Brief report

TLR4 Asp299Gly and *Thr399Ile* polymorphisms are very rare in the Chinese population

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Endotoxin is an important component of bio-aerosols that contribute to airway inflammation and airflow obstruction. Toll-like receptor 4 (TLR4) mediates the host response to bacterial lipopolysaccharide (LPS), and the *Asp299Gly* and *Thr399Ile* polymorphisms have been associated with the development of respiratory diseases. We hypothesized that *TLR4* polymorphisms may be associated with a decline of lung function in cotton workers who were exposed continuously to endotoxin. We investigated these two polymorphisms in our 20-year longitudinal cohort of Han Chinese cotton and silk textile workers. The two polymorphisms were genotyped using TaqMan and PCR-restriction fragment length polymorphism methods. No homozygous or heterozygous variant genotypes of the *Asp299Gly* and *Thr339Ile* polymorphisms were detected in 491 samples of this population. Our results suggest that the *TLR4 Asp299Gly* and *Thr339Ile* polymorphisms are very rare in the Chinese population.

Keywords: *TLR4* polymorphisms, *Asp299Gly, Thr399Ile*, Chinese population

INTRODUCTION

Endotoxin is an important component of bio-aerosols that contribute to airway inflammation and airflow obstruction. Inhaled endotoxin in bio-aerosols is associated with the development of acute and chronic decline of lung function in cotton textile workers.[1] The human homologues of the *Drosophila* Toll, termed Toll-like receptors (TLR), activate both innate and adaptive immune response in vertebrates. Among this family, TLR4 mediates the host response to bacterial lipopolysaccharide (LPS) by inducting of the NF-κB signaling pathway, and is involved in defense against Gram-negative bacteria.[2]

Two common co-segregating missense polymorphisms (*Asp299Gly* and *Thr399Ile*) in the extracellular domain of the human *TLR4* gene have been associated with acute airway response to inhaled endotoxins and altered ability of host response to LPS.[3] Our previous results have shown that cotton workers exposed to endotoxin had significantly greater annual decline in FEV_1 and FVC than did silk workers (controls).[4] We hypothesized that *TLR4* polymorphisms may be associated with a decline of lung function in cotton workers who were exposed continuously to endotoxin. We investigated these two polymorphisms in our 20-year longitudinal cohort of Chinese cotton and silk textile workers.

PATIENTS AND METHODS

The study was approved by the Institutional Review Boards of the Harvard School of Public Health and Shanghai Putuo District People's Hospital and The Human Resources Administration of China. This population-based 20-year longitudinal cohort study for respiratory disease among cotton textile workers was established

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Table 1. The frequencies of variant allele of the *Asp299Gly* and *Thr399Ile* in different ethnic populations

Population	*n*	*Asp299Gly (A299G)* *G* allele frequencies (%)	*Thr399Ile (C399T)* *T* allele frequencies (%)
Chinese (this study)	491	0	0
Japanese[7]	107	0	NA
American Caucasian (family-based cohorts)[6]	960	6.3	5.3
Canadian Caucasian (family-based cohorts)[6]	423	10	10

NA, not applicable.

in 1981. The initial study population consisted of 447 cotton textile workers (52% female) in Shanghai, China. They represented 90% of workers who had been employed for more than 2 years in the yarn preparation areas of two cotton textile mills. At the same time, 472 silk textile workers (58% female) who had worked for a minimum of 2 years in a silk-thread processing mill in the same city were involved in the study as a comparison population. All workers were of Han descent. Subjects were excluded from the cohort if they had a history of active tuberculosis or asthma preceding entry into the textile workforce. A total of 597 subjects donated blood samples in this study. We report genotype results for the fist 500 individuals in this cohort.

DNA was extracted from 3 ml of peripheral whole blood using the Puregene DNA Isolation Kit (Gentra Systems, Minneapolis, MN, USA). The *Thr399Ile* (*C399T*) polymorphism was detected using previously described PCR-restriction fragment length polymorphism (PCR-RFLP) methods.[5] The *Asp299Gly* (*A299G*) polymorphism was detected using the TaqMan method. Primers, probes, and reaction conditions are available upon request.

RESULTS AND DISCUSSION

Among the first 500 samples that were genotyped, 9 samples failed in the PCR reaction. In the 491 samples with genotype results, no homozygous or heterozygous variant genotypes of the *Asp299Gly* or *Thr339Ile* polymorphism were detected (Table 1). There was 100% concordance of randomly repeated samples. Because the *Asp299Gly* and *Thr339Ile* alleles were very rare in our study population, the rest of the DNA samples were not genotyped and the original analysis was not pursued.

Our results suggest that the genotype distribution of *TLR4* polymorphisms is different between Caucasians and Chinese.[6] As shown in Table 1, the frequencies of variant alleles of *TLR4* polymorphisms were very similar in different Caucasian populations, with the allele frequencies between 5–10%. Consistent with our results, the *G* allele of the *Asp299Gly* polymorphism was not detected in a Japanese population.[7] In contrast to the *TLR4* polymorphisms, the *Arg677Trp* polymorphism of *TLR2* was found to be common in Asian individuals (22% [10/45] in Korean lepromatous leprosy patients), and very rare in Caucasian populations (none in healthy Caucasian volunteers).[8,9] Further studies should investigate additional polymorphisms of *TLR4* and *TLR2* to understand the potential effects of innate immunity on health such as lung function in the Chinese population.[10]

This study population derived from the 20-year longitudinal cohort in two cotton mills and a silk mill in Shanghai, China. In the initial enrolment, subjects were excluded if they had a history of respiratory diseases, with only healthy subjects included in this cohort. Therefore, genotype distributions in our population are probably applicable to the general Chinese population. A number of other polymorphisms, including microsomal epoxide hydrolase, tumor necrosis factor alpha and beta, and myeloperoxidase were assessed in the study population, and the genotype distributions of these polymorphisms (unpublished) were very similar to those found in other studies of Chinese populations. For each single-nucleotide polymorphism we have assessed, the genotype distributions were in Hardy-Weinberg equilibrium.

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