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BANK1 functional variants are associated with susceptibility to diffuse systemic sclerosis in Caucasians

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Abstract

Objective—To investigate the possible association of the *BANK1* gene with genetic susceptibility to systemic sclerosis (SSc) and its subphenotypes.

Methods—A large multicentre case–control association study including 2380 patients with SSc and 3270 healthy controls from six independent case–control sets of Caucasian ancestry (American, Spanish, Dutch, German, Swedish and Italian) was conducted. Three putative functional *BANK1* polymorphisms (rs17266594 T/C, rs10516487 G/A, rs3733197 G/A) were selected as genetic markers and genotyped by Taqman 5´ allelic discrimination assay.

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Results—A significant association of the rs10516487 G and rs17266594 T alleles with SSc susceptibility was observed (pooled OR=1.12, 95% CI 1.03 to 1.22; p=0.01 and pooled OR=1.14, 95% CI 1.05 to 1.25; p=0.003, respectively), whereas the rs3733197 genetic variant showed no statistically significant deviation. Stratification for cutaneous SSc phenotype showed that the *BANK1* rs10516487 G, rs17266594 T and rs3733197 G alleles were strongly associated with susceptibility to diffuse SSc (dcSSc) (pooled OR=1.20, 95% CI 1.05 to 1.37, p=0.005; pooled OR=1.23, 95% CI 1.08 to 1.41, p=0.001; pooled OR=1.15, 95% CI 1.02 to 1.31, p=0.02, respectively). Similarly, stratification for specific SSc autoantibodies showed that the association of *BANK1* rs10516487, rs17266594 and rs3733197 polymorphisms was restricted to the subgroup of patients carrying anti-topoisomerase I antibodies (pooled OR=1.20, 95% CI 1.02 to 1.41, p=0.03; pooled OR=1.24, 95% CI 1.05 to 1.46, p=0.01; pooled OR=1.26, 95% CI 1.07 to 1.47, p=0.004, respectively).

Conclusion—The results suggest that the *BANK1* gene confers susceptibility to SSc in general, and specifically to the dcSSc and anti-topoisomerase I antibody subsets.

INTRODUCTION

B cells are important regulators of immune homoeostasis and have an essential role in humoral and cellular immunity.[1] Alteration of the mechanisms that control B-cell activation and function can lead to pathogenic autoantibody production and auto-immunity as occurs in different autoimmune diseases such as systemic lupus erythematosus (SLE) or rheumatoid arthritis (RA).[2]

Recent results have shown that the B-cell scaffold protein with ankyrin repeats gene (*BANK1*), which encodes a signalling molecule expressed exclusively in B cells, is implicated in genetic predisposition to SLE and RA.[3 4] Three functional polymorphisms affecting *BANK1* regulatory sites and key functional domains (rs17266594 T/C, rs10516487 G/A and rs3733197 G/A) were strongly associated with susceptibility to SLE and RA.[3 4]

Systemic sclerosis (SSc) is another autoimmune disease of unknown aetiology in which genetic factors are thought to have an important role.[5] In the pathogenesis of SSc the dysregulation of the immune response is a central event shown by the presence of lymphocyte infiltrates in affected tissues, increased levels of inflammatory mediators and the presence of specific SSc autoantibodies. B cells seem to be one of the key players of this response.[6 7] Analysis of gene expression patterns in patients with SSc discloses a strong expression of genes associated with B lymphocytes.[7] In addition, in SSc there are indicators of increased B-cell activation such as hypergammaglobulinaemia, enhanced expression of activation markers and autoantibody production.[6 8]

Thus, taking into consideration both the relevance of B cells in SSc pathogenesis and the hypothesis that different autoimmune conditions share common genetic markers,[9] we selected *BANK1* as an interesting functional candidate gene and investigated its implications in susceptibility to SSc, and its clinical and autoantibody subsets.

MATERIALS AND METHODS

Patients

We conducted a large multicentre case–control association study including a total of 2380 patients with SSc and 3270 healthy controls. Six independent case–control sets with Caucasian ancestry were analysed (USA cohort: 1053 patients with SSc and 696 controls; Spanish cohort: 588 patients with SSc and 1051 controls; Dutch cohort: 142 patients with SSc and 256 controls; Swedish cohort: 89 patients with SSc; German cohort: 183 patients with SSc; and Italian cohort:

325 patients with SSc and 340 controls). As control populations for the Swedish and German cohorts genotype and allelic frequencies of 565 and 362 controls, respectively, were derived from a literature report.[3]

All patients with SSc fulfilled the 1980 American College of Rheumatology classification criteria for SSc.[10] Patients were classified as having limited (lcSSc) or diffuse SSc (dcSSc). Patients with SSc with cutaneous involvement distal from the elbows and knees fulfilled definitions for lcSSc.[11] Those patients with SSc with cutaneous changes proximal to elbows and knees were classified as having dcSSc.12

For patients with SSc, main clinical data, age, gender, disease duration and presence of SSc-specific autoantibodies anti-topoisomerase (ATA; anti-Scl-70) and anticentromere (ACA) have been described previously.[13] 14 Data for SSc-specific autoantibody status was available for 1038 USA, 353 Spanish, 133 Dutch, 74 Swedish, 168 German and 272 Italian patients with SSc.

The study was approved by all local ethic committees and all participants were included after written informed consent.

BANK1 single nucleotide polymorphisms (SNPs) genotyping

Three putative functional *BANK1* polymorphisms (rs17266594 T/C, rs10516487 G/A, rs3733197 G/A) were selected as genetic markers and genotyped by Taqman 5´ allelic discrimination assay (see online supplementary material).

Statistical analysis

We tested Hardy–Weinberg equilibrium for each case–control set by using the program FINETI (http://ihg.gsf.de/cgi-bin/hw/hwa2.pl, accessed 31 December 2009). Significance was calculated by χ^2 test using Statcalc software (Epi Info 2002; Centers for Disease Control and Prevention, Atlanta, Georgia, USA). p Values <0.05 were considered as statistically significant. Analysis of the combined data from all populations was performed using the Stats Direct software. First, homogeneity of OR among cohorts was calculated using Breslow–Day and Woolf Q methods. We then performed a calculation of the pooled OR under a fixed-effects model (Mantel–Haenszel meta-analysis) or random effects (DerSimonian–Laird) when necessary.

The estimation of the power of the study was performed using the Quanto v 0.5 software (Department of Preventive Medicine University of Southern California, California, USA). For the pooled analysis of SSc (n=2380) and considering a medium minor allele frequency of 0.30 our study reached a 99% power to detect the effect of a polymorphism at an OR of 1.3, similar to that observed for the analysed *BANK1* polymorphisms in previous studies.3 [4] Under the same conditions, estimation of the power for the pooled analysis of SSc clinical analysis was 93% for lcSSc (n=1394), 73% for dcSSc (n=770), 71% for ACA (n=749) and 51% for ATA (n=435). Since the *BANK1*-analysed SNPs are in strong linkage disequilibrium, the Bonferroni correction for multiple testing was not applied.[15]

RESULTS

The distribution of the *BANK1*-analysed polymorphisms was found to be in Hardy–Weinberg equilibrium in all the patients with SSc and control groups.

First, we investigated the possible implication of *BANK1* rs17266594, rs10516487 and rs3733197 SNPs in SSc susceptibility. In the independent analysis, an increased frequency of the rs10516487 G and the rs17266594 T alleles among patients with SSc compared with

controls was observed, although these differences were not statistically significant except for the rs17266594 T allele in the Italian population (p=0.001, OR=1.54, 95% CI 1.20 to 2.0) (table 1). In spite of the different geographical origin of study case–control sets, the combin-ability test according to the Breslow–Day method showed no significant differences among them. Therefore, we performed a pooled OR analysis using the Mantel–Haenszel test under fixed effects that showed a significant association of the rs10516487 G and the rs17266594 T alleles with SSc susceptibility (pooled OR=1.12, 95% CI 1.03 to 1.22, p=0.01 and pooled OR=1.14, 95% CI 1.05 to 1.25, p = 0.003, respectively) (table 1). The analysis of the rs3733197 genetic variant did not show any statistically significant deviation in the independent and pooled analyses (table 1).

To further characterise the role of the *BANK1* gene in SSc susceptibility we investigated its possible implication in SSc subtype or clinical manifestations. In the stratification accordingly to SSc subtype, we found that the general trend was an increased frequency of the major alleles of *BANK1* rs10516487, rs17266594 and rs3733197 genetic variants in patients with dcSSc compared with healthy subjects (table 2). Only in the Spanish and Swedish populations the frequency of these alleles in patients with dcSSc was very similar to that observed in the control population (table 2). A meta-analysis of the six study populations showed that the *BANK1* rs10516487 G, rs17266594 T and rs3733197 G alleles were strongly associated with dcSSc susceptibility (pooled OR=1.20, 95% CI 1.05 to 1.37, p=0.005; pooled OR=1.23, 95% CI 1.08 to 1.41, p=0.001; and pooled OR=1.15, 95% CI 1.02 to 1.31, p=0.02, respectively) (table 2). In contrast, for limited SSc no significant association of the *BANK1* rs10516487, rs17266594 or rs3733197 polymorphisms was observed in the independent and pooled analysis (table 2).

Interestingly, the analysis of *BANK1* genetic variants according to specific SSc autoantibody status confirmed the findings from the subtype analysis since the associations of *BANK1* rs10516487, rs17266594 and rs3733197 polymorphisms were significant only in the subgroup of patients carrying anti-Scl-70 antibodies that are highly specific for dcSSc (pooled OR=1.20, 95% CI 1.02 to 1.41, p=0.03; pooled OR=1.24, 95% CI 1.05 to 1.46, p=0.01; pooled OR=1.26, 95% CI 1.07 to 1.47, p=0.004) (table 3). In contrast, in the ACA patient groups the distribution of *BANK1* rs10516487, rs17266594 and rs3733197 genotypes and allelic frequencies did not reach any statistically significant skewing with ORs near 1.00 for the three SNPs (table 3). Additionally, this analysis showed the same trend in all six study populations even in the Spanish and Swedish groups, in which the subtype stratification showed no risk effect of *BANK1* polymorphisms for the dcSSc form (table 3).

The analysis of the *BANK1* selected genetic markers considering SSc organ involvement (interstitial lung disease, pulmonary hypertension) did not show any statistically significant skewing (data not shown).

DISCUSSION

In this study we analysed for the first time the possible association of the *BANK1* gene in genetic predisposition to SSc susceptibility and subphenotypes. *BANK1* is a functionally excellent a priori candidate gene since it is implicated in the regulation of B-cell receptor-mediated activation and B cells are thought to have a relevant role in SSc susceptibility.[6 16 17]

We conducted a large association study which included six independent case–control sets of European Caucasian ancestry and observed that *BANK1* functional polymorphisms are associated with genetic predisposition to SSc, specifically with the diffuse form of the disease and the presence of ATA. Although our results strongly suggest that the *BANK1* gene confers susceptibility to dcSSc but not to lcSSc, it should be noted that owing to the lower prevalence of the diffuse form of the disease, the number of patients with dcSSc included in our study

(n=770) has only 70% power to detect an association and therefore further independent replication studies are needed to confirm *BANK1* as a genetic marker of dcSSc across populations.

The hypothesis that genetic differences may influence the development of different SSc clinical subsets is supported by a number of well-powered SSc genetic studies that are being conducted. In this vain, recent evidence suggest that the *STAT4* gene confers an increased risk for lcSSc susceptibility but not for dcSSc.[13] Similarly, the *BLK* gene has been shown to confer increased risk for lcSSc (unpublished observation). Meanwhile, other genetic factors, such as *PTPN22* or *MIF* genes, seem to predispose particularly to dcSSc/anti-Scl-70 positive subset.[18 19]

Thus, the association of *BANK1* with dcSSc reported in this work supports novel evidence for this hypothesis.

All the *BANK1* genetic markers analysed in this study have been found to exert functional relevance. The rs10516487 and rs17266594 SNPs associated with dcSSc susceptibility lead to a non-synonymous substitution (R61H) and to a branch point site, respectively.[3] The rs10516487 polymorphism lies within a region essential for binding to the calcium channel IP3R, a downstream protein that mediates *BANK1* activity.[3] The functional relevance of the rs17266594 SNP resides in its ability to alter the expression of the two different BANK1 isoforms since in subjects with the rs17266594 G risk allele the expression of the full-length BANK1 protein is decreased while the Δ2 isoform is upregulated.[3] Thus the presence of these risk alleles could lead to more efficient splicing of a more ‘active’ protein.

BANK1 is primarily expressed in CD19 B cells that are significantly overexpressed in patients with SSc.[3 20] Activation through the B-cell receptor leads to BANK1 phosphorylation and signalling.[17] For subjects carrying *BANK1* functional mutations, altered B-cell activation may occur that could contribute to the production of autoantibodies as well. This could be the case for SSc, in which disease-specific autoantibodies are produced. However, further functional studies are necessary to elucidate the exact molecular mechanisms by which BANK1 is implicated in the B-cell activation process and, more precisely, how it can lead to the development of dcSSc.

In summary, our results suggest that the *BANK1* gene is associated with dcSSc genetic susceptibility. This could in part provide an explanation for one of the best described pathogenic mechanisms of disease, the production of specific autoantibodies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Distribution of *BANK1* single nucleotide polymorphisms (SNPs) genotypic and allelic frequencies in patients with systemic sclerosis (SSc) and controls

Population		GG	GA	AA	G	A	p Value G allele	OR (95% CI)
rs10516487								
USA	SSc (n = 1050)	0.50	0.40	0.10	0.70	0.30	0.14	1.12 (0.97 to 1.29)
	Controls (n = 694)	0.46	0.44	0.10	0.68	0.32		
Spanish	SSc (n = 578)	0.54	0.40	0.07	0.74	0.26	0.44	1.06 (0.91 to 1.25)
	Controls (n = 1040)	0.52	0.40	0.08	0.72	0.28		
Dutch	SSc (n = 139)	0.50	0.44	0.06	0.72	0.28	0.65	1.07 (0.78 to 1.48)
	Controls (n = 255)	0.49	0.44	0.07	0.71	0.29		
Swedish	SSc (n = 89)	0.48	0.43	0.09	0.70	0.30	0.94	0.98 (0.69 to 1.38)
	Controls (n = 565)	0.49	0.42	0.09	0.70	0.30		
German	SSc (n = 179)	0.51	0.42	0.06	0.73	0.27	0.19	1.20 (0.91 to 1.59)
	Controls (n = 360)	0.46	0.45	0.09	0.69	0.31		
Italian	SSc (n = 327)	0.57	0.36	0.07	0.75	0.25	0.04	1.29 (1.02 to 1.65)
	Controls (n = 334)	0.49	0.41	0.10	0.70	0.30		
Pooled*	SSc (n = 2362)	0.52	0.40	0.08	0.72	0.28	**0.01**	**1.12 (1.03 to 1.22)**
	Controls (n = 3248)	0.49	0.42	0.09	0.70	0.30		
		TT	**CT**	**CC**	**T**	**C**	**p Value T allele**	
rs17266594								
USA	SSc (n = 1046)	0.50	0.40	0.10	0.70	0.30	0.10	1.13 (0.98 to 1.31)
	Controls (n = 695)	0.45	0.44	0.11	0.67	0.33		
Spanish	SSc (n = 572)	0.54	0.39	0.07	0.73	0.27	0.76	1.02 (0.87 to 1.21)
	Controls (n = 1048)	0.52	0.42	0.06	0.73	0.27		
Dutch	SSc (n = 142)	0.51	0.44	0.06	0.73	0.27	0.62	1.08 (0.78 to 1.49)
	Controls (n = 256)	0.48	0.45	0.07	0.71	0.29		
Swedish	SSc (n = 89)	0.48	0.43	0.09	0.70	0.30	0.83	0.96 (0.67 to 1.36)
	Controls (n = 416)	0.51	0.40	0.10	0.70	0.30		
German	SSc (n = 178)	0.52	0.42	0.06	0.73	0.27	0.07	1.29 (0.97 to 1.72)
	Controls (n = 335)	0.45	0.46	0.09	0.68	0.32		
Italian	SSc (n = 324)	0.57	0.36	0.07	0.75	0.25	0.001	1.54 (1.20 to 2.0)
	Controls (n= 307)	0.43	0.47	0.10	0.66	0.34		

Population		GG	GA	AA	G	A	p Value G allele	OR (95% CI)
Pooled*	SSc (n = 2351)	0.52	0.40	0.08	0.72	0.28	**0.003**	**1.14 (1.05 to 1.25)**
	Controls (n = 3231)	0.48	0.43	0.08	0.70	0.30		
		GG	**GA**	**AA**	G	A	**p Value G allele**	
rs3733197								
USA	SSc (n = 1053)	0.43	0.43	0.14	0.64	0.36	0.23	1.09 (0.95 to 1.25)
	Controls (n = 696)	0.39	0.47	0.14	0.62	0.38		
Spanish	SSc (n = 588)	0.49	0.44	0.07	0.71	0.29	0.50	1.05 (0.90 to 1.23)
	Controls (n = 1051)	0.49	0.42	0.10	0.70	0.30		
Dutch	SSc (n = 142)	0.40	0.50	0.10	0.65	0.35	0.63	1.08 (0.79 to 1.46)
	Controls (n = 250)	0.40	0.48	0.13	0.63	0.37		
Swedish	SSc (n = 89)	0.37	0.43	0.21	0.58	0.42	0.69	0.86 (0.62 to 1.20)
	Controls (n = 444)	0.37	0.50	0.14	0.62	0.39		
German	SSc (n = 183)	0.42	0.49	0.09	0.66	0.34	0.15	1.21 (0.92 to 1.57)
	Controls (n = 362)	0.41	0.42	0.17	0.62	0.38		
Italian	SSc (n = 325)	0.50	0.41	0.10	0.70	0.30	0.07	1.24 (0.98 to 1.56)
	Controls (n = 340)	0.41	0.49	0.10	0.65	0.35		
Pooled*	SSc (n = 2378)	0.52	0.40	0.08	0.72	0.28	0.48	0.96 (0.89 to 1.05)
	Controls (n = 3143)	0.42	0.45	0.12	0.65	0.35		

*Mantel–Haenszel test under fixed effect.

Table 2

Analysis of *BANK1* polymorphisms according to systemic sclerosis (SSc) subtype

Population		GG	AG	AA	G	A	p Value G allele	OR (95% CI)
rs10516487								
USA	Limited (n = 607)	0.48	0.41	0.12	0.68	0.32	0.85	1.02 (0.86 to 1.20)
	Diffuse (n = 389)	0.54	0.38	0.08	0.73	0.27	0.01	1.30 (1.07 to 1.58)
	Controls (n = 694)	0.46	0.44	0.10	0.68	0.32		
Spanish	Limited (n = 315)	0.55	0.40	0.05	0.75	0.25	0.20	1.14 (0.93 to 1.40)
	Diffuse (n = 150)	0.53	0.41	0.07	0.73	0.27	0.82	1.03 (0.78 to 1.35)
	Controls (n = 1040)	0.52	0.40	0.08	0.72	0.28		
Dutch	Limited (n = 103)	0.47	0.49	0.05	0.71	0.29	0.98	1.00 (0.70 to 1.42)
	Diffuse (n = 27)	0.63	0.30	0.07	0.78	0.22	0.28	1.37 (0.71 to 2.65)
	Controls (n = 255)	0.49	0.44	0.07	0.71	0.29		
Swedish	Limited (n = 58)	0.52	0.40	0.09	0.72	0.28	0.71	1.07 (0.70 to 1.62)
	Diffuse (n = 31)	0.42	0.48	0.10	0.66	0.34	0.53	0.82 (0.48 to 1.41)
	Controls (n = 565)	0.49	0.42	0.09	0.70	0.30		
German	Limited (n = 93)	0.52	0.44	0.04	0.74	0.26	0.19	1.26 (0.88 to 1.80)
	Diffuse (n = 86)	0.51	0.41	0.08	0.72	0.28	0.48	1.13 (0.78 to 1.63)
	Controls (n = 360)	0.46	0.45	0.09	0.69	0.31		
Italian	Limited (n = 218)	0.54	0.39	0.07	0.74	0.26	0.17	1.21 (0.92 to 1.58)
	Diffuse (n = 87)	0.61	0.31	0.08	0.76	0.24	0.08	1.39 (0.94 to 2.04)
	Controls (n = 334)	0.49	0.41	0.10	0.70	0.30		
Pooled*	Limited (n = 1394)	0.51	0.41	0.08	0.71	0.29	0.08	1.09 (0.98 to 1.21)
	Diffuse (n = 770)	0.54	0.38	0.08	0.73	0.27	**0.005**	**1.20 (1.05 to 1.37)**
	Controls (n = 3284)	0.49	0.42	0.09	0.70	0.30		
		TT	**CT**	**CC**	**T**	**C**	**p Value T allele**	
rs17266594								
USA	Limited (n = 602)	0.48	0.41	0.12	0.68	0.32	0.84	1.02 (0.86 to 1.20)
	Diffuse (n = 389)	0.55	0.38	0.08	0.73	0.27	0.004	1.33 (1.09 to 1.61)
	Controls (n = 695)	0.45	0.44	0.11	0.67	0.33		
Spanish	Limited (n = 315)	0.54	0.39	0.06	0.74	0.26	0.52	1.07 (0.87 to 1.30)
	Diffuse (n = 149)	0.52	0.41	0.07	0.73	0.27	0.95	1.00 (0.76 to 1.31)

Population		GG	AG	AA	G	A	p Value G allele	OR (95% CI)
	Controls (n = 1048)	0.52	0.42	0.06	0.73	0.27		
Dutch	Limited (n = 105)	0.48	0.48	0.05	0.71	0.29	0.89	1.02 (0.72 to 1.45)
	Diffuse (n = 28)	0.61	0.32	0.07	0.77	0.32	0.35	1.30 (0.68 to 2.46)
	Controls (n = 256)	0.48	0.45	0.07	0.71	0.29		
Swedish	Limited (n = 58)	0.52	0.40	0.09	0.72	0.28	0.71	1.07 (0.70 to 1.62)
	Diffuse (n = 31)	0.42	0.48	0.10	0.66	0.34	0.53	0.82 (0.48 to 1.41)
	Controls (n = 565)	0.49	0.42	0.09	0.70	0.30		
German	Limited (n = 93)	0.52	0.44	0.04	0.74	0.26	0.19	1.26 (0.87 to 1.80)
	Diffuse (n = 86)	0.51	0.41	0.08	0.72	0.28	0.48	1.13 (0.78 to 1.67)
	Controls (n = 360)	0.46	0.45	0.09	0.69	0.31		
Italian	Limited (n = 217)	0.54	0.39	0.07	0.74	0.26	0.013	1.41 (1.07 to 1.84)
	Diffuse (n = 85)	0.61	0.32	0.07	0.77	0.23	0.01	1.68 (1.14 to 2.49)
	Controls (n = 307)	0.43	0.47	0.10	0.66	0.34		
Pooled*	Limited (n = 1143)	0.51	0.41	0.09	0.71	0.29	0.05	1.10 (1.00 to 1.23)
	Diffuse (n = 613)	0.54	0.38	0.08	0.73	0.27	**0.001**	**1.23 (1.08 to 1.41)**
	Controls (n = 3231)	0.48	0.43	0.08	0.70	0.30		
		GG	**GA**	**AA**	**G**	**A**	**p Value G allele**	
rs3733197								
USA	Limited (n = 608)	0.42	0.43	0.15	0.64	0.36	0.55	1.05 (0.90 to 1.23)
	Diffuse (n = 390)	0.44	0.43	0.13	0.66	0.34	0.14	1.15 (0.96 to 1.38)
	Controls (n = 696)	0.39	0.47	0.14	0.62	0.38		
Spanish	Limited (n = 324)	0.50	0.44	0.06	0.72	0.28	0.26	1.11 (0.92 to 1.35)
	Diffuse (n = 153)	0.49	0.43	0.08	0.71	0.29	0.75	1.04 (0.80 to 1.35)
	Controls (n = 1051)	0.49	0.42	0.10	0.70	0.30		
Dutch	Limited (n = 105)	0.36	0.53	0.10	0.63	0.37	0.89	0.97 (0.70 to 1.36)
	Diffuse (n = 28)	0.50	0.43	0.07	0.71	0.29	0.23	1.40 (0.77 to 2.54)
	Controls (n = 250)	0.40	0.48	0.13	0.63	0.37		
Swedish	Limited (n = 47)	0.43	0.36	0.21	0.61	0.39	0.87	0.96 (0.62 to 1.47)
	Diffuse (n = 30)	0.40	0.33	0.27	0.57	0.43	0.46	0.81 (0.72 to 2.08)
	Controls (n = 444)	0.37	0.50	0.14	0.62	0.39		
German	Limited (n = 93)	0.40	0.53	0.08	0.66	0.34	0.30	1.19 (0.85 to 1.66)
	Diffuse (n = 86)	0.44	0.45	0.11	0.67	0.33	0.24	1.23 (0.57 to 1.16)

Population		GG	AG	AA	G	A	p Value G allele	OR (95% CI)
Italian	Controls (n = 362)	0.41	0.42	0.17	0.62	0.38		
	Limited (n = 217)	0.45	0.45	0.10	0.68	0.32	0.45	1.10 (0.85 to 1.42)
	Diffuse (n = 86)	0.56	0.35	0.09	0.73	0.27	0.05	1.44 (0.99 to 2.09)
Pooled*	Controls (n = 340)	0.41	0.49	0.10	0.65	0.35		
	Limited (n = 1158)	0.43	0.45	0.11	0.66	0.34	0.15	1.07 (0.97 to 1.18)
	Diffuse (n = 613)	0.47	0.42	0.12	0.67	0.33	**0.02**	**1.15 (1.02 to 1.31)**
	Controls (n = 3143)	0.42	0.45	0.12	0.65	0.35		

*Mantel–Haenszel test under fixed effect.

Table 3

Analysis of *BANK1* polymorphisms according to systemic sclerosis (SSc)-specific autoantibodies

Population		GG	GA	AA	Allele G	Allele A	p Value G allele	OR (95% CI)
rs10516487								
USA	ACA (n = 298)	0.48	0.41	0.11	0.68	0.32	0.85	1.02 (0.83 to 1.25)
	ATA (n = 173)	0.50	0.41	0.09	0.71	0.29	0.29	1.14 (0.88 to 1.48)
	Controls (n = 694)	0.46	0.44	0.10	0.68	0.32		
Spanish	ACA (n = 155)	0.46	0.45	0.09	0.69	0.31	0.18	0.84 (0.65 to 1.08)
	ATA (n = 68)	0.59	0.34	0.07	0.76	0.24	0.39	1.17 (0.78 to 1.75)
	Controls (n = 1040)	0.52	0.40	0.08	0.72	0.28		
Dutch	ACA (n = **37**)	0.50	0.42	0.07	0.71	0.29	0.85	1.03 (0.72 to 1.46)
	ATA (n = 28)	0.54	0.39	0.07	0.73	0.27	0.70	1.09 (0.59 to 2.01)
	Controls (n = 255)	0.49	0.44	0.07	0.71	0.29		
Swedish	ACA (n = 16)	0.56	0.38	0.06	0.75	0.25	0.54	1.20 (0.54 to 2.64)
	ATA (n = 11)	0.64	0.27	0.09	0.77	0.23	0.46	1.29 (0.49 to 3.40)
	Controls (n = 565)	0.49	0.42	0.09	0.70	0.30		
German	ACA (n = 66)	0.50	0.44	0.06	0.72	0.28	0.46	1.15 (0.76 to 1.73)
	ATA (n = 41)	0.51	0.41	0.07	0.72	0.28	0.55	1.14 (0.69 to 1.88)
	Controls (n = 360)	0.46	0.55	0.09	0.69	0.31		
Italian	ACA (n = 107)	0.50	0.42	0.07	0.71	0.29	0.63	1.08 (0.77 to 1.51)
	ATA (n = 114)	0.59	0.33	0.08	0.75	0.25	0.10	1.32 (0.94 to 1.86)
	Controls (n = 334)	0.49	0.41	0.10	0.70	0.30		
Pooled*	ACA (n = 749)	0.49	0.42	0.09	0.70	0.30	0.97	1.00 (0.88 to 1.40)
	ATA (n = 435)	0.54	0.37	0.08	0.73	0.27	**0.03**	**1.20 (1.02 to 1.41)**
	Controls (n = 3284)	0.49	0.42	0.09	0.70	0.30		
		TT	**CT**	**CC**	**Allele T**	**Allele C**	**p Value T allele**	
rs17266594								
USA	ACA (n = 294)	0.47	0.41	0.11	0.68	0.32	0.81	1.02 (0.83 to 1.26)
	ATA (n = 173)	0.50	0.41	0.09	0.71	0.29	0.235	1.16 (0.90 to 1.50)
	Controls (n = 695)	0.45	0.44	0.11	0.67	0.33		
Spanish	ACA (n = 161)	0.48	0.11	0.11	0.69	0.31	0.17	0.83 (0.64 to 1.07)
	ATA (n = 72)	0.57	0.41	0.08	0.74	0.26	0.67	1.07 (0.73 to 1.57)

Population		GG	GA	AA	Allele G	Allele A	p Value G allele	OR (95% CI)
	Controls (n = 1048)	0.52	0.42	0.06	0.73	0.27		
Dutch	ACA (n = 37)	0.54	0.41	0.05	0.74	0.26	0.54	1.15 (0.67 to 2.00)
	ATA (n = 28)	0.54	0.39	0.07	0.73	0.27	0.72	1.08 (0.59 to 2.00)
	Controls (n = 256)	0.48	0.45	0.07	0.71	0.29		
Swedish	ACA (n = 16)	0.56	0.38	0.06	0.75	0.25	0.54	1.20 (0.54 to 2.64)
	ATA (n = 11)	0.64	0.27	0.09	0.77	0.23	0.46	1.29 (0.49 to 3.40)
	Controls (n = 565)	0.49	0.42	0.09	0.70	0.30		
German	ACA (n = 66)	0.50	0.44	0.06	0.72	0.28	0.46	1.15 (0.76 to 1.73)
	ATA (n = 41)	0.51	0.42	0.07	0.72	0.28	0.55	1.14 (0.69 to 1.88)
	Controls (n = 360)	0.46	0.45	0.09	0.69	0.31		
Italian	ACA (n = 107)	0.51	0.40	0.08	0.72	0.29	0.161	1.27 (0.90 to 1.78)
	ATA (n = 112)	0.59	0.34	0.07	0.76	0.24	0.01	1.59 (1.12 to 2.24)
	Controls (n = 307)	0.43	0.47	0.10	0.66	0.34		
Pooled*	ACA (n = 632)	0.53	0.37	0.10	0.70	0.30	0.61	1.03 (0.91 to 1.18)
	ATA (n = 478)	0.50	0.43	0.07	0.73	0.27	**0.01**	**1.24 (1.05 to 1.46)**
	Controls (n = 3231)	0.48	0.43	0.08	0.70	0.30		
		GG	**GA**	**AA**	**Allele G**	**Allele A**	**p Value G allele**	
rs3733197								
USA	ACA (n = 299)	0.41	0.44	0.15	0.63	0.37	0.85	1.02 (0.84 to 1.24)
	ATA (n = 173)	0.50	0.42	0.08	0.71	0.29	0.004	1.45 (1.13 to 1.88)
	Controls (n = 696)	0.39	0.47	0.14	0.62	0.38		
Spanish	ACA (n = 164)	0.43	0.48	0.09	0.67	0.33	0.29	0.87 (0.68 to 1.12)
	ATA (n = 72)	0.44	0.46	0.10	0.67	0.33	0.56	0.89 (0.62 to 1.28)
	Controls (n = 1051)	0.49	0.42	0.10	0.70	0.30		
Dutch	ACA (n = 28)	0.39	0.54	0.07	0.66	0.34	0.69	1.10 (0.62 to 1.96)
	ATA (n = 37)	0.51	0.30	0.19	0.66	0.34	0.64	1.11 (0.67 to 1.86)
	Controls (n = 250)	0.40	0.48	0.13	0.63	0.37		
Swedish	ACA (n = 16)	0.38	0.50	0.13	0.63	0.38	0.91	1.01 (0.49 to 2.07)
	ATA (n = 11)	0.46	0.46	0.09	0.68	0.32	0.52	1.25 (0.52 to 3.03)
	Controls (n = 444)	0.37	0.50	0.14	0.62	0.39		
German	ACA (n = 76)	0.49	0.43	0.08	0.70	0.30	0.05	1.44 (0.99 to 2.10)
	ATA (n = 42)	0.45	0.45	0.10	0.68	0.32	0.29	1.27 (0.79 to 2.05)

Population		GG	GA	AA	Allele G	Allele A	p Value G allele	OR (95% CI)
Italian	Controls (n = 362)	0.41	0.42	0.17	0.62	0.38		
	ACA (n = 105)	0.44	0.41	0.15	0.64	0.36	0.79	0.95 (0.69 to 1.32)
	ATA (n = 112)	0.51	0.40	0.09	0.71	0.29	0.12	1.29 (0.93 to 1.79)
Pooled*	Controls (n = 340)	0.41	0.49	0.10	0.65	0.35		
	ACA (n = 688)	0.42	0.45	0.13	0.65	0.35	0.81	1.02 (0.89 to 1.15)
	ATA (n = 447)	0.49	0.42	0.10	0.70	0.30	**0.004**	**1.26 (1.07 to 1.47)**
	Controls (n = 3143)	0.42	0.45	0.12	0.65	0.35		

*Mantel–Haenszel test under fixed effects.

ACA, anticentromere antibodies; ATA, anti-topoisomerase antibodies.