

Correlation between polymorphisms of nicotine acetylcholine acceptor subunit CHRNA3 and lung cancer susceptibility

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Abstract. Both environmental and genetic factors participate in the pathogenesis of lung cancer. The aim of this study was to explore the association between CHRNA3 polymorphisms of the nicotinic acetylcholine receptor gene and lung cancer risk in a hospital-based, case-controlled study. Single nucleotide polymorphisms (SNPs) in CHRNA3 rs3743073 (A>G) were determined using the TaqMan-MGB probe technique in 600 lung cancer cases and 600 normal controls. The differences in genotype and allele frequency were compared between groups and their association with lung cancer. The genotype frequency of rs3743073 (A>G) demonstrated Hardy-Weinberg equilibrium ($P<0.05$). The genotype and allele frequencies were significantly different between the cancer and control groups ($P<0.05$). Compared with patients with the TT genotype, lung cancer incidence was increased in patients with the TG and GG genotypes (OR=1.68; 95% CI, 1.30-2.19; $P<0.05$; OR=1.30; 95% CI, 1.05-1.61; $P<0.05$, respectively). Patients with rs3743073G variant alleles (TG and GG) were at greater risk (OR=0.65; 95% CI, 0.50-0.84; $P<0.05$) of developing lung cancer. Increased risk associated with rs3743073G variant alleles was observed in male smokers over the age of 60 ($P<0.05$). In this cohort, the CHRNA3 gene rs3743073G variant genotype significantly increased lung cancer risk, especially in male smokers over the age of 60.

Introduction

Lung cancer is one of the most common malignant tumors in humans and is the most common cause of cancer-related mortality (1). For example, the United States had 219,440 new cases of lung cancer and 159,390 mortalities in 2009. China had 483,040 new cases of lung cancer and 420,411 mortalities

between 2004 and 2005 (2). Lung cancer is the main health threat in China, and greater research focus is needed towards the prevention and control of this disease.

Epidemiology studies attribute the occurrence of lung cancer to exposure to environmental risk factors. Chronic smoking, occupational exposure, air pollution and other factors are considered to be causes of lung cancer (3,4). Smoking causes over 80% of lung cancer cases (5). However, less than 20% of smokers develop lung cancer. The reasons for varied cancer susceptibility among smokers are unknown. Molecular epidemiology investigates the correlation between genetic factors, environmental factors and lung cancer incidence. Relevant clinical histories, such as COPD (6), and genetic factors, such as gene mutations, are correlated with lung cancer incidence. A previous meta-analysis (7) on family history and lung cancer showed that risk was increased with lung cancer occurrence within three generations compared with those without such a family history (8). This finding suggests that individual genetic background is closely correlated with lung cancer susceptibility.

Smoking is by far the main contributor to lung cancer incidence. Cigarette smoke contains numerous carcinogens, including tar and benzopyrene (9). These carcinogens activate signaling pathways that affect cell growth, differentiation and apoptosis. At present, nicotine is the main addictive component in cigarettes, and opinions differ about whether nicotine directly causes cancer. Nicotine is responsible for the dependence-forming properties of tobacco smoking and this addiction greatly exacerbates the cumulative health dangers of tobacco (10).

Nicotine acts on specific nicotinic acceptors *in vivo*. Activation of members of the nicotinic receptor family (CHRNA) by nicotine activates Akt signaling molecules to protect tumor cells from 'programmed cell death', or apoptosis. Avoiding apoptosis is a key early event in cancer initiation (11). CHRNA expression in a core region of the brain is closely correlated with nicotine addiction (12,13). Nicotinic receptors are present in lung epithelial cells and are involved in signal transduction that promotes cell proliferation and cancer metastasis (14-16).

Genome-wide association studies of lung cancer in Europe and the United States have identified sites on the acetylcholine receptor subunit that alter genetic susceptibility for lung cancer (17-19). *In vivo*, the acetylcholine receptor is activated

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by carcinogenic agents in tobacco, such as nicotine and nitrosamine. Nicotine-activated acetylcholine receptor promotes cell tumorigenic transformation, angiogenesis and cell growth, thereby promoting tumor development (20).

Allelic variation in one site in the acetylcholine receptor subunit CHRNA3 is closely associated with lung cancer. The frequency of this allele variation is higher in Asian populations (21). Therefore, it is necessary to explore polymorphisms associated with lung cancer susceptibility as affected by several demographic and environmental variables. This study aims to disclose the correlation between polymorphisms in CHRNA3 (rs3743073) and lung cancer in a cohort using a case-control association study.

Subjects and methods

Research subjects. Samples from cases of primary lung cancer (n=600) were collected from Jiangsu Cancer Hospital of Nanjing city between January 2008 and February 2011. Samples from a normal control population (n=600) included 444 males and 156 females. All patients in the cancer patient group had confirmed pathological diagnosis of lung cancer without other tumor disease histories. Control group subjects were randomly collected from healthy individuals during the same time period in one geographic region and without any tumor history. Subjects in the two groups were matched by age and gender. A peripheral venous blood sample (5 cc, sodium heparin anticoagulant) was obtained from all individuals after obtaining signed informed consent. This study was approved by the ethics committee of Jiangsu Cancer Hospital, Nanjing, China.

Epidemiology survey. The epidemiology survey included age, gender, individual history of occupational exposure, disease history, family history of tumor and nutrition status. Individual smoking status included smoking or non-smoking, daily number of cigarettes smoked, years of smoking and smoking situation for the primary residence. The definition of non-smokers was those who smoke <1 cigarette per day and had a cumulative smoking time of <1 year in a lifetime. Those who had quit smoking more than 1 year previously were considered non-smokers. Informed consent was obtained from subjects or from their family/caregiver.

DNA extraction. Genomic DNA was extracted from 2 ml of peripheral blood using the 0.1-20 ml blood genomic DNA extraction system (DP319-01, Tiangen Biotech Co. Ltd., Beijing, China).

Genotyping. The genome sequence of rs3743073 (T>G) in CHRNA3 was obtained by referencing a single nucleotide polymorphism (SNP) database (<http://www.ncbi.nlm.nih.gov/snp>). The CHRNA3 SNP genotype of rs3743073 (T>G) was detected using the TaqMan-MGB probe technology (Applied Biosystems, Foster City, CA, USA). Furthermore, the experimental results were separately observed and recorded by two researchers.

Statistical methods. SPSS 17.0 statistical software was used for statistical analysis. The t-test was used to compare age between

Table I. Comparison of basic characteristics between cancer and control groups.

Variable	Cases (n=600)	Controls (n=600)	t-test/ χ^2 test ^a	P-value
Age (mean \pm SD)	59.9 \pm 5.30	60.4 \pm 5.40	1.531	0.126
Gender (%)				
Male	466 (77.7)	444 (74.0)	2.201	0.138
Female	134 (22.3)	156 (26.0)		
Smoking status (%)				
Non-smokers	248 (41.3)	308 (51.3)	12.065	0.001
Smokers	352 (58.7)	292 (48.7)		
Family history of cancer (%)				
No	524 (87.3)	542 (90.3)	2.722	0.099
Yes	76 (12.7)	58 (9.7)		

^at-test for age, χ^2 test for other variables.

the two groups, and the χ^2 test was adopted to compare gender, smoking, tumor family history, genotypes and allele frequency. The correlation between gene polymorphism and the risk of lung cancer was analyzed using the odds ratio (OR), which was obtained by unconditional logistic regression analysis and a 95% confidence interval. The above analysis was performed with a two-sided test; the α level was 0.05, and P<0.05 was considered to indicate a statistically significant difference.

Results

Table I shows the distribution of age, gender, smoking status and tumor family history. The median age for the cancer patient group was 59.9 \pm 5.3 years and 60.4 \pm 5.4 years for the control group. Lung cancer was more frequently observed in males than females, with a ratio of approximately 3:1. The percentage of smokers in the cancer patient group was higher than that of the control (58.7 vs. 48.7%). There was no significant difference in age, gender distribution or tumor family history between the cancer patient and control groups (P>0.05), while there was a significant difference in smoking history between the cancer patient and control groups (P<0.05).

Genotypes and allele frequency. Table II shows that all rs3743073 allele frequencies in the control group were in compliance with Hardy-Weinberg equilibrium (P>0.05), therefore, the genotype frequencies in all sites were in balance. The difference between the genotype frequency at rs3743073 (T>G) sites and allele frequency was statistically significant (P<0.05).

Genotype frequencies and lung cancer. Unconditional logistic regression analysis showed (Table III) that the incidence of lung cancer for patients with genotype TG increased by 1.68-fold relative to patients with the genotype TT (95% CI, 1.30-2.19; P=0.01). The incidence of lung cancer for patients with genotype GG increased by 1.30 times (95% CI, 1.05-1.61;

Table II. Comparison of genotype and allele frequencies between cancer and control groups, no. (%).

Group	No.	Genotype frequency			Allele frequency	
		TT	TG	GG	A	G
Case	600	124 (20.7)	258 (43.0)	218 (36.3)	506 (96.2)	694 (3.8)
Control	600	186 (31.0)	291 (48.5)	123 (20.5)	663 (98.3)	537 (1.8)
Total		310 (25.8)	549 (45.8)	341 (28.4)	2333 (97.2)	67 (2.8)
χ^2 test			40.850		9.596	
P-value			0.001		0.002	

Table III. Association between genotype frequency and risk of lung cancer.

Variable	Genotype	Cases no. (%)	Controls no. (%)	OR (95% CI)	P-value
Total	TT	124 (20.7)	186 (31.0)	Ref.	
	TG	258 (43.0)	291 (48.5)	1.68 (1.30-2.19)	0.001
	GG	218 (36.3)	123 (20.5)	1.30 (1.05-1.61)	0.017
	TG+GG	476 (79.3)	414 (69.0)	0.65 (0.50-0.84)	0.001
≤60 years old	TT	96 (29.9)	86 (29.3)	Ref.	
	TG	143 (44.5)	148 (50.3)	0.97 (0.69-1.37)	0.859
	GG	82 (25.5)	60 (20.4)	1.12 (0.83-1.51)	0.457
	TG+GG	225 (70.1)	208 (70.7)	0.79 (0.54-1.16)	0.231
>60 years old	TT	28 (10.0)	100 (32.7)	Ref.	
	TG	115 (41.2)	143 (46.7)	4.35 (2.75-6.88)	0.001
	GG	136 (48.7)	63 (20.6)	1.52 (1.12-2.06)	0.008
	TG+GG	251 (90.0)	206 (67.3)	0.56 (0.40-0.80)	0.001
Male	TT	80 (17.2)	146 (32.9)	Ref.	
	TG	189 (40.6)	204 (45.9)	2.36 (1.73-3.23)	0.001
	GG	197 (42.3)	94 (21.2)	1.40 (1.09-1.79)	0.008
	TG+GG	386 (82.8)	298 (67.1)	0.62 (0.46-0.83)	0.001
Female	TT	44 (32.8)	40 (25.6)	Ref.	
	TG	69 (51.5)	87 (55.8)	0.71 (0.42-1.17)	0.179
	GG	21 (15.7)	29 (18.6)	0.98 (0.64-1.49)	0.918
	TG+GG	90 (67.2)	116 (74.4)	1.07 (0.57-2.00)	0.829
Non-smoker	TT	48 (19.4)	48 (15.6)	Ref.	
	TG	123 (49.6)	183 (59.4)	0.77 (0.50-1.20)	0.243
	GG	77 (31.0)	77 (25.0)	1.14 (0.85-1.54)	0.368
	TG+GG	200 (80.6)	260 (84.6)	0.77 (0.53-1.11)	0.160
Smoker	TT	76 (21.6)	138 (47.3)	Ref.	
	TG	135 (38.4)	108 (37.0)	3.25 (2.31-4.58)	0.001
	GG	141 (40.1)	46 (15.8)	1.43 (1.04-1.98)	0.028
	TG+GG	276 (78.4)	154 (52.7)	0.59 (0.40-0.86)	0.007

P=0.017) compared with the patients with the genotype TT. The incidence of lung cancer for patients with rs3743073G (TG and GG) increased by 0.65 times (95% CI, 0.50-0.84; P=0.01) relative to patients with the genotype TT. Male smokers >60 years of age with rs3743073G variant genotypes had significantly increased risk of lung cancer (P<0.05), while female non-smoking patients ≤60 years old with rs3743073G

variant genotypes had no obvious increased risk of lung cancer (P>0.05).

Discussion

It has been verified in multiple populations that there is a correlation between genetic variation of the nicotine receptor family

and lung cancer incidence (17-19). Polymorphism rs3743073 (T>G) of the CHRNA3 gene was the functional genetic variation site of peculiar relevance for the Chinese (22). This case-controlled study on 600 cases of lung cancer revealed a statistically significant difference in genotype frequency rs3743073 (T>G) and allele frequency between lung cancer patients and a normal control population. A significant increase of rs3743073G carrier frequency markedly increases the risk of lung cancer and was consistent with the previous results in Chinese population (22). These results indicate a role for this site as a biological susceptibility marker for lung cancer in the Chinese population. There was significant difference in SNP site frequency and haplotype module in rs3743073 (T>G), with no chain relationship with the high contact strength sites of lung cancer reported by previous studies in other ethnic groups. Therefore, greater clarity is needed on whether this site is correlated with lung cancer incidence in other populations.

A number of studies have verified that smoking is closely correlated with lung cancer (3-5). Our study also investigated the correlation of genetic variation of rs3743073 (T>G) and lung cancer incidence between smokers and non-smokers in a cohort. The results demonstrate that rs3743073G increases the risk of lung cancer for smokers but had no marked effect on non-smokers. Simultaneously, multiple studies have found that lung cancer incidence increased with increasing age, and nearly 70% of primary lung cancer patients were male (23,24). We found that age and gender had interactive effects with variant genotype rs3743073G; both increase the risk of lung cancer. In conclusion, subjects with the rs3743073G variant genotype of the CHRNA3 gene had significantly increased risk of lung cancer, particularly for male smokers over 60 years of age.

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