An allograft inflammatory factor 1 (AIF1) single nucleotide polymorphism (SNP) is associated with anticentromere antibody positive systemic sclerosis

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Objective. To identify genetic associations between allograft inflammatory factor 1 (AIF1) and systemic sclerosis (SSc), or its subsets, using a single nucleotide polymorphism (SNP) in a replicate case-control study.

Methods. The frequencies of alleles and genotypes of an SNP, rs2269475, for the AIF1 gene were examined in two large independent cohorts of SSc patients (n=1015 total), and compared with two groups of normal controls (n=893 total). Both cases and controls were stratified by ethnicity (Caucasian, African American and Hispanic) and by autoantibody status [anti-centromere antibodies (ACA) and antitopoisomerase I antibody (ATA)].

Results. The minor T allele and CT/TT genotype frequencies of the AIF1 SNP were not observed more frequently in SSc patients of the three ethnic groups (individually or combined) when compared with controls. On the other hand, T and CT/TT frequencies were significantly increased in ACA-positive Caucasian SSc patients, and all ACA-positive SSc patients (the three ethnic groups combined), when compared with ACA-negative SSc patients and with normal controls, with odds ratios of ~1.5.

Conclusion. The data demonstrate a genetic association between AIF1 and the ACA-positive subset of SSc. This polymorphism is a non-synonymous substitution and therefore likely to represent an important functional change in AIF1. Since vascular pathology is a prominent feature in ACA-positive SSc patients, the observed association with a vasculotrophic inflammatory gene is biologically plausible and warrants further research.

KEY WORDS: Allograft inflammatory factor 1, AIF1, Systemic sclerosis, Scleroderma, Genetics, Anticentromere antibodies, Autoantibodies.

Introduction

Allograft inflammatory factor-1 (AIF-1) is a 143 amino acid, 17 kDa, cytoplasmic calcium-binding protein, that is encoded within the HLA class III genomic region on chromosome 6p21, in close proximity to the tumour necrosis factor (TNF) cluster of genes [1]. It is a highly conserved and constitutively expressed molecule across different species suggesting that it plays an important role in inflammation. AIF-1 is produced by macrophages and its synthesis is mediated by several cytokines, including interferon-γ (INF-γ) and transforming growth factor β (TGFβ) [1].

One of the hallmarks of systemic sclerosis (SSc) is microvascular dysfunction that precedes clinical fibrosis. In this context, it is interesting to note that AIF-1 is not detected in normal arteries or in unstimulated cultured human vascular smooth muscle cells (VSMCs); however, it is readily detected after vascular injury [2], and after stimulation of cultured VSMCs with pro-inflammatory cytokines, such as tumour necrosis factor-alpha (TNF-α). AIF1 overexpression induces VSMC activation and proliferation [3, 4], and attenuation of its expression in macrophages induces apoptosis, and impedes migration [5]. Silencing of AIF1 with small interfering RNA (siRNA) in macrophages inhibits their proliferation and migration and, interestingly, these functions were restored after reintroduction of AIF1 using an adenovirus carrier [6]. AIF1 expression also has been reported in activated T lymphocytes, and has been found to remarkably enhance lymphocyte activation [7], which correlates with the severity of rejection after cardiac transplantation [2, 8].

Recently, we compared the transcriptional profiles of peripheral blood cells (PBCs) from individuals with early and progressing SSc with those from matched healthy controls using oligonucleotide microarrays and reverse transcription-polymerase chain reaction (RT-PCR) [9]. We identified 18 interferon-inducible genes with demonstrable transcript expression, as well as a group of more selective vasculotrophic genes, including AIF1, several selectins and integrins, suggesting their involvement in the vasculopathy of scleroderma. In addition, expression of a number of these genes, including AIF1, was also increased in whole skin biopsies from SSc patients [10], but not in cultured SSc fibroblasts [11], thus suggesting an origin from cell types in the skin other than fibroblasts.

A recent study by Del Galdo et al. [12] examined AIF1 expression in SSc, documenting its presence in affected blood vessels of the lung and skin from SSc patients. They also showed that AIF1 mRNA is overexpressed in affected skin lesions, specifically in T-cells, macrophages and endothelial cells and that TGFβ is able to stimulate AIF1 expression in peripheral mononuclear blood cells from SSc patients, further suggesting a potentially important role of this molecule in SSc pathogenesis.

A preliminary report by the same group [13] had suggested a genetic association of AIF1 SNPs with SSc, including those studied here: rs2269475 and rs4711274. The first SNP is a non-synonymous, encoding a tryptophan to arginine substitution at amino acid residue 15, located in exon 4 of the AIF1 gene. The second SNP is located in intron 2 of the gene. Thus, we undertook a study to identify these AIF-1 SNPs allele and genotype frequencies in SSc patients and compare them with normal controls in our two large cohorts of SSc patients and controls. We also assessed the AIF1 SNPs genotype frequencies of AIF1 using an adenovirus carrier [6]. AIF1 expression also has been reported in activated T lymphocytes, and has been found to remarkably enhance lymphocyte activation [7], which correlates with the severity of rejection after cardiac transplantation [2, 8].

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in two autoantibody subsets of SSc, ACA positive and ATA positive patients, hypothesizing that correlations of AIF-1 polymorphisms might be more predominant in one or more autoantibody subsets.

Materials and methods

Patient selection

The two multiethnic large SSc cohorts included: (i) the Scleroderma Family Registry and DNA Repository cohort of 548 SSc patients (or the ‘registry’); and (ii) the Genetics vs Environment in Scleroderma Outcomes Study (GENISOS) combined with the UTH Rheumatology Division cohort of 467 SSc patients (or the ‘division’). The two cohorts together contained 1015 SSc patients (763 Caucasians, 135 African Americans and 117 Hispanics). All patients fulfilled the American College of Rheumatology (ACR) criteria for the diagnosis of SSc [14]. The registry is a cross sectional cohort of SSc patients collected from across the US. GENISOS is a prospective longitudinal study of SSc patients with early onset disease (<5 yrs) from Southeast Texas, who have been enrolled and are being actively followed at our collaborating centres. GENISOS and UTH division SSc patients (who were not included in GENISOS because of longer disease duration or being no longer available) were combined. The characteristics of these cohorts have been presented elsewhere [15, 16]. The control groups consisted of 893 normal controls (582 Caucasians, 170 African Americans and 141 Hispanics). Registry controls were primarily spouse or friend controls, and division controls were healthy medical centre personnel or blood bank donors from the local Houston area. During recruitment, special effort was made to enroll comparable numbers of controls from each ethnicity to those enrolled in the SSc group; however, this was limited by availability. At the time of enrolment, all study subjects provided written informed consent that authorized future genetic testing. The study was approved by the UTH Committee for the Protection of Human Subjects and the other collaborating institutions’ respective institutional review boards. Peripheral blood samples had been collected at the time of enrolment and genomic DNA extracted and serum separated for further studies, including autoantibodies.

Autoantibody identification

All serum samples were tested for antinuclear antibodies (ANA) using indirect immunofluorescence (IIF) and HEp-2 cells as antigen substrate (Antibodies Inc., Davis, CA, USA). ACA were determined by their distinctive IIF pattern on HEp-2 cells. ATA were determined by passive immunofluorescence against calf thymus extract using commercially available kits (Inova Diagnostics, San Diego, CA, USA).

SNP genotyping

We analysed the allele and genotype frequencies of the SNP using an ABI TaqMan 5’-allele discrimination assay-by-design method on an ABI 7900HT sequence detection system. Automated allele calling was performed by allelic discrimination plots using SDS 2.2.2 software. Standard control DNA was added in replicates to minimize error and check genotyping quality.

Statistical analysis

Statistical analysis was performed using SAS 9.1.3 (http://www.sas.com). Chi-square or Fisher’s exact tests were used to compare allele or genotype frequencies between comparison groups. Mantel–Haenszel tests were conducted to compare allele or genotype frequencies between comparison groups to control the confounding effect of ethnicity. Stepwise logistic regression analyses were used to identify significant independent risk factors associated with SSc cases and autoantibody subsets.

Results

We analysed the allelic and genotype frequencies in both the registry and the division cohorts, first independently and then combined, for the selected SNPs, stratified by ethnic group and compared them with alleles and genotype frequencies in their respective controls. All genotype frequencies in cases and controls of both cohorts and in all ethnicities were found to be in Hardy–Weinberg equilibrium. We then analysed the SNP frequencies based on autoantibody status (ACA- or ATA-positive) and compared those with controls. Last, SNP frequencies of ACA-positive SSc patients were compared with those of ACA-negative patients. The intronic SNP (rs4711274) showed no association with SSc or any autoantibody subset thereof. The presented data below thus refer to the exonic SNP (rs2269475).

The number of Caucasian SSc patients and controls provided adequate statistical power in both cohorts, but definitive conclusions could not be obtained in the African American or Hispanic groups individually because of smaller sample sizes. We used the Mantel–Haenszel test to control for the confounding effects of ethnicity.

AIF1 SNP is associated with ACA-positive SSc

Compared with their respective controls, we found no association of the AIF1 SNP (rs2269475) alleles with all SSc patients in either cohort separately or combined, regardless of ethnicity (P = 0.17) (Table 1). There also was no association with the limited or diffuse subgroups (data not shown). Significantly higher frequencies of the minor T allele, however, were observed in the ACA-positive SSc subgroup when the allele frequencies were compared with controls in both cohorts (P = 0.025 and 0.04 for the registry and the division cohorts, respectively). The odds ratios in both cohorts were similar (between 1.53 and 1.56) with comparable 95% confidence intervals. The association was most significant when the two cohorts were combined; Caucasians (P = 0.006) and all ethnic groups combined (P = 0.002). In contrast, no association was found when the ATA-positive or other autoantibody positive subgroups were compared with controls (data not shown).

In keeping with the allele comparison results, we observed no AIF1 SNP genotype frequencies association when all SSc patients were compared with controls (P = 0.18) (Table 2). However, higher frequencies of the CT and TT genotypes (combined) were observed in the ACA-positive SSc subgroup when the allele frequencies were compared with controls in both cohorts (P = 0.036, 0.05 and 0.004 for the registry and the division cohorts, respectively). The odds of both cohorts were similar (between 1.53 and 1.56) with comparable 95% confidence intervals. The association was most significant when the two cohorts were combined; Caucasians (P = 0.98) and all ethnic groups combined (P = 0.98). In contrast, no association was found when the ATA-positive or other autoantibody positive subgroups were compared with controls (data not shown).

### Table 1. Differential frequencies of AIF1 alleles rs2269475 in Caucasians and among all three ethnic groups in the registry and division cohorts combined, compared with controls

<table>
<thead>
<tr>
<th>Allele</th>
<th>Caucasians</th>
<th>AIF1-positive SSc</th>
<th>AIF1-negative SSc</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>(n = 763)</td>
<td>(n = 205)</td>
<td>(n = 557)</td>
<td>(n = 582)</td>
</tr>
<tr>
<td>T</td>
<td>86.5%</td>
<td>82.4%</td>
<td>88.0%</td>
<td>87.8%</td>
</tr>
<tr>
<td></td>
<td>13.5%</td>
<td>17.6%</td>
<td>12.0%</td>
<td>12.2%</td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td>1.12 (0.89–1.42)</td>
<td>1.53 (1.11–2.11)</td>
<td>0.98 (0.76–1.28)</td>
<td></td>
</tr>
<tr>
<td>P&lt;</td>
<td>0.006</td>
<td>0.002</td>
<td>0.591</td>
<td></td>
</tr>
</tbody>
</table>

*by chi-square or Fisher’s exact test.

Statistically significant values are put in bold.
**Table 2. Differential frequencies of AIF1 genotypes for rs2269475 SNP in Caucasians and among all three ethnic groups in the registry and division cohorts combined, compared with controls**

<table>
<thead>
<tr>
<th></th>
<th>ACC-positive SSc</th>
<th>ACC-negative SSc</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>((n = 763))</td>
<td>((n = 557))</td>
<td>((n = 582))</td>
</tr>
<tr>
<td>CC</td>
<td>75.1%</td>
<td>77.4%</td>
<td>77.3%</td>
</tr>
<tr>
<td>CT/TT</td>
<td>24.9%</td>
<td>22.6%</td>
<td>22.7%</td>
</tr>
<tr>
<td>(p^a)</td>
<td>0.344</td>
<td>0.015</td>
<td>0.981</td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td>1.13 (0.87–1.47)</td>
<td>1.00 (0.75–1.33)</td>
<td></td>
</tr>
<tr>
<td>All ethnicities ((n = 1015))</td>
<td>0.18</td>
<td>0.727</td>
<td></td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td>1.15 (0.93–1.43)</td>
<td>1.61 (1.16–2.22)</td>
<td>1.04 (0.83–1.31)</td>
</tr>
</tbody>
</table>

\(a\)By chi-square or Fisher's exact test.

\(b\)By Mantel-Haenszel test.

**Table 3. Differential frequencies of AIF1 alleles for rs2269475 SNP in ACA-positive SSc Caucasians and among all three ethnic groups in the registry and division cohorts combined with ACA-negative SSc subjects**

<table>
<thead>
<tr>
<th></th>
<th>ACA-positive SSc</th>
<th>ACA-negative SSc</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>((n = 205))</td>
<td>((n = 557))</td>
</tr>
<tr>
<td>C</td>
<td>82.4%</td>
<td>86.0%</td>
</tr>
<tr>
<td>T</td>
<td>17.6%</td>
<td>12.0%</td>
</tr>
<tr>
<td>(p^a)</td>
<td>0.005</td>
<td></td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td>1.56 (1.13–2.15)</td>
<td></td>
</tr>
<tr>
<td>All ethnicities ((n = 233))</td>
<td>0.004</td>
<td></td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td>1.54 (1.16–2.04)</td>
<td></td>
</tr>
</tbody>
</table>

\(a\)By chi-square or Fisher's exact test.

\(b\)By Mantel-Haenszel test.

**Discussion**

In this study, we present evidence that an SNP of AIF1, a vasculotrophic gene that is overexpressed in SSc [9, 10, 12], correlates with ACA positivity. This association was demonstrated by both allele and genotype frequency comparisons, as well as logistic regression analysis using two relatively large, multiethnic and independent cohorts of SSc patients (Tables 1–3). Sample size remains a key factor in detecting a significant association and may explain the lack of significant association among African American and Hispanic SSc patients for this AIF1 SNP [19], whose sample sizes were relatively small. Also, this lack of association is not unexpected as it has been demonstrated that ACA positivity is more common in Caucasian than in African American or Hispanic SSc patients [20]. Nonetheless, it is noteworthy that the point estimates of the odds ratio for the African American and Hispanic groups were also >1.0 indicating that this might be due to a lack of statistical power rather than lack of a true association.

This particular AIF1 SNP results in a non-synonymous substitution and is very likely to cause an alteration of the function of the protein. Sequence analysis of this amino acid substitution showed that this SNP was predicted to be ‘damaging’ according to the PolyPhen (polymorphism phenotyping) database for prediction of functional importance of SNPs [21]. Furthermore, three splice variants (i.e. isoforms) have been identified for AIF1 and isoform 2 was recently found to be differentially overexpressed in SSc tissue relative to the other two isoforms, especially after stimulation with TGFB[12]. In this context, we find it intriguing that this SNP is exonic resulting in the non-synonymous substitution only in isoform 2 and not the other two isoforms. This might suggest that an alteration of the relative expression of the three isoforms could potentially be important in SSc pathogenesis.

Although our study was not designed to directly answer the question of the functional significance of this SNP in SSc subjects but rather to address the question of an underlying genetic association with SSc or its subsets, the finding of such an association is in and of itself noteworthy. This association of a vasculotrophic gene in ACA-positive (but not ATA or other autoantibody positive) SSc patients is potentially biologically relevant.

Vascular pathology is a feature of both diffuse and limited scleroderma, as well as ACA- and ATA-positive patients.
Nevertheless, the ACA-positive subset of SSc tends to have more prominent clinical manifestations of microvascular dysfunction [22]. In addition, vasculopathy appears to play a more prominent role in limited SSc patients who are ACA positive and is manifested by Raynaud’s phenomenon and nailfold capillary changes, which are some of the earliest clinical signs of SSc, usually predating fibrosis of the skin and internal organs [23].

Although the molecular basis for this vasculopathic process remains unclear, there is increasing evidence that endothelial damage results from autoantibodies directed against the endothelium, which induces apoptosis [24, 25]. The specificities of these autoantibodies are yet to be determined. In addition, refractory Raynaud’s phenomenon, recurrent digital ulceration and progressive late-onset pulmonary arterial hypertension are all well-recognized complications associated with ACA positivity [22].

In conclusion, the described AIF1 association with ACA positivity in SSc merits further research, especially in those SSc patients suffering from vascular complications who also have ACA. Moreover, it may further indicate that SSc is genetically heterogeneous and that different autoantibody subsets may be associated with distinct susceptibility genes, similar to what is observed in HLA associations. It also raises the question of whether the different autoantibody subsets of SSc may arise from different genetic susceptibility loci, as previously suggested by a variety of HLA associations with SSc autoantibodies.

**Rheumatology key messages**

- AIF1 is associated with ACA-positive SSc.
- AIF1 could be involved in the vasculopathy of SSc.

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**References**