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Macrophage Migration Inhibitory Factor is not Associated with Sarcoidosis Susceptibility or Severity in Whites or Blacks

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Abstract. *Background:* Macrophage migration inhibitory factor (MIF) is an inflammatory cytokine, and increased *MIF* expression has been associated with the development and severity of multiple granulomatous, autoimmune diseases. However, *MIF* association studies have been discordant in sarcoidosis. *Objective:* To evaluate associations between macrophage migration inhibitory factor (*MIF*) promoter polymorphisms and sarcoidosis susceptibility and severity. *Methods:* Three hundred and fifty one patients with sarcoidosis were recruited through the Genomic Research in Alpha-1 Antitrypsin Deficiency and Sarcoidosis (GRADS) study. Genomic DNA was isolated from serum, and the *MIF* -173G/C SNP [*rs755622*] and *MIF* -794 $CATT_{5-8}$ microsatellite repeat [*rs5844572*] were genotyped. Allelic frequencies were compared between cases and healthy controls and associations between *MIF* alleles and sarcoidosis severity were assessed. *Results:* The frequencies of the high expression -173C SNP and the low expression -794 $CATT_5$ containing genotypes in white and black sarcoidosis patients were the same as those of healthy controls. High expression *MIF* alleles were not associated with sarcoidosis severity. Associations between *MIF* alleles and extrapulmonary sarcoidosis phenotypes were limited by small sample sizes. *Conclusions:* High expression *MIF* genotypes were not associated with the susceptibility to or severity of pulmonary sarcoidosis in a large North American cohort. *(Sarcoidosis Vasc Diffuse Lung Dis 2020; 37 (3): e2020004)*

Key words: macrophage migration inhibitory factor, sarcoidosis, GRADS, susceptibility, severity

Introduction

Macrophage migration inhibitory factor (MIF) is an inflammatory cytokine that sustains macrophage activation and suppresses glucocorticoid signaling (1). *MIF* expression is associated with two commonly occurring promoter polymorphisms. The -794 CATT microsatellite has 5 to 8 repeats, and longer length results in increased gene expression due to enhanced binding of the transcription factor ICBP90 (2). The high expression allele, -794 $CATT_{7,}$ is in linkage disequilibrium with the -173C single nucleotide polymorphism (SNP)(1), and this SNP sometimes reveals associations because of reduced locus heterogeneity. Increased *MIF* expression has been associated with the development and severity of multiple granulomatous, autoimmune diseases including granulomatosis with polyangitis (3), hypersensitivity pneumonitis (4), and idiopathic pulmonary fibrosis (5).

Despite these reports, *MIF* association studies have been discordant in sarcoidosis. Amoli et al.

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identified a greater frequency of the -173C allele in patients with erythema nodosum (EN) due to sarcoidosis compared to those with EN due to other etiologies (6). A subsequent study reported that the *MIF* -173C allele is associated with Lofgren's syndrome but not with susceptibility to sarcoidosis (7). Plant et al. examined the functional -794 $CATT_{5\text{-}8}$ microsatellite and reported no association between the low expression $CATT_5$ allele and susceptibility to sarcoidosis, EN, or disease severity (8). These differing conclusions may be related to variable disease definitions, population stratification, or underpowered sample sizes.

The Genomic Research in Alpha-1 Antitrypsin Deficiency and Sarcoidosis (GRADS) study is an observational cohort study designed to examine the pathobiology of these two diseases. Patients with sarcoidosis were enrolled with the goal of identifying biomarkers and genotypes associated with the various sarcoidosis phenotypes. Patient data included detailed medical and exposure histories, blood samples, spirometry and radiology studies (9). We examined associations between sarcoidosis phenotypes and *MIF* promoter polymorphisms in these patients.

Methods

The GRADS study recruited 351 sarcoidosis patients from nine clinical centers across the United States, and 371 healthy controls were culled from a previously published registry (10). The relevant Institutional Review Boards approved this study, and all subjects gave signed informed consent. The easyDNA kit (Invitrogen) was used to isolate genomic DNA from serum samples and polymorphisms were identified as described previously (11). In short, the -173G/C SNP [*rs755622*] was analyzed with the established Taqman assay for allelic discrimination (Applied Biosystems, ABI), and the -794 $CATT_{5\text{–}8}$ microsatellite repeat [*rs5844572*] genotyping was performed by polymerase chain reaction with analysis by automated capillary electrophoresis (3730xl Genetic Analyzer, ABI).

Because *MIF* allelic frequencies vary by race (10), we stratified our cohort into self-identified white (including two Latinos) and black subjects. Asians and Native Americans were excluded due to small sample sizes. Race categories were confirmed by genetic admixture analysis in the control group. The -173G/C SNP conformed to Hardy-Weinberg equilibrium in both whites and blacks. Differences in genotype frequencies were assessed by logistic regression adjusting for age and sex. Pulmonary sarcoidosis severity was characterized by forced expiratory volume in one second (FEV1), forced vital capacity (FVC), diffusing lung capacity for carbon monoxide (DLCO) and Scadding stage. Differences in these measurements by genotype were assessed using a two-sample t-test for normally distributed samples and Mann-Whitney test for non-normally distributed two group comparisons. Chi-square tests for homogeneity were used to compare the proportions of white patients with each Scadding stage by genotype. Genotype frequencies in cardiac sarcoidosis patients versus healthy controls were compared using chi-square tests. Analyses were performed in RStudio v 1.2.1335 for Mac.

Results

After age and sex adjustment, there were no differences in the frequency of the low *MIF* expression -794 $CATT_5$ containing genotype between sarcoidosis subjects and healthy controls in whites (41% vs. 36% in controls, $p = 0.169$) or blacks (47% vs. 52% in controls, $p = 0.744$). The -173C containing genotype was not more common in the sarcoidosis patients versus controls regardless of race (whites: 31% vs 40% in controls, $p = 0.153$; blacks: 63% vs. 64% in controls, $p = 0.150$). All sarcoidosis patients had normal (> 80% predicted) FEV1 and FVC regardless of their -794 CATT and -173 SNP genotypes. However, while whites had normal DLCO, blacks had reduced DLCO. The severity of this impairment was not associated with genotype (black DLCO: -794 $CATT_5$ 71.4% predicted vs. -794 $CATT_{non\text{-}5}$ 66.6% predicted $p = 0.508$; -173G/G 66.6% predicted vs -173C 69.2% predicted, $p = 0.595$). The proportion of white patients with each Scadding stage did not vary by genotype (Table 1). While the sample sizes for black patients with each Scadding stage were too small for statistical analyses, the proportions of patients with each Scadding stage appeared similar regardless of genotype.

The potential role of MIF in extrapulmonary sarcoidosis phenotypes was examined. Sixteen pa-

Table 1. The number (%) of white (A) and black (B) subjects with each Scadding stage by genotype. Chi-square tests for homogeneity were used to compare the proportions of white subjects with each Scadding stage by genotype

A

Whites				
Scadding Stage n (%)	-794 $CATT_5$ (n = 104)	-794 $CATT_{non\text{-}5}$ (n = 151)	-173G/G (n = 176)	-173C (n = 78)
0	11 (10.6)	26 (17.6)	24 (13.7)	13 (17.1)
1	29 (27.9)	32 (21.6)	42 (24.0)	18 (23.7)
2	32 (30.8)	41 (27.7)	56 (32.0)	17 (22.4)
3	15 (14.4)	19 (12.8)	24 (13.7)	10 (13.2)
4	17 (16.3)	30 (20.3)	29 (16.6)	18 (23.7)
p		0.661		0.728

B

Blacks				
Scadding Stage n (%)	-794 $CATT_5$ (n = 37)	-794 $CATT_{non\text{-}5}$ (n = 42)	-173G/G (n = 29)	-173C (n = 49)
0	3 (8.1)	2 (4.8)	1 (3.4)	4 (8.2)
1	7 (18.9)	6 (14.3)	5 (17.2)	7 (14.3)
2	11 (29.7)	13 (31.0)	11 (37.9)	13 (26.5)
3	3 (8.1)	8 (19.0)	3 (10.3)	8 (16.3)
4	13 (35.1)	13 (31.0)	9 (31.0)	17 (34.7)

tients had sarcoidosis with neurologic involvement, 4 with CNS disease (2 black and 2 white) and 12 with peripheral nerve involvement (5 black and 7 white). Among the patients with neurologic sarcoid, the -794 $CATT_5$ containing genotype was common in both racial groups (6 of 9 white patients and 5 of 6 black patients with CATT genotyping available), while the -173C SNP was more common in blacks as compared to whites (83% vs 22%). Erythema nodosum occurred in 8 whites and 1 black patient, and Lofgren syndrome occurred in 5 whites and no black patients. Among the white patients, 6 of those with erythema nodosum and 3 of those with Lofgren's syndrome had the -794 $CATT_5$ containing genotype. In contrast, the high expression -173C SNP was present in 2 of those with erythema nodosum and 1 of those with Lofgren's syndrome. Cardiac sarcoidosis was reported in 53 white and 7 black subjects. There were no differences in the frequency of the -794 $CATT_5$ containing genotype between white cardiac sarcoidosis subjects and healthy controls (46% vs. 36% in controls, p = 0.231). The -173C SNP frequency was the same in white patients with this phenotype and healthy controls (-173C: 37% vs. 40% in healthy controls, p = 0.894).

Discussion

Because MIF is associated with other autoimmune (10) and granulomatous (4,10,12) diseases, some have hypothesized that variability in *MIF* expression may contribute to sarcoidosis susceptibility or severity (6–8). Examination of the GRADS co-

hort revealed no apparent relationship between either the -794 $CATT_5$ allele or the -173C SNP and sarcoidosis development, which is consistent with previous studies (7,8). However, two groups have reported associations between the *MIF* -173C allele and sarcoidosis-related erythema nodosum (6) and Lofgren's syndrome (7). These associations were not observed in our study, but our cohort was limited by small extrapulmonary sarcoidosis subgroups. In sum, this is the third study to examine *MIF* allelic frequencies in sarcoidosis and none have found associations between MIF and disease development. In contrast, previous work has reported increased frequency of the -173C SNP in patients with erythema nodosum and Lofgren's syndrome and further examination of MIF in these phenotypes is warranted. Our study was not powered to study these sarcoidosis subtypes.

Separately, it is well established that African Americans have more severe sarcoidosis presentations than Caucasian Americans (13), and blacks in our cohort had lower DLCO than whites, which is consistent with previous work (14). Because *MIF* allelic frequencies vary by race (10), we hypothesized that some of the increased sarcoidosis severity observed in blacks could be related to differences in *MIF* genotypes. However, our patients had the same *MIF* allelic frequencies as healthy controls regardless of race. Moreover, when stratified by disease severity, there were no differences in allelic frequencies between blacks.

Other studies have examined possible determinants of sarcoidosis severity and some may be associated with race including less physical activity and more mineral exposures in blacks compared to whites (13). Improving our understanding of the environmental and genetic factors that contribute to racial disparities in sarcoidosis will be critical to bolstering the health of the African American community. While MIF may play a role in the racial differences observed in other diseases given its strong population stratification (15,16), it does not appear central to pulmonary sarcoidosis pathogenesis.

Our findings are consistent with previous reports (7,8) suggesting that *MIF* expression is not associated with sarcoidosis susceptibility or severity of pulmonary disease regardless of race.

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