Strong association between lung cancer and the *AXIN2* polymorphism

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Abstract. Accumulated evidence suggests that alterations due to mutations or genetic polymorphisms in the *AXIN2* tumor suppressor gene, a component of the Wnt signaling pathway, contributes to carcinogenesis. The effect of the *AXIN2* exon 1 148 C→T polymorphism was recently investigated in a Japanese population, but has not been investigated in other populations. Additionally, other common polymorphisms of this gene have not been studied. In the present study, 8 polymorphisms of the *AXIN2* gene, including exon 1 148 C→T, were investigated in a Turkish population of 100 lung cancer patients using PCR-RFLP methods. For the exon 1 432 C→T, exon 5 1365 G→A, exon 5 1386 C→T, intron 5 1712+19 G→T, exon 7 2062 C→T and intron 7 2141+73 G→A single nucleotide polymorphisms of *AXIN2*, no significant association was found between the controls and the lung cancer patients. For exon 1 148 C→T, a statistically significant association between the controls and lung cancer patients was found. For this region, lung cancer patients with the TT genotype showed a decreased risk [odds ratio (OR_{TT}) 0.33, 95% confidence interval (CI) 0.12-0.89; p=0.032 (adjusted for age, gender and smoking status)] as compared with the controls with the CC genotype. Concerning histological tumor type, it has been found that exon 1 148 C→T SNP is associated with a significant decreased risk in squamous cell carcinoma patients (OR_{TT} 0.16; 95% CI 0.03-0.79; p=0.014). Male (OR_{TT} 0.19; 95% CI 0.04-0.77; p=0.015) and smoker (OR_{TT} 0.11; 95% CI 0.01-0.71; p=0.019) lung cancer patients with the TT genotype showed a decreased risk for the same region. Our results suggest that the risk of lung cancer in a Turkish population is related to polymorphisms of the *AXIN2* gene.

Introduction

Lung cancer is one of the leading causes of cancer-related death in males and females worldwide (1,2). Epidemiologic studies show that approximately 80% of all lung cancer cases are attributable to cigarette smoking (3), while the combined effect of environment and genetic factors is responsible for 20% of cases. Among genetic factors, molecular abnormalities such as mutations and deletions have been identified in oncogenes and tumor suppressor genes, and loss of heterozygosity has been detected in a number of chromosomal regions (4-6). In addition, single nucleotide polymorphism (SNP) studies have been carried out in a large number of populations in order to show the susceptibility of an individual to lung cancer. These studies focused mainly on genes encoding xenobiotic metabolizing and DNA repair enzymes, including NAT1 and 2, cytochrome P450 dependent monooxygenases, glutathione S transferases, XRCC1 and XPD (7-9). The association between tumor suppressor gene SNPs and lung cancer risk has also been studied (10-12).

AXIN2, a negative regulator of Wnt/β-catenin signaling, acts as a tumor suppressor gene (13,14). The loss of heterozygosity in the genomic locus containing *AXIN2* (15-17) and mutations in the gene itself have been observed in certain types of cancer, including hepatocellular (18), ovarian (19) and colorectal carcinomas (20) and medullablastomas (21), suggesting that this gene plays a role in carcinogenesis. Polymorphisms in the *AXIN2* gene, including exon 1 P50S, have recently been shown to be associated with lung (22) and breast (23) cancers, suggesting they play a role in lung cancer susceptibility.

To our knowledge, there have been no prior studies on the association between *AXIN2* SNPs and lung cancer susceptibility in a Turkish population. We therefore genotyped 200 Turkish individuals (100 controls and 100 lung cancer patients) for 8 SNPs of *AXIN2* in order to investigate their association with susceptibility to lung cancer. The SNPs included the exon 1 148 C→T (Pro50Ser) variant, exon 1 432 T→C, intron 2 956+16 A→G, exon 5 1365 G→A, exon 5 1386 C→T, intron 5 1712+19 G→T, exon 7 2062 C→T and intron 7 2141+73 G→A.

Materials and methods

Subjects. The study population consisted of 100 lung cancer patients admitted to the Respiratory Disease Department at Cumhuriyet University Hospital in Sivas (Central Anatolia) in 2006. Only patients with newly diagnosed lung cancer and no previous diagnosis of cancer or previous radiotherapy or chemotherapy were included in the study. There were no gender, age, histologic type or stage restrictions. The diagnosis of

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Table I. Conditions for the identification of *AXIN2* polymorphisms.

Polymorphism	Marker	Protein effect	Location	Primer sequence (5'-3') (forward and reverse)	Product (bp)	RFLP	Allele (bp)
148 C/T	rs2240308	Pro50Ser	exon 1	F: CCACGCCGATTGCTGAGAGG R: TTCCGCCTGGTGTTGGAAGAGACA**T**	242	*Mph1103I*	C (218, 24) T (242)
432 T/C	rs2240308	Ile144Ile	exon 1	F: CCTGGAGAGGGAGAAATGC R: CATCACCGACTGGATCTCG	253	*Esp3I*	T (253) C (145, 108)
956+16 A/G	-	-	intron 2	F: GGGTTTCTGGTGAGGGTCAGT R: CAAGCAAGCCCACGGAAGG	228	*MlsI*	A (205, 23) G (228)
1365 G/A	rs9915936	Pro455Pro	exon 5	F: ACGTCTTCCCTTTCAGGATG R: GGTACTGCGAATGGTGGTG	248	*MspI*	G (100, 80, 36, 32) A (116, 100, 32)
1386 C/T	rs1133683	Pro462Pro	exon 5	F: TGCGTAGGGAGCCGAATGTTG R: GTGGTCCGGGGAGCGGGA**T**C	294	*TaqI*	C (294) T (274, 20)
1712+19 G/T	-	-	intron 5	F: CACCACCACTACATCCACCAC R: GCTCCCACCTCACACCTG	249	*CpoI*	G (184, 65) T (249)
2062 C/T	-	Leu688Leu	exon 7	F: TCCTTCTGTTTTCTCTCTGCTCATTCC R: AGCGTGTTGGGTGGGGTC**G**	209	*TaqI*	C (209) T (191, 18)
2141+73 G/A	rs4072245	-	intron 7	F: GTGTCGAAGCCCCCAAAG R: CTTGATCCTCCATCTCACAGC	370	*BseRI*	G (370) A (260, 110)

lung cancer was histologically confirmed. The control group consisted of 100 healthy individuals. All cases and controls were born in Turkey of native Turkish parents, and were interviewed by means of a questionnaire including questions about family history of cancer, smoking and other lifestyle habits, and lifetime occupational history. The study was approved by the local university ethics committee on human research.

Genotyping. Prior to the initiation of chemotherapy or radiotherapy, 2 ml of blood was collected per sample. DNA extraction was performed as soon as the blood samples reached the laboratory using a genomic DNA isolation kit (MBI Fermentas, Lithuania) according to manufacturer's instructions. The samples were assayed without knowledge of the case-control status.

A total of 8 regions including 5 exons and 3 introns of the *AXIN2* gene were amplified by PCR over 30 cycles (Table I) using previously reported primers (24). PCR was performed in a reaction volume of 25 μl containing 100 ng of genomic DNA, 10 pmol of the appropriate amplification primers, 5 nmol each of 4 deoxynucleotide triphosphates (Fermentas), 2.5 Units of Taq DNA polymerase (Fermentas), 10 mmol/l Tris-HCl (pH 8.3 at 25˚C), 50 mmol/l KCl and 1.5 mmol/l $MgCl_2$. PCR conditions consisted of an initial denaturation at 94˚C for 5 min followed by 30 cycles of 94˚C for 30 sec, 60˚C for 30 sec and 72˚C for 60 sec, and lastly 1 cycle at 72˚C for 5 min.

In order to detect the 8 *AXIN2* gene SNPs, PCR products were digested with 5 Units of appropriate restriction endonuclease enzymes (Fermentas) (Table I) in a total reaction volume of 10 μl containing 1X reaction buffer (supplied with the enzyme) for 4 h at optimum temperature according to the manufacturer's instructions. Amplified and digested DNA fragment sizes are shown in Table I. Following digestion, DNA fragments were seperated on 2.5% agarose gels.

Statistical analysis. Statistical analysis was performed using the Statistical Package for Social Sciences Program (SPSS, version 16). Statistically significant departures from Hardy-Weinberg equilibrium for controls were assessed using the χ^2 test. For each polymorphism, unconditional logistic regression was used to calculate the odds ration (OR) and 95% confidence interval (CI) for the lung cancer and tumor types, adjusted for the study matching factors of age, gender and smoking habits. The relationship between genotypes and clinopathological charactersitics was examined by the χ^2 test. A p-value <0.05 was considered to be statistically significant.

Results

A total of 200 individuals (100 lung cancer patients and 100 healthy controls) were genotyped for 8 different SNPs, including exon 1 148 C→T of the *AXIN2* gene. The results of SNP analysis in the control population did not deviate from Hardy-Weinberg equilibrium. The distribution of demographic characteristics in the genotyped cancer cases as similar to the demographic distribution of the controls. The principal characteristics of the study population are presented in Table II. The mean ages of patients and controls were 59.22±9.63 and 57.01±7.89 years, respectively. Relative to the controls, there were proportionally more males among the lung cancer patients (56%). The proportion of smokers was higher among patients (40%) than among controls (30%).

Table III summarizes the main effects of the 8 SNPs of *AXIN2* that are involved in lung cancer. Genotype analysis carried out on exons and the exon-intron boundaries of *AXIN2* in lung cancer patients indicated that there was no significant association between genotype and risk of lung cancer for the following SNPs: exon 1 432 T→C, intron 2 956+16 A→G, exon 5 1365 G→A, exon 5 1386 C→T, intron 5 1712+19 G→T, exon 7

Table II. Characteristics of lung cancer patients and healthy controls.

	Patients (n=100) n (%)	Controls (n=100) n (%)	p-value[a]
Gender			0.157
Male	56 (56)	46 (46)	
Female	44 (44)	54 (54)	
Age (years ± SD)	59.22±9.63	57.01±7.89	
Smoking habit			0.138
Non-smoker	60 (60)	70 (70)	
Smoker	40 (40)	30 (30)	

[a]P-values were calculated using the χ^2 test.

2062 C→T and intron 7 2141+73 G→A. However, individuals carrying the 148 TT (Ser/Ser) genotype were found to have a decreased risk of lung cancer ($p<0.05$). For this region of *AXIN2*, the frequencies of the Pro50Pro (CC), Pro50Ser (C→T) and Ser50Ser (TT) genotypes were 32, 52 and 16% in the controls, respectively, and 45, 47 and 8% in the lung cancer patients, respectively. The calculated values for the 148 C→T polymorphism were OR_{TT} 0.35, 95% CI 0.13-0.93 and p=0.032. When age, gender and smoking status were included in the analysis, the estimated OR was found to be 0.33 and the 95% CI was 0.12-0.89.

The the χ^2 test was used to evaluate the association between the *AXIN2* polymorphisms and smoking habit, gender and histopatholgic type of lung cancer (Table IV). The most prevalent type of tumors were squamous cell carcinoma (46%) and small cell carcinoma (27%). Others included lung adenocarcinoma, and large cell and adenosquamous carcinoma. With the exception of the exon 1 148 C→T SNP, none of the remaining 7 *AXIN2* SNPs exhibited statistically significant results. For the 148 C→T SNP, squamous cell carcinoma patients (OR_{TT} 0.16, 95% CI 0.03-0.79; p=0.014), males (OR_{TT} 0.19, 95% CI 0.04-0.077; p=0.015) and smokers (OR_{TT} 0.11, 95% CI 0.01-0.71; p=0.019) with the TT genotype were fiound to exhibit a statistically significant decreased risk of lung cancer.

For the intron 2 956+16 A→G SNP, the p-value was found to be 0.044 for small cell carcinoma (OR_{AG} 0.28, 95% CI 0.80-1.02). However, as it was close to 0.05 it did not reach statistical significance, since the OR value was in the range of 95% CI.

Discussion

Molecular epidemiology studies indicate that genetic polymorphisms and mutations of xenobiotic metabolizing enzymes and DNA repair genes as well as tumor suppressor genes and oncogenes are among the risk factors for lung cancer (25). Polymorphisms of xenobiotic enzymes have been demonstrated to be a major risk factor for lung cancer by several case-control studies; however, the results of these studies are inconsistent (25). Several lines of evidence indicate that events that alter the tumor suppressor pathways affect lung and other types of cancer (26-31). These events include polymorphisms of *AXIN2*, which play an important role in Wnt/β-catenin signaling and function as a scaffolding protein for a protein complex including *AXIN2*, adenomatous polyposis coli (APC), glycogen synthase kinase 3 (GSK3) and β-catenin. This complex is required for the phosphorylation of β-catenin (32,33). In this complex is localized human chromosome 17q21-25, a region highly susceptible to allelic losses and chromosomal re-arrangements involved in several types of cancer (24,34,35). A recent study by Kanzaki *et al* in a Japanese population showed that the *AXIN2* SNP at codon 50 is associated with lung cancer (22). In another study, 5 *AXIN* SNPs were investigated in breast cancer patients and found to be associated with the disease (23). In this study, the effect of the *AXIN2* SNP at codon 50 as well as 7 other significant SNPs of *AXIN2* were investigated in a Turkish population in order to further analyse their role in lung cancer susceptibility.

The study comprised 100 lung cancer patients and 100 hospital-based controls. Although the frequency of males and smokers was higher among the lung cancer patients, there was no significant relationship between lung cancer and these factors. No association was observed between lung cancer and the 7 following SNPs of *AXIN2*: exon 1 432 T→C, intron 2 956+16 A→G, exon 5 1365 G→A, exon 5 1386 C→T, intron 5 1712+19 G→T, exon 7 2062 C→T and intron 7 2141+73 G→A.

In the study by Kanzaki *et al*, the SNP at codon 50 of the *AXIN2* gene encoding either proline (CCT) or serine (TCT) was found to be associated with lung cancer, but not with colorectal and head and neck cancers (22). The authors found that patients with the TT genotype showed a reduced risk of cancer (OR_{TT} 0.31, 95% CI 0.12-0.79). The results of the present study also indicate a decreased risk for lung cancer patients with the TT genotype (OR_{TT} 0.35, 95% CI 0.13-0.93). The CC, CT and TT allele frequencies were 45, 47 and 8% in lung cancer patients and 32, 52 and 16% in controls, respectively. These results are similar to those of Kanzaki *et al*, who found a CC, CT and TT genotype distribution of 50, 44 and 8% in lung cancer patients and 42, 52 and 15% in controls, respectively. The 17q21-25 region of *AXIN2* is very close to the functional motif where APC binds. *AXIN2* binding site disruption or mutations in APC result in human cancer (36). It is possible that this SNP of *AXIN2* changes the binding efficiency of APC, though this requires further investigation.

No prior study has examined lung cancer risk associated with 7 of the *AXIN2* polymorphic sites investigated in the present study; however, the intron 2 956+16 A→G and exon 7 2062 C→T polymorphisms were found to be associated with tooth agenesis (24). It has been proposed that this variant of *AXIN2* may exert a weak effect on splicing, since it creates an additional donor splicing site within the sequence of exon 2. This was not observed in the present study. Additionally, the SNP at exon 5 1365 G→A of the *AXIN2* gene has been reported to be A→G (dbSNP ID: rs9915936), but we did not identify any AA genotypes in our control and study groups (24). The GG genotype was therefore selected as the wild-type genotype for statistical testing.

This is the first study carried out in a Turkish population investigating the *AXIN2* polymorphism and its association with lung cancer. The data indicate that there is a significant association between *AXIN2* SNPs and lung cancer in this population. Further investigation in different populations with large case numbers is needed to support these results.

Table III. *AXIN2* genotypes in healthy controls and lung cancer patients.

AXIN2 genotype	Patients n (%)	Controls n (%)	p-value[a]	OR (95% CI)	
				Crude	Adjusted[b]
Exon 1 148 C/T					
CC	45 (45.0)	32 (32.0)	1	1 (Reference)	1 (Reference)
CT	47 (47.0)	52 (52.0)	0.148	0.64 (0.35-1.17)	0.62 (0.33-1.16)
TT	8 (8.0)	16 (16.0)	0.032	0.35 (0.13-0.93)	0.33 (0.12-0.89)
Allele frequencies					
C	137 (61.5)	116 (58.0)			
T	63 (38.5)	84 (42.0)			
Exon 1 432 T/C					
TT	96 (96.0)	95 (95.0)	1	1 (Reference)	1 (Reference)
TC	4 (4.0)	5 (5.0)	1.0	1.0 (0.24-4.11)	1.2 (0.28-5.12)
CC	0	0			
Allele frequencies					
T	196 (98.0)	195 (97.5)			
C	4 (2.0)	5 (2.5)			
Intron 2 956+16 A/G					
AA	77 (77.0)	64 (64.0)	1	1 (Reference)	1 (Reference)
AG	20 (20.0)	28 (28.0)	0.121	0.59 (0.30-1.15)	0.57 (0.29-1.13)
GG	3 (3.0)	8 (8.0)	0.08	0.31 (0.07-1.22)	0.30 (0.07-1.22)
Allele frequencies					
A	174 (87.0)	156 (78.0)			
G	26 (13.0)	44 (22.0)			
Exon 5 1365 G/A					
GG	91 (91.0)	88 (88.0)	1	1 (Reference)	1 (Reference)
GA	9 (9.0)	12 (12.0)	0.489	0.72 (0.29-1.80)	0.73 (0.29-1.86)
AA	0	0			
Allele frequencies					
G	191 (95.5)	188 (94.0)			
A	9 (4.5)	12 (6.0)			
Exon 5 1386 C/T					
CC	52 (52.0)	42 (42.0)	1	1 (Reference)	1 (Reference)
CT	38 (38.0)	50 (50.0)	0.102	0.61 (0.34-1.10)	0.57 (0.31-1.05)
TT	10 (10.0)	8 (8.0)	0.985	1.01 (0.36-2.78)	0.80 (0.27-2.30)
Allele frequencies					
C	142 (71.0)	134 (67.0)			
T	58 (29.0)	66 (33.0)			
Intron 5 1712+19 G/T					
TT	97 (97.0)	96 (96.0)	1	1 (Reference)	1 (Reference)
GT	3 (3.0)	4 (4.0)	0.352	1.80 (0.51-6.37)	1.98 (0.55-7.12)
GG	0	0			
Allele frequencies					
T	193 (96.5)	196 (98.0)			
G	7 (3.5)	4 (2.0)			
Exon 7 2062 C/T					
CC	91 (91.0)	86 (86.0)	1	1 (Reference)	1 (Reference)
CT	9 (9.0)	14 (14.0)	0.268	0.60 (0.25-1.47)	0.61 (0.24-1.52)
TT	0	0			
Allele frequencies					
C	191 (95.5)	186 (93.0)			
T	9 (4.5)	14 (7.0)			
Intron 7 2141+73 G/A					
GG	73 (73.0)	80 (80.0)	1	1 (Reference)	1 (Reference)
GA	27 (27.0)	20 (20.0)	0.243	1.47 (0.76-2.86)	1.63 (0.82-3.21)
AA	0	0			
Allele frequencies					
G	173 (86.5)	180 (90.0)			
A	27 (13.5)	20 (10.0)			

[a]p-values were calculated using the χ^2 test. [b]Adjusted for age, gender and smoking status.

Table IV. Association between the *AXIN2* genotype and the clinicopathological characteristics of the lung cancer patiens.

Region	Genotype			p-value	Odds ratio (95% CI)	P-value	Odds ratio (95% CI)
Exon 1 148 C/T	CC	CT	TT		CT		TT
Controls	32	52	16				
Patients	45	47	8	0.148	0.64 (0.35-1.17)	0.032	0.35 (0.13-0.93)
Male	25	27	4	0.082	0.45 (0.18-1.11)	0.015	0.19 (0.04-0.77)
Female	20	20	4	0.620	0.80 (0.34-1.88)	0.463	0.60 (0.15-2.36)
Non-smoker	28	26	6	0.243	0.64 (0.30-1.34)	0.380	0.59 (0.18-1.90)
Smoker	17	21	2	0.268	0.54 (0.18-1.61)	0.019	0.11 (0.01-0.71)
SQC	24	20	2	0.075	0.51 (0.24-1.07)	0.014	0.16 (0.03-0.79)
SCC	10	13	4	0.639	0.80 (0.31-2.03)	1.000	0.80 (0.21-2.95)
Other	11	14	2	0.596	0.78 (0.31-1.93)	0.310	0.36 (0.07-1.84)
Exon 1 432 T/C	TT	TC			TC		
Controls	96	4					
Patients	96	4		0.640	1.00 (0.24-4.11)		
Male	55	1		1.000	0.81 (0.05-13.4)		
Female	41	3		1.000	1.20 (0.23-6.49)		
Non-smoker	56	4		0.703	1.59 (0.34-7.42)		
Smoker	40	0		0.429	0.96 (0.90-1.03)		
SQC	44	2		1.000	1.09 (0.20-6.18)		
SCC	26	1		1.000	0.92 (0.10-8.61)		
Other	26	1		1.000	0.92 (0.09-8.61)		
Intron 2 956+16 A/G	AA	AG	GG		AG		GG
Controls	64	28	8				
Patients	77	20	3	0.121	0.59 (0.30-1.15)	0.080	0.31 (0.79-1.22)
Male	43	11	2	0.196	0.54 (0.21-1.37)	1.000	0.69 (0.10-5.23)
Female	34	9	1	0.367	0.64 (0.24-1.68)	0.113	0.16 (0.02-1.45)
Non-smoker	47	12	1	0.055	0.45 (0.20-1.02)	0.059	0.14 (0.01-1.25)
Smoker	30	8	2	0.747	1.20 (0.35-4.24)	1.000	0.76 (0.10-5.86)
SQC	31	13	2	0.916	0.95 (0.43-2.10)	0.500	0.51 (0.10-2.57)
SCC	24	3	0	0.044	0.28 (0.80-1.02)	0.195	0.88 (0.82-0.96)
Other	22	4	1	0.127	0.41 (0.13-1.31)	0.447	0.36 (0.04-3.07)
Exon 5 1365 G/A	GG	GA			GA		
Controls	88	12					
Patients	91	9		0.489	0.72 (0.29-1.80)		
Male	52	4		0.216	0.42 (0.11-1.56)		
Female	39	5		0.750	1.20 (0.33-4.65)		
Non-smoker	53	7		0.837	0.89 (0.31-2.56)		
Smoker	38	2		0.645	0.47 (0.07-3.03)		
SQC	43	3		0.390	0.51 (0.13-1.90)		
SCC	22	5		0.357	1.66 (0.53-5.22)		
Other	26	1		0.297	0.28 (0.03-2.27)		
Exon 5 1386 C/T	CC	CT	TT		CT		TT
Controls	42	50	8				
Patients	52	38	10	0.102	0.61 (0.34-1.10)	0.985	1.01 (0.36-2.78)
Male	31	22	3	0.201	0.59 (0.26-1.32)	1.000	0.96 (0.14-6.31)
Female	21	16	7	0.318	0.64 (0.27-1.52)	0.752	1.22 (0.35-4.23)
Non-smoker	28	23	9	0.491	0.77 (0.36-1.61)	0.386	1.66 (0.52-5.25)
Smoker	24	15	1	0.072	0.40 (0.14-1.09)	0.265	0.22 (0.02-2.80)
SQC	27	18	1	0.115	0.56 (0.27-1.15)	0.146	0.19 (0.02-1.64)
SCC	12	11	4	0.575	0.77 (0.38-1.92)	0.417	1.75 (0.45-6.82)
Other	13	9	5	0.257	0.58 (0.22-1.49)	0.306	2.01 (0.56-7.24)
Intron 5 1712+19 G/T	GG	GT			GT		
Controls	96	4					
Patients	93	7		0.352	1.80 (0.51-6.37)		
Male	52	4		0.688	1.69 (0.29-9.68)		
Female	41	3		0.654	1.90 (0.30-11.9)		
Non-smoker	53	7		0.080	4.50 (0.90-22.5)		
Smoker	40	0		0.180	0.93 (0.84-1.02)		
SQC	42	4		0.261	2.28 (0.54-9.5)		
SCC	27	0		0.578	0.96 (0.92-1.00)		
Other	24	3		0.165	3.00 (0.62-14.3)		

Table IV. Continued.

Region	Genotype		p-value	Odds ratio (95% CI)	P-value	Odds ratio (95% CI)
Exon 7 2062 C/T	CC	CT		CT		
Controls	86	14				
Patients	91	9	0.268	0.60 (0.25-1.47)		
Male	51	5	1.000	1.02 (0.26-4.07)		
Female	40	4	0.250	0.44 (0.12-1.51)		
Non-smoker	56	4	0.350	0.55 (0.15-1.93)		
Smoker	35	5	0.511	0.57 (0.15-2.08)		
SQC	42	4	0.365	0.58 (0.18-1.88)		
SCC	24	3	1.000	0.76 (0.20-2.89)		
Other	25	2	0.520	0.49 (0.10-2.30)		
Intron 7 2141+73 G/A	GG	GA		GA		
Controls	80	20				
Patients	73	27	0.243	1.47 (0.76-2.86)		
Male	43	13	0.469	1.40 (0.53-3.87)		
Female	30	14	0.285	1.60 (0.66-4.02)		
Non-smoker	40	20	0.183	1.68 (0.77-3.66)		
Smoker	33	7	0.747	1.37 (0.36-5.22)		
SQC	31	15	0.097	1.93 (0.88-4.25)		
SCC	22	5	0.864	0.90 (0.30-2.69)		
Other	20	7	0.504	1.40 (0.52-3.76)		

SQC, squamous cell carcinoma; SCC, small cell carcinoma.

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