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Original Article

CHRNA5 polymorphisms and risk of lung cancer in Chinese Han smokers

Chong-Ya Huang[1], Xiao-Jie Xun[2], A-Jing Wang[3], Ya Gao[1], Jing-Yuan Ma[1], Yuan-Tang Chen[1], Tian-Bo Jin[2], Peng Hou[3], Shan-Zhi Gu[1]

[1]College of Forensic Science, School of Medicine, Xian Jiaotong University, Xi'an, 710061, China; [2]School of Life Sciences, Northwest University, Xi'an, 710069, China; [3]The First Affiliated Hospital of Xian Jiaotong University, Xi'an, 710061, China

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Abstract: Lung cancer is the most frequent cancer among men in many countries. It is the result of interactions between genetic and environmental factors, among which tobacco smoking is a key environmental factor. *CHRNA5,* Cholinergic Receptor, Neuronal Nicotinic, Alpha Polypeptide-5, was previously reported to be associated with lung cancer risk. To identify the genetic susceptibility and tobacco smoking that influence lung cancer risk in Han population, we performed a case-control study in 228 patients and 301 controls. These data were compared using the χ^2-test, genetic model analysis, and haplotype analysis. rs495956, rs680244, rs601079, rs555018, 588765 and rs11637635 showed an increased risk of lung cancer in both allelic model and genetic mode analysis. The genotype G/A-A/A of rs11637635 was most strongly associated with a 2.17-fold increased risk of lung cancer in dominant model ($p = 0.018$). One SNP, rs684513, was associated with a 0.645-fold decreased risk ($p = 0.033$) in allelic model analysis. By haplotype association analysis, haplotype sequences CTTATCAAAGA and GA of *CHRNA5* were found to be associated with a 2.03-fold and 1.91-fold increased lung cancer risk ($p < 0.05$). Our results, combined with those from previous studies, suggest that genetic variation in *CHRNA5* may influence susceptibility to lung cancer among Han smokers.

Keywords: Lung cancer, smoking behavior, SNP, *CHRNA5*, case-control studies

Introduction

Lung cancer is one of the most common malignant tumors, and it causes the highest number of cancer-related deaths [1]. It appears to result from interactions between genetic susceptibility of the individual and risk factors in the environment. Among the latter, tobacco smoking is the primary risk factor for developing lung cancer [1-3], and this has prompted a search for susceptibility genes linking smoking behavior and nicotine dependence [4].

In recent genome-wide association studies, several genetic variants at chromosomal locus 15q25, including the cholinergic receptor nicotinic α5-encoding gene (*CHRNA5*), were found to be associated with lung cancer risk and nicotine dependence [5-7]. Most of the earlier studies had focused solely on European and American populations [8, 9]. The genetic structures of these loci differ between Asians and whites, however, indicating the need for additional studies in Asian populations. To examine whether *CHRNA5* may also contribute to lung cancer risk in a defined Eastern population, the Han population of northwest China was studied. We selected 22 single nucleotide polymorphisms (SNPs) in *CHRNA5* that were previously reported to be associated with lung cancer susceptibility [10-12] for a case-control study in this population.

Materials and methods

Study participants

Samples from cases of lung cancer (n = 228) were seen between January 2010 and February 2013 at the First Affiliated Hospital of the Medical College of Xi'an Jiaotong University. All patients were newly diagnosed with lung cancer and were characterized histologically. None of the patients had previous history of other can-

Table 1. Basic characteristics of case and control patients

	Lung cancer (n = 228)	Control (n = 301)	*p*
Age (yr)	58.7 ± 10	50.2 ± 8.1	< 0.001[a]
Sex			< 0.001[b]
Male	178 (78.1%)	188 (62.5%)	
Female	50 (21.9%)	113 (37.5%)	
Smoking status			< 0.001[a]
Smoker	165 (72.4%)	91 (30.2%)	
Nonsmoker	63 (27.6%)	210 (69.8%)	
Drinking Status			0.101[a]
Drinking	37 (16.2)	66 (21.9)	
Nondrinking	191 (83.8)	235 (78.1)	
Histology type			
SCLC	37 (16.2%)		
LAC	73 (32%)		
LSCC	80 (35.1%)		
LASC	11 (4.8%)		
Others	27 (32%)		

Abbreviations: SCLC, small-cell lung cancer; LAC, lung adenocarcinoma; LSCC, lung squamous cell carcinoma; LASC, lung adenosquamous carcinoma. [a]*p* values were calculated by Student t tests; [b]*p* values were calculated from two-sided chi-square tests.

cers, chemotherapy, or radiotherapy. All healthy individuals were received physical examination at First Affiliated Hospital were selected for a control group. The control group comprised 301 unrelated healthy individuals who had no known medical illness or hereditary disorders and who were not taking any medications. Participants were chosen without restrictions of age, gender, or disease stage. Basic characteristics of the participants, e.g., gender, age, and pathology, are listed in **Table 1**.

Clinical data and demographic information

We used a standard epidemiological questionnaire and in-person interview to collect personal data, including residential region, age, smoking status, gender, education status, and family history of cancer. Never smokers were defined as those who smoked less than 100 cigarettes in their lifetime (or before diagnosis for cases) and former smokers as those who quit smoking at least 1 year before the time of the survey. All of the smokers had at least a 10-pack-year history of smoking. The case information was collected through consultation with treating physicians or from medical chart review. All of the participants signed an informed consent agreement. The Human Research Committee for Approval of Research Involving Human Subjects, First Affiliated Hospital of Xi'an Jiaotong University, approved the use of human blood in this study.

Selection of SNPs and methods of genotyping

All of the 22 selected SNPs had been previously reported to be associated with lung cancer, with minor allele frequencies of > 5%, in the HapMap of the Chinese Han Beijing (CHB) population. Extraction of DNA from whole-blood samples was done with GoldMag-Mini Whole Blood Genomic DNA Purification Kits (GoldMag Co., Ltd.; Xi'an City, China), and DNA concentration was measured with a NanoDrop 2000 spectrophotometer. The multiplexed SNP MassEXTENDED assay was designed using SequenomMassARRAY Assay Design 3.0 Software [24]. Genotyping was done with the Sequenom MassARRAY RS1000 system using the standard protocol recommended by the manufacturer. Data management and analysis was done using SequenomTyper 4.0 Software [24, 25]. The software and kits for SNP analyses were obtained from Sequenom (Sequenom. San Diego, California, USA).

Statistical analysis

The SPSS17.0 statistical software and Microsoft Excel were used for statistical analysis. All *p* values presented in this study are two sided, and we used $p \leq 0.05$ as the cutoff value for statistical significance. An exact test was used to assess the variation of each SNP frequency from Hardy-Weinberg equilibrium (HWE) in the control subjects. Student t test was used to determine the differences in age at diagnosis, smoking and drinking status. Chi-square (Pearson's χ^2) test or Fisher's exact test was used where necessary to calculate the *P* values and corresponding odds ratios (ORs) with 95% confidence intervals (CIs) to determine the associations between genotypes and lung cancer risk [26]. Odds ratios (ORs) and 95% confidence intervals (CIs) were determined using unconditional logistic regression analysis with adjustments for age, gender and drinking status [27].

Associations between *CHRNA5* and risk of lung cancer were tested in genetic models (co-dominant, dominant, recessive, over-dominant, and

Table 2. Frequency distributions of *CHRNA5* alleles and their associations with lung cancer risk in smokers

SNP ID	Position	Alleles	MAF		H-W	Without adjustment			With adjustment		
		A[a]/B	case	control	*p*-value	ORs	95% CI	Allele *p*[b]	ORs	95% CI	Allele *p*[c]
rs10519205	78878791	C/T	0.061	0.038	0.703	1.623	0.673-3.916	0.277	1.371	0.510-3.687	0.532
rs12903839	78867042	G/C	0.009	0.005	0.958	1.671	0.172-16.180	0.939	0.725	0.104-5.070	0.746
rs16969948	78864786	G/A	0.064	0.038	0.703	1.699	0.708-4.077	0.230	1.384	0.516-3.713	0.519
rs17408276	78881618	C/T	0.235	0.167	0.255	1.532	0.959-2.448	0.073	1.741	1.013-2.991	0.045*
rs17486278	78867482	C/A	0.282	0.306	0.233	0.892	0.599-1.327	0.572	0.837	0.532-1.316	0.440
rs495956	78869930	G/A	0.280	0.187	0.208	1.697	1.089-2.645	0.019*	1.856	1.116-3.086	0.017*
rs601079	78869579	A/T	0.291	0.198	0.342	1.664	1.077-2.571	0.021*	1.787	1.086-2.940	0.022*
rs680244	78871288	A/G	0.291	0.194	0.282	1.700	1.096-2.636	0.017*	1.834	1.110-3.030	0.018*
rs7180002	78873993	T/A	0.033	0.044	0.661	0.750	0.296-1.900	0.543	0.687	0.238-1.985	0.488
rs951266	78878541	T/C	0.033	0.049	0.620	0.663	0.269-1.631	0.368	0.629	0.225-1.759	0.377
rs16969968	78882925	A/G	0.042	0.044	0.661	0.964	0.396-2.342	0.935	1.022	0.370-2.820	0.967
rs1875869	78868157	T/A	0.064	0.038	0.703	1.699	0.708-4.077	0.230	1.384	0.516-3.713	0.519
rs667282	78863472	C/T	0.430	0.500	0.753	0.755	0.525-1.086	0.130	0.759	0.502-1.147	0.190
rs11637635	78877150	A/G	0.215	0.143	0.328	1.645	1.006-2.688	0.046*	1.975	1.120-3.485	0.019*
rs12903575	78881087	A/G	0.009	0.011	0.915	0.817	0.135-4.932	0.803	1.213	0.113-13.012	0.873
rs16969949	78866964	T/C	0.064	0.038	0.703	1.699	0.708-4.077	0.230	1.384	0.516-3.713	0.519
rs684513	78858400	G/C	0.227	0.313	0.971	0.645	0.430-0.968	0.033*	0.652	0.411-1.035	0.070
rs2277550	78858263	A/C	0.015	0.022	0.830	0.685	0.181-2.582	0.833	0.473	0.100-2.231	0.344
rs555018	78879242	C/T	0.227	0.154	0.496	1.618	1.003-2.609	0.047*	1.883	1.086-3.263	0.024*
rs588765	78865425	T/C	0.227	0.154	0.496	1.618	1.004-2.609	0.047*	1.883	1.086-3.263	0.024*
rs7178897	78862975	C/T	0.061	0.038	0.845	1.623	1.005-3.916	0.277	1.266	0.466-3.442	0.644
rs692780	78876505	G/C	0.236	0.159	0.035#	–	–	–	–	–	–

[a]Minor allele. [b]*p* values were calculated from two-sided chi-square tests or Fisher's exact tests for either allele frequency. [c]*p* values were calculated by unconditional logistic regression adjusted for age and sex. **p* value ≤ 0.05 indicates statistical significance. #site with HWE *p* ≤ 0.05 is excluded. CI, confidence interval; HWE, Hardy-Weinberg Equilibrium; MAF, minor allele frequency; ORs, odds ratios; SNP, single nucleotide polymorphism.

log-additive) by analysis with SNP Stats software, obtained from http://bioinfo.iconcologia.net. Values of OR and 95% CI were calculated as above. Akaike's Information Criterion and Bayesian Information Criterion were applied to estimate the best-fit model for each SNP.

Haploview software version 4.2 was used to analyze the association between haplotype and lung cancer. Linkage disequilibrium (LD) analysis was done using genotype data from all the subjects. The pattern of LD was analyzed using two parameters, r^2 and D^0. Statistical significance was set at $p \leq 0.05$.

Results

A total of 529 participants, including 228 lung cancer cases (178 males, 50 females; mean age at diagnosis 58.7 ± 10 yr) and 301 controls (188 males, 113 females; mean age 50.8 ± 8.1 yr) were successfully genotyped for further analysis (**Table 1**). More smokers were observed in cases compared with subjects in the control group ($p < 0.001$). This result was expected because most lung cancers can be attributed to smoking. A total of 22 SNPs in *CHRNA5* were genotypedin lung cancer patients (smoker and nonsmoker) and healthy controls. The average SNP call rate was 98.5% in cases and controls. **Table 2** summarizes the basic information of *CHRNA5* SNPs in the smoker subpopulation. One SNP (rs692780) was excluded at the 5%-HWE *p* level.

We compared the differences in frequency distributions of alleles between cases and controls by χ^2-test or Fisher's exact test and found seven significant SNPs in the smoker subpopulation. The polymorphisms detected by rs495956 (OR = 1.70; 95% CI, 1.09-2.65, p = 0.019), rs680244 (OR = 1.70; 95% CI, 1.10-2.64, p = 0.017), rs601079 (OR = 1.66; 95% CI, 1.08-2.57, p = 0.021), rs11637635 (OR = 1.64; 95% CI, 1.01-2.69, p = 0.046), rs555018 (OR =

Table 3. Dominant model analysis of relationship between SNPs and lung cancer risk in the smoking versus nonsmoking subpopulations

SNP	Genotype	Smoking subpopulation		Nonsmoking subpopulation	
		OR[a] (95% CI)	*p*-value	OR[a] (95% CI)	*p*-value
rs495956	A/A	1.00		1.00	
	G/A-G/G	2.07 (1.12-3.82)	0.018*	0.90 (0.48-1.70)	0.75
rs601079	T/T	1.00		1.00	
	A/T-A/A	1.99 (1.09-3.64)	0.024*	0.82 (0.44-1.56)	0.55
rs680244	G/G	1.00		1.00	
	G/A-A/A	2.07 (1.12-3.81)	0.018*	0.82 (0.44-1.56)	0.55
rs588765	C/C	1.00		1.00	
	T/C-T/T	2.07 (1.09-3.93)	0.023*	0.89 (0.46-1.73)	0.74
rs555018	T/T	1.00		1.00	
	C/T-C/C	2.07 (1.09-3.93)	0.023*	1.00 (0.52-1.92)	0.99
rs11637635	G/G	1.00		1.00	
	G/A-A/A	2.17 (1.12-4.19)	0.018*	0.88 (0.45-1.72)	0.71

*A p value ≤ 0.05 indicates statistical significance; [a]adjusted by sex, age, and drinking status. Abbreviations: OR, odds ratio; CI, confidence interval.

1.62; 95% CI, 1.00-2.61, p = 0.047), and rs588765 (OR = 1.62; 95% CI, 1.00-2.61, p = 0.047) showed an increased risk of lung cancer. Only the rs684513 (OR = 0.64; 95% CI, 0.43-40.91, p = 0.033) was associated with a decreased risk. After adjusted by age, gender and drinking status, rs684513 was not statistically significant anymore (p = 0.07).

As summarized in Supplemental Table 3, we analyzed the SNPs in the context of various genetic models. In the smoker subpopulation, the genotypes G/A-G/G of rs495956 (OR = 2.07; 95% CI, 1.12-3.82, p = 0.018), A/T-A/A of rs601079 (OR = 1.99; 95% CI, 1.09-3.64, p = 0.024), G/A-A/A of rs680244 (OR = 2.07; 95% CI, 1.12-3.81, p = 0.018), G/A-A/A of rs11637635 (OR = 2.17; 95% CI, 1.12-4.19, p = 0.018), C/T-C/C of rs555018 (OR = 2.07; 95% CI, 1.09-3.93, p = 0.023), and T/C-T/T of rs588765 (OR = 2.07; 95% CI, 1.09-3.93, p = 0.023) in *CHRNA5* showed an increased risk of lung cancer in the dominant model (**Table 3**). However, no statistically significant evidence suggested that the polymorphisms tested were associated with lung cancer risk in the nonsmoker subpopulation.

The LD and haplotype analyses of the SNPs in the case and control samples for the smoker subpopulation were further studied. The LD of 22 SNPs distributed throughout CHRNA5 sequences was analyzed (D^0 and r^2). Thirteen SNPs were found to exist in 2 LD blocks: 11 SNPs (rs684513, rs7178897, rs667282, rs16969948, rs588765, rs16969949, rs17486278, rs1875869, rs601079, rs495956, and rs680244) in block 1, and 2 completely linked SNPs (rs692780 and rs11637635) in block 2 (**Figure 1**). The SNPs located in the same LD block were used for a haplotype-based association study among the individuals with a history of tobacco smoking. Results showing association between CHRNA5 haplotype and risk of lung cancer are summarized in **Table 4**. We found that the haplotype CTTATCAAAGA, composed of the variant alleles of rs16969948, rs588765, rs601079, rs495956, and rs680244 and the reference allele of rs684513, rs7178897, rs667282, rs16969949, rs17486278 and rs1875869 were found to be increased lung cancer risk by 2.03-fold (OR = 2.03; 95% CI, 1.07-3.88, p = 0.032); the haplotype GA of variant alleles rs692780 and rs11637635 also increased lung cancer risk, by 1.91-fold (OR = 2.03; 95% CI, 1.07-3.88, p = 0.032). Haplotype analysis for the CHRNA5 polymorphisms showed an association with lung cancer risk.

Discussion

In this study we investigated the associations between 22 selected *CHRNA5* SNPs and risk of lung cancer in the Han population from the northwest China. We did not find significant association with lung cancer in the whole population (Supplemental Table 1). However, 6 SNPs showed a significant association with increased risk of lung cancer, and 1 SNP contributed to a decreased risk of lung cancer, by individual SNP analysis in the smoker subpopulation. The results suggest that the polymorphisms of *CHRNA5* may play an important role in the risk of lung cancer in the subpopulation of Han individuals who smoke.

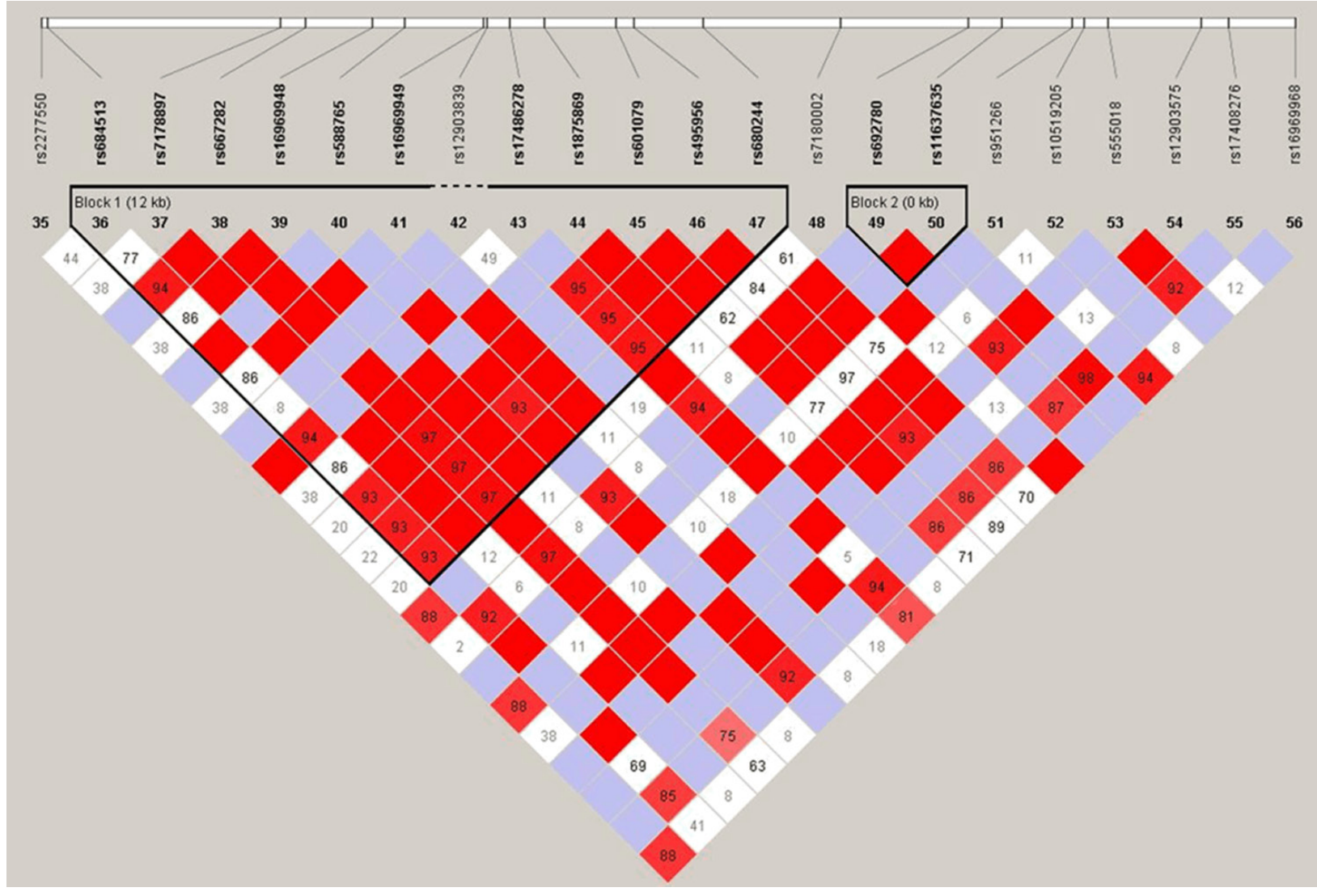

Figure 1. D′ linkage map of 22 SNPs in *CHRNA5*. A standard color scheme is used to display LD with bright red for very strong LD (LOD = 2, *D*′ = 1), white for no LD (LOD < 2, *D*′ < 1), pink red (LOD = 2, *D*′ < 1), and blue (LOD < 2, *D*′ = 1) for intermediate LD.

Table 4. *CHRNA5* haplotype frequencies and associations with the lung cancer risk in individuals who smokedin the cases and controls

Block	Haplotype	Frequency	OR	*p*-value
1	CTTACCCATAG	0.2834	1.00	---
	GTCACCAATAG	0.2482	0.84 (0.48-1.50)	0.57
	CTCACCAATAG	0.2050	1.39 (0.75-2.59)	0.3
	CTTATCAAAGA	0.1914	2.03 (1.07-3.88)	0.032*
	CCTGCTATAGA	0.0508	1.82 (0.59-5.66)	0.3
	rare	0.0214	1.21 (0.26-5.60)	0.81
2	CG	0.7910	1.00	---
	GA	0.1895	1.91 (1.10-3.33)	0.023*
	GG	0.0195	0.95 (0.24-3.72)	0.94

*A *p*-value of ≤ 0.05 indicates statistical significance. Abbreviations: OR, odds ratio; CI, confidence interval. Block 1: rs684513, rs7178897, rs667282, rs16969948, rs588765, rs16969949, rs17486278, rs1875869, rs601079, rs495956, rs680244; Block 2: rs692780, rs11637635.

Nicotine acetylcholine receptor genes are expressed in key regions of the brain and play an important role in controlling smoking behavior. Acetylcholine receptors can bind to tobacco carcinogens, such as nicotine and nitrosamines, in the body. Subsequently, activated nicotinic acetylcholine receptors can promote tumorigenic conversion of cells, angiogenesis, and cell growth, thereby contributing to tumor development [6]. The corresponding genes are located on chromosome 15q25 [10, 13], and the encoded receptors are the primary targets for nicotine in the brain, leading to strong downstream responses in that tissue [14, 15]. Smoking is the major environmental risk factor for lung cancer because tobacco smoke contains strong carcinogens, such as empyreumatic oil and benzo(a)pyrene [16]. In earlier studies, sequence variants in *CHRNA* SNPs have been associated with increased (self-reported) cigarette dose and nicotine dependence, and with increased risk of lung cancer in smokers [7, 8], whereas, no association in nonsmokers was reported [8].

In the present study, *CHRNA5* rs684513 polymorphisms were found to contribute to reduced risk of lung cancer. In contrast, the rs495956, rs601079, rs11637635, rs555018, rs588765, and rs680244 showed an increased risk in the smoker subpopulation. However, none of these *CHRNA5* polymorphisms were associated with lung cancer in Han individuals in our study who had never smoked (Supplemental Table 2). This result may be because *CHRNA5* encodes a polypeptide of the nicotinic acetylcholine receptor, and smoking behavior a priori will appear as an environmental risk factor for lung cancer most strongly in smokers. Previous findings generally suggested that that the aetiology, clinical characteristics, and prognosis of lung cancer in never smokers are substantially different to those in smokers [17]. In our study, rs684513, located in the first intron of *CHRNA5*, was the only SNP that show a decreased risk of lung cancer. This results show an consistency with previous studies in other population [11]. A study before showed that rs601079, rs11637635, rs555018, rs588765, and rs680244 were associated with mRNA expression level of *CHRNA5* [18]. Other study before showed that rs11637635 and rs588765 is associated with smokers' total puff volume per cigarette [19]. This may be the reason why these SNPs associated with an increased risk of lung cancer.

Some studies before showed that rs16969968 was associated with lung cancer risk in European-ancestry populations and Japanese population [15, 20-22]. In a study of African-Americans, *CHRNA5* rs17486278 was found to be associated with increased lung cancer risk [23]. However, in our study, the same SNPs were not associated with lung cancer risk. These differences may be primarily attributed to distinct genetic backgrounds and differences in specific environmental factors of the Han people compared to other populations.

In conclusion, our study has described the association between SNPs in *CHRNA5* and lung cancer risk in a group composed of the Han individuals of northwestern China for the first time. Our findings suggested that genetic variants and environmental factors especially tobacco smoking may play an important role in occurrence of lung cancer in this population.

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Disclosure of conflict of interest

None.

Address correspondence to: Dr. Shan-Zhi Gu, College of Forensic Science, School of Medicine, Xian Jiaotong University, Xi'an, 710061, China. Tel: +86-29-82655114; Fax: +86-29-82655114; E-mail: gushanzhi1@163.com

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Supplemental Table 1. Basic information of *CHRNA5* SNPs in this study

SNP ID	Position	Allele	MAF		HWE	ORs	95% CI		Allele
		A[a]/B	Case	Control	*p*-value				*p*[b]-value
rs2277550	78858263	A/C	0.015	0.018	0.747	0.838	0.322	2.178	0.716
rs684513	78858400	G/C	0.25	0.286	0.333	0.833	0.632	1.098	0.195
rs7178897	78862975	C/T	0.062	0.061	0.063	1.004	0.605	1.666	0.989
rs667282	78863472	C/T	0.447	0.487	0.279	0.854	0.669	1.09	0.204
rs16969948	78864786	G/A	0.064	0.055	0.314	1.171	0.7	1.959	0.547
rs588765	78865425	T/C	0.219	0.188	0.544	1.216	0.899	1.644	0.204
rs16969949	78866964	T/C	0.061	0.056	0.966	1.093	0.653	1.83	0.736
rs12903839	78867042	G/C	0.009	0.005	0.931	1.775	0.395	7.97	0.707
rs17486278	78867482	C/A	0.272	0.27	0.799	1.01	0.768	1.328	0.944
rs1875869	78868157	T/A	0.061	0.056	0.966	1.093	0.653	1.83	0.736
rs601079	78869579	A/T	0.281	0.246	0.321	1.197	0.908	1.578	0.201
rs495956	78869930	G/A	0.271	0.238	0.528	1.193	0.902	1.577	0.216
rs680244	78871288	A/G	0.281	0.248	0.642	1.181	0.897	1.556	0.236
rs7180002	78873993	T/A	0.033	0.032	0.572	1.044	0.524	2.077	0.903
rs692780	78876505	G/C	0.228	0.199	0.707	1.187	0.882	1.596	0.257
rs11637635	78877150	A/G	0.208	0.179	0.788	1.204	0.885	1.637	0.237
rs951266	78878541	T/C	0.033	0.035	0.531	0.941	0.48	1.847	0.86
rs10519205	78878791	C/T	0.059	0.054	0.872	1.114	0.658	1.888	0.687
rs555018	78879242	C/T	0.221	0.188	0.544	1.231	0.911	1.664	0.176
rs12903575	78881087	A/G	0.009	0.01	0.861	0.876	0.246	3.123	0.907
rs17408276	78881618	C/T	0.231	0.189	0.856	1.292	0.956	1.746	0.095
rs16969968	78882925	A/G	0.044	0.035	0.278	1.269	0.679	2.371	0.454

[a]Minor allele; [b]*p* values were calculated from two-sided chi-square tests or Fisher's exact tests for either allele frequency. Abbreviations: OR, odd ratio; CI, confidence interval; HWE, Hardy-Weinberg equilibrium; MAF, minor allelefrequency.

Supplemental Table 2. Basic information of *CHRNA5* SNPs in nonsmokersubpopulation

SNP ID	Position	Alleles	Frequency				H-W	ORs	95% CI		Allele
		A[a]/B	Case		Control		*p*-value		lower	upper	*p*[b]-value
rs10519205	78878791	C/T	0.056	0.944	0.060	0.940	0.764	0.915	0.386	2.169	0.841
rs12903839	78867042	G/C	0.008	0.992	0.005	0.995	0.945	1.672	0.150	18.594	0.792
rs16969948	78864786	G/A	0.063	0.937	0.062	0.938	0.339	1.027	0.453	2.330	0.948
rs17408276	78881618	C/T	0.222	0.778	0.199	0.801	0.369	1.153	0.710	1.874	0.564
rs17486278	78867482	C/A	0.246	0.754	0.255	0.745	0.618	0.955	0.602	1.514	0.843
rs495956	78869930	G/A	0.246	0.754	0.260	0.740	0.137	0.931	0.588	1.475	0.761
rs601079	78869579	A/T	0.254	0.746	0.267	0.733	0.082	0.936	0.594	1.477	0.777
rs680244	78871288	A/G	0.254	0.746	0.271	0.729	0.226	0.914	0.580	1.440	0.698
rs7180002	78873993	T/A	0.032	0.968	0.026	0.974	0.697	1.219	0.381	3.897	0.981
rs951266	78878541	T/C	0.032	0.968	0.029	0.971	0.670	1.115	0.353	3.519	0.908
rs16969968	78882925	A/G	0.048	0.952	0.031	0.969	0.066	1.565	0.583	4.207	0.371
rs1875869	78868157	T/A	0.056	0.944	0.064	0.936	0.880	0.856	0.364	2.016	0.722
rs667282	78863472	C/T	0.492	0.508	0.481	0.519	0.134	1.045	0.702	1.557	0.827
rs11637635	78877150	A/G	0.190	0.810	0.195	0.805	0.379	0.970	0.585	1.609	0.906
rs12903575	78881087	A/G	0.008	0.992	0.010	0.990	0.889	0.832	0.092	7.512	0.712
rs16969949	78866964	T/C	0.056	0.944	0.064	0.936	0.880	0.856	0.364	2.016	0.722
rs2277550	78858263	A/C	0.016	0.984	0.017	0.983	0.806	0.952	0.195	4.640	0.736
rs684513	78858400	G/C	0.310	0.690	0.274	0.726	0.258	1.189	0.770	1.836	0.435
rs555018	78879242	C/T	0.206	0.794	0.202	0.798	0.266	1.025	0.626	1.677	0.923
rs588765	78865425	T/C	0.198	0.802	0.202	0.798	0.266	0.976	0.593	1.606	0.922
rs7178897	78862975	C/T	0.063	0.937	0.071	0.929	0.045#	0.881	0.393	1.975	0.759
rs692780	78876505	G/C	0.206	0.794	0.217	0.783	0.376	0.940	0.576	1.534	0.804

#Site with HWE $p \leq 0.05$ is excluded; [a]Minor allele; [b]*p* values were calculated from two-sided chi-square tests or Fisher's exact tests for either allele frequency. Abbreviations: OR, odd ratio; CI, confidence interval; HWE, Hardy-Weinberg equilibrium.

Supplemental Table 3. Analysis of SNP distribution and models in the smoking versus nonsmoking-subpopulations

SNP	Model	Genotype	Smoking subpopulation		Nonsmoking subpopulation	
			OR[a] (95% CI)	*p*-value	OR[a] (95% CI)	*p*-value
rs495956	Co-dominant	A/A	1.00	0.057	1.00	0.82
		G/A	1.98 (1.03-.79)		0.85 (0.44-1.67)	
		G/G	2.56 (0.75-8.75)		1.21 (0.35-4.24)	
	Dominant	A/A	1.00	0.018*	1.00	0.75
		G/A-G/G	2.07 (1.12-3.82)		0.90 (0.48-1.70)	
	Recessive	A/A-G/A	1.00	0.24	1.00	0.68
		G/G	2.04 (0.61-6.83)		1.30 (0.38-4.40)	
	Over-dominant	A/A-G/G	1.00	0.068	1.00	0.59
		G/A	1.80 (0.95-3.40)		0.83 (0.43-1.61)	
	Log-additive		1.77 (1.08-2.89)	0.019	0.98 (0.59-1.63)	0.93
rs601079	Co-dominant	T/T	1.00	0.07	1.00	0.39
		A/T	1.89 (1.00-3.58)		0.72 (0.37-1.42)	
		A/A	2.57 (0.75-8.78)		1.62 (0.48-5.44)	
	Dominant	T/T	1.00	0.024*	1.00	0.55
		A/T-A/A	1.99 (1.09-3.64)		0.82 (0.44-1.56)	
	Recessive	T/T-A/T	1.00	0.24	1.00	0.31
		A/A	2.04 (0.61-6.84)		1.85 (0.57-6.04)	

	Over-dominant	T/T-A/A	1.00	0.087	1.00	0.25
		A/T	1.72 (0.92-3.20)		0.68 (0.35-1.32)	
	Log-additive		1.74 (1.07-2.82)	0.023	0.98 (0.59-1.64)	0.95
rs680244	Co-dominant	G/G	1.00	0.056	1.00	0.43
		G/A	1.98 (1.04-3.78)		0.73 (0.37-1.43)	
		A/A	2.56 (0.75-8.74)		1.52 (0.46-5.00)	
	Dominant	G/G	1.00	0.018*	1.00	0.55
		G/A-A/A	2.07 (1.12-3.81)		0.82 (0.44-1.56)	
	Recessive	G/G-G/A	1.00	0.25	1.00	0.36
		A/A	2.01 (0.60-6.72)		1.74 (0.54-5.53)	
	Over-dominant	G/G-A/A	1.00	0.065	1.00	0.27
		G/A	1.80 (0.96-3.38)		0.69 (0.36-1.34)	
	Log-additive		1.77 (1.09-2.89)	0.019	0.98 (0.59-1.62)	0.93
rs588765	Co-dominant	C/C	1.00	0.07	1.00	0.84
		T/C	1.98 (1.02-3.87)		0.85 (0.42-1.70)	
		T/T	2.83 (0.58-13.70)		1.22 (0.29-5.09)	
	Dominant	C/C	1.00	0.023*	1.00	0.74
		T/C-T/T	2.07 (1.09-3.93)		0.89 (0.46-1.73)	
	Recessive	C/C-T/C	1.00	0.28	1.00	0.72
		T/T	2.29 (0.48-10.91)		1.29 (0.32-5.28)	
	Over-dominant	C/C-T/T	1.00	0.06	1.00	0.6
		T/C	1.87 (0.96-3.61)		0.83 (0.42-1.66)	
	Log-additive		1.86 (1.08-3.20)	0.022	0.96 (0.56-1.65)	0.89
rs555018	Co-dominant	T/T	1.00	0.07	1.00	0.93
		C/T	1.98 (1.02-3.87)		0.95 (0.48-1.91)	
		C/C	2.83 (0.58-13.70)		1.27 (0.31-5.30)	
	Dominant	T/T	1.00	0.023*	1.00	0.99
		C/T-C/C	2.07 (1.09-3.93)		1.00 (0.52-1.92)	
	Recessive	T/T-C/T	1.00	0.28	1.00	0.72
		C/C	2.29 (0.48-10.91)		1.29 (0.32-5.28)	
	Over-dominant	T/T-C/C	1.00	0.06	1.00	0.85
		C/T	1.87 (0.96-3.61)		0.94 (0.47-1.86)	
	Log-additive		1.86 (1.08-3.20)	0.022	1.03 (0.60-1.77)	0.9
rs11637635	Co-dominant	G/G	1.00	0.058	1.00	0.82
		G/A	2.08 (1.04-4.15)		0.83 (0.41-1.69)	
		A/A	2.83 (0.58-13.70)		1.22 (0.29-5.06)	
	Dominant	G/G	1.00	0.018*	1.00	0.71
		G/A-A/A	2.17 (1.12-4.19)		0.88 (0.45-1.72)	
	Recessive	G/G-G/A	1.00	0.28	1.00	0.72
		A/A	2.29 (0.48-10.91)		1.29 (0.32-5.28)	
	Over-dominant	G/G-A/A	1.00	0.048	1.00	0.57
		G/A	1.96 (0.99-3.88)		0.82 (0.41-1.65)	
	Log-additive		1.91 (1.10-3.32)	0.019	0.95 (0.55-1.64)	0.87

*A p value ≤ 0.05 indicates statistical significance; [a]adjusted by sex, age,and drinking status. Abbreviations: OR, odds ratio; CI, confidence interval.