Original Article

Genetic Variants in *MMP9* and *TCF2* Contribute to Susceptibility to Lung Cancer

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ABSTRACT

Objective: The Wnt signaling pathway is crucial for pulmonary development and differentiation; dysregulation of the Wnt signaling pathway may impair lung function. Indeed, single nucleotide polymorphisms (SNPs) of Wnt pathway-related genes have been suggested as risk factors for certain types of cancers. In this study, we aimed to evaluate the influence of SNPs in Wnt-related genes (*TCF2*, *MMP9*) on susceptibility to lung cancer.

Methods: Polymorphisms of *TCF2* rs4430796, *MMP9* rs2250889, and *MMP*9 rs17576 were studied in Han Chinese subjects, including 135 patients with lung cancer and 176 controls, using the Sequenom MassARRAY platform. The association of genotypes with susceptibility to lung cancer was analyzed using odds ratio (OR), with 95% confidence interval (95% CI) and χ^2.

Results: The three SNPs (rs4430796, rs2250889, and rs17576) were found to be significantly associated with an increased risk of lung cancer. The AA genotype and AG+AA genotype of rs4430796 showed a significantly increased susceptibility to lung cancer compared with the GG genotype (adjusted OR=6.03, 95% CI: 1.30-28.09, *P*=0.022; 5.55, 95% CI: 1.20-25.58, *P*=0.028). Compared with the rs17576 GG genotype, the AG and AG+AA genotypes were also associated with a significant risk (adjusted OR=2.65, 95% CI: 1.60-4.37, *P*≤0.001; 2.57, 95% CI: 1.59-4.19, *P*≤0.001) whereas the rs2250889 CG and CG+GG genotypes had 2.97-fold (95% CI: 1.81-4.85; *P*≤0.001) and 2.80-fold increased associations with lung cancer (95% CI: 1.73-4.54; *P*≤0.001), respectively, compared with the rs2250889 CC genotype. Furthermore, the association of rs4430796 with lung cancer became insignificant (*P*>0.05) after adjusting for gender and rs2250889.

Conclusion: The three SNPs may play a role in the predisposition of members of the Han Chinese population to lung cancer.

Key words: Single nucleotide polymorphisms (SNPs); *TCF2*; *MMP9*; Susceptibility; Lung cancer

INTRODUCTION

Lung cancer is one of the most commonly diagnosed cancers worldwide and causes the highest rate of morbidity and mortality of all cancers[1]. Despite the advances in both diagnosis and treatment in the last few decades, the prognosis for someone diagnosed with lung cancer remains poor[1]. The development of lung cancer is considered as a multifaceted and polygenic event. Although smoking is the most prominent etiological factor for the development of lung cancer[2], single nucleotide polymorphisms (SNPs) have recently been identified as important factors for tumorigenesis. An increasing number of SNPs associated with susceptibility to lung cancer have been discovered, suggesting that SNP is an important mechanism underlying lung cancer development[3-6].

The Wnt signaling pathway is involved in regulating cell function and tumorigenesis. It also plays a critical role in pulmonary development and differentiation[7-10], and its activation is dysregulated in lung adenocarcinoma cells[11, 12]. Recently, SNPs of Wnt signaling genes have been reported to correlate with the risk of cancer[13, 14]. We therefore hypothesized that SNPs of the Wnt pathway-related genes *TCF2* and *MMP9* may be associated with an increased risk for the development of lung cancer.

TCF2, also known as *HNF1β*, is a downstream transcription activator of the Wnt signaling pathway that is widely expressed in a variety of tissues, and it is crucial in embryonic development[15, 16]. It also regulates the expression of many other tissue-specific genes by binding the proximal region of the promoter sequences of genes such as alpha 1-antitrypsin[17, 18], which, when overexpressed, is associated with lung cancer development[19]. Interestingly, the SNP of *TCF2* is associated with renal cancer and prostate

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cancer[20, 21], but the association with lung cancer remains unknown. Therefore, a better understanding of the polymorphism of *TCF2*, particularly its contribution to lung cancer development, is needed.

As a transcriptional target of Wnt signaling through tandem LEF/TCF binding sites, *MMP9*, plays an important role in regulating tumor cell behaviors, including growth, differentiation, apoptosis, migration, invasion, tumor angiogenesis, and immune surveillance[22-25]. *MMP9* overexpression is seen in lung cancer samples[26], suggesting that it may contribute to cancer development. SNPs of *MMP9* have been reported to correlate with susceptibility to lung cancer[26, 27], but the data are inconsistent.

The aim of the present study was to investigate whether genetic variations of *MMP9* and *TCF2* contribute to increased risk of lung cancer development. We focused on three polymorphisms, *TCF2* rs4430796, *MMP9* rs2250889, and *MMP9* rs17576, to evaluate the associations between the genotypes and the risk of lung cancer development.

MATERIALS AND METHODS

Subjects

The subjects were 135 unrelated lung cancer patients and 176 controls. All the subjects were genetically Han Chinese and were recruited from patients at the First Affiliated Hospital of Nanchang University, Nanchang, China. All lung cancer patients were confirmed by pathology tests based on clinical examination, and the control group was defined by having no history of cancer. The median ages of the patients and control group were 58.3 and 59.0 years old, respectively.

Genotyping

Genomic DNA was extracted from a 200 μl peripheral blood sample using a Genomic DNA Purification Kit (Tianamp Biotech, Beijing, China) according to the manufacturer's instructions and stored at -70°C until use. Three SNPs, rs4430796, rs17576, and rs2250889, were genotyped using SEQUENOM MassARRAY matrix-assisted laser desorption ionization-time of flight mass spectrometry platform (Sequenom, USA). Primers were designed using a semiautomated method (Assay Design3.1, Sequenom). The call rate for each assay was set at >90%.

Statistical Analysis

Hardy–Weinberg equilibrium (HWE) was examined using Haploview4.1. The characteristics of the subjects and the odds ratio (OR) were assessed using SPSS, version 13.0. The characteristics of the patients and the controls were compared using a two-sample *t*-test for continuous variables and Chi-square for categorical variable, with 95% confidence interval (95% CI). The associations between polymorphisms and lung cancer risk were estimated by computing the OR and 95% CI from binary logistic regression analyses with adjustment for age and gender. All the tests were two-sided, and $P<0.05$ was considered statistically significant.

RESULTS

The demographics of the patients and controls enrolled in this study are shown in Table 1. No significant difference in age distribution was observed between the case and control groups ($P=0.619$). However, gender was significantly different between the two groups ($P=0.011$). So, age and gender adjustment were carried in the subsequent statistical analyses. The observed genotype frequencies for rs17576, rs2250889, and rs4430796 were in Hardy-Weinberg equilibrium in the control group, and the *P* values were 0.878, 0.959, and 0.656, respectively. The frequency distribution of the alleles and genotypes for the three polymorphisms is presented in Tables 2 and 3.

Table 1. Characteristics of the study population

Variable	Control N=175, n (%)	Lung cancer N=136, n (%)	*P*
Gender			
Male	103 (58.9)	99 (72.8)	
Female	72 (41.1)	37 (27.2)	0.011
Age (years)*	59.03±15.71	58.30±10.88	0.619

*Age (years) is shown as $\bar{x}\pm s$, calculated by a two-sample *t*-test.

Table 2. Summary results for *TCF2* SNP showing a promising association with lung cancer risk

Genotypes	Cases (%)	Controls (%)	Adjusted OR (95% CI)	*P*
	rs4430796 (126 cases, 171 controls)			
GG	1.6	7.6	1.00 (reference)	
AG	38.9	44.4	4.85 (1.02-23.01)	0.047
AA	59.5	48.0	6.03 (1.30-28.09)	0.022
Per A allele			1.50 (1.02-2.20)	0.042
Recessive model				
GG+AG			1.00 (reference)	
AA			1.44 (0.89-2.31)	0.137
Dominant model				
GG			1.00 (reference)	
AG+AA			5.55 (1.20-25.58)	0.028

*Adjusted by gender and age, $P<0.05$ was considered significant.