Tuberculosis susceptibility genes in the chemokine cluster region of chromosome 17 in Hong Kong Chinese

Introduction

Tuberculosis (TB) is a major global health concern, particularly in the Asia Pacific region. Host genetic factors play an important role in the predisposition and prognosis for infectious diseases, in addition to factors such as the environment, pathogen virulence, and public health status.

Genetic variation of disease predisposition is largely determined by single-base variants in our genome, known as single nucleotide polymorphisms (SNPs). One third of the population has been exposed to Mycobacterium tuberculosis, but only 10% of them go on to develop the disease.\textsuperscript{1,2} Monozygotic twins were more likely to develop TB or not at all together (concordance) than dizygotic twins, indicating an underlying genetic trait of susceptibility to TB.\textsuperscript{3}

Several genes contributing to the susceptibility to TB have been identified, including \textit{SLC11A} (also known as \textit{NRAMP1}). This gene was first identified for its role in susceptibility to multiple intracellular pathogens in mice, and later mapped to chromosome 2q35 in humans. Its function has been reviewed.\textsuperscript{4} Other than \textit{SLC11A1}, genes involved in immune reaction and familial predisposition to TB are also potential susceptibility genes in sporadic TB. They include the signalling pathway of interferon gamma (IFN\gamma) and other cytokines.\textsuperscript{5} Other predisposing genes found in sporadic cases entail the vitamin D receptor, IL12B, and other cytokines/chemokines.\textsuperscript{4}

Familial studies suggest that one or more chemokine genes in the chemokine cluster regions in chromosome 17q11.2 might be associated with susceptibility to TB or mycobacterial disease. However, the exact identity and location of the predisposing gene is not known. To delineate the causative predisposing genet(s), genetic association studies in other populations are needed. We therefore used a tagging SNP approach to study the association between CCL chemokine genes and susceptibility to TB, and between the clinical phenotype and disease severity.

Methods

This genetic association study was conducted from 1 November 2005 to 31 October 2007. Tagging genetic polymorphisms (tagSNPs) were defined and genotyped in the whole sample set of 1040 TB cases and >1000 controls. Hong Kong Chinese attending the territory-wide Chest Clinic of the Tuberculosis and Chest Service with confirmed TB were invited to participate. Ethical approval was obtained from The Chinese University of Hong Kong and the Hong Kong Department of Health in 2002. Informed written consent was obtained from each subject. Blood samples were collected, and clinical parameters about the extent and severity of disease were recorded.

Inclusion criteria were smear and/or culture and/or clinical symptoms positive for TB, according to the diagnostic criteria of the International Union against Tuberculosis and Lung Diseases with clinical-radiological and histological evidence and clinical responses to treatment. Patients with HIV or other immunodeiciencies were excluded.
A tagging SNP approach was used to select informative SNPs for genotyping of the whole sample set. All SNP data were extracted from dbSNP and International HapMap Project both at the time when this project started in November 2005 and subsequently in January 2006 when the HapMap Project released the Phase II results. A spectral decomposition algorithm was used to identify the location of SNPs that are important to delineate the overall genetic variations in this region. A total of 30 factors (composition of SNPs) were identified at this stage covering the genomic regions, including coding and non-coding region of the genes. The SNPs entered into these 30 factors were genotyped by polymerase chain reaction (PCR)–restriction fragment length polymorphism (PCR-RFLP), allele-specific PCR (AS-PCR) or PCR-DNA sequencing.

Genomic DNA was extracted from peripheral blood using the DNA extraction kit according to the manufacturer’s instruction. Genotyping was performed with the FAM dye detected the Allele 1 sequence. By using two probes with different fluorescent dyes that bind to two alleles of the SNP. One probe labelled with VIC dye detected the Allele 1 sequence, and the other labelled with the FAM dye detected the Allele 2 sequence. By using the 5’ nuclease assay, the products were amplified and the specific SNP alleles could be detected in purified genomic DNA samples.

Sequencing of PCR products was performed by BigDye Terminator Cycle sequencing kits with an ABI-3100 autosequencer (Applied Biosystems, USA). The sequencing reaction cycles were performed according to the manufacturer’s instructions.

Statistical analysis of genotype distribution was performed by the Chi-square test for trend, assuming an additive model of allelic risk. The Hardy-Weinberg equilibrium test for genotype distribution was performed by the Chi-square test with 1 df. Univariate associations between categorical clinical features and genotypes were performed using the Chi-square test. Odds ratios (ORs) and 95% confident intervals (CIs) were also calculated. To correct for multiple testing, the Bonferroni correction was applied to provide a corrected P value at significant level of 0.05. Therefore, only those associations with empirical P values of <0.0016 (ie 0.05/30 tagSNPs or independent tests) were considered significant. Haplotype evaluation was performed with a haplview program. Spectral decomposition was performed using statistical software R.

**Results**

The mean age of the patients was 48.3 (standard deviation, 19.4) years. The male gender was predominant (695 males vs 345 females). About 88% of the patients were new cases. The smear positive group consisted of 344 patients. Among patients with pulmonary TB, the extent of lung involvement was minimal (less than the right upper lobe) in 67%, moderate (more than the right upper lobe) in 25%, and severe (more than one lung) in 8%, based on chest radiography at the time of diagnosis.

Within the proximity of the 14 candidate chemokines genes, the pattern of LD was evaluated by the Haploview program. There was LD within the following clusters of genes present inside different haploblocks: CCL2-CCL7-CCL11, CCL8, CCL13-CCL1, CCL5, CCL16, CCL14, CCL15-CCL23, CCL18, CCL3-CCL4. After application of the spectral decomposition algorithm for tagSNP selection, 30 tagSNPs were identified to represent most of the genetic variations in the candidate regions.

**Association between genetic polymorphisms and predisposition to tuberculosis**

In the whole sample set, a total of 30 tagSNPs were selected for genotyping. This set of tagSNPs provided a comprehensive coverage of the genetic variation of the chemokine gene clusters in chromosome 17q11.2. A borderline association was detected between an SNP (rs2282691) located in intron of CCL1 gene and TB predisposition using a single-point chi-square for trend analysis (P=0.006, Fig). The frequency of the minor allele was lower in the control group (0.27) than in the patient group (0.31). Nonetheless, the association was not significant after correction of multiple testing by the Bonferroni method (corrected P=0.16). The minor allele
carried a 1.19-fold increase in risk of TB (95% CI, 1.05-1.36).

As young TB patients may have a strong genetic predisposition, patients within the lowest tertile of onset age (ie <35 years) were defined as early onset, and the association with genotypes was examined again as categorical groups. The CCL1 was also associated with disease predisposition in early-onset patients (P trend=0.00013, Table 1). Genotypes followed the Hardy-Weinberg equilibrium in both groups.

**Association between clinical parameters and genotypes**
The CCL1 predisposing SNPs were also examined for potential association with disease phenotype and severity. The predisposing SNP (rs2282691) was not associated with smear positivity (P=0.8) when comparing the 344 smear positive patients with the remaining patients.

The extent of pulmonary TB was categorised into three grades to examine the association between genotypes and the extent of disease, using the Chi-square test for trend. The SNP was not associated with the extent of disease on radiographs (P for trend=0.1).

Among patients with and without pulmonary cavity, a moderate association was found (P trend=0.01, Table 2). This was consistent with the association with predisposition to TB infection that the “A” allele was also the high-risk allele for development of a cavity.

**Discussion**
The genetic association of the tagging SNP located inside intron 2 of CCL1 with TB was of borderline significance. This SNP was associated with predisposition in young-onset patients and with disease severity. It was also located in proximity to CCL13, in addition to CCL1. This suggested that both genes might be important mediators in the host defense against TB. Young-onset patients appeared to represent a more homogeneous group of patients under stronger genetic influence.
Both innate and adaptive immunity are activated in TB infection. Activation of the Toll-like receptor (TLR) system is the first key innate response. The TLR2, together with other TLRs, interacts with lipoprotein and other secreted antigens of TB and leads to induction of a battery of cytokines, including IL-6, IL-10, TNFα, and IL-12. These are followed by activation of acquired immunity, which includes CD4+ T cell involvement. In addition to signalling through the TLRs, additional receptors and signals also play important roles. Type 1 interferon receptors and STAT1 are also activated in macrophages leading to activation of a battery of chemokines, including RANTES, IP-10, and MIG. Subsequent induction of interferon γ in Th1 cells potentiates macrophage phagosomal lytic activity in an attempt to kill TB by expression of NOS2. Many interferon inducible genes and chemokines are activated as a result of this variety of host responses to TB. These findings support an essential role of chemokines in host defence against TB.

In animal models, mycobacteria-infected macrophages are induced to produce a whole panel of chemokines (CCL1 to CCL5, CCL7, CCL8, CCL13). These chemokines are also found in bronchial lavage of TB patients, confirming their functional importance in the immune reaction to TB in humans.

Chemokines are involved in the differentiation into Th1 and Th2 immune response. Different chemokines are activated when exposed to different antigens (mycobacteria and Schistosoma) and subsequently lead to formation Th1 or Th2 granulomas in mice. Induction of CCL1 is associated with the formation of Th2 granulomas. CCL1 is expressed and secreted by monocytes and binds to CCR8, which is highly expressed among polarised Th2 cells and regulatory T cells. Thus activation of CCR8 by CCL1 leads to a Th2 immune response. However, host defence against TB and autophagy requires induction by Th1, which is inhibited by Th2 cytokines. Therefore, we postulate that individuals with preferential activation/expression of CCL1 are likely to over-express a Th2 phenotype in response to pathogens (including TB) and inappropriately inhibit the required Th1 response. Future functional studies are required to confirm this hypothesis.

It is important to confirm this borderline significant association in another sample set, with much larger samples of cases and controls. Other sample sets with no bias on clinical diagnostic features may be useful to confirm the association with clinical disease severity. Functional genetic study is needed to elaborate on the mechanism underlying the predisposition or protective effect of this genetic variation.

Acknowledgements

This study was supported by the Research Fund for the Control of Infectious Diseases (#03040512), Food and Health Bureau, Hong Kong SAR Government. We thank all patients and health care workers who participated in this study.

References


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