Association of the *CHRNA3* Locus with Lung Cancer Risk and Prognosis in Chinese Han Population

Xiaomin Niu, MD, Zhiwei Chen, MD, PhD,* Shengping Shen, MD,* Yun Liu, PhD,†‡§ Daizhan Zhou, PhD,‡§ Jie Zhang, MD, PhD,‖ Ziming Li, MD,* Yongfeng Yu, MD,* Meilin Liao, MD,* Shun Lu, MD, PhD,* and Lin He, PhD†‡§*

Introduction: Recent genome-wide association studies in Caucasians revealed association with lung cancer risk of single nucleotide polymorphisms (SNPs) in the locus containing two nicotine acetylcholine receptor *CHRNA* genes. However, the reported risk SNPs are extremely rare in Asians. This study sought to identify other variants on *CHRNA3* associated with lung cancer susceptibility and to explore whether SNPs of *CHRNA3* are of prognostic factors in patients with non-small cell lung cancer (NSCLC) in Chinese Han population.

Methods: A case-control study of 529 cases and 567 controls was performed to study the association of three SNPs (rs3743076, rs3743078, and rs3743073) in *CHRNA3* with lung cancer risk in Chinese Han population using logistic regression models. The relationship between *CHRNA3* polymorphisms with overall survival among 122 patients with advanced stage (stage IIIb and IV) NSCLC were evaluated using Cox multiple model based on the International Association for the Study of Lung Cancer recommended tumor, node, metastasis new staging.

Results: Patients with genotypes TG or GG for the novel SNP rs3743073 in *CHRNA3* gene, compared with those with TT, showed an increased risk of lung cancer (adjusted odds ratio = 1.91; 95% confidence interval, 1.38–2.63; $p = 9.67 \times 10^{-5}$) and worst survival (adjusted hazard ratio = 2.35; 95% confidence interval, 1.05–5.26; $p = 0.04$) in patients with advanced stage NSCLC based on International Association for the Study of Lung Cancer recommended tumor, node, metastasis new staging.

Conclusions: These results suggest that the rs3743073 polymorphism in *CHRNA3* is predictive for lung cancer risk and prognostic in advanced stage NSCLC in Chinese Han population.

Key Words: Lung cancer, *CHRNA3*, Polymorphism, Case-control study, Survival.

(*J Thorac Oncol.* 2010;5: 658–666)

Lung cancer is the most common cause of cancer mortality worldwide.[1] Although smoking is known to be one of the most important risk factors, the fact that only a portion of smokers develop lung cancer during their lifetime suggests that other genetic and epigenetic factors are of importance in determining individual's susceptibility to lung cancer.[2]

Recently, the locus containing two genes encoding nicotine acetylcholine receptor (nAChR) subunits, *CHRNA3* and *CHRNA5*, was shown to be associated with lung cancer risk in Caucasians by three genome-wide association (GWA) studies.[3–5] *CHRNA* proteins are expressed in lung epithelial cells and bind nicotine, an addictive compound in cigarette smoke, and nitrosamines, potential lung carcinogens in cigarette smoke and foods.[6,7] Signal transduction through *CHRNA* proteins was suggested to cause cell proliferation and also to facilitate neoplastic transformation.[8,9] A decreased survival correlation with lung cancer has been shown[10,11] because the reduced treatment efficacy and the worse survival may be well explained by the nAChRs pathway through *CHRNA* proteins on tumor cell proliferation, apoptosis, epithelial-mesenchymal transition, and pro-invasive and angiogenic effects.[8,9]

These exciting results in the GWA studies encouraged us to investigate the association between the risk single nucleotide polymorphisms (SNPs) (rs8034191, rs1051730, and rs16969968) reported in Caucasians[3–5] with lung cancer risk in Chinese populations. However, by examining the HapMap data, these three risk SNPs are extremely rare in Asians, suggesting that the role these SNPs play in risk of lung cancer in Asian populations, if any, may not be as important as they do in Caucasians. Thus, we hypothesized that if *CHRNA* polymorphisms also play a role in susceptibility to lung cancer in Chinese populations, there must be other risk variants. Therefore, we conducted a case-control

*Department of Shanghai Lung Cancer Center, Shanghai Chest Hospital, Jiaotong University; †Institutes of Biomedical Sciences, Fudan University, Shanghai, PR China; ‡Institute for Nutritional Sciences, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shaghai, PR China; §Bio-X Center, Key Laboratory for the Genetics of Developmental and Neuropsychiatric Disorders (Ministry of Education), Shaghai Jiaotong University, Shaghai, PR China; and ‖Department of Surgery, Shanghai Cancer Hospital, Fudan University, Shanghai, China.

Disclosure: The authors declare no conflicts of interest.

Address for correspondence: Shun Lu, MD, PhD, Department of Shanghai Lung Cancer Center, Shanghai Chest Hospital, Shaghai Jiaotong University, 241 Huaihai West Road, Shanghai 200030, China. E-mail: shun_ lu@hotmail.com, or Lin He, PhD, Institutes of Biological Sciences, Fudan Univeristy, 138 Yixueyuan Road, Shanghai 200032, China; Institute for Nutritional Sciences, Shaghai Institutes for Biological Sciences, Chinese Academy of Sciences, 294 Taiyuan Road, Shanghai 200031, China; or Bio-X Center, Key Laboratory for the Genetics of Developmental and Neuropsychiatric Disorders (Ministry of Education), Shanghai Jiaotong University, 1954 Huashan Road, Shanghai 200030, China. E-mail: helin@bio-x.cn.

X.N. and Z.C. contributed equally to the study.

ISSN: 1556-0864/10/0505-0658

study about three novel SNPs, rs3743076, rs3743078, and rs3743073, located in *CHRNA3* based on the HapMap data and the reported studies,[12,13] to examine the association of *CHRNA3* polymorphisms with risks for three major histologic types of lung cancer, adenocarcinoma (ADC), squamous cell carcinoma (SQC), and small cell lung cancer (SCLC), in a Chinese Han population. We hypothesize further that *CHRNA3* polymorphisms may be associated with survival outcomes in lung cancer.

PATIENTS AND METHODS

Case-Control Study

All cases and controls were Chinese Han Population. The cases consisted of 356 ADC, 131 SQC, and 42 SCLC patients of Shanghai Chest Hospital affiliated with Shaghai Jiaotong University from 2005 to 2008. The controls consisting of 567 healthy residents were selected by random sampling from 11 independent communities in Shanghai Pudong New Area and Baoshan district during the same time period as the cases were recruited. All the lung cancer cases without a history of other cancer, from whom informed consents and blood samples were obtained, were consecutively included in this study. All the lung cancer cases were diagnosed as ADC, SQC, or SCLC by histologic examinations according to World Health Organization classification confirmed by two independent pathologists. Routine tumor, node, metastasis (TNM) staging was performed according to the new staging project initiated by the International Association for the Study of Lung Cancer in 2009.[14,15] All the control subjects were selected with a criterion of no history of any cancer. A standard informed consent of the control subjects was approved and obtained by the ethics committee of the Shanghai Institute for Biologic Sciences.

Smoking history of cases and controls was obtained through interview using a questionnaire. Smoking dose of each subject was expressed by "cigarettes per day," i.e., the number of cigarettes smoked per day on average on most days; smoking exposure of each subject was expressed by "pack-years," which was defined as the number of pack per day (i.e., cigarettes per day divided by 20) multiplied by years of smoking as in previous studies.[3–5] Smokers were defined as those who had smoked regularly for 12 months or longer at any time in their life, whereas nonsmokers were defined as those who had not. There were no individuals who had smoked regularly for less than 12 months.

Genotyping

Blood samples were collected from all study participants. DNA was extracted from peripheral blood samples using the Tiangen Biotech kit (DP318; Tiangen Biotech (Beijing) Co., Ltd., Beijing China) The three novel genotype SNPs, rs3743076, rs3743078, and rs3743073 in *CHRNA3* locus, were selected based on the fact that minor allele frequencies (MAFs) in the Chinese Han population are reported to be >10% in the dbSNP database (http://www.ncbi.nlm.nih.gov/SNP/) and the reported studies.[12,13] The *CHRNA3* polymorphisms were genotyped by the 5′-nuclease assay (TaqMan) using the ABI Prism 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA). Genotyping was performed by laboratory personnel blinded to sample status, and a random 5% of the samples were repeated to validate genotyping procedures. Two authors independently reviewed the genotyping results, data entry, and statistical analyses.

Outcome Collection

Survival analysis was carried out in patients with non-small cell lung cancer (NSCLC) (ADC and SQC) because of the limited statistical power of the relatively smaller numbers of patients with SCLC ($n = 42$). Data were collected from at least one of the following sources: the inpatient and outpatient records of Shanghai Chest Hospital, the follow-up registries of Shanghai Municipal Center for Disease Control and Prevention, and patient or family contact. Patients distribution is listed in Figure 1. Of all 487 patients with NSCLC, 44 (44 of the 487, 9.0%) patients were excluded from survival analyses because of no adequate blood for genotyping and 25 (25 of the 487, 5.1%) were excluded because of no survival data. There were no significant differences in clinical information between those who had blood available for genotyping and those who did not, and no differences between those who had survival data and those who did not.

Overall survival (OS) was calculated from date of a definite diagnosis to date of death or the last known date alive. The last follow-up date was November 6, 2009. A total of 296 patients with early-stage (stage I, II, and IIIa) NSCLC continued to be visited because of relatively short median follow-up time of 18.5 months. One hundred twenty-two patients with advanced stage (stage IIIb and IV) NSCLC were available for survival analysis with a median follow-up of 21.5 months (range, 12.3–57.0 months). The patients who received chemotherapy were defined as those who received first-line platinum-based chemotherapy for at least two cycles, whereas patients who did not receive chemotherapy were defined as those who had not received first-line platinum-based chemotherapy. Response Evaluation Criteria in Solid Tumors (RECIST) was introduced to standardize tumor response assessment. One hundred three of 122 (84.4%) patients with advanced-stage NSCLC receiving chemotherapy were assessable for efficacy evaluation. Efficacy results for the platinum-based chemotherapy were summarized as follows: progressive disease or its converse, disease control rate, consisting of complete response, partial response, and stable disease.

Statistical Methods

A Hardy-Weinberg equilibrium test was performed using the SHEsis software (http://www.nhgg.org/analysis).[16] Calculation of the D' and r^2 values as well as haplotype estimation was undertaken using the expectation-maximization algorithm of the same software.[16] The information was compared across genotype and clinical records using Pearson χ^2 tests (for categoric variables) and Kruskal-Wallis tests (for continuous variables), where appropriate. The strength of association of alleles with ADC, SQC, and SCLC risks was measured as crude odds ratios (ORs). To

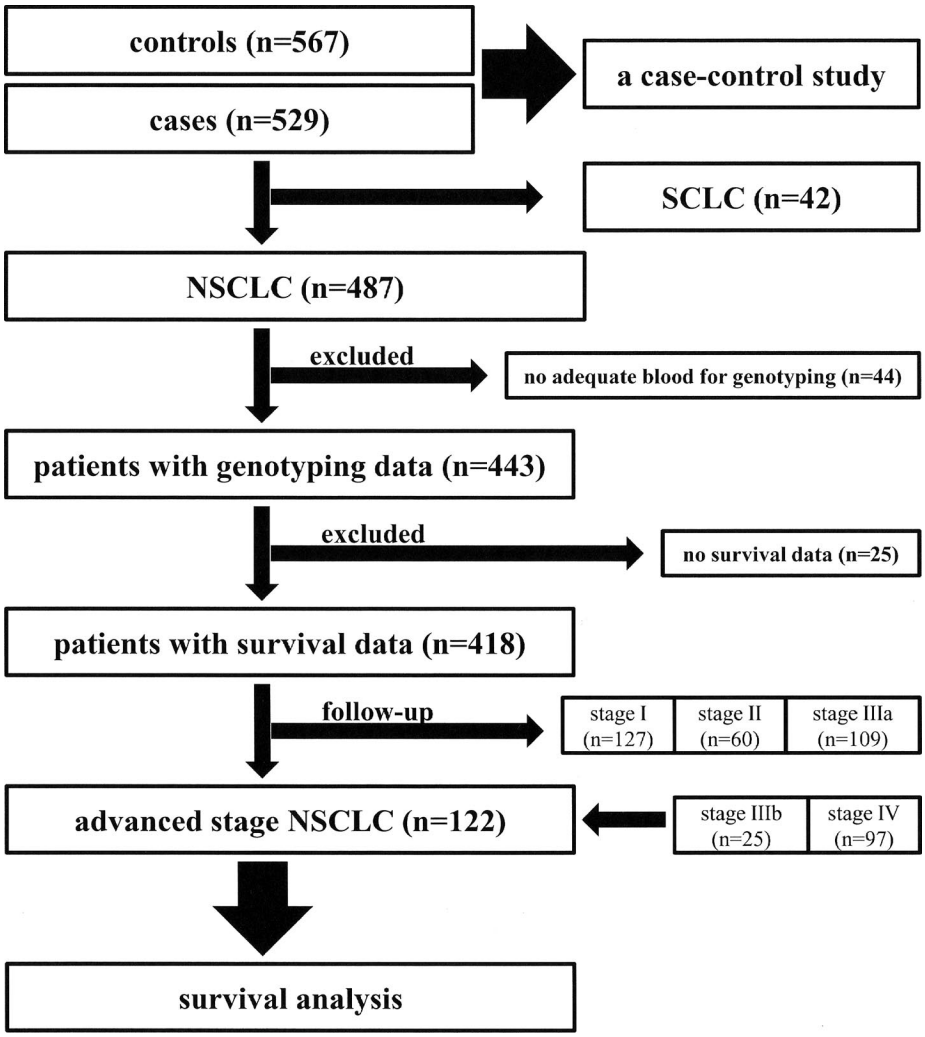

FIGURE 1. Patients distribution.

minimize the confounding effects from gender and smoking status, we performed the stratified analysis for each SNP on gender and smoking status individually. The strength of association of genotypes was further measured as ORs adjusted for additional parameters, including gender, age, and smoking status, using an unconditional multivariate logistic regression analysis, a second way to control for confounding effects. The associations between *CHRNA3* polymorphism status and survival were estimated using the method of Kaplan-Meier and assessed using the log-rank test. Cox regression models were used to adjust for potential confounders, with *CHRNA3* genotypes fitted as indicator variables. These statistical analyses were performed using SPSS 15.0 statistical software (SPSS Inc., Chicago, IL). A level of $p < 0.05$ for an odds ratio was considered significant.

RESULTS

Identification of a Lung Cancer Susceptibility Locus on 15q25

We conducted a case-control study consisting of 529 cases and 567 controls (Table 1). All the subjects were Chinese Han population. Most of the SQC and SCLC cases were male and smokers, whereas nearly 50% of ADC cases were smokers, as has been reported.[17] All the cases and controls were genotyped for the three SNPs (Table 2). All these SNPs were in Hardy-Weinberg equilibrium both in cases and controls ($p > 0.05$). One SNP, rs3743073 located in *CHRNA3* gene, showed significant allelic differentiations irrespective of histologic types, smoking status, and gender, whereas the other two SNPs, rs3743076 and rs3743078, did not show significant allelic differentiations (Table 2). Thus, it was indicated that SNP rs3743073 was associated with lung cancer risk in this population. To control the confounding effects further, adjusted ORs by age, gender, and smoking status for genotypes of rs3743073 were calculated (Table 3). Patients with genotypes TG or GG for the novel SNP rs3743073 in *CHRNA3* gene, compared with those with TT, showed an increased risk of lung cancer (adjusted odds ratio = 1.91; 95% confidence interval, 1.38–2.63; $p = 9.67 \times 10^{-5}$, Table 3). Meanwhile, ORs of heterozygotes and homozygotes for the reference genotype were consistently increased among populations irrespective of histologic types, smoking status, and gender (Table 3).

TABLE 1. Lung Cancer Cases and Controls Used for a Case-Control Study

		Cases			
Variable	**Control**	**All**	**ADC**	**SQC**	**SCLC**
Total	567	529	356	131	42
Age (mean ± SD, yr)	59 ± 9	58 ± 10	57 ± 10	61 ± 9	60 ± 10
Gender (%)	192 (33.9)	355 (67.1)	191 (53.7)	124 (94.7)	40 (95.2)
Male					
Female	375 (66.1)	174 (32.9)	165 (46.3)	7 (5.3)	2 (4.8)
Smoking status (%)	480 (84.7)	238 (45.0)	218 (61.2)	14 (10.7)	6 (14.3)
Nonsmokers					
Smokers	87 (15.3)	291 (55.0)	138 (38.8)	117 (89.3)	36 (85.7)
Pack-years (mean ± SD)[a]	27.9 ± 18.7	38.8 ± 22.5	32.8 ± 20.5	45.0 ± 20.5	41.9 ± 29.6
Cigarettes per day, CPD (mean ± SD)[a]	16.3 ± 8.7	23.9 ± 11.6	22.4 ± 11.8	25.5 ± 10.2	24.0 ± 14.3
Duration of smoking (mean ± SD, yr)[a]	34.3 ± 10.6	31.9 ± 10.4	28.7 ± 10.3	35.2 ± 8.8	33.6 ± 11.5

[a] Value for smokers.
ADC, adenocarcinoma; SQC, squamous cell carcinoma; SCLC, small cell lung cancer.

TABLE 2. Allele Differentiation between Controls and Cases

			Frequency (%)			
SNP	**Allele**	**Category**	**Control**	**Case**	**OR (95% CI)**	***p***
rs3743076	T	All	840 (74.6)	736 (72.7)	1.10 (0.91–1.34)	0.33
		ADC		487 (71.6)	1.16 (0.94–1.44)	0.16
		SQC		183 (72.6)	1.11 (0.81–1.51)	0.52
		SCLC		66 (82.5)	0.62 (0.35–1.13)	0.11
		Nonsmokers	705 (74.1)	326 (72.1)	1.10 (0.86–1.42)	0.44
		Smokers	135 (77.6)	410 (73.2)	1.27 (0.85–1.89)	0.25
		Male	283 (74.1)	496 (72.7)	1.07 (0.81–1.42)	0.63
		Female	557 (74.9)	240 (72.7)	1.12 (0.83–1.50)	0.46
rs3743078	G	All	271 (24.2)	240 (24.0)	1.01 (0.83–1.24)	0.90
		ADC		159 (23.6)	1.03 (0.83–1.29)	0.77
		SQC		60 (24.0)	1.01 (0.73–1.39)	0.95
		SCLC		21 (26.9)	0.87 (0.52–1.46)	0.59
		Nonsmokers	230 (24.3)	109 (24.3)	1.00 (0.78–1.30)	0.98
		Smokers	41 (23.8)	131 (23.6)	1.01 (0.68–1.51)	0.96
		Male	94 (24.7)	153 (22.8)	1.12 (0.83–1.50)	0.47
		Female	177 (23.9)	87 (26.4)	0.88 (0.65–1.18)	0.39
rs3743073	G	All	520 (45.9)	523 (54.3)	1.40 (1.18–1.66)	1.49×10^{-4}
		ADC		346 (53.7)	1.37 (1.13–1.66)	1.60×10^{-3}
		SQC		131 (54.1)	1.39 (1.05–1.84)	0.02
		SCLC		46 (59.0)	1.69 (1.06–2.70)	0.03
		Nonsmokers	449 (46.9)	230 (54.2)	1.34 (1.07–1.69)	0.01
		Smokers	71 (40.8)	293 (54.3)	1.72 (1.22–2.43)	2.03×10^{-3}
		Male	182 (47.4)	350 (53.7)	1.29 (1.00–1.66)	0.05
		Female	338 (45.2)	173 (55.4)	1.51 (1.16–1.97)	2.32×10^{-3}

Genotype data were missing for 27 subjects (27 of 1096, 2.46%) for rs3743076, 35 subjects (35 of 1096, 3.19%) for rs3743078, and 48 subjects (48 of 1096, 4.38%) for rs3743073.
CI, confidence interval; ADC, adenocarcinoma; SQC, squamous cell carcinoma; SCLC, small cell lung cancer.

We next examined linkage disequilibrium (LD) among these three SNPs located in one gene *CHRNA3*, and haplotypes were estimated. The LD structure (rs3743076/rs3743078/rs3743073) is shown in Figure 2. LD coefficients (D' and r^2) among the three variants (Figure 3) suggested that the degree of LD was relatively high. So, haplotypes were constructed with all three variants, and the frequencies of haplotypes were then analyzed by excluding the rare haplotypes (those <3% frequency in cases or controls). The result revealed a significant difference in the distribution of the global haplotypes between cases and controls ($p = 8.74 \times 10^{-12}$, Table 4). We also observed

TABLE 3. Genotype Differentiation for rs3743073 between Controls and Cases

SNP	Category	Genotype	No. of Controls (%)	No. of Cases (%)	Adjusted OR (95% CI)	Adjusted *p* Value
rs3743073	All[a]	TT	166 (29.3)	100 (20.7)	Reference	
		TG	280 (49.5)	241 (50.0)	1.79 (1.27–2.52)	0.001
		GG	120 (21.2)	141 (29.3)	2.16 (1.46–3.19)	1.20×10^{-4}
		TG + GG	400 (70.7)	382 (79.3)	1.91 (1.38–2.63)	9.67×10^{-5}
	ADC[a]	TT		70 (21.7)	Reference	
		TG		158 (49.1)	1.61 (1.12–2.31)	0.010
		GG		94 (29.2)	2.07 (1.38–3.13)	0.001
		TG + GG		252 (78.3)	1.75 (1.25–2.46)	0.001
	SQC[a]	TT		24 (19.8)	Reference	
		TG		63 (52.1)	2.32 (1.23–4.39)	0.010
		GG		34 (28.1)	2.31 (1.12–4.79)	0.024
		TG + GG		97 (80.2)	2.32 (1.27–4.22)	0.006
	SCLC[a]	TT		6 (15.4)	Reference	
		TG		20 (51.3)	2.99 (1.08–8.32)	0.036
		GG		13 (33.3)	3.63 (1.20–11.00)	0.023
		TG + GG		33 (84.6)	3.21 (1.22–8.48)	0.018
	Nonsmokers[b]	TT	133 (27.8)	38 (17.9)	Reference	
		TG	243 (50.7)	118 (55.7)	1.72 (1.12–2.64)	0.013
		GG	103 (21.5)	56 (26.4)	1.93 (1.18–3.14)	0.009
		TG + GG	346 (72.2)	174 (82.1)	1.78 (1.19–2.68)	0.005
	Smokers[b]	TT	33 (37.9)	62 (23.0)	Reference	
		TG	37 (42.5)	123 (45.6)	1.91 (1.07–3.39)	0.028
		GG	17 (19.5)	85 (31.5)	2.63 (1.33–5.17)	0.005
		TG + GG	54 (62.1)	208 (77.0)	2.15 (1.26–3.65)	0.005
	Male[c]	TT	58 (30.2)	73 (22.4)	Reference	
		TG	86 (44.8)	156 (47.9)	1.91 (1.18–3.11)	0.009
		GG	48 (25.0)	97 (29.8)	1.88 (1.10–3.24)	0.022
		TG + GG	134 (69.8)	253 (77.6)	1.90 (1.21–2.99)	0.005
	Female[c]	TT	108 (28.9)	27 (17.3)	Reference	
		TG	194 (51.9)	85 (54.5)	1.76 (1.07–2.88)	0.025
		GG	72 (19.3)	44 (28.2)	2.46 (1.40–4.33)	0.002
		TG + GG	266 (71.1)	129 (82.7)	1.95 (1.21–3.12)	0.006

[a] Adjusted for age, gender, and smoking status.
[b] Adjusted for age and gender.
[c] Adjusted for age and smoking status.
OR, odds ratio; CI, confidence interval; ADC, adenocarcinoma; SQC, squamous cell carcinoma; SCLC, small cell lung cancer.

that the TCT haplotype frequency was significantly lower in cases than in controls, whereas the TCG haplotype was significantly higher in cases than in controls (both $p < 0.0001$, Table 4). Subjects carrying the TCG haplotype had a 6.57-fold ($p = 5.19 \times 10^{-12}$, Table 4) increased lung cancer risk compared with the other haplotypes.

We further examined the association of the polymorphisms of rs3743073 with smoking status in lung cancer smokers. The values of cigarettes per day and pack-years were used as measures of smoking doses of subjects for this analysis because the values were commonly used in previous three GWA studies.[3–5] Values of cigarettes per day and pack-years of individuals with the risk allele of rs3743073 were higher than those without the risk allele in all cases and NSCLC group (ADC and SQC) and slightly lower in SCLC group; however, the difference did not reach a statistical significance ($p > 0.05$, Table 5).

Association of rs3743073 SNP with Advanced Stage NSCLC Prognosis

Patients characteristics are listed in Table 6. Given that the direction of effect was the same in the TG and GG genotypes of lung cancer susceptibility analysis, we combined the TG and GG genotypes for survival analysis. There were no significant differences by *CHRNA3* genotype status in patient-, tumor-, or treatment-related factors such as age, gender, smoking status, stage, histology, and chemotherapy status ($p > 0.05$, Table 6).

Univariate survival analysis demonstrated that OS was prolonged among the patients with the TT genotype compared with the TG/GG genotypes (log-rank $p = 0.037$, Figure 4). Cox multiple OS analysis showed that patients carrying the TG/GG genotype had a poorer survival than patients with the TT genotype (adjusted hazard ratio for death, 2.35; 95%

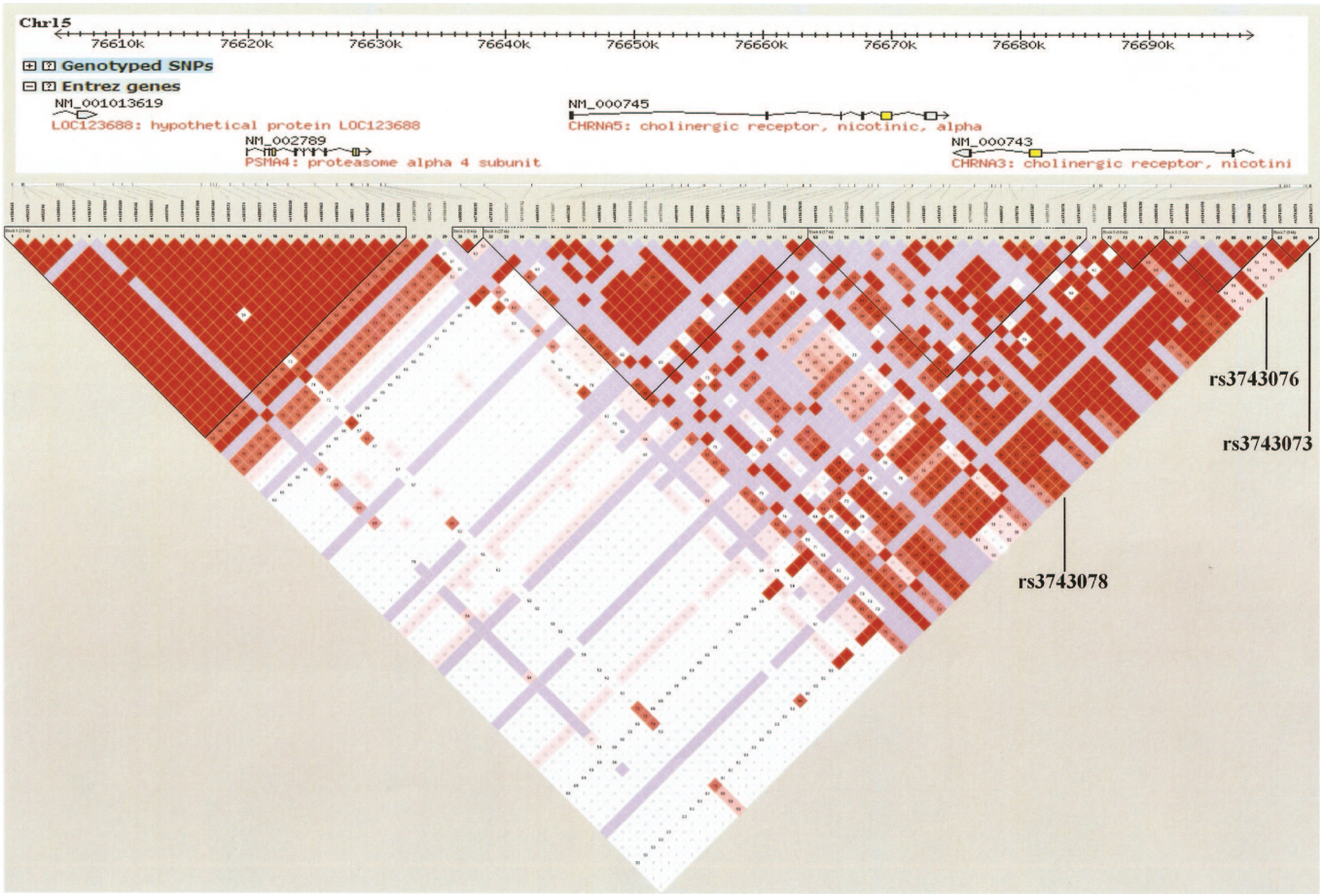

FIGURE 2. Linkage disequilibrium (LD) structure at 15q25 in individuals in CHB (Han Chinese in Beijing, China). Boxes are shaded according to *D′* derived in Haploview (v4.0).

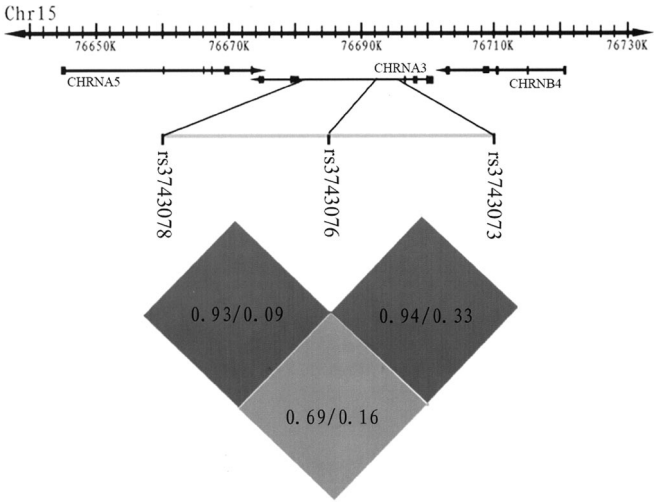

FIGURE 3. Linkage disequilibrium (LD) structure of the single nucleotide polymorphism (SNP) in CHRNA3 (D'/r^2 value). Pairwise *D′* values are color coded: high *D′* values are dark, low *D′* values are light (values were generated by SHEsis software[16]). The numbers shown at the top represent the degrees of the location of the region on chromosome 15 (Chr 15).

confidence interval, 1.05–5.26; $p = 0.04$, Table 7), with adjustment for important prognostic factors (age, gender, smoking status, stage, histology, and chemotherapy) between the two groups.

DISCUSSION

In this study, we examined whether the most significant SNPs on *CHRNA3* are also associated with risk of lung cancer in Chinese Han populations. For the fact that allele frequencies in Asians of reported risk SNPs, rs8034191, rs1051730, and rs16969968, were lower than that in Caucasians,[18] this study sought to identify other variants on *CHRNA3* associated with lung cancer susceptibility in Chinese. On the basis of analysis of 529 lung cancer cases and 567 controls derived from Chinese Han populations, we found that rs3743073G>T in the *CHRNA3* gene located on chromosome 15q25 were significantly associated with increased risk of lung cancer irrespective of histologic types, smoking status, and gender. In addition, we showed that carrying G allele of rs3743073 was associated with significantly worst survival among patients with advanced stage NSCLC. Together, our results support *CHRNA3* located on chromosome 15q25 as a susceptibility region for lung cancer

TABLE 4. Estimated Haplotype Frequencies and Association Significance

Haplotype[a]			*n* (%)				
rs3743076	rs3743078	rs3743073	Case	Control	χ^2	OR (95% CI)	*p*
A	C	G	249 (26.1)	272 (24.4)	1.07	1.11 (0.91–1.36)	0.30
T	C	G	66 (6.9)	13 (1.1)	47.69	6.57 (3.57–12.08)	5.19×10^{-12}
T	C	T	390 (41.0)	561 (50.3)	15.96	0.70 (0.59–0.83)	6.53×10^{-5}
T	G	G	202 (21.1)	216 (19.4)	1.28	1.13 (0.91–1.40)	0.26
T	G	T	30 (3.1)	47 (4.2)	1.57	0.74 (0.46–1.19)	0.21
Global					57.72		8.74×10^{-12}

[a] Those with frequency <0.03 in both control and case have been excluded.
OR, odds ratio; CI, confidence interval.

TABLE 5. Analyses between Genotypic Differentiation of rs3743073 with Cigarettes per Day and Pack-Years of Lung Cancer Smokers

	Genotype			*p* Value by Kruskal-Wallis Test
	TT	TG	GG	
No. of cigarettes/d (mean ± SD)				
All patients (*n* = 291)	22 ± 12	25 ± 11	24 ± 12	0.19
ADC (*n* = 138)	22 ± 13	23 ± 11	23 ± 11	0.67
SQC (*n* = 117)	22 ± 10	27 ± 11	26 ± 9	0.08
SCLC (*n* = 36)	28 ± 13	22 ± 10	24 ± 19	0.50
Pack-years (mean ± SD)				
All patients (*n* = 291)	34 ± 22	42 ± 22	39 ± 23	0.08
ADC (*n* = 138)	30 ± 19	36 ± 21	34 ± 21	0.41
SQC (*n* = 117)	38 ± 23	48 ± 20	47 ± 20	0.12
SCLC (*n* = 36)	50 ± 29	40 ± 26	37 ± 33	0.51

ADC, adenocarcinoma; SQC, squamous cell carcinoma; SCLC, small cell lung cancer.

TABLE 6. *CHRNA3* Genotype Distribution in 122 Advanced Stage NSCLC Patients

Characteristic	rs3743073 TT (*n* = 20)	rs3743073 TG + GG (*n* = 102)	*p*
Age, yr			0.40[a]
Median ± SD	54 ± 10	56 ± 11	
Range	34–67	22–78	
Gender			1.00[b]
Male	12 (60.0%)	62 (60.8%)	
Female	8 (40.0%)	40 (39.2%)	
Smoking status			0.81[b]
Smokers	10 (50.0%)	48 (47.1%)	
Nonsmokers	10 (50.0%)	54 (52.9%)	
IASLC recommended TNM new staging[c]			1.00[b]
Stage IIIb	4 (20.0%)	21 (20.6%)	
Stage IV	16 (80.0%)	81 (79.4%)	
Histology			0.73[b]
ADC	18 (90.0%)	86 (84.3%)	
SQC	2 (10.0%)	16 (15.7%)	
Platinum-based chemotherapy[d]			0.11[b]
DCR (RPs + SDs)	17 (85.0%)	60 (58.8%)	
PD	2 (10.0%)	24 (23.5%)	
Nonchemo	1 (5.0%)	18 (17.6%)	

[a] *P* values were calculated by Wilcoxon test.
[b] *P* values were calculated by Pearson χ^2 test.
[c] TNM denotes tumor-node-metastasis based on the International Association for the Study of Lung Cancer (IASLC) recommended TNM new staging.[14,15] Percentages may not total 100 because of rounding.
[d] The patients who received chemotherapy were defined as those who received first-line platinum-based chemotherapy for at least two cycles, whereas patients who did not receive chemotherapy were defined as those who had not received first-line platium-based chemotherapy. One hundred three of 122 (84.4%) patients with advanced-stage NSCLC receiving chemotherapy were assessable for efficacy evaluation.
DCR, disease control rate; PR, partial response; SD, stable disease; PD, progressive disease.

and indicated that risk allele G of rs3743073 may be as a worst prognostic indicator for patients with advanced stage NSCLC.

We consider these findings important because our data underscore the difference in genetic markers among different ethnic populations. Recently, a Japanese study reported that the three risk SNPs of rs8034191, rs1051730, and rs16969968, described in previous GWA studies in Caucasians,[3–5] were also associated with lung cancer risk in a Japanese population.[19] However, a recent Chinese study showed that these three risk SNPs were not significantly associated with lung cancer risk in Chinese populations.[12] Allele frequencies in Asians of rs8034191, rs1051730, and rs16969968 were lower than that in Caucasians.[18] For rs8034191, the MAFs were reported to be 0.42, 0.16, and 0.03 in whites, African Americans, and Chinese, respectively. Likewise, for rs1051730, the MAFs were 0.39, 0.15, and 0.04; for rs16969968, the MAFs were 0.39, 0.08, and 0.04, respectively, based on the HapMap data. The Japanese findings[19] inconsistent with that reported Chinese study[12] might also reflect the difference among different populations.

Effects of *CHRNA* SNPs according to histologic types of lung cancers were examined in a previous study[4] and were similar among ADC, SQC, and SCLC. In this study, the risk allele of rs3743073 also showed similarly and significantly increased ORs among different pathologic subtype (Tables 2 and 3), indicating that rs3743073 SNPs are associated with lung cancer risk irrespective of histologic types of cancer. In NSCLC and SCLC, the same nAchRα3-α5-α7-β4 subunits as mentioned for the human bronchial epithelium cells are expressed,[20,21] and NSCLC and SCLC cell lines can both

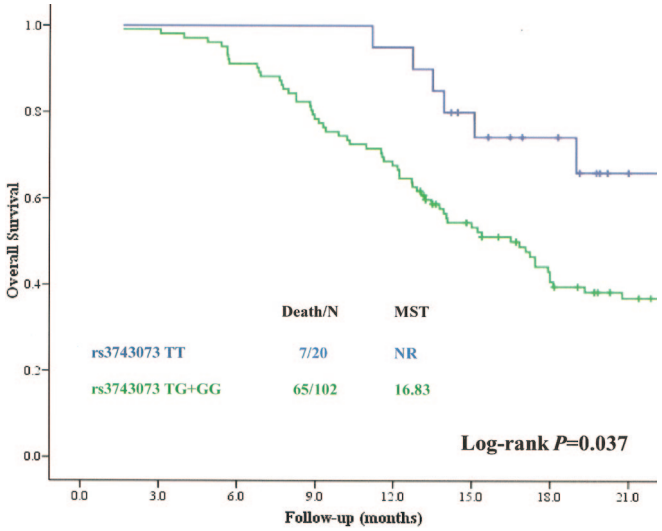

FIGURE 4. Kaplan-Meier survival curves about rs3743073 genotype in 122 patients with advanced stage non-small cell lung cancer (NSCLC). The curves of patients with CHRNA3 genotype TT (n = 20), TG + GG (n = 102) were shown as blue and green lines, respectively. Follow-up was shown truncated at 21 months. Log-rank analysis indicated significant differences between OS curves (p = 0.037). OS, overall survival; MST, median survival time; NR, not reach.

TABLE 7. Cox Multiple Analysis for Overall Survival in 122 Patients with Advanced Stage NSCLC

	OS		
Characteristic	**Adjusted HR**	**95% CI**	***p***
Age[a]	1.00	0.98–1.03	0.89
Gender			
Male	Reference		
Female	0.84	0.42–1.67	0.62
Smoking status			
Smokers	Reference		
Nonsmokers	0.57	0.30–1.12	0.10
IASLC recommended TNM new staging			
Stage IIIb	Reference		
Stage IV	1.93	1.04–3.58	0.04
Histology			
ADC	Reference		
SQC	1.87	0.96–3.63	0.06
Platinum-based chemotherapy			
DCR	Reference		
PD	0.83	0.47–1.46	0.51
Nonchemo	1.98	1.05–3.73	0.03
rs3743073			
TT	Reference		
TG + GG	2.35	1.05–5.26	0.04

[a] Age calculated as continuous variables.

OS, overall survival; HR, hazard ratio; ADC, adenocarcinoma; CI, confidence interval; SQC, squamous cell carcinoma; DCR, disease control rate; PD, progressive disease; IASLC, International Association for the Study of Lung Cancer; TNM, tumor, node, metastasis.

synthesize and release Ach.[22] Increased levels of Ach provide endogenous proliferative stimuli to nAchR, suggesting that an autocrine or a paracrine cholinergic pathway loop for nAchR in NSCLC and SCLC. The lung cancer cell proliferation, apoptosis, epithelial-mesenchymal transition, and pro-invasive and angiogenic effects are therefore reinforced by an autocrine or a paracrine loop.[8,9,23]

Studies investigating the *CHRNA* polymorphisms in relation to survival outcomes have been few. An Italian study reported that there is no significant association of the *CHRNA5* D398N polymorphism with the clinical stage of lung ADC cancer.[24] TNM staging is a prognostic factor for lung cancer; however, it does not account for survival differences within the same stage. Moreover, as the 13th World Conference on Lung Cancer was held in San Francisco from July 31, 2009, to August 4, 2009, a new TNM staging (7th edition) was published by the International Association for the Study of Lung Cancer because the previous TNM stage cannot predict OS accurately. Based on this TNM new staging project, we found worse survival among patients with advanced stage NSCLC carrying the variant G allele of rs3743073. The *CHRNA3* gene encodes different subunits of nicotinic acetylcholine receptors, which are members of a superfamily of ligand-gated ion channels that mediate rapid signal transmission at synapses. On one hand, nicotine after initiation may contribute to the progression phase and survival outcomes of cancer development[23]; on the other hand, it can cause resistance to gemcitabine, cisplatin, and paclitaxel in NSCLC by activation of Akt by DhβE-sensitive α3 nAChRs.[25] The interaction of nicotine with *CHRNA3* plays a key role in the worse survival and the reduced treatment efficacy by the nAChRs pathway. The expression of these receptors can be inhibited by nicotine receptor antagonists of α3 subunits, which implies possible chemoprevention opportunities for lung cancer.[26] Of interest will be whether nAChRs pathway polymorphisms can be used to predict benefit from nicotine receptor antagonists.

Association of *CHRNA* SNPs with lung cancer risk by dividing subjects into smokers and nonsmokers was examined in two GWA studies,[4,5] with values of cigarettes per day and pack-years investigated in three GWA studies.[3–5] In the Iceland, Spain, and The Netherland study, the individuals with one and two copies of the minor allele for the rs1051730 SNP were estimated to smoke approximately one and two more cigarette per day than those without.[3] Association with risk in smokers was commonly observed in other two GWA studies,[4,5] whereas association in nonsmoker was not.[5] In this study, the risk allele of rs3743073 showed similarly increased ORs in both smokers and nonsmokers (Tables 2 and 3), indicating that *CHRNA3* SNPs are associated with lung cancer risk irrespective of smoking, which was consistent with the study by Hung et al.[4] and the recent Japanese study.[19] In addition, no association of rs3743073 SNPs with cigarettes per day and pack-years in lung cancer cases was demonstrated in our data, consistent with the recent report about rs3743073 in European population.[13] Reasons underlying the racial difference in the genotype with smoking associations are unclear.[5] This discrepancy may be due to differences in

genetic and environmental backgrounds. Alternatively, other factors that have not been taken into account, such as food intake and passive smoking,[6,7] differentiate the mode of contribution of the *CHRNA* SNPs in nonsmokers. Because the results of these studies are inconsistent, further studies are still needed to draw a conclusion on this issue.

There are still several limitations to our study. First, because of the relative smaller number of SCLC cases in our group, a larger sample with sufficient histologic subtypes of lung cancer is needed to investigate for better understanding this issue among different histologic subtypes. In addition, a longer follow-up period is needed to investigate the *CHRNA* polymorphisms with lung cancer survival in all staging, especially in early stage. Second, the factors of gender and smoking status in the case and control groups were imbalanced. Considering this aspect, two methods were used in our analysis to control the imbalanced factors. (1) The stratified analysis on gender and smoking status were performed to ensure a balanced distribution of confounding factor, individually. (2) Unconditional multivariate logistic regression model, including gender, age, and smoking status, provided another way to control for confounders. Third, there is still debate regarding the true function of the polymorphisms. The candidate SNP in our study resides in the intron 3 of *CHRNA3* gene, 64 base pairs away from exon 4. It might function by altered binding of transcription factors and altered splicing of gene, or even through LD with truly functional sites, but so far unknown. Further functional experiments on this SNP are needed to explore the mechanism by which rs3743073 in *CHRNA3* affects lung cancer.

In conclusion, we identified a novel rs3743073 SNP in *CHRNA3* gene on chromosome 15q25 with lung cancer risk in Chinese Han populations, and the allele G of rs3743073 associated with an increased lung cancer risk was also associated with poor survival in patients with advanced stage NSCLC. Further studies are warranted to confirm these findings in different ethnic populations. We are planning to investigate the addition of nicotine receptor antagonists to the treatment of patients with lung cancer.

REFERENCES

1. Edwards BK, Brown ML, Wingo PA, et al. Annual report to the nation on the status of cancer, 1975–2002, featuring population-based trends in cancer treatment. *J Natl Cancer Inst* 2005;97:1407–1427.
2. Spitz MR, Wei Q, Dong Q, et al. Genetic susceptibility to lung cancer: the role of DNA damage and repair. *Cancer Epidemiol Biomarkers Prev* 2003;12:689–698.
3. Thorgeirsson TE, Geller F, Sulem P, et al. A variant associated with nicotine dependence, lung cancer and peripheral arterial disease. *Nature* 2008;452:638–642.
4. Hung RJ, Mckay JD, Gaborieau V, et al. A susceptibility locus for lung cancer maps to nicotinic acetylcholine receptor subunit genes on 15q25. *Nature* 2008;452:633–637.
5. Amos CI, Wu X, Broderick P, et al. Genome-wide association scan of tag SNPs identifies a susceptibility locus for lung cancer at 15q25.1. *Nat Genet* 2008;40:616–622.
6. Minna JD. Nicotine exposure and bronchial epithelial cell nicotinic acetylcholine receptor expression in the pathogenesis of lung cancer. *J Clin Invest* 2003;111:31–33.
7. Schuller HM. Nitrosamines as nicotinic receptor ligands. *Life Sci* 2007; 80:2274–2280.
8. West KA, Brognard J, Clark AS, et al. Rapid Akt activation by nicotine and a tobacco carcinogen modulates the phenotype of normal human airway epithelial cells. *J Clin Invest* 2003;111:81–90.
9. Dasgupta P, Chellappan SP. Nicotine-mediated cell proliferation and angiogenesis: new twists to an old story. *Cell Cycle* 2006;5:2324–2328.
10. Sardari Nia P, Weyler J, Colpaert C, et al. Prognostic value of smoking status in operated non-small cell lung cancer. *Lung Cancer* 2005;47: 351–359.
11. Tammemagi CM, Neslund-Dudas C, Simoff M, et al. Smoking and lung cancer survival: the role of comorbidity and treatment. *Chest* 2004;125: 27–37.
12. Wu C, Hu Z, Yu D, et al. Genetic variants on chromosome 15q25 associated with lung cancer risk in Chinese populations. *Cancer Res* 2009;69:5065–5072.
13. Saccone NL, Saccone SF, Hinrichs AL, et al. Multiple distinct risk loci for nicotine dependence identified by dense coverage of the complete family of nicotinic receptor subunit (CHRN) genes. *Am J Med Genet B Neuropsychiatr Genet* 2009;150B:453–466.
14. Goldstraw P, Crowley J, Chansky K, et al. The IASLC Lung Cancer Staging Project: proposals for the revision of the TNM stage groupings in the forthcoming (seventh) edition of the TNM Classification of malignant tumours. *J Thorac Oncol* 2007;2:706–714.
15. Goldstraw P. The 7th edition of TNM in lung cancer: what now? *J Thorac Oncol* 2009;4:671–673.
16. Shi YY, He L. SHEsis, a powerful software platform for analyses of linkage disequilibrium, haplotype construction, and genetic association at polymorphism loci. *Cell Res* 2005;15:97–98.
17. Yoshimi I, Ohshima A, Ajiki W, et al. A comparison of trends in the incidence rate of lung cancer by histological type in the Osaka Cancer Registry, Japan and in the Surveillance, Epidemiology and End Results Program, USA. *Jpn J Clin Oncol* 2003;33:98–104.
18. Schwartz AG, Cote ML, Wenzlaff AS, et al. Racial differences in the association between SNPs on 15q25.1, smoking behavior, and risk of non-small cell lung cancer. *J Thorac Oncol* In press.
19. Shiraishi K, Kohno T, Kunitoh H, et al. Contribution of nicotine acetylcholine receptor polymorphisms to lung cancer risk in a smoking-independent manner in the Japanese. *Carcinogenesis* 2009;30:65–70.
20. Chini B, Clementi F, Hukovic N, et al. Neuronal-type alpha-bungarotoxin receptors and the alpha 5-nicotinic receptor subunit gene are expressed in neuronal and nonneuronal human cell lines. *Proc Natl Acad Sci USA* 1992;89:1572–1576.
21. Tarroni P, Rubboli F, Chini B, et al. Neuronal-type nicotinic receptors in human neuroblastoma and small-cell lung carcinoma cell lines. *FEBS Lett* 1992;312:66–70.
22. Song P, Sekhon HS, Jia Y, et al. Acetylcholine is synthesized by and acts as an autocrine growth factor for small cell lung carcinoma. *Cancer Res* 2003;63:214–221.
23. Thunnissen FB. Acetylcholine receptor pathway and lung cancer. *J Thorac Oncol* 2009;4:943–946.
24. Falvella FS, Galvan A, Frullanti E, et al. Transcription deregulation at the 15q25 locus in association with lung adenocarcinoma risk. *Clin Cancer Res* 2009;15:1837–1842.
25. Dasgupta P, Kinkade R, Joshi B, et al. Nicotine inhibits apoptosis induced by chemotherapeutic drugs by up-regulating XIAP and survivin. *Proc Natl Acad Sci USA* 2006;103:6332–6337.
26. Russo P, Catassi A, Cesario A, et al. Development of novel therapeutic strategies for lung cancer: targeting the cholinergic system. *Curr Med Chem* 2006;13:3493–3512.