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Effects of polymorphisms in translesion DNA synthesis genes on lung cancer risk and prognosis in Chinese men

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Abstract

Purpose—Translesion DNA synthesis (TLS) plays an important role in promoting replication through DNA lesions. Genetic polymorphisms in TLS genes may have potential roles in lung cancer development in humans.

Methods—We evaluated the association between genetic variants in six TLS genes and the risk and survival of lung cancer in a case-control study in China. Included in the study are 224 lung cancer patients and 448 healthy controls.

Results—Carriers of the CG genotype of *POLK* rs5744724 had significantly reduced risk of lung cancer (odds ratio (OR) = 0.60, 95% confidence interval (CI): 0.42-0.87), comparing with those carrying the CC genotype, and the AA genotype of *PCNA* rs25406 was also associated with significantly decreased cancer risk compared with the major homozygote alleles (OR= 0.47, 95% CI: 0.25-0.86). Haplotype analysis showed that subjects with the *POLK* C-G (rs5744533-rs5744724) haplotype had decreased risk of lung cancer (OR = 0.69, 95% CI: 0.49-0.98), comparing with those carrying the C-C haplotype. Besides, the heterozygotes of *REVI* rs3087386 and rs3792136 were independent prognostic factors for lung cancer survival with hazard ratios (HR) 1.54 (95% CI: 1.12-2.12) and 1.44 (95% CI: 1.06-1.97) respectively.

Conclusions—Our findings suggested that genetic variants in *POLK* and *PCNA* genes may play roles in the susceptibility of lung cancer, and *REVI* gene may have roles in lung cancer survival in Chinese men.

Keywords

lung cancer; TLS pathway; genetic mutation; susceptibility; survival; men

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1. Introduction

Lung cancer is one of the most common cancers worldwide. It is the leading cause of death among all cancers with rapidly increasing incidence and mortality in China (1). Based on the estimation of IARC (GLOBOCAN 2008), about 522,000 new cases of lung cancer were diagnosed annually in China, and more than 452,000 Chinese died of lung cancer per year (2). The risk factors for lung cancer in China include cigarette smoking, indoor air pollution (e. g. cooking fumes, coal burning, etc.), family history of lung cancer and pulmonary tuberculosis (3-7). Cigarette smoking, the most important risk factor for lung cancer, contains 50–60 known carcinogens. Although only a small proportion of smokers eventually come down with lung cancer, it was responsible for approximately 90 percent of lung cancer deaths in men and approximately 60 percent of lung cancer deaths in women (8,9). Many chemical carcinogens and their metabolites in cigarette smoking, such as polycyclic aromatic hydrocarbons (PAHs) and N-nitrosamines, can bind to DNA to form carcinogen-DNA adducts, and consequently cause mutations in critical genes. These accumulated errors in the DNA may play a key role in the initiation of various types of cancers including lung cancer (10). Translesion DNA synthesis (TLS) is a DNA damage tolerance process, which contributes to cell survival by bypass of the unrepaired DNA lesions. TLS functions in an error-prone manner and sometimes can actively promote the generation of mutations (11). The key proteins in TLS are low fidelity DNA polymerases which were conserved in evolution in all kinds of organisms, including the Y-family DNA polymerases (POL η , POL κ , POL ι and REV1), B-family DNA polymerases (REV3 and REV7) and PCNA (12). Researches have shown that loss of Rev3L expression could contribute to genome instability during neoplastic transformation and progression in mammals (13), and depletion of REV3 can be applied for susceptible cancers (including lung cancer) as a potential monotherapy (14). Germline of POL κ (-/-) mice may result in replication fork pausing and affect expanded simple tandem repeat (ESTR) mutation rate (15).

To investigate the role of TLS genes in lung cancer etiology, we examined selected genetic polymorphisms in the *REV3* (rs465646, rs462779), *POL ι* (rs8305, rs3730668), *REV1* (rs3087386, rs2290257, rs3792136), *POL κ* (rs5744724, rs1018119, rs5744533), *PCNA* (rs25406, rs3729558) and *POL η* (rs6941583) genes in association with the risk and survival of lung cancer in Chinese men by conducting a case–control study nested in the SMHS (Shanghai Men's Health Study), a prospective cohort study on going in Shanghai, China.

2. Materials and Methods

2.1. Study subjects

Details of the study design of the SMHS have been reported elsewhere (16-18). Briefly, the SMHS is a population-based, prospective cohort study, designed to investigate the contribution of lifestyle and genetic susceptibility to risk of cancer and other chronic diseases. Recruitment of the study subjects was initiated in April 2002 and was completed in June 2006. A total of 83,125 male, residents of eight communities from a district in urban Shanghai, China, between the ages of 40 and 74 years were invited to take part in the study by trained interviewers through in-person contact. A total of 61,500 (74.0%) participants were enrolled in the study. Reasons for non-participation were refusals 17,523 (21.1 %), absence during the study period 2,553 (3.1%), and other miscellaneous reasons including poor health or hearing problems 1,561 (1.9%). The study protocol was approved by the Institutional Review Boards of the Shanghai Cancer Institute and the Vanderbilt University, and all participants provided written, informed consent.

During interview, 46,119 (75.0%) study participants donated a 10-ml blood. The cohort was followed up with home visits every 2 years and annual record linkage to databases of the population-based Shanghai Cancer Registry and the Shanghai Municipal Vital Statistics Unit to confirm the cause of death, the cancer diagnosed date and death date. Through December 31, 2008, 323 incident cases of lung cancer had been identified, of which were histopathologically confirmed or clinically diagnosed on the basis of radiography or computer-assisted tomography. Among these cases, 224 (69.3%) patients provided a blood sample at baseline. 448 controls who had no prior benign or malignant tumours who donated a blood sample in the baseline survey were matched to cases on age at interview (within 2 years), and timing of sample collection (month and year)

2.2. Genotyping

Total of 13 single-nucleotide polymorphisms (SNPs) in six candidate genes were selected in this study. Specifically, 7 SNPs were selected based on their potential role in cancers identified by review of literature (*POL* ι rs8305 and rs3730668, *REV3* rs462779, *POL* κ rs1018119, *REVI* rs3087386 and rs3792136, *PCNA* rs25406), and other 6 SNPs were functional SNPs that selected by searching Han Chinese data from the HapMap using the criteria of $r^2 > 0.9$ and MAF ≥ 0.05 (*POL* η rs6941583, *REV3* rs465646, *POL* κ rs5744724 and rs5744533, *REVI* rs2290257, *PCNA* rs3729558). Genomic DNA was extracted from buffy coat using DNA Extraction Kit (Promega, USA). Genotyping was performed using the TaqMan assay (Applied Biosystems, Foster City, CA), in 384-well format. Genotyping failure rate was $< 1.0\%$. The accuracy of the genotyping was evaluated by performing duplicate analysis of 34 DNA samples (5% of total samples) that were randomly selected from the whole population. The replicates were 100% concordant.

2.3. Statistical analysis

Distributions of selected characteristics, including age, education, body mass index (weight in kilograms per height in square meters), cigarette smoking (smoking at least one cigarette per day for 6 months), cigarettes per day and years of smoking were evaluated by χ^2 test. The associations between SNPs and lung cancer risk were measured by odds ratios (OR) and 95% confidence intervals (CI) estimated using unconditional logistic regression. The estimates were calculated for the heterozygous and homozygous variant genotypes relative to the common homozygous genotype (co-dominant model), as well as for the presence and absence of the variant allele (dominant model), using indicator variables in the regression model. An initial model was adjusted for age, and additional models were further adjusted for age, education, ever smoked, years of smoking, cigarettes per day and BMI. Hardy–Weinberg equilibrium for genotypic distribution and linkage disequilibrium between loci in genes were assessed by the HaploView (version 4.0) (19). Associations between haplotypes ($> 1\%$ frequency among controls) and the risks of lung cancer were evaluated by computing OR and 95% CI, using the SNPStats, assuming an additive model, and using the most common haplotype as the referent category. Global differences in haplotype frequencies between cases and controls were assessed for each gene using the Score test in the SNPStats (20).

Based on the data from our cohort to December 2010, there were 184 cases died of lung cancer. Overall survival time was calculated from the date of diagnosis to the date of death. Effects of the different genotypes on lung cancer survival were evaluated using Kaplan–Meier method and were compared using log-rank test. Univariate and multivariate Cox regression analysis was used to analysis different parameters for prognostic significance. P-values of less than 0.05 (two-sided probability) were considered as statistically significant.

3. Results

3.1. Population characteristics

Selected demographic and risk factors of the study subjects are shown in Table 1. Cases and controls were evenly matched by age. Higher education level had protective effect against the development of lung cancer, while ever smoked, years of smoking and cigarettes per day were risk factors for lung cancer development. Compared with those BMI <18.5 kg/m², people who had BMI 23.0–24.9 kg/m², and 25.0 kg/m² had reduced cancer risk. None of the other factors was associated with lung cancer risk.

3.2. Effects of Genotype on cancer susceptibility

The associations of the TLS gene variants with lung cancer risk are presented in Table 2. The genotype distributions of these variants showed no deviation from the expected Hardy–Weinberg equilibrium among controls ($P > 0.05$), except for rs8305 in the *POL* gene ($P_{HW}=0.007$) and rs1018119 in the *POLK* gene ($P_{HW}=0.03$), which were excluded from the following statistical analysis. MAFs of rs8305 and rs1018119 in our study population were 0.28 and 0.09, while MAFs in HapMap HCB population were 0.27 and 0.089. Of these SNPs, carriers of the CG genotype of *POLK* rs5744724 were associated with a reduced risk of lung cancer (OR=0.60, 95%CI = 0.42–0.87) compared with those carrying the CC genotype. This association was remained statistically significant after further adjusted for age, education, ever smoked, years of smoking, cigarettes per day and BMI (OR=0.60, 95%CI = 0.40–0.90). In the *PCNA* gene, the AA genotype of rs25406 and GG genotype of rs3729558 were also significantly associated with decreased cancer risk compared with GG and CC genotype (OR=0.47, 95%CI = 0.25–0.86; OR=0.46, 95%CI = 0.22–0.95), respectively. After adjustment for other risk factors, the rs25406 AA genotype remained significant (OR=0.49, 95%CI = 0.25–0.97), but not for rs3729558 GG genotype (OR=0.49, 95%CI = 0.22–1.06). None of the other SNPs examined was associated with the risk of lung cancer in our study.

3.3. Effects of Haplotype on cancer susceptibility

The two *POLK* SNPs, rs5744533 and rs5744724, are in linkage disequilibrium ($D'=1.0$, $r^2=0.10$), so were the *PCNA* SNPs, rs25406 and rs3729558 ($D'=0.98$, $r^2=0.72$) (21). Table 4 lists the associations between *POLK* and *PCNA* haplotypes and lung cancer risk. Carriers of the *POLK* (rs5744533-rs5744724) C-G haplotype had reduced risks of lung cancer (OR=0.69, 95%CI = 0.49–0.98) relative to carriers of the C-C haplotype. After adjustment for age, education, ever smoked, cigarettes per day, years of smoking and BMI, this association remained significant (OR=0.64, 95%CI = 0.44–0.93). The A-G and A-C haplotypes in the *PCNA* gene (rs25406- rs3729558) were associated with a non-significantly reduced risk of lung cancer (OR=0.80, 95%CI = 0.61–1.04; OR=0.70, 95%CI = 0.42–1.15), comparing with the G-C haplotype (Table 3).

3.4. Effect of Characteristics, Clinical data and Genotype on Survival Time

The univariate Cox regression analysis showed that the risk of death for lung cancer patients was significantly decreased in the people who received surgical operation (HR=0.47, 95%CI = 0.32–0.67). Although advanced clinical stage (III - IV) had increased risk of death (HR=1.51, 95%CI = 0.96–2.37) compared with early clinical stage (I – II), it did not reach statistical significance. None of the other characteristics and clinical data was associated with survival time (Table 4). When the Cox regression analysis was used, there were three genetic markers significantly associated with lung cancer survival (Table 5). Specifically, cases with the *REVI* rs3087386 AG had increased risk of death compared with the AA carriers (HR=1.54, 95%CI: 1.12–2.12). The *REVI* rs3792136 CT genotype was also found to

increase the lung cancer death relative to CC genotype (HR=1.44, 95%CI: 1.06-1.97). Besides, patients with the *POLK* rs5744724 CG genotype showed a lower survival time than the individuals with the CC genotype (HR=1.53, 95%CI: 1.08-2.15). To identify independent prognostic factors for lung cancer, we further adjusted for age, education, BMI, ever smoked, cigarettes per day and year of smoking, histological types, surgery, radiotherapy, chemotherapy, and clinical stage using multivariate models. The results showed that the *REVI* rs3087386 and rs3792136 were independent risk factors for lung cancer with adjusted HRs 1.92 (95%CI: 1.08-3.39) and 2.36 (95%CI: 1.30-4.25). None of the other SNPs was associated with survival time.

4. Discussion

In this nested case-control study, we found that two single-nucleotide polymorphisms in the *POLK* (rs5744724) and *PCNA* (rs25406) genes were associated with lung cancer risk, and mutations in *REVI* (rs3087386 and rs3792136) gene were associated with lung cancer survival.

Reports have shown that *POLK* overexpression was notably observed in lung cancer tissue, and this overexpression may result from increased spontaneous mutagenesis and pleiotropic alterations such as DNA breaks, genetic exchanges and aneuploidy (22,23). Few published studies have addressed lung cancer risk in relation to *POLK* gene polymorphism. Michiels et al. (24) previously investigated 70 genes involved in base excision repair, nucleotide excision repair, and mismatch repair pathways and in DNA synthesis among 151 Caucasian lung cancer cases and 172 controls. The results showed that *POLK* rs5744720 GC and rs1018119 GA genotypes had decreased risk (OR=0.20, 95% CI = 0.06-0.65; OR=0.36, 95% CI = 0.18-0.73) of lung cancer comparing to GG genotypes. In our study, mutation in rs5744724, which was in the 3' untranslated region (3'UTR) of the *POLK* gene, was associated with a reduced risk of lung cancer. And this finding has not been previously reported. The functional mechanism of this variant is unknown so far. We presume that it may affect gene expression, since it located in the 3'UTR of the gene.

PCNA exists in the nuclei of yeast, plant and animal cells, and plays an important role in cell cycle regulation and DNA replication. Studies have shown that interactions between *PCNA* and non-classical polymerases were essential for translesion synthesis (25,26). It functions as a sliding clamp for the polymerase *POLδ*, also interacts with the three other TLS polymerases (*POLη*, *POLι*, and *POLκ*) to initiate the translesion synthesis, when the replication fork stalled at the DNA damage site (27). It has been suggested that G178S *PCNA* mutant protein might block translesion synthesis (28). Zienolddiny et al. (29) previously investigated 44 SNPs in DNA repair genes, including nucleotide excision repair (NER) genes and double-strand break repair (DSB-R) genes with the risk of non-small cell lung cancer in Caucasians, and found that GG genotype in *PCNA* 1876A>G (rs25405) had 3.19-fold increased cancer risk (95% CI = 1.21-8.40). *PCNA* rs25406 has been reported to have excess risk of adult meningioma (OR_{CT+TT/CC} =2.1, 95% CI: 1.3-3.5) (30). But in our study we found that rs25406 AA genotype had decreased risk of lung cancer compared with the major genotype. The rs25406 variant was located in intron 2 of the *PCNA* gene, and the functional significance of this change is unclear.

Although we did not find any association between *POLη*, *POLι*, *REV3* and *REVI* variants and lung cancer risk in our study, we found *REVI* rs3087386 and rs3792136 were related to lung cancer survival, which had not been reported before. Rs3087386 was a missense mutation (Phe257Ser) that might affect the functional properties of the mutation protein, and rs3792136 was located in the intron region with unclear function. So, the mechanisms still need to be clarified. A previous study, included 752 adenocarcinoma cases, 250 squamous

cell carcinoma cases and 685 controls in Japanese population, found *REVI* rs3087386 was associated with the risk of squamous cell carcinoma of lung ($OR_{CC/TT} = 1.71$, 95% CI: 1.1-2.6) and *POL* rs8305 was associated with the risk of lung adenocarcinoma ($OR_{AG/AA} = 1.3$, 95% CI: 1.0-1.6) (31).

Strengths and limitations of our study should be noted. Strengths of our study include the population-based and prospective design in urban Shanghai, China, thereby minimizing selection bias; detailed review of cancer diagnosis, thereby minimizing disease misclassification. But our nested case-control sample was relatively small in size, which hampered our ability to evaluate specific gene-disease association by different histological types and provided limited power for evaluating gene-environment interactions. Besides, multiple testing for SNP data analysis may raise the potential for false positive results. Finally the explanation from our SNPs results should be cautious, and the findings would have to be validated in large population in the future.

In summary, our results showed that genetic polymorphisms in *POL* (rs5744724) and *PCNA* (rs25406) may play a role in the pathogenesis of lung cancer, and mutations in *REVI* (rs3087386 and rs3792136) gene may have relation with lung cancer survival.

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Table 1
Comparison of selected characteristics of subjects by case-control status

Selected characteristics	Cases, n (%)	Controls, n (%)	P-value
Age			
40-49	25(11.1)	48(10.7)	0.70
50-59	46(20.4)	92(20.5)	
60-69	73(32.5)	143(31.9)	
70-74	81(36.0)	165(36.9)	
Education			
Elementary or below	54(24.7)	64(14.7)	0.0025
Middle school	78(35.6)	151(34.7)	
High school	54(24.7)	113(26.0)	
College and above	33(15.0)	107(24.6)	
Income			
<1000	28(12.7)	37(8.6)	0.14
1000-	116(52.7)	232(53.8)	
2000-	69(31.4)	142(32.9)	
3000-	7(3.2)	20(4.7)	
Ever smoked			
Yes	197(87.9)	273(60.9)	<0.0001
No	27(12.1)	175(39.1)	
Cigarettes per day			
No	27(12.1)	175(39.1)	<0.0001
<10	14(6.2)	56(12.5)	
10-	49(21.9)	88(19.7)	
20-	99(44.2)	95(21.3)	
30-	35(15.6)	33(7.4)	
Years of smoking			
No	27(12.1)	175(39.1)	<0.0001
<20	14(6.2)	25(5.6)	
20-	28(12.5)	62(13.9)	
30-	55(24.6)	94(21.0)	
40-	100(44.6)	91(20.4)	
BMI			
<18.5	23(10.4)	13(3.2)	<0.0001
18.5-22.9	95(43.0)	140(33.9)	
23.0-24.9	44(19.9)	117(28.3)	
25.0	59(26.7)	143(34.6)	

Note. Total number of subjects may vary because of missing values.

Table 2
Associations of polymorphisms of TLS genes with lung cancer risk among Chinese men

Gene	SNP	Genotypes	Cases, n	Controls, n	OR (95% CI) [†]	OR (95% CI) [‡]
<i>REV3</i>	rs462779	GG	53	126	1.00	1.00
		GA	123	226	1.29 (0.88-1.91)	1.21 (0.79-1.85)
		AA	48	96	1.19 (0.74-1.91)	1.02 (0.60-1.73)
		<i>P</i> _{trend}			0.20	0.36
		GA+AA	171	322	1.26 (0.87-1.83)	1.15 (0.77-1.74)
	rs465646	AA	157	307	1.00	1.00
		AG	56	125	0.87 (0.60-1.26)	0.92 (0.61-1.38)
		GG	11	15	1.42 (0.64-3.17)	1.21 (0.50-3.02)
		<i>P</i> _{trend}			0.53	0.76
		AG+GG	67	140	0.93 (0.66-1.32)	0.95 (0.65-1.41)
<i>POL1</i>	rs3730668	TT	103	212	1.00	1.00
		TG	100	208	0.99 (0.71-1.38)	1.02 (0.71-1.48)
		GG	21	28	1.55 (0.84-2.86)	1.94 (0.97-3.89)
		<i>P</i> _{trend}			0.95	0.84
		TG+GG	121	236	1.05 (0.76-1.45)	1.12 (0.78-1.60)
<i>REV1</i>	rs2290257	GG	95	192	1.00	1.00
		GC	99	204	0.99 (0.70-1.39)	0.92 (0.63-1.35)
		CC	30	52	1.17 (0.70-1.96)	1.11 (0.63-1.98)
		<i>P</i> _{trend}			0.93	0.65
		GC+GG	129	256	1.02 (0.74-1.42)	0.96 (0.67-1.38)
	rs3087386	AA	86	175	1.00	1.00
		AG	105	208	1.03 (0.73-1.46)	0.94 (0.64-1.37)
		GG	33	65	1.04 (0.64-1.70)	1.08 (0.62-1.87)
		<i>P</i> _{trend}			0.87	0.68
		AG+GG	138	273	1.03 (0.74-1.44)	0.97 (0.67-1.39)
	rs3792136	CC	111	229	1.00	1.00
		CT	94	178	1.09 (0.78-1.53)	0.87 (0.60-1.27)
		TT	19	41	0.97 (0.54-1.74)	0.88 (0.46-1.71)

Gene	SNP	Genotypes	Cases, n	Controls, n	OR (95% CI) [†]	OR (95% CI) [‡]
<i>POLK</i>	rs5744533	<i>P</i> _{trend}			0.62	0.47
		CT+TT	113	219	1.07 (0.78-1.47)	0.88(0.61-1.25)
		CC	99	214	1.00	1.00
		CT	96	185	1.12 (0.80-1.58)	1.21(0.82-1.76)
	rs5744724	TT	29	49	1.27 (0.76- 2.14)	1.32(0.75-2.32)
		<i>P</i> _{trend}			0.51	0.33
<i>PCNA</i>	rs25406	CT+TT	125	234	1.15 (0.84-1.59)	1.23(0.86-1.76)
		CC	164	284	1.00	1.00
		CG	54	155	0.60 (0.42-0.87)	0.60(0.40-0.90)
		GG	5	9	0.96 (0.32-2.92)	0.51(0.15- 1.73)
	rs3729558	<i>P</i> _{trend}			0.007	0.01
		CG+GG	59	164	0.62 (0.44-0.89)	0.59(0.40-0.88)
<i>POLη</i>	rs6941583	GG	102	186	1.00	1.00
		GA	107	203	0.96 (0.69-1.35)	0.99(0.68-1.43)
		AA	15	59	0.47 (0.25-0.86)	0.49(0.25-0.97)
		<i>P</i> _{trend}			0.84	0.94
	rs3729558	GA+AA	122	262	0.85 (0.62-1.17)	0.90(0.63-1.28)
		CC	122	228	1.00	1.00
<i>POLη</i>	rs6941583	CG	92	179	0.96 (0.69-1.34)	0.89(0.67-1.42)
		GG	10	41	0.46 (0.22-0.95)	0.49(0.22-1.06)
		<i>P</i> _{trend}			0.75	0.91
		CG+GG	102	220	0.87 (0.63-1.20)	0.89(0.62-1.27)
	rs6941583	AA	208	411	1.00	1.00
		AT	16	36	0.87 (0.47-1.61)	0.89(0.45-1.76)
<i>POLη</i>	rs6941583	TT	0	1	—	—
		<i>P</i> _{trend}			0.64	0.64
<i>POLη</i>	rs6941583	AT+TT	16	37	0.85 (0.46-1.57)	0.83(0.42-1.64)
		<i>P</i> _{trend}			0.64	0.64

Note. Total number of subjects per SNP may vary because of missing values.

[†] Adjusted for age.

[‡] Adjusted for age, education, BMI, ever smoked, cigarettes per day and years of smoking.

Table 3
Associations of *PCNA* and *POLK* haplotypes with lung cancer risk among Chinese men

Haplotype	Cases, %	Controls, %	OR (95%CI) [†]	OR (95%CI) [‡]
<i>POLK</i> (rs5744533-rs5744724)				
C-C	51.4	49.1	1.00	1.00
T-C	34.3	31.5	1.03(0.80-1.32)	1.04(0.79-1.37)
C-G	14.3	19.4	0.69(0.49-0.98)	0.64(0.44-0.93)
<i>PCNA</i> (rs25406- rs3729558)				
G-C	69.3	64.0	1.00	1.00
A-G	24.9	28.8	0.80(0.61-1.04)	0.81(0.61-1.09)
A-C	5.8	7.2	0.70(0.42-1.15)	0.70(0.40-1.23)

[†] Adjusted for age.

[‡] Adjusted for age, education, BMI, ever smoked, cigarettes per day and years of smoking.

Table 4

Univariate analysis of survival for lung cancer patients

Selected characteristics	Cases, n	Deaths, n	MST	HR (95% CI)	Log-rank
Age					
40-49	25	14	8.8	1.00	
50-59	45	34	9.0	0.85(0.45-1.62)	
60-69	73	61	7.6	0.94(0.52-1.69)	
70-74	81	75	6.3	0.96(0.54-1.73)	0.99
Education					
Elementary or below	55	51	8.0	1.00	
Middle school	78	64	6.5	1.14(0.79-1.66)	
High school	54	41	7.6	1.30(0.85-1.99)	
College and above	33	25	11.8	0.75(0.46-1.22)	0.13
BMI					
< 18.5	23	22	7.3	1.00	
18.5-22.9	96	97	7.7	0.91(0.57-1.46)	
23.0-24.9	44	33	8.0	0.91(0.52-1.58)	
25.0	59	48	5.9	0.92(0.55-1.53)	0.97
Ever smoked					
Yes	196	162	6.6	1.00	
No	27	22	13.2	0.68(0.43-1.07)	0.09
Cigarettes per day					
No	27	22	13.2	1.00	
<10	14	13	6.7	1.23(0.62-2.46)	
10-	49	41	8.1	1.26(0.75-2.13)	
20-	99	80	6.4	1.49(0.93-1.40)	
30-	35	28	2.8	2.07(1.17-3.67)	0.11
Years of smoking					
No	27	22	13.2	1.00	
<20	14	13	4.4	1.21(0.60-2.41)	
20-	28	14	8.3	1.24(0.62-2.49)	
30-	55	45	7.2	1.48(0.87-2.50)	

Selected characteristics	Cases, n	Deaths, n	MST	HR (95% CI)	Log-rank
40-	100	90	5.8	1.57(0.97-2.55)	0.44
Histological types					
Squamous cell carcinoma	40	30	12.5	1.00	
Adenocarcinoma	56	41	8.0	1.30(0.80-2.11)	
Small cell carcinoma	13	13	6.8	1.95(0.98-3.90)	
Large cell carcinoma	3	3	7.7	2.85(0.83-9.78)	
Others	25	16	9.0	1.34(0.72-2.51)	0.26
Surgery					
No	151	120	5.7	1.00	
Yes	51	42	12.7	0.47(0.32-0.67)	<0.0
Chemotherapy					
No	108	84	5.9	1.00	
Yes	94	78	10.3	0.85(0.62-1.16)	0.29
Radiotherapy					
No	178	139	8.0	1.00	
Yes	24	23	4.3	1.35(0.87-2.11)	0.18
Clinical stage					
I-II	51	24	11.5	1.00	
III-IV	103	93	5.5	1.51(0.96-2.37)	0.06

Note. Total number of subjects may vary because of missing values.

Table 5
Association between genotypes of *REV1* and *POLK* genes and survival of lung cancer patients

SNP	Genotypes	Cases, n	Deaths, n	MST	HR (95%CI) [†]	Adjusted HR (95%CI) [‡]
<i>REV1</i> rs3087386	AA	86	69	9.5	1.00	1.00
	AG	105	87	6.0	1.54(1.12-2.12)	1.92(1.08-3.39)
	GG	33	28	5.4	1.41(0.89-2.24)	1.53(0.64-3.64)
						0.03
rs3792136	CC	111	92	9.0	1.00	1.00
	CT	94	75	5.8	1.44(1.06-1.97)	2.36(1.30-4.25)
	TT	19	17	8.0	1.41(0.83-2.40)	1.58(0.64-3.95)
						0.004
<i>POLK</i> rs5744724	CC	164	134	9.0	1.00	1.00
	CG	54	44	4.2	1.53(1.08-2.15)	1.49(0.79-2.79)
	GG	5	5	4.8	1.57(0.64-3.85)	0.57(0.11-2.89)
						0.01
						0.27

MST: median survival time.

[†] Adjusted for age.

[‡] Adjusted for age, education, ever smoked, cigarettes per day, years of smoking, BMI, histological types, surgery, radiotherapy, chemotherapy, and clinical stage.