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SHORT COMMUNICATION

Evidence of association of macrophage migration inhibitory factor gene polymorphisms with systemic lupus erythematosus

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*The aim of this study was to evaluate the potential association of functional polymorphisms of macrophage migration inhibitory factor with systemic lupus erythematosus. Our study includes 711 systemic lupus erythematosus (SLE) patients and 755 healthy controls. We genotyped the migration inhibitory factor (MIF) −173G/C using a polymerase chain reaction (PCR) system with predeveloped TaqMan allelic discrimination assay and the MIF −794 $CATT_n$ microsatellite polymorphism using a PCR-fluorescent method. A statistically significant difference in the distribution of the MIF −173*C allele between SLE patients and controls (P = 0.004, OR = 1.34, 95% CI = 1.05–1.27) was observed. In addition, the frequency of the MIF −173*C/C genotype was higher in SLE patient (P = 0.002, OR = 2.58, 95% CI = 1.32–5.10). No differences in the distribution of $CATT_n$ were found. However, the haplotypes analyses showed that only the $CATT_7$-MIF −173*C haplotype was associated with a higher susceptibility to SLE (P = 0.001, OR 1.84, 95% CI 1.35–2.79). No association with clinical features was detected in any case. These results suggest that both, MIF −173*C allele and $CATT_7$-MIF −173*C haplotype, confer susceptibility to SLE in our population.*

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Introduction

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease with a complex pathogenesis involving multiple genetic and environmental factors. The genetic background of SLE is complex and involves multiple genes encoding different molecules with significant functions in the regulation of the immune system.[1–4] In this respect, several studies support that cytokines play an important role in the development and progression of the autoimmune diseases.[5,6]

Macrophage migration inhibitory factor (MIF) is an immunoregulatory cytokine that has proinflammatory, hormonal and enzymatic activities, and it is expressed in a wide variety of cell types including macrophages, B and T cells.[7,8] There are several lines of evidence supporting the *MIF* gene as a good candidate in inflammatory disorders, such as SLE.[9] *MIF* plays a critical role in regulation of T-cell activation, and it has been demonstrated that alterations in this pathway lead to the development of SLE in animal models[10] *MIF* may provide signals for B-cell proliferation that could maintain the hyperactivity of B cells showed in SLE patients.[11] *MIF* might also contribute to the chronic inflammatory injury in SLE, due to, induction of proinflammatory cytokines, such as interleukin (IL)-1β, tumor necrosis factor α (TNF-α), IL-2, IL-6, IL-8 and interferon γ (IFN-γ), which seem to be associated with the severity of SLE.[7] *MIF* is able to increase the nitric oxide (NO) production, which can directly mediate the inflammatory process. In addition, *MIF* inhibited p53 expression,[12] and it has been demonstrated that p53 has apoptotic effects *in vitro* and *in vivo*, which is consistent with the possible impairment of apoptosis in SLE. Finally, MIF serum levels are significantly increased in SLE patients[13] and functional polymorphisms of the human *MIF* gene have been associated with increased susceptibility to inflammatory and autoimmune diseases.[14]

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The *MIF* gene maps to chromosome 22q11.2. Four polymorphisms have been reported in the human MIF gene,[15,16] including a microsatellite consisting of a different number of CATT repeats located in the promoter region of the gene (beginning at position −794) and three single-nucleotide polymorphism (SNP) at positions −173 (rs755622), +254 (rs2096525) and +656 (rs2070766). The rs2096525 and rs2070766 are located in introns whereas rs755622 is located in the position −173 within the promoter region of the gene and the −173C allele (MIF −173*G/C) has been associated with a higher production of MIF protein.[16] On the other hand, the allele $CATT_7$ of the −794 microsatellite polymorphism has been associated with higher levels of MIF gene transcription *in vitro*.[16,17] The aim of this study was to investigate the potential association of the functional *MIF* −173G/C and −794 $(CATT)_n$ polymorphisms with the susceptibility and/or clinical features of SLE.

Our study includes 711 SLE patients and 755 blood donors. Both patients and donors were recruited from five Spanish hospitals: Hospital Virgen de las Nieves and Hospital Clinico (Granada), Hospital Virgen del Rocio (Seville), Hospital Carlos-Haya (Malaga) and Hospital Xeral-Calde (Lugo). The patient and control groups were of Spanish Caucasian origin and were matched for age and sex. Eighty-seven percent of the SLE patients were women, the mean age of SLE patients at diagnosis was 43 ± 13.3 years and the mean age at disease onset of SLE symptoms was 32 ± 15 years. The SLE clinical manifestations studied were articular involvement (76%), renal affectation (37%), cutaneous lesions (62%), hematopoietic alterations (73%), photosensivity (51%), neurological disease (17%) and serositis (28%). The study was approved by all local ethical committees from the corresponding hospitals.

Results and discussion

The genotype frequencies were not found to be significantly different from those predicted by the Hardy–Weinberg equilibrium in healthy controls and SLE patients for the polymorphisms studied. The homogeneity between five Spanish populations was calculated using a Pearson χ^2 goodness-of-fit test, then we decided combine the groups.

With regard to the *MIF −173G/C* polymorphism, the genotype and allele distribution in SLE patients and healthy controls are shown in Table 1. Statistically significant differences in the distribution of the genotypes were observed comparing SLE patients and healthy subjects ($P = 0.005$ by χ^2 on 2×3 contingency table). Frequency of the C/C genotype was higher in the SLE patient group than in the healthy control group (4.6 versus 1.8%, $P = 0.002$, OR = 2.58, 95% CI = 1.32–5.10). Differences in the distribution of the allele frequencies were also observed, being the −173*C allele overrepresented in SLE patients (17 versus 13.2%, $P = 0.004$, OR = 1.34, 95% CI = 1.05–1.27).

Table 2 shows the genotype and allele distribution of the CATT repeat polymorphism in SLE patients and healthy controls. Four alleles having from five to eight repeats were detected in our control population with similar frequencies to those reported in other white

Table 1 Frequency of *MIF −173G/C* alleles and genotypes among SLE Spanish patients and healthy controls

MIF −173G/C Genotype	*SLE* n = *711 (%)*	*Controls* n = *755 (%)*	P-*value*	*OR (95% CI)*
G/G	503 (70.8)	570 (75.5)	NS	
G/C	175 (24.6)	171 (22.7)	NS	
C/C	33 (4.6)	14 (1.8)	0.002	2.58 (1.32–5.10)
Allele	2n = *1422 (%)*	2n = *1510 (%)*		
G	1181 (83)	1311 (86.8)		
C	241 (17)	199 (13.2)	0.004	1.34 (1.05–1.27)

MIF −173 genotyping was performed using a pre-developed TaqMan allelic discrimination assay (part number: C_2213785_10, Applied Biosystems, Foster City, CA, USA). The PCR was carried out with mixes consisting of 8 ng of genomic DNA, 2.5 μl of Taqman master mix, 0.125 μl of 20 × assay mix and ddH_2O up to 5 μl of final volume. The amplification protocol used was 50°C for 2 min and initial denaturation at 95°C for 10 min followed by 50 cycles of denaturation at 92°C for 15 s, and annealing/extension at 60°C for 1 min. After PCR, the genotype of each sample was automatically attributed by measuring the allele-specific fluorescence in the ABI Prism 7900 Sequence Detection System, using the SDS 2.2.2 software for allele discrimination (Applied Biosystems, Foster City, CA, USA). To confirm the genotype obtained by predeveloped TaqMan allelic discrimination assay, direct sequencing using ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) had been performed. For Hardy–Weinberg equilibrium and statistical analysis to compare allelic and genotypic distributions, we used the χ^2-test. Odds ratio (OR) with 95% confidence intervals (95% CI) were calculated using the software StatCalc program (Epi Info 2002; Centers of Disease Control and Prevention, Atlanta, GA, USA).

Table 2 Frequency of *MIF −794 $(CATT)_{5–8}$* alleles and genotypes among SLE Spanish patients and healthy controls

MIF −794 $(CATT)_{5–8}$ Genotype	*SLE* n = *711 (%)*	*Controls* n = *755 (%)*
5/5	67 (9.4)	55 (7.3)
5/6	212 (29.8)	254 (33.6)
5/7	29 (4.1)	37 (5)
5/8	0	1 (0.1)
6/6	305 (42.9)	318 (42.1)
6/7	84 (11.8)	81 (10.7)
7/7	14 (2)	9 (1.2)
Allele	2n = *1422 (%)*	2n = *1510 (%)*
5	375 (26.4)	402 (26.6)
6	906 (63.7)	971 (64.3)
7	141 (9.9)	136 (9)
8	0	1 (0.1)

MIF CATT repeats genotyping was carried out by PCR using a primer labeled with a fluorescent dye as previously described.[18] In brief, we used the following primers: 5′-TTG CAC CTA TCA GAG ACC-3′ as forward primer 5′ labeled with 6-FAM and 5′-TCC ACT AAT GGT AAA CTC G-3′ as reverse. After capillary electrophoresis on an automated DNA sequencer (ABI Prism 3100 Genetic Analyzer, Applied Biosystems), alleles of the CATT repeat element were identified using Genotyper 3.7 software (Applied Biosystems). Some selected samples were sequenced to confirm the length of each allele.

Table 3 Frequencies of estimated CATT-MIF −173 haplotypes among SLE Spanish patients and healthy controls[a]

Haplotypes	*SLE* 2n = *1090 (%)*	*Controls* 2n = *1098 (%)*
$CATT_5$-MIF −173*G	259 (23.7)	276 (25.1)
$CATT_6$-MIF −173*G	631 (58)	669 (60.9)
$CATT_7$-MIF −173*G	18 (1.6)	25 (2.3)
$CATT_5$-MIF −173*C	17 (1.5)	27 (2.5)
$CATT_6$-MIF −173*C	69 (6.3)	55 (5)
$CATT_7$-MIF −173*C	96 (8.8)	52 (4.7)

[a]Comparison of $CATT_7$-MIF −173*C haplotype versus all other haplotypes combined in SLE patients and controls, $P = 0.001$, OR = 1.94, and 95% CI = 1.35–2.79. The haplotype analysis was constructed using the UNPHASED software.

populations.[14] No statistically significant differences between SLE patients and healthy controls were observed when the −794 $(CATT)_{5–8}$ genotype and allele distributions were compared.

Table 3 shows data of haplotype frequencies in SLE patients and healthy controls. After analysis only four common haplotypes with frequencies >4% in our control population were found: $CATT_5$-MIF −173*G, $CATT_6$-MIF −173*G, $CATT_6$-MIF −173*C and $CATT_7$-MIF −173*C. When the haplotype frequencies were compared, a significant deviation was found ($P = 0.007$) (the results were not corrected for multiple comparisons). This significant deviation corresponded mainly to an increased frequency of the haplotype $CATT_7$-MIF −173*C in SLE patients (8.8 versus 4.7%, $P = 0.001$, OR = 1.94, 95% CI = 1.35–2.79).

In addition, available clinical features of patients with SLE were analysed for possible association with the different alleles or genotypes of these *MIF* polymorphisms. When we stratified SLE patients according to the presence of renal involvement, no statistically significant differences were observed in the distribution of *MIF* polymorphisms between SLE patients with and without lupus nephritis. Similarly, no significant differences were observed between −173G/C, −794CATT and haplotypes variants and the following variables: sex, age at onset, articular involvement, cutaneous lesions, photosensitivity, hematological alterations, neurological disorders and serositis (data not shown).

This study constitutes the first attempt to determine the potential implication of the *MIF* gene polymorphisms in SLE. Our results show that in Spanish population the *MIF* −173*C allele was associated with an increased risk of SLE (OR = 1.34). In addition, we demonstrated that homozygosity for the *MIF* −173*C risk allele increased significantly the susceptibility to SLE (OR = 2.58). This implies that the effect of *MIF* gene on SLE predisposition is dose-dependent. Furthermore, the MIF −173*C haplotype with the – $CATT_7$ allele was found to confer twofold increased risk of SLE susceptibility. This haplotype has been found to be associated with juvenile idiopathic arthritis (JIA),[16] rheumatoid arthritis (RA)[17] and psoriasis.[18]

The allele frequencies found in the Spanish population are similar to those reported in other white population from North America and Europe,[14] and differ significantly from those observed in other populations of different ethnic origin.[19,20] Variations in the distribution of *MIF* alleles among racial groups may suggest the existence of selective pressure acting on the *MIF* locus. Therefore, it might be of major interest to investigate the role of the *MIF* gene variants in other populations, such as African-American patients with SLE.

Several studies have reported a functional significance of *MIF* promoter polymorphism. A study performed in healthy individuals found significantly higher MIF protein serum levels in individuals carrying the *MIF* −173*C allele.[16] In addition, using a human T lymphoblast cell line a higher reporter luciferase activity for both the *MIF* −173*C allele and the *MIF* −173*C-$CATT_7$ haplotype was found, although cell type-specific differences were also reported in this study.[16] These results provide biological support for the observed association of both the *MIF* −173*C allele and the *MIF* −173*C $CATT_7$ haplotype with SLE.

Elevated levels of MIF have been correlated with indices of organ damage in SLE patients.[13] We do not currently have sufficient data to exclude the influence of the *MIF* polymorphism in the severity of SLE. In other autoimmune diseases, the influence of *MIF* polymorphism in disease severity is controversial. In this regard, the *MIF* −794 $CATT_n$ was reported to be associated with disease severity in patient with RA in the United States.[17] Recently, a correlation of *MIF* variants with high levels of radiological joint damage in RA was also reported.[21] However, a study on RA patients in the UK population supported association with susceptibility but not with disease severity.[22] In yet other studies, association of MIF with susceptibility to JIA and psoriasis is not restricted to the most severe clinical subgroups.[16,18] These observations suggest that the MIF gene may be associated with predisposition rather than with severity. These discrepancies could be explained, at least in part, by the small sample size of some clinical subgroups resulting in the lack of power to detect an association. Alternatively, *MIF* may be involved in the persistence, but no in the magnitude of the inflammatory response. Further studies using SLE clinical cohorts assessed early after the onset of the disease should be undertaken to determine the potential effect of *MIF* on SLE outcome.

There is accumulating evidence to suggest the presence of common genetic factor that predispose to autoimmunity.These findings show another piece of evidence that support the hypothesis that different genetics components may be shared between autoimmune diseases. Furthermore, the association of regulators of T-cell activation, *CTLA4*,[23] *PTPN22*[24,25] and *MIF*,[14] with autoimmune diseases indicates the importance of the regulation of T-cell response in the development of the autoimmune response. The data support the development of novel anti-MIF therapies in SLE patients[26] like the use of anti-TNF and anti-IL-6 currently being used in other autoimmune diseases.

In conclusion, our findings support that both the MIF −173*C allele and in particular the MIF −173*C-$CATT_7$ haplotype confer risk in the susceptibility to SLE.

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References

1 Wakeland EK, Liu K, Graham RR, Behrens TW. Delineating the genetic basis of systemic lupus erythematosus. *Immunity* 2001; **15**: 397–408.
2 Tsao BP. Update on human systemic lupus erythematosus genetics. *Curr Opin Rheumatol* 2004; **16**: 513–521.
3 Croker JA, Kimberly RP. Genetics of susceptibility and severity in systemic lupus erythematosus. *Curr Opin Rheumatol* 2005; **17**: 529–537.
4 Prokunina L, Alarcon-Riquelme M. The genetic basis of systemic lupus erythematosus – knowledge of today and thoughts for tomorrow. *Hum Mol Genet* 2004; **13** (Spec No 1): R143–R148.
5 Illei GG, Tackey E, Lapteva L, Lipsky PE. Biomarkers in systemic lupus erythematosus. I. General overview of biomarkers and their applicability. *Arthritis Rheum* 2004; **50**: 1709–1720.
6 Illei GG, Tackey E, Lapteva L, Lipsky PE. Biomarkers in systemic lupus erythematosus: II. Markers of disease activity. *Arthritis Rheum* 2004; **50**: 2048–2065.
7 Calandra T, Roger T. Macrophage migration inhibitory factor: a regulator of innate immunity. *Nat Rev Immunol* 2003; **3**: 791–800.
8 Donn RP, Ray DW. Macrophage migration inhibitory factor: molecular, cellular and genetic aspects of a key neuroendocrine molecule. *J Endocrinol* 2004; **182**: 1–9.
9 Gregersen PK, Bucala R. Macrophage migration inhibitory factor, MIF alleles, and the genetics of inflammatory disorders: incorporating disease outcome into the definition of phenotype. *Arthritis Rheum* 2003; **48**: 1171–1176.
10 Bacher M, Metz CN, Calandra T, Mayer K, Chesney J, Lohoff M *et al.* An essential regulatory role for macrophage migration inhibitory factor in T-cell activation. *Proc Natl Acad Sci USA* 1996; **93**: 7849–7854.
11 Chesney J, Metz C, Bacher M, Peng T, Meinhardt A, Bucala R. An essential role for macrophage migration inhibitory factor (MIF) in angiogenesis and the growth of a murine lymphoma. *Mol Med* 1999; **5**: 181–191.
12 Leech M, Lacey D, Xue JR, Santos L, Hutchinson P, Wolvetang E *et al.* Regulation of p53 by macrophage migration inhibitory factor in inflammatory arthritis. *Arthritis Rheum* 2003; **48**: 1881–1889.
13 Foote A, Briganti EM, Kipen Y, Santos L, Leech M, Morand EF. Macrophage migration inhibitory factor in systemic lupus erythematosus. *J Rheumatol* 2004; **31**: 268–273.
14 Renner P, Roger T, Calandra T. Macrophage migration inhibitory factor: gene polymorphisms and susceptibility to inflammatory diseases. *Clin Infect Dis* 2005; **41** (Suppl 7): S513–S519.
15 Donn RP, Shelley E, Ollier WE, Thomson W. A novel 5′-flanking region polymorphism of macrophage migration inhibitory factor is associated with systemic-onset juvenile idiopathic arthritis. *Arthritis Rheum* 2001; **44**: 1782–1785.
16 Donn R, Alourfi Z, De Benedetti F, Meazza C, Zeggini E, Lunt M *et al.* Mutation screening of the macrophage migration inhibitory factor gene: positive association of a functional polymorphism of macrophage migration inhibitory factor with juvenile idiopathic arthritis. *Arthritis Rheum* 2002; **46**: 2402–2409.
17 Baugh JA, Chitnis S, Donnelly SC, Monteiro J, Lin X, Plant BJ *et al.* A functional promoter polymorphism in the macrophage migration inhibitory factor (MIF) gene associated with disease severity in rheumatoid arthritis. *Genes Immun* 2002; **3**: 170–176.
18 Donn RP, Plant D, Jury F, Richards HL, Worthington J, Ray DW *et al.* Macrophage migration inhibitory factor gene polymorphism is associated with psoriasis. *J Invest Dermatol* 2004; **123**: 484–487.
19 Nohara H, Okayama N, Inoue N, Koike Y, Fujimura K, Suehiro Y *et al.* Association of the −173 G/C polymorphism of the macrophage migration inhibitory factor gene with ulcerative colitis. *J Gastroenterol* 2004; **39**: 242–246.
20 Hizawa N, Yamaguchi E, Takahashi D, Nishihira J, Nishimura M. Functional polymorphisms in the promoter region of macrophage migration inhibitory factor and atopy. *Am J Respir Crit Care Med* 2004; **169**: 1014–1018.
21 Radstake TR, Sweep FC, Welsing P, Franke B, Vermeulen SH, Geurts-Moespot A *et al.* Correlation of rheumatoid arthritis severity with the genetic functional variants and circulating levels of macrophage migration inhibitory factor. *Arthritis Rheum* 2005; **52**: 3020–3029.
22 Barton A, Lamb R, Symmons D, Silman A, Thomson W, Worthington J *et al.* Macrophage migration inhibitory factor (MIF) gene polymorphism is associated with susceptibility to but not severity of inflammatory polyarthritis. *Genes Immun* 2003; **4**: 487–491.
23 Ueda H, Howson JM, Esposito L, Heward J, Snook H, Chamberlain G *et al.* Association of the T-cell regulatory gene CTLA4 with susceptibility to autoimmune disease. *Nature* 2003; **423**: 506–511.
24 Criswell LA, Pfeiffer KA, Lum RF, Gonzales B, Novitzke J, Kern M *et al.* Analysis of families in the multiple autoimmune disease genetics consortium (MADGC) collection: the PTPN22 620W allele associates with multiple autoimmune phenotypes. *Am J Hum Genet* 2005; **76**: 561–571.
25 Orozco G, Sanchez E, Gonzalez-Gay MA, Lopez-Nevot MA, Torres B, Caliz R *et al.* Association of a functional single-nucleotide polymorphism of PTPN22, encoding lymphoid protein phosphatase, with rheumatoid arthritis and systemic lupus erythematosus. *Arthritis Rheum* 2005; **52**: 219–224.
26 Hoi AY, Morand EF, Leech M. Is macrophage migration inhibitory factor a therapeutic target in systemic lupus erythematosus? *Immunol Cell Biol* 2003; **81**: 367–373.