are referred to the total number of lcSSc and dcSSc cohorts, respectively, with PF data.

Table 1

Main clinical features of the female systemic sclerosis patients included in the study. Data are referred to the total of analysed individuals

Clinical feature	Spain	USA	Germany	The Netherlands	UK
lcSSc	0.70	0.66	0.63	0.71	0.76
dcSSc	0.30	0.34	0.37	0.29	0.24
ACA positive	0.47	0.33	0.40	0.27	0.41
ACA negative	0.49	0.66	0.56	0.71	0.57
ATA positive	0.22	0.18	0.28	0.24	0.15
ATA negative	0.72	0.81	0.68	0.74	0.82
PF positive *	0.22	0.09	0.26	0.36	0.19
PF negative *	0.70	0.37	0.60	0.49	0.18
SSc patients without PF data *	0.08	0.54	0.14	0.15	0.63

ACA, anticentromere antibody; ATA, antitopoisomerase antibody; dcSSc, diffuse cutaneous SSc; lcSSc, limited cutaneous SSc; PF, pulmonary fibrosis; SSc, systemic sclerosis.

*
Pulmonary fibrosis data were obtained by high resolution CT (HRCT) in the European cohorts and by pulmonary function test (FVC<70%) in the US cohort.

Table 2

Combined analysis of the allele frequencies of Xq28 genetic variants in Caucasian systemic sclerosis patients and unaffected controls from Spain, USA, Germany, The Netherlands and UK

Locus	rs#	Gene	1/2	Subgroup (N)	Genotype, N (%)			MAF (%)	M-H Allele test		
					1/1	1/2	2/2		p Value*	p_{FDR}[†]	OR (95% CI)[‡]
ChrX: 152937386	rs1059702	*IRAK1*	A/G	Controls (n=2530)	70 (2.77)	599 (23.68)	1861 (73.56)	14.60			
				SSc (n=2890)	107 (3.70)	729 (25.22)	2054 (71.07)	16.31	0.028	0.070	1.13 (1.01 to 1.26)
				lcSSc (n=1969)	67 (3.40)	476 (24.17)	1426 (72.42)	15.49	0.298	0.429	1.07 (0.95 to 1.20)
				dcSSc (n=921)	40 (4.34)	253 (27.47)	628 (68.19)	18.08	1.65E-03	4.12E-03	1.27 (1.09 to 1.47)
				ACA+ (n=1145)	24 (2.10)	279 (24.37)	842 (73.54)	14.28	0.734	0.734	0.98 (0.84 to 1.13)
				ATA+ (n=610)	24 (3.93)	169 (27.70)	417 (68.36)	17.79	0.017	0.087	1.23 (1.04 to 1.46)
ChrX: 152957662	rs3027935	*MECP2*	T/C	Controls (n=2539)	13 (0.51)	229 (9.02)	2297 (90.47)	5.02			
				SSc (n=3003)	12 (0.40)	322 (10.72)	2669 (88.88)	5.76	0.311	0.389	1.09 (0.92 to 1.30)
				lcSSc (n=2056)	7 (0.34)	227 (11.04)	1822 (88.62)	5.86	0.343	0.429	1.09 (0.91 to 1.32)
				dcSSc (n=947)	5 (0.53)	95 (10.03)	847 (89.44)	5.54	0.778	0.778	1.04 (0.81 to 1.32)
				ACA+ (n=1178)	1 (0.08)	134 (11.38)	1043 (88.54)	5.77	0.469	0.596	1.08 (0.87 to 1.35)
				ATA+ (n=625)	4 (0.64)	52 (8.32)	569 (91.04)	4.80	0.348	0.435	0.87 (0.65 to 1.17)
ChrX: 152965174	rs17435	*MECP2*	T/A	Controls (n=2508)	99 (3.95)	744 (29.67)	1665 (66.39)	18.78			
				SSc (n=2990)	170 (5.69)	952 (31.84)	1868 (62.47)	21.61	5.36E-04	2.68E-03	1.19 (1.08 to 1.31)
				lcSSc (n=2044)	106 (5.19)	638 (31.21)	1300 (63.60)	20.79	0.027	0.133	1.13 (1.01 to 1.26)
				dcSSc (n=946)	64 (6.77)	314 (33.19)	568 (60.04)	23.36	1.05E-04	5.26E-04	1.30 (1.14 to 1.48)
				ACA+ (n=1176)	48 (4.08)	368 (31.29)	760 (64.63)	19.73	0.459	0.596	1.05 (0.92 to 1.19)
				ATA+ (n=626)	38 (6.07)	199 (31.79)	389 (62.14)	21.96	0.035	0.088	1.18 (1.01 to 1.38)
ChrX: 152983236	rs5987201	*MECP2*	T/C	Controls (n=2449)	6 (0.24)	176 (7.19)	2267 (92.57)	3.84			
				SSc (n=2985)	8 (0.27)	241 (8.07)	2736 (91.66)	4.31	0.468	0.468	1.07 (0.88 to 1.31)
				lcSSc (n=2036)	6 (0.29)	162 (7.96)	1868 (91.75)	4.27	0.728	0.728	1.04 (0.84 to 1.29)
				dcSSc (n=949)	2 (0.21)	79 (8.32)	868 (91.46)	4.37	0.641	0.778	1.06 (0.81 to 1.39)
				ACA+ (n=1181)	1 (0.08)	104 (8.81)	1076 (91.11)	4.49	0.477	0.596	1.09 (0.85 to 1.40)
				ATA+ (n=619)	2 (0.32)	40 (6.46)	577 (93.21)	3.55	0.297	0.435	0.84 (0.60 to 1.18)
ChrX: 153011951	rs5945175	*MECP2*	C/T	Controls (n=2550)	3 (0.12)	157 (6.16)	2390 (93.73)	3.20			
				SSc (n=2991)	7 (0.23)	242 (8.09)	2742 (91.68)	4.28	0.063	0.104	1.22 (0.99 to 1.50)

Locus	rs#	Gene	1/2	Subgroup (N)	Genotype, N (%)			MAF (%)	M-H Allele test		
					1/1	1/2	2/2		p Value[*]	p_FDR[†]	OR (95% CI)[‡]
				lcSSc (n=2038)	5 (0.25)	166 (8.15)	1867 (91.61)	4.32	0.065	0.162	1.23 (0.99 to 1.55)
				dcSSc (n=953)	2 (0.21)	76 (7.97)	875 (91.82)	4.20	0.270	0.450	1.17 (0.88 to 1.55)
				ACA+ (n=1167)	3 (0.26)	97 (8.31)	1067 (91.43)	4.41	0.036	0.178	1.32 (1.02 to 1.71)
				ATA+ (n=628)	1 (0.16)	39 (6.21)	588 (93.63)	3.26	0.590	0.590	0.91 (0.64 to 1.30)

[*] All p values have been calculated for the allelic model.

ACA, anticentromere antibodies; ATA antitopoisomerase antibodies; dcSSc, diffuse cutaneous SSc; MAF, minor allele frequency; M-H, Mantel–Haenszel test under fixed effect; lcSSc, limited cutaneous SSc; SSc, systemic sclerosis.

[†] Benjamini & Hochberg (1995) step-up FDR control correction.

[‡] OR for the minor allele.

Table 3

Combined analysis of the allele frequencies of Xq28 genetic variants comparing limited cutaneous systemic sclerosis patients against diffuse cutaneous systemic sclerosis patients from Spain, USA, Germany, The Netherlands and UK

				M-H Allele test	
rs#	Gene	Subgroup (N)	MAF (%)	p Value[*]	OR (95% CI)[†]
rs1059702	*IRAK1*	lcSSc (n=1969)	15.49		
		dcSSc (n=921)	18.08	0.022	1.19 (1.02 to 1.37)
rs17435	*MECP2*	lcSSc (n=2044)	20.79		
		dSSc (n=946)	23.36	0.026	1.16 (1.02 to 1.32)

[*]
All p values have been calculated for the allelic model.

[†]
OR for the minor allele.

lcSSc, limited cutaneous systemic sclerosis; dcSSc, diffuse cutaneous systemic sclerosis; MAF, minor allele frequency; M-H, Mantel-Haenszel test under fixed effects; NA, not applicable.

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Table 4

Conditional logistic regression analysis for *IRAK1/MECP2* polymorphisms accordingly with diffuse cutaneous systemic sclerosis considering the populations as covariate

SNP	Gene	Uncorrected p value	Conditioned p value	Linkage disequilibrium (r²)					
				Spain	USA	Germany	Netherlands	UK
rs1059702	*IRAK1*	1.65E-03	0.786	0.69	0.67	0.60	0.68	0.53
rs17435	*MECP2*	1.05E-04	0.049					

SNP, single-nucleotide polymorphism.

Table 5

Combined analysis of the allele frequencies of Xq28 genetic variants accordingly with pulmonary fibrosis in the study cohorts from Spain, Germany, The Netherlands and UK

rs#	Gene	Comparison	N	MAF (%)	M-H Allele test	
					p Value[*]	OR (95% CI)[†]***
rs1059702	*IRAK1*	PF+ versus controls	461/2043	18.33/14.46	8.46E-03[‡]	1.30 (1.07 to 1.58)
		PF+ versus PF−	461/1149	18.33/15.54	0.025	1.26 (1.03 to 1.55)
rs17435	*MECP2*	PF+ versus controls	481/2035	22.66/18.72	0.015[§]	1.24 (1.04 to 1.48)
		PF+ versus PF−	481/1151	22.66/21.33	0.346	1.09 (0.91 to 1.31)

MAF, minor allele frequency; M-H, Mantel–Haenszel test under fixed effect; PF+, patients *with* pulmonary fibrosis; PF−, patients *without* pulmonary fibrosis.

[*]
All p values have been calculated for the allelic model.

[†]
OR for the minor allele.

[‡]
P_{FDR}=0.039.

[§]
P_{FDR}=0.039.