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Association Study of *ITGAM*, *ITGAX*, and *CD58* Autoimmune Risk Loci in Systemic Sclerosis: Results from 2 Large European Caucasian Cohorts

BAPTISTE COUSTET, SANDEEP K. AGARWAL, PRAVITT GOURH, MICKAEL GUEDJ, MAUREEN D. MAYES, PHILIPPE DIEUDE, JULIEN WIPFF, JEROME AVOUAC, ERIC HACHULLA, ELISABETH DIOT, JEAN LUC CRACOWSKI, KIET TIEV, JEAN SIBILIA, LUC MOUTHON, CAMILLE FRANCES, ZAHIR AMOURA, PATRICK CARPENTIER, OLIVIER MEYER, ANDRE KAHAN, CATHERINE BOILEAU, FRANK C. ARNETT, and YANNICK ALLANORE

Université Paris Descartes, Rhumatologie A, Hôpital Cochin, APHP, Paris; INSERM U1016, Université Paris Descartes, Hôpital Cochin, Paris; Division of Rheumatology and Clinical Immunogenetics, Department of Internal Medicine, University of Texas Health Science Center at Houston, Houston, Texas, USA; UMR CNRS-8071/INRA-1152, Université d'Evry Val d'Essonne, Evry; Université Paris 7, Rhumatologie, Hôpital Bichat, Paris; Université Lille II, Médecine Interne, Lille; INSERM EMI-U 00-10, Médecine Interne, CHU Bretonneau, Tours; INSERM CIC3, CHU Grenoble, Grenoble; Université Pierre et Marie Curie, Hôpital Saint Antoine, Paris; Université Louis Pasteur, Rhumatologie, Hôpital Hautepierre, Strasbourg; Université Paris Descartes, Médecine Interne, Hôpital Cochin, APHP, Paris; Université Paris 6, Dermatologie, Hôpital Tenon, Paris; Université Paris 6, Médecine Interne, Pitié Salpêtrière, Paris; Clinique Universitaire de Médecine Vasculaire, Pôle Pluridisciplinaire de Médecine, Centre Hospitalier Universitaire, Grenoble; and Université Versailles Saint Quentin Yvelines, Laboratoire de Biochimie Hormonale et Génétique, Hôpital Ambroise Paré, APHP, Boulogne, France

Abstract

Objective—Accumulating evidence shows that shared autoimmunity is critical for the pathogenesis of many autoimmune diseases. Systemic sclerosis (SSc) belongs to the connective tissue disorders, and recent data have highlighted strong associations with autoimmunity genes shared with other autoimmune diseases. To determine whether novel risk loci associated with systemic lupus erythematosus or multiple sclerosis may confer susceptibility to SSc, we tested single-nucleotide polymorphisms (SNP) from *ITGAM*, *ITGAX*, and *CD58* for associations.

Methods—SNP harboring associations with autoimmune diseases, *ITGAM*rs9937837, *ITGAX*rs11574637, and *CD58*rs12044852, were genotyped in 2 independent cohorts of European Caucasian ancestry: 1031 SSc patients and 1014 controls from France and 1038 SSc patients and 691 controls from the USA, providing a combined study population of 3774 individuals. *ITGAM*rs1143679 was additionally genotyped in the French cohort.

Results—The 4 polymorphisms were in Hardy-Weinberg equilibrium in the 2 control populations, and allelic frequencies were similar to those expected in European Caucasian populations. Allelic and genotypic frequencies for these 3 SNP were found to be statistically similar in SSc patients and controls. Subphenotype analyses for subgroups having diffuse

cutaneous subtype disease, specific autoantibodies, or fibrosing alveolitis did not reveal any difference between SSc patients and controls.

Conclusion—These results obtained through 2 large cohorts of SSc patients of European Caucasian ancestry do not support the implication of *ITGAM*, *ITGAX*, and *CD58* genes in the genetic susceptibility of SSc, although they were recently identified as autoimmune disease risk genes.

Key Indexing Terms

SYSTEMIC SCLEROSIS; SYSTEMIC LUPUS ERYTHEMATOSUS; AUTOIMMUNITY
SINGLE NUCLEOTIDE POLYMORPHISM; ITGAM; ITGAX; CD58

Systemic sclerosis (SSc) is a chronic autoimmune disease with a complex pathogenesis that is driven by a combination of genetic risk factors and environmental events¹.

Accumulating data have demonstrated shared autoimmunity pathways and genetic susceptibility factors among various autoimmune diseases. Most of these genetic susceptibility factors are frequently replicated in different diseases such as insulin-dependent diabetes mellitus, multiple sclerosis (MS), systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), juvenile idiopathic arthritis, celiac disease, and others².

Regarding SSc, recent data have shown that the main shared genetic factors that contribute the most to susceptibility are the major histocompatibility complex (MHC), *IRF5*, *STAT4*, *BANK1*, *PTPN22*, and *TNFAIP3*^{3,4}. These genes and pathways are, strikingly, all known to contribute to susceptibility to SLE.

In SLE, some new risk loci have recently been identified. Among these, *ITGAM* and *ITGAX* were identified first in association studies performed in large cohorts. Proteins encoded by these latter genes all belong to the integrin family. Among integrin molecules, very late antigen-4 (VLA-4) and lymphocyte function associated-1 (LFA-1) have been implicated in both SLE and SSc^{5,6}. Some hypotheses suggest their participation in maintaining the pathogenic cells in targeted tissues, thereby promoting tissue damage⁷.

ITGAM, also known as CD11b, is located in chromosome 16p11.2 and encodes for the α -chain of the $\alpha_M\beta_2$ - integrin. This leukocyte-specific integrin regulates cell activation and adhesion of neutrophils and monocytes, permitting endothelium stimulation and phagocytosis of complement-coated particles. Particular involvement in immune complex clearance can be linked to the demonstrated impairment of this function in SLE⁸. Indeed, a deficiency of *ITGAM* leads to enhanced production of interleukin 6 by antigen-presenting cells⁹. rs9937837 and other genetic variants located on chromosome 16p11.12 near the gene *ITGAM* were found to contribute to SLE susceptibility in a genome-wide association study (OR 1.28, $p = 7 \times 10^{-7}$)¹⁰. At the same time, another strong genetic variant of *ITGAM* rs1143679 was reported to be associated with SLE (OR 1.78, $p = 1.7 \times 10^{-17}$)¹¹. A metaanalysis strengthened these results and a strong association of *ITGAM* rs9937837 (OR 0.47) and rs1143679 (OR 3.04) was shown to influence disease severity^{12,13,14}. This latter finding was convincingly reported as the putative causal variant¹⁵.

ITGAX encodes the integrin α -X chain protein, which forms by association with β -chain, another leukocyte-specific integrin that overlaps the properties of *ITGAM*. *ITGAM* variants have been found to be associated with SLE (OR 1.3). However, linkage disequilibrium data with *ITGAM* have questioned its role as an independent signal¹⁰. This assumption was emphasized by the role of rs1143679¹⁵.

MS and RA also share genetic susceptibility factors with other autoimmune diseases. Another new risk locus shown to be associated with MS is *CD58*, also known as *LFA-3*. It encodes a member of the immunoglobulin superfamily, a ligand of the T lymphocyte CD2 protein, and has been implicated in adhesion and activation of T lymphocytes. The rs12044852 variant of *CD58* was found to be associated in a large genome-wide association study (OR 1.48)¹⁶ in MS and was independently replicated¹⁷. Another *CD58* variant was also found to be associated with RA (OR 1.14)¹⁸.

Taking into account (1) the autoimmune background of SSc; (2) the contribution of shared autoimmunity in this condition; (3) the recent report of new autoimmune susceptibility risk factors belonging to integrin genes for autoimmune diseases¹⁰; and (4) implication of integrins in SSc, we investigated whether *ITGAM*, *ITGAX*, and *CD58* variants may confer susceptibility to SSc.

MATERIALS AND METHODS

We performed a large case-control association study in 2 independent cohorts of European Caucasian ancestry: a French cohort consisting of 1031 SSc patients compared to 1014 healthy unrelated controls and a US cohort including 1038 SSc patients and 691 controls. For all SSc patients, LeRoy's cutaneous subtype was determined¹⁹ and phenotypic assessment was carried out, as recommended²⁰. These cohorts have been described in detail^{4,21}.

The study was approved by all the necessary local institutional review boards, and written informed consent was obtained from all subjects. *Genotyping*. We selected the following 3 SNP, for which the most convincing association signals have been reported in autoimmune diseases: rs9937837 *ITGAM*¹⁰, rs11574637 *ITGAX*¹⁰, and rs12044852 *CD58*¹⁶. Another SNP at the *ITGAM* locus, nonsynonymous rs1143679 (R77H) located in exon 3¹², was studied only in the French cohort, as being more recently reported, and was not initially included in this study. These SNP were genotyped using a competitive allele-specific polymerase chain reaction system (Kaspar Genotyping, Kbioscience, Hoddeston, UK) for the French cohort, as reported²², and a predesigned TaqMan SNP genotyping assay (Applied Biosystems, Foster City, CA, USA) in the US cohort²¹. The average genotype completeness for these SNP polymorphisms was 99% for the SSc samples and the controls. The accuracy was > 99%, according to duplicate genotyping of 10% of all samples using the Taqman SNP genotyping assay-allelic discrimination method (Applied Biosystems).

Statistical analyses

The statistical analyses were performed using the R software (version 2.10.0). The level of significance for all the tests corresponds to a type I error-rate $\alpha = 5\%$. Tests for conformity with Hardy-Weinberg equilibrium were performed using a standard chi-square test (1 degree of freedom). Individual analyses of association of the 4 SNP with SSc were performed by comparing cases and controls by Fisher's exact test on genotype distribution. The same procedure was applied in subgroups stratified according to SSc phenotypes. Bonferroni's correction was applied to all tests of SNP marker associations with the disease (the p value multiplied by n SNP) and to all "hypothesis-generating steps" when comparing the SSc subgroups and control (10 phenotypic subsets). P values adjusted for multiple testing are indicated in the tables and identified as P_{adj} in the text.

In case an association signal was detected in one population, the combined data for the 2 populations were analyzed by calculating the homogeneity of odds ratios between cohorts by the Breslow-Day and Woolf Q methods; and by calculating the pooled odds ratios under a fixed-effects model (Mantel-Haenszel metaanalysis) or a random-effects model

(DerSimonian-Laird), as appropriate. The linkage disequilibrium structure of the loci of interest was scrutinized and linkage disequilibrium blocks defined using the expectation-maximization (EM) algorithm, as implemented in the haplo.stats R library.

Power calculation

Statistical power was assessed by a standard noncentral chi-square approximation, as described²³. For *ITGAM*, taking into account the expected frequency of the rare allele of rs9937837, the set has a power of 98% and 99%, respectively, in French and combined cases for detecting an association between SSc and this variant, with an OR of 1.5 at the 5% significance level. For the other SNP of *ITGAM* rs1143679, the French set has a power of 88% with an OR of 1.5. Similarly, the power for *ITGAX* rs11574637 was at 96% in the French SSc set and 99% in the combined populations. Finally, statistical power was 77% and 99% in French and combined populations for *CD58* rs12044852, respectively.

RESULTS

Demographic data and disease characteristics of SSc patients and controls are shown in Table 1.

All the SNP were in Hardy-Weinberg equilibrium in the control populations. Allelic frequencies were found to be in good agreement with those previously reported in the European population^{10,11,17}.

In the French cohort, the *ITGAM* rs9937837 G allele was found on 30% of chromosomes of SSc patients compared to 27.8% of controls ($p =$ nonsignificant; Table 2). The *ITGAM* rs1143679, the other SNP tested in only the French cohort, did not show genotypic or allelic associations to SSc (allelic frequency 13.5% in SSc cases, 12.6% in controls). The *ITGAX* rs11574637 C minor allele frequency was found, respectively, on 20.4% and 18.9% of SSc case and control chromosomes (Table 3). Regarding the third locus, the *CD58* rs12044852 A allele was found on 10.3% of chromosomes from SSc cases compared to 9.5% from controls.

Very congruent results were obtained in the US cohort. Indeed, the G allele of *ITGAM* rs9937837 was found on 29.9% of SSc cases versus 27.4% on controls ($p =$ non-significant; Table 2). The C minor allele of *ITGAX* rs11574637 was found in 19.4% of SSc case chromosomes and did not deviate from the frequency observed in the controls (17%; $p =$ nonsignificant; Table 3). For the third locus, the *CD58* rs12044852 A allele was found on 18.3% of chromosomes from SSc cases compared to 23.3% of controls.

Therefore, no significant evidence of allelic or genotypic association was detected for the *ITGAM*, *ITGAX*, and *CD58* SNP (Tables 2, 3, and 4). Secondary analyses with adjustment for age and sex did not show any signal of association. Regarding linkage disequilibrium structure at the *ITGAM/ITGAX* locus, we found that r^2 between the *ITGAM* rs9937837 and *ITGAX* rs11574637 was 0.26 and 0.25, respectively, between the 2 *ITGAM* rs9937837 and rs1143679 SNP in the French control population. Further, regarding SSc subphenotypes, intracohort comparisons also failed to detect any association. One signal for association was suggested in the US anti-topoisomerase I subset for *ITGAM* rs9937837, but this was not confirmed in the French sample. This led us to perform a metaanalysis, which also did not find any association ($p = 0.31$). Regarding *CD58*, the minor allele frequency differed between the 2 cohorts with respect to controls as well as SSc patients (Table 4). Although a trend for allelic association was observed for rs12044852 in the US cohort, the association signal was dropped after correction for multiple testing. There was no association in the French cohort (Table 4).

DISCUSSION

Although rare, SSc presents a major medical challenge, being one of the most severe connective tissue disorders in terms of its prognosis²⁴. Shared autoimmunity pathways among SSc, SLE, and other autoimmune diseases are well illustrated by many common genetic susceptibility factors²⁵. This led us to test for associations of these new autoimmune loci in SSc. Our results from 2 large independent cohorts showed that the studied polymorphisms of *ITGAM*, *ITGAX*, and *CD58* do not contribute to susceptibility to SSc or its subphenotypes in European Caucasians. Some concerns have been raised regarding the linkage disequilibrium structure of the *ITGAM/ITGAX* locus, leading us to genotype another SNP of *ITGAM*, rs1143679, that could be the causal variant in different ethnic groups^{11,13,15,26}. The calculation in our sample did not reveal linkage disequilibrium between *ITGAM*rs9937837 and rs1143679 or between *ITGAM*rs9937837 and *ITGAX*rs11574637, suggesting that they represent independent loci. The functional SNP rs1143679 that may be the causal variant of association in lupus was not associated with SSc in our study; although the genotyping was restricted to the French population, the statistical power reasonably suggests the lack of association in this sample. Furthermore, similar data were reported in RA²⁷, suggesting a specific role of *ITGAM* in SLE. However, more dense SNP genotyping is required before association with SSc can be definitely ruled out.

Methodological limitations of genetic studies must always be considered. Appropriate sample sizes for case and control cohorts are critical to provide sufficient statistical power²⁸. In our study, the 2 large sample sizes of the cohorts provided a strong rationale for ruling out type II statistical bias. Further, the genetic background of the studied populations should be as homogeneous as possible, thereby limiting bias by population stratification. To avoid this bias, ethnicity was taken into account and we focused on Caucasian individuals. Moreover, the 2 cohorts were very homogeneous in particular for proportions of SSc subphenotypes including autoantibodies. Available genetic data in SSc and autoimmune disease suggest that some critical immune factors contribute to autoimmunity, and these findings support the evolving concept that common risk genes underlie multiple autoimmune disorders. However, they also highlight that further specific, downstream biological mechanisms must be involved to generate the respective phenotypes. Integrins are central in maintaining the homeostasis of the cellular microenvironment permitted by extracellular matrix. They are also critical in mediating specific signaling events and function as master effectors of transforming growth factor- β activation that play a central role in any fibrotic process^{29,30}. However, our results suggest that genetic variants of integrin coding genes may not be involved in SSc, despite playing a role in some autoimmune diseases.

The genotyping of 3 risk loci for autoimmune diseases (*ITGAM*, *ITGAX*, and *CD58*) in 2 large cohorts of European Caucasian ancestry from France and the USA did not reveal any allelic or genotypic association with SSc or its main subphenotypes.

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B. Coustet, Fellow, Université Paris Descartes, Rhumatologie A, Hôpital Cochin, APHP, INSERM U1016; S.K. Agarwal, MD, PhD; P. Gourh, MD, Division of Rheumatology and Clinical Immunogenetics, Department of

Internal Medicine, University of Texas Health Science Center at Houston; M. Guedj, PhD, UMR CNRS-8071/INRA-1152, Université d'Evry Val d'Essonne; M. Mayes, MD, Division of Rheumatology and Clinical Immunogenetics, Department of Internal Medicine, University of Texas Health Science Center at Houston; P. Dieude, MD, PhD, Université Paris 7, Rhumatologie, Hôpital Bichat; J. Wipff, MD, PhD; J. Avouac, MD, PhD, Université Paris Descartes, Rhumatologie A, Hôpital Cochin, APHP, INSERM U1016; E. Hachulla, MD, PhD, Université Lille II, Médecine Interne; E. Diot, MD, INSERM EMI-U 00-10, Médecine Interne, CHU Bretonneau; J.L. Cracowski, MD, PhD, INSERM CIC3, CHU Grenoble; K. Tiev, MD, PhD, Université Pierre et Marie Curie, Hôpital Saint Antoine; J. Sibilia, MD, PhD, Université Louis Pasteur, Rhumatologie, Hôpital Hautepierre; L. Mouthon, MD, PhD, Université Paris Descartes, Médecine Interne, Hôpital Cochin, APHP; C. Frances, MD, Université Paris 6, Dermatologie, Hôpital Tenon; Z. Amoura, MD, PhD, Université Paris 6, Médecine Interne, Pitié Salpêtrière; P. Carpentier, MD, Clinique Universitaire de Médecine Vasculaire, Pôle Pluridisciplinaire de Médecine, Centre Hospitalier Universitaire Grenoble; O. Meyer, MD, PhD, Université Paris 7, Rhumatologie, Hôpital Bichat; A. Kahan, MD, PhD, Université Paris Descartes, Rhumatologie A, Hôpital Cochin, APHP; C. Boileau, PharmD, PhD, Université Versailles Saint Quentin Yvelines, Laboratoire de Biochimie Hormonale et Génétique, Hôpital Ambroise Paré, APHP; F.C. Arnett, MD, Division of Rheumatology and Clinical Immunogenetics, Department of Internal Medicine, University of Texas Health Science Center at Houston; Y. Allanore, MD, PhD, Université Paris Descartes, Rhumatologie A, Hôpital Cochin, APHP, INSERM U1016.

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Table 1

Clinical and serologic characteristics of patients with systemic sclerosis.

Characteristic	French Caucasian N = 1014 (%)	US Caucasian N = 691 (%)
Controls		
Sex		
Female	646 (64.2)	329 (50.5)
Male	360 (35.8)	322 (49.5)
Systemic sclerosis		
Sex	N = 1031 (%)	N = 1038 (%)
Female	875 (85.7)	918 (88.4)
Male	146 (14.3)	120 (11.6)
Skin involvement	N = 971 (%)	N = 983 (%)
Limited systemic sclerosis	650 (66.9)	600 (61.0)
Diffuse systemic sclerosis	321 (33.1)	383 (39.0)
Autoantibodies	N = 928 (%)	N = 653 (%)
Anti-centromere	377 (40.6)	293 (44.9)
Anti-topoisomerase I	253 (27.3)	172 (26.3)

Table 2

Distribution of *ITGAM* rs9937837 in patients with SSs and healthy controls.

	No.	Genotype, %			G Allele, %	P	Allelic Association OR (95% CI)
		GG	GT	TT			
French Caucasian							
Controls	971	8.5	38.7	52.8	27.8		
Patients with SSs	1001	9.2	41.7	49.1	30.0	0.13	1.1 (0.97–1.3)
Limited SSs	630	8.4	42.7	48.9	29.8	0.23	1.1 (0.9–1.3)
Diffuse SSs	312	10.9	40.7	48.4	31.3	0.10	1.18 (0.97–1.4)
Autoantibodies							
Anti-centromere	363	10.5	40.8	48.7	30.9	0.12	1.16 (0.96–1.4)
Anti-topoisomerase I	248	8.5	47.2	44.3	32.1	0.06	1.23 (0.99–1.5)
US Caucasian							
Controls	574	8.7	37.5	53.8	27.4		
Patients with SSs	1029	8.0	43.9	48.1	29.9	0.14	1.13 (0.96–1.3)
Limited SSs	591	8.5	43.7	47.9	30.3	0.13	1.15 (0.96–1.4)
Diffuse SSs	384	7.0	45.1	47.9	29.6	0.31	1.11 (0.9–1.4)
Autoantibodies							
Anti-centromere	290	9.7	42.4	47.9	30.9	0.14	1.18 (0.9–1.5)
Anti-topoisomerase I	170	5.3	50.6	44.1	30.6	0.26	1.17 (0.9–1.5)

Table 3Distribution of *ITGAX* rs11574637 in patients with SSc and healthy controls.

	No.	Genotype, %		G Allele, %	Allelic Association	
		GG	GT	TT	P	OR (95% CI)
French Caucasian						
Controls	993	3.3	31.1	65.6	18.9	
Patients with SSc	1011	4.0	32.9	63.1	20.4	0.22 1.1 (0.9–1.3)
Limited SSc	636	3.9	33.5	62.6	20.7	0.21 1.1 (0.9–1.3)
Diffuse SSc	315	3.8	32.1	64.1	19.8	0.59 1.06 (0.85–1.3)
Autoantibodies						
Anti-centromere	367	4.4	33.2	62.4	21.0	0.22 1.1 (0.9–1.4)
Anti-topoisomerase I	246	4.1	30.9	65.0	19.5	0.75 1.0 (0.8–1.3)
US Caucasian						
Controls	561	3.0	28.0	69.0	17.0	
Patients with SSc	1013	3.9	30.9	65.2	19.4	0.1 1.17 (0.97–1.4)
Limited SSc	584	3.9	31.7	64.4	19.8	0.09 1.2 (0.97–1.5)
Diffuse SSc	377	4.2	30.0	65.8	19.2	0.22 1.16 (0.9–1.5)
Autoantibodies						
Anti-centromere	287	2.4	32.8	64.8	18.8	0.36 1.13 (0.9–1.5)
Anti-topoisomerase I	166	5.4	33.1	61.4	22.0	0.039 1.37 (1.0–1.9)

Table 4

Distribution of *CD58* rs12044852 in patients with SSc and healthy controls.

	No.	Genotype, %			A Allele, %	P	Allelic Association	
		AA	AC	CC		Corrected P_{adj}	OR (95% CI)	
French Caucasian								
Controls	995	1.2	16.5	82.3	9.5			
Patients with SSc	1015	1.0	18.5	80.5	10.3	0.40	NA	1.1 (0.89–1.3)
Limited SSc	640	0.8	19.4	79.8	10.5	0.34	NA	1.1 (0.89–1.4)
Diffuse SSc	316	1.3	17.4	81.3	10.0	0.70	NA	1.1 (0.79–1.4)
Autoantibodies								
Anti-centromere	373	0.6	18.2	81.2	9.7	0.87	NA	1.0 (0.77–1.4)
Anti-topoisomerase I	247	2.0	17.8	80.2	10.9	0.32	NA	1.2 (0.85–1.6)
US Caucasian								
Controls	691	1.6	20.1	78.3	23.3			
Patients with SSc	1038	0.9	16.6	82.6	18.3	0.017	0.051	0.76 (0.6–0.96)
Limited SSc	600	1.0	17.0	82.0	19.0	0.08	0.8	0.80 (0.6–1.03)
Diffuse SSc	383	0.5	15.7	83.8	16.7	0.017	0.17	0.69 (0.5–0.95)
Autoantibodies								
Anti-centromere	293	1.0	14.3	84.6	16.4	0.023	0.23	0.68 (0.5–0.96)
Anti-topoisomerase I	172	1.7	14.5	83.7	18.0	0.16	NA	0.75 (0.5–1.1)