Letters to the Editors

No evidence for association between the *CCR5/Delta32CCR5* polymorphism and systemic sclerosis

Sirs,

Systemic sclerosis (SSc), also known as scleroderma, is a complex polygenic autoimmune disease (AID) of unknown aetiology characterised by extensive fibrosis, fibroproliferative vasculopathy and presence of auto-antibodies against nuclear selfantigens. It is known that genetic factors play an important role in the susceptibility and clinical features of this AID, especially those related to the immune response (1). A 32-base pair deletion mutation in the CC chemokine receptor 5 gene (CCR5), known as $\triangle 32CCR5$, produces a non-functional receptor by modifying the second extracellular loop of the protein. A protective effect of this mutant variant on the development and progression of different autoimmune disorders has been proposed (2, 3). To explore the possible role of $\triangle 32CCR5$ in the pathogenesis and clinical phenotype of scleroderma, a total of 844 Spanish Caucasian SSc patients, who met the 1980 American College of Rheumatology (ACR) classification criteria for this disease (4), were included in the study. Approval from the local ethical committees and written informed consent from all participants were obtained. To perform the phenotype analysis, patients were further subdivided into limited cutaneous SSc (lcSSc) and diffuse cutaneous SSc (dcSSc) subgroups, and by autoantibody profile based on the presence of anti-topoisomerase (ATA) and anti-centromere (ACA) autoantibodies. Measurement of autoantibodies was performed using standard procedures. Clinical features of the patients have been described before (5). The data from the control population was obtained from (3), since this set matched geographically and ethnically our SSc cohort. Polymerase chain reaction (PCR) was performed to genotype samples using a couple of primers that span the 32-base pair deletion in the *CCR5* gene (GenBank Accession#: AF009962): 5'-CAAAAA-GAAGGTCTTCATTACACC-3' (sense), and 5'-CCTGTGCCTCTTCTTCTCATT-TCG-3' (antisense). The size of the amplified wild-type and deleted DNA fragments was 189 bp and 157 bp, respectively. The statistical power of the global analy-

sis was 90% to detect associations with OR=1.5 at the 5% statistical level, according to Power Calculator for Genetic Studies 2006 software (http://www.sph.umich.edu/csg/abecasis/CaTS/). However, it should be noted that this study is underpowered to detect lower ORs, and this may be considered a limitation. No significant differences were observed between the $\Delta 32CCR5$ allele frequencies of the different case sets and that of the control population, as well as when subgroups were compared one to another (Table I).

Abnormal chemokine and chemokine receptor expressions, which lead to an excessive infiltration of leukocytes, seem to play key roles in the fibrosis and vascular alterations of SSc patients (6). This study represents the first attempt to evaluate the possible implication of the functional variant $\Delta 32CCR5$ of the chemokine receptor CCR5 in the pathophysiology of SSc up to now. This receptor is highly expressed on the surface of T lymphocytes, and plays an important role during the recruitment of this cell type towards inflammation regions (7). Although some evidences about a possible involvement of the $\triangle 32CCR5$ mutant allele in autoimmunity have been reported (2, 3), recent studies demonstrate a lack of association of this CCR5 variant with several AIDs, including systemic lupus erythematosus, rheumatoid arthritis, inflammatory bowel disease, and type 1 diabetes (8-10). Specially controversial are the results published in relation to SLE and RA (2). Our data show no significant association of the CCR5/A32CCR5 polymorphism with the susceptibility and clinical manifestations of scleroderma, in spite of the well-defined and large-sized SSc cohort that we have included in the analysis. In any case, additional

studies are needed to draw firm conclusions about the exact role of the *CCR5/32CCR5* polymorphism in the susceptibility and clinical spectrum of autoimmunity.

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Table I. Distribution of the $CCR5/\Delta 32CCR5$ polymorphism in SSc patients and the phenotypic subgroups compared to healthy controls, and accordingly with presence or absence of specific features of the disease.

	Genotype, n. (%)			Allele (%)		Cases vs. Controls		Subgroup comparisons	
	CCR5/CCR5	CCR5/Δ32	Δ32/Δ32	CCR5	Δ32	p-value	OR [CI 95%]*	<i>p</i> -value	OR [CI 95%]*
Controls (n=815)**	707 (86.75)	101 (12.39)	7 (0.86)	92.94	7.06				
SSc (n=844)	738 (87.44)	103 (12.20)	3 (0.36)	93.54	6.46	0.4926	0.91 [0.69-1.19]		
lcSSc (n=457)	403 (88.18)	53 (11.60)	1 (0.22)	93.98	6.02	0.3146	0.85 [0.61-1.18]		
dcSSc (n=231)	194 (83.98)	35 (15.15)	2 (0.87)	91.56	8.44	0.3138	1.23 [0.85-1.80]	0.0923ª	0.69 [0.45-1.06]
ACA+ (n=343)	306 (89.21)	36 (10.50)	1 (0.29)	94.46	5.54	0.1799	0.79 [0.54-1.14]		
ATA+ (n=171)	143 (83.63)	27 (15.79)	1 (0.58)	91.52	8.48	0.3573	1.25 [0.82-1.90]		
ACA- (n=411)	354 (86.13)	55 (13.38)	2 (0.49)	92.82	7.18			0.1966 ^b	0.77 [0.50-1.16]
ATA- (n=579)	515 (88.95)	62 (10.71)	2 (0.35)	94.30	5.70			0.0636°	1.56 [0.99-2.45]

Statcalc software (Epi Info 2002; Centers for Disease Control and Prevention, Atlanta, GA, USA) was used to perform 2x2 contingency tables and χ^2 test and/or Fisher's exact test, when appropriate, and to calculate *p*-values, odds ratios (OR), and 95% confidence intervals (CI). *OR for the $\Delta 32CCR5$ allele. **From Gomez-Reino *et al.* (1999). *IcSSc vs. dcSSc; ^bACA+ vs. ACA-; ^cATA+ vs. ATA-.

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