A Functional Polymorphism on Chromosome 15q25 Associated with Survival of Early Stage Non–Small-Cell Lung Cancer

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Introduction: The 15q25 region has been associated with lung-cancer risk and might also be associated with the prognosis of lung cancer. This study was conducted to determine the impact of a functional polymorphism in the CHRNA3 gene on chromosome 15q25 in the survival of patients with early-stage non–small-cell lung cancer (NSCLC).

Methods: Five hundred and eighty-three consecutive patients with surgically resected NSCLC were enrolled. The rs6495309C > T polymorphism in the promoter of the *CHRNA3* gene was investigated. The association between genotype and overall survival (OS) and disease-free survival (DFS) was analyzed.

Results: Patients with the rs6495309 CT or TT genotype had a significantly better OS and DFS than the rs6495309 CC genotype (adjusted hazard ratio for OS = 0.56, 95% confidence interval = 0.41–0.75, $p = 0.0001$; and adjusted hazard ratio for DFS = 0.61, 95% confidence interval = 0.48–0.79, $p = 0.0001$). An association between the rs6495309C > T polymorphism and survival outcome was demonstrated in smokers and never-smokers, and in squamous-cell carcinomas and adenocarcinomas.

Conclusion: The *CHRNA3* rs6495309C > T polymorphism may affect survival in patients with early-stage NSCLC. Analysis of the rs6495309C > T polymorphism can help identify patients at high risk of a poor disease outcome.

Key Words: CHRNA3, Polymorphism, Non–small-cell lung cancer, Survival.

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Single nucleotide polymorphisms (SNPs) are the most common form of human genetic variation. Several studies have demonstrated that some of these SNPs affect the expression or activities of enzymes; therefore, they are associated with the risk of cancer.[1–3] In addition, genetic polymorphisms are increasingly studied as potential prognostic factors in a variety of cancers including lung cancer.[4–7]

Recently, genome-wide association studies (GWASs) have identified hundreds of genetic variants influencing the risk of complex human diseases, including lung cancer.[8–10] In addition, these GWASs have highlighted the significance of understanding the diverse molecular pathways underlying specific human diseases.[9–11] The 15q25 region that contains the nicotine acetylcholine receptor α subunit 5 and 3 (*CHRNA5* and *CHRNA3*) genes and the nicotine acetylcholine receptor β subunit 4 (*CHRNB4*) gene has been identified as a lung-cancer susceptibility locus in GWASs conducted in populations of European ancestry.[12–14] This locus has also been shown to be associated with lung-cancer risk in several replication studies conducted in diverse populations.[15–17] In addition, there is a growing body of evidence that nicotinic acetylcholine receptors (nAChRs) are involved in the etiology and progression of lung cancer through nicotine dependence and activation of downstream signaling networks that promote cell proliferation, survival, migration, invasion, and angiogenesis.[18–20] Based on the biological significance of nAChRs in lung-cancer carcinogenesis, it is possible that functional genetic variants in the 15q25 region contribute to the prognosis of lung cancer.

Three SNPs (rs8034191, rs16969968, and rs1051730) that map to a region of strong linkage disequilibrium in the *CHRNA5-CHRNA3-CHRNB4* gene cluster have been reported to be strongly associated with the risk of lung cancer in Caucasian populations.[12–14] However, these three SNPs are extremely rare in Asian populations, according to the HapMap database. In addition, Wu et al.[15] have reported that these three SNPs are very rare (minor allele frequencies < 0.05) and are not associated with the risk of lung cancer in Chinese populations.[15] Interestingly, Wu et al.[15] reported that the rs6495309C > T polymorphism in the promoter of the *CHRNA3* gene affects the binding ability of the transcriptional factor, Oct-1, resulting in alteration of the *CHRNA3* expression.

In addition, Wu et al.[15] reported that individuals with higher production of the rs6495309C allele for CHRNA3 were at a significantly increased risk of lung cancer. Therefore, we evaluated the prognostic effect of the rs6495309C > T polymorphism in patients with surgically resected non–small-cell lung cancer (NSCLC).

PATIENTS AND METHODS

Patients

This study included all patients (n = 583) with stage I, II, or IIIA (micro-invasive N2) NSCLC, who underwent curative surgical resection at the Kyungpook National University Hospital (KNUH, Daegu, Korea) between December 1997 and October 2009. Tumor and corresponding nonmalignant lung-tissue specimens were provided by the National Biobank of Korea, KNUH, which is supported by the Ministry of Health, Welfare and Family Affairs. All materials derived from the National Biobank were obtained under Institutional Review Board approved protocols. All of the patients included in this study were ethnic Koreans. None of the patients included in this study received chemotherapy or radiotherapy before surgery. The histologic types of lung cancers were as follows: 285 cases (48.9%) were squamous-cell carcinomas (SQs); 283 cases (48.5%) were adenocarcinomas (ACs); and 15 cases (2.6%) were large-cell carcinomas. The pathologic staging of the tumors, which was determined according to the International System for Staging Lung Cancer,[21] was as follows: 317 patients (54.4%) had stage-I disease; 114 patients (19.6%) had stage-II disease; and 152 patients (26.1%) had stage-IIIA disease. This study was approved by the Institutional Review Board of the KNUH.

Genotype Determination

Genomic DNA was extracted from tissues via proteinase K digestion, followed by phenol/chloroform extraction. The genotype of rs6495309C > T polymorphism was determined by polymerase chain reaction and melting-curve analysis using fluorescence-labeled hybridization probes (LightCycler 480, Roche Diagnostics; Mannheim, Germany). For quality control, the genotype analysis was performed blind with respect to the subjects. In addition, approximately 10% of the samples were randomly selected for repeat genotyping by a different investigator, and the results were 100% concordant. Furthermore, to confirm the genotyping results, approximately 10% of the samples were randomly selected to be genotyped again by selected polymerase chain reaction-amplified DNA sequencing, and these results were also 100% concordant.

Statistical Analysis

Demographic and clinical information were compared across genotypes and stages using chi-square tests for categorical variables. The Hardy–Weinberg equilibrium was tested by comparing the observed and expected genotype frequencies using a goodness-of-fit chi-square test. The primary outcomes used for the present study were overall survival (OS) and disease-free survival (DFS). OS was measured from the day of surgery until the date of death or to the date of the last follow-up. DFS was calculated from the day of surgery until recurrence or death from any cause. The survival estimates were calculated using the Kaplan–Meier method. The differences in OS and DFS across different genotypes were compared using the log-rank test. Hazard ratios (HRs) and 95% confidence intervals (CIs) were estimated using multivariate Cox proportional hazard models, with adjustment for age (≤ 64 years versus > 64 years), sex (female versus male), smoking status (never-smokers versus ever-smoker), pathologic stage (I versus II-IIIA), and adjuvant therapy (yes versus no). A homogeneity test was performed to compare the difference between genotype-related HRs of the different groups. All analyses were performed using Statistical Analysis System for Windows, version 9.1 (SAS Institute, Cary, NC).

RESULTS

Patient Characteristics and Clinical Predictors

The clinical and pathologic characteristics of the patients and the association with OS and DFS are shown in Table 1. There were 198 deaths (34.0%), and the estimated 5-year OS and DFS for all of the patients was 55% (95% CI = 46%–61%) and 39% (95% CI = 37%–51%), respectively. The pathologic stage was significantly associated with the OS and DFS based on univariate analysis (log-rank p [$p_{L\text{-}R}$] < 0.0001 for OS and DFS). There was no significant difference in survival outcomes by age, sex, smoking status, histologic subtype, or adjuvant therapy.

Genotype Frequency and Association with Clinicopathological Factors

The *CHRNA3* rs6495309C > T genotypes were successfully obtained from all the patients enrolled, and the frequencies of the genotypes and alleles are listed in Table 2. The frequency of the rs6495309C allele among the patients was 0.551, which was comparable with the Chinese and Japanese lung-cancer cases (0.572 and 0.518).[15,22]. The frequency of the rs6495309C allele was significantly associated with smoking status (p_{trend} = 0.02); specially, the frequency of the rs6495309C allele was significantly higher in ever-smokers (current- and former-smokers) than in never-smokers (0.569 versus 0.503, p = 0.046). In addition, the rs6495309C allele was more frequent in men and SQs than in women and ACs, respectively (p = 0.02, and p = 0.01, respectively).

Effect of the rs6495309C > T Polymorphism on Survival Outcomes

The rs6495309C > T polymorphism was significantly associated with OS and DFS (under a dominant model for the variant T allele; $p_{L\text{-}R}$ for OS = 0.0004 and $p_{L\text{-}R}$ for DFS = 0.005; Table 3 and Fig. 1). Based on multivariate analysis, the rs6495309 CT or TT genotype exhibited a better OS and DFS than the rs6495309 CC genotype (adjusted HR [aHR] for OS = 0.56, 95% CI = 0.41–0.75, p = 0.0001; and aHR for DFS = 0.61, 95% CI = 0.48–0.79, p = 0.0001).

The association of the rs6495309C > T genotypes with the survival of the patients was further examined after categorizing

TABLE 1. Univariate Analysis for Overall Survival by Demographics, Smoking Status, Histological Type, and Pathologic Stage

		Overall Survival			Disease-Free Survival		
Variables	**No. of Cases**	**No. of Death (%)[a]**	**5Y-OSR (%)[b]**	**Log-Rank *P***	**No. of Event (%)[a]**	**5Y-DFSR (%)[b]**	**Log-Rank *P***
Overall	583	198 (34.0)	55		298 (51.1)	39	
Age (yrs)							
≤64	284	91 (32.0)	59	0.14	140 (49.3)	43	0.18
<64	299	107 (35.8)	51		158 (52.8)	36	
Sex							
Male	427	158 (37.0)	53	0.13	225 (52.7)	39	0.86
Female	156	40 (25.6)	63		73 (46.8)	38	
Smoking status							
Never	155	41 (26.5)	60	0.40	76 (49.0)	33	0.56
Former	133	46 (34.6)	50		73 (54.9)	32	
Current	295	111 (37.6)	54		149 (50.5)	44	
Pack-years[c]							
<20	45	11 (24.4)	65	0.29	20 (44.4)	47	0.85
20–< 35	140	55 (39.3)	47		73 (52.1)	38	
35–< 50	117	40 (34.2)	55		60 (51.3)	43	
≥50	126	51 (40.5)	55		69 (54.8)	42	
Histological type[d]							
Squamous-cell ca.	285	97 (34.0)	57	0.86	134 (47.0)	46	0.04
Adenoca.	283	95 (33.6)	52		154 (54.4)	32	
Pathologic stage							
I	317	79 (24.9)	63	< 0.0001	115 (36.3)	52	< 0.0001
II	114	48 (42.1)	48		74 (64.9)	28	
IIIA	152	71 (46.7)	45		109 (71.7)	22	
Adjuvant therapy[e]							
No	131	61 (46.6)	48	0.88	94 (71.8)	28	0.63
Yes	135	58 (43.0)	43		89 (65.9)	20	

[a] Row percentage.
[b] Five-year OSR and 5-year DFSR, proportion of survival derived from Kaplan-Meier analysis.
[c] In never-smokers.
[d] Fifteen large cell carcinomas were excluded from this analysis.
[e] In pathologic stage II-IIIA; 111 cases received chemotherapy, 5 cases received radiotherapy, and 19 cases received both chemotherapy and radiotherapy.
OSR, overall survival rate; DFSR, disease-free survival rate.

the patients by smoking status and tumor histology (Table 4). In ever-smokers, the rs6495309C >T genotype was significantly associated with OS and DFS (under a dominant model for the variant T allele; aHR for OS = 0.59, 95% CI = 0.42–0.82, $p = 0.002$; and aHR for DFS = 0.58, 95% CI = 0.44–0.77, $p = 0.0002$). In never-smokers, the rs6495309C > T genotype had a significant effect on OS under a dominant model for the T allele (aHR = 0.41, 95% CI = 0.20–0.81, $p = 0.01$). The effects of the rs6495309C > T genotype on OS and DFS were not significantly different between ever- and never-smokers (p value of test for homogeneity [p_H] for OS = 0.35 and p_H for DFS = 0.54). When categorized by tumor histology, the rs6495309 CT or TT genotypes exhibited a significantly better OS and DFS in patients with SQs and ACs compared with the rs6495309 CC genotype (in patients with SQs, p for OS and DFS = 0.002 and 0.02, respectively; and in patients with ACs, p for OS and DFS = 0.05 and 0.01, respectively; p_H for OS and DFS = 0.54 and 0.81, respectively).

Based on a multivariate survival analysis using a Cox's proportional hazard model, the rs6495309C > T genotype, tumor histology (HR for OS for AC/SQ = 1.41, 95% CI = 1.01–1.97, $p = 0.04$; HR for DFS for AC/SQ = 1.52, 95% CI =1.15–2.00, $p = 0.004$), and the pathologic stage (HR for OS for stage IIIA/II/I = 2.01, 95% CI = 1.43–2.81, $p < 0.0001$; and HR for DFS for stage IIIA/II/I = 2.81, 95% CI = 2.13–3.71, $p < 0.0001$) were independent prognostic factors for the survival of patients (Table 5).

DISCUSSION

Several GWASs have mapped a lung-cancer susceptibility locus to chromosome 15q25 containing the *CHRNA5, CHRNA3*, and *CHRNB4* genes. In the present study, we determined the prognostic effect of a functional SNP, rs6495309C > T, in the promoter of the *CHRNA3* gene in patients with surgically resected NSCLC. Our study demonstrated that the rs6495309 > T polymorphism was significantly associated with OS and DFS in the patients. In addition, multivariate logistic regression analysis revealed that the rs6495309C > T polymorphism was an independent prognostic factor after adjusting for clinicopathologic factors, including pathologic

TABLE 2. Genotype and Allele Frequencies of rs6495309C > T Polymorphism According to Clinicopathologic Factors

		Genotypes, n (%)				Allele frequency		
Variables	**No. of Cases**	**CC**	**CT**	**TT**	***P***	**C**	**T**	***P***
Age (yrs)								
≤64	284	82 (28.9)	153 (53.9)	49 (17.3)	0.80	0.558	0.442	0.66
>64	299	85 (28.4)	156 (52.2)	58 (19.4)		0.545	0.455	
Sex								
Male	427	129 (30.2)	230 (53.9)	68 (15.9)	0.04	0.571	0.429	0.02
Female	156	38 (24.4)	79 (50.6)	39 (25.0)		0.497	0.503	
Smoking status								
Never	155	39 (25.2)	78 (50.3)	38 (24.5)	0.14	0.503	0.497	0.08
Former	133	36 (27.1)	72 (54.1)	25 (18.8)		0.541	0.459	
Current	295	92 (31.2)	159 (53.9)	44 (14.9)		0.581	0.419	
p trend		0.16[a]	0.51[a]	0.01[a]		0.02[b]	0.02[b]	
Pack-years of smoking[c]								
<20	45	12 (26.7)	23 (51.1)	10 (22.2)	0.77	0.522	0.478	0.56
20–<35	140	42 (30.0)	79 (56.4)	19 (13.6)		0.582	0.418	
35–<50	117	39 (33.3)	61 (52.1)	17 (14.5)		0.594	0.406	
≥50	126	35 (27.8)	68 (54.0)	23 (18.3)		0.548	0.452	
Histological type[d]								
Squamous-cell ca.	285	95 (33.3)	148 (51.9)	42 (14.7)	0.02	0.593	0.407	0.01
Adenoca.	283	69 (24.4)	152 (53.7)	62 (21.9)		0.512	0.488	
Pathologic stage								
I	317	94 (29.7)	166 (52.4)	57 (18.0)	0.98	0.558	0.442	0.88
II	114	32 (28.1)	60 (52.6)	22 (19.3)		0.544	0.456	
IIIA	152	41 (27.0)	83 (54.6)	28 (18.4)		0.543	0.457	

[a] Cochran-Armitage trend test.
[b] Mantel–Haenszel linear trend test.
[c] In never-smokers.
[d] Fifteen large cell carcinoma cases were excluded for this analysis.

TABLE 3. Overall Survival and Disease-Free Survival According to the XHPNA3 rs6495309C>T Genotypes

		Overall Survival					Disease-Free Survival				
Polymorphism/ Genotype	**No. of Cases (%)[a]**	**No.of deaths (%)[b]**	**5Y-OSR (%)[c]**	**Log-rank *p***	**HR (95% CI)[d]**	***P*[d]**	**No. of events (%)[b]**	**5Y-DFR (%)[c]**	**Log-rank *p***	**HR (95% CI)[d]**	***P*[d]**
Rs6495309											
CC	167 (28.6)	70 (41.9)	51	0.002	1.00		93 (55.7)	34	0.007	1.00	
CT	309 (53.0)	93 (30.1)	57		0.53 (0.38–0.72)	<0.0001	147 (47.6)	44		0.57 (0.44–0.75)	<0.0001
TT	107 (18.4)	35 (32.7)	54		0.66 (0.44–1.00)	0.05	58 (54.2)	33		0.74 (0.53–1.04)	0.08
CC	167 (28.6)	70 (41.9)	51	0.0004	1.00		93 (55.7)	34	0.005	1.00	
CT + TT	416 (71.4)	128 (30.8)	56		0.56 (0.41–0.75)	0.0001	205 (49.3)	41		0.61 (0.48–0.79)	0.0001

[a]Column percentage.
[b]Row percentage.
[c]Five-year survival rate, proportion of survival derived from Kaplan-Meier analysis.
[d]HRs, 95% CIs and their corresponding *P* values were calculated using multivariate Cox proportional hazard models, adjusted for age, sex, smoking status, tumor histology, pathologic stage and adjuvant therapy.
5Y-OSR, 5-year overall survival rate; 5Y-DFSR, 5-year disease free survival rate; HR, hazard ratio; CI, confidence interval.

stage. This finding suggests that the chromosome 15q25 region affects prognosis of patients with lung cancer, and modulates susceptibility to lung cancer. Indeed, this is the first study to investigate whether or not a genetic variant in the 15q25 region has a prognostic effect on the survival outcome of patients with cancer.

In the present study, the *CHRNA3* rs6495309T allele was associated with a better survival outcome. This finding is biologically plausible, especially in light of the putative function of this SNP. It has been demonstrated that the rs6495309C allele has significantly higher promoter activity compared with the rs6495309T allele, resulting in up-regulation of the *CHRNA3* expression.[15] Therefore, it is reasonable to expect that patients with a lower-production T allele for CHRNA3 have a better survival outcome. In addition, this finding is consistent with the results of a previous study in which the *CHRNA3*

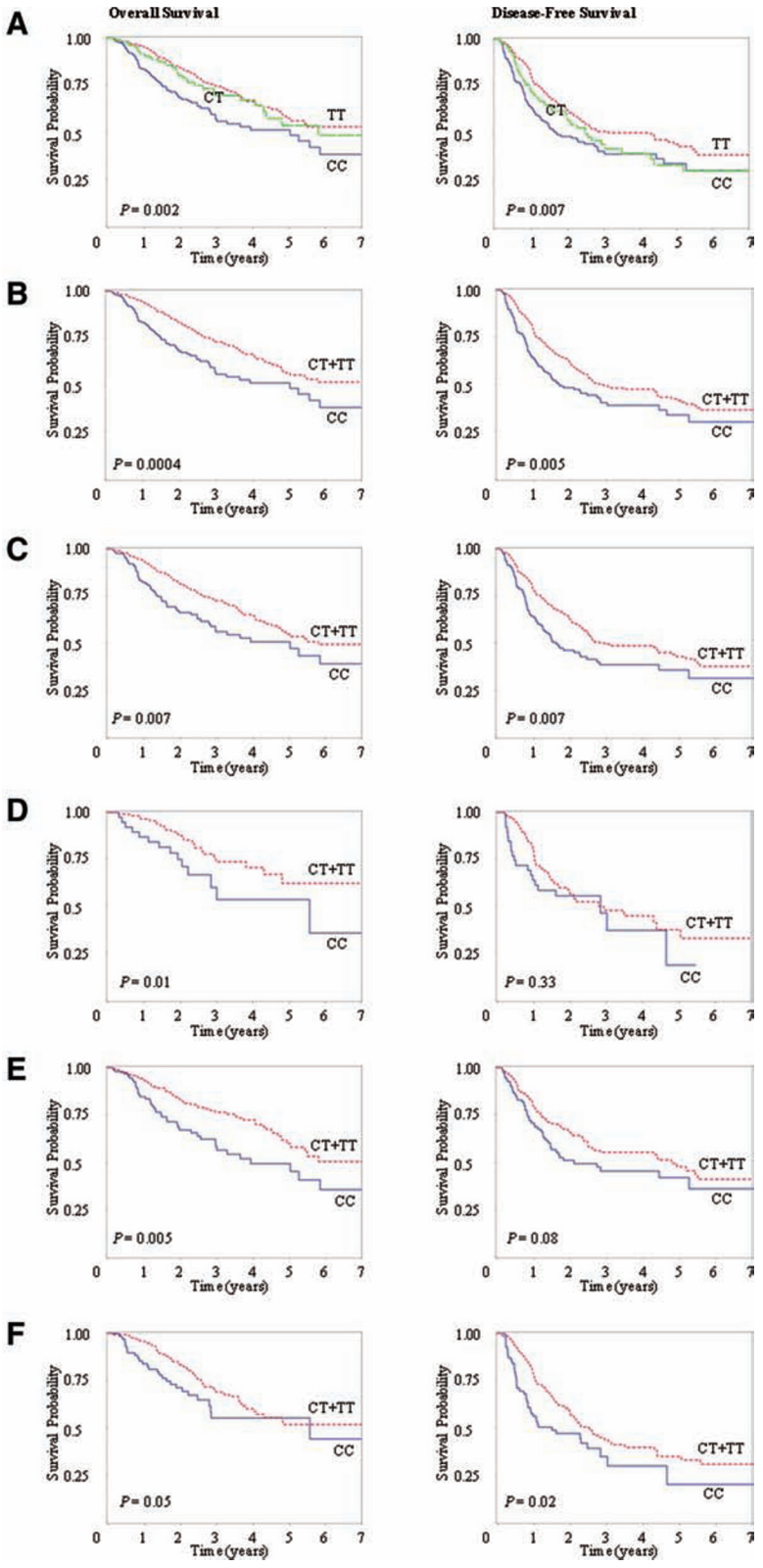

FIGURE 1. Kaplan–Meier plot of overall survival and disease-free survival by the CHRNA3 rs6495309C > T genotypes: under a referent model, in all patients (A); and under a dominant model for the rs6495309T allele: in all patients (B), in ever-smokers (C); in never-smokers (D); in squamous-cell carcinomas (E); and in adenocarcinomas (F). *P* values, from log-rank test.

TABLE 4. Overall Survival and Disease-Free Survival According to the *CHRNA3* Polymorphism Genotypes in Smoking Status, Histological Types, and Stage

		Overall Survival					Disease-Free Survival				
Polymorphism/ Genotype	No. of Cases (%)[a]	No. of Deaths (%)[b]	5Y-OSR (%)[c]	Log-rank *p*	HR (95% CI)[d]	*p*[d]	No. of Events (%)[b]	5Y-DFR (%)[c]	Log-rank *p*	HR (95% CI)[d]	*P*[d]
Smoking status											
Never											
CC	39 (25.2)	15 (38.5)	53	0.03	1.00		20 (51.3)	19	0.53	1.00	
CT	78 (50.3)	14 (18.0)	71		0.37 (0.17–0.79)	0.01	35 (44.9)	48		0.72 (0.40–1.29)	0.27
TT	38 (24.5)	12 (31.6)	50		0.47 (0.21–1.07)	0.07	21 (55.3)	23		0.65 (0.35–1.23)	0.19
CC	39 (25.2)	15 (38.5)	53	0.01	1.00		20 (51.3)	19	0.33	1.00	
CT+TT	116 (74.8)	26 (22.4)	62		0.41 (0.20–0.81)	0.01	56 (48.3)	38		0.70 (0.41–1.19)	0.19
Ever											
CC	128 (29.9)	55 (43.0)	51	0.03	1.00		73 (57.0)	36	0.01	1.00	
CT	231 (54.0)	79 (34.2)	54		0.57 (0.40–0.81)	0.002	112 (48.5)	44		0.53 (0.39–0.72)	<0.0001
TT	69 (16.1)	23 (33.3)	56		0.66 (0.40–1.08)	0.09	37 (53.6)	39		0.78 (0.52–1.16)	0.22
CC	128 (29.9)	55 (43.0)	51	0.007	1.00		73 (57.0)	36	0.007	1.00	
CT+TT	300 (70.1)	102 (34.0)	55		0.59 (0.42–0.82)	0.002	149 (49.7)	43		0.58 (0.44–0.77)	0.0002
Histological type											
Squamous-cell ca.											
CC	95 (33.3)	40 (42.1)	49	0.02	1.00		48 (50.5)	42	0.14	1.00	
CT	148 (51.9)	43 (29.1)	60		0.51 (0.33–0.79)	0.003	64 (43.2	48		0.63 (0.43–0.92)	0.02
TT	42 (14.7)	14 (33.3)	57		0.59 (0.32–1.10)	0.10	22 (52.4)	44		0.73 (0.43–1.23)	0.24
CC	95 (33.3)	40 (42.1)	49	0.005	1.00		48 (50.5)	42	0.08	1.00	
CT + TT	190 (66.7)	57 (30.0)	60		0.53 (0.35–0.80)	0.002	86 (45.3)	47		0.65 (0.46–0.94)	0.02
Adenoca.											
CC	69 (24.4)	28 (40.6)	55	0.14	1.00		42 (60.9)	20	0.05	1.00	
CT	152 (53.7)	47 (30.9)	52		0.60 (0.38–0.98)	0.04	78 (51.3)	39		0.57 (0.39–0.84)	0.005
TT	62 (21.9)	20 (32.3)	51		0.73 (0.40–1.32)	0.30	34 (54.8)	23		0.71 (0.45–1.13)	0.15
CC	69 (24.4)	28 (40.6)	55	0.05	1.00		42 (60.9)	20	0.02	1.00	
CT+TT	214 (75.6)	67 (31.3)	52		0.64 (0.41–1.00)	0.05	112 (52.3)	35		0.61 (0.43–0.88)	0.01

[a] Column percentage.
[b] Row percentage.
[c] Five-year survival rate, proportion of survival derived from Kaplan-Meier analysis.
[d] HRs, 95% CIs and their corresponding P-values were calculated using multivariate Cox proportional hazard models, adjusted for age, sex, smoking status, tumor histology, pathologic stage and adjuvant therapy. 5Y-OSR, 5-year overall survival rate; 5Y-DFSR, 5-year disease free survival rate; HR, hazard ratio; CI, confidence interval.

rs6495309C allele was associated with a significantly increased risk of lung cancer as compared with the rs6495309T allele.[15] Moreover, these observations suggest that a lower-production genotype for CHRNA3 not only decreases the risk of lung-cancer development, but also leads to good survival outcome in patients with surgically resected early-stage NSCLC.

It has been demonstrated that nAChRs can be triggered by nicotine and nicotine-derived nitrosamines in cigarette smoke, the most important causative carcinogens for lung AC.[23] Therefore, it can be assumed that genetic variants in the *CHRNA* genes may have a pronounced association with the prognosis of lung cancer in smokers, and AC in smokers. In contrast to this assumption, however, we observed that the rs6495309C > T polymorphism was associated with survival outcome in both never- and ever-smokers, and in SQs and ACs. In fact, this finding is supported by several reports that showed that genetic variants in the *CHRNA* genes affect lung-cancer risk independent of smoking status[13,15,20,22] and the histologic type of lung cancer.[13,17,20,22] Although the mechanism by which SNPs in *CHRNA* genes contribute to lung carcinogenesis in never-smokers remains to be elucidated, other factors, such as food intake and passive smoking, differentiate the mode of contribution of the CHRNA SNPs in never-smokers. A recent report pointed out the structural similarities between acetylcholine and the carcinogenic nitrosamine, *N*-nitrosodiethylamine (DEN), and identified DEN as a high-affinity ligand for nAChRs in lung-cancer cells.[24] Because DEN and similar nitrosamines are contained in numerous foods, beverages, and cosmetics,[24–26] this family of agents may contribute to nontobacco-related modulations of nAChRs.[20]

Several studies have reported that genetic variants in the *CHRNA5–CHRNA3* region on chromosome 15q25 are responsible for tobacco nicotine dependence.[14,15,19,27] In agreement with the results of a previous study,[15] the frequency of the rs6495309C allele was associated with smoking status, suggesting an impact of the rs6495309C > T polymorphism on nicotine dependence. However, different levels of exposure to smoking made no significant difference to genotype and allele frequencies.

Validation of genotype–phenotype association studies requires replication using an independent data set.[28] Although

TABLE 5. Multivariate Analyses of the Prognostic

	Overall Survival		Disease-Free Survival	
Variables	**HR (95% CI)**	***p***	**HR (95% CI)**	***p***
rs6495309 (CT + TT/CC)	0.56 (0.41–0.75)	0.0001	0.61 (0.48–0.79)	0.0001
Age (>64/≤64 yrs)	1.23 (0.93–1.64)	0.15	1.11 (0.88–1.41)	0.36
Sex (female/male)	0.80 (0.50–1.30)	0.37	0.80 (0.55–1.16)	0.24
Smoking status (never/ever)	1.14 (0.88–1.46)	0.32	0.93 (0.76–1.13)	0.45
Histology (AC/SQ)	1.41 (1.01–1.97)	0.04	1.52 (1.15–2.00)	0.004
Pathologic stage (II+IIIA/I)	2.01 (1.43–2.81)	<0.0001	2.81 (2.13–3.71)	<0.0001
Adjuvant therapy (Yes/No)	1.05 (0.73–1.51)	0.80	0.92 (0.69–1.24)	0.59

HR, hazard ratio; CI, confidence interval; SQ, squamous-cell carcinoma; AC, adenocarcinoma.

our finding of a significant association between the *CHRNA3* rs6495309C > T polymorphism and survival outcome was not examined in an independent sample, our finding is supported by several lines of evidence. This SNP has been reported to be associated with the risk of lung cancer.[15] Furthermore, our finding is biologically plausible in light of the putative function of the SNP. Therefore, it is unlikely that the association occurred by chance.

In conclusion, the *CHRNA3* rs6495309C > T SNP was shown to be an independent prognostic marker for patients with surgically resected NSCLC. Consequently, in addition to the pathologic stage, testing for the presence of the *CHRNA3* SNP may help identify patient subgroups at high risk for a poor disease outcome, thereby helping to refine therapeutic decisions in the treatment of NSCLC. However, because this study is the first study to investigate the association between *CHRNA* gene polymorphisms and survival outcomes in cancer patients, additional studies are required to confirm the findings in diverse ethnic populations.

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