Macrophage inhibitory factor (MIF) gene polymorphisms are associated with disease susceptibility and with circulating MIF levels in active non-segmental vitiligo in patients from western Mexico

Alejandra García-Orozco¹ ² | Itzel Alejandra Martínez-Magaña³ | Annie Riera-Leal² | José Francisco Muñoz-Valle⁴ | Marco Alonso Martínez-Guzman¹ | Ricardo Quiñones-Venegas³ | Gabriela Athziri Sánchez-Zuno² ⁴ | Mary Fafutis-Morris¹

¹Centro de Investigación en Inmunología y Dermatología/Instituto Dermatológico de Jalisco “Dr. José Barba Rubio”, Centro Universitario de Ciencias de la Salud, Universidad de Guadalajara, Zapopan, Mexico
²Doctorado en Ciencias Biomédicas con Orientación en Inmunología, Departamento de Fisiología, Centro Universitario de Ciencias de la Salud, Universidad de Guadalajara, Guadalajara, Mexico
³Instituto Dermatológico de Jalisco “Dr. José Barba Rubio”, Secretaría de Salud Jalisco, Zapopan, Mexico
⁴Instituto de Investigación en Ciencias Biomédicas, Centro Universitario de Ciencias de la Salud, Universidad de Guadalajara, Guadalajara, Mexico

Correspondence
Mary Fafutis-Morris, Centro de Investigación en Inmunología y Dermatología/Instituto Dermatológico de Jalisco “Dr. José Barba Rubio”, Centro Universitario de Ciencias de la Salud, Universidad de Guadalajara, Av. Federalismo Norte 3102, Col. Atemajac del Valle, Zapopan, Jalisco 45190, Mexico.
Email: mfafutis@gmail.com

Abstract

Background: The macrophage migration inhibiting factor (MIF) is a protein that promotes the activation of immune cells and the production of other proinflammatory cytokines such as TNF-α, IL-1β, and IFN-γ, which have proposed to play an essential role in the pathogenesis of vitiligo. The study aimed to assess the association between MIF polymorphisms (−794 CATT⁺⁻́∞⁻ and −173 G>C), MIF in situ expression, and MIF serum concentrations with susceptibility and disease activity in patients with non-segmental vitiligo (NSV) from western Mexico.

Methods: The study included 111 patients with NSV and 201 control subjects. Genotyping was performed by conventional PCR (−794 CATT₅ subst and −173 G>C) and PCR-RFLP (−173 G>C) methods. MIF mRNA expression was quantified by real-time PCR and MIF serum concentrations were determined by ELISA kit. Histopathological samples were analyzed by automated immunohistochemistry.

Results: The MIF polymorphisms were associated with NSV susceptibility. Serum concentrations of MIF were higher in patients with active NSV and correlated negatively with the years of evolution. The depigmented skin from patients with active vitiligo showed a high expression of MIF.

Conclusion: MIF polymorphisms increase the risk of NSV in the western Mexican population. The serum concentrations of MIF and in situ expression are associated with active NSV.

KEYWORDS

genetic susceptibility, MIF, non-segmental vitiligo, polymorphisms
1 | INTRODUCTION

Vitiligo affects 0.5% to 2% of the world population, placing it as the most common skin depigmenting disorder resulting from a selective loss of epidermal melanocytes (Boniface, Seneschal, Picardo, & Taieb, 2018; Rodrigues, Ezzedine, Hamzavi, Pandya, & Harris, 2017). Even though it is a complex disease, that combines genetic and environmental factors with metabolic and immune alterations, a significant role of silent inflammation and autoimmunity is demonstrable, in particular, during the progressive phase of the disease (Boniface et al., 2018; Rodrigues et al., 2017; Speeckaert, Speeckaert, De Schepper, & van Geel, 2017).

Depigmentation of the skin and hair follicles is the clinical hallmark of vitiligo, and the extension of the patches allows us to classify the pathology in localized or segmental vitiligo and non-segmental vitiligo (NSV) (Rodrigues et al., 2017). Vitiligo Global Issues Consensus Conference characterizes non-segmental vitiligo as an acquired chronic pigmentation disorder with white patches, most often symmetrical increasing in size progressively or during flares with time, corresponding histologically to a substantial loss of functioning epidermal pigment cells and, usually in a second time, of hair follicle melanocytes (Boniface et al., 2018; Ezzedine et al., 2012).

The immunopathogenesis of vitiligo starts with intrinsic abnormalities of melanocytes and keratinocytes, leading up to the activation of the innate immune response and subsequently, the adaptive immunity (Laddha et al., 2013; Picardo et al., 2015). The recruitment of natural killer cells and inflammatory dendritic cells has described when melanocytes from vitiligo patients develop cellular stress and release inflammatory signals (Rashighi & Harris, 2017). Also, an increased number of cytotoxic CD8+ T cells in the blood and epidermis in the affected skin of patients has been seen and the infiltration correlates with the disease severity (Le Poole, van den Wijngaard, Westerhof, & Das, 1996; van den Boorn et al., 2009). Recently, the release of the chemokine ligands CXCL16 by keratinocytes, and CXCL12 and CCL5 by melanocytes in vitiligo under oxidative stress stand out among the physiopathological mechanisms involved in the recruitment of T cells (Li et al., 2017; Rezk et al., 2017).

Another of the pro-inflammatory cytokines that have recently been associated with an increased risk of vitiligo is the Macrophage migration inhibitory factor (MIF) (Farag, Habib, Kamh, Hammam, & Elhaidany, 2018; Ma et al., 2013; Serarslan et al., 2009). It has been shown to play a crucial role in several types of immune and autoimmune diseases. MIF is stored in the cytoplasm in vesicle-like structures and it is secreted in response to several stimuli including lipopolysaccharide (LPS), tumor necrosis factor (TNF)-α, hypoxia, hydrogen peroxide (H2O2), thrombin, and angiotensin II (Jankauskas, Wong, Bucala, Djudjaj, & Boor, 2019). Also, MIF secretion was induced by oxidative stress and DNA damage, both common mediators of a variety of stimulators of MIF secretion (Gupta, Pasupuleti, Du, & Welford, 2016). Also, it is characterized by the fact that it allows the activation of immune cells and the production of proinflammatory cytokines such as TNF-α, IL-1β, and IFN-γ, which have proposed to play an essential role in the pathogenesis of vitiligo (Ma et al., 2013).

Polymorphism in the number of CATT microsatellite repeats at the position −794 of MIF promoter affects its transcription (rs5844572). The presence of more than five CATT repeats genotypes is associated with a higher MIF promoter activity and has been associated with higher severity of autoimmune diseases (Baugh et al., 2002). SNP −173 G>C (rs755622) located in the promoter region of MIF gene (OMIM: 153620) strongly correlates with the severity and susceptibility to several inflammatory and autoimmune pathologies (Castañeda-Moreno et al., 2018; De la Cruz-Mosso et al., 2014; Illescas, Gomez-Verjan, García-Velázquez, Govezensky, & Rodriguez-Sosa, 2018; Llamas-Covarrubias et al., 2013).

The combination of oxidative stress and inflammatory mediators in the pathogenesis of vitiligo and within the physiological mechanisms of MIF release, analyze the polymorphisms that affect the promoter region of the MIF gene an important determinant of risk factors for the disease, which may lead to improved prevention and treatment options. That is why in this study, the influence of −794 CATT5,8 and −173 G>C MIF polymorphisms in non-segmental vitiligo and its correlation with serum concentrations, histopathological levels of the protein of patients samples, and disease activity was evaluated.

2 | MATERIALS AND METHODS

2.1 | Ethical compliance

The present study was approved by the Ethical Investigation and Biosafety Committee of the University Center of Health Sciences at the University of Guadalajara (Reference Number CUCS/CINV/0170/17; Guadalajara, Mexico). All research was performed according to the Brazil 2013 amendment of the Declaration of Helsinki (World Medical Association, 2013) and Mexico regulations for studies on human health. Informed consent was signed by all the individuals included in our study.

2.2 | Subjects

The 312 participants in the study were classified into two groups: 111 patients from the Dermatological Institute of
GARCIA-OROZCO et Al.

Jalisco “Dr. José Barba Rubio” with a clinical diagnosis of NSV and 201 clinically healthy subjects (CS). None of the CS or their relatives had any evidence of vitiligo. All participants were at least 18 years old of any gender, Mexican mestizo, and native to western Mexico for at least three generations. For the purpose of this study, a Mexican mestizo was defined as a person that was born in Mexico, with the last name of Spanish origin and whose previous three ascending generations were also born in Mexico (Gorodezky et al., 2001). Patients and CS with a body mass index >30, smoking habit, personal history of infectious, immunological, autoimmune/inflammatory or immunosuppressive diseases, pregnant, and lactating women were excluded from the study. None of the patients were having systemic steroid therapy, photo(chemo)therapy, or immunosuppressant treatments.

Patients with NSV were also subclassified according to the vitiligo disease activity (VIDA) score. This score uses a six-point scale ranging from −1 to +4 based on the presence of new lesions or expansion of existing lesions, where the higher the score represents, the more activity (Ibrahim, Ghaly, El-Tatawy, Khalil, & El-Batch, 2014).

2.3 Genotyping of −794 CATT₅₋₈ and −173 G>C MIF polymorphisms

Genomic DNA was extracted from all subjects from peripheral blood leukocytes by the salting-out method (Noguera, Tallano, Bragós, & Milaní, 2000). The −794 CATT₅₋₈ (NC_000022.11:g.23893563_23893564insATTC) MIF polymorphism was analyzed by end-point polymerase chain reaction (PCR) and polyacrylamide gel electrophoresis using the primers reported by Radstake et al. (Radstake et al., 2007). The −173G>C (NC_000022.11:g.23894205G>C) MIF polymorphism was genotyped by the PCR-restriction fragment length polymorphism (RFLP) technique. Amplification of the polymorphic fragment was performed using the primers reported by Makhija et al. (Makhija, Kingsnorth, & Demaine, 2007); 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. The PCR protocols used in both polymorphisms were as reported by De la Cruz-Mosso et al. (De la Cruz-Mosso et al., 2014).

2.4 RNA extraction and quantitative real-time PCR

Total RNA was extracted from 5 ml of peripheral blood of 15 NSV patients and 15 CS, according to Chomczynski–Sacchi technique (Chomczynski & Sacchi, 1987). One microgram of total RNA was converted to cDNA using oligo-dT and M-MLV reverse transcriptase (Promega Corp., Madison, WI, USA).

MIF mRNA quantification was determined by quantitative real-time PCR (qPCR) using UPL hydrolysis probes (Roche Applied Science, Germany) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) used as a reference housekeeping gene (Cat. No. 05190541001). The PCR reaction was performed on a LightCycler Nano System (Roche Applied Science, Germany). All samples were run in triplicate using the conditions indicated in the UPL Gene Expression Assay protocol in a LightCycler Nano System (Roche Applied Science). After the validation of reaction efficiency, relative expression analysis was performed by the 2−ΔΔCq and 2−ΔCq methods.

2.5 Quantification of serum MIF concentrations

Serum was obtained from all individuals at the time of inclusion; cytokine levels were quantified in a subset of 111 NSV patients and 103 control subjects. The determination of serum MIF concentrations was performed by kit LEGEND MAX™ Human Active MIF ELISA (BioLegend®, San Diego, CA, EUA) according to manufacturer’s instructions. The MIF assay sensitivity was 17.4 ± 9.2 pg/ml.

2.6 Histopathological samples and immunohistochemistry

The histopathological samples were taken from 25 vitiligo patients and 10 control subjects. The samples of the control subjects were obtained from the perilesional area of the lipoma or benign cyst samples from individuals who came to the Institute for their extraction. Additionally, patients with vitiligo were divided into two groups: 15 samples from patients with active disease and 10 patients with stable disease. The biopsies of the patients contained fragments of depigmented skin and perilesional skin in equal parts. Based on the fact that Sutton’s nevus shares physiopathological, clinical, and histopathological characteristics with vitiligo, we decided to include five samples from patients with this diagnosis. Written informed consent was signed from all the participants included, to collect and use the tissue specimens for research purposes. After collection, tissues were fixed in 4% formalin and embedded in paraffin.

Serial sections of 4 μm were obtained and fixed in positively charged slides (Cat. No. 6776214; Thermo Scientific). Immunohistochemistry processing and staining were performed using the BenchMark ULTRA automated system (Cat. No. N750-BMKU-FS 05342716001; Ventana Medical Systems, Inc, Roche, Tucson, USA). Rabbits and polyclonals primaries antibodies against MIF (Cat. No. sc-20121 RRID: AB_648587) and CD74 (Cat. No. sc-20082 RRID: AB_2075501), both from
Santa Cruz Biotechnology, Inc., TX, USA; were prepared at a concentration of 1:50 in a 1x concentrated solution of Tris-buffered saline with Tween 20 (TBS-T). As a negative control, in some samples, the primary antibody was omitted. The Ventana OptiView DAB IHC detection and OptiView Amplification kit from Ventana Medical Systems, Inc., were used for the staining and amplification of the stain, respectively.

2.7 | Analysis of the protein expression

Digital image files were obtained at 10x and 40x magnifications using an optical microscope (Carl Zeiss AG, Oberkochen, Germany), coupled with a digital camera CoolSNAP (Photometrics, Tucson, USA). The digital analysis was performed using the cell counter function of the Image-pro Plus 6.0® software (Media Cybernetics, Inc., Rockville, MD, USA). The optical densities of the brown color were calculated for five fields of each sample.

2.8 | Statistical analysis

The descriptive analysis, nominal variables were expressed as frequencies; continuous variables with nonparametric distribution were expressed as medians, percentile 10–90, and interquartile ranges 25–75. The genotypic and allelic frequencies of the polymorphisms were performed by direct counting. Hardy–Weinberg equilibrium in control subjects was determined by the chi-square test ($X^2$). The distribution of the genotypes and allele frequencies of the polymorphisms in both groups (NSV patients and control subjects) were analyzed by $X^2$ test with 3x2 and 2x2 contingency tables, respectively. To compare nonparametric quantitative determinations, the Mann–Whitney U test, Odds ratio (OR), and 95% confidence interval (95% CI) were used to analyze the risk for NSV associated with the $MIF$ gene polymorphisms. To evaluate the effect of both polymorphisms on NSV, we performed dominant inheritance genetic models. For correlation analysis of continuous variables with nonparametric distribution, we used the Spearman correlation test. Obtained data were analyzed with the statistical software SPSS v 23 and GraphPad Prism v 7, considering $p \leq 0.05$ as statistically significant and 80% statistical power.

3 | RESULTS

3.1 | Clinical and demographic characteristics

The clinical and demographic features of the study subjects are summarized in Table 1. The median age of patients was 42 years, and 55% were female. All the participants had a normal weight with a body mass index of less than 25. Approximately 10% of the patients had a family history of vitiligo. Forty-five patients suffered some degree of disease activity, while the most had a stable state. Most patients (64%) were used topical treatment at the time of the study: 26.2%, medium-potency corticosteroids, 15.3% a calcineurin inhibitor medication, and 22.5% combination of topical treatment. None of the participants were using systemic therapy. All the patients did not present any other comorbidity at the time of the study and 100% presented negative thyroid function tests (data not shown).

### Table 1 Clinical and demographic characteristics of NSV patients and CS

| Variable                  | NSV $n = 111$ (%) | CS $n = 201$ (%) |
|---------------------------|-------------------|------------------|
| **Sociodemographic characteristics** |                   |                  |
| Age (years)$^a$           | 42 (18–82)        | 38 (18–89)       |
| Gender$^b$                |                   |                  |
| Female                    | 61 (55)           | 107 (53.2)       |
| Male                      | 50 (45)           | 94 (46.8)        |
| BMI (kg/m$^2$)$^c$        | 22.7 (19.1–23.6)  | 20.9 (18.7–24.5) |
| **Clinical characteristics** |                   |                  |
| Family history of vitiligo$^b$ | 11 (9.9)          | –                |
| Activity$^b$              |                   |                  |
| Active                    | 45 (40.5)         | –                |
| Stable                    | 66 (59.5)         | –                |
| Treatment$^b$             |                   |                  |
| Treated                   | –                 | –                |
| TCs                       | 29 (26.2)         | –                |
| TCIs                      | 17 (15.3)         | –                |
| TCs + TCIs                | 25 (22.5)         | –                |
| Non-treated               | 40 (36)           | –                |

*Data are expressed as median and (p5–p95).

*Data are expressed as the number of individuals and percentage (%).

### Table 1Clinical and demographic characteristics of NSV patients and CS

Abbreviations: BMI, body mass index; CS, control subjects; NSV, non-segmental vitiligo; TCIs, topical calcineurin inhibitors; TCs, topical corticosteroids.

3.2 | Association between NSV and $MIF$ gene polymorphisms

The genotypic and allelic frequencies of $–794\ CATT_{5,8}$ and $–173\ G>C$ $MIF$ polymorphisms were determined in patients and CS. No deviation from Hardy–Weinberg equilibrium was observed in both cases ($p > 0.05$). A significant association in the genotype distribution of the $–794\ CATT_{5,8}$ $MIF$ polymorphism in NSV patients ($p = 0.02$) was found, with an OR of 3.16 (CI = 1.33–7.40; $p = 0.01$) for the 5,7 repeats heterozygote genotype, and an OR value of 1.89 (IC = 1.28–2.79, $p = 0.001$) for the $–794\ CATT_7$ allele. This evidence indicates
that the −794 CATT<sub>7</sub> allele may increase the risk of NSV in the western Mexican population. According to the dominance model, a statistically significant difference between both groups (p = 0.001) and an OR of 2.20 (IC = 1.37–3.53) were found, which suggests that the heterozygous genotypes with allele (−,7 + 7,7) have 2.20-fold more susceptibility to present NSV compared with the subjects without the risk allele. Following these observed patterns, we concluded that a single copy of the −794 CATT<sub>7</sub> allele is enough to increase the risk (Table 2).

Similar to −794 CATT<sub>5-8</sub>, the analysis of the genotype distribution evidenced a significant association (p = 0.05) with an OR equal to 2.08 (1.29–3.36; p = 0.003) of the −173 G>C MIF polymorphism with NSV diagnosis. However, for the heterozygote genotype GC, no significant difference in the allelic frequency was found. According to the dominance model, the carriers of the −173*C allele (GC + CC) showed 1.93-fold risk (OR = 1.93; CI = 1.20–3.08, p = 0.008) to develop NSV than carriers of the −173*G allele (GG), indicating that a single copy of C allele is enough to increase the risk (Table 2).

### 3.3 | MIF mRNA expression and serum MIF concentrations in NSV patients, association with the disease activity

The relative mRNA expression of MIF was evaluated in all groups (Figure 1a). The analysis by the 2<sup>−ΔΔCq</sup> method showed that MIF mRNA expression in NSV patients and active NSV was 0.54- and 0.21-fold, respectively, less compared to MIF mRNA expression in CS. Moreover, the MIF mRNA expression
in stable NSV was 1.90-fold higher than the CS. When these data were analyzed by the $2^{-\Delta\Delta C_q}$ method, these differences were not statistically significant ($p = 0.246$, data not shown).

We determined if there were differences in serum MIF concentrations between patients and CS. The comparison of the levels between both groups was not significant. Nevertheless, when we stratified the serum MIF concentrations according to the activity index of the patients, a higher MIF concentration was found in active patients in comparison with the stable ones [5.83 ng/ml (4.39–7.64) vs. 4.16 ng/ml (3.75–5.35); $p = 0.0001$]; and with the CS [5.83 ng/ml (4.39–7.64) vs. 4.94 ng/ml (3.05–7.84); $p = 0.033$] (Figure 1b). Moreover, patients with active vitiligo and a score of the activity index equal to or greater ($\geq$) than +3 had higher concentrations of MIF [$p <0.0001$ compared to +2 punctuation and $p = 0.0008$ compared to +1 score]; (Figure 1c).

To evaluate if the use of topical treatment influenced the serum MIF concentrations and the interpretation of our results, we compared the mean values of both groups. Any significant difference was found ($p = 0.445$) (Figure 1d).

### 3.4 Circulating levels of MIF correlated with the time of evolution and activity of the disease

Correlation analysis revealed that the MIF serum concentrations correlated negatively with the years of evolution ($r = -0.222$, $p = 0.019$; Figure 2a) and with the activity of the disease ($r = -0.426$, $p = 0.004$; Figure 2b).

### 3.5 High circulating levels of MIF were associated with MIF-polymorphisms in active vitiligo

The MIF serum concentration in patients was compared by genotypes grouped according to the dominance model proposed for each polymorphism (Figure 3). For $-794$ CATT$_{5-8}$ MIF polymorphism, active NSV patients’ carriers of genotypes with the $-794$ CATT$_{7}$ allele risk showed a significant increase in MIF serum concentrations compared with the...
stable NSV patients carriers of genotypes without the allele risk (-,-) (6.23 vs. 4.45 ng/ml; p = 0.026) and with the stable NSV patients carriers of genotypes with −794 CATT7 allele risk (6.23 vs. 4.10 ng/ml; p = 0.0001) (Figure 3a).

The analysis of −173 G>C MIF polymorphism revealed that stables NSV patients with GG genotype had 4.29 ng/ml of MIF, while actives who are carrying genotypes with the −173*C risk allele (GC + CC) had an increase of 5.81 ng/ml; this difference was statistically significant (p = 0.038). Moreover, active NSV patients carrying the genotypes GC and CC showed a significant increase in MIF levels (5.83 ng/ml vs. 4.29 ng/ml; p = 0.006) and active NSV patients carriers of GG genotype (4.14 ng/ml; p = 0.001) (Figure 3b).

3.6 | In situ involvement of MIF

The depigmented skin from patients with active vitiligo showed a high expression of MIF, which was significantly higher than the appearance of the protein in the rest of the groups (p < 0.05 in all cases). Also, perilesional skin samples of active patients showed significantly higher levels...
of MIF compared to the perilesional skin of stable patients \((p = 0.04)\). Both the depigmented skin and perilesional skin of the 25 patients with vitiligo had higher values of MIF expression compared to the skin sample of control subjects \((p < 0.01)\). The Sutton nevus samples had high MIF levels compared to control subjects \((p = 0.002)\); however, the average optical densities were significantly lower compared to the samples from the active vitiligo lesions \((p = 0.03)\) (Figure 4).

4 | DISCUSSION

Vitiligo is a skin disease mediated by autoreactive CD8\(^{+}\)T cells that destroy melanocytes, the pigment-producing cells, resulting in disfiguring depigmented macules and patches. Even though the precise etiology of vitiligo remains obscure and many factors have been implicated in the development of the disease, the involvement of the immune system is a decisive factor. Macrophage migration inhibitory factor (MIF) is known to participate in immune-mediated diseases, including rheumatoid arthritis, systemic lupus erythematosus, and some autoimmune skin diseases. However, its role in the pathophysiology of vitiligo has been little explored. Although there are at least 200 genetic polymorphisms described within the MIF gene (Donn et al., 2002), only two identified in the promoter region appear to have functional importance: the \(-794 \text{ CATT}_{5,8}\) and \(-173 \text{ G>C}\) polymorphisms. Both have been associated with increased serum MIF circulation levels in different populations.

This is the first study that describes the presence of the \(-794 \text{ CATT}_{5,8}\) and \(-173 \text{ G>C}\) polymorphisms associated with an increased risk of presenting non-segmental vitiligo. Previous studies have determined the frequency of the MIF promoter polymorphisms and their contribution for systemic autoimmune diseases such as rheumatoid and juvenile idiopathic arthritis (Donn et al., 2004; Llamas-Covarrubias et al., 2013), systemic lupus erythematosus (De la Cruz-Mosso et al., 2014; Sreih et al., 2011), sarcoidosis, and multiple sclerosis (Castañeda-Moreno et al., 2018); or infectious pathologies such as active pulmonary tuberculosis (Kuai et al., 2016) and malaria (Awandare, Martinson, Were, Ouma, & Gregory, 2009). There are reported associations between high-expression MIF alleles and improved outcomes in pneumonia and meningococcal meningitis (Kuai et al., 2016; Renner et al., 2012). Besides, the main impact of high-expression of MIF alleles on the severity of the clinical phenotype in asthma (Mizue et al., 2005), systemic sclerosis (Castañeda-Moreno et al., 2018), and inflammatory bowel disease (Nohara et al., 2004), have been described.

Despite several previous studies that reported an increased risk and severity of inflammatory diseases and reduced response to glucocorticoid medication (Barton et al., 2003); the genetic contribution of MIF promoter polymorphisms to some pathologies susceptibility and phenotype is unclear. To clarify, as definitively as possible, the contribution of MIF promoter polymorphisms to vitiligo risk and phenotype in this study, we also determined the relationship between \(-794 \text{ CATT}_{5,8}\) and \(-173 \text{ G>C}\) polymorphisms and the serum levels of MIF. In the first instance, there were no
significant differences between patients and controls in terms of serum MIF levels. Moreover, serum MIF concentrations were not related to the presence of polymorphisms in patients with vitiligo.

MIF has been named as “an incriminating agent in dermatological disorders” (Pazyar, Feily, & Yaghoobi, 2013). However, few studies analyze its role in the pathophysiology of dermatological diseases. In vitiligo, this statement is based only on the results of two independent studies. Serarslan and cols. Evaluated the serum of 30 patients and 30 control subjects. The authors concluded that MIF would have an important role in vitiligo since the mean serum MIF level of patients was higher than that of controls.

Interestingly, the author found a significant difference between patients with generalized vitiligo and those who had the localized variant, still, there was no correlation between MIF levels and the disease activity (Serarslan et al., 2009). The other study conducted by Ma et al. analyzed the serum MIF concentrations and mRNA levels in PBMCs of 44 vitiligo vulgaris patients and 32 controls. Both MIF serum levels and mRNA were significantly higher in PBMCs from patients than controls. Also, there was a significant difference between progressive and stable patients, and the vitiligo area severity index score (VASI) of patients correlated positively with changes in both serum MIF concentrations and mRNA levels (Ma et al., 2013). More recently, Farag carried out a study with 50 patients with different degrees of vitiligo severity and 15 healthy controls. Serum MIF concentrations and MIF mRNA levels were significantly higher in patients with vitiligo vulgaris compared to controls, in generalized vitiligo compared to the localized one, and it was a positive correlation with the vitiligo type, duration, and severity (Farag et al., 2018).

The previously exposed evidence presents severe limitations to be able to define the role of MIF in vitiligo. At first glance, all three studies analyzed a small sample of patients. The selection criteria of the patients were not uniform among the three studies and factors that can modify MIF concentrations such as body mass index and cigarette smoke were not considered. The role of MIF in human adipose tissue was first explained by Skurk et al., who demonstrated that adipocytes, as well as preadipocytes, released significant amounts of MIF, and the protein could be localized in the cytoplasm of both the cell types (Skurk et al., 2005). Later, the stronger association of MIF in obese individuals and the relationship with some specifics MIF genotypes have been evidenced by other authors (Kim, Pallua, Bernhagen, & Bucala, 2015; Nishihira & Sakaue, 2012; Sakaue et al., 2006). The capacity of cigarette smoke to alter MIF expression and the susceptibility to suffer from human chronic lung diseases has been proven, and in the murine model, chronic cigarette smoke exposure resulted in decreased MIF mRNA and protein expression in the intact lung (Fallica et al., 2014, 2016). Other deficiencies in published studies about MIF and vitiligo include: Differences in the measurement of vitiligo lesions by different evaluators, different criteria to evaluate the activity index and the progressive or stable character of vitiligo, and only one study defined MIF mRNA levels directly in a minimal number of histopathological skin samples of patients (Ma et al., 2013).

Then, we decided to stratify the patients according to the activity index of vitiligo. Similar to Ma and Farag, a significant difference in MIF concentrations between the active vitiligo patients compared to the stable ones was found. Even more significant, the degree of activity of vitiligo showed a correlation with the presence of −794 CATT5−8 and the −173 G>C polymorphisms and the serum concentrations of MIF. These data suggested that even when there is a genetic risk to suffer the disease, MIF has dynamic changes during different disease status. Eating habits, lifestyle, and other epidemiological factors could intervene in the analysis of MIF participation in the pathophysiology of diseases. Even when evaluating the evolution of the activity of the disease in months, we found that patients with more significant activity and shorter “active” progression have higher levels of serum MIF. These results have also been observed in other autoimmune diseases by our research group (Llamas-Covarrubias et al., 2012, 2013). A possible explanation for this is that MIF is involved in the early stages of the disease-promoting proinflammatory synthesis. The prognostic utility for MIF in predicting acute states of different pathologies has been described (Grieb, Merk, Bernhagen, & Bucala, 2010). It has been proposed as a biomarker in central nervous system infection (Östergaard & Benfield, 2009), acute pancreatitis and pancreatic necrosis (Rahman, Menon, Holmfield, McMahon, & Guillou, 2007), acute pyelonephritis (Ouksel et al., 2009), and different causes of severe sepsis (Östergaard & Benfield, 2009) Thus, MIF appeared to be a biomarker for acute pathologies such as active vitiligo and clinical illness.

Since its discovery, MIF has been assumed an important role as a pro-inflammatory cytokine; however, at present, MIF is also believed to control the inflammatory “set point” by regulating the release of other pro-inflammatory mediators (Kuai et al., 2016). MIF secretion was induced rather than inhibited by glucocorticoid hormones, and this system controls inflammatory and immune responses (Calandra et al., 1995; Calandra & Roger, 2003). MIF normally circulates in plasma, and its levels rise together with adrenocorticotropic hormone in response to stress or invasive stimuli, the hormone stimulates adrenal glucocorticoid production (Flaster, Calandra, & Roger, 2003). Also, MIF is expressed constitutively by several cell types, including the epithelial lining of tissues in direct contact with the external environment, positioning MIF as a regulator of host responses (Calandra & Roger, 2003). It is considered that it could have a fundamental role in the
differentiation of the normal epithelium in the skin (Shimizu, Ohkawara, Nishihira, & Sakamoto, 1996). This evidence and our results open new avenues about the possible role of MIF in vitiligo. Molecular studies are required to evaluate whether MIF has a protective role in patients with active vitiligo and short evolution trying to regulate the inflammatory response or if MIF is a part of the courtship of pro-inflammatory cytokines that cause the clinical manifestations.

Since most of our patients were receiving treatment, we wanted to evaluate if this variable affected the interpretation of our results by modifying the MIF concentrations. There were no differences in the serum concentrations of MIF between patients with vitiligo in treatment and without treatment. Commonly used repigmentation therapies for vitiligo that are supported by data from randomized controlled trials include topical agents such as corticosteroids and calcineurin inhibitors (Boniface et al., 2018). In the last decades, corticosteroid derivatives have been designed to simultaneously improve efficacy without reaching the systemic circulation to avoid systemic adverse effects (Gual, Pau-Charles, & Abeck, 2015). Esterification, increase lipophilicity, and improvement of the characteristics of the vehicle, together with the proper indication and use, have been reported to enhance potency, while improving the safety profile of the molecule (Chi et al., 2017; Gual et al., 2015). Another treatment frequently used are calcineurin inhibitors (Cavalié et al., 2015). They offered alternative to topical corticosteroids, since for the treatment of some diseases, like vitiligo, they are as effective or more effective than mild topical corticosteroids with fewer adverse effects (Carr, 2013; Sisti, Sisti, & Oranges, 2016).

It should be noted that the global values of MIF in both study groups were not found above the standard reference values reported in serum (2–6 ng/ml) (Petrovsky et al., 2003). This could indicate a possible in situ and non-systemic involvement of MIF. That is why, in this study, we also evaluate the presence of MIF protein in histopathological samples of patients with vitiligo and compare them with the expression in healthy tissues. Significantly higher levels of MIF protein were found in the samples from patients with vitiligo, especially in the depigmented skin of patients with disease activity. According to our results, Ma et al., 2013 found that MIF mRNA levels were significantly higher in lesional than in healthy skin (Ma et al., 2013). A limitation of this study is that the analysis of mRNA was only carried out in 15 patients and 15 subjects, so new studies are required to ascertain our results. In conclusion, the MIF gene polymorphisms increase the risk of NSV in the western Mexican population. However, further studies with a large sample are necessary to verify the association between NSV and the MIF gene polymorphisms. Moreover, the serum concentrations of MIF and in situ expression are associated with active NSV.

ACKNOWLEDGMENT
We thank and appreciate the participation of every person in this study.

CONFLICTS OF INTEREST
No conflict of interest was declared by the authors.

AUTHOR CONTRIBUTIONS
Alejandra Garcia-Orozco performed the genotyping, statistical analysis, and drafted the manuscript. Itzel Alejandra Martínez-Magaña and Ricardo Quíñones-Venegas diagnosed and treated the patients. Annie Riera-Leal participated in sample collection, sample processing, and provided immunohistochemical assistance. Gabriela Athziri Sánchez-Zuno performed mRNA extraction and real-time PCR experiments. Marco Alonso Martínez-Guzman participated in the interpretation of results and statistical analysis of data. Mary Fafutis-Morris and José Francisco Muñoz-Valle designed the study, co-supervised the work, and revised the manuscript. All the authors have read and approved the final manuscript.

ORCID
José Francisco Muñoz-Valle https://orcid.org/0000-0002-2272-9260
Mary Fafutis-Morris https://orcid.org/0000-0002-5384-1175

REFERENCES
Awandare, G. A., Martinson, J. J., Were, T., Ouma, C., & Gregory, C. (2009). Macrophage Migration Inhibitory Factor (MIF) promoter polymorphisms and susceptibility to severe malarial anemia. *The Journal of Infectious Diseases*, 200(4), 629–637. https://doi.org/10.1086/600894
Barton, A., Lamb, R., Symmonds, D., Silman, A., Thomson, W., Worthington, J., & Donn, R. (2003). Macrophage migration inhibitor factor (MIF) gene polymorphism is associated with susceptibility to but not severity of inflammatory polyarthritis. *Genes and Immunity*, 4(7), 487–491. https://doi.org/10.1038/sj.gene.6364014
Baugh, J. A., Chitnis, S., Donnelly, S. C., Monteiro, J., Lin, X., Plant, B. J., … Bucala, R. (2002). A functional promoter polymorphism in the macrophage migration inhibitory factor (MIF) gene associated with disease severity in rheumatoid arthritis. *Genes and Immunity*, 3(3), 170–176. https://doi.org/10.1038/sj.gene.6363867
Boniface, K., Seneschal, J., Picardo, M., & Tabet, A. (2018). Vitiligo: Focus on clinical aspects, immunopathogenesis, and therapy. *Clinical Reviews in Allergy & Immunology*, 54(1), 52–67. https://doi.org/10.1007/s12016-017-8622-7
Calandra, T., Bernhagen, J., Metz, C. N., Spiegel, L. A., Bacher, M., Donnelly, T., … Bucala, R. (1995). MIF as a glucocorticoid-induced modulator of cytokine production. *Nature*, 377(6544), 68–71. https://doi.org/10.1038/377068a0
Calandra, T., & Roger, T. (2003). Macrophage migration inhibitory factor: A regulator of innate immunity. *Nature Reviews Immunology*, 3(10), 791–800. https://doi.org/10.1038/nri1200
Carr, W. W. (2013). Topical calcineurin inhibitors for atopic dermatitis: Review and treatment recommendations. *Pediatric Drugs*, 15(4), 303–310. https://doi.org/10.1007/s40272-013-0013-9

Castañeda-Moreno, V. A., De la Cruz-Mosso, U., Torres-Carrillo, N., Macías-Islas, M. A., Padilla-De la Torre, O., Mireses-Ramírez, M. A., … Muñoz-Valle, J. (2018). MIF functional polymorphisms (-794 CATT 5–8 and -173 G>C) are associated with MIF serum levels, severity and progression in male multiple sclerosis from western Mexican population. *Journal of Neuroimmunology*, 320, 117–124. https://doi.org/10.1016/j.jneuroim.2018.04.006

Cavalié, M., Ezzedine, K., Fontas, E., Montaudie, H., Castela, E., Bahadoran, P., … Passeron, T. (2015). Maintenance therapy of adult vitiligo with 0.1% tacrolimus ointment: A randomized, double blind, placebo-controlled study. *Journal of Investigative Dermatology*, 135(4), 970–974. https://doi.org/10.1016/j.jid.2014.527

Chi, C.-C., Kirtschig, G., Aberer, W., Gabbud, J.-P., Lipozenčić, J., Kärpäti, S., … Zuberbier, T. (2017). Updated evidence-based (S2e) European dermatology forum guideline on topical corticosteroids in pregnancy. *Journal of the European Academy of Dermatology and Venereology*, 31(5), 761–773. https://doi.org/10.1111/jdv.14101

Chomczynski, P., & Sacchi, N. (1987). Single-step method of RNA isolation by acid guanidinium thiocyanate–phenol–chloroform extraction. *Analytical Biochemistry*, 162(1), 156–159. https://doi.org/10.1016/0003-2697(87)99999-7

De la Cruz-Mosso, U., Bucala, R., Palafoux-Sánchez, C. A., Parra-Rojas, I., Padilla-Gutiérrez, J. R., Pereira-Suárez, A. L., … Muñoz-Valle, J. F. (2014). Macrophage migration inhibitory factor: Association of −794 CATT5–8 and −173 G>C polymorphisms with TNF-α in systemic lupus erythematosus. *Human Immunology*, 75(5), 433–439. https://doi.org/10.1016/j.humimm.2014.02.014

Donn, R., Alourfi, Z., De Benedetti, F., Meazza, C., Zeggini, E., Lunt, M., … Ray, D. (2002). 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 and Rheumatism*, 46(9), 2402–2409. https://doi.org/10.1002/art.10492

Donn, R., Alourfi, Z., Zeggini, E., Lamb, R., Jury, F., Lunt, M., … Ray, D. (2004). A functional promoter haplotype of macrophage migration inhibitory factor is linked and associated with juvenile idiopathic arthritis. *Arthritis and Rheumatism*, 50(5), 1604–1610. https://doi.org/10.1002/art.10718

Ezzedine, K., Lim, H. W., Suzuki, T., Katayama, I., Hamzavi, I., Lan, C. C. E., … Taieb, A. (2012). Revised classification/nomenclature of vitiligo and related issues: The Vitiligo Global Issues Consensus Conference. *Pigment Cell & Melanoma Research*, 25(3), E1–E13. https://doi.org/10.1111/j.1755-148X.2012.00997.x

Fallica, J., Boyer, L., Kim, B. O., Serebreni, L., Varela, I., Hamdan, O., … Damico, R. (2014). Macrophage migration inhibitory factor is a novel determinant of cigarette smoke-induced lung damage. *American Journal of Respiratory Cell and Molecular Biology*, 51(1), 94–103. https://doi.org/10.1165/rcmb.2013-0371OC

Fallica, J., Varela, L., Johnston, L., Kim, B. O., Serebreni, L., Wang, L., … Damico, R. (2016). Macrophage migration inhibitory factor: A novel inhibitor of apoptosis signal-regulating kinase 1–p38-xanthine oxidoreductase-dependent cigarette smoke-induced apoptosis. *American Journal of Respiratory Cell and Molecular Biology*, 54(4), 504–514. https://doi.org/10.1165/rcmb.2014-0403OC

Farag, A. G. A., Habib, M. S., Kamh, M. E., Hammam, M. A., & ElNaidany, N. F. (2018). Macrophage migration inhibitory factor as an incriminating agent in vitiligo. *Anais Brasileiros de Dermatologia*, 93(2), 191–196. https://doi.org/10.1590/abd1806-4841.20186068

Flaster, H., Calandra, T., & Bucala, R. (2007). The macrophage migration inhibitory factor–glucocorticoid dyad: Regulation of inflammation and immunity. *Molecular Endocrinology*, 21(6), 1267–1280. https://doi.org/10.1210/me.2007-0065

Gordezky, C., Alaez, C., Vázquez-García, M. N., de la Rosa, G., Infante, E., Balladares, S., … Muñoz, L. (2001). The genetic structure of Mexican Mestizos of different locations: Tracking back their origins through MHC genes, blood group systems, and microsatellites. *Human Immunology*, 62(9), 979–991. https://doi.org/10.1016/S0198-8859(01)00296-8

Grieb, G., Merk, M., Bernhagen, J., & Bucala, R. (2010). Macrophage migration inhibitory factor (MIF): A promising biomarker. *Drug News & Perspectives*, 23(4), 257–264. https://doi.org/10.1538/dnp.2010.23.4.1453629

Gual, A., Pau-Charles, I., & Abeck, D. (2015). Topical corticosteroids in dermatology: From chemical development to galenic innovation and therapeutic trends. *Journal of Clinical & Experimental Dermatology Research*, 6(2), 1–5. https://doi.org/10.4172/2155-9554.1000269

Gupta, Y., Pasupuleti, V., Du, W., & Welford, S. M. (2016). Macrophage migration inhibitory factor secretion is induced by ionizing radiation and oxidative stress in cancer cells. *PLoS One*, 11(1), 1–11. https://doi.org/10.1371/journal.pone.0146482

Ibrahim, Z. A., Ghaly, N. R., El-Tatawy, R. A., Khalil, S. M., & El-Batch, M. M. (2014). A proliferation-inducing ligand in atopic dermatitis and vitiligo. *International Journal of Dermatology*, 53(9), 1073–1079. https://doi.org/10.1111/ijd.12176

Illescas, O., Gomez-Verjan, J. C., Garcia-Velázquez, L., Govezensky, T., & Rodriguez-Sosa, M. (2018). Macrophage migration inhibitory factor -173 G/C polymorphism: A global meta-analysis across the disease spectrum. *Frontiers in Genetics*, 9, 1–14. https://doi.org/10.3389/fgene.2018.00055

Jankauskas, S. S., Wong, D. W. L., Bucala, R., Djudjaj, S., & Boor, P. (2019). Evolving complexity of MIF signaling. *Cellular Signalling*, 57, 76–88. https://doi.org/10.1016/j.cellsig.2019.01.006

Kim, B. S., Pallua, N., Bernhagen, J., & Bucala, R. (2015). The macrophage migration inhibitory factor protein superfamily in obesity and wound repair. *Experimental and Molecular Medicine*, 47(5), e161. https://doi.org/10.1038/emm.2015.26

Kuai, S.-G., Ou, Q.-F., You, D.-H., Shang, Z.-B., Wang, J., Liu, J., … Huang, L.-H. (2016). Functional polymorphisms in the gene encoding macrophage migration inhibitory factor (MIF) are associated with active pulmonary tuberculosis. *Infectious Diseases*, 48(3), 222–228. https://doi.org/10.3109/23744235.2015.1107188

Laddha, N. C., Dwivedi, M., Mansuri, M. S., Gani, A. R., Ansarullah, M. D., Ramachandran, A. V., … Begum, R. (2013). Vitiligo: Interplay with active pulmonary tuberculosis. *Experimental Pathology*, 48(4), 1219–1228. https://doi.org/10.1159/000349531

Le Poole, I. C., van den Wijngaard, R. M., Westerhof, W., & Das, P. K. (1996). Presence of T cells and macrophages in inflammatory vitiligo skin parallels melanocyte disappearance. *The American Journal of Pathology*, 148(4), 1219–1228.

Li, S., Zhu, G., Yang, Y., Jian, Z., Guo, S., Dai, W., … Li, C. (2017). Oxidative stress drives CD8+ T-cell skin trafficking in patients with vitiligo through CXCL16 upregulation by activating the unfolded protein response in keratinocytes. *Journal of Allergy and Clinical Immunology*, 140(1), 177–189. https://doi.org/10.1016/j.jaci.2016.10.013
Primers Nature Reviews Disease https://doi.org/10.1046/j.0818-9641.2002.01148.x

and activity in vitiligo vulgaris. Brazilian Journal of Medical and Biological Research, 46(5), 460–464. https://doi.org/10.1590/S0100-879X201207500152

Ma, L., Xue, H., Guan, X., Shu, C., Zhang, J.-H., & An, R.-Z. (2013). Relationship of macrophage migration inhibitory factor levels in PBMCs, lesional skin and serum with disease severity and activity in vitiligo vulgaris. Brazilian Journal of Medical and Biological Research, 46(5), 460–464. https://doi.org/10.1590/S0100-879X201207500152

Makhija, R., Kingsnorth, A., & Demaine, A. (2007). Gene polymorphisms of the macrophage migration inhibitory factor and acute pancreatitis. Journal of the Pancreas, 8(3), 289–295. https://doi.org/10.5281/ZENODO.30883

Mizue, Y., Ghani, S., Leng, L., McDonald, C., Kong, P., Baugh, J., ... Bucala, R. (2005). Role for macrophage migration inhibitory factor in asthma. Proceedings of the National Academy of Sciences, 102(40), 14410–14415. https://doi.org/10.1073/pnas.0507189102

Nishihira, J., & Sakaue, S. (2012). Overview of macrophage migration inhibitory factor (MIF) as a potential biomarker relevant to adiposity. Journal of Traditional and Complementary Medicine, 2(3), 186–191. https://doi.org/10.1016/S2225-4110(16)30098-0

Noguer, N. I., Tallano, C. E., Bragós, I. M., & Milani, A. C. (2000). Modified salting-out method for DNA isolation from newborn cord blood nucleated cells. Journal of Clinical Laboratory Analysis, 14(6), 280–283. https://doi.org/10.1002/1098-2825(20001 212)14:6<280::AID-JCLA6>3.0.CO;2-0

Nohara, H., Okayama, N., Inoue, N., Koike, Y., Fujimura, K., Suehiro, N., ... Hinoda, Y. (2004). Association of the -173G/C polymorphism of the macrophage migration inhibitory factor gene with ulcerative colitis. Journal of Gastroenterology, 39(3), 242–246. https://doi.org/10.1007/s00535-003-1284-7

Östergaard, C., & Benfield, T. (2009). Macrophage migration inhibitor factor in cerebrospinal fluid from patients with central nervous system infection. Critical Care, 13(3), 1–8. https://doi.org/10.1186/cc7933

Otokesh, H., Fereshtehnejad, S.-M., Hoseini, R., Hekmat, S., Chalian, H., Chalian, M., ... Mahdavi, S. (2009). Urine macrophage migration inhibitory factor (MIF) in children with urinary tract infection: A possible predictor of acute pyelonephritis. Pediatric Nephrology, 24(1), 105–111. https://doi.org/10.1007/s00467-008-0969-9

Pazyar, N., Feily, A., & Yaghoobi, R. (2013). Macrophage migration inhibitory factor as an incriminating agent in dermatological disorders. Indian Journal of Dermatology, 58(2), 157. https://doi.org/10.4103/0019-5154.108068

Petrovsky, N., Socha, L., Silva, D., Grossman, A. B., Metz, C., & Bucala, R. (2003). Macrophage migration inhibitory factor exhibits a pronounced circadian rhythm relevant to its role as a glucocorticoid counter-regulator. Immunology and Cell Biology, 81(2), 137–143. https://doi.org/10.1046/j.0818-9641.2002.01148.x

Picardo, M., Dell’Anna, M. L., Ezzedine, K., Hamzavi, I., Harris, J. E., Parsad, D., & Taieb, A. (2015). Vitiligo. Nature Reviews Disease Primers, 1, 1–16. https://doi.org/10.1038/nrdp.2015.11

Radstake, T., Fransen, J., Toonen, E. J. M., Coenen, M. J. H., Eijsbouts, A. E., Donn, R., ... Van Riel, P. L. C. M. (2007). Macrophage migration inhibitory factor polymorphisms do not predict therapeutic response to glucocorticoids or to tumour necrosis factor α-neutralising treatments in rheumatoid arthritis. Annals of the Rheumatic Diseases, 66(11), 1525–1530. https://doi.org/10.1136/ard.2006.064394

Rahman, S. H., Menon, K. V., Holmfield, J. H. M., McMahon, M. J., & Guillou, J. P. (2007). Serum macrophage migration inhibitory factor is an early marker of pancreatic necrosis in acute pancreatitis. Annals of Surgery, 245(2), 282–289. https://doi.org/10.1097/01.sla.0000245471.33987.4b

Rashighi, M., & Harris, J. E. (2017). Vitiligo pathogenesis and emerging treatments vitiligo cellular stress autoimmunity chemokines targeted therapy melanogenesis. Dermatologic Clinics, 35(2), 257–265. https://doi.org/10.1016/j.det.2016.11.014

Renner, P., Roger, T., Bochud, P.-Y., Sprong, T., Sweep, F. C. G. J., Bochud, M., ... Calandra, T. (2012). A functional microsatellite of the macrophage migration inhibitory factor gene associated with meningococcal disease. The FASEB Journal, 26(2), 907–916. https://doi.org/10.1096/fj.11-195065

Rezk, A. F., Kemp, D. M., El-Domyati, M., El-Din, W. H., Lee, J. B., Uitto, J., ... Aleaxe, V. (2017). Misbalanced CXCL12 and CCL5 chemotactic signals in vitiligo onset and progression. Journal of Investigative Dermatology, 137(5), 1126–1134. https://doi.org/10.1016/j.jid.2016.12.028

Rodrigues, M., Ezzedine, K., Hamzavi, I., Pandya, A. G., & Harris, J. E. (2017). New discoveries in the pathogenesis and classification of vitiligo. Journal of the American Academy of Dermatology, 77(1), 1–13. https://doi.org/10.1016/j.jaad.2016.10.048

Sakaue, S., Ishimaru, S., Hizawa, N., Ohtsuka, Y., Tsurino, I., Honda, T., ... Nishimura, M. (2006). Promoter polymorphism in the macrophage migration inhibitory factor gene is associated with obesity. International Journal of Obesity, 30(2), 238–242. https://doi.org/10.1038/sj.ijo.0803148

Serarslan, G., Yönden, Z., Söğüt, S., Savaş, N., Çelik, E., & Arpaci, A. (2009). Macrophage migration inhibitory factor in patients with vitiligo and relationship between duration and clinical type of disease. Clinical and Experimental Dermatology, 35, 487–490. https://doi.org/10.1111/j.1365-2230.2009.03617.x

Shimizu, T., Ohkawara, A., Nishihira, J., & Sakamoto, W. (1996). Identification of macrophage migration inhibitory factor (MIF) in human skin and its immunohistochemical localization. FEBS Letters, 381(3), 199–202. https://doi.org/10.1016/0014-5793(96)00120-2

Sisti, A., Sisti, G., & Oranges, C. M. (2016). Effectiveness and safety of topical tacrolimus monotherapy for repigmentation in vitiligo: A comprehensive literature review. Anais Brasileiros de Dermatologia, 91(2), 187–195. https://doi.org/10.1590/abd18 06-4841.20164012

Skurk, T., Herder, C., Kräft, I., Müller-Scholze, S., Hauner, H., & Kolb, H. (2005). Production and release of macrophage migration inhibitory factor from human adipocytes. Endocrinology, 146(3), 1006–1011. https://doi.org/10.1210/en.2004-0924

Speckaert, R., Speckaert, M., De Schepper, S., & van Geel, N. (2017). Macrophage migration inhibitory factor gene on the development and severity of human pancreatitis. Critical Care, 3(3), 1–8. https://doi.org/10.1101/sf.0000245471.33987.4b
systemic lupus erythematosus. *Arthritis and Rheumatism*, 63(12), 3942–3951. https://doi.org/10.1002/art.30624

van den Boorn, J. G., Konijnenberg, D., Dellemijn, T. A. M., Wietze van der Veen, J. P., Bos, J. D., Melief, C. J. M., … Luiten, R. M. (2009). Autoimmune destruction of skin melanocytes by perilesional T cells from vitiligo patients. *Journal of Investigative Dermatology*, 129(9), 2220–2232. https://doi.org/10.1038/jid.2009.32

World Medical Association. (2013). World Medical Association declaration of Helsinki ethical principles for medical research involving human subjects. *JAMA*, 310(20), 2191–2194. https://doi.org/10.1001/jama.2013.281053

**How to cite this article:** Garcia-Orozco A, Martinez-Magaña IA, Riera-Leal A, et al. Macrophage inhibitory factor (MIF) gene polymorphisms are associated with disease susceptibility and with circulating MIF levels in active non-segmental vitiligo in patients from western Mexico. *Mol Genet Genomic Med*. 2020;8:e1416. https://doi.org/10.1002/mgg3.1416