ORIGINAL RESEARCH

Polymorphism in lncRNA AC008392.1 and its interaction with smoking on the risk of lung cancer in a Chinese population

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Purpose: To evaluate the association between rs7248320 in lncRNA AC008392.1 and the risk of lung cancer, this case–control study was carried out in a Chinese population. This study also evaluated the gene–environment interaction between rs7248320 and exposure to smoking status on the risk of lung cancer.

Patients and methods: We conducted a hospital-based case–control study including 512 lung cancer cases and 588 healthy controls. The association between rs7248320 and the risk of lung cancer was analyzed, and the gene–environment interaction was estimated on an additive scale.

Results: The variant genotype of rs7248320 was significantly related to the risk of non-small-cell lung cancer (NSCLC). Individuals carrying homozygous GG genotype had decreased risk of NSCLC, compared with individuals carrying the homozygous wild AA genotype/heterozygote GA genotype (adjusted odds ratio [OR] =0.653, 95% confidence interval [CI] =0.442–0.966, P=0.033). Moreover, in the subgroup of ages, there were statistically significant associations between rs7248320 and the risk of lung cancer and NSCLC in the population over 60 years of age. Compared with individuals carrying genotypes AA/GA, individuals with genotype GG had the lower risk of lung cancer and NSCLC (adjusted ORs were 0.579 and 0.433, 95% CIs were 0.338–0.994 and 0.231–0.811, P-values were 0.048 and 0.009, respectively). Compared with homozygote AA, the homozygote GG was associated with a decreased risk in NSCLC (OR =0.456, 95% CI =0.235–0.887, P=0.021). There were no statistically significant results in gene–environment interactions on an additive scale.

Conclusion: These findings suggest that lncRNA AC008392.1 rs7248320 may be involved in genetic susceptibility to NSCLC in a Chinese population.

Keywords: lung cancer, single-nucleotide polymorphism, lncRNA, susceptibility, CARD8

Introduction

Lung cancer, remaining an important public health burden worldwide, was a leading cause of cancer-related morbidity and mortality around the world, especially in China.[1,2] Data in the International Agency for Research on Cancer by Region (2008–2012) indicated that lung cancer was one of the most common form of malignancy affecting human worldwide.

The increasing number of studies have shown that the tumorigenesis of lung cancer is a complex process involving multiple genetic, environmental factors, and the interaction of them.[3,4] The development of high-throughput DNA sequencing and array projects, including the Encyclopedia of DNA Elements,[5] 29 Mammals Project,[6] and Health Roadmap Epigenomics Project,[7] leads to the identification of non-coding RNAs

(ncRNAs).[8] Of the two types of human genome sequences, ncRNAs and protein coding RNAs, ~99% are ncRNAs, which also play an important role in regulating transcription.[9,10] As the regulators of protein coding genes, ncRNAs include small nuclear RNA, microRNA (miRNA), small interfering RNA, long non-coding RNA (lncRNA), and so on.[11] In the past years, most studies focused on miRNAs that influenced various cellular processes including inhibiting cell proliferation, inducing cell apoptosis, and so on.[12–15]

In human genome, lncRNAs are vital parts of "dark matter."[9] As the vast majority of ncRNAs, lncRNAs are becoming the new focus of science studies.[16–18] The length of lncRNAs are >200 nucleotides,[19] and lncRNAs can influence the pathologic processes, comprising disease and carcinogenesis.[8,20–22] Furthermore, due to the attention on lncRNAs, many studies found that lncRNAs played important roles in cellular processes such as cell growth,[23] cell apoptosis,[24] cell differentiation,[25] cell proliferation,[26] cell metastasis, cancer progression, and autophagy.[27,28] Accumulated evidence has shown that the single-nucleotide polymorphisms (SNPs) are the most common genetic variants in miRNAs and lncRNAs.[12,29]

As a member of caspase recruitment domain family, caspase recruitment domain family member 8 (CARD8), which is involved in the interleukin-1β (IL-1β) processing pathway,[30,31] protein–protein interaction modules, cell apoptosis,[32] the suppressor of caspase-1, and the activation of nuclear factor kappa-B (NF-κB).[33] NF-κB is a key regulator for gene transcription and tumor genesis.[34] And the activation of NF-kB was effectively suppressed by CARD8 through inflammatory mediators.[35] The defects of apoptosis are seen in many forms of cancer.[36] CARD8 has influence on cancers by decreasing the cell apoptosis.[30,37] The expression of CARD8 was related with poor prognosis in colon cancer cases and the high expression level of CARD8 could reduce the survival time of colon cancer cases.[32,37,38] The overexpression of CARD8 was found in non-small-cell lung cancer (NSCLC) cells rather than small-cell lung cancer (SCLC).[39] Recently, a pilot study has shown that homozygous mutations of CARD8 might conduce to the higher susceptibility of cancers including lung cancer.[39,40]

LncRNA AC008392.1, located in the upstream region of CARD8 in the long arm of the nineteenth chromosome, is one of the recently identified lncRNAs. The expression of lncRNA AC008392.1 is found in many cell lines including human B lymphocyte and human cervical cancer cells.[41] Some studies have demonstrated that rs7248320 in AC008392.1 may represent the expression quantitative trait loci (eQTL) for

CARD8. Hence, it may change the expression of CARD8.[41,42] Yin et al reported that the variant genotype of rs7248320GG increased the risks of hepatocellular carcinoma (HCC) and cervical cancer.[41] Therefore, rs7248320 may relate with the risks of cancers by influencing the interaction between lncRNA AC008392.1 and CARD8. And then, we assumed that the SNP rs7248320 may alter the risk of lung cancer by influencing the expression of CARD8. To evaluate the role of rs7248320 in lncRNA AC008392.1 on the susceptibility of lung cancer, we carried out a case–control study including 512 lung cancer patients and 588 controls.

Materials and methods
Study subjects
This study is a hospital-based case–control study, which is carried out in Shenyang City, northeast China. We recruited 512 lung cancer patients and 588 healthy controls. The inclusion criteria for cases were: 1) newly confirmed as lung cancer patients, 2) no treatment (both chemotherapy and radiotherapy), and 3) capability to have a 1.5 h interview. The cases were without any previous cancer or metastatic cancer. The inclusion criteria for healthy controls were: 1) without history of cancer, 2) selected from the same hospital during the same period, and 3) matched to case subjects by age (±5 years). Participants with unrelated kinship were Chinese Han population. This case–control study was approved by the Institutional Review Board of China Medical University, and each participant signed an informed consent. For each participant, an interview was scheduled to get the information on demographic data and environmental exposure status when she or he was admitted to hospital, and all the participants donated ~10 mL of venous blood sample for SNP detection. An individual who smoked <100 cigarettes in his lifetime was defined as non-smoker, otherwise was categorized as smoker. The minor allele frequency (MAF) of our selected SNP was >5% in Chinese population. Genomic DNA samples were extracted from venous blood by phenol–chloroform method. An Applied Biosystems 7500 FAST Real-Time PCR System using Taqman® allelic discrimination was used for SNP genotyping. Negative control was included in each genotyping. More than 10% of samples which were tested twice by two persons were randomly selected, and the results showed that the concordance rate was 100%.

Statistical analysis
Student's *t*-test and χ² test were respectively used to calculate the differences of demographic variables and genotype distribution of SNP rs7248320 in continuous and categorical

variables between the case group and the control group. The goodness-of-fit χ^2 test was performed to analyze the Hardy–Weinberg equilibrium (HWE) of the SNP rs7248320. Unconditional logistic regression analyses calculated the odds ratios (ORs) and their 95% confidence intervals (CIs) to estimate the associations between SNP rs7248320 and the risk of lung cancer and NSCLC. Statistical significance was set as $P<0.05$, and all tests were two-sided, and all of the statistical analyses were performed by using SPSS (version 20.0).

Results

This ongoing epidemiologic study included 512 cases of lung cancer and 588 controls of healthy population, whose characteristics are described in Table 1. There were no statistically significant differences in the proportion of age and sex status between the lung cancer case group (mean age 58.04±11.626 years and 66.8% females) and the control group (mean age 56.52±15.781 years and 68.20% females). However, the distribution of smoking status was significantly higher in lung cancer cases than in healthy controls ($P=0.006$). In addition, tumor node metastasis (TNM) stage (17.8% of TNM stage I and II, 40.4% of TNM stage III, 12.5% of TNM stage IV, and 29.3% were missing) and pathological type (50.4% of lung adenocarcinoma [(AD], 21.5% of lung squamous cell carcinoma [SQ], 23.0% of small cell lung cancer [SCC], and 5.1% of others) of the cases were listed. The frequency of genotype was expected under HWE in controls ($P=0.11332>0.05$). Table 2 shows the observed allele and genotype frequency distribution for rs7248320 SNP in case subjects and control

Table 1 Distribution of demographic variables in lung cancer cases and controls

Variables	Cases (N=512)	Controls (N=588)	P-value
Age (mean ± SD)	58.04±11.626	56.52±15.781	0.071
Gender			0.621
Female	342 (66.8%)	401 (68.20%)	
Male	170 (33.2%)	187 (31.8%)	
Smoking status			0.006
Ever	144 (28.1%)	121 (20.6%)	
Never	368 (71.9%)	467 (79.4%)	
TNM stage			
I, II	91 (17.8%)		
III	207 (40.4%)		
IV	64 (12.5%)		
Other	150 (29.3%)		
Pathological type			
AD	258 (50.4%)		
SQ	110 (21.5%)		
SCC	118 (23.0%)		
Other	26 (5.1%)		

Abbreviations: AD, lung adenocarcinoma; SCC, small-cell lung cancer; SQ, lung squamous cell carcinoma.

Table 2 The association of rs7248320 with lung cancer risk and non-small-cell lung cancer risk

Genotype	Controls (%) (n=588)	Lung cancer					Non-small-cell lung cancer				
		Cases (%) (n=512)	OR (95% CI)	P-value	OR^a (95% CI)	P^a-value	Cases (%) (n=377)	OR (95% CI)	P-value	OR^a (95% CI)	P^a-value
AA	235 (40.0)	195 (38.1)	1.00 (ref)				145 (38.5)	1.00 (ref)			
GA	259 (44.0)	252 (49.2)	1.173 (0.907–1.516)	0.225	1.159 (0.895–1.502)	0.263	190 (50.4)	1.189 (0.899–1.572)	0.224	1.164 (0.879–1.541)	0.290
GG	94 (16.0)	65 (12.7)	0.833 (0.576–1.205)	0.333	0.816 (0.563–1.182)	0.282	42 (11.1)	0.724 (0.476–1.100)	0.131	0.710 (0.466,1.081)	0.110
GG+GA vs AA			1.082 (0.849–1.380)	0.524	1.068 (0.836–1.363)	0.600		1.065 (0.817–1.389)	0.641	1.043 (0.798–1.362)	0.759
GG vs GA+AA			0.764 (0.543–1.075)	0.122	0.753 (0.534–1.061)	0.105		0.659 (0.446–0.972)	0.036	0.653 (0.442–0.966)	0.033
A allele	729 (62.0)	642 (62.7)	1.00 (ref)				480 (63.7)	1.00 (ref)			
G allele	447 (38.0)	382 (37.3)	0.970 (0.816–1.154)	0.733			274 (36.3)	0.931 (0.770–1.25)	0.459		

Note: ^aAdjusted for age, gender, and smoking.
Abbreviations: CI, confidence interval; OR, odds ratio; ref, reference.

subjects. The associations between the SNP rs7248320 and risk of lung cancer and NSCLC are also shown in Table 2. There were no statistically significant associations between rs7248320 polymorphism and risk of lung cancer in this present study in four models (GA vs AA: OR =1.159, 95% CI =0.895–1.502, P=0.263; GG vs AA: OR =0.816, 95% CI =0.536–1.182, P=0.282; GG+GA vs AA: OR =1.068, 95% CI =0.836–1.363, P=0.600; GG vs GA+AA: OR =0.753, 95% CI =0.534–1.061, P=0.105; adjusted for age, sex, and smoking status). We further performed subgroup analysis stratified by pathological type of lung cancer to investigate whether rs7248320 relate with the risk of NSCLC, SQ, and AD. We found that individuals carrying homozygous GG genotype had decreased risk of NSCLC by 0.653-fold (adjusted OR =0.653, 95% CI =0.442–0.966, P=0.033) compared with individuals carrying the homozygous wild AA genotype/the heterozygote GA genotype. The associations of rs7248320 with SQ and AD are listed in Table 3. We failed to find the statistically significant association between rs7248320 and SQ. The same results were also found in AD group. Table 4 shows the associations between rs7248320 and lung cancer and NSCLC in female and male, respectively. In the subgroup of sex, there were no statistically significant associations between rs7248320 polymorphism and overall risk of lung cancer and NSCLC in female. The same results existed in male. We performed a subgroup-stratified analysis by smoking status, but we failed to find any statistically significant associations, and the results were shown in Table 5. In the subgroup analysis of ages, we found the significant association between rs7248320 and lung cancer risk in the second group (>60 years). Compared with the individuals carrying genotypes AA/GA, individuals carrying genotype GG had the lower lung cancer risk by 0.579-fold (adjusted 95% CI =0.338–0.994, P=0.048). The same results existed in NSCLC in Table 6. Compared with homozygote AA, the homozygote GG was associated with a decreased risk in NSCLC (adjusted OR =0.456, 95% CI =0.235–0.887, P=0.021). Individuals with GG genotype also had a reduced susceptibility of NSCLC compared with homozygote AA and genotype GA (adjusted OR =0.433, 95% CI =0.231–0.811, P=0.009). In further analysis, we wanted to know about the interaction between the SNP rs7248320 and smoking exposure on the susceptibility of lung cancer, NSCLC, AD, and SQ (Tables 7–9). Compared to individuals with both GA/AA genotypes and exposure to smoking status, individuals with homozygous GG genotype and never smoking status had the increased risk of lung cancer and NSCLC (adjusted ORs were 2.319 and 1.963, 95% CIs were 1.416–3.798 and

Table 3 The association of rs7248320 with lung adenocarcinoma risk and lung squamous cell carcinoma risk

Genotype	Controls (%) (n=588)	Lung adenocarcinoma						lung squamous cell carcinoma					
		Cases (%) (n=258)	OR (95% CI)	P-value	OR^a (95% CI)	P^a-value		Cases (%) (n=110)	OR (95% CI)	P-value	OR^a (95% CI)	P^a-value	
AA	235 (40.0)	94 (36.4)	1.00 (ref)					49 (44.5)	1.00 (ref)				
GA	259 (44.0)	132 (51.2)	1.274 (0.927–1.751)	0.135	1.236 (0.893–1.709)	0.201		51 (46.4)	0.941 (0.614–1.452)	0.794	0.917 (0.588–1.431)	0.703	
GG	94 (16.0)	32 (12.4)	0.851 (0.533–1.358)	0.499	0.839 (0.522–1.349)	0.469		10 (9.1)	0.510 (0.248–1.049)	0.067	0.504 (0.241–1.053)	0.068	
GG+GA vs AA			1.161 (0.858–1.572)	0.332	1.131 (0.831–1.540)	0.435			0.829 (0.550–1.250)	0.370	0.807 (0.528–1.234)	0.322	
GG vs GA+AA			0.744 (0.484–1.145)	0.179	0.746 (0.481–1.156)	0.189			0.526 (0.265–1.044)	0.066	0.527 (0.262–1.062)	0.073	
A allele	729 (62.0)	320 (62.0)	1.00 (ref)					149 (67.7)	1.00 (ref)				
G allele	447 (38.0)	196 (38.0)	0.999 (0.807–1.236)	0.992				71 (32.3)	0.777 (0.572–1.056)	0.106			

Note: ^aAdjusted for age, gender, and smoking.
Abbreviations: CI, confidence interval; OR, odds ratio; ref, reference.

Table 4 The association of rs7248320 with lung cancer risks and non-small-cell lung cancer risks in female and male populations

Genotype and gender	Controls (%) (n=588)	Lung cancer					Non-small cell lung cancer				
		Cases (%) (n=512)	OR (95% CI)	P-value	ORa (95% CI)	Pa-value	Cases (%) (n=377)	OR (95% CI)	P-value	ORa (95% CI)	Pa-value
Female											
AA	152 (37.9)	136 (39.8)	1.00 (ref)				107 (38.2)	1.00 (ref)			
GA	184 (45.9)	165 (48.2)	1.002 (0.733–1.370)	0.989	1.006 (0.735–1.376)	0.973	141 (50.4)	1.089 (0.782–1.515)	0.615	1.108 (0.795–1.545)	0.544
GG	65 (16.2)	41 (12.0)	0.705 (0.448–1.110)	0.131	0.708 (0.449–1.116)	0.136	32 (11.4)	0.699 (0.428–1.142)	0.153	0.705 (0.431–1.152)	0.163
GG+GA vs AA			0.925 (0.688–1.243)	0.604	0.928 (0.689–1.248)	0.620		0.987 (0.721–1.352)	0.953	1.002 (0.731–1.374)	0.989
GG vs GA+AA			0.704 (0.462–1.072)	0.102	0.706 (0.463–1.075)	0.105		0.667 (0.424–1.050)	0.08	0.666 (0.422–1.049)	0.08
A allele	488 (60.8)	437 (63.9)	1.00 (ref)				355 (63.4)	1.00 (ref)			
G allele	314 (39.2)	247 (36.1)	0.878 (0.711–1.085)	0.228			205 (36.6)	0.897 (0.718–1.122)	0.341		
Male											
AA	83 (44.4)	59 (34.7)	1.00 (ref)				38 (39.2)	1.00 (ref)			
GA	75 (40.1)	87 (51.2)	1.632 (1.036–2.571)	0.035	1.554 (0.957–2.524)	0.075	49 (50.5)	1.427 (0.843–2.415)	0.185	1.299 (0.740–2.283)	0.362
GG	29 (15.5)	24 (14.1)	1.164 (0.617–2.198)	0.639	1.018 (0.520–1.992)	0.958	10 (10.3)	0.753 (0.333–1.701)	0.495	0.649 (0.276–1.530)	0.324
GG+GA vs AA			1.501 (0.979–2.303)	0.063	1.397 (0.886–2.202)	0.15		1.239 (0.752–2.042)	0.4	1.110 (0.651–1.892)	0.702
GG vs GA+AA			0.896 (0.499–1.609)	0.712	0.803 (0.433–1.491)	0.488		0.626 (0.291–1.346)	0.23	0.566 (0.253–1.263)	0.165
A allele	241 (64.4)	205 (60.3)	1.00 (ref)				125 (64.4)	1.00 (ref)			
G allele	133 (35.6)	135 (39.7)	1.193 (0.881–1.616)	0.253			69 (35.6)	1.000 (0.696–1.437)	0.999		

Note: [a]Adjusted for age, gender, and smoking.
Abbreviations: CI, confidence interval; OR, odds ratio; ref, reference.

1.134–3.399, P-values were 0.001 and 0.016, respectively). Similarly, the same results existed in SQ (adjusted OR =4.846, 95% CI =1.892–12.416, P=0.001). However, the quantitative analyses suggested that the interactions between rs7248320 in lncRNA AC008392.1 and exposure to smoking on risks of lung cancer, NSCLC, AD, and SQ were not significant on an additive scale (Table 9). In additive model interaction, three measures (relative excess risk due to interaction and the attributable proportion due to interaction, and the synergy index) with their 95% CI were used to show the relationship, and the criterion of these three measures was just as in our previous study.[43]

Discussion

During the period from 2008 to 2012, the Cancer Registry of the Center of China counted 13,155 new cases of pulmonary diseases (trachea, bronchus, and lung cancer). The crude incidence rate of cancer was 44.9 per 100,000s and the age-standardized incidence rate (ASR) world was 52.7 per 100,000s for men during the study period. Then, there were 28.2 new cancer cases per 100,000s, and the ASR world was 28.4 per 100,000s during the study period. Moreover, lung cancer is one of the cancers that is most difficult to diagnose at the early stage, and most lung cancer cases are too late to be treated.[8,28] In recent years, lncRNAs were identified by progressive science and technology; the functions of lncRNAs are becoming the hotspot in different diseases including tumor. The dysregulations of lncRNAs may influence the level of gene expression, thus potentially playing an essential role on susceptibility to lung cancer. Many studies have shown that lncRNAs were deregulated in lung cancer, such as MALAT1 (NEAT2),[44,45] HOTAIR,[46] SOX2-OT,[47] and H19.[48] MALAT1 could be associated with prognostic parameter for poor survival[49] and cell migration[50] in lung cancer cases. Compared with normal lung tissue, the expression of HOTAIR was higher in lung cancer tissues.[51] SOX2-OT could play a vital role in regulating cell proliferation and become a novel indicator for lung cancer.[52]

LncRNA AC008392.1, located in the upstream region of CARD8, may affect the normal expression of CARD8.[41] CARD8 may influence the tumor biology by inhibiting cell apoptosis[32,53] and involving the NF-kB pathway.[54] In dbSNP database, the MAF of rs7248320 in the Chinese population was 0.402 (MAF >0.05). The relationship between rs7248320 and other cancers risk have been reported in the past years. Yin et al[41] reported that the variant genotype of GG increased the risk of HCC (adjusted OR =1.28, 95% CI =1.03–1.61, P=0.028) as compared with individuals with

Table 5 Stratified analyses of rs7248320 with lung cancer risks and non-small-cell lung cancer risks by smoking status

Genotype and smoking status	Controls (%) (n=588)	Lung cancer					Non-small-cell lung cancer				
		Cases (%) (n=512)	OR (95% CI)	P-value	OR^a (95% CI)	P^a-value	Cases (%) (n=377)	OR (95% CI)	P-value	OR^a (95% CI)	P^a-value
Never smoking											
AA	188 (40.3)	142 (38.6)	1.00 (ref)				113 (37.9)	1.00 (ref)			
GA	204 (43.7)	182 (49.5)	1.181 (0.879–1.588)	0.27	1.180 (0.876–1.588)	0.277	151 (50.7)	1.231 (0.899–1.686)	0.194	1.213 (0.883–1.666)	0.234
GG	75 (16.1)	44 (12.0)	0.777 (0.505–1.196)	0.251	0.776 (0.503–1.197)	0.252	34 (11.4)	0.754 (0.473–1.204)	0.237	0.742 (0.463–1.190)	0.216
GG+GA vs AA			1.072 (0.811–1.419)	0.624	1.071 (0.808–1.419)	0.633		1.103 (0.819–1.487)	0.519	1.086 (0.803–1.469)	0.591
GG vs GA+AA			0.710 (0.476–1.059)	0.093	0.710 (0.475–1.061)	0.095		0.673 (0.436–1.039)	0.074	0.668 (0.431–1.035)	0.071
A allele	580 (62.1)	466 (63.3)	1.00 (ref)				377 (63.3)	1.00 (ref)			
G allele	354 (37.9)	270 (36.7)	0.949 (0.777–1.159)	0.61			219 (36.7)	0.952 (0.769–1.177)	0.649		
Smoking											
AA	47 (38.8)	53 (36.8)	1.00 (ref)				32 (40.5)	1.00 (ref)			
GA	55 (45.5)	70 (48.6)	1.129 (0.666–1.914)	0.653	1.047 (0.600–1.826)	0.873	39 (49.4)	1.041 (0.567–1.914)	0.896	0.971 (0.507–1.859)	0.928
GG	19 (15.7)	21 (14.6)	0.980 (0.470–2.043)	0.957	0.915 (0.426–1.967)	0.821	8 (10.1)	0.618 (0.242–1.584)	0.316	0.600 (0.223–1.612)	0.311
GG+GA vs AA			1.091 (0.663–1.795)	0.733	1.012 (0.599–1.710)	0.964		0.933 (0.523–1.665)	0.814	0.874 (0.472–1.621)	0.670
GG vs GA+AA			0.917 (0.467–1.798)	0.8	0.893 (0.443–1.799)	0.751		0.605 (0.251–1.458)	0.263	0.610 (0.242–1.535)	0.294
A allele	149 (61.6)	176 (61.1)	1.00 (ref)				103 (65.2)	1.00 (ref)			
G allele	93 (38.4)	112 (38.9)	1.020 (0.718–1.448)	0.914			55 (34.8)	0.856 (0.564–1.299)	0.464		

Note: ^{a}Adjusted for age, gender, and smoking.
Abbreviations: CI, confidence interval; OR, odds ratio; ref, reference.

Table 6 Stratified analyses of rs7248320 with lung cancer risk and non-small-cell lung cancer risk by age

Genotype and age	Controls (%) (n=614)	Lung cancer					Non-small-cell lung cancer				
		Cases (%) (n=434)	OR (95% CI)	P-value	OR^a (95% CI)	P^a-value	Cases (%)	OR (95% CI)	P-value	OR^a (95% CI)	P^a-value
≤60 years											
AA	129 (40.4)	106 (37.6)	1.00 (ref)		1.00 (ref)		69 (35.9)	1.00 (ref)		1.00 (ref)	
GA	142 (44.5)	135 (47.9)	1.157 (0.816–1.640)	0.412	1.104 (0.770–1.583)	0.590	96 (50.0)	1.264 (0.855–1.868)	0.24	1.122 (0.747–1.685)	0.578
GG	48 (15.0)	41 (14.5)	1.040 (0.637–1.696)	0.877	0.916 (0.553–1.519)	0.735	27 (14.1)	1.052 (0.604–1.831)	0.859	0.917 (0.516–1.629)	0.768
GG+GA vs AA			1.127 (0.811–1.566)	0.475	1.055 (0.751–1.482)	0.757		1.210 (0.836–1.752)	0.312	1.070 (0.728–1.572)	0.732
GG vs GA+AA			0.960 (0.612–1.509)	0.861	0.868 (0.545–1.384)	0.553		0.924 (0.555–1.538)	0.761	0.859 (0.508–1.455)	0.573
A allele	400 (62.7)	347 (61.5)	1.00 (ref)				234 (60.9)	1.00 (ref)			
G allele	238 (37.3)	217 (38.5)	1.051 (0.832–1.327)	0.676			150 (39.1)	1.077 (0.830–1.398)	0.575		
>60 years											
AA	106 (39.4)	89 (38.7)	1.00 (ref)				76 (41.1)	1.00 (ref)			
GA	117 (43.5)	117 (50.9)	1.191 (0.814–1.743)	0.368	1.138 (0.769–1.682)	0.518	94 (50.8)	1.121 (0.751–1.673)	0.578	1.104 (0.733–1.663)	0.636
GG	46 (17.1)	24 (10.4)	0.621 (0.352–1.097)	0.101	0.621 (0.348–1.111)	0.108	15 (8.1)	0.455 (0.237–0.874)	0.018	0.456 (0.235–0.887)	0.021
GG+GA vs AA			1.030 (0.718–1.478)	0.871	0.993 (0.685–1.438)	0.970		0.933 (0.637–1.366)	0.720	0.921 (0.624–1.361)	0.681
GG vs GA+AA			0.565 (0.333–.958)	0.034	0.579 (0.338–0.994)	0.048		0.428 (0.231–0.792)	0.007	0.433 (0.231–0.811)	0.009
A allele	329 (61.2)	295 (64.1)	1.00 (ref)				246 (66.5)	1.00 (ref)			
G allele	209 (38.8)	165 (35.9)	0.880 (0.681–1.139)	0.333			124 (33.5)	0.793 (0.602–1.047)	0.101		

Note: ^aAdjusted for age, gender, and smoking.
Abbreviations: CI, confidence interval; OR, odds ratio; ref, reference.

Table 7 Relationship of interaction between rs7248320 and smoking with lung cancer risk and non-small-cell lung cancer risk

Genotype	Controls (%) (n=588)	Smoking status	Lung Cancer			Non-small cell lung cancer		
			Cases (%) (n=512)	OR[a] (95% CI)	P[a]-value	Cases (%) (n=377)	OR[a] (95% CI)	P[a]-value
GG	75 (12.8)	Never	44 (8.6)	1.00 (ref)		34 (9.0)	1.00 (ref)	
GA+AA	392 (66.7)	Never	324 (63.3)	1.420 (0.951–2.121)	0.086	264 (70.0)	1.499 (0.969–2.319)	0.069
GG	19 (3.2)	Ever	21 (4.1)	2.110 (0.998–4.459)	0.050	8 (2.1)	1.176 (0.457–3.026)	0.736
GA+AA	102 (17.3)	Ever	123 (24)	2.319 (1.416–3.798)	0.001	71 (18.8)	1.963 (1.134–3.399)	0.016

Note: [a]Adjusted for age, gender, and smoking.
Abbreviations: CI, confidence interval; OR, odds ratio; ref, reference.

Table 8 Relationship of interaction between rs7248320 and smoking with lung adenocarcinoma risk and lung squamous cell carcinoma risk

Genotype	Controls (%) (n=588)	Smoking status	Lung adenocarcinoma			Lung squamous cell carcinoma		
			Cases (%) (n=258)	OR[a] (95% CI)	P[a]-value	Cases (%) (n=110)	OR[a] (95% CI)	P[a]-value
GG	75 (12.8)	Never	28 (10.9)	1.00 (ref)		6 (5.5)	1.00 (ref)	
GA+AA	392 (66.7)	Never	203 (78.7)	1.377 (0.860–2.204)	0.182	55 (50.0)	1.810 (0.749–4.375)	0.188
GG	19 (3.2)	Ever	4 (1.6)	0.863 (0.261–2.849)	0.809	4 (3.6)	2.369 (0.591–9.502)	0.224
GA+AA	102 (17.3)	Ever	23 (8.9)	0.970 (0.496–1.896)	0.928	45 (40.9)	4.846 (1.892–12.416)	0.001

Note: [a]Adjusted for age, gender, and smoking.
Abbreviations: CI, confidence interval; OR, odds ratio; ref, reference.

AA/GA genotypes. Similarly, they also found that rs7248320 GG genotype increased the cervical cancer risk by 1.34-fold (95% CI =1.09–1.66, P=0.006). In the past studies, they found that rs7248320GG genotype was a risk factor for HCC and cervical cancer.

Perhaps the pathogenic mechanisms were diverse in different cancers; hence, the effects of rs7248320GG genotype were different. We found that rs7248320GG genotype could be a protecting factor in NSCLC. In this ongoing case–control study, we evaluated the association between rs7248320 polymorphism in lncRNA AC008392.1 and the susceptibility of lung cancer in a Chinese population of 512 lung cancer cases and 588 cancer-free controls. To our knowledge, this is the first study to investigate the relationship between rs7248320 and lung cancer risk. The results of our study suggest that the rs7248320GG genotype for lncRNA AC008392.1 compared with GA/AA genotypes was associated with deceased risk of NSCLC (adjusted P=0.033). Moreover, in the subgroup of ages, individuals with GG genotype had a lower risk on lung cancer or NSCLC compared with individuals with GA/AA genotypes (adjusted P=0.048 or P=0.009, respectively). The homogeny GG could play an important role in reducing the susceptibility of NSCLC (adjusted P=0.021). This study may help to identify people who are more susceptible to NSCLC. Individuals with dangerous genotypes should pay more attention to the occurrence and development of NSCLC. However, we failed to find the statistically significant

association between rs7248320 and SQ. The same results were also found in AD group. NSCLC includes large cell lung cancer, AD, SQ, and so on. SQ, mostly originated from the larger bronchi, is often central lung cancer, and AD is mostly peripheral lung cancer. The sample size in the present study may be too small to get significant results in AD and SQ. The same problem also exists in the subgroup analysis.

Few studies investigated the function of gene–environment interaction on the risk of lung cancer. Therefore, in the case-control study, we investigated the interaction between exposure to smoking status and the SNP rs7248320 on lung cancer risk. The crossover analysis in this study qualitatively suggests the meaningful interaction between rs7248320 and smoking exposure on the susceptibility of lung cancer, NSCLC, and SQ in Chinese population. Then, the quantitative analyses in this study have shown that the interaction between this SNP rs7248320 and smoking status exposure did not have any statistical significance on an additive scale. These results might be due to the small sample size.

Several limitations should be noted in the present case–control study. First, the study is based on the hospital from which the cases and controls were selected, which might lead to Berkson's bias. In order to reduce the Berkson's bias, we chose the cases and controls from several different hospitals in Shenyang. Second, we collected the data of smoking exposure in participants which may result in recall bias. Third, the sample size in the present study may be too small

Table 9 Interaction measures between rs7248320 in lncRNA and smoking exposure in lung cancer, NSCLC, lung adenocarcinoma, and lung squamous cell carcinoma

Measure	Lung cancer		Measure	NSCLC		Measure	Lung adenocarcinoma		Measure	Lung squamous cell carcinoma	
	Estimate	95% CI		Estimate	95% CI		Estimate	95% CI		Estimate	95% CI
RERI	-0.237	-1.62 to 1.146	RERI	0.121	-0.872 to 1.114	RERI	-0.347	-1.264 to 0.57	RERI	2.129	-1.465 to 5.724
AP	-0.115	-0.788 to 0.557	AP	0.079	-0.569 to 0.727	AP	-0.575	-2.054 to 0.905	AP	0.386	-0.199 to 0.971
S	0.816	0.279 to 2.391	S	1.292	0.113 to 14.764	S	8.086	0 to 1458742883.487	S	1.893	0.476 to 7.524

Abbreviations: AP, attributable proportion due to interaction; CI, confidence interval; NSCLC, non-small-cell lung cancer; RERI, relative excess risk due to interaction; S, synergy index.

to get significant results; hence, the relationship between the SNP rs7248320 in lncRNA AC008392.1 and lung cancer risk need to be validated by further large size studies. Finally, we did not experiment to determine whether the association between the genotypes of rs7248320 and the expression of the lncRNA AC008392.1 existed. However, the study reported that LncRNA AC008392.1 rs7248320 may represent the eQTL for CARD8 by bioinformatics analyses. It has been shown that there is a significant association between the genotypes of eQTL SNP and the expression of the corresponding lncRNA.[55] Hence, further studies are needed.

Conclusion

In this study, we found the association between rs7248320 A>G polymorphism in lncRNA AC008392.1 and the susceptibility to lung cancer in Chinese population. The interactions between rs7248320 and smoking exposure were not statistically significant.

Acknowledgment

Disclosure

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