NLRP1 influences the systemic sclerosis phenotype: a new clue for the contribution of innate immunity in systemic sclerosis-related fibrosing alveolitis pathogenesis

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► Supplementary data are published online only. To view these files please visit the journal online (<http://ard.bmj.com>).

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Accepted 1 November 2010
Published Online First
13 December 2010

ABSTRACT

Background Recent evidence has highlighted a potential role of interleukin 1 β (IL-1 β) in systemic sclerosis (SSc). *NLRP1* provides a scaffold for the assembly of the inflammasome that promotes the processing and maturation of pro-IL-1 β . In addition, *NLRP1* variants were found to confer susceptibility to autoimmune disorders.

Objective To study a possible association of the *NLRP1* rs6502867, rs2670660 and rs8182352, rs12150220 and rs4790797 with SSc in the European Caucasian population.

Methods *NLRP1* single nucleotide polymorphisms were genotyped in 3227 individuals comprising a discovery set (870 SSc patients and 962 controls) and a replication set including individuals from Germany (532 SSc patients and 324 controls) and Italy (527 SSc patients and 301 controls), all individuals being of European Caucasian origin.

Results Conditional analyses revealed a significant association for the *NLRP1* rs8182352 variant with both anti-topoisomerase-positive and SSc-related fibrosing alveolitis (FA) subsets under an additive model: $p=0.0042$, OR 1.23 (95% CI 1.07 to 1.41) and $p=0.0065$ OR 1.19 (95% CI 1.05 to 1.36), respectively. Logistic regression analysis showed an additive effect of *IRF5* rs2004640, *STAT4* rs7574865 and *NLRP1* rs8182352 risk alleles on SSc-related FA.

Conclusions Our results establish *NLRP1* as a new genetic susceptibility factor for SSc-related pulmonary fibrosis and anti-topoisomerase-positive SSc phenotypes. This provides new insights into the pathogenesis of SSc, underlining the potential role of innate immunity in particular in the FA-positive SSc subphenotype, which represents a severe subset of the disease.

Several lines of evidence have recently raised the paradigm of the contribution of innate immune mechanisms to autoimmunity. The concept of innate immunity as a primitive system, being non-specific in most aspects of both recognition and response, has been transformed by the identification of the membrane-associated Toll-like receptors and the cytosol-expressed Nod-like receptors (NLR).^{1,2} The NLR family, pyrin domain containing 1 (*NLRP1*), is a member of the NLR family, which are cytoplasmic proteins that sense endogenous

microbial products and metabolic stresses, thereby stimulating innate immunity. NLR associated with other proteins form multiprotein cytoplasmic complexes, the so-called inflammasomes. *NLRP1* provides a scaffold for the assembly of the inflammasome that activates caspases 1 and 5, which subsequently promote the processing and maturation of the inflammatory cytokines, pro-interleukin 1 β (IL-1 β), IL-18 and IL-33.^{3,4}

Some studies have highlighted a potential role of IL-1 β in fibrotic disorders.^{5,6} In systemic sclerosis (SSc), the prototypic fibrotic connective tissue disorder, IL-1 β has been reported to induce the fibrogenic phenotype of SSc fibroblasts and to increase strikingly in vitro the production of extracellular matrix by these cells.^{7,8} SSc is a chronic autoimmune disease with a complex pathogenesis driven by a combination of genetic risk factors and environmental events that lead to a break in immunological self-tolerance and systemic fibrosis.^{9,10} Two of the SSc susceptibility genes recently identified play a pivotal role in innate immunity: *IRF5* and *STAT4*.^{11–14} In addition, other genes involved in innate immunity have been found to be associated with various autoimmune or auto-inflammatory disorders. *NOD2* have thus been associated with a risk of Crohn's disease.¹⁵ Mutations of *NLRP3* (also known as cryopyrin) were reported to cause three Mendelian dominant inflammatory disorders.¹⁶ Finally, *NLRP1* has been found to confer a risk for extended autoimmune/inflammatory disorders^{17–19} and more recently to autoimmune Addison's disease and type I diabetes.^{20,21}

Taking into consideration: (1) the potential role of IL-1 β in the SSc fibrosing process; (2) the identification of SSc susceptibility genes encoding proteins involved in innate immunity; and (3) common genetic variants shared by different autoimmune diseases, we hypothesised that the previously identified *NLRP1* risk variants may be involved in the susceptibility of SSc.

PATIENTS AND METHODS

Study population and study design

We performed a case-control association study including a replication step. The discovery

sample included 870 SSc patients (83.6% women with a mean age ($\pm$ SD) of 57.2 ± 12.8) and 962 controls coming from the French network (82.8% women with a mean age ($\pm$ SD) of 53.8 ± 9.7).^{11 12 22 23} The replication step included individuals from Germany: 532 SSc patients (84.2% women with a mean age ($\pm$ SD) of 56.1 ± 8.3) and 324 controls (81.8% women with a mean age ($\pm$ SD) of 56.3 ± 7.1), and Italy: 527 SSc patients (86.1% women with a mean age ($\pm$ SD) of 53.6 ± 10.9) and 301 controls (80.2% women with a mean age ($\pm$ SD) of 53.8 ± 9.4). All SSc patients were classified according to LeRoy's cutaneous subtypes²⁴ and phenotypically assessed as recommended.^{25 26} The characteristics of SSc patients are summarised in table 1.

The control groups consisted of healthy unrelated individuals who were ethnically matched to the SSc cases (all individuals were of European Caucasian ancestry). All local institutional review boards approved the study, and written informed consent was obtained from all subjects in the study. SSc patients were tested for antinuclear antibodies using indirect immunofluorescence and HEp-2 cells as antigen substrate (Antibodies, Davis, California, USA). Specific SSc antibodies were systematically assessed; anticentromere antibodies were determined by their distinctive indirect immunofluorescence pattern on HEp-2 cells. Anti-topoisomerase I (Topo) antibodies were determined by counter immunoelectrophoresis. Regarding lung fibrosis, fibrosing alveolitis (FA) was defined as the presence of typical features on chest high-resolution CT, which was collected for all SSc patients.

Genotyping

We selected the following five single nucleotide polymorphisms (SNP) for which convincing association was reported with vitiligo and/or autoimmune diseases: rs6502867, rs2670660 and rs8182352, rs12150220 and rs4790797.^{17 18 20 21} The five *NLRP1* SNP were genotyped using a competitive allele specific PCR system (Kaspar genotyping; KBioscience, Hoddesdon, UK) as previously described.^{11 12 22 23} The average genotype completeness for both *NLRP1* rs2670660, rs6502867, rs8182352, rs12150220 and rs4790797 polymorphisms was 98% for the SSc and the control samples. The accuracy was greater than 99%, according to duplicate genotyping of 10% of all samples using the Taqman SNP genotyping assay-allelic discrimination method (Applied Biosystems, Foster City, California, USA).

Statistical analysis

Statistical analyses were performed using the R computer package software (version 2.6.0). 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 χ^2 test (1 degree of freedom). Many methods for addressing the problem of multiple comparisons invoke a conservative approach, namely a standard Bonferroni correction, which assumes the independence of all tests performed. In many association studies, markers are not independent because they are in linkage disequilibrium (LD), and so a standard Bonferroni correction would be overly conservative.²⁷ Therefore, we applied a correction for multiple testing for all the tests performed for a 'generating hypothesis step' when comparing SSc subgroups and controls (10 phenotypic subsets) in the 'discovery' sample. p Values remaining significant after this adjustment for multiple testing are indicated in the tables and termed ' p_{adj} ' in the text. In the case of a defined hypothesis for any particular SSc phenotype detected in the discovery sample, we thereafter tested it in the relevant SSc samples (German and Italian sets) without correction for multiple testing. All the OR are provided with their 95% CI. Individual association analyses of *NLRP1* SNP with SSc were performed by comparing cases and controls with a Fisher's exact test on genotype distribution. The corresponding OR were assessed using a standard logistic regression analysis with the homozygous genotype for the most frequent allele, in the control population, taken as the reference. The same procedure was applied in subgroups stratified according to SSc phenotypes.

Finally, we investigated whether the *NLRP1* rs8182352 variant was statistically associated with a specific SSc subset. The main association concerned both SSc pulmonary fibrosis and Topo-positive subsets; so we performed multivariate logistic models including *NLRP1* rs8182352, the Topo-positive status and the SSc diffuse cutaneous subset in order to assess the respective independent effect of these three genetic and phenotypic factors on pulmonary fibrosis.

Meta-analysis on *NLRP1* rs8182352

The analysis of the combined data including the three populations was performed: (1) by the calculation of homogeneity of OR among cohorts using Breslow-Day and Woolf Q methods; (2) by calculation of the pooled OR under a fixed-effects model (Mantel-Haenszel meta-analysis) or random effects (DerSimonian-Laird) when necessary assessed by logistic regression analysis genetic effects under three modes of inheritance: additive, dominant and recessive. The meta-analysis was performed using logistic regression analysis adjusted for the three populations investigated.

Power calculation

Power calculations are driven through an asymptotic non-central χ^2 approach as previously described.²⁸ The discovery set of 870 SSc cases and 962 controls provides a power greater than 80% to detect the association between SSc and the *NLRP1* rs8182352 risk allele with an OR of 1.30 at the 5% significance level.

RESULTS

Discovery set

Genotype frequencies were in Hardy-Weinberg equilibrium in the control population for all the *NLRP1* SNP investigated.

Single marker analysis

The case-control association study performed in the discovery sample ($n=870$ SSc patients and 962 controls) did not reveal any association between the five *NLRP1* SNP and the SSc population as a whole. When SSc subphenotypes were queried we observed a significant increase in the frequency of the rare *NLRP1*

Table 1 Characteristics of the SSc patients in the three European populations investigated

	French population (n=870)	German population (n=532)	Italian population (n=527)
Female, %	83.6	84.2	86.1
Mean age, years $\pm$ SD	57.2 ± 12.8	56.1 ± 8.3	53.6 ± 10.9
Mean disease duration, years $\pm$ SD	10.9 ± 8.7	10.7 ± 8.1	11.5 ± 8.9
Limited cutaneous subtype, %	64.5	58.5	69.9
Diffuse cutaneous subtype, %	35.5	34.9	22.4
Anti-Topo-positive patients, %	27.3	30.3	29.9
ACA-positive patients, %	40.3	35.1	42.1
FA on CT scan, %	42.0	29.5	33.0

ACA, anti-centromere antibodies; Anti-Topo, anti-topoisomerase I antibodies; SSc, systemic sclerosis.

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rs8182352 C allele in the Topo-positive SSc subset compared with the controls: $p_{\text{adj}}=0.047$, OR 1.35, 95% CI 1.09 to 1.66). Analysis of the genotype distributions also revealed a strong increase in frequency of the homozygous CC genotype in SSc patients compared with controls: $p_{\text{adj}}=0.038$, OR 1.83, 95% CI 1.21 to 2.76 (table 2). We also observed a strong increase in frequency of both the rs8182352 C risk allele and the homozygous CC genotype in the subset of SSc patients having FA, however, it did not reach statistical significance (table 2).

Regarding the *NLRP1* rs2670660 SNP, we observed an increased frequency of the rare allele in both Topo-positive and FA-positive SSc subsets, however, not reaching statistical significance after correction for multiple testing. In agreement with the previously reported LD analysis, *NLRP1* rs2670660 and rs8182352 were found to be in relatively high LD ($r^2=0.71$). Conditional analyses between rs2670660 and rs8182352 revealed a significant association restricted to the *NLRP1* rs8182352 risk allele ($p=0.014$) (table 2 and supplementary file 1, available online only).

No association between SSc and the *NLRP1* rs6502867, rs12150220 and rs4790797 SNP was detected with any of the subphenotypes investigated (supplementary file 1, available online only). In agreement with the previously reported LD analysis, *NLRP1* rs2670660 and rs8182352 were found to be in LD ($r^2=0.709$), whereas rs6502867 was not in LD with both rs2670660 and rs8182352 ($r^2=0.057$ and 0.056 , respectively) (figure 1). The pairwise correlation coefficient found rs12150220 in LD with rs2670660 ($r^2=0.59$) and rs8182352 ($r^2=0.56$), whereas rs6502867 and rs4790797 were found to belong to distinct LD blocks (supplementary figure 1, available online only).

Replication set

Following the results obtained in the discovery set, we selected the *NLRP1* rs8182352 SNP to be investigated in Topo-positive SSc subset. Genotype frequencies of rs8182352 were in Hardy–Weinberg equilibrium in the control population. No association was detected in the German population. Conversely, the association between *NLRP1* rs8182352 and the Topo-positive SSc subset was replicated in the Italian population (table 3). Taking into account the striking differences in frequency of the *NLRP1* rs8182352 risk allele when comparing SSc FA-positive with the controls and an elevated OR (1.41 for the homozygous risk genotype) for a given complex disease, although not reaching significance, we decided to follow this association in the replication sample. We assume that the lack of significance in the first set relates to an insufficient statistical power, and considering the critical weight of FA on SSc morbidity and mortality, was another argument to follow the association. Interestingly, as observed in the French population we detected a strong increase in frequency of the risk allele in the FA-positive SSc subset in both German and Italian populations; however, not reaching statistical significance (table 3).

Meta-analysis

The combinability test according to the Breslow–Day method showed no significant differences among them. Therefore, we performed a meta-analysis using the Mantel–Haenszel test under fixed effects.

The *NLRP1* rs8182352 SNP was found to confer susceptibility to both Topo-positive SSc and SSc-related FA, under an additive model: pooled OR 1.23 (95% CI 1.07 to 1.41) and 1.19 (95% CI 1.05 to 1.35), respectively. These results are detailed in table 4 shown in figure 1 and supplementary data, available online only.

Intra-cohort analyses found that the *NLRP1* rs8182352 C allele and CC genotype frequencies were significantly increased in Topo-positive SSc compared with Topo-negative SSc: 50.4% versus 46.3%, $p=0.025$ and 24.8% versus 21.6%, $p=0.027$, respectively. In addition, both the C allele and CC genotype were increased in FA-positive compared with FA-negative SSc: 49.7% versus 46.2%, $p=0.046$ and 25.4% versus 20.9%, $p=0.041$, respectively. As FA is strongly linked to the presence of anti-topoisomerase,²⁹ we next performed multivariate logistic regression analysis including *NLRP1* rs8182352 and Topo and FA status in order to assess the respective independent effect of *NLRP1* rs8182352 on pulmonary fibrosis and/or Topo-positive subsets. Regarding the association of *NLRP1* rs8182352 with the FA adjusted for Topo status and with Topo adjusted for FA, no association was detected: OR 1.14 (95% CI 0.97 to 1.34), $p=0.11$ and OR 1.13 (95% CI 0.95 to 1.34), $p=0.17$, respectively. Multivariate logistic regression analysis adjusted for the dcSSc status found similar results (data not shown). Therefore, *NLRP1* contribute to both Topo-positive and FA-positive SSc phenotypes, which are strongly linked.

Joint effects of the *IRF5* rs20046640, *STAT4* rs7574865 and *NLRP1* rs8182352 risk alleles on SSc-related FA susceptibility

As we have recently reported that carrying at least three *IRF5* rs2004640/*STAT4* rs7574865 risk alleles is an independent marker of SSc-related FA,¹² we decided to investigate the potential joint effects of the risk alleles of *NLRP1*, *IRF5* and *STAT4* in the French sample of SSc patients and controls using an additive logistic regression model. When gene–gene interaction between *NLRP1* and *IRF5* and *STAT4* was queried, no evidence for dominance or interactions was observed between *IRF5* rs2004640, *STAT4* rs7574865 and *NLRP1* rs8182352 risk alleles using multiple logistic regression analysis ruling out epistatic effects. We observed an additive effect of risk alleles on the FA-positive SSc susceptibility. The OR for FA-positive SSc were 1.82, 95% CI 1.25 to 2.65; $p=0.001$ for carriers of three risk alleles among these three SNP, 2.47, 95% CI 1.62 to 3.75; $p<0.0001$ for carriers of four risk alleles, and 2.91, 95% CI 1.58 to 5.36; $p<0.0001$ for carriers of five or six risk alleles.

Intracohort analysis comparing FA-positive and FA-negative subsets revealed that SSc patients carrying at least four *IRF5*, *STAT4*, *NLRP1* risk alleles were at increased risk of FA. The OR for FA-positive SSc were 1.68, 95% CI 1.05 to 2.67; $p=0.019$ for carriers of four risk alleles, 2.03, 95% CI 1.03 to 4.04; $p=0.033$ for carriers of five or six risk alleles.

In these settings, figure 2 shows OR for FA-positive SSc individuals carrying three, four, five or six risk alleles, using the individuals with no or one risk allele as reference. The OR increases as a function of the number of risk alleles in a multiplicative manner, with a 1.33-fold increase in OR for each additional risk allele.

DISCUSSION

In this study our aim was to test for association of *NLRP1* with SSc. We first detected in the discovery set an association between *NLRP1* rs2670660 and rs8182352 with SSc Topo-positive and FA subsets and Topo-positive subgroup, respectively. Conditional analysis suggested that the association with this region could best be explained by the *NLRP1* rs8182352 variant. Indeed, we next investigated the *NLRP1* rs8182352 polymorphism in two European Caucasian populations. The association was replicated in the Italian population but not in the German. The weak sample size of the German sample may partly account

Table 2 Genotype and allele distribution of the *NLRP1* rs8182352 SNP in SSc patients and controls in the French Caucasian population

	CC (%)	CT (%)	TT (%)	C (%)		p Value*	p _{adj} Value†	OR (95% CI)
SSc, n=870	21.7	48.1	30.2	45.8	C	0.32		1.07 (0.94 to 1.22)
					CC	0.27		1.16 (0.89 to 1.51)
					CT	0.86		0.98 (0.79 to 1.21)
					TT			1 (ref)
dcSSc, n=294	22.4	49.7	27.9	47.3	C	0.18		1.14 (0.94 to 1.37)
					CC	0.17		1.30 (0.89 to 1.89)
					CT	0.54		1.01 (0.81 to 1.49)
					TT			1 (ref)
lcSSc, n=535	20.9	47.7	31.4	44.8	C	0.73		1.03 (0.88 to 1.19)
					CC	0.63		1.08 (0.79 to 1.45)
					CT	0.60		0.94 (0.74 to 1.20)
					TT			1 (ref)
ACA-positive SSc, n=322	21.7	48.1	30.1	45.8	C	0.45		1.07 (0.89 to 1.28)
					CC	0.41		1.16 (0.81 to 1.67)
					CT	0.92		0.99 (0.74 to 1.32)
					TT			1 (ref)
ACA-negative SSc, n=477	21.2	49.0	29.8	45.7	C	0.42		1.07 (0.91 to 1.25)
					CC	0.39		1.15 (0.84 to 1.57)
					CT	0.89		1.02 (0.79 to 1.31)
					TT			1 (ref)
Topo-positive SSc, n=217	27.7	47.9	24.4	51.6	C	0.0047	0.047	1.35 (1.09 to 1.66)
					CC	0.0038	0.038	1.83 (1.21 to 2.76)
					CT	0.29		1.21 (0.84 to 1.74)
					TT			1 (ref)
Topo-negative SSc, n=577	19.4	48.7	31.9	43.8	C	0.98		0.98 (0.85 to 1.14)
					CC	0.91		0.98 (0.73 to 1.32)
					CT	0.94		0.94 (0.75 to 1.19)
					TT			1 (ref)
FA-positive SSc, n=343	24.2	48.1	27.7	48.3	C	0.062		1.18 (0.99 to 1.41)
					CC	0.052		1.41 (0.99 to 1.99)
					CT	0.64		1.07 (0.80 to 1.44)
					TT			1 (ref)
FA-negative SSc, n=469	20.3	48.8	30.9	44.7	C	0.76		1.02 (0.88 to 1.19)
					CC	0.71		1.06 (0.77 to 1.46)
					CT	0.98		0.98 (0.76 to 1.26)
					TT			1 (ref)
Controls, n=962	19.1	50.0	30.9	44.1				

Allelic *NLRP1* rs8182352 p value conditional on rs2670660=0.014‡*By χ^2 test or Fisher's exact test.

†p Value <0.05 adjusted after Bonferroni's correction for multiple comparisons.

‡Conditional analysis that corresponds to the case-control association test performed in the Topo-positive SSc subset.

ACA, anti-centromere antibodies; dcSSc, diffuse cutaneous subtype; FA, fibrosing alveolitis; lcSSc, limited cutaneous subtype; SSc, systemic sclerosis; Topo, anti-topoisomerase I.

Figure 1 Association between *NLRP1* rs8182352 and systemic sclerosis (SSc) in the European Caucasian population: meta-analysis.

for this unexpected finding. However, some data showed that FA may also occur as a limited cutaneous disease and this can also account for the discrepancy between the association with Topo or FA.³⁰ Nonetheless, the meta-analysis that is corrected

for population stratification further strengthens this association and supports its soundness, showing that the *NLRP1* rs8182352 rare allele acts with a risk effect, under an additive model on both SSc-related pulmonary fibrosis and Topo-positive subsets.

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Table 3 Association study of *NLRP1* rs8182352 with SSc in both German and Italian Caucasian populations

	CC (%)	CT (%)	TT (%)	C (%)		p Value*	OR (95% CI)
German							
SSc, n=532	22.7	48.7	28.6	47.1	C	0.84	1.02 (0.84 to 1.24)
					CC	0.82	1.05 (0.71 to 1.55)
					CT	0.84	0.97 (0.70 to 1.34)
					TT		1 (ref)
SSc Topo-positive, n=161	19.2	52.7	28.6	45.3	C	0.71	0.95 (0.73 to 1.24)
					CC	0.67	0.89 (0.51 to 1.54)
					CT	0.87	1.04 (0.67 to 1.61)
					TT		1 (ref)
SSc FA-positive, n=157	25.5	50.9	23.6	50.9	C	0.20	1.19 (0.91 to 1.56)
					CC	0.20	1.42 (0.82 to 2.45)
					CT	0.39	1.23 (0.77 to 1.96)
					TT		1 (ref)
Controls, n=324	21.6	50.0	28.4	46.6			
Italian							
SSc, n=527	22.4	52.4	25.2	48.6	C	0.31	1.11 (0.97 to 1.35)
					CC	0.40	1.18 (0.79 to 1.75)
					CT	0.017	1.49 (1.07 to 2.09)
					TT		1 (ref)
SSc Topo-positive, n=158	26.6	54.4	19.0	53.8	C	0.025	1.37 (1.04 to 1.79)
					CC	0.028	1.87 (1.07 to 3.27)
					CT	0.003	2.01 (1.27 to 3.38)
					TT		1 (ref)
SSc FA-positive, n=174	27.6	47.7	24.7	51.4	C	0.11	1.24 (0.95 to 1.36)
					CC	0.13	1.48 (0.89 to 2.48)
					CT	0.15	1.39 (0.94 to 2.17)
					TT		1 (ref)
Controls, n=301	23.9	44.2	31.9	46.0			

*By χ^2 test or Fisher's exact test.

ACA, anti-centromere antibodies; dcSSc, diffuse cutaneous subtype; FA, fibrosing alveolitis; lcSSc, limited cutaneous subtype; SSc, systemic sclerosis; Topo, anti-topoisomerase I.

Table 4 Meta-analysis of the *NLRP1* rs8182352 variant in the European Caucasian population

	CC (%)	CT (%)	TT (%)	C (%)		p Value*	OR (95% CI)
SSc, n=1929	22.2	49.4	28.4	46.7	C	0.19	1.06 (0.97 to 1.17)
					CC	0.21	1.13 (0.93 to 1.37)
					CT	0.56	1.05 (0.88 to 1.25)
					TT		1 (ref)
SSc Topo-positive, n=536	24.8	51.1	24.1	50.4	C	0.0042	1.23 (1.07 to 1.41)
					CC	0.0049	1.50 (1.13 to 1.99)
					CT	0.32	1.14 (0.88 to 1.46)
					TT		1 (ref)
SSc FA-positive, n=674	25.4	48.7	25.9	49.7	C	0.0065	1.19 (1.05 to 1.36)
					CC	0.0063	1.43 (1.10 to 1.84)
					CT	0.09	1.22 (0.97 to 1.52)
					TT		1 (ref)
Controls, n=1587	20.5	48.9	30.6	45.0			

	Additive		Recessive		Dominant	
	p Value	OR (95% CI)	p Value	OR (95% CI)	p Value	OR (95% CI)
SSc Topo-positive	0.0042	1.23 (1.07 to 1.41)	0.0059	1.38 (1.09 to 1.73)	0.059	1.25 (0.99 to 1.58)
SSc FA-positive	0.0064	1.19 (1.05 to 1.35)	0.033	1.24 (1.02 to 1.53)	0.019	1.29 (1.04 to 1.59)

*Mantel-Haenszel meta-analysis under a fixed-effects model.

ACA, anti-centromere antibodies; dcSSc, diffuse cutaneous subtype; FA, fibrosing alveolitis; lcSSc, limited cutaneous subtype; SSc, systemic sclerosis; Topo, anti-topoisomerase I.

Multivariate analysis revealed that *NLRP1* was associated with both SSc subsets, illustrating the high correlation of Topo positivity and SSc-related pulmonary fibrosis.

We then investigated the *NLRP1* rs8182352 SNP, a polymorphism with no predicted functional role. Both allele and genotype frequencies were in agreement with those previously reported in Caucasian populations,^{17 18} supporting the accuracy of this genotyping. The restricted association of *NLRP1* rs8182352 with both FA-positive and Topo-positive SSc subsets suggests that this variant could contribute to a disease-specific

phenotype, and may be regarded as a marker of SSc severity, lung disease being actually the primary cause of SSc-related death.³¹ No other *NLRP1* variant investigated was found to be associated with SSc; notably, the non-synonymous *NLRP1* rs12150220 polymorphism, reported to confer susceptibility to autoimmune diseases.^{20 21} Interestingly, this risk variant is located in the same *NLRP1* LD block tagged by rs8182352.²⁰ Therefore, taking into account that the putative functional role of the *NLRP1* rs8182352 SNP is unknown, re-sequencing of the LD block tagged by rs8182352 may be essential in order to

Figure 2 Additive effect of *IRF5* rs2004640, *STAT4* rs7574865 and *NLRP1* rs8182352 risk alleles on systemic sclerosis (SSc)-related fibrosing alveolitis susceptibility.

identify one or multiple SSc causal variants. In addition, future functional analyses of the candidate *NLRP1* causal variants are mandatory to obtain a better understanding of the contribution of *NLRP1* in the pathogenesis of SSc.

NLRP1 is believed to mediate inflammation and autoimmunity partly due to its ability to form the inflammasome that plays a pivotal role in the activation of pro-IL-1 β .³² Several lines of evidence have raised the contribution of IL-1 β in SSc pathogenesis, including an increased expression of IL-1 β by fibroblasts detected in skin biopsies, high IL-1 β serum concentrations and overexpression of IL-1 β and IL-1ra genes in bronchoalveolar lavage fluid of SSc patients compared with controls.^{33–36} Nevertheless, it remains to be determined whether IL-1 β may induce the fibrotic process in the lung or contribute to the chronicity and severity of involvement. In addition, it has previously been reported that silica exposure, which is a well-known SSc environmental factor, leads to the local release of IL-1 β , and may contribute to abnormal connective tissue deposition by stimulating fibroblast proliferation.^{37–39}

Of most interest, we found an additive effect of the rare variants of these genes on SSc-related FA, with a 1.33-fold increase in OR for each additional risk allele. Furthermore, intra-cohort analysis revealed that SSc patients carrying at least five *IRF5*, *STAT4*, *NLRP1* risk alleles were at a twofold increased risk of having FA. The identification of *IRF5*, *STAT4* and *NLRP1* as new SSc-related pulmonary fibrosis genetic susceptibility factors raises the question of the contribution of innate immunity in SSc pathogenesis and in the fibrotic process, notably through IL-1 β and the type I interferon pathway. However, this model, observed in the French Caucasian population, needs to be replicated in other populations to be validated.

In the present study we show for the first time that the *NLRP1* rs8182352 variant plays a significant role in SSc-related FA and

SSc Topo-positive susceptibility. These findings were obtained through large samples and replication in an independent cohort. The fact that genotyping provided similar frequencies in both control groups underlines the consistency of the reported results. Intracohort analyses revealed an additive effect of *NLRP1* with *IRF5* and *STAT4* risk variants on SSc-related pulmonary fibrosis susceptibility. These data provide new insights into the pathogenesis of SSc, underlying the pivotal key role of innate immunity at least in the FA SSc subphenotype.

Acknowledgements The authors would like to thank Dr Joëlle Benessiano (Centre de Ressource Biologique, Hôpital Bichat Claude-Bernard, Paris France) and staff members of the Établissement Français du Sang for their assistance and Mrs Carole Desbas for expert secretarial assistance. The authors also thank Professor Bernard Grandchamp and Dr Nadem Soufir (Hôpital Bichat Claude-Bernard) for assistance in setting up the French Caucasian control sample.

Funding ANR (grant R070994KS), SFR, ASF, GFRS, INSERM. The German Network for Systemic Sclerosis was funded by the German Federal Ministry for Education and Research (grant nos 01 GM 0310 and 01 GM 0634 to IM, GR, NH and IT).

Competing interests None.

Patient consent Obtained.

Ethics approval This study was conducted with the approval of the Comité de Protection des Personnes of Cochin Hospital, Paris, France.

Provenance and peer review Not commissioned; externally peer reviewed.

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REFERENCES

1. Fritz JH, Girardin SE. How Toll-like receptors and Nod-like receptors contribute to innate immunity in mammals. *J Endotoxin Res* 2005;**11**:390–4.
2. Martinon F, Tschopp J. NLRs join TLRs as innate sensors of pathogens. *Trends Immunol* 2005;**26**:447–54.
3. Martinon F, Hofmann K, Tschopp J. The pyrin domain: a possible member of the death domain-fold family implicated in apoptosis and inflammation. *Curr Biol* 2001;**11**:R118–20.
4. Martinon F, Tschopp J. Inflammatory caspases: linking an intracellular innate immune system to autoinflammatory diseases. *Cell* 2004;**117**:561–74.
5. Gasse P, Mary C, Guenon I, et al. IL-1R1/MyD88 signaling and the inflammasome are essential in pulmonary inflammation and fibrosis in mice. *J Clin Invest* 2007;**117**:3786–99.

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6. **Kolb M**, Margetts PJ, Anthony DC, *et al*. Transient expression of IL-1 β induces acute lung injury and chronic repair leading to pulmonary fibrosis. *J Clin Invest* 2001;**107**:1529–36.
7. **Kawaguchi Y**, McCarthy SA, Watkins SC, *et al*. Autocrine activation by interleukin 1 α induces the fibrogenic phenotype of systemic sclerosis fibroblasts. *J Rheumatol* 2004;**31**:1946–54.
8. **Kawaguchi Y**, Harigai M, Suzuki K, *et al*. Interleukin 1 receptor on fibroblasts from systemic sclerosis patients induces excessive functional responses to interleukin 1 β . *Biochem Biophys Res Commun* 1993;**190**:154–61.
9. **Allanore Y**, Wipff J, Kahan A, *et al*. Genetic basis for systemic sclerosis. *Joint Bone Spine* 2007;**74**:577–83.
10. **Allanore Y**, Dieude P, Boileau C. Genetic background of systemic sclerosis: autoimmune genes take centre stage. *Rheumatology (Oxford)* 2010;**49**:203–10.
11. **Dieudé P**, Guedj M, Wipff J, *et al*. Association between the IRF5 rs2004640 functional polymorphism and systemic sclerosis: a new perspective for pulmonary fibrosis. *Arthritis Rheum* 2009;**60**:225–33.
12. **Dieudé P**, Guedj M, Wipff J, *et al*. STAT4 is a genetic risk factor for systemic sclerosis having additive effects with IRF5 on disease susceptibility and related pulmonary fibrosis. *Arthritis Rheum* 2009;**60**:2472–9.
13. **Ito I**, Kawaguchi Y, Kawasaki A, *et al*. Association of a functional polymorphism in the IRF5 region with systemic sclerosis in a Japanese population. *Arthritis Rheum* 2009;**60**:1845–50.
14. **Rueda B**, Broen J, Simeon C, *et al*. The STAT4 gene influences the genetic predisposition to systemic sclerosis phenotype. *Hum Mol Genet* 2009;**18**:2071–7.
15. **Schreiber S**, Rosenstiel P, Albrecht M, *et al*. Genetics of Crohn disease, an archetypal inflammatory barrier disease. *Nat Rev Genet* 2005;**6**:376–88.
16. **Stojanov S**, Kastner DL. Familial autoinflammatory diseases: genetics, pathogenesis and treatment. *Curr Opin Rheumatol* 2005;**17**:586–99.
17. **Jin Y**, Birlea SA, Fain PR, *et al*. Genetic variations in NALP1 are associated with generalized vitiligo in a Romanian population. *J Invest Dermatol* 2007;**127**:2558–62.
18. **Jin Y**, Mailloux CM, Gowan K, *et al*. NALP1 in vitiligo-associated multiple autoimmune disease. *N Engl J Med* 2007;**356**:1216–25.
19. **Jin Y**, Riccardi SL, Gowan K, *et al*. Fine-mapping of vitiligo susceptibility loci on chromosomes 7 and 9 and interactions with NLRP1 (NALP1). *J Invest Dermatol* 2010;**130**:774–83.
20. **Magitta NF**, Bøe Wolff AS, Johansson S, *et al*. A coding polymorphism in NALP1 confers risk for autoimmune Addison's disease and type 1 diabetes. *Genes Immun* 2009;**10**:120–4.
21. **Zurawek M**, Fichna M, Januszkiewicz-Lewandowska D, *et al*. A coding variant in NLRP1 is associated with autoimmune Addison's disease. *Hum Immunol* 2010;**71**:530–4.
22. **Dieudé P**, Guedj M, Wipff J, *et al*. The PTPN22 620W allele confers susceptibility to systemic sclerosis: findings of a large case-control study of European Caucasians and a meta-analysis. *Arthritis Rheum* 2008;**58**:2183–8.
23. **Dieudé P**, Wipff J, Guedj M, *et al*. BANK1 is a genetic risk factor for diffuse cutaneous systemic sclerosis and has additive effects with IRF5 and STAT4. *Arthritis Rheum* 2009;**60**:3447–54.
24. **LeRoy EC**, Black C, Fleischmajer R, *et al*. Scleroderma (systemic sclerosis): classification, subsets and pathogenesis. *J Rheumatol* 1988;**15**:202–5.
25. **Valentini G**, Matucci Cerinic M. Disease-specific quality indicators, guidelines and outcome measures in scleroderma. *Clin Exp Rheumatol* 2007;**25**(6 Suppl 47):159–62.
26. **Hunzelmann N**, Genth E, Krieg T, *et al*. The registry of the German Network for Systemic Scleroderma: frequency of disease subsets and patterns of organ involvement. *Rheumatology (Oxford)* 2008;**47**:1185–92.
27. **Chanock SJ**, Manolio T, Boehnke M, *et al*. Replicating genotype-phenotype associations. *Nature* 2007;**447**:655–60.
28. **Guedj M**, Della-Chiesa E, Picard F, *et al*. Computing power in case-control association studies through the use of quadratic approximations: application to meta-statistics. *Ann Hum Genet* 2007;**71**:262–70.
29. **Steen VD**, Owens GR, Fino GJ, *et al*. Pulmonary involvement in systemic sclerosis (scleroderma). *Arthritis Rheum* 1985;**28**:759–67.
30. **Clements PJ**, Roth MD, Elashoff R, *et al*. Scleroderma lung study (SLS): differences in the presentation and course of patients with limited versus diffuse systemic sclerosis. *Ann Rheum Dis* 2007;**66**:1641–7.
31. **Steen VD**, Medsger TA. Changes in causes of death in systemic sclerosis, 1972–2002. *Ann Rheum Dis* 2007;**66**:940–4.
32. **Martinson F**, Burns K, Tschopp J. The inflammasome: a molecular platform triggering activation of inflammatory caspases and processing of proIL- β . *Mol Cell* 2002;**10**:417–26.
33. **Kawaguchi Y**, Hara M, Wright TM. Endogenous IL-1 α from systemic sclerosis fibroblasts induces IL-6 and PDGF-A. *J Clin Invest* 1999;**103**:1253–60.
34. **Atamas SP**, White B. Cytokine regulation of pulmonary fibrosis in scleroderma. *Cytokine Growth Factor Rev* 2003;**14**:537–50.
35. **Sasaki M**, Kashima M, Ito T, *et al*. Differential regulation of metalloproteinase production, proliferation and chemotaxis of human lung fibroblasts by PDGF, interleukin-1 β and TNF- α . *Mediators Inflamm* 2000;**9**:155–60.
36. **Luzina IG**, Atamas SP, Wise R, *et al*. Gene expression in bronchoalveolar lavage cells from scleroderma patients. *Am J Respir Cell Mol Biol* 2002;**26**:549–57.
37. **Schmidt JA**, Oliver CN, Lepe-Zuniga JL, *et al*. Silica-stimulated monocytes release fibroblast proliferation factors identical to interleukin 1. A potential role for interleukin 1 in the pathogenesis of silicosis. *J Clin Invest* 1984;**73**:1462–72.
38. **Sarih M**, Souvannavong V, Brown SC, *et al*. Silica induces apoptosis in macrophages and the release of interleukin-1 α and interleukin-1 β . *J Leukoc Biol* 1993;**54**:407–13.
39. **Srivastava KD**, Rom WN, Jagirdar J, *et al*. Crucial role of interleukin-1 β and nitric oxide synthase in silica-induced inflammation and apoptosis in mice. *Am J Respir Crit Care Med* 2002;**165**:527–33.

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Ann Rheum Dis 2011 70: 668-674 originally published online December 13, 2010

doi: 10.1136/ard.2010.131243

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