

# Association of Interleukin 23 Receptor Polymorphisms with Anti-Topoisomerase-I Positivity and Pulmonary Hypertension in Systemic Sclerosis

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**ABSTRACT. Objective.** *IL23R* has been identified as a susceptibility gene for development of multiple autoimmune diseases. We investigated the possible association of *IL23R* with systemic sclerosis (SSc), an autoimmune disease that leads to the development of cutaneous and visceral fibrosis.

**Methods.** We tested 9 single-nucleotide polymorphisms (SNP) in *IL23R* for association with SSc in a cohort of 1402 SSc cases and 1038 controls. *IL23R* SNP tested were previously identified as SNP showing associations with inflammatory bowel disease.

**Results.** Case-control comparisons revealed no statistically significant differences between patients and healthy controls with any of the *IL23R* polymorphisms. Analyses of subsets of SSc patients showed that rs11209026 (Arg381Gln variant) was associated with anti-topoisomerase I antibody (ATA)-positive SSc ( $p = 0.0011$ ) and rs11465804 SNP was associated with diffuse and ATA-positive SSc ( $p = 0.0001$ ,  $p = 0.0026$ , respectively). These associations remained significant after accounting for multiple comparisons using the false discovery rate method. Wild-type genotype at both rs11209026 and rs11465804 showed significant protection against the presence of pulmonary hypertension (PHT). ( $p = 3 \times 10^{-5}$ ,  $p = 1 \times 10^{-5}$ , respectively).

**Conclusion.** Polymorphisms in *IL23R* are associated with susceptibility to ATA-positive SSc and protective against development of PHT in patients with SSc. (First Release Nov 15 2009; *J Rheumatol* 2009;36:2715–23; doi:10.3899/jrheum.090421)

## Key Indexing Terms:

SYSTEMIC SCLEROSIS  
POLYMORPHISM

SCLERODERMA  
AUTOANTIBODIES

*IL23R*  
PULMONARY HYPERTENSION

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Systemic sclerosis (scleroderma, SSc) is a chronic multisystem disease clinically characterized by autoimmunity leading to progressive fibrosis in the skin and internal organs<sup>1</sup>. Pathologically, SSc exhibits 3 cardinal features: inflammation and autoimmunity, vasculopathy, and excessive extracellular matrix production and deposition<sup>1</sup>. It remains to be determined how the disease process is triggered, but current models indicate immune dysregulation as a central process in the pathogenesis of SSc.

Skin biopsies of early scleroderma skin demonstrate perivascular infiltrates of mononuclear inflammatory cells, including CD4+ T cells, which produce cytokines and chemokines that induce tissue damage, promote fibrosis, and recruit additional inflammatory cells<sup>2</sup>. Several subsets of effector CD4+ T cells have been identified based on their distinct cytokine profiles<sup>3</sup>. In the presence of interleukin 12 (IL-12), naive CD4+ T cells differentiate into Th1 cells characterized by the production of interferon- $\gamma$  (IFN- $\gamma$ )<sup>4</sup>. In the presence of IL-4, naive CD4+ T cells differentiate into Th2 cells characterized by the production of IL-4, IL-5, and IL-13<sup>4</sup>. A novel subset of T cells, Th17, characterized by the

production of IL-17, has been identified<sup>5</sup>. Th17 cells differentiate from naive CD4+ T cells upon T cell receptor activation in the presence of IL-6 and transforming growth factor  $\beta$  (TGF- $\beta$ )<sup>6,7</sup>.

Current models suggest Th17 cells as key mediators in the pathogenesis of a variety of autoimmune diseases, such as rheumatoid arthritis, inflammatory bowel disease (IBD), psoriasis, and ankylosing spondylitis (AS)<sup>8</sup>. IL-23 and the IL-23 receptor have been implicated in the development of autoimmune diseases such as multiple sclerosis<sup>9</sup>. While IL-23 is not required for Th17 differentiation, it is an important cytokine that promotes expansion of the Th17 population by inducing proliferation of Th17 cells<sup>10</sup>. IL-23 signals through the heterodimeric IL-23 receptor, composed of the IL-12R $\beta$ 1 chain and a unique subunit called IL-23R<sup>9</sup>. Genome-wide association studies have demonstrated that single-nucleotide polymorphisms (SNP) in the *IL23R* gene confer a significant risk for development of multiple autoimmune diseases including IBD, psoriasis, and AS<sup>11-16</sup>. These studies implicate IL-23 and Th17 cells in the immune pathogenesis of these diseases.

It has been hypothesized that Th17 cells might play a role in the pathogenesis of SSc. Indeed, plasma concentrations of IL-17 and IL-23 have been reported to be elevated in Japanese SSc patients from 2 cohorts<sup>17,18</sup>. Further, peripheral blood T cells from patients with SSc have been reported to produce increased levels of IL-17<sup>17</sup>. These studies point to an increase in Th17 cytokine activity in SSc. Given the potential role of Th17 cells in the immunopathogenesis of SSc, we investigated if polymorphisms in *IL23R* are associated with SSc susceptibility and clinical phenotypes in a large cohort of North American SSc patients compared to healthy controls.

## MATERIALS AND METHODS

**SSc patients and controls.** In total, 1402 SSc patients and 1038 healthy control subjects from the Scleroderma Family Registry and DNA Repository<sup>19</sup> and the University of Texas Rheumatology Division<sup>20</sup>, dating from 1986 to present, including the Genetics versus Environment in Scleroderma Outcomes Study (GENISOS)<sup>21</sup> formed the study cohort. All SSc patients fulfilled American College of Rheumatology (ACR; formerly American Rheumatism Association) preliminary criteria for disease classification<sup>22</sup> (n = 1122, 80%) or had at least 3 of the 5 CREST features (calcinosis, Raynaud's phenomenon, esophageal dysfunction, sclerodactyly, and telangiectasias) (n = 280, 20%)<sup>23,24</sup>.

Clinical data were obtained from the database. Ethnicity was self-defined by the cases and controls. Patients were classified as having limited or diffuse cutaneous SSc disease according to published criteria<sup>25</sup>. Clinical data also were obtained from the database to determine visceral involvement, including fibrosing alveolitis, pulmonary hypertension (PHT), and scleroderma renal crisis. Fibrosing alveolitis was defined as the presence of typical findings on chest high-resolution computerized tomography (CT), regular chest CT or radiograph, or restrictive pattern on pulmonary function testing. PHT was defined as estimated peak right-ventricular systolic pressure  $\geq$  40 mm Hg on echocardiography or pulmonary arterial systolic pressure  $\geq$  40 mm Hg by right-heart catheterization. Scleroderma renal crisis was characterized by the presence of new-onset accelerated systemic hypertension with evidence of renal impairment.

All subjects provided written informed consent and the study was approved by the Committee for the Protection of Human Subjects of The University of Texas Health Science Center at Houston.

**Autoantibody analysis.** All SSc patients were tested for antinuclear antibodies using indirect immunofluorescence (IIF) and HEp-2 cells as antigen substrate (Antibodies Inc., Davis, CA, USA). Anticentromere antibodies (ACA) were determined by their distinctive IIF pattern on HEp-2 cells<sup>26</sup>. Autoantibodies to topoisomerase I (ATA) were determined by passive immunodiffusion (Inova Diagnostics, San Diego, CA, USA). Anti-RNA polymerase III antibodies (ARA) were determined by enzyme-linked immunoassay (MBL Co. Ltd., Nagoya, Japan). For ARA, the cutoff was defined as 2.5 standard deviations above the mean of 40 healthy controls.

**SNP selection and genotyping.** SNP in the *IL23R* gene were selected based on *IL23R* variants identified in 2 independent genome-wide association studies in IBD<sup>11,12</sup>. The following *IL23R* SNP were tested: intronic SNP rs1884444, rs1004819, rs11805303, rs7530511, rs10489629, rs11465804, and rs1343151; exonic 3'-untranslated region SNP rs10889677; and non-synonymous SNP rs11209026 (Arg381Gly).

Genomic DNA was extracted from peripheral blood with the PureGene genomic DNA isolation kit (Gentra Systems, Minneapolis, MN, USA). The SNP were genotyped using an ABI-TaqMan SNP genotyping assay (Applied Biosystems ABI, Foster City, CA, USA). The ABI 7900HT real-time thermocycler was used to perform the plate reading. Automated allele calling was performed by allelic discrimination plots using SDS 2.3 software (ABI). Multiple positive (CEPH samples) and negative controls were used in each genotyping assay and allele calls were verified with HAPMAP data for validation.

**Statistical analysis.** Statistical analyses were performed using SAS 9.1.3 (SAS Institute Inc., Cary, NC, USA). Hardy-Weinberg equilibrium testing was performed using the exact test. Allelic and genotypic associations were calculated using standard Pearson's chi-square test or, when appropriate, Fisher's exact test. To account for multiple comparisons, we used a false discovery rate approach<sup>27</sup>, less conservative than a traditional Bonferroni correction. We used alpha = 0.05 level of significance and accounted for 135 potential multiple comparisons that were performed. These comparisons include each of the 9 SNP tested for different ethnic groups and different subtypes of SSc. Using these values for alpha and the number of tests performed, a point-wise significance of 0.0051 is considered significant for multiple comparisons. Measures of pairwise linkage disequilibrium were determined using Haploview (Whitehead Institute for Biomedical Research, Cambridge, MA, USA).

## RESULTS

**Demographic data.** A candidate gene association study was conducted using SSc patients and healthy controls from the Scleroderma Family Registry and DNA Repository combined with subjects evaluated at the University of Texas Houston Rheumatology Division. Clinical and serological information of patients are presented in Table 1. In total, 1402 SSc patients and 1038 healthy controls were enrolled for study belonging to Caucasian, African American, and Hispanic ethnic groups. Seven hundred thirty patients had diffuse scleroderma and 595 had limited scleroderma. Of 1402 patients, 341 (24%) were positive for the ACA, 256 (18%) for ATA, and 261 (19%) for the ARA.

**Linkage disequilibrium and haplotype block structure of *IL23R* gene healthy controls.** Pairwise linkage disequilibrium was calculated by both  $D'$  and  $r^2$  for the 9 *IL23R* SNP typed in Caucasian, African American, and Hispanic controls (Figure 1). The *IL23R* SNPs- rs11209026-rs11465804

Table 1. Clinical and serologic characteristics of patients with systemic sclerosis (SSc)\* and healthy controls. Data are n (%).

Characteristic	Caucasian	African American	Hispanic
Controls	698*	201*	139
Female	334 (50.8)	142 (71.0)	80 (57.6)
Male	323 (49.2)	58 (29.0)	59 (42.4)
SSc	1059	177	166
Female	935 (88.3)	151 (85.3)	148 (89.2)
Male	124 (11.7)	26 (14.7)	18 (10.8)
Skin involvement	1004	164	157
Limited SSc	611 (60.9)	53 (32.3)	66 (42.0)
Diffuse SSc	393 (39.1)	111 (67.7)	91 (58.0)
Antibodies	667	86	105
Anticentromere	300 (45.0)	12 (14.0)	29 (27.6)
Anti-topoisomerase I	174 (26.1)	43 (50.0)	39 (37.2)
Anti-RNA polymerase III	193 (28.9)	31 (36.0)	37 (35.2)

\* Missing gender information on 41 white and 1 black samples.

and rs1004819-rs11805303 showed tight LD in the Caucasian population ( $r^2 > 0.90$ ). The *IL23R* SNP rs1004819-rs11805303 were in complete linkage disequilibrium in the African American control population and rs11209026-rs11465804 were in complete linkage disequilibrium in the Hispanic control population ( $r^2 = 1.0$ ).

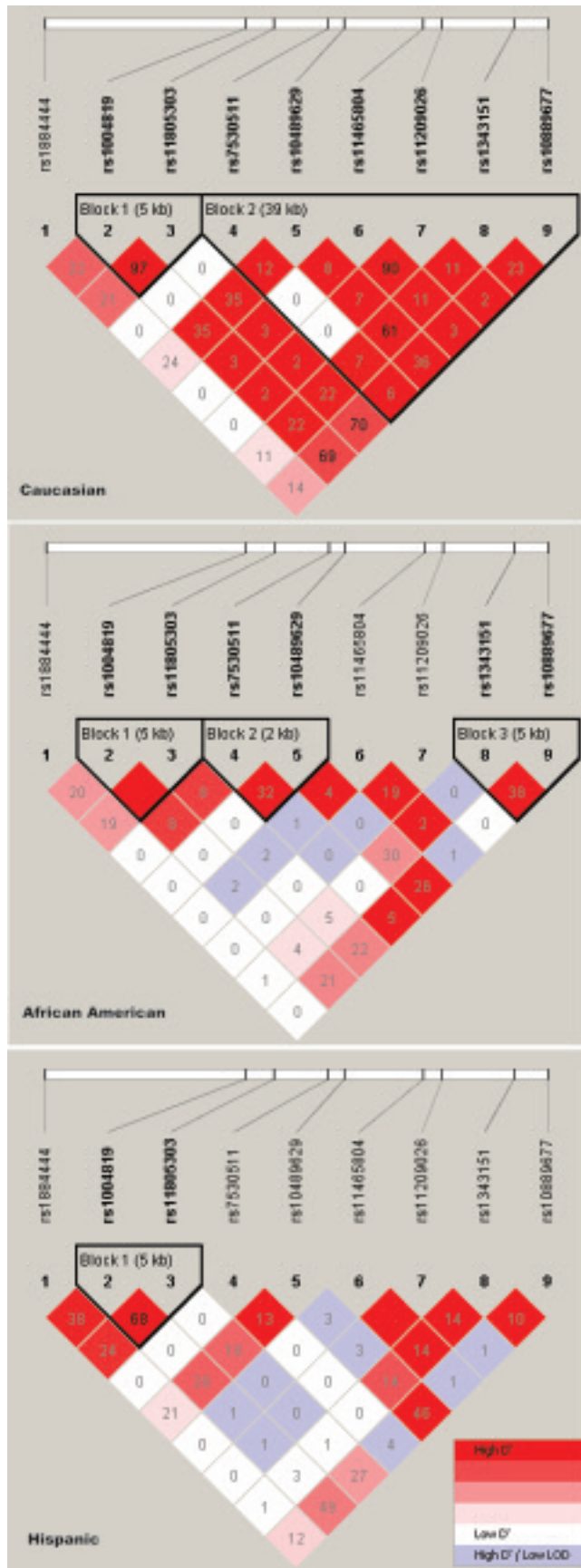
*IL-23R polymorphisms and association with SSc.* A total of 9 SNP in the *IL23R* gene were genotyped in our cohorts. All SNP studied in the *IL23R* gene were in Hardy-Weinberg equilibrium in Caucasian, African American, and Hispanic controls (Table 2). Allelic frequencies of the polymorphisms were similar between sexes in cases and controls. Case-control comparisons for genotypes using the chi-square test did not reach statistical significance in the Caucasian population for all the 9 *IL23R* SNP (Table 3). In African Americans, there was a genotypic association of the rs1884444 SNP with SSc susceptibility ( $p = 0.004$ ). In Hispanics, a minor allelic association with SSc susceptibility was observed with the rs1343151 SNP ( $p = 0.047$ ), but this was not significant after accounting for multiple comparisons.

*IL-23R polymorphisms and association with SSc subsets.* SSc is a clinically heterogeneous disease, divided into 2 main subsets based on the extent of cutaneous involvement: limited and diffuse<sup>25</sup>. The presence of SSc-associated autoantibodies such as ACA, ATA, and ARA has been shown to be associated with distinct clinical subsets characterized by extent of cutaneous involvement and pattern of organ involvement, such as PHT, pulmonary fibrosis, or SSc renal crisis, respectively<sup>28</sup>. Several studies have recently shown that certain genetic polymorphisms may have stronger associations with particular phenotypic subsets<sup>29,30</sup>. Therefore, we compared the frequency of *IL23R* SNP within the clinical and autoantibody subsets of SSc in Caucasians.

Of the 9 *IL23R* SNP, 2 (rs11209026 and rs11465804) were associated with susceptibility to specific subsets of

SSc in Caucasians (Table 4a, 4b). Genotypic comparison revealed that GG homozygosity at the *IL23R* SNP rs11209026 (Arg381Gln) was found more frequently in patients with diffuse SSc compared to controls (Table 4a), although this did not remain significant after accounting for multiple comparisons ( $p = 0.027$ ). Using the SSc-associated autoantibodies to subcategorize SSc patients, we found a significant association of the GG genotype at the *IL23R* SNP rs11209026 in ATA-positive patients relative to controls ( $p = 0.001$ ), which was significant using the point-wise threshold of 0.0051 accounting for multiple comparisons. The *IL23R* SNP rs11209026 was more associated with ATA-positive SSc patients relative to ATA-negative SSc patients ( $p = 0.007$ ), but this did not remain significant after correcting for multiple comparisons. The association of rs11209026 with ATA-positive SSc was also observed after deleting patients who had only 3 of 5 CREST features but did not meet the ACR preliminary criteria for SSc<sup>22</sup> ( $p = 0.0003$ ). No associations were found between limited scleroderma and the ACA-positive or ARA-positive patients. Haplotype analyses was performed but did not show any additional associations with susceptibility to ATA-positive SSc. No associations of *IL23R* SNP rs11209026 with subsets of SSc were observed in African American or Hispanic SSc patients (data not shown).

Similar associations were observed with the *IL23R* SNP rs11465804 (Table 4b). Genotypic comparison demonstrated that TT homozygosity at the *IL23R* SNP rs11465804 was more frequent in patients with diffuse SSc compared to controls (Table 4a), and this remained significant after accounting for multiple comparisons ( $p = 0.002$ ). Using SSc-associated autoantibodies to subcategorize SSc patients, we observed a significant association of the TT genotype at the *IL23R* SNP rs11465804 in ATA-positive patients relative to controls ( $p = 0.0001$ ) and relative to ATA-negative SSc patients ( $p = 0.0026$ ), both of which remained significant



after accounting for multiple comparisons using a point-wise threshold of 0.0051. The association of rs11465804 with ATA-positive SSc was also observed after deleting patients who had only 3 of 5 CREST features but did not meet the ACR criteria for SSc ( $p < 0.0001$ ). No associations were found between limited scleroderma and ACA-positive or ARA-positive patients. No associations of *IL23R* SNP rs11465804 with subsets of SSc were observed in African American or Hispanic SSc patients (data not shown).

Together these data demonstrate that even after controlling for multiple comparisons, there was a significant association of 2 *IL23R* polymorphisms (rs11209026 and rs11465804) with susceptibility to ATA-positive SSc in North American Caucasians.

*Association of IL23R polymorphisms with visceral involvement of SSc.* Additional analyses were performed to determine if the 2 susceptibility *IL23R* SNP (rs11209026 and rs11465804) were associated with visceral involvement of SSc, such as fibrosing alveolitis, PHT, or SSc renal crisis (Table 5a, 5b). Interestingly, the major allele at both *IL23R* SNP (rs11209026 and rs11465804) that was associated with ATA-positive SSc demonstrated statistically significantly decreased frequency with the presence of PHT (by genotypic analyses,  $p = 3 \times 10^{-5}$  for rs11209026, and  $p = 1 \times 10^{-5}$  for rs11465804; by allelic analyses,  $p = 0.005$  for rs11209026, and  $p = 0.001$  for rs11465804). These associations remained significant after correcting for multiple comparisons using the false discovery rate method. The major allele frequencies of these 2 SNP (G at rs11209026 and T at rs11465804) were decreased in SSc cases with PHT, compared to SSc cases without PHT. The protective association of the *IL23R* polymorphisms was found to be independent of age, sex, and the presence of SSc-associated autoantibodies including ACA, ATA, and ARA by logistic regression (data not shown). Thus, the *IL23R* SNP rs11209026 and rs11465804 were protective with regard to development of PHT in SSc. Chi-square analyses showed no associations with fibrosing alveolitis or SSc renal crisis. Together, these data show that the wild-type genotype for the 2 *IL23R* SNP is negatively associated with development of PHT in SSc.

## DISCUSSION

We demonstrated that *IL23R* polymorphisms are associated with diffuse SSc and ATA-positive SSc, but show no associations with limited SSc or ACA-positive or ARA-positive SSc. In addition to being a susceptibility factor for the devel-

*Figure 1.* Linkage disequilibrium (LD) and haplotype structure of *IL23R* gene. LD between SNP across the *IL23R* gene region is shown within healthy controls. Blocks connecting pairs of SNP are shaded according to the strength of the LD between the SNP, from 0.0 (white) to 1.0 (bright red), as measured by the disequilibrium coefficient  $D'$ . The blue blocks indicate pairs of markers for which  $D'$  is equal to 1.0, but the LOD score is less than 2.0. As an additional measure of strength of LD,  $r^2$  values are given as numerical values within each box.

Table 2. Hardy-Weinberg equilibrium of *IL23R* gene single-nucleotide polymorphisms (SNP) in healthy controls.

IL23R	IL23R SNP	Position, bp	p, Chi-square		
			Caucasian	African American	Hispanic
1	rs1884444	67406400	0.59	0.07	0.09
2	rs1004819	67442801	0.67	0.58	0.93
3	rs11805303	67448104	0.70	0.46	0.83
4	rs7530511	67457975	0.99	0.62	0.76
5	rs10489629	67460937	0.56	0.74	0.52
6	rs11465804	67475114	0.24	0.46	0.66
7	rs11209026	67478546	0.29	0.89	0.66
8	rs1343151	67491717	0.48	0.89	0.66
9	rs10889677	67497708	0.95	0.23	0.53

Table 3. Distribution of the *IL23R* genotype in SSc and controls.

SNP (minor allele)	Chr	Position (bp)	No. Controls/SSc	MAF, %		Allelic	P* Genotypic
				Controls	SSc		
Caucasian							
rs1884444 (G)	1	67406400	693/1047	46.8	47.7	0.58	0.78
rs1004819 (A)	1	67442801	694/1046	30	29.3	0.62	0.56
rs11805303 (T)	1	67448104	695/1047	30.1	29.4	0.68	0.75
rs7530511 (T)	1	67457975	694/1051	12.6	11.8	0.45	0.53
rs10489629 (C)	1	67460937	695/1054	46.1	48.2	0.24	0.34
rs11465804 (G)	1	67475114	691/1030	6.4	6.8	0.64	0.1
rs11209026 (A)	1	67478546	693/1052	6.1	6.7	0.5	0.21
rs1343151 (A)	1	67491717	696/1049	35.4	34.8	0.73	0.78
rs10889677 (A)	1	67497708	696/1048	30.2	29.5	0.63	0.48
African American							
rs1884444 (G)	1	67406400	198/176	48.5	48.3	0.38	0.004
rs1004819 (A)	1	67442801	199/176	31.2	29.3	0.57	0.41
rs11805303 (T)	1	6744804	198/175	30.8	30.3	0.91	0.41
rs7530511 (T)	1	67457975	198/176	20	25	0.1	0.22
rs10489629 (C)	1	67460937	200/175	43.5	44.9	0.7	0.88
rs11465804 (G)	1	67475114	200/175	5	3.7	0.39	0.38
rs11209026 (A)	1	67478546	199/176	1	1.7	0.4	0.4
rs1343151 (A)	1	67491717	199/177	33.7	36.2	0.49	0.75
rs10889677 (A)	1	67497708	199/175	16.8	15.1	0.53	0.08
Hispanic							
rs1884444 (G)	1	67406400	137/162	46	41.1	0.23	0.43
rs1004819 (A)	1	67442801	136/163	26.8	25.5	0.73	0.91
rs11805303 (T)	1	67448104	135/163	20	21.2	0.72	0.94
rs7530511 (T)	1	67457975	138/164	9.8	10.4	0.81	0.95
rs10489629 (C)	1	67460937	138/164	44.9	48.5	0.38	0.26
rs11465804 (G)	1	67475114	138/163	3.6	4.9	0.66	0.66
rs11209026 (A)	1	67478546	138/160	3.6	4.7	0.52	0.62
rs1343151 (A)	1	67491717	137/165	21.2	28.2	0.047	0.15
rs10889677 (A)	1	67497708	138/161	27.5	32.6	0.16	0.18

\* p values considered significant after correcting for multiple comparisons using the false discovery method and point-wise significance = 0.0051. SNP: single-nucleotide polymorphism; Chr: chromosome; MAF: minor allele frequency.

opment of specific subsets of SSc, *IL23R* SNP exert a protective effect for the development of PHT. These data add to the growing list of immune-associated genes that are associated with susceptibility to SSc that includes MHC class II, *PTPN22*, *AIF1*, and *IRF5* and thus position immune dysregulation as being pivotal to the pathogenesis of SSc<sup>29-32</sup>.

SSc is a heterogeneous autoimmune disease that is clinically categorized based on the degree of cutaneous involvement<sup>1,25</sup>. Limited SSc is defined by skin thickening in areas distal to the elbows and knees. In contrast, diffuse SSc is defined by the presence of skin thickening proximal and distal to the elbow and knees. The SSc-associated autoantibod-

Table 4A. Distribution of the *IL23R* SNP rs11209026 genotype in North American Caucasian patients with SSc and healthy controls.

	No.	Genotypic Comparison			p <sup>†</sup>
		AA	AG %	GG	
Controls	693	0.1	12.0	87.9	
Patients with SSc	1052	0.8	11.9	87.4	0.21
Limited	608	0.5	12.8	86.7	0.47
Diffuse	389	1.3	9.5	89.2	0.027
Antibodies					
Anticentromere	298	0.7	12.4	86.9	0.37
Anti-topoisomerase I	173	2.3	7.5	90.2	0.001
Anti-RNA polymerase III	191	0.5	12.6	86.9	0.60
ATA+ vs ATA- <sup>††</sup>	170	2.3	7.5	90.2	0.007
	852	0.5	12.6	86.9	

<sup>†</sup> p values considered significant after correcting for multiple comparisons using the false discovery method and point-wise significance = 0.0051. <sup>††</sup> Controls used as reference for all comparisons except this one.

Table 4B. Distribution of the *IL23R* SNP rs11465804 genotype in North American Caucasian patients with SSc and healthy controls.

	No.	Genotypic Comparison			p <sup>†</sup>
		GG	GT %	TT	
Controls	691	0.1	12.6	87.3	
Patient with SSc	1030	1.0	11.7	87.3	0.10
Limited	593	0.5	12.8	86.7	0.51
Diffuse	383	1.8	9.1	89.0	0.002
Antibodies					
Anticentromere	292	0.7	13.0	86.3	0.36
Anti-topoisomerase I	170	2.9	7.1	90.0	0.0001
Anti-RNA polymerase III	186	1.1	11.8	87.1	0.15
ATA+ vs ATA- <sup>††</sup>	170	2.9	7.1	90.0	0.0026
	852	0.6	12.6	86.9	

<sup>†</sup> p values considered significant after correcting for multiple comparisons using the false discovery method and point-wise significance = 0.0051. <sup>††</sup> Controls used as reference for all comparisons except this one.

ies such as ACA, ATA, and ARA have also been used to categorize patients<sup>28,33</sup>. The presence of these antibodies is also associated with the degree of cutaneous involvement as well as visceral involvement. For example, ACA is found almost exclusively in patients with limited SSc and helps identify patients at higher risk for development of PHT and lower risk for development of interstitial lung disease<sup>33</sup>. In contrast, presence of ATA is often associated with diffuse SSc as well as a higher risk of interstitial lung disease and lower risk of PHT<sup>33</sup>. The association of *IL23R* SNP with risk of developing diffuse SSc and ATA-positive SSc is consistent with these observations. Accordingly, we hypothesized that *IL23R* would be associated with fibrosing alveolitis; however, no such association was observed. In contrast, a protective association of *IL23R* SNP with PHT was observed. It is important to note that PHT was not confirmed by right-heart catheterization in all patients unless this was deemed medically necessary by the practicing physician. These data

suggest that *IL23R* may help to identify a unique subset of patients with ATA-positive SSc who are at risk for developing PHT without interstitial lung disease.

Recently, a large number of studies have identified genes that are associated with development of multiple autoimmune diseases<sup>29,31,34-36</sup>. Results from these studies point to shared genes, often with functions involved in immune regulation, as risk factors for development of autoimmunity and, in turn, autoimmune diseases. For example, *PTPN22* has been associated with development of type I diabetes mellitus, rheumatoid arthritis, systemic lupus erythematosus (SLE), and SSc<sup>31,34,35</sup>. *IRF5*, a susceptibility gene for SLE, has been associated with development of diffuse SSc<sup>29,36</sup>. Our report identifies *IL23R* as a susceptibility factor for the ATA-positive subset of SSc. Other studies have shown that *IL23R* SNP are associated with a group of immune-mediated illnesses commonly thought to share similar immune alterations, including IBD, psoriasis, and AS<sup>11-16,37</sup>.

Table 5A. Distribution of the *IL23R* SNP rs11209026 genotype and alleles in North American Caucasian patients with SSc and healthy controls. Odds ratios (95% CI) are for carriage of the minor allele.

	No.	Genotypic Comparison			p <sup>†</sup>	No.	Allelic Comparison			OR (95% CI)
		AA	AG %	GG			A	G %	p <sup>†</sup>	
Controls	693	0.1	12.0	87.9		1162	5.80	94.20		
Patients with SSc	1052	0.7	11.9	87.4	0.21	1968	7.00	93.00	0.17	0.81 (0.6–1.1)
Pulmonary fibrosis +SSc	266	1.5	12.0	86.5	0.03	532	7.50	92.50	0.17	0.75 (0.5–1.1)
Pulmonary hypertension (PHT) +SSc	133	3.8	14.3	82	0.00003	266	10.90	89.10	0.005	0.53 (0.3–0.8)
Systemic renal crisis +SSc	34	0	17.6	82.4	0.6	68	8.80	91.20	0.3	0.63 (0.3–1.7)
Pulmonary fibrosis –SSc	440	0.9	11.8	87.3	0.17	880	6.80	93.20	0.33	0.83 (0.6–1.3)
Pulmonary hypertension –SSc	556	0.5	11.5	87.9	0.46	1112	6.30	93.70	0.6	0.91 (0.6–1.3)
Systemic renal crisis –SSc	652	1.2	11.8	87	0.05	1304	7.10	92.90	0.17	0.79 (0.6–1.1)
PHT+ vs PHT– <sup>††</sup>										
PHT+	133	3.8	14.3	82	0.005	266	10.90	89.10	0.009	0.55 (0.3–0.9)
PHT–	556	0.5	11.5	87.9		1112	6.30	93.70		

<sup>†</sup> p values considered significant after correcting for multiple comparisons using the false discovery method and point-wise significance = 0.0051. <sup>††</sup> Controls used as reference for all comparisons except this one.

Table 5B. Distribution of the *IL23R* SNP rs11465804 genotype and alleles in North American Caucasian patients with SSc and healthy controls. Odds ratios (95% CI) are for carriage of the minor allele.

	No.	Genotypic Comparison			p <sup>†</sup>	No.	Allelic Comparison			OR (95% CI)
		GG	GT %	TT			G	T %	p <sup>†</sup>	
Controls	691	0.1	12.6	87.3		1158	6.00	94.00		
Patients with SSc	1030	1.0	11.7	87.3	0.14	1924	7.20	92.80	0.23	0.83 (0.6–1.1)
Pulmonary fibrosis +SSc	260	1.5	11.9	86.5	0.03	520	7.50	92.50	0.26	0.79 (0.5–1.3)
Pulmonary hypertension (PHT) +SSc	129	3.9	16.3	79.8	0.00001	258	12.00	88.00	0.001	0.50 (0.3–0.8)
Systemic renal crisis +SSc	33	0	15.2	84.8	0.9	66	7.60	92.40	0.38	0.79 (0.3–2.5)
Pulmonary fibrosis –SSc	431	1.2	12.8	86.1	0.07	862	7.50	92.50	0.06	0.79 (0.6–1.1)
Pulmonary hypertension –SSc	546	0.7	11.7	87.5	0.25	1092	6.60	93.40	0.59	0.91 (0.6–1.3)
Systemic renal crisis –SSc	639	1.4	12.2	86.4	0.03	1278	7.50	92.50	0.15	0.79 (0.6–1.1)
PHT+ vs PHT– <sup>††</sup>										
PHT+	129	3.9	16.3	79.8	0.006	258	12.00	88.00	0.003	0.52 (0.3–0.8)
PHT–	546	0.7	11.7	87.5		1092	6.60	93.40		

<sup>†</sup> p values considered significant after correcting for multiple comparisons using the false discovery method and point-wise significance = 0.0051. <sup>††</sup> Controls used as reference for all comparisons except this one.

Together, these studies suggest that common biological pathways of immune dysregulation can lead to the development of a variety of autoimmune and inflammatory diseases, but are not able to explain why certain individuals preferentially develop one autoimmune disease over another. Investigation of how genetic variations regulate these biological pathways will improve our understanding of the pathogenesis of these complex polygenic disorders.

In contrast to our findings, it has been reported that *IL23R* SNP do not confer risk for development of SSc<sup>38,39</sup>. The factors responsible for these discrepant results could be differences in the *IL23R* SNP in each study, differences in the LD of *IL23R* SNP in various populations, lack of power to detect the change due to smaller sample size, or variations in the minor allele frequencies between different ethnic groups based on the genetic drift and isolation of that ethnicity in their population history. In our study, Caucasian,

African American, and Hispanic populations showed associations of different *IL23R* SNP with SSc. This suggests that there are differences in the *IL23R* SNP in these 3 cohorts, which might explain the lack of association in the Dutch and Spanish populations compared to our North American cohorts. Perhaps differences in the frequencies of clinical features and SSc-associated autoantibodies (ACA, ATA, ARA) among the 2 studies along with the small sample size of the former<sup>38</sup> may be responsible for the lack of association. No clinical information on the SSc patients was reported in the Hungarian cohort<sup>39</sup>. The combined Dutch and Spanish cohorts comprised 518 SSc patients, but only 100 (19%) were positive for ATA<sup>38</sup>. Our cohort had a similar percentage of ATA-positive patients, but the total number of ATA-positive SSc patients was 256. As the association of *IL23R* was found only in ATA-positive patients in our study, it is reasonable to postulate that the previous studies<sup>38,39</sup>

were not sufficiently powered to detect an association in the ATA-positive subset of SSc patients. Unfortunately, a meta-analysis of previous studies and the current report could not be performed because the allelic and genotypic frequencies of the clinical and autoantibody subsets of SSc were not described in those studies. Finally, the previous studies did not test for *IL23R* SNP associations with visceral involvement (e.g., PHT) in patients with SSc.

It has been hypothesized that Th17 cells might play a role in the pathogenesis of SSc. Indeed, plasma concentrations of IL-17 and IL-23 were reported to be elevated in Japanese patients with SSc from 2 cohorts<sup>17,18</sup>. Further, peripheral blood T cells from SSc patients have been reported to produce increased levels of IL-17<sup>17</sup>. These studies point to an increase in Th17 cytokine activity in SSc. The link between PHT and Th17 cells or IL-23 is less clear. Neither IL-17 nor IL-23 has been reported to be elevated in PHT. However, cytokines such as IL-6 and TGF- $\beta$  that promote a Th17 response are increased in PHT<sup>40</sup>. There is some evidence to support the cross-regulation of IL-17/IL-23 and some of the molecular mediators of PHT such as nitric oxide, endothelin, and prostaglandin E<sub>2</sub>; however, this remains speculative<sup>41-43</sup>. Studies are needed to determine how the polymorphisms in the *IL23R* alter *IL23R* signaling and Th17 development and how this may relate to the pathogenesis of SSc and SSc-associated PHT.

Despite our large cohort (> 1400 SSc patients), our study is limited due to the absence of a replication cohort. Efforts are under way to determine if these SNP also are associated with SSc susceptibility in other populations. Our findings emphasize that these studies need to be sufficiently powered with ATA-positive patients, as *IL23R* SNP are not associated with ACA- or ARA-positive SSc. Another limitation of our study is that our findings are directly applicable to a North American Caucasian population. The ethnicity we reported was self-defined and the possibility of mixed populations within this group should be considered when interpreting these findings. We found no associations of *IL23R* SNP with subsets of SSc in African Americans or Hispanics or upon combination of the 3 cohorts by Mantel-Haenszel test. It is possible that there may be multiple SNP on the *IL23R* gene involved in SSc susceptibility that are unique to each ethnic/regional population but are in LD with the undetected causal SNP. Higher resolution genotyping or DNA sequencing of the *IL23R* gene would be required to identify the SNP involved in SSc pathogenesis, and to determine the variation in genetic structure of this gene in various populations.

We identified *IL23R* as a susceptibility gene for development of ATA-positive SSc that is protective against the development of pulmonary hypertension. These data extend our understanding of the complex immunopathogenesis of SSc and provide support for a potential role of Th17 cells in the immunopathogenesis of SSc. The association of *IL23R*

SNP and ATA-positive SSc may have important implications in the identification of potential therapeutic targets in SSc.

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