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Association between STR -794 $CATT_{5-8}$ and SNP -173 G/C polymorphisms in the *MIF* gene and Lepromatous Leprosy in Mestizo patients of western Mexico

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ABSTRACT

Lepromatous Leprosy (LL) is the most common presentation of leprosy in Mexico. LL patients are unable to activate an effective inflammatory response against *Mycobacterium leprae* probably due to the genetics of the host. Macrophage Migration Inhibitory Factor (MIF) is important to trigger inflammation processes. Two polymorphisms have been reported for human *MIF*: STR -794 $CATT_{5-8}$ and SNP -173 G/C. 7-8 CATT repeats at -794 and the C allele at -173 increase the expression of MIF. We aim to determine the association between the polymorphisms in *MIF* gene and LL. We carried a case and controls study with 100 Mexican LL patients and 100 healthy subjects (HS). PCR was used for genotyping of STR -794 $CATT_{5-8}$ polymorphism and PCR-RFLP for -173 G/C. We found that LL patients possess high -794 CATT repeats (47.1%) more often than HS (32.7%). In conclusion, a *MIF* polymorphism is associated with susceptibility to LL in Western Mexican population.

1. Introduction

Leprosy is a chronic infectious disease in humans caused by the intracellular pathogen *Mycobacterium leprae*, which infects macrophages and Schwann cells, thus damaging peripheral nerves and giving rise to loss of sensitivity. The clinical presentation of the disease is diverse and depends on the immune response of the host, ranging from Tuberculoid Leprosy (TT) to Lepromatous Leprosy (LL) poles through intermediate Borderline Tuberculoid (BT), Borderline Borderline (BB), and Borderline Lepromatous (BL) poles. Cellular immune response present in TT and BT is mediated by macrophages and Th1 lymphocytes; however, in BB, BL, and mainly in LL, the immune response is antibody-mediated accompanied by ineffective foamy infected macrophages, whose bactericidal activities are further hindered by the Th2 cytokine profile [1]. Both poles may be distinguished by bacterial density in histopathological smears: a maximum of 10 bacilli in 100 microscopic fields is observed in samples of patients with TT and BT, equivalent to a Bacterial Index (BI) of 1+; in BB, BL, and LL, from 1 to >1000 bacilli can be observed in a single microscopic field, representing from a score of 2+ to 6+ on the BI [2].

Due to its status as a Neglected Tropical Disease (NTD), the World Health Organization (WHO) has proposed treating contagious patients with polychemotherapy to stop the transmission of leprosy. Despite the significant reduction in worldwide prevalence with this strategy, new cases continue to present constantly

Abbreviations: STR, short tandem repeats; SNP, Single Nucleotide Polymorphism; MIF, Macrophage Migration Inhibitory Factor; HS, Healthy Subjects; PCR, Polymerase Chain Reaction; RFLP, Restriction Fragment Length Polymorphism; TT, Tuberculoid Leprosy; LL, Lepromatous Leprosy; BT, Borderline Tuberculoid; BB, Borderline Borderline; BL, Borderline Lepromatous; BI, Bacterial Index; NTD, Neglected Tropical Disease; WHO, World Health Organization; ROS, Reactive Oxygen Species; TNF-α, Tumor Necrosis Factor alpha; AP-4, Activator Protein 4; WT, Wild-Type; EDTA, ethylenediaminetetraacetic acid; DNA, deoxyribonucleotide acid; DNTP, dioxynucleotide triphosphate; SD, Standard Deviations; OR, Odds Ratios; SE, Standard Errors; 95% CI, 95% Confidence Intervals; ICBP90, inverted CCAAT box-binding protein of 90 kDa; HIV, human immunodeficiency virus; HBV, hepatitis B virus; bp, Base Pairs.

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[3]. However, this approach ignores that the role of genetic factors in the risk of contracting and combating the disease has been proven. *IL-12B*, *HLA-DRB1*, *TNF*, *LTA*, *IL10*, *PARK2*, *NOD2*, *RIPK2*, and *LACC1* are some of the genes previously associated with the disease. Because leprosy is a complex disease and that final outcomes largely depend on host immunological factors, it is necessary to maintain the investigation of genetic markers of susceptibility by means of molecular techniques in an ongoing manner [4,5].

The Macrophage Migration Inhibitory Factor (MIF) enhances the production of Reactive Oxygen Species (ROS) by macrophages and their migration to infection. Unlike other cytokines, MIF is usually maintained in vesicles in its mature and active form, which confers upon it the capacity to be one of the first cytokines to be released on infectious stimuli. Furthermore, MIF promotes production and establishes a network of positive feedback with Tumor Necrosis Factor alpha (TNF-α) [6].

MIF gene is located in chromosome 22 (22q11.2) and contains two clinically relevant polymorphisms within the promoter region [6] that have been associated with susceptibility to several diseases [7,8]. A Short Tandem Repeat (STR) polymorphism is located at *locus* -794, for which seven or eight repeats of CATT have been associated with higher MIF production and greater susceptibility to inflammatory diseases when compared with five or six CATT repeats [7]. Likewise, the C allele of a Single Nucleotide Polymorphism (SNP) at position -173 creates a binding site for Activator Protein 4 (AP-4), and its presence is associated with higher severity and inflammation in autoimmune diseases than in the Wild-Type (WT) G allele [9,10].

The prevalence of leprosy cases has diminished in Mexico due to the recommended polychemotherapy treatment [3,11]. Four regions of distinct prevalence can be localized in this country, among which the western region presents the highest number of cases, perhaps because of higher susceptibility to the disease. In this region, LL comprises the main presentation of leprosy [12]. Considering that genetic background is one of the most relevant factors for developing LL and that *MIF* polymorphisms have been associated with susceptibility to several diseases, the aim of this study was to study the association of STR -794 $CATT_{5-8}$ and SNP -173 G/C polymorphisms in the *MIF* gene and the development of LL in the population of western Mexico.

2. Materials and methods

2.1. Ethical considerations

We designed the present study according to the Declaration of Helsinki, as last reviewed at Fortaleza, Brazil, in 2013. Patients and Healthy Subjects (HS) were informed about the objective of this research and they voluntarily signed an informed consent letter in agreement (Universidad de Guadalajara ethical committee number CI-02515).

2.2. Characteristics of patients and HS

We included 100 patients with a clinical, histopathological, and bacilloscopic diagnosis of LL who were seen at an external appointment at the Dr. José Barba Rubio Dermatological Institute of Jalisco. 100 HS were paired with patients regarding age and gender. Both HS and patients were genealogically native to western Mexico for at least three generations.

2.3. DNA extraction

We obtained 8 mL peripheral blood from both study groups using EDTA as anticoagulant, and DNA was purified by the salting-out technique. Briefly, erythrocytes were lysed using hypotonic ammonium solution, leukocytes membrane was disrupted using SDS for 48 h at 37 °C and proteinase K., proteins were precipitated by increasing salt concentration and finally DNA was purified by cold absolute ethanol precipitation followed by 2 washes with ethanol 70% [13].

2.4. Genotyping of the STR -794 $CATT_{5-8}$ polymorphism

To analyze the STR -794 $CATT_{5-8}$ polymorphism, we amplified polymorphic fragments by end-point Polymerase Chain Reaction (PCR), for which we used the protocol previously reported by Muñoz-Valle group [7,14]. The reaction mix contained Buffer *A* 1X, *TaqPol* 0.032 U/μl (Vivantis Technologies Sdn. Bhd., USA), $MgCl_2$ 5 mM, an equimolar mix of dioxynucleotide triphosphate (dNTP) 0.1 mM and primers 300 nM (Invitrogen, USA), and 500 ng of DNA as substrate. The amplification protocol included an initial denaturation at 95 °C for 4 min, 30 denaturation cycles at 95 °C for 30 s, alignment at 60 °C for 30 s, and an extension at 72 °C for 30 s, followed by a final extension at 72 °C for 2 min. As a result, fragments of 208, 212, 216, or 220 base pairs (bp) were obtained for alleles of 5, 6, 7, or 8 CATT repetitions, respectively. The PCR products were visualized on 10% polyacrylamide gels 29:1 (Sigma-Aldrich, USA) stained with 0.2% $AgNO_3$ (Caledon, Canada) after electrophoresing at 60V for 25 h. Genetic dominance analysis was performed considering as dominant the seven and eight CATT repetitions high repeats (HR).

2.5. Genotyping the SNP -173 G/C polymorphism

We determined the genotypes of the SNP -173 G/C polymorphism by PCR-Restriction Fragment Length Polymorphism (RFLP). For PCR, we employed a modification to the primers as reported by Makihja et al. [15] as follows: forward primer 5′-ACT-AAG-AAA-GAC-CCG-AGG-3′ and reverse primer 5′-GGG-GCA-CGT-TGG-TGT-TTA-CG-3′. The reaction mix contained Buffer *A* 0.93X, *TaqPol* 0.112 U/μl (Vivantis Technologies), $MgCl_2$ 4.7 mM, equimolar mix of dNTP 0.09 mM, primers 84 nM (Invitrogen, USA) and betaine 1.12 M (Sigma-Aldrich, USA), and 100 ng of DNA as substrate. The amplification protocol included initial denaturation at 95 °C for 4 min, 30 denaturation cycles at 95 °C for 30 s, alignment at 60 °C for 30 s, and an extension at 72 °C for 30 s, followed by a final extension at 72 °C for 2 min, which resulted in a 366-bp-long fragment. Finally, we digested the amplified products using 0.032 U/μl of *AluI* enzyme (New England Biolabs, USA) for 16 h at 37 °C, and the products were observed on 6% polyacrylamide gels 29:1 (Sigma-Aldrich, USA) stained with 0.2% $AgNO_3$ (Caledon, Canada) after electrophoresis at 150 V for 1 h. As a result, we obtained 268-bp fragments for G/G subjects, two fragments of 206 and 62 bp for C/C subjects, and all three fragments were observed for G/C subjects; additionally, a 98-bp fragment was observed in all subjects due to the presence of another restriction site in amplified products. Additionally, 10 random samples were sequenced in order to confirm the results obtained by RFLP. Genetic dominance analysis was performed considering as dominant the C allele.

2.6. Statistical analysis

Regarding the clinical and demographic characteristics of patients and HS, qualitative variables were expressed as frequencies and quantitative variables, as means and Standard Deviations (SD). Analyses were carried out with IBM SPSS Statistics ver. 20 and Microsoft Excel 2010 in Windows 7. Genotype and allele distribution in the study groups was determined by direct counting and was expressed as frequencies with Standard Errors (SE), and their association with the disease was studied using Odds Ratios (OR) and 95% Confidence Intervals (95% CI) computed by χ^2.