The role of the *NLRP1* gene in systemic sclerosis: a replication study

Sirs,
Systemic sclerosis (SSc) is an autoimmune disease of the connective tissue characterised by excessive fibrosis of the dermis and vascular damage (1), in which environmental and genetic factors are involved in its susceptibility. Despite the fact that the whole genetic component of SSc remains unknown, in the last decade several genes have been described to influence SSc predisposition and main clinical characteristics (2). In this regard, the *NLRP1* (NLR family, pyrin domain containing 1) gene, a regulator of the innate response, has been recently reported as a new SSc susceptibility locus, mainly as a risk factor for SSc-related lung fibrosis and anti-topoisomerase positive SSc phenotypes (3). Given the importance to validate the association report through independent replication studies to establish new SSc susceptibility loci, we sought to replicate the *NLRP1* rs8182352 variant in an independent large European population.

A total of 2,000 SSc patients and 3,802 controls from four European populations (Spain, The Netherlands, Germany and Italy) were included. All the patients fulfilled the classification criteria for SSc by LeRoy *et al.* (4). The main features of all the populations have been described previously (5, 6).

Genotype frequencies were in HWE in cases and controls. No significant differences were detected between rs8182352 and SSc susceptibility or SSc-related subphenotypes (Table I). A combined meta-analysis was subsequently performed including the previous results reported by Dieudé *et al.* (3), comprising a total of 3,929 SSc patients and 5,731 controls. Similarly, no significant differences between the allele frequencies of cases and controls were observed in the pooled analysis (P_{Pooled} =0.55 OR=1.02, 95% CI= 0.96 to 1.08, P_{BD}= 0.23).

In addition, we analysed whether rs8182352 was a susceptibility locus for ATA production and the development of fibrosing alveolitis, as described previously (3). Our results showed heterogeneous differences between populations in both comparisons; hence, the combined OR was calculated under random effects. However, the overall allelic distribution in the combined analysis revealed no evidence of association with the presence of ATA and lung fibrosis (Table I). Additionally, we compared SSc patients positive for ATA or lung fibrosis with those patients without these clinical features. The meta-analysis including the French population showed no evidence of association for ATA under random model (P_{Pooled} =0.535, OR=1.08 95% CI=0.85-1.36, P_{BD}=0.01) and for fibrosis alveolitis under fixed model (P_{Pooled}=0.940, OR=0.99 95% CI=0.88-1.12, P_{BD}=0.212).

Table I. Combined analyses of the NLRP1 rs8182352 variant.

Sample sets	Statistical Power[‡]	T/T (%)	T/C (%)	C/C (%)	C (%)	*p*-value**	OR [95% CI]**	P_BD[††]
a) Meta-analysis of the four European populations* †								
SSc (n=2,000)	100	28.7	48.0	23.4	47.3	0.684	0.98 [0.91–1.06]	0.12
lcSSc (n=1,361)	98	28.1	48.2	23.7	47.8	0.983	1.00 [0.92–1.09]	0.15
dcSSc (n=631)	85	30.1	47.1	22.8	46.4	0.402	0.95 [0.84–1.07]	0.52
ACA + (n=784)	91	28.7	47.8	23.5	47.4	0.726	0.98 [0.88–1.09]	0.28
ATA + (n=524)	79	30.9	45.4	23.7	46.4	0.353	0.94 [0.83–1.07]	0.20
PF + (n=569)	82	29.3	49.6	21.1	49.9	0.258	0.93 [0.82–1.06]	0.15
Controls (n=3,802)		27.5	49.4	23.1	47.8			
b) Meta-analysis including the previous study from Dieudé *et al.* (3)								
SSc (n=3,929)	100	28.0	49.8	22.2	47.1	0.549	1.02 [0.96–1.08]	0.23
ATA + (n=1,059)	97	26.6	49.9	23.4	48.4	0.345	1.07 [0.90–1.27]	0.01
PF + (n=1,243)	98	27.1	49.9	22.9	47.9	0.361	1.07 [0.93–1.22]	0.04
Controls (n=5,731)		28.1	49.8	22.1	47.0			

All comparisons are against controls.
*Independent analysis in each population is showed in Supplementary Table I.
†All the patients fulfilled the classification criteria for SSc by LeRoy *et al.* (4). ACAs were determined by their characteristic distinctive pattern on HEP 2 cells, while ATAs was detected by passive immunodiffusion against calf thymus extract (Inova Diagnostics, Davis, California, USA). Pulmonary fibrosis was diagnosed by High Resolution Computed Tomography (HRCT).
‡ The estimation of the power was calculated with OR of 1.2 at the 5% significance level, assuming a SSc prevalence of 0.01% and considering a minor allele frequency (MAF) of 0.48.
**Combined ORs were calculated according to a fixed-effects model (Mantel-Haenszel meta-analysis) or random effects (DerSimonian-Laird meta-analysis), when necessary. *p*-values lower than 0.05 were considered as statistically significant.
††The homogeneity of OR among all populations was calculated by Breslow-Day test.
SSc: sclerosis systemic; lcSSc: limited cutaneous subtype; dcSSc: diffuse cutaneous subtype; ACA: anti-centromere antibodies; ATA: anti-topoisomerase I antibodies; PF: pulmonary fibrosis; +: positive; OR: odds ratio; CI: confidence intervals; P_BD: *p*-value by Breslow-Day method; HWE: Hardy-Weinberg Equilibrium.

In agreement with previous data (3), our results firmly establish that *NLRP1* gene does not confer risk to SSc or the two major clinical forms of the disease: limited cutaneous (lcSSc) and diffuse cutaneous (dcSSc). In addition, we can not confirm the recent reported association of the *NLRP1* rs8182352 variant with SSc-related fibrosis alveolitis or anti-topoisomerase-positive SSc, which is probably due to the differences in the phenotype measurement, difference in the genotype and population stratification. A homogeneous genetic background is necessary in order to limit the possibility of this bias. However, SSc patients show a high phenotypical heterogeneity between populations (2) and, a new revision of the current classification criteria for SSc would be desirable to better characterise this disease and its major subtypes/specific clinical features (2).

In summary, our results do not support the previously suggested role of the *NLRP1* rs8182352 in the development of specific features of SSc.

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