Correction

Alizadeh BZ, Broen J, Rueda B, Hesselstrand R, Wuttge D, Simeon C, Ortego-Centeno N, Gonzalez-Gay M, Pros A, Herrick A, Worthington J, Denton C, Fonseca C, Riemekasten G, Vonk MC, Van Den Hoogen F, Guiducci S, Matucci-Cerinic M, Scorza R, Beretta L, Airó P, Coenen M, Martin J, Koeleman BPC, Radstake TRDJ, and EUSTAR Coauthors. Functional variants of Fc gamma receptor (FCGR2A) and FCGR3A are not associated with susceptibility to systemic sclerosis in a large European study (EUSTAR). J Rheumatol 2010 Jun 15 [Epub ahead of print]. The correct name of the eighth author is Miguel A. Gonzalez-Gay. We regret the error.

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Functional Variants of Fc Gamma Receptor (FCGR2A) and FCGR3A Are Not Associated with Susceptibility to Systemic Sclerosis in a Large European Study (EUSTAR)

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ABSTRACT. ***Objective.*** To investigate the possible role of *FCGR2A* 519A>G and *FCGR3A* 559A>C functional polymorphisms in the genetic predisposition to susceptibility to systemic sclerosis (SSc) or clinical phenotype.

Methods. A total of 1566 patients with SSc and 2271 geographically matched controls were included in our study. We analyzed the genotype and allele frequencies of the *FCGR2A* 519A>G and *FCGR3A* 559A>C functional variants in 6 independent European cohorts of white patients with SSc, and white controls. The cohorts comprised 165 Dutch patients with SSc and 1326 controls, 236 Spanish patients with SSc and 257 controls, 267 German patients with SSc and 270 controls, 202 Swedish patients with SSc and 261 controls, 416 Italian patients with SSc and 157 controls, and additionally 280 English patients with SSc. Genotyping was performed using Taqman 5′ allelic discrimination assay. The study reached a 99% power to detect the effect of a polymorphism at an OR of 1.3.

Results. Neither *FCGR2A* 519A>G nor *FCGR3A* 559A>C was significantly associated with susceptibility to SSc. We did not find an association with specific disease phenotypes, limited or diffuse cutaneous involvement, autoantibody profiles, or pulmonary involvement.

Conclusion. Our study strongly suggests the lack of a role for the *FCGR2A* 519A>G and *FCGR3A* 559A>C polymorphisms in SSc susceptibility or clinical phenotype in 6 independent European cohorts. (First Release June 15 2010; J Rheumatol 2010;37:1673–9; doi:10.3899/jrheum.091259)

Key Indexing Terms:
FC GAMMA RECEPTORS SYSTEMIC SCLEROSIS GENETICS ASSOCIATION STUDY

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Systemic sclerosis (SSc) is a systemic connective tissue disorder characterized by fibrosis of the skin and visceral organs. SSc is a complex autoimmune disease whose pathogenic hallmarks are endothelial cell death and immune aberrations including the presence of activated T cells and antibody production by B cells. The etiology of SSc remains obscure, but the disease is likely to result from the interplay of genetic and environmental factors.

In SSc, many cell types have been proposed as key players in the complex network of proinflammatory mediators. For instance, fibroblasts are involved in the production of the extracellular matrix and the fibrosis-inducing cytokine transforming growth factor ß (TGF-ß), while antigen-presenting cells (APC) such as dendritic cells (DC), macrophages, and B cells have been demonstrated to support the profibrotic environment and autoantibody production. Further, the potential role of APC in SSc has been suggested by several observations. First, APC infiltration is one of the early features of skin pathology in patients with SSc[1,2]. APC accumulate perivascularly, where they become activated and produce a wide array of chemotactic factors, which results in the chemoattraction of many other immune effector cells, including monocytes, DC, T cells, and fibroblasts. Second, the degree of mononuclear cell infiltration in the skin of patients with SSc correlates well with both the degree and progression of skin thickening[3].

How APC become activated is unknown but several mechanisms have been proposed, including binding of Fc gamma receptors (FcγR). In men, FcγR can be divided into 3 classes. The FcγRI is a high-affinity receptor that mainly binds monomeric IgG, while FcγRII and FcγRIII interact preferentially with immune complexes[4,5,6,7]. Following ligand binding, FcγRI, FcγRIIa, and FcγRIII are activating receptors while FcγRIIb is the only inhibitory receptor.

Several studies have shown that FcγR triggering by immune complexes determines APC phenotype and behavior in numerous autoimmune conditions as well as in tumor immunity[8,9,10,11]. Genetic studies have focused on these receptors and have associated the functional variant in FcγR genes with several autoimmune diseases, demonstrating a role in susceptibility or clinical phenotype of rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), vasculitis, and Sjögren's syndrome[12,13]. Two polymorphisms are of special interest: *FCGR2A* 519A>G and *FCGR3A* 559A>C. They pose a change in amino acids, subsequently affecting the binding affinity of the *FCGR2A* and *FCGR3A*[7]. This might have implications in SSc pathology. Both high-affinity alleles may increase capture of IgG opsonized pathogens or IgG immune complexes and process them directly into the antigen-processing pathway, which results in more efficient presentation of self-antigens. On the other hand, the low-affinity alleles bind fewer immune complexes and could therefore reduce inflammatory response. In addition, *FCGR2A* is able to respond to surface-bound IgG by enhancing leukocyte attachment. In SSc, IgG against endothelial cells is present; subjects carrying the high-affinity allele of the *FCGR2A* would be at more risk for a sustained inflammation that follows enhanced leukocyte adhesion[14,15]. On the basis of the functional evidence for involvement of FcγR in multiple autoimmune diseases and a possible role in SSc pathology, we investigated whether the functional variants of the activating *FCGR2A* and *FCGR3A* genes are involved in SSc disease susceptibility and/or clinical phenotype, using a large cohort of patients with SSc collected within the EULAR (European League Against Rheumatism) Scleroderma Trials and Research consortium (EUSTAR).

MATERIALS AND METHODS

Study population. We performed our study within the framework of a large cohort of patients with SSc (EUSTAR). The local ethical committee from each center approved our study. Both patients and controls were included in our study after providing written informed consent. Our study population was composed of a total of 1566 white patients from 6 independent cohorts of European ancestry and 2271 ethnically matched controls (unrelated healthy individuals recruited in the same region as the patients with SSc). The Dutch cohort had 165 patients with SSc and 1326 controls; the Spanish, 236 patients and 257 controls; the German, 267 patients and 270 controls; the British, 280 patients; the Italian, 416 patients and 157 controls; and the Swedish, 202 patients and 261 controls. For the UK cohort, we did not have control data available. All the patients fulfilled the 1980 American College of Rheumatology classification criteria for SSc[16].

Clinical characterization of patients. All patients included in our study were classified as having limited or diffuse SSc. When patients with SSc had cutaneous involvement distal to elbows, knees, and clavicles, they fulfilled definitions for limited scleroderma[17]. Those patients with SSc with proximal cutaneous changes were classified as having diffuse SSc[18]. Data regarding selective autoantibody status were not available in all patients with SSc. A total of 983 patients were assessed for the presence of anti-topoisomerase (ATA) I (anti-Scl-70) antibodies and 902 for anticentromere antibodies (ACA). Involvement of the lungs was assessed in 750 patients with SSc according to the international guidelines[19]. The presence of pulmonary fibrosis was investigated by a computed tomography scan. Restrictive syndrome and diffusion capacity of the lungs was defined as a forced vital capacity < 75% of the predicted value and a diffusion capacity of the lung for carbon monoxide < 75% of predicted value.

FcGR genotyping. *FCGR2A* has 2 isoforms, which are encoded by a G to A substitution at nucleotide 519 of the *FCGR2A* (*FCGR2A* 519A>G; National Center for Biotechnology Information single-nucleotide polymorphism (SNP) identification number rs1801274)[20]. The *FCGR2A* 519G allele encodes high-binding allele to IgG2 with a histidine at position 131 in the protein, while the *FCGR2A* 519A encodes the low-binding isoform where the histidine is replaced by an arginine[20]. The *FCGR3A* 559A>C polymorphism (rs396991) results in the expression of 2 receptor isoforms, i.e., an isoform with valine or phenylalanine at position 158 in the protein. The *FCGR3A* 559C allele encodes the valine isoform (i.e., V158 isoform) that is a high-binding allele to IgG1 and IgG3, while the *FCGR3A* 559A allele encodes the 158 low-binding phenylalanine isoform (i.e., F158)[21,22]. Genotyping was performed using Taqman SNP genotyping assays (Applied

Biosystems, Foster City, CA, USA) for *FCGR2A* 519A>G (ABI assay identification number C_9077561_20) and for *FCGR3A* 559A>C (C_25815666_10). All assays were performed according to the manufacturer's protocol.

Data analysis. Genotype and allele frequencies were calculated by direct counting and were tested for Hardy-Weinberg equilibrium in each case-control set by using the FINETI program (http://ihg.gsf.de/cgi-bin/hw/hwa2.pl). Because a large proportion of previous studies and meta-analyses showed an association between *FCGR2A* 519GG and *FCGR3A* 559CC genotypes and susceptibility to different autoimmune diseases, we decided *a priori* to specifically compare the frequency of *FCGR2A* 519GG and *FCGR3A* 559CC genotypes of controls and patients[23,24], using the chi-squared test. We used the Mantel-Haenszel (MH) test to estimate strata-weighted chi-squared test in a pooled analysis of all subjects and to calculate pooled OR and the corresponding 95% CI. Homogeneity of OR were tested using Breslow-Day and Woolf Q methods. When there was a significant heterogeneity we applied random effects using the DerSimonian-Laird test to calculate the confidence limit for pooled OR. Regression analysis was used to estimate the age-adjusted effect of *FCGR2A* 519A>G and *FCGR3A* 559A>C alleles on SSc or its clinical phenotypes, while controlling for population differences. Data analysis was performed using SPSS version 15.0.

Estimation of the power of our study was performed using the Quanto v 0.5 software (Department of Preventive Medicine, University of Southern California, Los Angeles, CA, USA). For the pooled analysis of SSc 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. Under the same conditions, estimation of the power for the pooled analysis of SSc clinical analysis was 93% for limited cutaneous SSc (lcSSc; n = 1108), 73% for diffuse cutaneous SSc (n = 458), 77% for ACA (n = 1219), and 61% for ATA (n = 347).

RESULTS

General findings. In the whole cohort, comprising 1566 patients with SSc, 82.7% were women, which was similar across the different study populations (Table 1). The age of disease onset in patients with SSc was also comparable across different study cohorts. Similarly, the distribution of disease duration was comparable among the study cohorts, except for the Swedish cohort, who had shorter disease duration than the other cohorts. The Swedish cohort contained the most patients with lcSSc compared to the other study cohorts. The Swedish cohort also had the highest frequency of autoantibodies and pulmonary involvement compared to the other cohorts.

FCGR2A variant does not confer risk to SSc susceptibility. Overall, the frequency of the FCGR2A genotype distribution was quite similar among most populations investigated, both in controls and in patients with SSc (Table 2). The frequency of the *FCGR2A* 519AA genotype was clearly lower in the Swedish control and SSc populations compared to all other populations (p = 0.005 in SSc and p = 0.0021 in controls). However, for all 6 populations investigated, the *FCGR2A* 519GG genotype was equally distributed among patients with SSc and controls in the Dutch (p = 0.38), Spanish (p = 0.07), German (p = 0.28), Swedish (p = 0.38), and Italian (p = 0.57) populations (Table 2). Pooled analysis also did not reveal a difference in the frequency of *FCGR2A* alleles between patients with SSc and controls. Also, there was no difference in the frequency of the *FCGR2A* variant in patients with SSc and controls in a pooled analysis of the cohorts using the MH test (p = 0.45; Figure 1).

The FCGR3A variant does not confer risk to SSc susceptibility. For the *FCGR3A* polymorphism, no significant difference was observed in the frequency of *FCGR3A* polymorphism genotype between patients with SSc and controls in the Dutch (p = 0.83), Spanish (p = 0.90), German (p = 0.93), Swedish (p = 0.45), and Italian (p = 0.92) populations (Table 2). This finding persisted when we performed a pooled analysis of all the study populations (MH chi-squared = 0.22; p = 0.62; Table 2). Also, there was no difference in the frequency of the *FCGR3A* 559CC genotype in patients with SSc and controls in the Dutch (p = 0.50), Spanish (p = 0.92), German (p = 0.78), Swedish (p = 0.21), Italian (p = 0.77), or English (p = 0.63) populations, or when data were pooled together (MH chi-squared = 0.001; p = 0.99). Similarly, we found no difference in the

Table 1. Basic and clinical characteristics of the 6 European SSc cohorts.

Characteristic	The Netherlands	Spain	Germany	Sweden	Italy	United Kingdom
Patients with SSc, n	165	236	267	202	416	280
Female, %	71.7	83.8	87.4	76.9	95.5	81.3
Age, yrs, SD	56 (13)	58 (13)	56 (12)	56 (15)	55 (13)	59 (12)
Disease duration, mo (SD)	133 (87)	144 (90)	110 (109)	81 (73)	141 (138)	154 (91)
Limited phenotype, %	77.5	70	52.5	82.1	70.7	74.6
Positive ANA, (%)	97.1	—	94.4	83.1	74.8	78.4
Positive Scl70, (%)	20.3	18.4	24.7	23.4	46.9	6.3
Positive ACA, (%)	26.8	46.7	40.4	46.8	48.1	38.5
Pulmonary fibrosis CT scan, %	30.4	30.7	36.2	44.9	38	43
Low FVC (< 75% predicted), %	24.3	29.1	18.1	19.2	13.2	30
Low DLCO (< 75% predicted), %	36.5	45.1	50.8	35.9	78	11.4

ANA: antinuclear antibodies; Scl70: antitopoisomerase antibodies; ACA: anticentromere antibodies; FVC: forced vital capacity; DLCO: diffusion capacity of the lung for carbon monoxide; SSc: systemic sclerosis; CT: computed tomography.

Table 2. Association analysis of the *FCGR2A* and *FCGR3A* genotypes and alleles with SSc in 6 European populations.

Nationality		*FCGR2A* Controls	*FCGR2A* SSc	p		*FCGR3A* Controls	*FCGR3A* SSc	p
Dutch	n	1324	157		n	1326	157	
	AA	0.29	0.29	0.38	AA	0.4	0.43	0.83
	AG	0.49	0.44		AC	0.46	0.45	
	GG	0.23	0.27		CC	0.14	0.12	
Spanish	n	251	231		n	253	229	
	AA	0.25	0.35	0.07	AA	0.41	0.43	0.90
	AG	0.49	0.42		AC	0.47	0.45	
	GG	0.26	0.23		CC	0.12	0.12	
German	n	261	231		n	270	224	
	AA	0.23	0.35	0.28	AA	0.419	0.433	0.93
	AG	0.48	0.42		AC	0.448	0.442	
	GG	0.29	0.23		CC	0.135	0.123	
Sweden	n	149	195		n	271	194	
	AA	0.18	0.19	0.38	AA	0.47	0.46	0.45
	AG	0.55	0.6		AC	0.46	0.44	
	GG	0.27	0.21		CC	0.07	0.1	
Italian	n	157	385		n	150	395	
	AA	0.35	0.34	0.57	AA	0.29	0.29	0.92
	AG	0.51	0.48		AC	0.48	0.5	
	GG	0.14	0.18		CC	0.23	0.22	
British	n	—	236		n	—	247	
	AA	—	0.19	—	AA	—	0.43	—
	AG	—	0.53		AC	—	0.45	
	GG	—	0.28		CC	—	0.12	

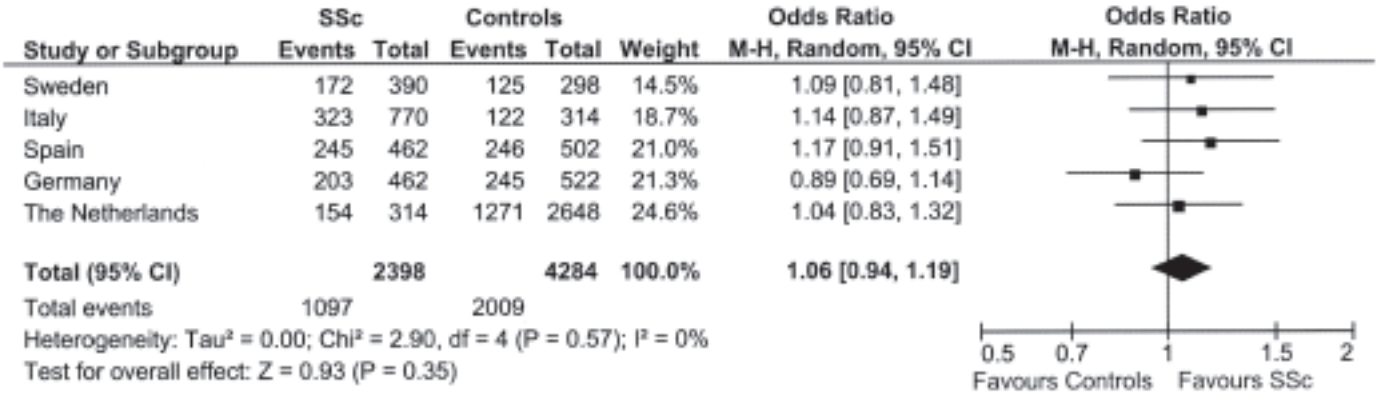

Figure 1. Effect of the FCγRIIa minor allele (G) in 5 European populations, comparing healthy controls with patients with SSc, using the Mantel-Haenszel test for overall effect under random effects.

frequency of *FCGR3A* 559C allele in patients with SSc and controls in the Dutch (p = 0.36), Spanish (p = 0.75), German (p = 0.85), Swedish (p = 0.44), and Italian (p = 0.95) populations. Pooling data yielded similar results (MH p = 0.38; Figure 2). Although there were no controls available from the UK, the distribution of both the genotypes as well as alleles in the patients with SSc was similar compared to that observed in the Dutch, Swedish, Spanish, and German populations, suggesting that a role for this genotype in SSc susceptibility is unlikely.

SSc phenotype is not associated with FCGR2A or FCGR3A genotype. Several reports indicate that FcγR are involved in disease severity rather than susceptibility[25,26]. We tested this hypothesis and found no association of either *FCGR2A* or *FCGR3A* genotype with the patients' clinical characteristics, including the extent of skin involvement (Table 3, Table 4), the presence of autoantibodies, and pulmonary involvement (data not shown).

DISCUSSION

By studying one of the largest SSc cohorts, we found no association between the functional variants in *FCGR2A* and

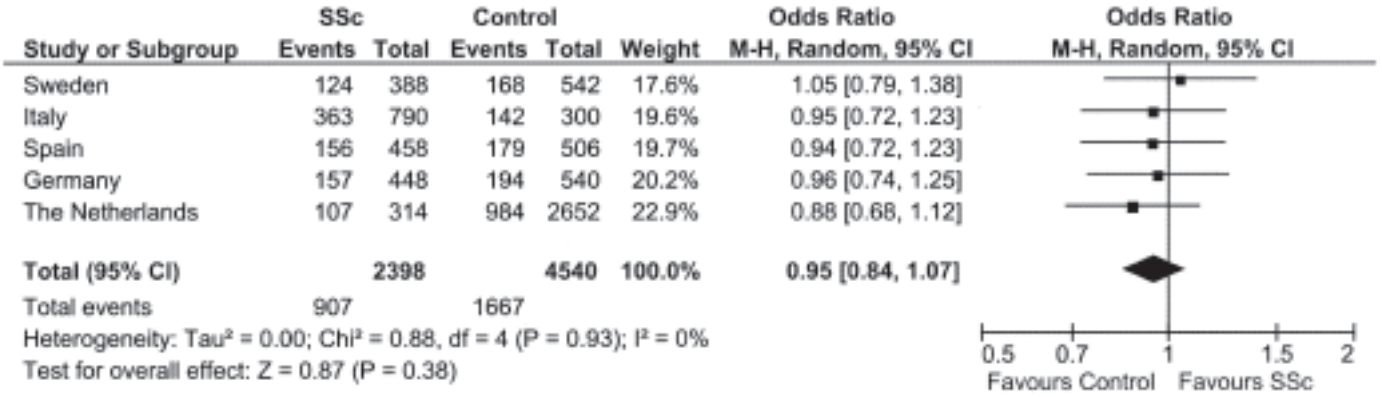

Figure 2. Effect of the FCγRIIIa minor allele (C) in 5 European populations, comparing healthy controls with patients with SSc, using the Mantel-Haenszel test for overall effect under random effects.

Table 3. Genotype frequencies of *FCGR2A* polymorphisms in 6 European SSc cohorts, comparing limited with diffuse cutaneous phenotypes.

Population	Phenotype	Genotype Frequencies AA	AG	GG	p
Spanish	Limited, n = 133	0.248	0.459	0.293	
	Diffuse, n = 59	0.203	0.492	0.305	0.794
Dutch	Limited, n = 101	0.277	0.475	0.248	
	Diffuse, n = 30	0.167	0.466	0.367	0.313
Swedish	Limited, n = 140	0.207	0.586	0.207	
	Diffuse, n = 55	0.200	0.636	0.164	0.775
German	Limited, n = 103	0.359	0.369	0.272	
	Diffuse, n = 88	0.341	0.443	0.216	0.523
Italian	Limited, n = 227	0.335	0.476	0.189	
	Diffuse, n = 93	0.430	0.419	0.151	0.263
British	Limited, n = 170	0.182	0.524	0.294	
	Diffuse, n = 59	0.203	0.576	0.220	0.55

Table 4. Genotype and allele frequencies of *FCGR3A* polymorphisms in 6 European SSc cohorts, comparing limited with diffuse phenotypes.

Population	Phenotype	Genotype Frequencies AA	AC	CC	p
Spanish	Limited, n = 132	0.416	0.47	0.114	
	Diffuse, n = 57	0.474	0.421	0.105	0.767
Dutch	Limited, n = 101	0.386	0.465	0.149	
	Diffuse, n = 29	0.586	0.379	0.034	0.089
Swedish	Limited, n = 141	0.468	0.404	0.128	
	Diffuse, n = 53	0.434	0.528	0.038	0.107
German	Limited, n = 97	0.464	0.423	0.113	
	Diffuse, n = 88	0.466	0.409	0.125	0.964
Italian	Limited, n = 232	0.302	0.509	0.189	
	Diffuse, n = 98	0.245	0.449	0.306	0.066
British	Limited, n = 179	0.447	0.447	0.106	
	Diffuse, n = 61	0.410	0.475	0.115	0.88

FCGR3A genes and SSc susceptibility or clinical characteristics in 6 populations throughout Europe. The role of *FCGR* genes has been thoroughly investigated globally in many diseases with often inconsistent results, which can be attributed to fairly small study populations.

Several lines of evidence suggest a role for FCGR polymorphisms in the pathogenesis of SSc. Boros, *et al* described the presence of anti-FcγR antibodies in sera from tight-skin mice and patients with SSc that suggests a dysfunction of the macrophage phagocytic system and inappro-

priate stimulation of FcγR-bearing cells as one of the pathogenic mechanisms in SSc[27,28,29]. Moreover, SSc-associated autoantibodies bind to FcγR, thereby influencing the outcome of the immune response. For instance, ATA and ACA are IgG antibodies and are thought to form immune complexes in SSc, thus potentially crosslinking FcγR and inducing cell signaling[27,28,29]. More recently, SSc-associated antibodies against platelet-derived growth factor receptor have been found to mediate enhanced leukocyte function by activating FcγR[30]. In addition, it was demonstrated that FcγRIIa functions in concert with Toll-like receptor (TLR) 9 to take up immune complexes and stimulate plasmacytoid DC in SLE. Because DC were found to be activated by SSc serum, a phenomenon that was dependent upon the presence of TLR9, it is tempting to speculate that FcγRIIa is involved in SSc[31].

We found no association of the 2 polymorphisms *FCGR2A* and *FCGR3A* in SSc. Several biases could mask an existing significant relationship between a genotype and a disease in association studies. However, it less likely that these biases played a role in our study. First, all samples were genotyped in 1 center, which lowers the chance of false-positive associations. Further, pooled analysis of the study cohorts provided sufficient power to detect a mild risk increase of 1.2 for any of the tested genotypes and SSc. And finally, patients were carefully characterized for both subjective and objective clinical features, each of which was further analyzed in relation to *FCGR2A* or *FCGR3A* genotypes. Therefore a role for bias due to heterogeneity among the patient groups is unlikely to cause a null finding. Despite various efforts, no healthy control DNA samples from the UK were available to include in our study. However, the genotype distribution in the English patients with SSc resembled those in the other SSc populations, and was similar to control populations reported in previous studies incorporating healthy controls from the UK, making a contribution of these variants to SSc in this cohort very unlikely[32,33]. We persistently found no association of SSc as a clinical diagnosis or any of its clinical characteristics with the *FCGR2A* or *FCGR3A* genotypes. These results indicate that our findings form a true negative, and suggest that genetic alterations in the *FCGR2A* or *FCGR3A* genes do not play a key role in the immune aberrations observed in SSc.

Although *FCGR2A* and *FCGR3A* have been involved with several other immune diseases, our study suggests that the role of the 2 investigated variants in the *FCGR2A* and *FCGR3A* might be limited in SSc. Several explanations might be put forward to explain this. From a genetic point of view, we performed a direct testing strategy and examined only 2 common functional variants with probably a relatively low effect size on a relatively rare disease in the population. It might be that other variants in the *FCGR2A* and *FCGR3A* do play roles in SSc. On the other hand, it is still not clear to what extent common variants can explain SSc. To date, no genome-wide association study has been performed for SSc. Genome-wide association studies in other complex disorders such as RA and diabetes do indicate that common variants play a role in them. The upcoming genome-wide association studies in SSc might put more light on this challenging dilemma. We focused on the functional variants in only 2 *FCGR* genes. However, there are other *FCGR* genes and other types of genetic variations (e.g., copy number variation) that also cluster on chromosome 1q21-q24, and are associated with several autoimmune diseases. Interestingly, associations between copy number variations of *FCGR2B* and lupus nephritis, SLE, and Wegener's granulomatosis have been reported[34,35]; we did not investigate these. Therefore, it remains unclear whether other types of genetic variations in the *FCGR* genes can affect susceptibility to SSc. From an immunological viewpoint, FcγRIIa and FcγRIIIa are only part of a very complex family of multiple FcγR subtypes. For instance, there is still debate about the exact contribution of FcγRIIIb in immune processes that recently gained even more interest after the identification of a copy number variant in this gene that was highly associated with SLE and RA[36]. In addition, the inhibitory FcγRIIb has been shown to play a crucial role in many diseases. Finally, FcγRIIc was recently identified as a single and independent entity. These observations indicate that the absence of association between *FCGR2A* and *FCGR3A* with SSc justifies further research into the role of *FCGR3B*, *2B*, and *2C*, and possible interactions between these receptors in SSc.

The *FCGR2A* and *FCGR3A* genes are not associated with SSc susceptibility and/or clinical phenotype. The rationale for the role of FcγR in SSc warrants further investigation into the potential role of other FcγR subtypes in this condition.

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APPENDIX. Other collaborators.

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