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Toll-Like Receptor 2 Polymorphisms and Nontuberculous Mycobacterial Lung Diseases

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To investigate the occurrence of the Toll-like receptor 2 (TLR2) polymorphisms in patients with pulmonary disease caused by nontuberculous mycobacteria (NTM), TLR2 Arg677Trp and Arg753Gln polymorphisms were examined. TLR2 polymorphisms do not appear to be responsible for host susceptibility to NTM lung disease, at least in the Korean population.

The incidence of pulmonary disease caused by nontuberculous mycobacteria (NTM) has been increasing, and a substantial proportion of these patients have no preexisting lung disease and no demonstrable immunodeficiency (2, 13). These patients are predominantly nonsmoking elderly women (2, 13). High-resolution computed tomography scans revealed the characteristic findings of multifocal bronchiectasis combined with multiple small nodules (2, 4, 9, 10, 14). NTM are ubiquitous environmental organisms. Because exposure to these organisms is universal and the occurrence of the disease is rare, normal host defense mechanisms must be effective enough to prevent the infection (8). Recently, we found an association between NTM lung disease and the polymorphisms of the natural resistance-associated macrophage protein 1 gene (12). This finding could raise the possibility of genetic susceptibility for patients with NTM lung disease.

Toll-like receptors (TLRs) are a central part of the innate immune response to mycobacterial infection (1, 18). TLR2 in particular has been implicated in the recognition of mycobacterial infection. Recently, an increased susceptibility to virulent *Mycobacterium avium* infection in TLR2 knockout mice was reported (7). Further, MyD88-deficient mice also showed augmented sensitivity to the *M. avium* infection (5). Therefore, these data suggest that infection with opportunistic *M. avium* species is controlled in a TLR2- and MyD88-dependent manner.

The importance of TLRs in human mycobacterial diseases has recently been shown in studies of polymorphisms in TLR2. The Arg677Trp polymorphism of TLR2 is associated with lepromatous leprosy in Korean patients (11) and pulmonary tuberculosis in Tunisian patients (3). The Arg753Gln polymorphism of TLR2 is also associated with tuberculosis in Turkish patients (16). However, whether genetic polymorphism of TLR2 determines susceptibility to NTM lung disease is unknown. The purpose of this study was to investigate the occurrence of the TLR2 polymorphisms in adult patients with NTM lung disease compared to that in healthy controls.

A total of 80 patients (15 men and 65 women) with the nodular bronchiectatic form of NTM lung disease were consecutively enrolled at the Samsung Medical Center (Seoul, Korea) from March 2002 to October 2005. The diagnosis of NTM lung disease was made when the patients fulfilled the diagnostic criteria of the American Thoracic Society (2). Patients ranged in age between 23 and 82 years, with an average age of 58 years. Of 80 patients, 41 patients were identified as having *M. avium* complex infection (*Mycobacterium intracellulare*, 23 patients; *M. avium*, 18 patients), and 39 patients were identified as having *Mycobacterium abscessus* infection.

The control subjects were 84 unrelated healthy volunteers, with an average age of 27 years (range, 20 to 48 years). All patients and control participants originated from Korea. This study was approved by the guidelines of the Institutional Review Board at our institution, and written informed consent was obtained from each participant.

PCR and restriction fragment length polymorphism analysis were used to type polymorphisms of the TLR2 Arg677Trp and Arg753Gln genes, as described in a previous study (6). DNA samples were extracted from whole blood (G-DEX kits; iNtRON Biotechnology, Sungnam, Korea) and then amplified using forward (5′-CCCCTTCAAGTTGTGGCTTCATAAG-3′) and reverse (5′-AGTCCAGTTCATACTTGCACCAC-3′) primers which span the region containing the Arg677Trp polymorphism. In order to characterize the Arg753Gln polymorphism, forward (5′-CATTCCCCAGCGCTTCTGCAAGCTCC-3′) and reverse (5′-GGAACCTAGGACTTTATCGCAGCTC-3′) primers were used. PCR amplification was performed in 50-μl reaction mixture volumes containing 200 ng of genomic DNA, 1.5 mM/liter $MgCl_2$, 1.0 mM deoxynucleotide triphosphate, 1 μmol of each primer, and 2 U *Taq* DNA polymerase (Promega, Madison, WI). PCR was performed in a thermal cycler (model 9600; PerkinElmer, Branchburg, NJ) under the following conditions: 30 s of initial denaturation at 94°C and

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then 35 cycles of denaturation for 30 s at 94°C, annealing for 30 s at 65°C, and extension for 30 s at 72°C. Final extension was carried out for 10 min at 72°C. The restriction assay contained 1× restriction buffer, 12.5 units of restriction enzyme MwoI for Arg677Trp or MspI for Arg753Gln (New England Biolabs, Beverly, MD), and 10 μl of the PCR product. After overnight incubation at 60°C (MwoI) or 37°C (MspI), the digestion products were subjected to electrophoresis on gels containing a mixture of 4% metaphor Tris-borate-EDTA agarose (Promega, Madison, WI) which were stained with ethidium bromide and then visualized under UV light.

In the Arg677Trp genotyping, the presence of wild-type homozygotes resulted in two bands (130 and 22 bp), heterozygotes resulted in three bands (152, 130, and 22 bp), and mutant homozygotes resulted in one band (152 bp). In the Arg753Gln genotyping, the presence of wild-type homozygotes resulted in two bands (104 and 25 bp), heterozygotes resulted in three bands (129, 104, and 25 bp), and mutant homozygotes resulted in one band (129 bp). The Arg677Trp or Arg753Gln mutant allele was not found in any of the patients, nor was it found in any of the control subjects. These findings were also confirmed by direct sequencing. Direct sequencing of the region containing the polymorphic site of interest did not detect C→T replacement (Arg677Trp) or G→A replacement (Arg753Gln) in any study participant.

This is the first study to evaluate whether the TLR2 polymorphisms are associated with human susceptibility to NTM lung disease. However, the Arg677Trp and the Arg753Gln mutant alleles were not detected in any members of our study population. This is consistent in part with the results of previous studies which identified no individuals carrying the Arg677Trp polymorphism, including Caucasians (19, 20), East Indians (15), and healthy Koreans (11). Another study also confirmed that the Arg677Trp and Arg753Gln polymorphisms were absent among Korean populations (17). In addition, Yim et al. recently reported that none of nine previously reported TLR2 missense mutations, including Arg753Gln, was polymorphic in 176 tuberculosis patients and 191 controls in Koreans (21). Interestingly, those authors demonstrated an association between guanine-thymine (GT) repeat polymorphisms in intron II of the TLR2 gene and the development of clinical tuberculosis in Koreans (21).

In conclusion, the TLR2 Arg677Trp and Arg753Gln polymorphisms do not appear to be responsible for host susceptibility to NTM lung disease, at least in the Korean population.

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