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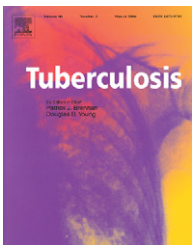

Matrix metalloproteinase-1 polymorphism in Taiwanese patients with endobronchial tuberculosis

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Summary
Endobronchial tuberculosis (TB) often leads to some degree of tracheobronchial stenosis. Because matrix metalloproteinases (MMPs) play an essential role in tissue remodeling in the airways, we investigated the role of *MMP-1* polymorphism in patients with endobronchial TB. One hundred and one cases of pulmonary TB in Taiwanese patients were genotyped for the 1G/2G polymorphism of *MMP-1* promoter (−1607 bp). Bronchoscopic examination was performed to determine the presence of endobronchial involvement. Levels of MMP-1 in peripheral blood monocytes and in bronchial biopsies were also determined. 1G genotypes of *MMP-1* polymorphism, containing at least one 1G allele, were associated with the presence of endobronchial TB. Using multivariate analysis, 1G genotypes and female gender were independent predictors of the development of endobronchial TB. Endobronchial TB patients with 1G genotypes had a 9.86-fold greater risk of developing tracheobronchial stenosis. IL-1β increased levels of MMP-1 in peripheral blood monocytes of TB patients with 1G genotypes. MMP-1 activity was also present in the endobronchial TB granuloma from patients with 1G/1G genotype. 1G genotypes of *MMP-1* polymorphism were associated with a greater risk of developing tracheobronchial stenosis through up-regulation of MMP-1 activity.

Introduction

Despite extensive global control, tuberculosis (TB) continues to ravage the developing world. Endobronchial involvement by TB is present in 10–40% of patients with active pulmonary TB.[1–3] To develop endobronchial TB, the infectious process

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with the accompanying inflammatory changes must invade through the bronchial wall, and the lining mucosa, ultimately leading to ulceration by granulation tissue.[4,5]

The matrix metalloproteinase (MMP) family comprises of more than 20 enzymes that are associated with degradation of the extracellular matrix, including basement membrane. *Mycobacterium tuberculosis* specifically upregulates MMP-1 expression in a cellular model of human infection and airway epithelial cells adjacent to TB granulomas express high level of MMP-1.[6,7] Since endobronchial TB is caused by local spread from adjacent TB lymph nodes or active TB lesions into endobronchial sites, we speculated that MMPs may play a role in disruption of the extracellular matrix in endobronchial TB.

A 1G/2G polymorphic site has been found to be located in a core recognition sequence of the binding sites for transcription factors that controls the level of MMP-1 expression.[8] The aim of this study was to investigate whether *MMP-1* polymorphism in the promoter region may be associated with a more aggressive matrix degradation, thereby, facilitating *M. tuberculosis* invasion through the bronchial wall, leading to the development of endobronchial TB.

Methods

Study patients

During the period between July 2002 and June 2003, 101 cases of pulmonary TB (59 males and 42 females) aged 51.0 ± 18.2 (mean ± SD) years diagnosed by positive sputum culture for *M. tuberculosis* and receiving chemotherapy for the first time, consented to participate in this study. They underwent bronchoscopic examination for investigation of possible endobronchial involvement. Among these patients, 38 patients (37.6%) were diagnosed as having endobronchial TB (14 males and 24 females) aged 49.4 ± 19.9 (mean ± SD) years. The diagnosis of endobronchial TB was based on the positive bronchial brushing smear or culture for *M. tuberculosis*, and typical bronchoscopic and pathological findings.[1] Sixty-three pulmonary TB patients (45 males and 18 females) aged 51.6 ± 17.3 (mean ± SD) years did not have endobronchial TB by bronchoscopic examination, and were defined as the pulmonary TB only group. All TB patients were negative for HIV infection. All endobronchial TB patients received a further bronchoscopic study to evaluate whether there was tracheobronchial stenosis 1–1.5 years after completion of successful chemotherapy. Tracheobronchial stenosis is defined as luminal narrowing to less than 50% of the original luminal area. This study was approved by the Chang Gung Memorial Hospital Ethics Committee. Informed consent was obtained from all subjects.

DNA extraction and MMP-1 (G-1607GG) genotyping

Genomic DNA was extracted from peripheral blood samples using a standard phenol/chloroform protocol.[9] The primer used to amplify the MMP-1 DNA (G-1607 GG) sequence single nucleotide polymorphisms (sense and anti-sense) was: (5′-TGCCACTTA-GATGACCAAATTG-3′ and 5′-GATTCCTGTTTTCTT-TCTGCGT-3′).[10] Amplification was performed in a 20 μl volume containing 100–200 ng of genomic DNA, 7.5 μM of each primer, 2.5 μM dNTPs, 10 × polymerase chain reaction (PCR) buffer and 1 U of *Taq* polymerase. The solution was incubated for 1.5 min at 94 °C, followed by 30 PCR cycles (0.5 min each at 94 °C), 30 s at 56 °C and 30 s at 72 °C, with a final extension for 5 min at 72 °C. PCR products were electrophoresized on a non-denaturing polyacrylamide gel stained with DNA Silver Staining Kit (Amersham Biosciences AB, Sweden). Method validity was checked by sequence analysis, using an Applied Biosystems 3730 series DNA Analyzers (Applied Biosystems, Foster City, CA) (Figure 1).

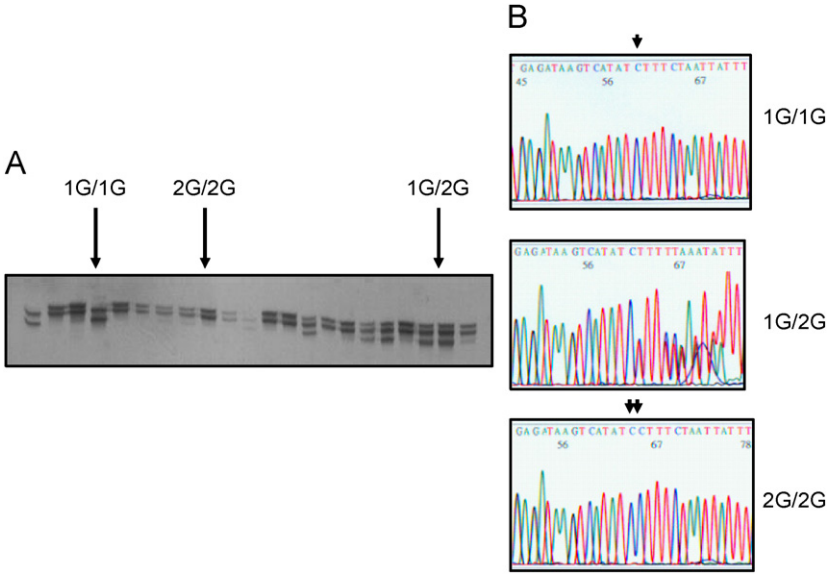

Figure 1 Genotyping of *MMP-1* promotor polymorphism: (A) Representative of DNA silver staining gel of PCR-amplified genomic DNA. (B) Representative sequence of the three genotypes using reverse primer.