Association of the macrophage migration inhibitory factor promoter polymorphisms with benign lymphoepithelial lesion of lacrimal gland

Qin-Jian Li¹, Peng-Xiang Zhao¹, Xu-Juan Zhang¹, Yang Yi¹, Dan-Ying Cheng², Jian-Min Ma³, Xue-Mei Ma¹

¹College of Life Science and Bio-engineering, Beijing University of Technology, Beijing 100124, China
²Institute of Infectious Diseases, Beijing Ditan Hospital, Capital Medical University, Beijing 100015, China
³Beijing Tongren Eye Center, Beijing Tongren Hospital, Capital Medical University, Beijing Ophthalmology & Vision Science Key Lab, Beijing 100730, China

Correspondence to: Peng-Xiang Zhao and Xue-Mei Ma. College of Life Science and Bio-engineering, Beijing University of Technology, Beijing 100124, China. zpx@bjut.edu.cn and xmma@bjut.edu.cn; Jian-Min Ma. Beijing Tongren Eye Center, Beijing Tongren Hospital, Capital Medical University, Beijing Ophthalmology & Vision Science Key Lab, Beijing 100730, China.

Co-first authors: Qin-Jian Li and Peng-Xiang Zhao

INTRODUCTION

Benign lymphoepithelial lesion (BLEL)¹, also referred to as Mikulicz disease², is a relatively rare disease, with the major clinical manifestations being symmetrical and painless enlargement of the bilateral lacrimal glands and/or the salivary glands³⁻⁴. The cause and pathogenesis of BLEL remain unclear. Clinically, BLEL can be treated with glucocorticoid therapy, but glucocorticoid resistance is a frequent occurrence⁵. Macrophage migration inhibitory factor (MIF) is a pro-inflammatory cytokine first identified in 1966 during studies of the delayed-type hypersensitivity reaction and characterized as a soluble product of activated T lymphocytes that inhibits macrophage migration in vitro⁶⁻⁷. MIF has been shown to act as a critical mediator of host defence with a role in septic shock and chronic inflammatory and autoimmune diseases⁸⁻⁹. Furthermore, MIF has the unique ability to override the inhibitory effects of glucocorticoid on the immune system¹⁰⁻¹¹. MIF gene is located in chromosome 22 (22q11.2) and contains two clinically relevant polymorphisms within the promoter region that have been associated with susceptibility to several diseases¹²⁻¹⁶. A short tandem repeat (STR) polymorphism is located at locus -794 (rs5844572), which is a microsatellite repetition of cytosine-adenine-thymine-thymine (CATT), and the repeat length (5 to 8 repetitions) which correlates with increased gene expression and with circulating MIF levels¹². Likewise, the single nucleotide polymorphism (SNP) -173 G>C MIF (rs755622) has been found at location -173 of the MIF gene with a change from guanine (G) to cytosine (C). Similar to the functions of STR polymorphism above, this -173 G>C

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The -794 CATT(5-8) MIF polymorphism was analyzed by conventional polymerase chain reaction (PCR) and amplification of a 346 bp fragment was completed using the primers as follows: forward primer 5'-AATGGTAAACTCGGGGAC-3' and reverse primer 5'-TGCAGGAACCAATACCCATAG G-3'. Cycling conditions were as follows: initial denaturing at 94°C for 5 min followed by 30 cycles of 30s at 94°C , 30s at 58°C , and 30s at 72°C and then a final extension of 5min at 72°C.

The -794 CATT(5-8) MIF polymorphism was analyzed by conventional polymerase chain reaction (PCR) and amplification of a 497 bp fragment was completed using the primers as follows: forward primer 5’-CCGCCG CTG AGC TAC GTG CC-3’ and reverse primer 5’-CCCCGC CCC ATC TCA AAC ACA-3’ and reverse primer 5’-TGCAGGAACCAATACCCATAG G-3'. Cycling conditions were as follows: initial denaturing at 94°C for 5 min followed by 30 cycles of 30s at 94°C, 30s at 58°C and 30s at 72°C and then a final extension of 5min at 72°C.

In this study, we evaluated the association of the -794 CATT(5-8) and -173G>C MIF polymorphisms with glucocorticoid susceptibility to lacrimal gland BLEL.

SUBJECTS AND METHODS

Participants or Samples  A total of 40 patients with lacrimal gland BLEL and 40 HS from Beijing Tongren Hospital and University Hospital of Beijing University of Technology are prepared for this trial. The study protocol was approved by the Ethics Committee of Beijing University of Technology and adhered to the tenets of the Declaration of Helsinki. Written informed consent was obtained from all participants before their enrollment (Table 1).

Pretreatments  Whole blood was centrifuged at 2000 rpm for 10min; the upper layer was then carefully removed into a clean tube and stored at -20°C ; whole blood DNA was at last extracted from the left blood cells by using Genomic DNA Extraction from blood system (TIANGEN® China).

Genotyping of the SNP-173G>C and STR -794 CATT(5-8) Polymorphism  To analyze the SNP-173G>C MIF polymorphism, we amplified polymorphic fragments by conventional polymerase chain reaction (PCR). Amplification of a 497 bp fragment was completed using the primers as follows: forward primer 5’-CCGCCG CTG AGC TAC GTG CC-3’ and reverse primer 5’-CCCCGC CCC ATC TCA AAC ACA-3’. Cycling conditions were as follows: initial denaturing at 94°C for 5 min followed by 30 cycles of 30s at 94°C , 30s at 58°C , and 30s at 72°C and then a final extension of 5min at 72°C .

Amplification products were sequenced by Sangon Biotech (Shanghai, China), and the sequential peaks showed genotyping results of the SNP-173G>C MIF and STR -794 CATT(5-8) MIF polymorphism.

Enzyme-linked Immunosorbent Assay for Macrophage Migration Inhibitory Factor  The determination of MIF plasma levels was performed by commercial ELISA Kits (RayBio® USA) according to manufacturer’s instructions. The sensitivity of MIF detection was 6 pg/mL.

Statistical Analysis  Data analysis was performed using IBM SPSS Statistics ver.20, GraphPad Prism6 software and Revman 5.3. Student’s t-test for parametric variables (data presented as mean±SD), and Mann-Whitney U test for nonparametric variables (data presented as median and 5th to 95th percentiles). 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). The genotype and allele frequencies were calculated by the Chi-square test. P<0.05 was considered statistically significant.

RESULTS

We analyzed the association of the SNP-173 G>C MIF and STR -794 CATT(5-8) MIF polymorphism with the susceptibility to lacrimal gland. The -173 G>C MIF polymorphism was significantly associated with lacrimal gland BLEL, with a significantly higher frequency of the C allele in lacrimal gland BLEL patients (22/80; 27.5%) compared with HS (11/80; 13.75%) (OR=2.38, 95%CI=1.07-5.31, P=0.032). Furthermore, we found that the G/G genotype was more frequent in HS (11/80; 13.75%) (OR=2.38, 95%CI=1.07-5.31, P=0.032). Otherwise, there was no statistically significant correlation existed between MIF-CATT(5-8) and the morbidity risk rate of lacrimal gland BLEL (Table 2).

The MIF level in plasma was significantly higher in patients of lacrimal gland BLEL (mean 11.07 ng/mL, range 2.01-33.41 ng/mL) versus HS (mean 1.71 ng/mL, range 0.98-2.71 ng/mL) (P<0.001; Figure 1). As shown in Figure 2, a total of 40 patients with lacrimal gland BLEL were genotyped for the -173 polymorphism of the MIF gene and evaluated for MIF levels in plasma. We found that patients carrying the MIF-173*C allele had higher MIF levels of serological MIF, which were significantly higher than those of patients with the GG genotype (P=0.0041). Although the plasma level of
MIF in patients with MIF-794 CATT (7/x) was elevated, but no significant difference was observed.

DISCUSSION

Lacrimal glands BLEL is characterized by unilateral or symmetric bilateral swelling of the lacrimal glands, the etiology and pathogenesis of which remain ill defined, is relatively less studied over the last decade.

In this study, we investigated the association of -173G>C MIF and -794 CATT(5-8) MIF polymorphism with the risk of BLEL of lacrimal gland in Beijing population. The patients with BLEL in this study were mainly middle-aged females with a male-to-female ratio of 1:3. The median age was 47y (range 23-63y). The enlargement of the lacrimal glands in patients with BLEL was found to be uncongested as well as asymptomatic and nontender to palpation. We found that -173 G to C mutations located in promoter region might be a potential risk factor. However, we did not find a significant association between -794 CATT(5-8) MIF polymorphism with the risk of BLEL of lacrimal gland.

MIF-173 G to C mutations are increasingly recognized causes of immune-system disorders, including acute myeloid leukemia, erythema nodosum and psoriatic arthritis[21-23]. We found the similar situation in BLEL of lacrimal gland. Besides, we found that the carriage rate of the MIF -173C was related to higher plasma levels of MIF in the BLEL of lacrimal gland. There were already massive evidences that elevated MIF overcomes the inhibitory effects of glucocorticoids on TNF-alpha, IL-6 and IL-8 production, restores IL-2 and IFN-gamma production, and antagonizes the glucocorticoid inhibition of the production of several enzymes and cell surface molecules[24].

However, glucocorticoid therapy is the main method of drug treatment in the BLEL of lacrimal gland. In line with this, higher carried allele MIF -173C and higher plasma levels of MIF in patients with BLEL of lacrimal gland were consistent with poorer response to glucocorticoid treatment, with a higher risk of local recurrence. Thereby, the detection of MIF -173G/C polymorphism could be a good index that can determine the...
curative effect of glucocorticoid therapy in BLEL of lacrimal gland.

In summary, we investigated for the first time the association between the functional MIF polymorphisms and BLEL of lacrimal gland. Our results suggested that MIF-173 G to C mutations played an insidious role in susceptibility of BLEL of lacrimal gland, and plasma MIF expression. Further studies are still needed to deeply reveal the mechanism of its mighty function.

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