

Single-nucleotide polymorphism and haplotype analysis of macrophage migration inhibitory factor gene and its correlation with serum macrophage migration inhibitory factor levels in North Indian psoriatic patients with moderate disease severity: A cross-sectional study

Seema Chhabra, Nirmalya Banerjee, Tarun Narang¹, Swati Sood², Anuradha Bishnoi¹, Shubham Goel, Frainey Bansal, Sukhwinder Singh, Kumari Priyanka, Ranjana Walker Minz, Sunil Dogra¹

Departments of Immunopathology, ¹Dermatology, Venereology and Leprosy, ²Cytology and Gynaecological Pathology, Postgraduate Institute of Medical Education and Research, Chandigarh, India

Abstract

Background: Psoriasis is associated with significant morbidity and impaired quality of life. Identification of the host genes that influence disease susceptibility and can potentially guide future, targeted therapy is the need of the hour.

Aims: The aim of the study was to investigate the associations of macrophage migration inhibitory factor (MIF) gene polymorphisms, that is, a 5–8-CATT tetra nucleotide repeats at -794 (-794*CATT₅₋₈) and a single-nucleotide polymorphism at -173 (-173*G/C) with the risk of chronic plaque psoriasis and to observe the correlation, if any, of disease determinants with genetic functional variants and circulating MIF levels.

Methods: Five hundred and seventeen individuals (265 psoriasis patients and 252 controls) were genotyped for MIF gene polymorphisms. Data were analyzed with respect to disease susceptibility, serum MIF levels, disease severity, age at onset, disease duration and presence of comorbidities.

Results: The presence of co-morbidities was more frequently noted in patients with late onset disease ($P = 0.01$). No statistically significant differences were observed either in genotype ($P = 0.680$) or allele frequency ($P = 0.69$) with respect to distribution of MIF-173*G/C polymorphism between patients and controls. The frequencies of genotypes -794*CATT 5/7 and 7/7 were significantly lower in patients ($P = 0.027^*$ and 0.038^* , respectively). CATT₅/MIF-173*C haplotype occurred at a higher frequency in patients (odds ratio 3.03, 95% confidence intervals 1.09–8.47, $P = 0.02$). The mean serum MIF levels were significantly higher in patients as compared to controls ($P < 0.001$). The presence of either extended MIF -794*CATT repeats or C allele did not reveal any significant association with serum MIF levels or age at onset. Analysis of effect of various disease determinants revealed no significant association with genetic variants and serum MIF levels.

Limitations: The lesional expression of MIF could not be studied.

Conclusion: Our results showed that CATT₅/MIF-173*C haplotype is associated with increased susceptibility to psoriasis vulgaris.

Key words: Macrophage migration inhibitory factor, psoriasis, single-nucleotide polymorphism

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Corresponding author: Prof. Sunil Dogra, Department of Dermatology, Venereology and Leprosy, Postgraduate Institute of Medical Education and Research, Chandigarh, India. sundogra@hotmail.com

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Introduction

Psoriasis is a common chronic inflammatory proliferative skin disease with prevalence varying from region to region due to various environmental and genetic factors.^{1,2} Macrophage migration inhibitory factor (MIF) is a potent pro-inflammatory cytokine involved in the pathogenesis of many inflammatory diseases, including psoriasis; a role suggested by its activity profile which includes induction of tumor necrosis factor- α secretion and promotion of T-cell activation.³⁻⁵ Polymorphisms in the promoter region of MIF gene, that is, CATT tetranucleotide repeat at -794 (*rs5844572*) or guanine (G) to cytosine (C) transition at -173 (-173*G/C, *rs755622*) have been reported to be associated with increased susceptibility to, or severity of, numerous chronic inflammatory, and autoimmune disorders in different populations.⁴⁻¹⁶ Given these data, the pharmacological or immunological modulation of MIF activity might offer new therapeutic options for patients suffering from these diseases.⁶

We planned this study to see whether MIF gene polymorphisms affect the susceptibility to and severity of chronic plaque psoriasis. Few studies have demonstrated MIF gene polymorphisms with psoriasis in different ethnic populations across the globe; however, no studies have documented the impact of MIF promoter variations on the risk of chronic plaque psoriasis in North Indian population.^{17,18} The main objective was to investigate the association between these two functional MIF polymorphisms, that is, -794*CATT₅₋₈ and -173*G/C and chronic plaque psoriasis and to observe a correlation, if any, between susceptibility and genetic functional MIF variants.

Methods

A total of 265 consenting patients of chronic plaque psoriasis were included in the study. None of the recruited patients had received any systemic or topical treatment for the last 4 weeks and 2 weeks respectively, before enrolment. None of the patients had any significant renal, hepatic, or other systemic disease (confounding conditions such as atopic dermatitis, lupus, systemic sclerosis and rheumatoid arthritis that can elevate MIF levels were excluded from the study). The disease severity was assessed by calculating the psoriasis area severity index (PASI) scores. Peripheral blood samples were obtained from patients and 252 age, sex, ethnically matched, healthy and unrelated controls. Genomic DNA was isolated from EDTA blood and stored. Serum MIF levels were measured by enzyme linked immunosorbent assay, ELISA (R&D Systems, Quantikine). The study was approved by the institutional ethics committee PGIMER, Chandigarh (PGI/IEC/2011/606-07 dated 05/12/2011).

Migration inhibitory factor-173*G/C genotyping (*rs755622*)

This single-nucleotide polymorphism was genotyped using polymerase chain reaction, followed by restriction fragment length analysis.

Migration inhibitory factor-794*CATT₅₋₈ genotyping (*rs5844572*)

This polymorphism was genotyped using polymerase chain reaction, followed by capillary gel electrophoresis.

Statistical analysis

The sample-size was determined based on the frequency of single-nucleotide polymorphisms as reported in literature and using software Quanto version 1.2, (<http://hydra.usc.edu/gxe>). Total sample-size calculated was 500 (250 patients and 250 controls) with 5% alpha error and 95% power. Considering a 6% drop out, this translated to 265 patients. The Hardy Weinberg equilibrium was analyzed for each polymorphism online, using <http://www.oege.org/software/hwe-mr-calc.shtml>. Statistical analysis was performed using IBMSPSS (Statistical Package for the Social Sciences) version 20. All statistical tests were two-sided and were performed at a significance level of $\alpha=0.05$. Tests of association were performed on full genotype and following simplification to 55, 5X or XX, where X = CATT₆₋₈ as previously described.^{15,19-21} The haplotype analysis was performed using PHASE, version v2.1 (<http://www.stat.washington.edu/stephens/software.html>), Haplo.score and Haplo.glm: <http://www.mayo.edu/research/labs/statistical-genetics-genetic-epidemiology/software>).

Logistic regression analysis was performed to test for any association between the genetic variants and various disease determinants such as sex (male vs. female), age at onset {early (≤ 40 years) vs. late (>40 years)}, disease duration {short (≤ 5 years) vs. long (>5 years)}, disease severity {mild-to-moderate (PASI ≤ 10) vs. severe (PASI >10)}, presence of comorbidities (present vs. absent) and serum MIF levels. Receiver-operating characteristics (ROC) curve analysis was performed to calculate the cutoff values of the serum MIF levels and to predict their specificity and sensitivity.

Results

Patient characteristics

Males outnumbered females by a ratio of 2.6:1. The mean age of onset of psoriasis was 32.6 \pm 15.5 years. The majority of patients (n=185, 69.8%) had early onset (≤ 40 years) disease. Disease associated comorbidities were seen in 70 (26.4%) patients, while 152 (57.4%) had disease exacerbation at the time of presentation. The demographic characteristics of both groups (patients and controls) are summarized in Table 1.

A significant association was observed between age at onset and presence of comorbidities ($P = 0.01^{**}$) adjusted for age and sex. The presence of comorbidities was more frequently noted in patients with late onset disease (odds ratio=2.579).

Analysis of allele and genotype frequencies of migration inhibitory factor-173*G/C and migration inhibitory factor-794*CATT₅₋₈ polymorphisms

There were no deviations from Hardy Weinberg equilibrium in patients or controls for either MIF-173 or the CATT

repeat polymorphism ($p > 0.05$). No linkage disequilibrium was observed between the studied alleles. GG genotype was most commonly encountered among both patients (144, 54.3 %) and controls (139, 55.1%). MIF-173 genotype and allele frequencies were comparable between the two groups ($P = 0.680$) {G/C vs. G/G, Odds ratio=1.11, 95% confidence intervals (0.77–1.59), $P = 0.55$; C/C vs. G/G, Odds ratio=0.51, 95% confidence intervals (0.21–1.25), $P = 0.13$ }. Carriage of the -173*C polymorphism (GC+CC vs.GG) in patients did not reveal an association with disease (Odds ratio=1.03, 95% confidence intervals 0.73–1.46, $P = 0.85$). The frequency of G allele was over-represented in both patients (76%) and controls (75 %). No significant association was detected between MIF-173 SNP and risk of psoriasis vulgaris using all models of inheritance [Table 2].

Genotype frequencies of CATT repeats element were compared between both groups ($P = 0.033^*$). The most commonly observed genotype among patients and controls was 6/6 [Figure 1]. The frequency of -794*CATT_{5/7} and -794*CATT_{7/7} genotypes were significantly lower in patients ($P = 0.027^*$ and 0.038^* , respectively), compared to the controls. When carriage of MIF CATT₇ (5/7+ 6/7 + 7/7 vs. 5/5 + 5/6 + 6/6) was compared between patients and controls, statistically significant differences were noted, with carriage of MIF CATT₇ being lower in patients ($P = 0.01^*$). The allelic distribution shows that the allele -794CATT₇ was significantly underrepresented in patients {Odds ratio=0.52, 95% confidence intervals (0.34–0.79), $P = 0.002^*$ }. None

Table 1: Basic demographic profile of the patients and healthy controls

Variables	Patients (n=265)	Controls (n=252)	P
Age at onset (years) (mean, range)	32.6±15.5, 1–73	NA	
Male: female ratio	2.6	2.1	0.216
Disease onset (years)			
<40	24.2±9, 1–40	NA	
>40	51.9±8.8, 41–73		
Age of onset in males (years)	32.96±15	NA	0.545
Age of onset in females (years)	31.65±16.8	NA	
Psoriasis area severity index (mean, range)	6.04±6.2, 0–59	NA	
Serum MIF levels (mean)* (ng/ml)	15.68±5.86	7.75±2.72	<0.001
Duration of psoriasis in years (mean, range)	8.7±7.1, 1 month–40 years	NA	
Number of patients with comorbidity	70 (26.4%)	NA	
Body surface area (mean, range)	10.01±11.54, 0%–90%	NA	

*Serum MIF ELISA was done in randomly chosen 100 patients and 100 healthy controls. MIF: Migration inhibitory factor, NA: Not available, ELISA: Enzyme-linked immunosorbent assay

of the CATT alleles (5, 6, 7) individually were found to be associated with increased risk of developing disease ($p > 0.05$). We grouped MIF-794 CATT₆₋₈ repeats and termed them X allele. X allele frequency was compared between two groups and it showed an almost equal distribution ($P = 0.080$).

Migration inhibitory factor haplotype analyses

The haplotype 6G was the most frequent amongst both patients and controls [Table 3]. The CATT₅-MIF-173*C haplotype was observed to be significantly higher in patients with an Odds ratio of 3.03 (95% confidence intervals =1.09–8.47). A significant evidence for association with disease was observed ($P = 0.02$), thereby suggesting the importance of this haplotype in susceptibility to psoriasis in our cohort.

Distribution of allele and genotype frequencies between various psoriatic subcategories

No significant differences were observed in the distribution of alleles and genotypes of the MIF-173*G/C polymorphism and MIF-794*CATT tetra-nucleotide repeats number variation in male versus female patients; in early versus late onset disease subjects; mild-to-moderate versus severe disease; in patients with short, intermediate, or longer duration of disease and in patients with or without any disease-associated comorbidities ($p > 0.05$) [Table 4].

Serum migration inhibitory factor concentration

The mean serum MIF levels were significantly higher in patients (15.68±5.86 ng/ml vs. 7.75±2.72 ng/ml; $P < 0.001$). In receiver-operator characteristics curve analysis, the cutoff value was 9.8 ng/ml with a sensitivity and specificity of 88% and 76%, respectively [Figure 2]. No statistically significant correlation was found between the mean serum MIF levels and gender ($P = 0.297$), age of onset ($P = 0.192$) [Figure 3a], disease severity ($P = 0.735$) [Figure 3b], and presence or absence of comorbidities ($P = 0.879$). Although mean serum MIF levels were found to be minimally elevated in males, in patients with severe psoriasis and with early-onset psoriasis, in -173C allele carriers (GC+CC genotypes; n=45), and in patients with extended (>5) CATT repeats, that is, 6/6+6/7+6/8+7/7 genotypes (n=45), none of the results were statistically significant.

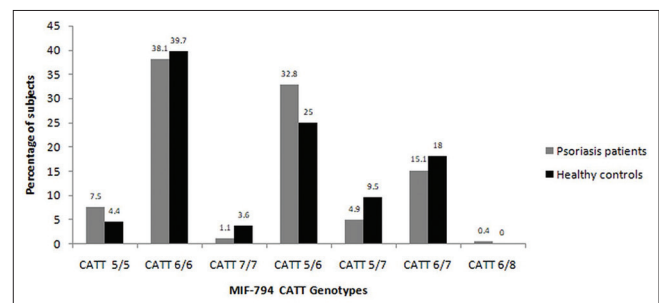


Figure 1: Distribution of MIF-794*CATT tetranucleotide repeats (genotypes) in patients and healthy controls

Table 2: Frequencies of migration inhibitory factor -173G/C and -794CATT alleles and genotypes among psoriasis patients and healthy controls

Polymorphism	Genotypes and alleles	Patients (n=265), n (%)	Controls (n=252), n (%)	OR (CI)	P
-173 G/C	G	401 (75.6)	376 (74.6)	Reference	Reference
	C	129 (24.3)	128 (25.3)	0.94 (0.71–1.25)	0.69
	G/G	144 (54.3)	139 (55.1)	Reference	Reference
	G/C	113 (46.6)	98 (38.9)	1.11 (0.77–1.59)	0.55
	C/C	8 (3.0)	15 (5.9)	0.51 (0.21–1.25)	0.13
	G/C+C/C	121 (45.6)	113 (44.8)	1.03 (0.73–1.46)	0.85
-794 CATT	5	140 (26.4)	109 (21.6)	Reference	Reference
	6	330 (62.2)	308 (61.1)	0.83 (0.62–1.12)	0.23
	7	59 (11.1)	87 (17.3)	0.52 (0.34–0.79)	0.002
	8	1 (0.2)	-	-	-
	Grouped				
	5	140 (26.4)	109 (21.6)	Reference	Reference
	>5 (X allele)	390 (73.5)	395 (78.3)	0.76 (0.57–1.02)	0.080
	5/5	20 (7.5)	11 (4.4)	Reference	Reference
	6/6	101 (38.1)	100 (39.7)	0.55 (0.25–1.21)	0.17
	7/7	3 (1.1)	9 (3.6)	0.18 (0.04–0.82)	0.038
	5/6	87 (32.8)	63 (25)	0.75 (0.33–1.69)	0.55
	5/7	13 (4.9)	24 (9.5)	0.29 (0.10–0.80)	0.027
	6/7	40 (15.1)	45 (18)	0.48 (0.20–1.14)	0.14
	6/8	1 (0.4)	-	-	-
	5/5+5/6+6/6	208 (78.5)	174 (69.0)	Reference	Reference
	5/7+6/7+7/7+6/8	57 (21.5)	78 (31.0)	0.61 (0.41–0.90)	0.01

OR: Odds ratio, CI: Confidence interval

Table 3: Frequencies of migration inhibitory factor CATT-173G/C haplotypes in psoriasis patients and healthy controls

Haplotypes	Psoriasis patients (2n=530), n (%)	Healthy controls (2n=504), n (%)	OR (95% CI)	P
MIF CATT ₅ -173G	122 (23)	103 (20.4)	Reference	Reference
MIF CATT ₅ -173C	18 (3.3)	5 (1)	3.03 (1.09–8.47)	0.02*
MIF CATT ₆ -173G	273 (51.5)	255 (50.6)	0.90 (0.66–1.23)	0.57
MIF CATT ₆ -173C	57 (10.8)	54 (10.7)	0.89 (0.56–1.40)	0.64
MIF CATT ₇ -173G	6 (1.1)	17 (3.4)	0.29 (0.11–0.78)	0.01
MIF CATT ₇ -173C	53 (10.0)	71 (14.0)	0.63 (0.40–0.98)	0.04
MIF CATT ₈ -173C	1 (0.2)	0	NA	NA

The MIF CATT₅-173G haplotype was selected as the baseline for comparisons (no associated OR, 95% CI or P value). NA: Not applicable (no test was done for uncommon haplotypes because the low frequencies are not reliable for comparison), OR: Odds ratio, CI: Confidence interval, MIF: Migration inhibitory factor

Discussion

The severity and course of illness differ across psoriatic patients and so does its management. Hence, it is important to classify patients into groups who might require aggressive and longer courses of therapy and those who can be managed more conservatively. The age of onset is one such marker which defines two subpopulations of patients. Early-onset (type I) psoriasis typically presents before the age of 40 years, with a peak onset at 16–22 years; approximately 70% of all psoriasis

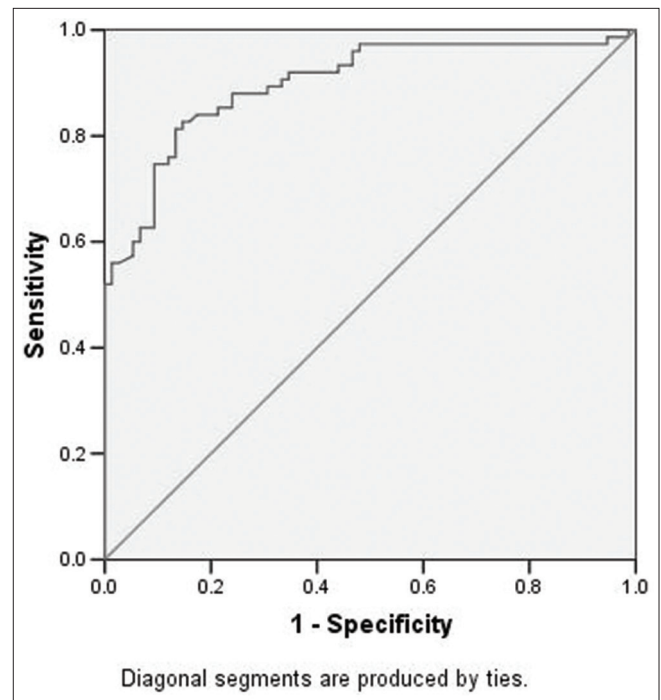


Figure 2: Receiver-operator characteristics (ROC) curve analysis depicting area under the curve (0.899), sensitivity (88%) and specificity (76%) for serum MIF levels.

patients fall in this category. Late-onset (type II) psoriasis usually presents after 40 years, with a peak onset between 57–60 years.^{21,22} The results of this Indian cohort corroborate

Table 4: Clinical data of 265 psoriasis patients according to the genotype for migration inhibitory factor -794CATT repeats

Clinical variable	MIF-794 CATT genotypes (n = 265)							P
	5/5 (n = 20)	6/6 (n = 101)	7/7 (n = 3)	5/6 (n = 87)	5/7 (n = 13)	6/7 (n = 40)	6/8 (n = 1)	
Age at onset (years) (mean, range)	40 ± 15, 18–73	31.4 ± 15.7, 1–69	34 ± 24, 15–61	33.45 ± 14, 1–65	28 ± 16, 8–63	31 ± 17.3, 1–71		0.125
Duration of disease (years) (mean, range)	7.5 ± 4.8, 0.5–20	8.8 ± 7, 0.25–40	7 ± 3, 4–10	8 ± 7, 0.08–34	12.1 ± 6.2, 3–26	8.7 ± 7.5, 0.50–35		0.290
PASI scores (mean)	5.3 ± 4.2	6.1 ± 7.2	6.5 ± 8.2	5.8 ± 4.1	3.4 ± 1.8	7.1 ± 8.5		0.422
Comorbidities (n = 70; 26.4%), n (%)	5 (7.1)	23 (32.9)	2 (2.9)	22 (31.4)	6 (8.6)	11 (15.7)	1 (1.4)	0.881
Serum MIF values* (mean, range)	13.2 ± 5.3	14.3 ± 6.6	-	12.4 ± 5.6	16.7 ± 4.9	13.4 ± 6.5	-	1.000

*Serum MIF values were available in 100/265 patients. MIF: Migration inhibitory factor, PASI: Psoriasis area severity index

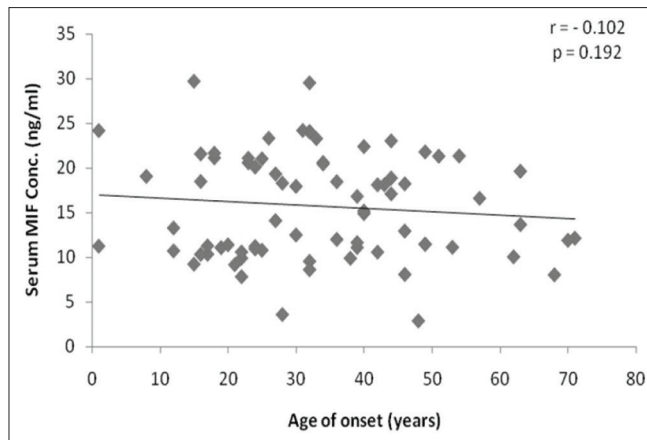


Figure 3a: Scatter plot with linear regression analysis showing correlation between serum MIF levels (ng/ml) and age of onset of chronic plaque psoriasis

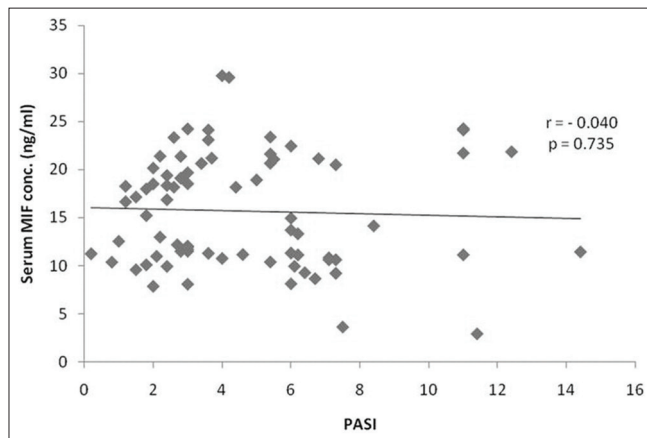


Figure 3b: Scatter plot with linear regression analysis showing correlation between serum MIF levels (ng/ml) and clinical severity of psoriasis in terms of PASI scores.

these findings as 69.8% of our patients had early-onset psoriasis. Early-onset psoriasis shows a strong association with Class I human leukocyte antigen (HLA) alleles, specifically HLA-C*06, whereas late-onset psoriasis is more sporadic, has an unclear genetic background, and evinces a greater association with disease associated comorbidities.²²⁻²⁵ In this study also, we found that the presence of comorbidities was more frequently noted in patients with late-onset disease ($P = 0.01$).

MIF is a pivotal regulator of innate immunity that promotes pro-inflammatory activities of various immune cells.^{26,27} Promoter polymorphisms in MIF gene influence basal as well as disease-associated MIF expression and its serum levels, which in turn have been shown to have enormous effects on disease phenotype in experimental models.^{28,29}

The choice of single-nucleotide polymorphisms tested in the present study was principally based on earlier reports.^{9-12,14,15,18,28} Baugh *et al.* identified a functionally significant polymorphism in the human MIF gene, consisting of CATT repeats at position -794 of the MIF promoter.¹⁵ The 5-CATT repeat display lowers promoter activity when compared to the 6-, 7-, or 8 repeat alleles. Another polymorphism in the MIF promoter region is -173*G/C. In all populations studied, the -173*G allele (75%–90%) has been found to be far more common than the -173*C allele (15%–20%). MIF-173*C allele introduces activating enhancer binding protein 4 (AP-4) transcription factor binding site which in turn affects MIF gene expression, increasing MIF production.¹⁶

The previous association studies investigating these polymorphisms in various diseases across a variety of populations have given discordant results.^{9-12,14-17} Thus, it appears that the role of MIF is highly variable, depending on the populations, disease, and cell-types being studied. The genotype and haplotype frequencies of MIF polymorphisms in controls were found to be consistent with similar published studies from other South East Asian countries as already observed in our earlier study (unpublished data, in communication).^{10-12,16-18}

This is the first report on the frequencies of these polymorphisms in North Indian psoriatic patients. In this study, 74% patients carried extended MIF -794*CATT repeats (>5, i.e., 6, 7, 8) which is expected to be associated with increased pro-inflammatory activity. Furthermore, the CATT₅-MIF-173*C haplotype conferred an odds ratio of 3.03 for the risk of susceptibility to psoriasis. Donn *et al.* found that MIF-CATT₇-173*C haplotype was important in susceptibility to psoriasis in a Caucasian population while Wu *et al.* did not observe any significant differences in the

distribution of alleles, genotypes or haplotypes between patients and controls in the Han Chinese population.^{17,18} These differences in haplotype frequencies may be due to ethnic or genetic differences in, or environmental influences on, psoriasis among the populations analyzed.

Mallon *et al.*³⁰ and Burden *et al.*³¹ observed an association between gender and psoriasis susceptibility. Wu *et al.* suggested a preliminary association between the MIF-173*C allele and male psoriasis and late-onset psoriasis in Han population from China.^{30,31} However, we did not find any such correlation.

We found that the serum MIF levels in patients were significantly elevated ($P < 0.001$), a finding similar to that of Shimizu *et al.*³² Shimizu *et al.* reported more severe disease in their cohort of 48 patients with a mean psoriasis area severity index of 17.9 ± 1.4 (range 3.2–41.4), and noted that severity of disease showed a linear correlation with serum MIF levels, which was not observed by us.³² Most patients in our cohort had mild-to-moderate disease severity (mean psoriasis area severity index score 5.45 ± 4.63), with only 13 patients having psoriasis area severity index score > 10 . We suggest that it would be an oversimplification to expect a linear correlation between serum MIF levels and disease severity, since there might be numerous other factors that determine the latter. In addition, our results reiterate the effect of genetic heterogeneity among different ethnic populations on the pathogenesis of psoriasis.

Limitations

There are some limitations to this analysis. First, serum MIF levels were not measured in all patients, and second the lesional expression of MIF could not be studied due to financial constraints.

We attempted to evaluate genetic susceptibility in patients with chronic plaque psoriasis, using the candidate gene approach. We observed that MIF promoter variants have an impact on susceptibility to psoriasis. Association of CATT5-MIF-173*C haplotype with chronic plaque psoriasis was found to be significant and could be used as a genetic marker to identify individuals at high risk for developing psoriasis. This is a preliminary attempt to identify the phenotype of disease susceptible groups and it must be stressed that our results need to be validated in larger population samples.

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Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent.

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Conflicts of interest

There are no conflicts of interest.

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