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.

Table 3. Summary results for *MMP9* SNPs showing a promising association with lung cancer risk

	Genotypes	Cases (%)	Controls (%)	Adjusted OR (95% CI)	*P*
rs17576 (122 cases, 166 controls)					
	GG	37.7	60.8	1.00 (reference)	
	AG	55.7	33.7	2.65 (1.60-4.37)	≤0.001
	AA	6.6	5.5	2.12 (0.75-5.93)	0.155
	Per A allele			1.86 (1.28-2.70)	0.001
	Recessive model				
	GG+AG			1.00 (reference)	
	AA			1.33 (0.49-3.60)	0.580
	Dominant model				
	GG			1.00 (reference)	
	AG+AA			2.57 (1.59-4.19)	≤0.001
rs2250889 (131 cases, 167 controls)					
	CC	45.0	69.5	1.00 (reference)	
	CG	52.7	27.5	2.97 (1.81-4.85)	≤0.001
	GG	2.3	3.0	1.23 (0.28-5.42)	0.789
	Per G allele			2.01 (1.35-2.99)	0.001
	Recessive model				
	CC+CG			1.00 (reference)	
	GG			0.78 (0.18-3.40)	0.741
	Dominant model				
	CC			1.00 (reference)	
	CG+GG			2.80 (1.73-4.54)	≤0.001

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

TCF2 Polymorphism and Lung Cancer

The genotype frequencies of rs4430796 were 1.6% (GG), 38.9% (AG), and 59.5% (AA) in lung cancer patients, which were significantly different from those observed in the control group. Compared with the GG genotype, the AA and AG genotypes were associated with lung cancer (adjusted OR=6.03, 95% CI: 1.30–28.09; 4.85, 95% CI: 1.02–23.01, respectively). Under the dominant model of inheritance, the AG+AA genotype had an adjusted OR (95% CI) of 5.55 (1.20–25.58; P=0.028) as compared with the homozygous GG genotype (Table 2). The A allele frequency of rs4430796 was higher in patients with lung cancer than in controls (OR=1.50; 95% CI: 1.02–2.20), as shown in Table 2.

MMP9 Polymorphism and Lung Cancer

The genotype distributions of *MMP9* rs17576 and rs2250889 among lung cancer patients and healthy controls are listed in Table 3. Significant increases in the frequency of the AG genotype and A allele of rs17576 were observed in patients with lung cancer compared with controls (OR=2.65; 95% CI: 1.60-4.37; OR=1.86; 95% CI: 1.28-2.70, respectively). Under the dominant model of inheritance, we observed a significant increase in the frequency of the AG+AA genotype of rs17576 (OR=2.57; 95% CI: 1.59-4.19) in patients with lung cancer. For rs2250889, significantly increased frequencies of the CG genotype (OR=2.97; 95% CI: 1.81–4.85) and of the G allele (OR=2.01; 95% CI: 1.35–2.99) were observed in the patients with lung cancer. Under the dominant model of inheritance, the CG+GG genotype correlated with an increased risk for lung cancer (OR=2.80; 95% CI: 1.73-4.54).

Linkage Disequilibrium and Interactions Analysis in *TCF2* and *MMP9* Gene Polymorphisms

We used Haploview 4.1 to calculate the pairwise r^2 between SNPs. The *MMP9* rs2250889 polymorphism was in linkage disequilibrium (LD) with rs17576 (r^2=0.59). The *TCF2* rs4430796 was not in LD with *MMP9* rs2250889 (r^2=0). Statistical differences in SNP–SNP interactions between *TCF2* and *MMP9* were examined (Table 4). The relationship between rs4430796 and the risk of lung cancer became

insignificant when rs2250889 was considered as a covariable.

Table 4. Multivariate logistic regression analysis for significant SNPs

SNP2 \ SNP1		*TCF2*	*MMP9*	
		rs4430796	rs17576	rs2250889
TCF2	rs4430796	-	≤0.001	≤0.001
MMP9	rs17576	0.025	-	0.015
	rs2250889	0.055	0.325	-

*Multivariate logistic regression analysis was performed. We used the data in case-control study to examine the residual effects of SNP1, while using SNP2 as a covariate, and we adjusted the results for gender and age. $P<0.05$ was considered significant.

DISCUSSION

SNPs are the most common inherited genetic markers, accounting for 90% of heterogeneity among individuals[28]. Recent reports have emphasized that some SNPs of Wnt signaling genes are related to the risk of cancer[13, 14]. In this study, we investigated the effects of three SNPs in two Wnt signaling genes on the development of lung cancer, including one in *TCF2* (rs4430796) and two in *MMP9* (rs2250889 and rs17576). The results indicated that rs4430796, rs2250889, and rs17576 were significantly associated with the occurrence of lung cancer.

TCF2 rs4430796 lies in intron 2 of the *TCF2* gene; an A-to-G substitution in this region had no obvious effect on the expression of protein[21]. Recently, some papers have reported that rs4430796 is associated with prostate cancer[21, 29] and type 2 diabetes[30]. In the present study, we show an association between rs4430796 and susceptibility to lung cancer. The location of rs17576 is in the catalytic domain of *MMP9*, particularly in the fibronectin type II domain, which plays important roles in substrate binding[31]. For the homozygous genotype (A/A) of rs17576, Wu, et al. found a significantly increased risk for esophageal squamous cell carcinoma (ESCC) (OR=1.70, 95% CI: 0.62–4.66)[32]; Jin, et al. reported a higher risk of death for non-small-cell lung cancer(NSCLC) (adjusted HR=1.62, 95% CI: 1.08–2.44)[27]. Our results also showed that A, as a risk allele of rs17576, increased the risk for lung cancer. The finding that the SNP rs17576 has a potential effect on the activity of this enzyme suggests a possible mechanism for the development or predisposition to lung cancer[26]. The rs2250889 is located in the haemopexin-like domain, which is an important site for binding with a metalloproteinase inhibitor[33]. The effect of the SNP rs2250889 in these functional domains has not been fully determined. Wu, et al. found that the rs2250889 GG genotype was associated with a significantly increased risk for ESCC (OR=4.08, 95% CI: 1.58–10.52)[32]. Our study indicated that G, as a risk allele of rs2250889, increased the risk for lung cancer. However, Hu, et al. found that rs17576 G and rs2250889 C as the risk alleles significantly increased the risk of lung cancer[26]. The differences in outcomes between studies may be due to differences in the ethnicity of the patients studied.

Wu, et al. reported that *MMP9* is a transcriptional target of the Wnt pathway and that the β-catenin/LEF/TCF transcription factor complex can regulate the expression of *MMP9* by binding its proximal promoter sequences[22]. From multivariate logistic regression analyses, we found that the effect of *TCF2* rs4430796 became insignificant for the risk for lung cancer when *MMP9* rs2250889 was considered as a covariate. LD was not found between *MMP9* rs2250889 and *TCF2* rs4430796; however, we did find an association between the two SNPs. The association between the genes caused by polymorphism may be related to the complex regulation of the signaling pathway, but the exact mechanism needs to be studied further.

In conclusion, the current study provides evidence that *TCF2* rs4430796, *MMP9* rs2250889, and *MMP9* rs17576 may be associated with susceptibility to lung cancer, and we report an association between the rs4430796 SNP and susceptibility to lung cancer. Our findings are an important addition to previously published work on the association between the Wnt pathway and lung cancer susceptibility. Further validation with meta-analysis, functional studies of genetic variants, and mechanical studies of the association between rs4430796 and rs2250889 are necessary.

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