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Genetic polymorphisms of IFNG and IFNGR1 in association with the risk of pulmonary tuberculosis

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ABSTRACT

Objective: Genetic host factors play an important role in controlling individual's susceptibility to the pathogen. This study aims to explore the single and joint effect of genetic polymorphisms of interferon-gamma (IFNG) and its receptor (IFNGR1) in association with the pulmonary tuberculosis in a Chinese Han population.
Methods: This population-based case control study consisted of 1434 pulmonary tuberculosis patients and 1412 healthy controls. Six tag SNPs in IFNG/IFNGR1 were genotyped using TaqMan allelic discrimination technology. The logistic regression model was carried out to analyze the associations between the genotypes and haplotypes and the risk of tuberculosis by calculating the odds ratio (OR) and 95% confidence interval (CI).
Results: After the Bonferroni correction for multiple comparisons, three SNPs (rs2234711, rs1327475 and rs7749390) in IFNGR1 gene were observed to be significantly associated with the altered risks of tuberculosis. For the SNP rs2234711, individuals carrying C allele (vs. T) showed a decreased risk, with the adjusted OR(95% CI) of 0.82(0.76–0.91). The additive model revealed that each additional allele contributed about 14% decreased risk (OR: 0.86, 95% CI: 0.77–0.95). Moreover, we observed a strong linkage disequilibrium between rs2234711 and rs3799488. Compared with the common rs2234711C–rs3799488C haplotype, the haplotype rs2234711T–rs3799488C contributed to a significant increase in the risk of tuberculosis (adjusted OR: 1.24, 95% CI: 1.09–1.41).
Conclusions: Our results suggest that genetic polymorphisms in IFNGR1 gene are involved in the risk of tuberculosis in the Chinese population. Future studies should include a comprehensive sequencing analysis to identify the specific causative sequence variants underlying the observed associations.

1. Introduction

Tuberculosis (TB) has been declared as a global public health emergency by the World Health Organization. It was estimated that almost 8.6 million people developed active TB and 1.3 million died from it in 2012, mostly in developing countries (WHO, 2013). Although nearly one-third of the world's population has been potentially infected with the pathogen *Mycobacterium tuberculosis* (MTB) (Young et al., 2008), only 5–10% will develop clinical TB during their lifetimes (Frieden et al., 2003). The outcome of infection is influenced by many factors, such as malnutrition, co-infection with other pathogens, exposure to environmental microbes, and previous vaccination (van de Vosse et al., 2004). It is clear that genetic host factors also play an important role in controlling individual's susceptibility to the pathogen (Altare et al., 1998; Newport et al., 1996). It was estimated that the contribution of genetic factors to the phenotypic variation and immune responses in the population infected with TB ranges up to 71% (Moller and Hoal, 2010).

In the past decades, there has been dramatic progress in our understanding of the innate and adaptive immunity in the human host defense to TB (Leandro et al., 2009). Current studies highlight a complex molecular network in antimycobacterial immunity, centered on interferon-gamma (IFNG) signaling pathway (Thakur et al., 2012). Individuals with inherited disorders of IFNG mediated immunity appear to be specifically vulnerable to MTB infections (Dupuis et al., 2000). The IFNG gene is located on chromosome 12q24.1. Studies have reported that IFNG can activate murine macrophages to inhibit MTB growth (Ehrt et al., 2001). It is up-regulated and secreted as a major cytokine to activate macrophages during MTB infection (Lee and Kornfeld,

Abbreviations: TB, tuberculosis; MTB, *Mycobacterium tuberculosis*; IFNG, interferon-gamma; IFNGR, interferon-gamma receptor; SNP, *single nucleotide polymorphism*; MAF, minor allele frequency; PCR, polymerase chain reaction; OR, odds ratio; CI, confidence interval; SD, standard deviation; LD, linkage disequilibrium; Th1, T-helper 1; UTR, untranslated region; ENCODE, Encyclopedia of DNA Elements.

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2010). The human receptor of IFNG (IFNGR) is a heterodimer of IFNGR1 and IFNGR2. The IFNGR1 gene is located on chromosome 6q23.3, which encodes the ligand-binding chain (alpha) of the interferon gamma receptor. Defects in IFNGR1 have been reported as a cause of Mendelian susceptibility to mycobacterial disease, also known as familial disseminated atypical mycobacterial infection (Jouanguy et al., 1999).

Previous studies mainly focused on the potentially functional polymorphisms of IFNG like +874 A/T in the intron 1 and adjacent CA repeats. He et al. analyzed seven functional SNPs in IFNG/IFNGR1 gene among a group of Chinese population, and data implied the involvement of IFNGR1 gene in susceptibility to TB (He et al., 2010). But the number of study participants of this study was relatively small (222 cases and 188 controls), resulting in lower statistical power (He et al., 2010). Considering the critical role of IFNG/IFNGR1 signaling pathway in the antimycobacterial immunity, we performed a population-based case control study with a large sample size in a Chinese Han population, with aims to systematically explore the single or joint effect of genetic polymorphisms of IFNG/IFNGR1 in the risk of pulmonary TB.

2. Methods

2.1. Study population

A total of 1434 pulmonary TB patients and 1412 controls were recruited from Jiangsu province of China. All of them were genetically-unrelated Chinese Han population. Patients were diagnosed with the following criteria: (1) sputum smear or culture positive for MTB pathogen; and/or (2) clinical–radiological and histological evidence of TB. We recruited controls from a pool of individuals who participated in the local community-based health examination program. Controls were frequency matched to the cases by sex and age. All cases and controls had no prior HIV positive history. Each subject was individually interviewed in local health facilities by using a structured questionnaire and donated a blood sample for genotyping analysis. Informed consents were obtained from all participants and the study protocol has been approved by the Institutional Review Board of Nanjing Medical University.

2.2. SNP selection and genotyping

Genomic DNA was extracted from leukocytes in the peripheral blood by proteinase K digestion and phenol/chloroform extraction. We identified taq SNPs in the IFNG and IFNGR1 gene through HapMap database (http://www.hapmap.org/) based on the following criteria: (1) minor allele frequency (MAF) ≥0.05 in the Chinese Han population; and (2) P value for Hardy–Weinberg equilibrium test ≥0.05. As a result, six SNPs were selected for genotyping, including one SNP in IFNG (rs1861494) and five SNPs in IFNGR1 (rs1327475, rs2234711, rs3799488, rs7749390 and rs9376267). We genotyped these SNPs using TaqMan allelic discrimination technology on the ABI 7900 RealTime PCR System (Applied Biosystems, Foster City, CA, USA) (Teuber et al., 2009). The primer and probe sequences for each SNP were designed by Nanjing Steed BioTechnologies Co. (Table 1).

2.3. Statistical analysis

Data were entered with EpiData 3.1 software (Denmark) and analyzed by using STATA 10.0 (StataCorp, College Station, TX, USA). Student *t*-test (for continuous variables) and χ^2-test (for categorical variables) were used to analyze the differences in demographic variables and potential risk factors between cases and controls. Hardy–Weinberg equilibrium was tested using a goodness-of-fit χ^2-test by comparing the observed genotype frequencies with the expected ones among the controls to make sure that alleles were independently segregated. Logistic regression model was carried out to analyze the associations between the genotypes and the risk of TB by calculating the odds ratio (OR) and 95% confidence interval (CI). To control the potential confounding factors, we adjusted the OR(95% CI) for age, sex, tobacco smoking and alcohol drinking. To analyze the effect of SNPs comprehensively, we applied three different genetic models: additive model, dominant model and recessive model (Lettre et al., 2007; Salanti et al., 2009). We further performed a haplotype analysis by constructing haplotypes using phase 2.1 software. Considering the potential false positive rate incurred by multiple comparisons of SNPs, we used the Bonferroni correction method to adjust the P value.

3. Results

3.1. General characteristics

The basic characteristics of the cases and controls are shown in Table 2. This study included 1434 TB cases (73.90% males and 26.10% females) and 1412 controls (72.03% males and 27.97 females). The mean (±SD) age was 52.04(±17.57) years for cases and 52.27 ± (17.09) years for controls, respectively. Due to the prior frequency matching, there was no significant difference in the distribution of age and sex between the two groups. The proportion of ever smokers was 53.19% among cases, which was significantly higher than that among controls (34.21%) ($P < 0.001$). As to the history of alcohol drinking, the proportion in cases (22.52%) was significantly lower than that in controls (26.54%) ($p = 0.014$).

3.2. Genotype analysis

The genotype distributions of six SNPs were all in Hardy–Weinberg equilibrium in the controls ($P = 0.37$ for rs1861494, $P = 0.60$ for rs1327475, $P = 0.47$ for rs2234711, $P = 0.09$ for rs3799488, $P = 0.78$ for rs7749390, and $P = 0.11$ for rs9376267). As shown in Table 3, after the Bonferroni correction for multiple comparisons, three SNPs

Table 1
Primers and probes designed for genotyping.

SNPs	Primer (5′–3′)	Probe
rs1861494	F-AGCAAGGGACAATGAGAGAACTG	C: FAM-TACTCCCCGCTTCT-MGB
C > T	R-GTAAAGACAGGTGAGTTGACAAATCC	T: HEX-TACTCCCTGCTTCTT-MGB
rs1327475	F-GTGGCCATAATATAATTGTGATAATGTAATAAA	T: FAM-AAGCAGATGTTTTTGAAG-MGB
C > T	R-CTCCATATTTAAATTGGAATTGGAGAAG	C: HEX-AAGCAGATGCTTTTGAA-MGB
rs2234711	F-AAAGAGGAGAGCCATGCTGCTA	A: FAM-AGCCCAGCACTGC-MGB
C > T	R-CGGTGACGGAAGTGACGTAA	G: HEX-CAGCCCAGCGCTG-MGB
rs3799488	F-TTTTGAGGGTAGGCACTTAAGCTT	G: FAM-TTCTCCCCGTAGATCT-MGB
C > T	R-TGGCTGGTATGACGTGATGAG	A: HEX-TTCTCCCCATAGATCT-MGB
rs7749390	F-GGTGTGAGCAGGGCTGAGAT	C: FAM-TACCGTCGCTCGC-MGB
A > G	R-CTAGGGCGACCTCGGAGAA	T: HEX-TACCGTCGTTCGC-MGB
rs9376267	F-GATTGAACAATGGAGCCACACA	C: FAM-CATCAACACTCTGCTCT-MGB
C > T	R-GCAAGAAGAAATGTTGGGTATGTTT	T: HEX-TCATCAACATTCTGCTCT-MGB