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Macrophage migration inhibitory factor (MIF): genetic evidence for participation in early onset and early stage rheumatoid arthritis

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Abstract

Macrophage migration inhibitory factor (MIF) is an upstream pro-inflammatory cytokine that is associated with the pathogenesis of autoimmune inflammatory diseases including rheumatoid arthritis (RA). Two polymorphisms in the upstream region exist in the *MIF* gene and are associated with RA susceptibility or severity in different populations. In this case-control study, we investigated whether *MIF* polymorphisms are associated with RA susceptibility or activity in a western Mexican population. The relationship of MIF levels with clinical features of disease also was assessed. Genotyping of the -794 CATT_{5,8} (*rs5844572*) and the -173 G>C (*s755622*) polymorphisms was performed by PCR and PCR-RFLP respectively on 226 RA patients and 210 healthy subjects. Serum MIF levels were determined by ELISA. We found a significant association between the -794 CATT_{5,8} 6,7 *MIF* genotype with RA. Moreover, we detected an association between the -794 CATT₇ allele with early onset RA. The -794 CATT₇ and -173*C alleles, which are in linkage disequilibrium, were associated with high disease activity on RA patients. A positive correlation between circulating MIF levels and C-reactive protein, erythrocyte sedimentation rate, rheumatoid factor, anti-citrullinated protein/peptides antibodies and TNF α was detected. MIF levels appear to be associated with disease progression rather than disease activity, which is distinct from the established relationship between disease activity and TNF α levels. In conclusion, the *MIF* gene and protein are associated with RA in a western Mexican population, with a main contribution onto early onset and early stages of disease.

Keywords

Rheumatoid arthritis; macrophage migration inhibitory factor; tumor necrosis factor α ; early onset; disease activity

1. Introduction

Rheumatoid arthritis (RA) is an autoimmune disease affecting approximately 1% of the population worldwide. It is characterized by chronic and symmetric inflammation of synovial joints; leading to joint destruction, loss of function and disability. The immunopathogenesis of RA is recognized to be multifactorial, with a significant contribution of genetic factors; approximately 60% of disease susceptibility has been estimated to be due to genetics [1].

Immune cytokines are central regulators of the inflammatory process and their role in the pathogenesis of RA has been well established in the past decade. Macrophage migration inhibitory factor (MIF) was first postulated to be an important contributor to RA pathophysiology based on murine and clinical investigations [2, 3]. Discovered over 50 years ago by its ability to inhibit the random migration of macrophages [4], the molecular identify of MIF remained unknown until its unambiguous cloning in 1993 [5]. MIF now is recognized to be a key regulator of the innate and adaptive immune responses [6]; it promotes the expression and secretion of downstream pro-inflammatory mediators such as: TNF α , IL-1 β , IL-2, IL-6, IL-8, IL-12, adhesion molecules, chemokines and matrix metalloproteinases [7-13]. Moreover, MIF inhibits p53 dependent apoptosis [14], and favors T cell activation and proliferation [11]. Of note, MIF also is an endogenous counter-regulator of the immunosuppressive action of glucocorticoids. Once secreted; it overrides the anti-inflammatory effects of glucocorticoids, creating a regulatory loop whereby MIF and glucocorticoids regulate both the setpoint and the expression of inflammation and immunity [8, 15].

The serum and synovial fluid of RA patients exhibit high levels of MIF that correlate with disease evolution, which suggest a role of MIF in disease progression [3, 16, 17]. Moreover, in animal models of arthritis, the role of MIF on disease pathogenesis is well established. Treatment with anti-MIF antibodies or a DNA vaccine delays the onset of disease and leads to a marked decline in incidence and severity [18-20].

Two promoter polymorphisms have been identified on *MIF* gene: a CATT₅₋₈ tetranucleotide repeat at -794 and a G>C transversion at -173 [21]. The presence of the C allele on -173 may facilitate the binding of the AP4 transcription factor and *in vitro* and *in vivo* studies suggest that this allele is associated with increased *MIF* gene expression and protein levels [21, 22]. With respect to the -794 CATT₅₋₈ polymorphism, gene reporter assays have demonstrated that the CATT₅ allele has the lowest transcriptional activity [23] while carriers of higher repeats (CATT₆, CATT₇, CATT₈) alleles show higher circulating MIF levels than non-carriers [22, 24, 25].

An association between these *MIF* promoter polymorphisms with susceptibility [26, 27] or severity [22, 23] of RA and other autoimmune and inflammatory diseases has been reported in several populations, as well as strong linkage disequilibrium between the -794 CATT₇ and -173*C allelic variants. Consistent with the proposed role of MIF on early stages of RA, Martinez *et al* recently showed an association of the -173*C allele with RA susceptibility, mainly on early onset Spanish patients [27].

The aim of this study was to investigate the potential association of these *MIF* genetic variants with RA susceptibility and activity as well as with MIF serum levels in a western Mexican population. Mexican mestizo population is composed of an admixture of Amerindian, European and African genes, and shows significant heterogeneity and shows an asymmetric genetic distribution throughout the country [28], the genetic contribution of the *MIF* gene to autoimmune diseases has not been previously analyzed in this population.

Furthermore, the association of serum MIF levels with clinical biomarkers of disease was also determined.

2. Subjects and methods

2.1. Subjects

The study group was composed of 226 RA patients recruited from two centers in Jalisco, Mexico (Hospital Civil de Guadalajara “Fray Antonio Alcalde”, Guadalajara and Hospital Valentín Gómez Farías, Zapopan) and classified according to the 1987 American College of Rheumatology (ACR) criteria [29]. As a control group, 210 unrelated, healthy subjects (HS) as identified by self-report and recruited from western Mexico general population were included. Only those individuals who for three generations, including their own, had been born in western Mexico were considered. In addition, their participation in the study was voluntary and all signed a written informed consent. The study was performed according the ethical principles for experiments involving humans stated on the Declaration of Helsinki, and it was also evaluated and approved by the Ethics Committees from both Hospital Civil de Guadalajara “Fray Antonio Alcalde” and Centro Universitario de Ciencias de la Salud, Universidad de Guadalajara.

A clinical assessment was performed in all the individuals, including determinations of: erythrocyte sedimentation rate (ESR), C reactive protein (CRP), rheumatoid factor (RF) and anti-citrullinated protein/peptides antibodies (ACPA) by conventional methods. Disease activity was evaluated in the RA group by means of the DAS28 index [30].

2.2. Determination of MIF and TNF α serum levels

Serum was obtained from all individuals, cytokine levels were determined only on subjects that were not being treated with glucocorticoids by the time of inclusion. 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. MIF assay sensitivity was 6 pg/ml while TNF α assay sensitivity was 1.7 pg/ml.

2.3. Genotyping of the *MIF*-794CATT₅₋₈ and -173G>C polymorphisms

Genomic DNA was extracted from peripheral white blood cells by means of the Miller technique [31]. Genotyping of the -794CATT₅₋₈ polymorphism was achieved by conventional PCR and polyacrylamide gel electrophoresis using the primers reported by Radstake et al. [22]. Cycling conditions were: initial denaturing 95°C for 4 min followed by 30 cycles of 30s at 95°C, 30s at 60°C and 30 s at 72°C, then a final extension of 2 min at 72 °C. Amplification products were further electrophoresed on a 19:1 7% polyacrylamide gel at 150 V during 15 hours and stained with AgNO₃. Automatized sequencing of one random sample of each homocygote genotype allowed confirmation of results (Applied Biosystems, USA).

The -173G>C polymorphism was genotyped by PCR-RFLP technique. Amplification of the polymorphic fragment was accomplished by using the primers reported by Makhija et al [32]. Forty cycles and an annealing temperature of 60 °C were used. The 366 bp fragment obtained was further digested with *Alu I* restriction endonuclease (New England Biolabs, Ipswich, MA, USA) by overnight incubation at 37 °C. Finally, the digest was resolved on a 29:1 6% polyacrylamide gel stained with 0.02% AgNO₃. The G allele resulted in a 268 bp and a 98 bp fragments while the C allele was represented by a 206 bp, 98 bp and a 62 bp fragments. Results were confirmed by the automatized sequencing of one random sample of each genotype (Applied Biosystems, USA).

2.4. Statistical analysis

Hardy-Weinberg equilibrium test and comparisons of allele and genotype distributions between groups were performed by chi-square test and Fisher exact test when appropriate. Odds ratios (OR) and 95% confidence intervals (95% CI) were calculated to determine the likelihood of genotype and allele frequencies to be associated with RA. Haplotype inference was performed using EMHAPFREQ software and linkage disequilibrium was estimated by means of the Lewontin D' measure. Haplotypic frequencies were also compared by means of chi-square and Fisher exact tests. MIF and TNF α serum levels were compared among groups by student T and ANOVA tests for MIF and U Mann Whitney and Kruskal-Wallis tests for TNF α , correlations were investigated by the Spearman correlation test. Statistical analysis was performed through SPSS v 10.0, GraphPad Prism v 5.0, GDA [33] and RxC [34] software; p values were considered significant at a level of <0.05.

3. Results

Patients

The clinical characteristics of the 226 RA patients in this study are shown in Table 1. The median age of RA patients was 47 years and 94% were female. In general, the RA patients had longstanding disease with a median disease duration of 8 years as well as moderate disease activity scores as evaluated by DAS28 [30], and mild disability as assessed through the Spanish HAQ-DI index [35].

Distribution of the MIF -794CATT₅₋₈ and -173G>C polymorphisms

No deviation from Hardy-Weinberg equilibrium was detected for the polymorphisms in the control group (-794CATT₅₋₈ p=0.159 and -173G>C p=0.220). Allelic frequencies of both *MIF* variants in our HS population were detected to be similar to those reported by Mitterski et al in a German population [36], but differed from reports from Spain (p=0.016 and p=0.02 for the STR and the SNP, respectively) [27], United Kingdom (p=0.001 and p=0.011 for the STR and the SNP, respectively) [26], and The Netherlands (p=0.05 and p=0.016 for the STR and the SNP, respectively) [22]. The population in our study showed a higher frequency of the -794 CATT₇ and the -173*C alleles in comparison to the populations mentioned above.

Additionally, a strong linkage disequilibrium among 7-CATT and -173*C alleles was detected (Normalized LD= 0.87; p<0.0001). The magnitude of LD detected was similar to that determined for Spanish population [27].

Table 2 shows genotypic, allelic and haplotypic frequencies for the *MIF*-794CATT₅₋₈ and -173G>C polymorphisms in the RA and healthy subjects. We detected a borderline significant association of the high expression -794CATT₆/CATT₇ genotype (*MIF* 6, 7) with RA susceptibility (p=0.048); nevertheless when analyzing the allelic frequencies for this polymorphism we could not establish an association with disease susceptibility. For the -173G>C polymorphism, we observed a higher frequency of the GC genotype and the -173*C allele in the RA group but this result did not reach statistical significance (Table 2). With respect to haplotypic frequencies, a trend for the -794 CATT₇-MIF-173*C haplotype to be associated with disease susceptibility was detected (p=0.085). Moreover, the -794CATT₇-MIF-173*G haplotype also showed a tendency toward association, however, the number of individuals on this last group was short and statistical power cannot be reliable.

Association of MIF -794CATT₅₋₈ polymorphism with early onset RA

Previous studies have shown an association between *MIF* gene polymorphisms and juvenile idiopathic arthritis (JIA) [18, 30]. Martinez *et al* [27] also determined that the -173*C allele

was associated with early onset RA in a Spanish population. In attempting to replicate that association, we subgrouped RA patients according to tertiles of age at disease onset. The groups were as follows: early onset <31 years; middle age onset 32-42 years and late onset ≥43 years. A significant difference among the early onset group and HS was found with regards to the -794 CATT₅₋₈ polymorphism, and corresponded to an increased frequency of the -794 CATT₇ allele on the early onset group (Table 3; $p=0.034$). No differences were found between the healthy controls and the rest of the age at onset groups as well as within the groups. With respect to the -173G>C genotypes or alleles, no differences were detected among subgroups (data not shown).

Association of MIF polymorphisms with disease activity

To determine if the *MIF* promoter polymorphisms are associated with RA clinical activity we classified patients according to DAS28 score in: $2.6 < \text{DAS28} < 3.2$ (low activity) and $\text{DAS28} \geq 5.1$ (high activity) [30] and compared genotypic and allelic frequencies of both polymorphisms among groups. As shown in Table 4, a significantly higher frequency of the -794 CATT₇ and the -173*G alleles was found on the high activity group ($p=0.037$ and $p=0.025$, respectively), which is in agreement with previous reports in a US [23] or European population study [22].

Analysis of MIF serum levels according to MIF promoter polymorphisms and correlation with clinical features of RA

To determine if *MIF* promoter polymorphisms are associated with circulating protein levels, we quantified MIF levels in the serum of 85 RA patients and 110 HS. Significantly higher levels of MIF were detected in RA patients (9.53 ± 11.61 vs 5.38 ± 4.22 ng/mL; $p < 0.0001$; data not shown). Nevertheless, when grouping individuals according to genotypes or alleles of both *MIF* promoter polymorphisms, no significant differences were detected with MIF serum levels (data not shown).

Furthermore, we investigated the association of MIF serum levels with clinical features of disease. In concordance with a previous report on our research group in a smaller sample [17], a significant positive correlation between MIF serum levels and CRP as well as RF was determined ($r=0.308$, $p > 0.0001$; and $r=0.399$, $p < 0.0001$; respectively. Figure 1A and 1C). Additionally, a positive correlation of MIF levels with ESR ($r=0.242$; $p=0.0001$) and ACPA ($r=0.416$; $p < 0.001$) was detected in this study (Figure 1B and 1D). MIF is expressed upstream of TNF α , and important contribution of MIF to TNF α has been previously reported [3, 7]. We investigated the correlation between MIF and TNF α serum levels in our study individuals. As depicted on Figure 1E, a positive correlation for MIF and TNF α levels was determined ($r=0.235$; $p=0.001$).

In a previous analysis of this study group, circulating MIF levels were shown to negatively correlate with years of disease duration, and that correlation was replicated on this bigger sample ($r=-0.263$; $p=0.016$; Figure 2A), thus confirming previous results. Circulating TNF α levels did not correlate with years of disease evolution however ($r=0.083$; $p=0.317$; Figure 2C).

Finally, as shown in Figure 2D TNF α levels positively correlate with disease activity ($r=0.365$; $p < 0.0001$) whilst MIF levels did not show any association with DAS28 ($r=0.003$; $p=0.980$; Figure 2B), and this last finding is in contrast to the report of Radstake et al [22].

4. Discussion

Autoimmune disorders are complex pathologies that involve both genetic and environmental factors. The investigation of gene polymorphisms involved in the pathogenesis of this sort of

diseases has been increasing over the last years. With respect to RA several genetic *loci* have been described to be associated with disease susceptibility and severity in Caucasian populations, with the best established association to be at the *HLA-DR* locus (OR>30) [1]. Other associated genes comprise *PTPN22*(OR:1.3–16.2 [37]), *PADI4*(OR:1.01–1.55 [38]), and *CTLA4*(OR:1.1 [39]) for example.

Although several studies have reported on the association between the *MIF* gene and RA susceptibility or clinical severity, *MIF*'s precise contribution is unclear. An association with disease susceptibility but not with severity has been detected in a Caucasian [26], as well as in Spanish [27] population. On the other hand, Radstake [22] and Baugh [23] reported that the *MIF* polymorphisms are not associated with RA susceptibility; instead, a contribution of *MIF* variants to radiologic joint damage and clinical severity were noted. It is unknown whether different conclusions regarding susceptibility versus severity are due to genetic differences in the populations studied or to statistical differences related to the sample sizes studied.

Herein, we report a borderline association of the 6, 7 genotype of the *MIF*-794 CATT₅₋₈ polymorphism with RA susceptibility on a western Mexican population. With respect to the contribution of the -173G>C polymorphism, a trend for a relationship of the -173 CC genotype and the C allele with disease susceptibility was detected but statistical significance was not achieved. According to former reports [21, 26–27], a significant linkage disequilibrium among *MIF* variants is present in our population; but when comparing haplotypic frequencies among groups, we could only perceive a trend of 7-CATT/-173*C haplotype to be associated with susceptibility.

The association of *MIF* polymorphisms with disease activity also was investigated. We detected that carriers of the -173*C allele were more prone than carriers of the -173*G allele to present with high disease activity as classified by a DAS28 score higher than 5.1. With respect to the -794CATT₅₋₈ polymorphism, an association of the high expression -794 CATT₇ allele with high RA disease activity was found. These conclusions nevertheless must be interpreted with caution because disease activity is highly susceptible to modification by treatment and our study group was heterogeneous in that respect.

A role for the *MIF* gene in the development of juvenile idiopathic arthritis has been reported on the literature [21, 40] and Martinez *et al.* showed an association of the -173*C allele with early onset RA [27]. These findings, together with the association of *MIF* serum levels with disease evolution on RA previously reported by our research group [17] and the association of *MIF* with disease severity reported by Radstake *et al* [22] in European Caucasian, may be considered in combination with *MIF*'s upstream function in the cytokine cascade [3, 41] to support a central role of *MIF* in the earliest stages of RA pathogenesis. In agreement with the notion, we identified an association of the high expression -794 CATT₇ allele with early onset RA in our western Mexican population, further supporting a role for *MIF* on early disease.

Gene reporter analyses have demonstrated that *MIF* promoter polymorphisms play a role on the gene transcriptional regulation in a cell-type dependent manner. The -173*C allele has been shown to favour transcription on a human T lymphoblast cell line (CEMC7A) whilst the -173*G allele promotes transcription on a lung epithelial cell line (A549) [21]. On the other hand, the -794 CATT₅ allele has been associated with lower basal and stimulated transcriptional activity in three cell lines [23]. In line with these reports, Radstake observed that RA patients that carried the -173*C or -794 CATT₇ alleles exhibited higher *MIF* circulating levels [22]. High expression *MIF* alleles (-794 CATT₇) also have been associated

with higher circulating MIF levels in Caucasian and African-American SLE populations [42].

Nonetheless, we were not able to replicate the association of any *MIF* promoter polymorphism with MIF serum levels, which may be related to either the limitations of our sample size or to differences in the genetic structure of our populations that may have obscured this effect of the *MIF* locus. Recently, it was demonstrated that the transcriptional repressor HMG box-containing protein 1 (HBP1) inhibits *MIF* gene transcription by interacting with -811 to -792 bp of the *MIF* promoter [43], which coincides with the polymorphic region for the CATT repeat. Nevertheless, the effect of the -794 CATT₅₋₈ alleles on this interaction needs to be experimentally assessed. On the other hand, a novel cell-type intronic enhancer of the *MIF* gene has recently been described [44], which could have a previously unexpected effect on MIF levels independently of *MIF* promoter polymorphisms. Further studies are required to elucidate the integral regulation of the *MIF* gene in health and disease states. MIF is a structurally unique cytokine with significant pro-inflammatory properties. It can promote the expression and secretion of numerous inflammatory mediators such as cytokines [7-9], cytokine receptors (IL-1R and p55TNFR) [45], phospholipase A2 and ciclooxigenase 2 [46]. MIF also promotes matrix metalloproteinases expression [12-13] and it is considered as a necessary component on T cell activation and proliferation as well as on antibody production [9]. Moreover, MIF can induce synovial fibroblasts proliferation independently on TNF α and IL-1 β [47]. In experimental animal models of arthritis, MIF neutralization markedly reduces disease incidence, severity and mortality and prolongs onset of disease. Immunoneutralization of MIF also decreases TNF α production [18-20]. In line with these findings, MIF levels are significantly increased on serum and synovial fluid of RA patients [3, 16] and correlate with disease activity as determined by CRP [48]. Our findings also showed a positive correlation of MIF serum levels with the clinical biomarkers of disease CRP, ESR, RF and ACPA, supporting a role for MIF in the inflammatory and autoantibody response in RA. With respect to the later it is notable that there is also evidence for a role for MIF in B cell survival and homeostasis [42, 49].

We observed a negative correlation between MIF levels with RA disease evolution and a positive correlation with TNF α levels. TNF α levels further correlated with disease activity but not with disease evolution. These findings suggest that on one hand, MIF may be implicated in the early stages of RA by promoting TNF α synthesis (consistent with genetic knockout studies in mice [50]), but that once disease activity becomes established RA is more dependent on TNF α than on MIF. It may be postulated that therapeutic MIF neutralization may be of greater importance during early RA to prevent disease progression, but that anti-TNF α treatment would be more efficacious on established RA patients.

In conclusion, we have provided evidence that *MIF* is involved in RA in a western Mexican population, with the added finding that its contribution may be more important in early onset or the early stages of disease evolution. There is a close relationship between MIF and TNF α , although TNF α expression appears to be more closely associated with disease activity rather than onset, while the opposite appears to be the case for MIF. These findings need to be extended and confirmed in additional RA cohorts, but they provide the first suggestion that MIF or TNF neutralization may be more efficaciously employed according to disease stage.

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Abbreviations

MIF	macrophage migration inhibitory factor
RA	rheumatoid arthritis
CRP	C reactive protein
ESR	erythrocyte sedimentation rate
RF	rheumatoid factor
ACPA	anti-citrullinated protein/peptides antibodies
AgNO₃	Silver nitrate
DMARDs	disease modifying antirheumatic drugs
NSAIDs	non-steroidal anti-inflammatory drugs

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Figure1. MIF levels correlation with clinical features of RA

MIF serum levels positively correlated with C reactive protein (A), erythrocyte sedimentation rate (B), rheumatoid factor (C), anti-citrullinated protein/peptides (D) and TNF α serum levels (E).

Figure 2. Correlation of MIF and TNF α serum levels with disease activity and years of RA evolution

MIF serum levels showed a negative correlation with years of disease evolution (A), indicating a possible role on early RA while no correlation was detected with disease activity (B). On the other hand, TNF α serum levels did not correlate with years of disease evolution (C) but correlated positively with disease activity (D), suggesting participation on active and established RA rather than on early RA.

Table 1

Clinical features of RA patients

Parameter	
n	226
Age (years)	47 (18-86)
Gender	
<i>Female</i>	212
<i>Male</i>	14
Age at disease onset	36.93 ± 13.27
Years of disease evolution	8 (0.11-52)
Spanish HAQ-DI score	0.61 (0.00-2.83)
DAS28 score	4.80±1.51
RF (UI/mL)	245.5 (6.7-4280)
ACPA (U/mL)	149.17 (1.54-727)
Treatment	
<i>DMARDS^a</i>	156/226 (69.0%)
Methotrexate	117/156 (75.0%)
Chloroquine	92/156 (59.0%)
Hydroxychloroquine	9/156 (5.8%)
Sulfasalazine	51/156 (32.7%)
Leflunomide	4/156 (2.6%)
<i>Biologics</i>	7/226 (3.1%)
Etanercept	6/7 (85.7%)
Infliximab	1/7 (14.3%)
<i>NSAIDs^b</i>	150/226 (66.4%)
<i>Steroids</i>	42/226 (18.6%)

Data are expressed as average ± standard deviation for normally distributed variables and median (range) for variables that showed not normal distribution.

^aDMARDS, Disease modifying anti-reumatic drugs

^bNSAIDs, Non-steroidal anti-inflammatory drugs

Table 2

Genotypic, allelic and haplotypic frequencies of -794CATT₅₋₈ and -173G>C *MIF* promoter polymorphisms in RA patients and HS

	RA (n=226) % (n)	HS (n=210) % (n)	p	OR	95% CI
-794CATT₅₋₈ Genotype					
5,5	3 (7)	2 (4)	0.346	2.12	0.59-7.00
5,6	24 (55)	26 (56)	0.507	1.19	0.71-1.98
5,7	8 (19)	8 (17)	0.420	1.35	0.64-2.84
6,6 ^a	25 (57)	33 (69)	1.000	-	-
6,7	32 (71)	25 (52)	0.048	1.65	1.00-2.72
7,7	7 (15)	6 (12)	0.330	1.51	0.66-3.49
7,8	1 (2)	0 (0)	0.211	-	-
-794CATT₅₋₈ Allele					
5	19.47 (88)	19.29 (81)	0.549	1.11	0.78-1.58
6 ^a	53.10 (240)	58.57 (246)	1.000	-	-
7	26.99 (122)	22.14 (93)	0.072	1.34	0.97-1.86
8	0.44 (2)	0.00 (0)	0.509	-	-
-173G>C Genotype					
GG ^a	43 (98)	53 (110)	1	-	-
GC	49 (111)	42 (89)	0.090	1.40	0.95-2.07
CC	8 (17)	5 (11)	0.170	1.73	0.78-3.88
-173G>C Allele					
G ^a	68 (307)	74 (309)	1	-	-
C	32 (45)	26 (111)	0.067	1.31	0.98-1.76
-794CATT₅₋₈ / -173G>C Haplotype					
6G ^a	45.27 (205)	52.86 (222)	1.000	-	-
5C	0.35 (2)	0.00 (0)	0.232	-	-
5G	19.12 (86)	19.29 (81)	0.446	1.15	0.80-1.65
6C	7.83 (35)	5.95 (25)	0.134	1.52	0.88-2.62
7C	23.46 (106)	20.23 (85)	0.085	1.35	0.96-1.90
7G	3.53 (16)	1.67 (7)	0.054	2.48	0.99-6.14

	RA (n=226) % (n)	HS (n=210) % (n)	p	OR	95% CI
8C	0.44 (2)	0.00 (0)	0.446	-	-

^aReference genotype, allele or haplotype

Allelic frequencies of *MIF* -794CATT₅₋₈ polymorphism in RA patients according to age at disease onset

Table 3

	Early onset RA (n=158)	HS (n=420)	p	OR	95% CI
5	20.25 (32)	19.29 (81)	0.371	1.25	0.77-2.02
6 ^a	49.37 (78)	58.57 (246)	1.000	-	-
7	29.75 (47)	22.14 (93)	0.034	1.59	1.03-2.46
8	0.63(1)	0.00 (0)	0.243	-	-

^aReference allele

Table 4

Allelic frequencies of *MIF* -794CATT₅₋₈ and -173G>C polymorphism in RA patients stratified according to DAS28 score.

	DAS28	5.1 % (n)	2.6<DAS28≤3.2 % (n)	p	OR	95% CI
-794CATT₅₋₈						
5-CATT	16.29 (29)	28.26 (13)	0.302	0.64	0.30-1.40	
6-CATT ^a	54.50 (97)	60.87 (28)	1.000	-	-	
7-CATT	28.09 (50)	10.87 (5)	0.037	2.89	1.05-7.93	
8-CATT	1.12 (2)	0.00 (0)	1.000	-	-	
-173G>C						
G ^a	62.92 (112)	80.43 (37)	1.000	-	-	
C	37.08 (66)	19.57 (9)	0.025	2.42	1.10-5.33	

^aReference genotype or allele.