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Original Article

Effects of *TERT* gene polymorphism and environmental factor interactions on lung cancer risk in the Xi'an Han population

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Abstract: Background: Genetic polymorphisms of the telomerase reverse transcriptase (TERT) gene influence an individual susceptibility to lung cancer. We examined the relationship between TERT single nucleotide polymorphisms (SNPs) and lung cancer occurrence and determined if smoking or alcohol consumption affects this relationship in lung cancer patients. Methods: Ten SNPs and inferred TERT haplotypes associated with increased risk of lung cancer were evaluated in a Han Chinese population-based study including 228 lung cancer patients and 310 healthy control individuals. Relationships between the patients' genotypes and risk factors were investigated by estimating odds ratios (ORs) and 95% confidence intervals (CIs) using unconditional logistic regression analysis. Results: Minor allelesrs2853672 and rs2736098 conferred significantly increased lung cancer risk in both allelic and genotypic association analyses (codominant, recessive, and log-additive models), while rs4246742 showed a protective effect against lung cancer in codominant and recessive models. Smoking increased the effect of rs2736098 on lung cancer risk, but the interaction between rs2736098 and smoking was only significant in the overdominant model (P=0.03). No evidence was found that alcohol consumption influenced or interacted with TERT polymorphism on lung cancer risk. Conclusions: Our results confirmed that TERT genetic polymorphisms contribute to lung cancer susceptibility in Chinese populations and of ten interact with smoking as a lung cancer risk factor. However, these associations should be further explored in larger and varying populations.

Keywords: *TERT*, genetic polymorphism, lung cancer, case-control study, Han

Introduction

In many countries, including China, the incidence and mortality rates of lung cancer are greater than those from any other type of cancer [1]. Despite considerable therapeutic progress, the prognosis of non-small cell lung cancer (NSCLC) patients remains poor, and their five-year survival rates are less than 15% [2]. Finding markers associated with lung cancer risk is vital to early detection and discovery of novel chemo-preventive agents.

Lung cancer development appears to result from a complex interaction between environmental exposures and genetic factors. At present, cigarette smoking is the most important risk factor for lung cancer [3], and recent epidemiological studies [4, 5] indicate alcohol consumption plays a potential role in lung carcinogenesis. In addition, genome-wide association (GWA) studies have determined common variations at three separate chromosomal regions (5p15, 6p21, and 15q25) influence the risk of developing lung cancer [6-9]. Genes encoding telomerase reverse transcriptase (TERT) and cleft lip and palate transmembrane1-like protein, located in region 5p15.33, a crucial genomic region for telomere biology, showed associations with lung cancer risk [6, 7, 9, 10].

The TERT enzyme is the catalytic subunit of telomerase that elongates telomeres and is a key regulator of telomerase activity [11]. TERT is not expressed in most normal cells but is significantly overexpressed in cells involved in

85%-90% of human cancers, indicating telomerase activation is necessary for healthy cells to transform into malignant ones [12, 13]. Telomerase activation, which depends on TERT expression, has been linked to tumor development in many types of cancer and is the rate-limiting step of regulating telomerase activity involved in tumorigenesis [14]. Many GWA studies have shown that approximately 30 single-nucleotide polymorphisms (SNPs)in the *TERT* gene affect an individual's susceptibility of developing many tumor types [6, 7, 9, 10, 15, 16]. More specifically, two comprehensive meta-analyses studying SNP rs2736100 confirmed its significantly positive association with lung cancer risk in whites and Asians, especially with respect to adenocarcinoma, the most common type of lung cancer [16, 17]. Additionally, the association between rs2736100 and adenocarcinoma development was stronger in females and in individuals who had never smoked [9, 18]. Another *TERT* locus, rs2736098, may also lead to significantly increased risk for lung cancer development in white and Asian populations [19-23].

Unlike rs2736100 and rs2736098, associations between other *TERT* loci (rs2853676, rs2242652, rs2736122, and rs2075786) and lung cancer risk in previous studies are inconsistent and conflicting, likely due to the different ethnicities and patient sample sizes investigated in those studies. For example, rs2075786, an intronic *TERT* gene polymorphism, confers an increased risk of developing NSCLC on African-American females, but not white [24]. However, Hsiung *et al*. negated its association with lung cancer risk in non-smoking Asian females [9]. Another SNP, rs2853677, exerted a significantly increased effect on lung cancer development in white and African-American females, but this association was not observed in Asians [24, 25]. Moreover, though many studies have shown environmental risk factors may interact with susceptibility loci to induce lung carcinogenesis, specific interactions between *TERT* genetic polymorphisms and environmental exposures remain unreported.

Therefore, to comprehensively assess the effect of *TERT* genetic polymorphisms on lung cancer risk, we conducted a Han Chinese population-based study including 228 lung cancer patients and 301 unrelated healthy individuals. We focused on the interactions between 10 common but relatively uncharacterized variants of the *TERT* gene and cigarette smoking or alcohol consumption to explore the genetic and environmental risk factors of lung cancer development.

Materials and methods

Study populations

This study included 228 lung cancer patients and 301 healthy control patients. To exclude the possible effects of ethnicity, all subjects in this study were genetically unrelated ethnic Han Chinese individuals from Xi'an, the capital of Shaanxi province. The eligible patients were recruited at the Department of Respiration from the First Affiliated Hospital of the Medical College of Xi'an Jiaotong University from October 2011 to September 2012.

All patients were recently diagnosed with lung cancer identified by histological observation. No patients had a previous history of other cancers, chemotherapy, or radiotherapy, and the patients were chosen regardless of age, gender, or disease stage. In addition, 310 healthy unrelated individuals were selected during the same time period from the medical examination center of Xi'an Jiaotong University based on standard recruitment and exclusion criteria to ensure they had no chronic or severe endocrinological, metabolic, or nutritional diseases.

Demographic data collection

A standardized epidemiological questionnaire was completed for each patient and control individual by a trained interviewer to collect demographic data (age, sex, ethnicity, and education level) and clinical information (smoking habits, alcohol use, and family history of cancer). Smokers and drinkers were classified into two categories ever and never. For smoking, those individuals who smoked < 2 cigarettes per week or < 100 cigarettes per year were defined as non-smokers, otherwise, the individuals were considered as smokers. For alcohol drinking, persons who were ingesting more than once per week in the past 6 months were considered to be drinkers, otherwise, the individuals were considered as non-drinkers. Participants were informed of the purpose and

Table 1. General characteristics of the study population

Variables	Patients N (%)	Controls N (%)	*P* value
Age (years)			
Mean ± SD	58.7±10	50.2±8.1	< 0.001[a]
≤50	49 (21.5)	166 (55.1)	< 0.001[b]
>50	179 (78.5)	135 (44.9)	
Sex			
Male	178 (78.1)	188 (62.5)	< 0.001[b]
Female	50 (21.9)	113 (37.5)	
Smoking status			
Ever-smokers	165 (72.4)	91 (30.2)	< 0.001[b]
Non-smokers	63 (27.6)	210 (69.8)	
Alcohol consumption			
Ever-drinkers	37 (16.2)	66 (21.9)	0.101[b]
Non-drinkers	191 (83.8)	235 (78.1)	
TOTAL	228	301	

[a]*P* values were calculated by Student's t tests. [b]*P* values were calculated from two-sided Chi-squared tests.

experimental procedures of the study, and written informed consent was obtained from each participant. This study was conducted under the approval of the Human Research Committee of the First Affiliated Hospital of Xi'an Jiaotong University and Northwest University.

SNP selection and genotyping

Based on previous GWA studies of lung cancer, 10 susceptibility loci in the *TERT* gene were selected as candidate SNPs. The SNPs include common variations with minor allele frequencies (MAF) greater than 0.05 in the HapMap Chinese Han Beijing population.

Blood samples were collected from all participants at the time of recruitment. Genomic DNA was extracted from peripheral blood obtained from each participant using GoldMag-Mini Whole Blood Genomic DNA Purification Kit (GoldMag Co. Ltd., Xi'an City, China) according to the manufacturer's protocol. DNA concentration was measured by aNanoDrop 2000 (Gene Company Ltd., Hong Kong, China). Polymerase chain reaction and extension primers (Table S1) were designed using MassARRAY Assay Design 3.0 software (Sequenom, Inc., San Diego, CA, USA). SNP genotyping procedures were performed according to the manufacturer's iPLEX Application Guide (Sequenom, Inc.).

Statistical analysis

The MAF, pairwise linkage disequilibrium (LD) across all SNPs, and the Hardy-Weinberg equilibrium for each SNP in the control individuals were measured by using HAPLOVIEW v4.2 (Daly Lab, Cambridge, MA, USA). The haplotypes and their frequencies were estimated based on a Bayesian algorithm using SNPstats [26] (http://bioinfo.iconcologia.net/snpstats/start.htm).

The statistical analyses were performed using SPSS16.0 (SPSS Inc, USA), Plink v1.07 (http://pngu.mgh.harvard.edu/~purcell/plink/) and SNPstats. A Chi-squared test was used to examine differences in the distributions of demographic characteristics among cancer patients and controls, and the Student's t test was performed to evaluate the any significant differences in age. Unconditional logistic regression analysis was used to calculate the adjusted odds ratios (ORs) and 95% confidence intervals (CIs) of the relationship between *TERT* SNPs and lung cancer risk, and participants' age was transformed into a binary covariate by using 50 years as the threshold age. Plink software performed the allele-based association test across the overall population. We also performed a stratified analysis by age, sex, smoking status, and alcohol use to further explore the association between SNPs and the risk of lung cancer in each stratum. Moreover, SNPstats were utilized to estimate ORs and CIs of the link between cancer risk and patient genotypes and haplotypes. A genotypic association analysis was performed under the following genetic models: codominant, dominant, recessive, overdominant, and log-additive. Further, potential interactions between genotypes and either smoking or alcohol consumption with respect to lung cancer were analyzed by a crossover table method using SNPstats. All analyses were performed with adjustments made for potential confounding factors such as age, gender, smoking status, or alcohol use. All tests were two-sided, and $P < 0.05$ was considered significant.

Results

Population characteristics

The distribution of selected characteristics of study subjects (n=529; 228 patients and 301

Table 2. Genotypic information of selected *TERT* SNPs and their minor allelic associations with lung cancer risk

SNP No.	SNP ID	Location	Allele A[a]/B	MAF		HWE *p*	OR[b]	95% CI	*P* value
				Case	Control				
1	rs2736122	Intron13	T/C	0.057	0.042	0.49	1.86	0.94-3.68	0.074
2	rs2736114	Intron10	A/G	0.057	0.042	0.43	1.92	0.97-3.83	0.063
3	rs2075786	Intron10	C/T	0.145	0.146	0.79	1.10	0.72-1.68	0.666
4	rs4246742	Intron9	T/A	0.307	0.36	0.22	0.75	0.55-1.02	0.069
5	rs10069690	Intron4	T/C	0.211	0.166	0.26	1.32	0.91-1.91	0.140
6	rs2242652	Intron4	T/C	0.206	0.161	0.33	1.35	0.93-1.96	0.112
7	rs34829399	Intron2	T/C	0.07	0.05	0.36	1.25	0.67-2.32	0.484
8	rs2853676	Intron2	A/G	0.185	0.166	0.11	1.12	0.77-1.63	0.549
9	rs2853672	Intron2	G/T	0.5	0.446	0.14	1.37	1.03-1.83	0.032
10	rs2736098	Exon2	A/G	0.377	0.323	0.27	1.47	1.07-2.02	0.017

[a]Minor allele; [b]Adjusted by sex, age, and smoking or drinking status. SNP: single nucleotide polymorphism; MAF: minor allele frequency; HWE: Hardy-Weinberg equilibrium.

healthy control individuals) are summarized in **Table 1**. Because control subjects were not selected based on age and sex, we observed significant differences in age ($P < 0.001$) and sex ratios ($P < 0.001$) between lung cancer patients and controls. Specifically, the mean age of the patients was greater than that of the control group (58.7±10 years vs. 50.2±8.1 years, respectively), and males were more prevalent among patients than controls (78.1% vs. 62.5%, respectively). In addition, subjects older than 50 years were more common in the patient group than in the control group (78.5% vs. 44.9%; $P < 0.001$).

We also observed significant differences in smoking status between patients and controls. More smokers were represented in the lung cancer patients compared with controls (72.4% vs. 30.2%, respectively; $P < 0.001$). By contrast, no significant difference was found between the patients and controls with regards to alcohol consumption ($P=0.101$).

Genetic polymorphisms and linkage disequilibrium

The MAFs of tested *TERT* polymorphisms and their allelic associations in patients and controls are presented in **Table 2**. The genotype distribution for the 10 SNPs was in Hardy-Weinberg equilibrium ($P > 0.05$) in the control group. In control individuals, only SNPs rs2736122 and rs2736114 exhibited a MAF slightly lower than 0.05. Pairwise LD values between SNPs were also calculated, which are shown in Figure S1. In the controls, paired loci rs2736122 and rs2736114 (D'=1; r^2=0.97), rs2242652 and rs10069690 (D'=0.99; r^2=0.96) were almost completely linked; rs2853672 and rs2736098 (D'=0.98; r^2=0.58), rs2736122 and rs2075786 (D'=1; r^2=0.22), rs2736114 and rs2075786 (D'=1, r^2=0.24), andrs2853672 and rs2853676(D'=1, r^2=0.23) were also highly linked (Figure S1A). Similar LD pattern among the above loci were also observed in patients (Figure S1B). However, we also found different LD relationship among other SNPs between patients and controls. In the control group, only weak linkage was existed between rs4246742 and rs2242652 or rs10069690 (D' < 0.3), but in patient groups their linkage was increased (D'=0.7).

Association of SNPs with lung cancer

We investigated if the minor allele of each SNP was a cancer risk factor compared with the wild-type allele. Only rs2853672 (adjusted OR=1.37, CI: 1.03-1.83; P=0.032) and rs2736098 (adjusted OR=1.47, CI: 1.07-2.02; P=0.017) conferred significantly elevated risk in this model (**Table 2**).

Subsequent stratified analysis data using covariates (sex, age, smoking status, and alcohol consumption) are shown in Table S2. Highly-linked SNPs rs2853672 and rs2853676 were significantly associated with increased risk of lung cancer among males, subjects older than 50 years, ever-smokers, and non-drinkers ($P < 0.05$). Other highly-linked SNPs, rs2736122

Table 3. Logistic regression analysis of the association between *TERT* genetic polymorphisms and lung cancer risk

SNP	Model	Genotype	Group=Control	Group=Patient	OR[a] (95% CI)	*P* value
rs4246742	Codominant	A/A	127 (42.3%)	109 (47.8%)	1.00	0.09
		T/A	129 (43%)	98 (43%)	0.91 (0.58-1.43)	
		T/T	44 (14.7%)	21 (9.2%)	0.46 (0.23-0.94)	
	Dominant	A/A	127 (42.3%)	109 (47.8%)	1.00	0.26
		T/A-T/T	173 (57.7%)	119 (52.2%)	0.78 (0.51-1.20)	
	Recessive	A/A-T/A	256 (85.3%)	207 (90.8%)	1.00	0.031
		T/T	44 (14.7%)	21 (9.2%)	0.48 (0.25-0.95)	
	Overdominant	A/A-T/T	171 (57%)	130 (57%)	1.00	0.77
		T/A	129 (43%)	98 (43%)	1.07 (0.69-1.64)	
	Log-additive	---	---	---	0.74 (0.54-1.02)	0.065
rs2853672	Codominant	T/T	97 (32.5%)	59 (25.9%)	1.00	0.021
		G/T	135 (45.3%)	110 (48.2%)	1.93 (1.15-3.25)	
		G/G	66 (22.1%)	59 (25.9%)	2.03 (1.12-3.68)	
	Dominant	T/T	97 (32.5%)	59 (25.9%)	1.00	0.0055
		G/T-G/G	201 (67.5%)	169 (74.1%)	1.97 (1.21-3.19)	
	Recessive	T/T-G/T	232 (77.8%)	169 (74.1%)	1.00	0.24
		G/G	66 (22.1%)	59 (25.9%)	1.35 (0.82-2.21)	
	Overdominant	T/T-G/G	163 (54.7%)	118 (51.8%)	1.00	0.13
		G/T	135 (45.3%)	110 (48.2%)	1.39 (0.90-2.14)	
	Log-additive	---	---	---	1.43 (1.07-1.93)	0.016
rs2736098	Codominant	G/G	132 (44.3%)	88 (38.6%)	1.00	0.02
		A/G	139 (46.6%)	108 (47.4%)	1.74 (1.09-2.78)	
		A/A	27 (9.1%)	32 (14%)	2.29 (1.11-4.72)	
	Dominant	G/G	132 (44.3%)	88 (38.6%)	1.00	0.007
		A/G-A/A	166 (55.7%)	140 (61.4%)	1.84 (1.17-2.87)	
	Recessive	G/G-A/G	271 (90.9%)	196 (86%)	1.00	0.12
		A/A	27 (9.1%)	32 (14%)	1.70 (0.87-3.34)	
	Overdominant	G/G-A/A	159 (53.4%)	120 (52.6%)	1.00	0.095
		A/G	139 (46.6%)	108 (47.4%)	1.45 (0.94-2.24)	
	Log-additive	---	---	---	1.58 (1.13-2.20)	0.0061

[a]Adjusted by sex, age, and smoking or drinking status.

and rs2736114 (similar to rs2736098), exhibited an approximately four-fold increased risk of lung cancer among ever-smokers ($P < 0.05$). In contrast, rs4246742 was inversely associated with lung cancer risk in ever-smokers (adjusted OR=0.58, CI: 0.38-0.58; P=0.011) and alcohol drinkers (adjusted OR=0.51, CI: 0.26-1.0; P=0.049).

Genotypic model analysis showed that rs4246742, rs2853672, and rs2736098 were associated with lung cancer risk (**Table 3**). Using the AA and TA genotypes combined as a reference, the TT genotype of rs4246742 was significantly associated with a decreased risk of lung cancer (adjusted OR=0.48, CI: 0.25-0.95; P=0.031, recessive model). With the AA genotype as a reference, the OR for the TT genotype was 0.46 (CI: 0.23-0.94; P=0.09, codominant model). Conversely, rs2853672 and rs2736098 were associated with an increased risk of lung cancer in the codominant, dominant, and log-additive models. Compared to the homozygous major allele carriers of these two variants, risks of heterozygous and homozygous minor allele carriers of rs2853672 (GT:

Table 4. Haplotype distributions of *TERT* polymorphisms and their association with lung cancer risk

Haplotype	SNP1	SNP2	SNP3	SNP4	SNP5	SNP6	SNP7	SNP8	SNP9	SNP10	Total	Control	Patient	OR[a] (95% CI)	*P* value
1	C	G	T	A	C	C	C	G	T	G	0.3044	0.3175	0.2991	1	---
2	C	G	T	T	C	C	C	G	T	G	0.1219	0.132	0.1	0.66 (0.34-1.31)	0.24
3	C	G	T	T	C	C	C	G	G	A	0.0971	0.0888	0.1096	2.12 (1.10-4.09)	0.025
4	C	G	T	A	C	C	C	G	G	A	0.0885	0.0964	0.0886	1.59 (0.61-4.15)	0.34
5	C	G	T	A	T	T	C	A	G	G	0.0513	0.0341	0.0763	3.17 (1.22-8.24)	0.018
6	C	G	T	A	T	T	C	A	G	A	0.027	0.0288	0.0272	0.87 (0.26-2.88)	0.82
7	C	G	C	T	C	C	C	G	T	G	0.0269	0.0345	0.018	0.20 (0.03-1.31)	0.094
8	C	G	T	A	T	T	C	G	G	A	0.0243	0.0223	0.0261	1.78 (0.44-7.24)	0.42
9	C	G	T	A	C	C	T	G	T	G	0.0226	0.0225	0.0126	0.75 (0.15-3.79)	0.72
10	C	G	T	A	C	C	C	A	G	G	0.0205	0.0162	0.0255	1.15 (0.30-4.42)	0.84
11	C	G	T	T	C	C	C	A	G	A	0.017	0.0207	0.0126	0.84 (0.22-3.23)	0.81
12	C	G	C	A	C	C	C	G	T	G	0.015	0.0201	0.0067	2.03 (0.09-44.21)	0.65
13	C	G	T	A	C	C	T	G	G	A	0.0143	0.0086	0.0144	1.04 (0.16-6.60)	0.97
14	C	G	C	T	C	C	C	G	G	A	0.0132	0	0.02	3.02 (0.43-21.45)	0.27
15	T	A	C	A	C	C	C	G	G	A	0.0129	0.0119	0.0137	3.04 (0.14-67.31)	0.48
16	C	G	T	T	T	T	C	A	G	G	0.0128	0.0193	0	0.21 (0.02-1.96)	0.17
17	C	G	T	T	C	C	C	G	G	G	0.0115	0.0169	0	-	-
18	C	G	C	A	T	T	C	A	G	G	0.0112	0.0132	0.0058	-	-
Rare	-	-	-	-	-	-	-	-	-	-	0.1076	0.0962	0.1438	-	-

[a]Adjusted by sex, age, and smoking or drinking status.

Table 5. Interaction between rs2736098 genotypes and smoking with respect to lung cancer

Genetic model	Genotype	Non-smoker			Ever-smoker			*P* value, interaction
		Controls	Patients	OR (95% CI)	Controls	Patients	OR (95% CI)	
Codominant	G/G	86	26	1	46	62	11.35 (4.97-25.93)	0.11
	A/G	103	26	1.02 (0.51-2.03)	36	82	31.00 (12.87-74.65)	
	A/A	20	11	1.88 (0.72-4.90)	7	21	29.04 (8.51-99.06)	
Dominant	G/G	86	26	1	46	62	11.68 (5.11-26.70)	0.065
	A/G-A/A	123	37	1.18 (0.62-2.24)	43	103	31.60 (13.42-74.39)	
Recessive	G/G-A/G	189	52	1	82	144	17.60 (8.97-34.53)	0.78
	A/A	20	11	1.85 (0.77-4.48)	7	21	26.76 (8.50-84.18)	
Overdominant	G/G-A/A	106	37	1.00	53	83	11.53 (5.35-24.85)	0.03
	A/G	103	26	0.87 (0.46-1.65)	36	82	26.35 (11.41-60.85)	

adjusted OR=1.93, CI: 1.15-3.25; TT: adjusted OR=2.03, CI: 1.12-3.68; *P*=0.021) and rs-2736098 (GA: adjusted OR=1.74, CI: 1.09-2.78; AA: adjusted OR=2.29, CI: 1.11-4.72; *P*=0.02) were all significantly increased. When heterozygous and homozygous carriers of the minor alleles of rs2853672 and rs2736098 were combined, their risks for lung cancer were all significantly elevated (GT+TT: adjusted OR=1.97, CI: 1.21-3.19, *P*=0.0055; GA+AA: adjusted OR=1.84, CI: 1.17-2.87, *P*=0.007, respectively).

Association of haplotypes with lung cancer

Based on SNP genotypic information, we used the maximum expectation method to estimate *TERT* gene haplotype distribution frequencies in the population. We identified 74 haplotypes, 18 of which exhibited frequencies greater than 0.01 in the study group (**Table 4**) and accounted for approximately 89% of all haplotypes observed. The most common haplotypes were 1, 2, 3, 4, and 5, with frequencies of 0.3044, 0.1219, 0.0971, 0.0885, and 0.0513, respectively, accounting for 66.3% of all haplotypes inferred. Of the 18 haplotypes, only the distributions of haplotypes 3 and 5 significantly differed in cancer patients vs. controls (*P*=0.025 and 0.018, respectively). Using subjects carrying haplotype 1 as the reference group, carriers with haplotype 3 or haplotype 5 showed a two-fold (OR=2.12, CI: 1.10-4.09, *P*=0.025) or three-fold (OR=3.17, CI: 1.22-8.24, *P*=0.018)

significantly increased risk of lung cancer. Haplotype 3 was composed of variant alleles of rs4246742, rs2853676, and rs2736098 and the reference allele of all other SNPs. Haplotype 5 consisted of the variant alleles of rs10069690, rs2242652, rs2853676, and rs2853672 and the reference allele of all other SNPs.

Effect of genetic-environmental interactions on lung cancer risk

Possible interaction effects of genetic polymorphisms and either cigarette smoking or alcohol consumption on lung cancer risk were analyzed. We found a significant interaction between rs2736098 and smoking (**Table 5**). Risks of smokers with any genotype of rs2736098 were higher than non-smokers with corresponding genotypes. Among smokers, risks for subjects carrying the variant genotype of rs2736098 were all higher than those carrying the reference genotype in dominant (A/G or A/Avs. G/G), codominant (A/G-A/A vs. G/G), and recessive models (A/Avs. G/Gvs. A/G). In the overdominant model, using non-smokers with non-A/G genotypes as the reference group, ever-smokers with the G/G-A/A (adjusted OR=11.53, CI=5.35-24.85) or A/G (adjusted OR=26.35, CI=11.41-60.85) genotype were associated with an increased risk of lung cancer. The multiplicative interaction between rs2736098 genotypes and smoking only reached statistical significance in the over dominant model (P=0.03). To date, we have not identified any *TERT* gene SNP that significantly interacted with alcohol consumption to affect lung cancer risk (data not shown).

Discussion

In this study, we explored the association between genetic variants of the *TERT* gene and lung cancer in a Chinese population and analyzed interactions between *TERT* polymorphisms and environmental risk factors, such as smoking and alcohol consumption. We found that *TERT* SNPs rs4246742, rs2853672, and rs2736098 were significantly associated with lung cancer risk. Among the 10 SNPs analyzed, only rs2736098 significantly interacted with smoking, as ever-smokers showed a stronger risk of lung cancer.

To date, more than 30 *TERT* variants associated with cancer susceptibility have been identified by GWA studies [6, 7, 9, 10, 15, 16]. Based on their allelic distributions in the HapMap Chinese population, we selected 10 common (frequencies greater than 0.05), but relatively less-studied SNPs to investigate their relationship with lung cancer risk. Genotypic data showed the frequencies of the 10 SNPs in the control group were comparable to those in the HapMap Chinese population. All alleles were at Hardy-Weinberg equilibrium, indicating the control genotypes in this study are representative of the general population. From the pairwise LD analysis, we determined some of the SNPs were highly linked, such as rs2736122/rs2736144 and rs2242652/rs10069690, suggesting one SNP of each pair can indicate the presence of the other SNP in genetic association studies. We also analyzed *TERT* haplotype structure and identified 18 haplotypes with frequencies above 0.01. Association analysis between *TERT* genetic polymorphisms and lung cancer risk was then performed at both single variant and haplotype levels.

First, we investigated the effect of a single *TERT* SNP on lung cancer risk in allelic and genotypic models. The results showed that three common variants, rs4246742, rs2853672, and rs2736098 were significantly associated with lung cancer risk. Locus rs2736098, a very common synonymous SNP (A305A) located in exon 2 of the *TERT* gene, is associated with telomere extension but not TERT expression [10]. Considerable studies have shown rs2736098 increased cancer susceptibility, including risk of lung cancer [6, 10, 19, 21, 22], bladder cancer [10, 27], prostate cancer [10], nasopharyngeal carcinoma [28], glioma [29], hepatocellular carcinoma [30], and cervical cancer [31]. However, the same SNP conferred a protective effect against breast cancer [32] and squamous cell carcinoma of the head and neck [33]. Other meta-analyses confirmed its strong correlation with lung cancer and bladder cancer but not with prostate cancer and CNS tumors [16, 20, 23]. Consistent with previous studies, our data demonstrated the minor allele of rs2736098 conferred increased risk of lung cancer in both allelic and genotypic analysis (codominant, recessive, log-additive models). Similarly to rs2736098, rs2853672 showed an increased effect on lung cancer risk in this study, whereas few data regarding this SNP were previously available. Several studies re-

ported rs4246742 did not significantly affect lung cancer risk in white [6] or Asian non-smoking females [9]. However, our data showed the risk of homozygous minor allele (T) carriers was significantly lower in the recessive model, compared to carriers of the major allele (A), indicating the TT genotype provided a beneficial impact against lung carcinogenesis. Therefore, further large-scale studies are needed to verify the role of rs2853672 and rs4246742 in lung cancer development. Other SNPs tested in our study did not significantly affect lung cancer risk. These data support previous stud= ies, which determined rs2853676, rs2242652, and rs2736122 are not associated with increased lung cancer risk in white [6, 7] or never-smoking females in Asia [9]. In contrast, SNPs rs2075786 and rs2853677 conferred an increased risk in Africa-American females, but not white females [24].

Secondly, we investigated possible associations between *TERT* haplotypes and lung cancer risk by focusing on any additive effects of carrying multiple variants of *TERT*. Haplotypes 3 and 5 were significantly associated with a two-fold and three-fold increased risk of lung cancer, respectively. Haplotype 3 was composed of variant alleles of rs4246742, rs2853676, and rs2736098, and haplotype 5 consisted of the variant alleles of rs10069690, rs2242652, rs2853676, and rs2853672. The risks associated with either of these two haplotypes were stronger than that of any single SNP. Interestingly, the protective effect of rs4246742 in haplotype 3 was reduced when other covariants occurred, suggesting *TERT* haplotyping may provide a more precise and comprehensive evaluation of genetic susceptibility to cancer than single variant analysis.

Finally, we assessed interactions between *TERT* polymorphism and environmental factors for lung cancer. Only rs2736098 exhibited a significant multiplicative interaction with smoking in the over dominant model. Specifically, we sometimes observed ever-smokers with a heterozygous or homozygous mutant genotype had a higher risk for lung cancer than corresponding non-smokers or ever-smokers with the homozygous reference genotype, suggesting cigarette smoking could intensify the effect of rs2736098 on lung cancer development. Subgroup analysis consistently showed that the impact of rs2736098 on lung cancer risk was significantly higher in ever-smokers.

The role of alcohol consumption in lung cancer development was inconclusive in various studies and may be confounded by cigarette smoking [4]. In our study, alcohol consumption was not a risk factor for lung cancer, and no significant interactions were observed between *TERT* genetic polymorphisms and alcohol consumption. However, due to relatively small subgroups, findings from these gene-environment interaction analyses must be interpreted with caution.

Several limitations of this study should be mentioned, such as the small study size. For example, the number of subjects with some SNP homozygous variant genotypes may have been too few for accurately testing associations with recessive or log-additive models. In addition, the statistical power of our stratified and gene-environment interaction analysis could be influenced by the small samples size of some subgroups. The second limitation involves the age and sex of patients vs. control individuals, which were not matched during subject selection. Therefore, we observed significant differences in both age and sex distributions between patients and controls. However, these variables were adjusted in our subsequent regression analysis.

In conclusion, our data confirmed certain *TERT* gene polymorphisms are associated with increased risk of lung cancer in Chinese individuals, and lung carcinogenesis may be affected by interactions between cigarette smoking and *TERT* genetic variants. Future population-based studies with larger sample sizes, and detailed environmental exposure information is needed to further explore these gene-environment interactions and their effects on lung cancer development.

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Disclosure of conflict of interest

None.

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References

[1] Jemal A, Bray F, Center MM, Ferlay J, Ward E and Forman D. Global cancer statistics. CA Cancer J Clin 2011; 61: 69-90.

[2] Ramalingam SS, Owonikoko TK and Khuri FR. Lung cancer: New biological insights and recent therapeutic advances. CA Cancer J Clin 2011; 61: 91-112.

[3] Cornfield J, Haenszel W, Hammond EC, Lilienfeld AM, Shimkin MB and Wynder EL. Smoking and lung cancer: recent evidence and a discussion of some questions. 1959. Int J Epidemiol 2009; 38: 1175-1191.

[4] Bandera EV, Freudenheim JL and Vena JE. Alcohol consumption and lung cancer: a review of the epidemiologic evidence. Cancer Epidemiol Biomarkers Prev 2001; 10: 813-821.

[5] Freudenheim JL, Ritz J, Smith-Warner SA, Albanes D, Bandera EV, van den Brandt PA, Colditz G, Feskanich D, Goldbohm RA, Harnack L, Miller AB, Rimm E, Rohan TE, Sellers TA, Virtamo J, Willett WC and Hunter DJ. Alcohol consumption and risk of lung cancer: a pooled analysis of cohort studies. Am J Clin Nutr 2005; 82: 657-667.

[6] McKay JD, Hung RJ, Gaborieau V, Boffetta P, Chabrier A, Byrnes G, Zaridze D, Mukeria A, Szeszenia-Dabrowska N, Lissowska J, Rudnai P, Fabianova E, Mates D, Bencko V, Foretova L, Janout V, McLaughlin J, Shepherd F, Montpetit A, Narod S, Krokan HE, Skorpen F, Elvestad MB, Vatten L, Njolstad I, Axelsson T, Chen C, Goodman G, Barnett M, Loomis MM, Lubinski J, Matyjasik J, Lener M, Oszutowska D, Field J, Liloglou T, Xinarianos G, Cassidy A, Vineis P, Clavel-Chapelon F, Palli D, Tumino R, Krogh V, Panico S, Gonzalez CA, Ramon Quiros J, Martinez C, Navarro C, Ardanaz E, Larranaga N, Kham KT, Key T, Bueno-de-Mesquita HB, Peeters PH, Trichopoulou A, Linseisen J, Boeing H, Hallmans G, Overvad K, Tjonneland A, Kumle M, Riboli E, Zelenika D, Boland A, Delepine M, Foglio M, Lechner D, Matsuda F, Blanche H, Gut I, Heath S, Lathrop M and Brennan P. Lung cancer susceptibility locus at 5p15.33. Nat Genet 2008; 40: 1404-1406.

[7] Landi MT, Chatterjee N, Yu K, Goldin LR, Goldstein AM, Rotunno M, Mirabello L, Jacobs K, Wheeler W, Yeager M, Bergen AW, Li Q, Consonni D, Pesatori AC, Wacholder S, Thun M, Diver R, Oken M, Virtamo J, Albanes D, Wang Z, Burdette L, Doheny KF, Pugh EW, Laurie C, Brennan P, Hung R, Gaborieau V, McKay JD, Lathrop M, McLaughlin J, Wang Y, Tsao MS, Spitz MR, Krokan H, Vatten L, Skorpen F, Arnesen E, Benhamou S, Bouchard C, Metspalu A, Vooder T, Nelis M, Valk K, Field JK, Chen C, Goodman G, Sulem P, Thorleifsson G, Rafnar T, Eisen T, Sauter W, Rosenberger A, Bickeboller H, Risch A, Chang-Claude J, Wichmann HE, Stefansson K, Houlston R, Amos CI, Fraumeni JF Jr, Savage SA, Bertazzi PA, Tucker MA, Chanock S and Caporaso NE. A genome-wide association study of lung cancer identifies a region of chromosome 5p15 associated with risk for adenocarcinoma. Am J Hum Genet 2009; 85: 679-691.

[8] Wang Y, Broderick P, Webb E, Wu X, Vijayakrishnan J, Matakidou A, Qureshi M, Dong Q, Gu X, Chen WV, Spitz MR, Eisen T, Amos CI and Houlston RS. Common 5p15.33 and 6p21.33 variants influence lung cancer risk. Nat Genet 2008; 40: 1407-1409.

[9] Hsiung CA, Lan Q, Hong YC, Chen CJ, Hosgood HD, Chang IS, Chatterjee N, Brennan P, Wu C, Zheng W, Chang GC, Wu T, Park JY, Hsiao CF, Kim YH, Shen H, Seow A, Yeager M, Tsai YH, Kim YT, Chow WH, Guo H, Wang WC, Sung SW, Hu Z, Chen KY, Kim JH, Chen Y, Huang L, Lee KM, Lo YL, Gao YT, Liu L, Huang MS, Jung TH, Jin G, Caporaso N, Yu D, Kim CH, Su WC, Shu XO, Xu P, Kim IS, Chen YM, Ma H, Shen M, Cha SI, Tan W, Chang CH, Sung JS, Zhang M, Yang TY, Park KH, Yuenger J, Wang CL, Ryu JS, Xiang Y, Deng Q, Hutchinson A, Kim JS, Cai Q, Landi MT, Yu CJ, Tucker M, Hung JY, Lin CC, Perng RP, Boffetta P, Chen CY, Chen KC, Yang SY, Hu CY, Chang CK, Fraumeni JF Jr, Chanock S, Yang PC, Rothman N and Lin D. The 5p15.33 locus is associated with risk of lung adenocarcinoma in never-smoking females in Asia. PLoS Genet 2010; 6.

[10] Rafnar T, Sulem P, Stacey SN, Geller F, Gudmundsson J, Sigurdsson A, Jakobsdottir M, Helgadottir H, Thorlacius S, Aben KK, Blondal T, Thorgeirsson TE, Thorleifsson G, Kristjansson K, Thorisdottir K, Ragnarsson R, Sigurgeirsson B, Skuladottir H, Gudbjartsson T, Isaksson HJ, Einarsson GV, Benediktsdottir KR, Agnarsson BA, Olafsson K, Salvarsdottir A, Bjarnason H, Asgeirsdottir M, Kristinsson KT, Matthiasdottir S, Sveinsdottir SG, Polidoro S, Hoiom V, Botella-Estrada R, Hemminki K, Rudnai P, Bishop DT, Campagna M, Kellen E, Zeegers MP, de Verdier P, Ferrer A, Isla D, Vidal MJ, Andres R, Saez B, Juberias P, Banzo J, Navarrete S, Tres A, Kan D, Lindblom A, Gurzau E, Koppova K, de Vegt F,

Schalken JA, van der Heijden HF, Smit HJ, Termeer RA, Oosterwijk E, van Hooij O, Nagore E, Porru S, Steineck G, Hansson J, Buntinx F, Catalona WJ, Matullo G, Vineis P, Kiltie AE, Mayordomo JI, Kumar R, Kiemeney LA, Frigge ML, Jonsson T, Saemundsson H, Barkardottir RB, Jonsson E, Jonsson S, Olafsson JH, Gulcher JR, Masson G, Gudbjartsson DF, Kong A, Thorsteinsdottir U and Stefansson K. Sequence variants at the TERT-CLPTM1L locus associate with many cancer types. Nat Genet 2009; 41: 221-227.

[11] Bianchi A and Shore D. How telomerase reaches its end: mechanism of telomerase regulation by the telomeric complex. Mol Cell 2008; 31: 153-165.

[12] Shay JW and Bacchetti S. A survey of telomerase activity in human cancer. Eur J Cancer 1997; 33: 787-791.

[13] Wu TC, Lin P, Hsu CP, Huang YJ, Chen CY, Chung WC, Lee H and Ko JL. Loss of telomerase activity may be a potential favorable prognostic marker in lung carcinomas. Lung Cancer 2003; 41: 163-169.

[14] Blasco MA. Telomeres and human disease: ageing, cancer and beyond. Nat Rev Genet 2005; 6: 611-622.

[15] Baird DM. Variation at the TERT locus and predisposition for cancer. Expert Rev Mol Med 2010; 12: e16.

[16] Mocellin S, Verdi D, Pooley KA, Landi MT, Egan KM, Baird DM, Prescott J, De Vivo I and Nitti D. Telomerase reverse transcriptase locus polymorphisms and cancer risk: a field synopsis and meta-analysis. J Natl Cancer Inst 2012; 104: 840-854.

[17] Timofeeva MN, Hung RJ, Rafnar T, Christiani DC, Field JK, Bickeboller H, Risch A, McKay JD, Wang Y, Dai J, Gaborieau V, McLaughlin J, Brenner D, Narod SA, Caporaso NE, Albanes D, Thun M, Eisen T, Wichmann HE, Rosenberger A, Han Y, Chen W, Zhu D, Spitz M, Wu X, Pande M, Zhao Y, Zaridze D, Szeszenia-Dabrowska N, Lissowska J, Rudnai P, Fabianova E, Mates D, Bencko V, Foretova L, Janout V, Krokan HE, Gabrielsen ME, Skorpen F, Vatten L, Njolstad I, Chen C, Goodman G, Lathrop M, Benhamou S, Vooder T, Valk K, Nelis M, Metspalu A, Raji O, Chen Y, Gosney J, Liloglou T, Muley T, Dienemann H, Thorleifsson G, Shen H, Stefansson K, Brennan P, Amos CI, Houlston R and Landi MT. Influence of common genetic variation on lung cancer risk: meta-analysis of 14 900 cases and 29 485 controls. Hum Mol Genet 2012; 21: 4980-4995.

[18] Wang Y, Broderick P, Matakidou A, Eisen T and Houlston RS. Role of 5p15.33 (TERT-CLPTM1L), 6p21.33 and 15q25.1 (CHRNA5-CHRNA3) variation and lung cancer risk in never-smokers. Carcinogenesis 2010; 31: 234-238.

[19] Choi JE, Kang HG, Jang JS, Choi YY, Kim MJ, Kim JS, Jeon HS, Lee WK, Cha SI, Kim CH, Kam S, Jung TH and Park JY. Polymorphisms in telomere maintenance genes and risk of lung cancer. Cancer Epidemiol Biomarkers Prev 2009; 18: 2773-2781.

[20] Li C, Yin Z, Wu W, Li X and Zhou B. Genetic variants in TERT-CLPTM1L genetic region associated with several types of cancer: a meta-analysis. Gene 2013; 526: 390-399.

[21] Pande M, Spitz MR, Wu X, Gorlov IP, Chen WV and Amos CI. Novel genetic variants in the chromosome 5p15.33 region associate with lung cancer risk. Carcinogenesis 2011; 32: 1493-1499.

[22] Wu H, Qiao N, Wang Y, Jiang M, Wang S, Wang C and Hu L. Association between the Telomerase Reverse Transcriptase (TERT) rs2736098 Polymorphism and Cancer Risk: Evidence from a Case-Control Study of Non-Small-Cell Lung Cancer and a Meta-Analysis. PLoS One 2013; 8: e76372.

[23] Zhang XJ, Xu Z, Gong YL, Tang CJ and Chen JF. Association of TERT rs2736098 polymorphism with cancer risk: a meta-analysis. Asian Pac J Cancer Prev 2012; 13: 4943-4946.

[24] Van Dyke AL, Cote ML, Wenzlaff AS, Abrams J, Land S, Iyer P and Schwartz AG. Chromosome 5p Region SNPs Are Associated with Risk of NSCLC among Women. J Cancer Epidemiol 2009; 2009: 242151.

[25] Hosgood HD 3rd, Menashe I, Shen M, Yeager M, Yuenger J, Rajaraman P, He X, Chatterjee N, Caporaso NE, Zhu Y, Chanock SJ, Zheng T and Lan Q. Pathway-based evaluation of 380 candidate genes and lung cancer susceptibility suggests the importance of the cell cycle pathway. Carcinogenesis 2008; 29: 1938-1943.

[26] Sole X, Guino E, Valls J, Iniesta R and Moreno V. SNPStats: a web tool for the analysis of association studies. Bioinformatics 2006; 22: 1928-1929.

[27] Gago-Dominguez M, Jiang X, Conti DV, Castelao JE, Stern MC, Cortessis VK, Pike MC, Xiang YB, Gao YT, Yuan JM and Van Den Berg DJ. Genetic variations on chromosomes 5p15 and 15q25 and bladder cancer risk: findings from the Los Angeles-Shanghai bladder case-control study. Carcinogenesis 2011; 32: 197-202.

[28] Fachiroh J, Sangrajrang S, Johansson M, Renard H, Gaborieau V, Chabrier A, Chindavijak S, Brennan P and McKay JD. Tobacco consumption and genetic susceptibility to nasopharyngeal carcinoma (NPC) in Thailand. Cancer Causes Control 2012; 23: 1995-2002.

[29] Chen H, Chen Y, Zhao Y, Fan W, Zhou K, Liu Y, Zhou L, Mao Y, Wei Q, Xu J and Lu D. Association

of sequence variants on chromosomes 20, 11, and 5 (20q13.33, 11q23.3, and 5p15.33) with glioma susceptibility in a Chinese population. Am J Epidemiol 2011; 173: 915-922.

[30] Zhang C, Tian YP, Wang Y, Guo FH, Qin JF and Ni H. hTERT rs2736098 genetic variants and susceptibility of hepatocellular carcinoma in the Chinese population: a case-control study. Hepatobiliary Pancreat Dis Int 2013; 12: 74-79.

[31] Wang S, Wu J, Hu L, Ding C, Kan Y, Shen Y, Chen X, Shen H, Guo X and Hu Z. Common genetic variants in TERT contribute to risk of cervical cancer in a Chinese population. Mol Carcinog 2012; 51 Suppl 1: E118-122.

[32] Savage SA, Chanock SJ, Lissowska J, Brinton LA, Richesson D, Peplonska B, Bardin-Mikolajczak A, Zatonski W, Szeszenia-Dabrowska N and Garcia-Closas M. Genetic variation in five genes important in telomere biology and risk for breast cancer. Br J Cancer 2007; 97: 832-836.

[33] Liu Z, Li G, Wei S, Niu J, Wang LE, Sturgis EM and Wei Q. Genetic variations in TERT-CLPTM1L genes and risk of squamous cell carcinoma of the head and neck. Carcinogenesis 2010; 31: 1977-1981.

Table S1. Primers used to amplify TERT genetic polymorphisms analyzed in this study

SNPs	Forward primer for PCR (5'-3')	Reverse Primer for PCR (5'-3')	Primer for extension reactions (5'-3')
rs4246742	ACGTTGGATGCCTTGATCCACTGTACATGC	ACGTTGGATGAGGCCACACAGCCATTTCTC	AGGAAAAACCCGGAG
rs10069690	ACGTTGGATGACCCCGTCATCTGAGGAGA	ACGTTGGATGATGTGTGTTGCACACGGGAT	cGAGGTGGACAGAGGT
rs2736098	ACGTTGGATGTTTGGAGGGTGCGCTCTCTG	ACGTTGGATGTGGTGGCCGCGATGTGGAT	gtCCGCCAGCACCACGC
rs2853672	ACGTTGGATGGGGTGGGAGGTAAGGGTT	ACGTTGGATGCTGGCCGTCAACACACAATT	gaAGGTAAGGGTTTTGCAG
rs2242652	ACGTTGGATGAGGCTCTGAGGACCACAAGA	ACGTTGGATGACAGCAGGACACGGATCCAG	cccctAGGACCACAAGAAGCAGC
rs2736122	ACGTTGGATGAAAGGAGTCAAGCAGCTTGC	ACGTTGGATGTCTCTCTCTGCCTCAAATC	cccccCGTCAGGGAGATGCAAAC
rs2853676	ACGTTGGATGACTAAGACCCAAGAGGGAAG	ACGTTGGATGAGGTTGGAGTGTCTCTGTCT	ggatAGGGAAGTCTGACGAAGGC
rs2736114	ACGTTGGATGGCTGTGCTTTAGACAAAGGG	ACGTTGGATGAAGCTGACGACTGGTGTTGC	GTTCCTGCTCAGGCG
rs2075786	ACGTTGGATGCAGGTTACACACGTGGTGAG	ACGTTGGATGAGCCGCCACTCTTGACTTTC	gtggGTGCAGGCGGTGACC

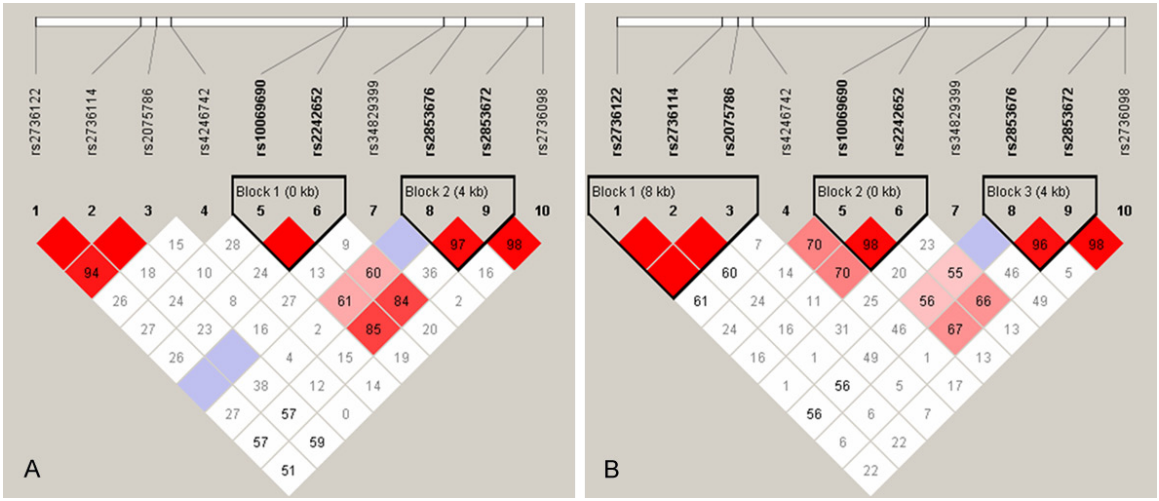

Figure S1. Linkage disequilibrium (LD) of polymorphic sites in the *TERT* gene in controls (A) and lung cancer patients (B). The LD values are coded with bright red for very strong LD (LOD=2, *D*'=1), white for no LD (LOD < 2, *D*' < 1), and pink (LOD=2, *D*' < 1) or blue (LOD < 2, *D*'=1) for intermediate LD.

Table S2. Stratified analysis of allelic associations of selected TERT minor alleles with respect to lung cancer risk

SNP	Population	MAF		ORs[a]	95% CI		*P* value	HWE *P*
		Cases	Controls		Lower	Upper		
rs2736122	Female	0.05	0.044	1.85	0.55	6.25	0.321	0.623
	Male	0.059	0.04	1.96	0.84	4.59	0.121	0.184
	Age ≤ 50	0.066	0.054	1.62	0.62	4.26	0.328	0.438
	Age > 50	0.063	0.026	2.32	0.83	6.47	0.109	0.756
	Ever-smokers	0.061	0.017	4.15	1.11	15.52	0.035	0.872
	Non-smokers	0.048	0.052	1.23	0.48	3.18	0.664	0.556
	Drinkers	0.054	0.023	2.71	0.45	16.42	0.278	0.849
	Non-drinkers	0.058	0.047	1.91	0.89	4.08	0.096	0.478
rs2736114	Female	0.05	0.04	1.98	0.92	4.29	0.081	0.659
	Male	0.059	0.04	1.96	0.84	4.59	0.121	0.182
	Age ≤ 50	0.065	0.051	1.71	0.65	4.53	0.279	0.363
	Age > 50	0.063	0.026	2.31	0.83	6.46	0.11	0.756
	Ever-smokers	0.061	0.016	4.17	1.11	15.65	0.034	0.873
	Non-smokers	0.048	0.05	1.3	0.5	3.36	0.595	0.49
	Drinkers	0.054	0.023	2.76	0.45	16.79	0.27	0.85
	Non-drinkers	0.058	0.045	1.98	0.92	4.29	0.081	0.417
rs2075786	Female	0.12	0.115	1.2	0.55	2.65	0.644	0.641
	Male	0.152	0.165	1.07	0.65	1.77	0.8	0.953
	Age ≤ 50	0.166	0.144	1.47	0.75	2.88	0.258	0.329
	Age > 50	0.144	0.149	0.85	0.5	1.44	0.546	0.503
	Ever-smokers	0.155	0.121	1.13	0.64	1.99	0.683	0.508
	Non-smokers	0.119	0.157	0.97	0.51	1.83	0.925	0.923
	Drinkers	0.108	0.144	0.53	0	1.45	0.216	0.714
	Non-drinkers	0.152	0.147	1.26	0.79	2.03	0.337	0.626
rs4246742	Female	0.27	0.341	0.73	0.42	1.25	0.246	0.104
	Male	0.317	0.372	0.73	0.49	1.07	0.105	0.77
	Age ≤ 50	0.304	0.38	0.63	0.37	1.05	0.078	0.111
	Age > 50	0.328	0.336	0.74	0.5	1.1	0.138	0.965
	Ever-smokers	0.312	0.412	0.58	0.38	0.88	0.011	0.27
	Non-smokers	0.294	0.338	0.89	0.57	1.41	0.625	0.538
	Drinkers	0.257	0.371	0.51	0.26	1	0.049	0.125
	Non-drinkers	0.317	0.357	0.77	0.54	1.09	0.135	0.575
rs10069690	Female	0.21	0.19	1.17	0.63	2.17	0.625	0.244
	Male	0.212	0.152	1.5	0.92	2.43	0.103	0.7
	Age ≤ 50	0.215	0.174	1.11	0.61	2.03	0.732	0.603
	Age > 50	0.212	0.157	1.58	0.96	2.57	0.069	0.264
	Ever-smokers	0.21	0.165	1.51	0.86	2.63	0.148	0.719
	Non-smokers	0.214	0.167	1.25	0.75	2.09	0.388	0.116
	Drinkers	0.189	0.174	1.51	0.64	3.55	0.346	0.997
	Non-drinkers	0.216	0.164	1.36	0.9	2.06	0.147	0.2
rs2242652	Female	0.22	0.181	1.1	0.37	3.22	0.867	0.417
	Male	0.202	0.149	1.29	0.6	2.79	0.51	0.596
	Age ≤ 50	0.204	0.168	1	0.31	3.15	0.993	0.866
	Age > 50	0.203	0.153	1.46	0.68	3.13	0.336	0.189
	Ever-smokers	0.2	0.157	1.94	0.77	4.89	0.158	0.872

	Non-smokers	0.222	0.163	0.87	0.35	2.2	0.772	0.21
	Drinkers	0.189	0.164	3.84	0.87	16.9	0.076	0.8
	Non-drinkers	0.209	0.16	0.99	0.49	2	0.977	0.334
rs34829399	Female	0.08	0.054	1.18	0.62	2.23	0.611	0.549
	Male	0.067	0.048	1.12	0.7	1.79	0.645	0.491
	Age ≤ 50	0.065	0.042	1.15	0.64	2.07	0.648	0.571
	Age > 50	0.078	0.06	1.1	0.68	1.78	0.704	0.462
	Ever-smokers	0.073	0.044	1	0.6	1.69	0.992	0.661
	Non-smokers	0.063	0.053	1.32	0.79	2.23	0.292	0.422
	Drinkers	0.095	0.03	0.74	0.34	1.59	0.439	0.8
	Non-drinkers	0.065	0.056	1.3	0.84	2	0.241	0.368
rs2853676	Female	0.22	0.174	1.1	0.68	1.78	0.7	0.087
	Male	0.175	0.16	1.56	1.08	2.26	0.018	0.519
	Age ≤ 50	0.183	0.163	1.27	0.78	2.07	0.335	0.137
	Age > 50	0.186	0.169	1.47	1.03	2.11	0.036	0.462
	Ever-smokers	0.174	0.189	1.58	1.05	2.39	0.029	0.885
	Non-smokers	0.214	0.156	1.22	0.81	1.84	0.335	0.038
	Drinkers	0.176	0.223	1.01	0.53	1.91	0.981	0.584
	Non-drinkers	0.187	0.15	1.51	1.09	2.09	0.014	0.155
rs2853672	Female	0.49	0.496	1.09	0.65	1.82	0.752	0.019
	Male	0.503	0.417	1.72	1.14	2.6	0.01	0.93
	Age ≤ 50	0.482	0.482	1.23	0.71	2.12	0.464	0.653
	Age > 50	0.496	0.402	1.72	1.15	2.57	0.008	0.106
	Ever-smokers	0.497	0.416	1.82	1.14	2.92	0.013	0.114
	Non-smokers	0.508	0.46	1.27	0.82	1.98	0.284	0.461
	Drinkers	0.473	0.445	1.48	0.72	3.04	0.288	0.243
	Non-drinkers	0.505	0.447	1.52	1.06	2.17	0.022	0.281
rs2736098	Female	0.35	0.35	1.85	0.55	6.25	0.321	0.622
	Male	0.385	0.306	1.96	0.84	4.59	0.121	0.059
	Age ≤ 50	0.394	0.359	1.62	0.62	4.26	0.328	0.011
	Age > 50	0.496	0.402	2.32	0.83	6.47	0.109	0.106
	Ever-smokers	0.376	0.281	4.15	1.11	15.52	0.035	0.991
	Non-smokers	0.381	0.34	1.23	0.48	3.18	0.664	0.182
	Drinkers	0.365	0.269	2.71	0.45	16.42	0.278	0.856
	Non-drinkers	0.38	0.338	1.91	0.89	4.08	0.096	0.172