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BRIEF COMMUNICATION

SNP rs4331426 in 18q11.2 is associated with susceptibility to tuberculosis among female Han Taiwanese

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A single nucleotide polymorphism (SNP) rs4331426 located in a gene-poor region on chromosome 18q11.2 has been associated with tuberculosis (TB) by genome-wide association studies in Ghana and Gambia. In this study, we analyzed the SNP rs4331426 for its association with the risk of TB in the Taiwanese population. The SNP rs4331426 was genotyped in a case-control design that included 377 Han Taiwanese (200 TB patients and 177 controls) and was associated with TB (marginally significant $p = 0.078$). An increasingly significant association was observed after adjusting for sex in the logistic regression analysis ($p = 0.029$). Furthermore, the G carrier (AG genotype) conferred the risk of TB in females ($p = 0.011$), but not in males. These findings indicate that the SNP rs4331426 associated with TB in the Han Taiwanese population, especially in females. Further investigations on its role and that of the genomic region surrounding it are warranted.

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Introduction

It is known that pathogens and environmental factors contribute to the development of tuberculosis (TB). However, there is increasing evidence that genetic factors may also play an important role in determining susceptibility to disease and disease severity. A study on twins has established the importance of genetic components as a factor in TB susceptibility.[1] Linkage studies[2–4] and a genome-wide association study[5] have investigated many candidate genes.[6]

In 2011, there were 12,634 TB cases (55 cases per 100,000 population) and 638 TB-related deaths (2.8 cases per 100,000 population) in Taiwan.[7] Previous studies in Taiwan have demonstrated a five-fold higher incidence of TB among the aborigines compared to the Han Chinese.[8] In addition, polymorphisms of the natural resistance-associated macrophage protein 1 (NRAMP1, also known as solute carrier family 11 member 1) gene appear to be associated with susceptibility to TB among the aborigines, but not among the Han Chinese population.[8] The genetic susceptibility to TB of the Han Chinese in Taiwan remains unknown.

Recently, a single nucleotide polymorphism (SNP), rs4331426, which is located in a gene-poor region on chromosome 18q11.2, has been associated with TB by genome-wide association studies in Ghana and Gambia.[5] The SNP rs4331426 and six other tag SNPs were tested for their association with TB in the Chinese population. None of the genetic polymorphisms but haplotypes on chromosome 18q11.2 contributed to an individual's susceptibility among the Chinese population.[9]

This study examined the association between SNP rs4331426 and TB susceptibility in a Han Taiwanese population. Genotyping experiments for SNP rs4331426 were conducted and the relationship with TB susceptibility in a Taiwanese population was evaluated.

Methods

Two hundred Han Taiwanese patients who were treated for active TB at General Taoyuan Hospital (Taoyuan, Taiwan) between 2007 and 2008 were consecutively surveyed. The inclusion criteria were adult age, newly diagnosed active TB, with evident TB lesions on plain X-rays and computed tomography, and with positive results of sputum smears and cultures for mycobacteria. As controls, 177 volunteer subjects without active TB or a history of TB were enrolled. The controls were selected from the same communities as the patients.

The Ethics Committee of Taoyuan General Hospital approved the study protocol (TYGH 100010), which conformed to the ethical guidelines of the 1975 Declaration of Helsinki. All of the participants provided written informed consent.

Genomic DNA was extracted from oral swabs collected from the 200 TB patients and 177 non-TB subjects using a QIAamp DNA Mini Kit (QIAGEN, Valencia, CA, USA) according to the manufacturer's instructions. The extracted genomic DNA was analyzed using agarose gel electrophoresis, quantitatively determined by spectrophotometry, and then stored at −80°C until use.

The SNP was genotyped using a high throughput, 384-microtiter plate (San Diego, CA, USA), MassARRAY System, SEQUENOM according to the manufacturer's instructions. Briefly, the DNA containing the SNP site of interest was amplified, followed by the homogenous MassEXTEND assay in which label-free primer extension chemistry was used to generate allele-specific diagnostic products. The allele-specific diagnostic products had a unique molecular weight and this was distinguished by the application of matrix-assisted laser desorption ionization time-of-flight mass spectrometry.

The quality of the genotyped data was evaluated by the Hardy–Weinberg equilibrium (HWE) proportion tests. Associations were tested by the χ^2 test. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated from contingency tables. Logistic regression analysis was performed after adjusting for age and sex. All statistical analyses were performed using the SPSS version 17.0 software (SPSS Inc., Chicago, IL, USA).

Results

The male-to-female ratio was 137/63 in TB patients and 81/96 in non-TB subjects. There were significant differences in sex between the groups, with males being more prevalent in the case group [137 (68.5%) males vs. 63 (31.5%) females] ($p < 0.0001$, by χ^2 test). The average ($\pm$ standard deviation, SD) age was 55.74 $\pm$ 11.08 years in TB patients and 55.74 $\pm$ 18.69 years in non-TB controls. There was no significant difference in average age between the two groups ($p = 0.199$, by t test).

The genotype distributions of all study subjects of the tested SNP did not deviate from the HWE (data not shown). After analysis with the χ^2 test, the SNP rs4331426 showed different genotype frequencies (marginally significant) between the TB patients and non-TB subjects. The genetic effects of SNP rs4331426 on susceptibility to TB was further adjusted for the influence of age and sex by logistic regression. The strength of the association was clearly increased by adjusting for age and sex, and the carriers of the G allele (AG genotype) of SNP rs4331426 had a higher risk of TB than those with the AA variant (adjusted OR: 2.82; 95% CI: 1.11–7.18; $p = 0.029$) (Table 1).

Furthermore, the association between SNP rs4331426 and TB in males and females were also analyzed. There were differences in the association between SNP rs4331426 and TB between males and females ($p = 0.632$ and $p = 0.011$, respectively) (Table 1). OR analysis showed that the AG genotype of SNP rs4331426 conferred the risk of TB in females (OR: 4.34, $p = 0.017$).

Discussion

The study by Thye et al[5] in Ghana and Gambia shows that SNP rs4331426 is highly associated with TB. They identified a new non-MHC (major histocompatibility complex) locus caused by a highly polymorphic pathogen. In the present study, the genetic association of SNP rs4331426 and TB was investigated in a Taiwanese population but the results are similar, suggesting that SNP rs4331426 of 18q11.2 also confers the risk of TB among Han Taiwanese.

Table 1 Genotyping frequencies of SNP rs4331426 and results of the χ^2 test in the TB and control groups

Genotype	TB	Non-TB	OR (95% CI)	Adj. OR (95% CI)
AA (ref.)	183	170		
AG	17	7	2.26 (0.91, 5.58)	2.82 (1.11, 7.18)
	$\chi^2 = 3.254$	$p = 0.071$	$p = 0.078$	$p = 0.029$
Male				
AA (ref.)	130	78		
AG	7	3	ND	
		$p = 0.632$		
Female				
AA (ref.)	53	92		
AG	10	4	4.34 (1.30, 14.52)	
		$p = 0.011$	$p = 0.017$	

Adj. = adjusted for sex by logistic regression; CI = confidence interval; ND = not detected; OR = odds ratio; ref. = reference genotype; SNP = single nucleotide polymorphism; TB = tuberculosis.

Moreover, SNP rs4331426 with the G allele is highly susceptible to *Mycobacterium tuberculosis* infection in females. SNP rs4331426 is located in a genomic region devoid of protein-coding genes and the mechanism regarding its effect on TB risk is enigmatic. The results here are not the same as those of a previous study in a Chinese population.[9] The discrepancy may reflect genetic and environmental differences between eastern Chinese population and Han Taiwanese. In terms of genetic background, the study subjects here may be more similar to southeastern Chinese than the eastern Chinese population.

Some genome-wide association studies have demonstrated that SNPs highly susceptible to some diseases are located at the non-coding region of chromosomes. For example, chromosome 8p deletions and 8q gains are relatively rare in early-stage prostate cancer. Further studies are needed to identify the mechanism that drives these alterations.[10] The results of this study suggest that SNP rs4331426, located in the intergenic genomic region, has an unrevealed function or link to real causative polymorphism(s) related to TB.

In conclusion, rs4331426 polymorphism may be a genetic factor for susceptibility to *M. tuberculosis* among Han Taiwanese, especially in females. Further investigations of the functional role of SNP rs4331426 and the genomic region surrounding it are warranted.

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