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

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***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. (J Rheumatol First Release July 1 2017; doi:10.3899/jrheum.161369)

*Key Indexing Terms:*
SYSTEMIC SCLEROSIS *MIF* rs755622 PULMONARY ARTERIAL HYPERTENSION

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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 antitopoisomerase (anti-topo I) and anticentromere 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[1]. These pulmonary complications can be present separately as interstitial lung disease (ILD) or isolated PAH, or combined[2].

In recent years, great advances have been made in the determination of the genetic component of SSc[3,4]. Nevertheless, we are still far from its complete understanding, especially regarding pulmonary involvement, in which only a few associated genes have been described[3,4].

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*[5,6]. 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[7,8,9]. This SNP is in high linkage disequilibrium (LD) with a functional polymorphism, a –794 CATT$_{5,6,7,8}$ microsatellite repeat (rs5844572) also located at the promoter region of the gene[10]. Interestingly, increased MIF protein levels have been reported in patients with idiopathic pulmonary fibrosis, pulmonary hypertension, and in individuals affected by SSc-associated PAH[11,12,13].

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*[7], 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[7] was 30%. All patients were classified as having dcSSc or lcSSc, following the criteria described by LeRoy, *et al*[14]. 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 ≥ 25 mmHg at the time of a right heart catheterization, pulmonary artery wedge pressure ≤ 15 mmHg, and FVC > 60%[15,16]. 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, de la Santa Creu i Sant Pau Hospital, Carlos Haya Hospital, San Carlos Hospital, Bellvitge University Hospital, Virgen del Rocio Hospital, San Jorge General Hospital, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinic Foundation, Università degli Studi di Verona University, Spedali Civili Hospital, Oslo University Hospital, Josefs-Hospital, University of Lübeck, Hannover Medical School, University of Cologne, Radboud University Nijmegen Medical Centre, VU University Medical Center, Leiden University Medical Center, Glasgow Biomedical Research Centre, Manchester University, and University Medical Center Utrecht). Patients and controls gave written informed consent for their participation in the present study.

*Genotyping*. DNA extraction was performed using standard methods. *MIF* rs755622 was genotyped using a TaqMan SNP genotyping assay (assay ID: C_2213785_10) in a Light Cycler 480 Real-Time PCR System (Roche Applied Science). We also used available *MIF* rs755622 genotyping data from a previously published study based on the Immunochip[17], a genotyping platform that was specifically designed for the study of the genetic component of immune-mediated diseases. Supplementary Table 1 (available with the online version of this article) shows the number of samples

genotyped in each platform. The genotyping call rate (the fraction of called samples per SNP over the total number of samples in the dataset) was 97% for the TaqMan assay and 99% for the Immunochip data. The genotype-genotype concordance was evaluated in 1253 samples with genotyping data from both platforms. A 100% of concordance was observed between both genotyping platforms.

*Statistical methods*. Using the Power Calculator for Association Studies (CaTS) software 2006 (Center for Statistical Genetics, The University of Michigan), and assuming an effect size of OR 1.2, we estimated that the statistical power of the study was 99% for the overall analysis of the whole cohort (SSc cases vs controls), 98% for the analysis of dcSSc and PF phenotypes, 61% for the analysis of patients who were PAH+, and 5% for the analysis with patients with dcSSc who have PAH. Because the statistical power depends on the OR of the SNP, we also calculated the statistical power for this last comparison assuming the observed effect size in our study, obtaining a 97% of statistical power. Additionally, no deviation from Hardy-Weinberg equilibrium was detected in our datasets.

All the statistical analyses were carried out with PLINK v1.07 (pngu.mgh.harvard.edu/purcell/plink). Association statistics for the 6 cohorts were calculated in each population by 2 × 2 contingency tables and the chi-square test. P values lower than 0.05 were considered statistically significant. The inverse-variance–weighted fixed-effects metaanalysis method was used for the pooled analyses.

## 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/autoantibody 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).

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

| Subgroup | MAF, % | p* | OR (CI 95%)** |
|---|---|---|---|
| Controls, n = 16,591 | 15.93 | | |
| SSc, n = 4392 | 16.32 | 0.093 | 1.06 (0.99–1.14) |
| LcSSc, n = 3058 | 15.89 | 0.331 | 1.04 (0.96–1.12) |
| DcSSc, n = 1334 | 17.25 | 0.032 | 1.13 (1.01–1.26) |
| ACA+, n = 1814 | 15.93 | 0.301 | 1.05 (0.96–1.17) |
| Anti-topo I+, n = 1079 | 16.68 | 0.071 | 1.12 (0.99–1.27) |
| PF+, n = 1274 | 16.25 | 0.257 | 1.07 (0.95–1.19) |
| PAH+, n = 230 | 19.13 | 0.0219 | 1.32 (1.04–1.67) |

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 the disease and for each phenotype comparison. ** OR for the minor allele. SSc: systemic sclerosis; MAF: minor allele frequency; lcSSc: limited cutaneous SSc; dcSSc: diffuse cutaneous SSc; ACA: anticentromere antibodies; anti-topo I: antitopoisomerase antibodies; PF: pulmonary fibrosis; PAH: pulmonary arterial hypertension.

## 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 disease[7,8]. 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 2*. Pooled analyses of *MIF* rs755622 with the SSc subtype and PAH status.

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

Six white European cohorts are included: Spain, Germany, the Netherlands, Italy, he 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[18,19]. 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[20], 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[5]. 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[13,21]. Moreover, Le Hiress, *et al* showed through *in vitro* studies that the MIF receptor (CD74) is overexpressed in individuals with idiopathic PAH[22]. 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[22]. MIF is a pleotropic 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)[23]. Increased levels of IL-6 have been described in serum and lungs of patients with idiopathic PAH and those with dcSSc compared with lcSSc[24,25], and IL-6 has a proved effect on the generation and development of PAH *in vivo*[24]. Moreover, allelic combinations of SNP in the *IL-6* gene have been suggested as susceptibility factors for SSc[26]. 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.

## APPENDIX 1.

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## ONLINE SUPPLEMENT

Supplementary material accompanies the online version of this article.

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