An MIF Promoter Polymorphism Is Associated with Susceptibility to Pulmonary Arterial Hypertension in Diffuse Cutaneous Systemic Sclerosis


ABSTRACT. Objective. Systemic sclerosis (SSc) is a fibrotic immune-mediated disease of unknown etiology. Among its clinical manifestations, pulmonary involvement is the leading cause of mortality in patients with SSc. However, the genetic factors involved in lung complication are not well defined. We aimed to review the association of the MIF gene, which encodes a cytokine implicated in idiopathic pulmonary hypertension among other diseases, with the susceptibility and clinical expression of SSc, in addition to testing the association of this polymorphism with SSc-related pulmonary involvement.

Methods. A total of 4392 patients with SSc and 16,591 unaffected controls from 6 cohorts of European origin were genotyped for the MIF promoter variant rs755622. An inverse variance method was used to metaanalyze the data.

Results. A statistically significant increase of the MIF rs755622*C allele frequency compared with controls was observed in the subgroups of patients with diffuse cutaneous SSc (dcSSc) and with pulmonary arterial hypertension (PAH) independently (dcSSc: p = 3.20E–2, OR 1.13; PAH: p = 2.19E–02, OR 1.32). However, our data revealed a stronger effect size with the subset of patients with SSc showing both clinical manifestations (dcSSc with PAH: p = 6.91E–3, OR 2.05).

Conclusion. We reviewed the association of the MIF rs755622*C allele with SSc and described a phenotype-specific association of this variant with the susceptibility to develop PAH in patients with dcSSc. (First Release July 1 2017; J Rheumatol 2017;44:1453–7; doi:10.3899/jrheum.161369)

Key Indexing Terms:
SYSTEMIC SCLEROSIS MIF rs755622 PULMONARY ARTERIAL HYPERTENSION
Systemic sclerosis (SSc) is a complex disease of unknown etiology influenced by both genetic and environmental factors. It is characterized by a progressive tissue fibrosis along with vascular anomalies and dysfunction, and the presence of autoantibodies directed to different cellular structures, mainly antitopoiso- merase (anti-topo I) and anticientromere antibodies (ACA). Depending on the extent of the skin implication, SSc is classified as diffuse cutaneous SSc (dcSSc) or limited cutaneous SSc (lcSSc). Among the clinical manifestations, pulmonary fibrosis (PF) and pulmonary arterial hypertension (PAH) are the leading causes of death in patients with SSc. These pulmonary complications can be present separately as interstitial lung disease (ILD) or isolated PAH, or combined.

In recent years, great advances have been made in the determination of the genetic component of SSc. Nevertheless, we are still far from its complete understanding, especially regarding pulmonary involvement, in which only a few associated genes have been described.

Macrophage migration inhibitory factor (MIF) gene encodes a constitutively expressed protein that seems to have an important role in autoimmune and inflammatory processes. Infections, proinflammatory cytokines, and antigen-specific activation can also lead to an increased expression of MIF. The promoter region of this gene contains a single-nucleotide polymorphism (SNP) at position -173 (rs755622) that has been associated with several immune-mediated diseases, including SSc and systemic lupus erythematosus. This SNP is in high linkage disequilibrium (LD) with a functional polymorphism, a $\sim$794 CATT microsatellite repeat (rs5844572) also located at the promoter region of the gene.

In our present study, we have analyzed for the first time, to our knowledge, the possible involvement of the MIF rs755622 polymorphism in the susceptibility to develop PF and PAH in patients with SSc.

**MATERIALS AND METHODS**

Samples. Overall, 4392 patients with SSc and 16,591 unaffected controls from Spain, Germany, the Netherlands, Italy, the United Kingdom, and Norway were included in our present study. The cohorts included in our study were partially overlapping with the cohorts in Bossini-Castillo, et al, based on the presence of pulmonary involvement clinical data. In total, the overlap between our global study cohort and the one included in Bossini-Castillo, et al’s study was 50%. All patients were classified as having dcSSc or lcSSc, following the criteria described by LeRoy, et al. Patients were additionally classified accordingly with the presence or absence of ACA and anti-topo I. PF was diagnosed by the presence of interstitial abnormalities in high-resolution computed tomography (HRCT) and forced vital capacity (FVC) lower than 60%. Pulmonary hypertension was considered PAH if FVC was higher than 60% or there was no moderate-severe extent of ILD in HRCT. Thus, patients were defined as PAH if they showed a mean resting pulmonary artery pressure $\geq$ 25 mmHg at the time of a right heart catheterization, pulmonary artery wedge pressure $\leq$ 15 mmHg, and FVC > 60%.

The control population consisted of unrelated healthy individuals recruited in the same geographic regions as the patients with SSc.

The local ethical committees from all the contributing centers approved the project in compliance with the Helsinki Agreement (Valle de Hebron Hospital, 12 de Octubre University Hospital, San Cecilio University Hospital, Hospital de la Santa Creu i Sant Pau, Carlos Haya Hospital, San Carlos Hospital, Bellvitge University Hospital, Virgen del Rocio Hospital, San Jorge General Hospital, Fondazione IRCCS Ca’ Granda Ospedale Maggiore Policlinico Foundation, Università degli Studi di Verona University, Spedali Civili Hospital, Osaka University Hospital, Josefs-Hospital, Hannover Medical School, University of Cologne, Radboud University Nijmegen Medical Centre, University of Dusseldorf, University of Milan; C. Lunardi, MD, Clinic of Rheumatology, University of Lübeck; T. Witte, MD, Klinik für Immunologie und Rheumatologie, Hannover Medical School; N. Hunzelmann, MD, Department of Dermatology, University of Cologne; M.C. Vönk, MD, Department of Rheumatology, Radboud University Nijmegen Medical Centre; A.E. Voskuyl, MD, VU University Medical Center; J. de Vries-Bouwstra, MD, Department of Rheumatology, Leiden University Medical Center; P. Shieh, MD, Glasgow Biomedical Research Centre, University of Glasgow; A. Herrick, MD, Arthritis Research UK Epidemiology Unit, The University of Manchester, Manchester Academic Health Science Centre; J. Worthington, PhD, Arthritis Research UK Epidemiology Unit, The University of Manchester, Manchester Academic Health Science Centre; T.R. Radstake, MD, Department of Rheumatology and Clinical Immunology, University Medical Center Utrecht; J. Martin, MD, PhD, Instituto de Parasitología y Biomedicina López-Neyra, IBPLN-CSIC. L. Bossini-Castillo, D. Campillo-Davó, and E. López-Iscà contributed equally to this study.

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RESULTS

We first investigated whether there were significant differences between the rs755622 allele frequencies of the whole SSc group or the different stratified groups (by clinical/auto-antibody phenotypes) and those of the control set. Supplementary Table 2 (available with the online version of this article) summarizes the results for the analyses of the independent cohorts and Table 1 shows the results of the pooled analysis. As observed in Table 1, a significant p value was obtained when the dcSSc group was compared against the control set in the metaanalysis (p = 3.20E–2, OR 1.13, 95% CI 1.01–1.26). Moreover, there was a significant increase of the minor allele frequency in the group of patients with SSc affected by PAH compared with controls (p = 2.19E–02, OR 1.32, 95% CI 1.04–1.67). However, no association was observed between rs755622 and the PF-positive SSc group (PF+ vs controls: p = 0.257; PF+ vs PF−: p = 0.737).

To dissect the genetic association of rs755622*C with the SSc clinical phenotypes, we classified the patients into 4 additional subgroups based on the SSc subtype and the presence/absence of PAH. Then we carried out a pooled analysis of the 6 cohorts by comparing these new subsets against controls (Table 2; see Supplementary Table 3 for the results of each cohort independently, available with the online version of this article). A considerably significant difference in the rs755622 frequencies between controls and the subgroup of patients with both dcSSc and PAH phenotypes was observed (dcSSc with PAH vs controls: p = 6.91E–03, OR 2.05, 95% CI 1.30–4.05). However, no association was evident in the analysis of the other subgroups against controls (lcSSc with PAH vs controls: p = 0.178; dcSSc without PAH vs controls: p = 0.086; lcSSc without PAH vs controls: p = 0.436). We also did not find evidence of association between MIF rs755622 and SSc-related PF and dcSSc-related PF (data not shown).

DISCUSSION

Our study, which consists of the largest cohort of patients with SSc-associated PAH analyzed to date, clearly suggests that the MIF rs755622*C variant is a firm genetic risk factor for the susceptibility to PAH in patients with dcSSc. Previous reports described an association of this SNP with the diffuse form of the disease7,8. Consistent with this, we also observed a significant increase in the MIF rs755622*C frequency in the dcSSc subgroup compared with the control set. However, subphenotype analyses indicated that the significant associations detected in dcSSc and the PAH+ subgroups may rely on the presence of subjects with both phenotypes in each dataset. Therefore, those carrying the MIF rs755622*C allele are at a higher risk of having dcSSc with PAH rather than PAH or dcSSc independently. The strongest effect size of MIF rs755622*C was observed in the subset of patients

Table 1. Pooled analyses of MIF rs755622 in patients with SSc, SSc phenotypic and serological subgroups, and unaffected controls.

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>MAF, %</th>
<th>p*</th>
<th>OR (CI 95%)**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls, n = 16,591</td>
<td>15.93</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SSC, n = 4392</td>
<td>16.32</td>
<td>0.093</td>
<td>1.06 (0.99–1.14)</td>
</tr>
<tr>
<td>lcSSc, n = 3058</td>
<td>15.89</td>
<td>0.331</td>
<td>1.04 (0.96–1.12)</td>
</tr>
<tr>
<td>dcSSc, n = 1334</td>
<td>17.25</td>
<td>0.032</td>
<td>1.13 (1.01–1.26)</td>
</tr>
<tr>
<td>ACA+, n = 1814</td>
<td>15.93</td>
<td>0.301</td>
<td>1.05 (0.96–1.17)</td>
</tr>
<tr>
<td>Anti-topo I+, n = 1079</td>
<td>16.68</td>
<td>0.071</td>
<td>1.12 (0.99–1.27)</td>
</tr>
<tr>
<td>PF+, n = 1274</td>
<td>16.25</td>
<td>0.257</td>
<td>1.07 (0.95–1.19)</td>
</tr>
<tr>
<td>PAH+, n = 230</td>
<td>19.13</td>
<td>0.0219</td>
<td>1.32 (1.04–1.67)</td>
</tr>
</tbody>
</table>

Six white European cohorts are included: Spain, Germany, the Netherlands, Italy, the United Kingdom, and Norway. * Unaffected controls are used as reference for the global set of disease and for each phenotype comparison. ** OR for the minor allele. SSc: systemic sclerosis; MAF: minor allele frequency; lc SSC: limited cutaneous SSc; dcSSc: diffuse cutaneous SSc; ACA: antientromere antibodies; anti-topo I: antitopoisomerase antibodies; PF: pulmonary fibrosis; PAH: pulmonary arterial hypertension.

Table 2. Pooled analyses of MIF rs755622 with the SSc subtype and PAH status.

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>MAF, %</th>
<th>p*</th>
<th>OR (CI 95%)**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls, n = 16,589</td>
<td>15.99</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DcSSc with PAH, n = 34</td>
<td>27.94</td>
<td>6.91E–03</td>
<td>2.05 (1.30–4.05)</td>
</tr>
<tr>
<td>LcSSc with PAH, n = 196</td>
<td>17.6</td>
<td>0.178</td>
<td>1.20 (0.92–1.56)</td>
</tr>
<tr>
<td>DcSSc without PAH, n = 951</td>
<td>16.39</td>
<td>0.086</td>
<td>1.12 (0.98–1.28)</td>
</tr>
<tr>
<td>LcSSc without PAH, n = 2013</td>
<td>15.76</td>
<td>0.436</td>
<td>1.04 (0.95–1.14)</td>
</tr>
</tbody>
</table>

Six white European cohorts are included: Spain, Germany, the Netherlands, Italy, the United Kingdom, and Norway. * Unaffected controls are used as reference in each comparison. ** OR for the minor allele. SSc: systemic sclerosis; PAH: pulmonary arterial hypertension; MAF: minor allele frequency; dcSSc: diffuse cutaneous SSc; lcSSc: limited cutaneous SSc.
having both dcSSc and PAH (OR 2.05), which clearly supports this idea. Therefore, our findings may suggest MIF rs755622*C as a marker for patients with dcSSc at risk of developing PAH.

In our set, the percentages of PAH in the different phenotype subsets, dcSSc or lcSSc, were 2.55% and 6.41%, respectively. That is, 14.78% of PAH+ cases were patients with dcSSc and 85.22% corresponded to lcSSc cases, in line with previous reports in SSc patient cohorts. However, as mentioned above, we found that the association observed between MIF rs755622 and the PAH+ group remained significant only under a dcSSc phenotype. In this regard, gene expression patterns specific for SSc, SSc-related PF, and SSC-related PAH have been described, thus indicating that each SSc phenotype may develop under a distinct molecular environment. The high specificity of the MIF rs755622 association with a particular subset of patients with SSc may be related to this fact.

MIF rs755622 is a promoter polymorphism that has been linked to the upregulation of the MIF expression in immune-mediated diseases. As mentioned, this SNP is in high LD with the functional CATT5-8 polymorphism, and it is still not established whether rs755622 influences MIF promoter activity by itself or whether the observed effect may be linkage to CATT5-8. Interestingly, elevated protein levels of this gene have been detected in the sera of patients with dcSSc affected by PAH. Moreover, Le Hiress, et al showed through in vitro studies that the MIF receptor (CD74) is overexpressed in individuals with idiopathic PAH. These authors also provided evidence for the effect of an MIF antagonist and anti-CD74–neutralizing antibodies on the reversion of PH in a rat model. MIF is a pleiotropic protein expressed in several human cell types that can act as a cytokine, hormonal, and immune modulatory factor. As a cytokine, MIF induces the expression and secretion of several immune mediators, including interleukin 6 (IL-6). Increased levels of IL-6 have been described in serum and lungs of patients with idiopathic PAH and those with dcSSc compared with lcSSc, and IL-6 has a proved effect on the generation and development of PAH in vivo. Moreover, allelic combinations of SNP in the IL-6 gene have been suggested as susceptibility factors for SSC. Based on the above, we speculate that MIF rs755622 could be influencing PAH development in dcSSc-affected individuals by promoting IL-6 secretion, which could contribute to the obstruction of small pulmonary vessels that leads to hypoxia, and eventually to PAH.

Here we provide novel insights into the genetic background of SSc-related PAH. We have shown that the MIF allele rs755622*C is associated with higher risk of being affected by this severe condition in patients with dcSSc, and our results may be helpful for a better evaluation of the prognosis in SSc and for the development of more effective personalized treatments.
REFERENCES