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Macrophage migration inhibitory factor: Association of -794 $CATT_{5-8}$ and -173 G>C polymorphisms with TNF-α in systemic lupus erythematosus

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Abstract

Macrophage migration inhibitory factor (MIF) is an upstream immunoregulatory cytokine associated with the pathogenesis of autoimmune inflammatory diseases. There is evidence that MIF functions in a positive feedback loop with TNF-α that could perpetuate the inflammatory process in systemic lupus erythematosus (SLE). In this case-control study we investigated whether commonly occurring functional *MIF* polymorphisms are associated with SLE as well as with MIF and TNF-α serum levels in a Mexican-Mestizo population. Genotyping of the -794$CATT_{5-8}$(rs5844572) and -173G>C(rs755622) *MIF* polymorphisms was performed by PCR and PCR-RFLP respectively in186 SLE patients and 200 healthy subjects. MIF and TNF-α serum levels were determined by ELISA. A significant increase of MIF and TNF-α levels was found in SLE patients. According to a genetic model, we found a significant association of genotypes carrying the -794$CATT_7$ and -173*C risk alleles with susceptibility to SLE and with a significant increase of TNF-α . In conclusion, *MIF* gene polymorphisms are associated with SLE susceptibility and with an increase of TNF-α serum levels in a Mexican-Mestizo population.

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Keywords

Systemic lupus erythematosus; association; macrophage migration inhibitory factor; tumor necrosis factor alpha; polymorphism

1. Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disease that leads to progressive end-organ damage and is characterized by the presence of autoantibodies directed against nuclear and cytoplasmic antigens [1–3]. Globally, the incidence rate of SLE varies from 1-10 per 100,000 person-years and the prevalence rate varies from 20-70 per 100,000 person-years [4]. SLE mainly affects women of reproductive age in a 1:10 ratio [1].

The phenotypic expression of the disease varies between individuals from different populations and its development is influenced by factors such as ethnicity, genetic susceptibility, environment and gender. In particular, genetic predisposition influences the development of SLE ~15% [2,5,6].

SLE is characterized by a significant humoral response with altered proinflammatory cytokine production, suggesting a critical role for cytokines in its pathogenesis [5,7–11]. The cytokine macrophage migration inhibitory factor (MIF) is distinguished functionally by its ability to counter-regulate glucocorticoid immunosuppression and sustain pro-inflammatory activation by inhibiting activation-induced apoptosis [12]. MIF further co-stimulates T and B lymphocytes and upregulates the production of interleukin-6, interferon-γ, and tumor necrosis factor alpha (TNF-α) by a feed-forward, positive feedback loop [13–15]. Notably, these is evidence that TNF-α levels correlate with the exacerbation of the inflammatory response, with subsequent tissue damage and SLE disease activity [16]. Ten polymorphic sites have been described within the *MIF* gene [17] . Of these, only two polymorphisms identified in the promoter region appear to have functional importance, the first is the short tandem repeat (STR) −794 $CATT_{5\text{-}8}$ *MIF* (rs5844572) which is a microsatellite repetition of cytosine-adenine-thyminethymine (CATT) at position −794 bp, in which the repeat length (5 to 8 repetitions) correlates with increased gene expression and with serum MIF circulation levels [18,19]. The second polymorphism is a single nucleotide polymorphism (SNP) -173 G>C *MIF* (rs755622) at position −173 of the *MIF* gene in which there is a change from guanine (G) by cytosine (C). The −173*C allele is associated with increased MIF levels in circulation [18,20] in several populations, most likely by linkage disequilibrium with the −794 $CATT_7$ high expression allele [18,21,22]. Both polymorphisms also occur commonly in different populations, with minor allele frequencies of > 5% [23]. Previously, both functional *MIF* polymorphisms have been associated with several diseases with an autoimmune component [24-30].

Based on this knowledge, polymorphisms in the *MIF* locus are candidate genetic determinants that could contribute to the susceptibility or clinical severity of SLE. Therefore, we designed this study to investigate the association of −794 $CATT_{5\text{-}8}$ and −173 G>C *MIF* polymorphisms with SLE susceptibility and clinical variables as well as with MIF and TNF-α serum levels in a Mexican-Mestizo population.

2. Material and methods

2.1. Subjects

A case-control study was conducted with two study groups; the first group consisted of 186 SLE patients classified according to the 1982 American College of Rheumatology (ACR) criteria for SLE [31] and enrolled from the Rheumatology Department of the Hospital General de Occidente in Zapopan, Jalisco, Mexico. Mexican-Systemic Lupus Erythematosus-Disease Activity Index (Mex-SLEDAI) and Systemic Lupus International Collaborating Clinics (SLICC) indexes were applied to patients [32,33]. For the control study group, 200 healthy subjects identified by self-report and recruited from the general population in the same geographic area were matched for analysis. All subjects were from an unrelated Mexican-Mestizo population with a family history of ancestors, at least back to the third generation.

2.2. Ethical considerations

Informed written consent was obtained from all patients and subjects before enrollment to the study, according to the ethical guidelines of the 2008 Declaration of Helsinki and the investigation was approved by the ethical, investigation, and biosecurity committee of the Centro Universitario de Ciencias de la Salud, Universidad de Guadalajara (C.I.084-2012).

2.3. Quantification of MIF and TNF-α serum levels

Serum was obtained from all individuals at the time of inclusion, cytokine levels were quantified in a subset of 135 SLE patients that were not being treated with glucocorticoids and matched by age with 200 control subjects. The determination of MIF and TNF-α serum levels was performed by commercial ELISA kits (RayBio®, USA and Invitrogen™, USA, respectively) according to manufacturer's instructions. The MIF assay sensitivity was 6 pg/mL and the TNF-α assay sensitivity was 1.7 pg/mL.

2.4. Genotyping of -794 $CATT_{5-8}$ and -173 G>C MIF polymorphisms

Total genomic DNA (gDNA) was isolated from peripheral blood leukocytes by the salting out method [34]. The −794 $CATT_{5–8}$ *MIF* polymorphism was analyzed by conventional polymerase chain reaction (PCR) and polyacrylamide gel electrophoresis using the primers reported by Radstake *et al* [18]. Cycling conditions were: initial denaturing 95°C for 4 min followed by 35 cycles of 30s at 95°C, 30s at 60°C and 30s at 72°C, then a final extension of 2 min at 72°C. Amplification products were further electrophoresed on a 29:1 10% polyacrylamide gel at 120V during 16h and stained with 0.02% AgNO3.

The −173G>C *MIF* polymorphism was genotyped by the PCR- restriction fragment length polymorphism (RFLP) technique. Amplification of the polymorphic fragment was done using the primers reported by Makhija *et al* [35]; 35 cycles and an annealing temperature of 60°C were used. The 366 bp fragment obtained was further digested with the *Alu I* restriction endonuclease (New England Biolabs, Ipswich, MA, USA) by overnight incubation at 37°C. Finally, the digestion was resolved on a 29:1 6% polyacrylamide gel stained with 0.02% AgNO3. The −173*G allele resulted in a 268 bp and a 98 bp fragment while the 173*C allele was represented by 206 bp, 98 bp and 62 bp fragments. To confirm

the results, genotyping of both polymorphisms was done in duplicate in all cases and confirmed by automatized sequencing of a randomly selected subset of −794 $CATT_{5-8}$ and −173 G>C *MIF* genotypes (Applied Biosystems, USA).

2.5. Immunoassay

dsDNA, Sm and Sm/RNP antibodies IgG type were measured in serum samples from SLE patients by ELISA kits (The Binding Site Ltd, Birmingham, UK). The cutoff level was of >10 U/mL for Sm and Sm/RNP antibodies and >75 U/mL for dsDNA antibody. The Immunoassays were performed following the instructions of manufacturer.

2.6. Statistical analysis

Statistical analysis was performed using the statistical software STATA v 9.2 and GraphPad Prism v 5.0. The statistical power was evaluated according to the calculation of sample size using the Kelsey's formula for proportions in case-control studies[36]. For the descriptive analysis, nominal variables were expressed as frequencies, continuous variables with nonparametric distribution were expressed as medians, percentile 5-95 and interquartile ranges 25-75. We determined genotype and allele frequencies for the polymorphisms −794 $CATT_{5-8}$ and −173 G>C *MIF* gene by direct counting. We performed chi-square test to compare proportions between groups, to compare the genotype and allele frequencies and to evaluate the Hardy-Weinberg equilibrium. To compare nonparametric quantitative determinations we used the U Mann-Whitney test, Odds ratio (OR) and 95% confidence interval (95% CI) were used to analyze the risk for SLE associated with the *MIF* gene polymorphisms. In order to evaluate the effect of both polymorphisms on SLE and clinical variables we performed dominant inheritance genetic models as well as linear regression models adjusted by gender. For correlation analysis of continuous variables with nonparametric distribution we used the Spearman correlation test. Differences were considered significant at $p<0.05$.

3. Results

3.1. Clinical and demographic characteristics

The clinical characteristics of the 186 SLE patients in this study are shown in Table 1. The median age of patients was 33 years; 94% were female and 6% male. The average evolution time of the disease was 5 years, and the subjects had moderate activity and chronicity scores evaluated by Mex-SLEDAI and SLICC-ACR indexes, respectively. The control group included 200 healthy subjects comprised of 65% women and 35% men with a median age of 30 (19-55) years.

3.2. MIF and TNF-α serum levels

A significant increase in MIF (10.74 vs 4.47 ng/mL; $p <0.001$; Fig. 1a) and TNF-α serum levels (23.01 vs 9.75 pg/mL, $p <0.01$; Fig. 1b) was found in the SLE group compared with control subjects.

Previous experimental studies have reported MIF's role in enhanced TNF-α expression and a positive feedback loop between these two cytokines [37]; accordingly, we determined the

correlation between MIF and TNF-α serum levels in our study groups. Fig. 1c shows a positive correlation between serum levels of MIF and TNF- ($r = 0.43$, $p < 0.001$) for both study groups.

Regarding the relationships between MIF or TNF- serum levels with SLE clinical activity, we did not find a significant correlation with the evolution time of the disease, activity and chronicity as evaluated by Mex-SLEDAI and SLICC indexes, respectively (data not shown).

3.3. Distribution of −794 $CATT_{5-8}$ and −173 G>C MIF polymorphisms in SLE patients and healthy subjects

In this study, both *MIF* promoter polymorphisms evaluated were in Hardy-Weinberg equilibrium in the control group (−794 $CATT_{5-8}$ p=0.47 and −173 G>C p= 1.0). The distribution of −794 $CATT_{5-8}$ and −173 G>C *MIF* polymorphisms in SLE patients and control subjects are shown in Table 2.

We found significant differences in the distribution of genotype frequencies of the -794 $CATT_{5-8}$ *MIF* polymorphism by study group ($p = 0.02$) with an OR of 1.83 (CI 1.04-3.19, $p = 0.02$) for the 6,7 repeat heterozygote genotype and an OR of 1.5 (CI 1.05-2.13, $p = 0.02$) for the 7 repeat allele (−794 $CATT_7$), indicating that the −794 $CATT_7$ allele carriers have a 1.5 fold greater susceptibility to present SLE. For the 6,7 repeats heterozygote genotype carriers, the susceptibility increases to 1.83 fold.

Applying a genetic model of dominant inheritance where the genotypes that contain the −794 $CATT_7$ risk allele were grouped, we found significant differences in the comparison of genotypic proportions by study group ($p < 0.01$). We also determined an OR of 1.86 (CI 1.22-2.84, $p < 0.01$) for genotypes carrying −794 $CATT_7$ risk allele (-, 7 +7.7) which indicates that subjects who are carriers of genotypes that contain the −794 $CATT_7$ risk allele have 1.86 fold more susceptibility to present SLE compared with subjects who are carriers of genotypes without the −794 $CATT_7$ risk allele.

For the −173 G>C *MIF* polymorphism, no significant differences in the distribution of the genotype frequencies by study group were observed, nevertheless a significant difference in the distribution of allele frequencies was found ($p = 0.03$). We determined an OR of 1.40 (CI 1.01-1.93, $p = 0.03$) for the −173*C allele, which indicates that subjects who are −173*C allele carriers have 1.40 fold more susceptibility to present SLE.

Following a similar genetic model of dominant inheritance we found a significant difference in the comparison of genotypic proportions by study group ($p = 0.01$); we also determined an OR of 1.64 (CI 1.08-2.48, $p = 0.01$) for the −173*C risk allele (GC+CC) carriers which indicates that subjects who are carriers of genotypes that contain the −173*C risk allele have 1.64 folds more susceptibility to present SLE compared with subjects who are GG genotype carriers (Table 2).

3.4. Association of TNF-α serum levels with MIF promoter polymorphisms in SLE

MIF and TNF-α serum levels were compared by genotypes grouped according to the dominant genetic model proposed for each polymorphism (Fig. 2).

For STR −794 $CATT_{5-8}$ *MIF*, SLE patients with genotypes without the −794 $CATT_7$ risk allele (-,-) had 10.6 ng/mL of MIF while patients carrying genotypes with −794 $CATT_7$ allele risk (-, 7 +7,7) showed a slight increase of 10.7 ng/mL of MIF, this difference was not significant (p = 0.52) (Fig. 2a). Regarding the levels of TNF-α , SLE patients with genotypes without the −794 $CATT_7$ risk allele (-,-) showed lower levels of TNF-α compared to genotypes with −794 $CATT_7$ allele risk (-,7+7.7), which showed a significant increase of TNF-α (13.7 vs 17.6 pg/mL, p <0.01) (Fig. 2b).

In the case of SNP -173 G>C *MIF*, SLE patients with GG genotype had 9.41 ng/mL of MIF while patients carrying genotypes with the −173*C risk allele carriers (GC+CC) had a slight increase of 10.78 ng/mL, this difference was not significant (p = 0.84) (Fig. 2C). Regarding the levels of TNF-α , SLE patients with GG genotype showed lower TNF-α serum levels compared to genotypes of −173*C risk allele carriers (GC+CC) which had a significant increase of TNF-α (14.01 vs 17.09 pg/mL, p <0.01) (Fig. 2d).

To estimate the contribution of both *MIF* polymorphisms to serum levels of TNF-α, multiple linear regression models were used. After adjustment by gender, it was determined that the genotype −173 GC *MIF* was associated with a significant increase of TNF-α (β1=9.35; IC95%=1.55-17.14; R^2 = 0.04; *p*= 0.01). However, we did not find a relationship of the −794 $CATT_{5-8}$ *MIF* genotype with TNF-α (data not shown).

4. Discussion

In the present study we evaluated the association of the functionally relevant −794 $CATT_{5-8}$ and -173 G>C *MIF* polymorphisms with SLE as well as with MIF and TNF- α serum levels in Mexican Mestizo patients. Regarding the genotype and allele frequencies, both polymorphisms were distributed similarly to those reported in our previous study of a Mexican-Mestizo population by Llamas-Covarrubias *et al.* [22] Unlike this previous study, in the case of the −794 $CATT_{5-8}$ *MIF* polymorphism we did not observe the presence of genotypes with the −794 $CATT_8$ high expression allele which was reported in low frequency (1%) in Mexican Mestizo patients with rheumatoid arthritis (RA). When we compared genotype and allele frequencies of both polymorphisms with frequencies reported in European Caucasians populations in the Netherlands [18], a Spanish population, [27] Caucasian Americans and African Americans populations [28] we observed that the genotype frequencies in our population were distributed similarly to those reported in these previous studies. However, in our study we observed a higher frequency of −794 $CATT_6$,−794 $CATT_7$, and −173*C *MIF* alleles.

The differences in the distribution of allele frequencies in both *MIF* gene polymorphisms may be attributed to the sample size and inclusion criteria in each study; besides these differences may be attributed to the racial influence, which is important in the global population stratification of the *MIF* locus [23]. It is known that the Mexican population originated from a mixture of European (60-64%), African (15%), and Amerindian groups (21-25%), giving origin to the Mexican-Mestizo population which has a higher genetic diversity in the distribution of these and other polymorphisms [38]. It has also been determined in previous studies that the STR −794 $CATT_{5-8}$ *MIF* is distributed in

relationship to the migration patterns of human populations, with an increase in high-expressing alleles accordingly as human populations become more diverse [23,39], which may explain the observed differences in the present study.

As an important finding in our study we found that the −794 $CATT_7$ allele carriers are 1.5 fold more susceptible to present with SLE and for 6,7 repeats genotype carriers the susceptibility increases 1.83 fold.

By applying a dominant inheritance genetic model, these associations were confirmed, with an OR of 1.86 for genotypes carrying the −794 $CATT_7$ risk allele for SLE compared with subjects who are carriers of genotypes without the risk allele. In the case of −173 G>C *MIF* polymorphism significant differences in the distribution of allele frequencies were found, −173*C allele carriers present 1.40 fold more susceptibility to SLE. By applying a similar genetic model of dominant inheritance we highlighted differences by study group, finding an OR of 1.64 for the −173*C risk allele carriers to present with SLE.

Nevertheless, the associations found in our study differ from those reported by Sreih *et al* in Caucasians and African Americans with SLE [28], because in these populations a protective effect of −794 $CATT_7$ and −173*C high expression alleles in the incidence of SLE has been attributed. In this study, the results shown in Caucasian and African Americans populations highlight the dual effect of MIF in the pathogenesis of SLE, where it has been proposed that high expression alleles are associated with reduced susceptibility to SLE in early stages of the disease, possibly by increasing the activity of macrophages in the clearance of infectious agents or immune complexes, but once the disease is established the low expression alleles protect against organ damage and the presence of high expression alleles are pathogenic and contribute to the manifestation of the disease at inflammation sites [28,39].

Several studies support the role of the −794 $CATT_7$ and −173*C high expression alleles in the pathogenesis of autoimmune diseases and in genetic studies with autoimmune diseases, the high expression alleles has been associated with the severity of the clinical manifestations of diseases such as scleroderma, rheumatoid arthritis and psoriasis [18,29,40]. In our previous study by Llamas-Covarrubias *et al*, the association of the −794 $CATT_7$ and −173*C high expression alleles with early onset of RA and disease activity was reported in a Mexican-Mestizo population, where both high expression alleles presented a strong linkage disequilibrium (LD = 0.87, $p < 0.001$) which indicates that both alleles are segregated in block from one generation to another and may confer a similar risk [22]. In a Spanish population with SLE, Sánchez *et al* also reported the association of −794 $CATT_7$ and −173*C high expression alleles with increased susceptibility to present SLE (OR = 2, $p < 0.01$) [27]. This association is similar to that reported in our study in SLE, which differs significantly with the reported for Caucasians and African Americans in the study of Sreih *et al* [28]. These differences between populations highlight the role of racial influence in the susceptibility to SLE, and the role of genetic factors in the predisposition to autoimmunity.

Experimental studies have reported the functional effect of polymorphisms in the *MIF* promoter. With respect to the −794$CATT_{5\text{-}8}$ polymorphism gene reporter assays have demonstrated that the $CATT_5$ allele has the lowest transcriptional activity [30] while carriers

of higher repeats alleles (6 to 8 repeats) show higher MIF levels [18–20]. These findings provide a biological support to the associations observed in our study.

MIF is functionally distinguished from other cytokines by its upstream immunoregulatory action on immune cells and to the positive feedback loop between MIF and pro-inflammatory cytokine TNF-α , which is a primary mediator of damage to vascular endothelial cells of organs [4]. In our study, serum levels of TNF-α (23.01 pg/mL) and MIF (10.74 ng/mL) were significantly increased in the group of patients with SLE and a positive correlation was observed between serum levels of these two cytokines (r = 0.43); this finding also was consistent with previously reported studies [22,28,42].

The increase of TNF-α in SLE patients was consistent with the reports in Mexican-Mestizo, Caucasian and African American populations [22,28]. In other Caucasian populations, elevated MIF levels have been correlated with indices of organ damage in patients with SLE [42], however we could not replicate these associations in our population.

Serum MIF levels in SLE patients were found to be above the normal values reported for clinically healthy subjects (2-6 ng/mL) [43]. In our previous study in a Mexican-Mestizo population Llamas-Covarrubias *et al* reported values of 9.5 ng/mL of MIF in RA patients [41] which is consistent with our results in SLE patients. However, the results of our study group contrast with those reported in Caucasian American and African Americans with SLE which on average show normal values of MIF (4.6 and 5.6 ng/mL, respectively) [28]. The factors by which these variations in MIF serum levels between populations could be attributed to racial differences in additional risk loci and differences in the clinical characteristics of the studied groups, including the duration of disease, disease activity, and the effects of immunosuppressive therapy including glucocorticoid administration [42].

To evaluate the association of *MIF* polymorphisms with MIF and TNF-α levels, we stratified our data according to the dominant inheritance genetic models proposed for both *MIF* polymorphisms. However both polymorphisms did not show significant differences with MIF levels. This differs with reports in European, Caucasian American and African Americans populations [18,19,28,44]. In the previous study by our research group in RA [22] and in the present study in SLE, we were not able to replicate the association of *MIF* polymorphisms with MIF serum levels, possibly due to differences in the genetic structure of our population which may influence activity at the *MIF* gene locus. Recently, it was demonstrated that the transcriptional repressor HBP1 inhibits *MIF* gene transcription by interacting with −811 to −792 bp of the *MIF* promoter [44], which coincides with the polymorphic region for the CATT repeat. Nevertheless, the effect of this transcriptional repressor in the −794 $CATT_{5-8}$ *MIF* alleles has not been evaluated and further studies are required to demonstrate the functional regulation of the *MIF* gene in different immunologic contexts.

An important finding in SLE patients with genotypes without the −794 $CATT_7$ risk allele are lower levels of TNF-α compared with genotypes with the −794 $CATT_7$ risk allele (13.7 vs 17.6 pg/mL, respectively). In the case of SNP -173 G>C, a similar pattern was observed, SLE patients with the −173 GG genotype showed lower levels of TNF-α compared with

genotypes with the −173*C risk allele (14.01 vs 17.09 pg/mL, respectively) and the −173 GC genotype containing the −173*C risk allele was associated with an increase of 9.35 pg/mL of TNF-α. Accordingly, 4% of the variability in TNF- serum levels may be attributed to the presence of GC genotype.

We describe for the first time in a Mexican-Mestizo population that *MIF* polymorphism may contribute to a significant increase of TNF-α serum levels. A possible explanation for this finding could be the well-documented positive feedback loop between MIF and pro-inflammatory cytokine such as TNF-α reported in several studies [18,22,28,29,40,42]. In murine models, it has been reported that immunoneutralization of MIF decreases the production of TNF-α [45–47] and *in vitro* studies have shown that MIF is a potent inducer of TNF-α in macrophages [13,37,48].

The relationship between genotypes carrying the −794 $CATT_7$ and −173*C high expression alleles with TNF-α serum levels is partly explained by the positive feedback loop between MIF and TNF-α . SLE is mainly mediated by immune complexes; the role of MIF in the pathogenesis of SLE could include activation and migration of macrophages at sites of inflammation [15]. Deposition of immune complexes in the microvasculature tends to induce the production of high levels of TNF-α in macrophages, which promotes inflammation and subsequent tissue damage [16]. The presence of the −794 $CATT_7$ and −173*C high expression alleles therefore may contribute to exacerbate this inflammatory response.

One of the limitations in our study was heterogeneity in terms of therapy, and the duration and stage of disease evolution in patients with SLE. Furthermore, the quantification of cytokines in serum by ELISA may not accurately reflect the increased expression of MIF in inflammation sites, so these finding must be interpreted with caution.

In conclusion, our findings in a Mexican-Mestizo population provide evidence that *MIF* polymorphisms are associated with susceptibility to SLE and MIF serum levels may contribute to disease persistence by the induction of TNF-α to promote inflammatory responses that are characteristic of SLE.

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Abbreviations

SLE	Systemic lupus erythematosus
MIF	macrophage migration inhibitory factor
TNF-α	tumor necrosis factor alpha
STR	short tandem repeat
SNP	single nucleotide polymorphism

bp	base pair
ACR	American College of Rheumatology
Mex-SLEDAI	Mexican-systemic lupus erythematosus-disease activity index
SLICC	systemic lupus international collaborating clinics
gDNA	genomic DNA
PCR	polymerase chain reaction
RFLP	restriction fragment length polymorphism
AgNO3	silver nitrate

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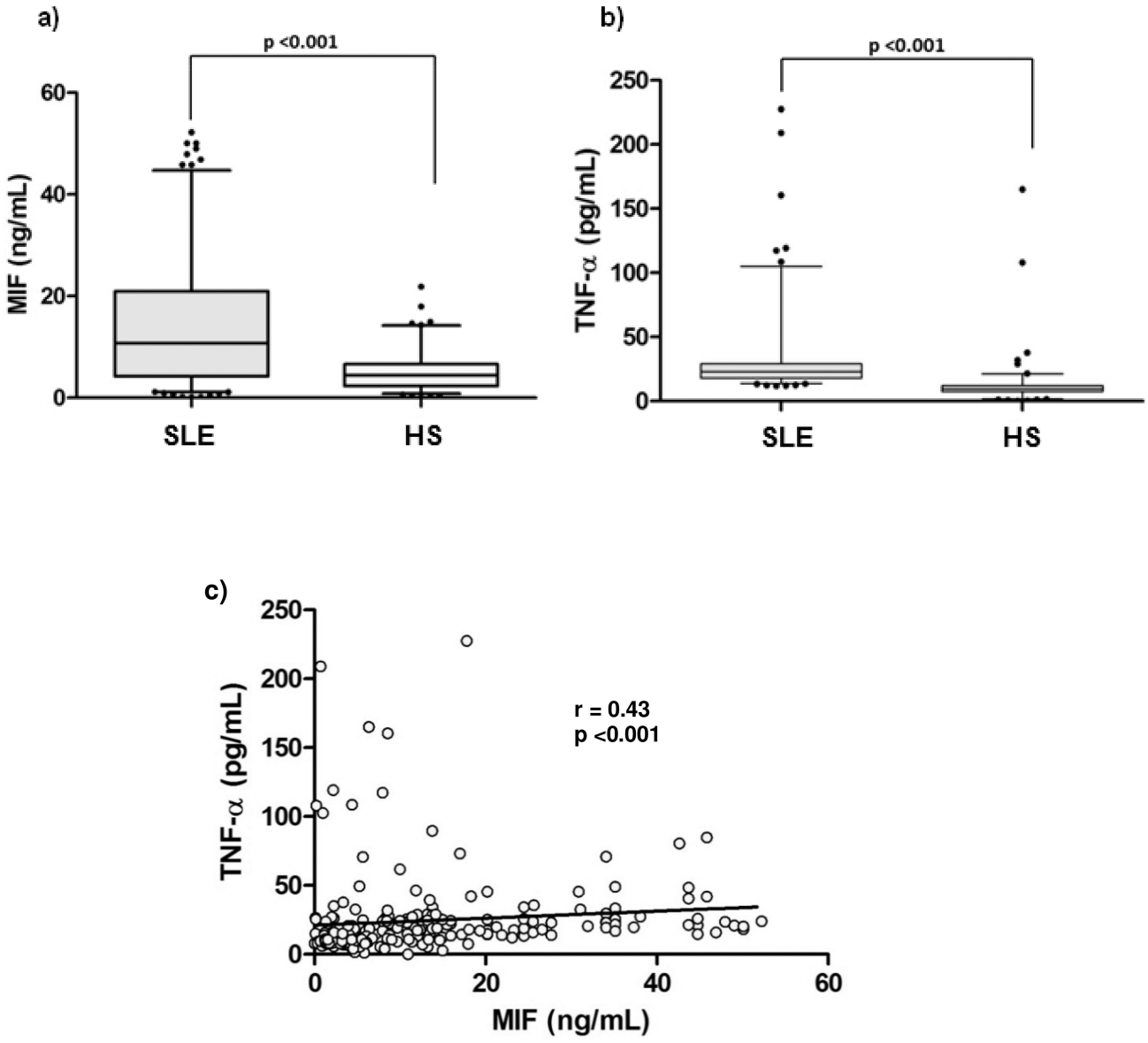

Figure 1.

Serum levels by study groups **and correlation** of MIF and TNF-**α.** 1a) Comparison of MIF serum levels by study groups; 1b) comparison of TNF-α serum levels by study groups. Data provided in median (p5-p95). U Mann-Whitney test. 1c) Positive correlation of serum levels of MIF and soluble TNF-α . Spearman's correlation test.

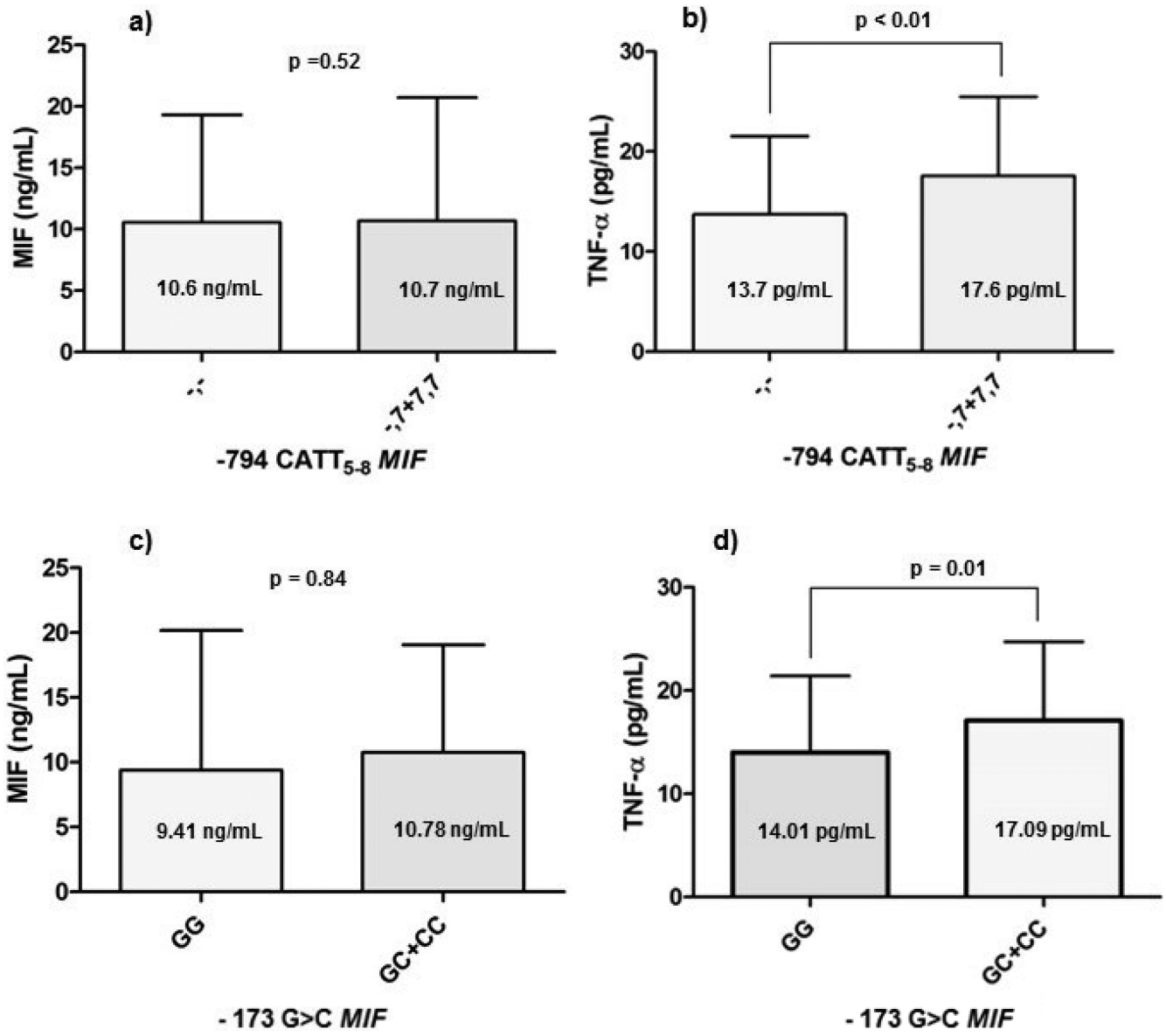

Figure 2. MIF and TNF-α concentrations by −794 $CATT_{5-8}$ −173 G>C *MIF* genotypes according to a dominant genetic model

. a) MIF serum levels 10.6 ng/mL (4.80-19.32) vs. 10.7 ng/mL (4.06-20.18) by −794 $CATT_{5-8}$ MIF genotypes b) TNF-α serum levels 13.7 pg / mL (8.90-21.51) vs 17.6 pg/mL (10.7-25.48) by −794 $CATT_{5-8}$ *MIF* genotypes. c) MIF serum levels 9.41 ng/mL (4.85-20.18) vs 10.78 ng/mL (4.27-18.81) by −173 G>C *MIF* genotypes d) TNF-α serum levels 14.01 pg/mL (8.78-21.32) vs 17.09 pg/mL (10.64-24.64) by 173 G>C *MIF* genotypes. Data provided in median (p25-p75). U Mann-Whitney test.

Table 1

Clinical features of SLE patients.

Variable	*n*=186
Demographics	
Age (years) [a]	33 (17-65)
Gender % (*n*)	
Male [b]	6 (11)
Female [b]	94 (175)
Disease status	
Disease evolution (years) [a]	5(0.5-17)
Clinical assessment	
Mex-SLEDAI[a]	2 (0-9)
SLICC-ACR[a]	1 (0-2)
Renal activity % (*n*)	5 (9)
CRF % (*n*)	4 (7)
Autoantibodies % (*n*)	
ANAs[b]	49 (92)
Anti-dsDNA [b]	33 (62)
Anti-La [b]	4 (8)
Anti-Ro [b]	13 (25)
Anti-Sm [b]	6 (11)
Anti-RNP [b]	12 (23)
Treatment	
NSAIDs [b]	28 (53)
Glucocorticoids [b]	
Prednisone	27 (51)
DMARDS[b]	
Azathioprine	27 (51)
Chloroquine	20 (38)
Methotrexate	6 (12)

[a] Data provided in median (p5-p95).

[b] Data provided in percentages and *n.* ANAs= anti-nuclear antibodies; CRF=chronic renal failure , NSAIDs= non-steroidal anti-inflammatory drugs , DMARDS=disease modifying antirheumatic drugs.

Table 2

Genotype and allele frequencies of -794 $CATT_{5-8}$ and -173 G>C *MIF* polymorphisms in cases and controls

Polymorphism	SLE %(*n* =186)	CS %(*n* =200)	*p value**	OR (CI 95%)	*p value**
		-794 $CATT_{5-8}$ *MIF*			
Genotype			**0.02**		
5,5	4 (7)	2 (4)		2.36 (0.56-11.6)	0.17
5,6	17 (31)	27 (54)		0.78 (0.42-1.44)	0.40
5,7	13 (24)	8 (16)		2.03 (0.92-4.56)	0.05
6,6§	26 (48)	33 (65)		1	-
6,7	35 (66)	24 (49)		**1.83 (1.04-3.19)**	**0.02**
7,7	5 (10)	6 (12)		1.13 (0.4-3.11)	0.79
Allele			0.06		
5	18 (69)	20 (78)		1.06 (0.72-1.60)	0.73
6§	52 (193)	58 (233)		1	-
7	30 (110)	22 (89)		**1.5 (1.05-2.13)**	**0.02**
		Genetic model			
Do			**< 0.01**		
-,-§	46 (86)	62 (123)		1	-
-,7 + 7,7	54 (100)	38 (77)		**1.86 (1.22-2.84)**	**<0.01**
		-173 G>C *MIF*			
Genotype			0.08		
GG§	43 (80)	53 (106)		1	-
GC	49 (91)	43 (85)		1.42 (0.92-2.19)	0.09
CC	8 (15)	4 (9)		2.2 (0.85-6.01)	0.07
Allele			**0.03**		
G§	67 (251)	74 (297)		1	-
C	33 (121)	26 (103)		**1.40 (1.01-1.93)**	**0.03**
		Genetic model			
Do			**0.01**		
GG§	43 (80)	53 (106)		1	-
GC+ CC	57 (116)	47 (94)		**1.64 (1.08-2.48)**	**0.01**

* Chi square test χ^2; OR: odds ratio; CI: confidence interval; Do: dominant inheritance genetic model (-,- = genotypes without risk allele ; -,7 = heterozygous genotypes with allele risk)

§: reference category.