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LncRNA NEAT1 polymorphisms and lung cancer susceptibility in a Chinese Northeast Han Population: A case-control study

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ABSTRACT

Background: Long non-coding RNA Nuclear Paraspeckle Assembly Transcript 1 (NEAT1) is a novel lncRNA localized specifically to nuclear paraspeckles. The study analyses the association between NEAT1 genetic polymorphisms and the susceptibility of lung cancer in a Chinese Northeast Population.

Methods: The NEAT1 rs512715 and rs2239895 genetic polymorphisms were genotyped in 462 lung cancer cases and 559 controls by a Real-Time Polymerase Chain Reaction (PCR) with the TaqMan discrimination assay.

Results: Our study found that the polymorphisms of two SNPs increased or decreased the risk of lung cancer were not obvious, but statistical significance in non-small cell lung cancer and lung squamous cell carcinoma can be observed. Compared with homozygous CC genotype carriers, the GC genotype of rs2239895 was positively related to the risk of lung squamous cell carcinoma (OR 1.805, 95% CI, 1.168–2.789, $P = 0.008$). Similarly, associations between rs2239895 and lung squamous cell carcinoma risk were found (CC + GC vs. GG, OR 1.668, 95%CI, 1.093–2.545, $P = 0.018$) in dominant model. In stratified analysis for age, rs2239895 GC genotype was observed to increase the risk of non-small-cell lung cancer compared with CC genotype (OR 1.562, 95%CI, 1.029–2.371, $P = 0.036$). However, the study showed that negative correlation the lung cancer risk and rs512715 polymorphisms. There was no remarkable relationship between the both additive and multiplicative model about the two SNPs.

Conclusions: The polymorphisms rs2239895 were associated with the risk of lung squamous cell carcinoma. The interaction between the two SNPs and the cigarette smoking was no notable difference.

1. Introduction

Lung cancer causes a major family and social burden on humans, and it has a high morbidity and mortality among malignant tumors. According to GLOBOCAN database (September 2018) and the latest published Chinese cancer monitoring data (2015), the lung cancer is widely distributed all over the world, especially in China [1,2] Cigarette smoking is a kind of being proved environmental risk factors leading to lung cancer. Cigarette smoking is closely related to lung cancer, but it is estimated that half of new cases comes from the population with no cigarette exposure history or quitting smoking several years [3]. And other study demonstrates that about a quarter of the lung cancer cases were nonsmokers [4]. In addition, epidemiological studies have shown that lung cancer susceptibility is a crucial genetic role except environmental factors [5–7].

In recent years, non-coding RNAs (ncRNAs) are a new class of transcripts with no coding functions. Particularly, long non-coding RNAs, a kind of ncRNAs and more than 200 nt in length, play critical roles in regulating chromatin dynamics, gene expression, growth, differentiation and development [8]. In more than half of the mammals, the non-coding transcript is composed of lncRNA [9]. Genome-wide association studies (GWAS) have determined that numerous single nucleotide polymorphisms (SNPs) are associated with disease or traits.

LncRNA NEAT1 (nuclear enriched abundant transcript 1) is a

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member of the lncRNA family on 11q13.1 about 4 kb in length. NEAT1 is an indispensable component of paraspeckles in the nucleus [10,11]. LncRNA NEAT1 up-regulates or inhibits the pathogenesis and expression of various diseases, such as lung cancer, liver cancer, glioma, ovarian cancer, breast cancer, prostate cancer and colorectal cancer [12–28]. And paraspeckles are involved in cell proliferation, migration and carcinogenesis. Currently, one published article has investigated the association between polymorphisms in NEAT1 and cervical cancer (CC) susceptibility. Fang Jing et al. showed a statistical association between rs512715 polymorphisms and risk of cervical cancer (CC) [29]. Therefore, the present study will be the first to reveal the relationship between NEAT1 polymorphisms and the risk of lung cancer. In summary, we selected rs512715 and rs2239895 to investigate the relationship between polymorphisms and lung cancer by carrying out a case-control study in a Chinese Northeast Han Population.

2. Material and methods

2.1. Study subjects and data collection

This hospital-based case–control study was performed in Shenyang, Liaoning Province and all the subjects were come from the Han population in Northeast of China. The inclusion and exclusion criterion for study subjects were described in our previous studies [30–32]. All patients (n = 462) were diagnosed with lung cancer for the first time by histological diagnosis before radiotherapy and chemotherapy. The healthy controls (n = 559) were from several medical examination centers of hospitals in Shenyang, matched by sex and age ± 5 years. Individuals who smoke less than 100 cigarettes a lifetime were defined as non-smokers. Two investigators who received unified training conducted an epidemiological survey of the respondents by using a unified questionnaire, and the data were double input to ensure the accuracy of these contents. After a rigorous examination, the Institutional Review Board of the China Medical University approved the case–control study. All members had signed an informed consent.

2.2. DNA collection and extraction

10 ml of venous blood from each of the 1021 participants was collected and stored at -20 °C. In this study, phenol-chloroform method was used to extract genomic DNA.

2.3. SNPs selection and genotyping

Two websites and a software had been used, including dbSNP database (https://www.ncbi.nlm.nih.gov/snp/), the Ensembl Project (http://grch37.ensembl.org/Homo_sapiens/Tools/VcftoPed?db=core;tl=FTJ3BsVq05WiKPMA-4169739) and Haploview 4.2 software. Among the SNPs of NEAT1, the minimum allele frequencies (MAF) of three SNPs (rs512715, rs680413 and rs2239895) were more than 0.05 in Chinese Han population (CHB). Moreover, there is a linkage disequilibrium between rs680413 and rs2239895 ($r^2 = 0.964$). Meanwhile, we considered the previous study and selected rs521715 and rs2239895. Genetic polymorphisms were detected by a Real-Time Polymerase Chain Reaction (PCR) with the TaqMan assay. PCR Taqman probe, primer and Taqman Master Mix were designed and synthesized by Applied Biosystems (ABI). To ensure quality, 10% of the samples were randomly selected for repeated genotyping. The results showed that the two genotyping results were consistent.

2.4. Statistical analysis

All the statistical analyses were two-sided and were performed using *SPSS* software 20.0 (IBM SPSS, Inc., Chicago, IL, USA). Differences in gender and cigarette smoking were compared by chi-square test (χ^2), and *t*-test was used to assess age differences. Hardy-Weinberg equilibrium (HWE) was estimated by a goodness-of-fit χ^2 (rs512715: $P = 0.343$, rs2239895: $P = 0.248$). Unconditional logistic regression analysis was performed to analysis the experimental data by calculating odds ratios (ORs) and 95% confidence intervals (CIs), adjusted by gender, age, and cigarette smoking. The three indicators were used to explain the interactions, namely the synergy index (S), the attributable proportion due to interaction (AP), and the relative excess risk due to interaction (RERI). *P*-value < 0.05 was defined as statistically significant.

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

Risk factor	Lung cancer (N = 462)	Controls (N = 559)	*P*-value
Age(mean ± SD)	59.80 ± 10.646	58.43 ± 14.553	0.093
Gender			0.954
Male	243 (52.6%)	293 (52.4%)	
Female	219 (47.4%)	266 (47.6%)	
Cigarette smoking			0.000
ever	239 (51.7%)	137 (24.5%)	
never	223 (48.3%)	422 (75.5%)	
Pathological type			
AD	235 (50.9%)		
SQ	159 (34.4%)		
SCC	60 (13.0%)		
Else	8(1.7%)		

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

3. Results

3.1. Subject characteristics

This study had 1021 samples, including 462 cases and 559 healthy controls. The demographic variables of these researchers were shown in Table 1. The mean ages of the case group and the control group were 58.43 mean (SD) 14.553 and 59.80 mean (SD) 10.646 ($P = 0.093$). The gender ratio was balanced between groups, and the difference was not statistically significant ($P = 0.954$). Naturally, the proportion of cigarette smoking in the case group is higher than that in the control group ($P < 0.001$). For pathological type, nearly half of the cases were lung adenocarcinoma (AD), 34.4% were lung squamous cell carcinoma (SQ), lung small cell carcinoma (SCC) was accounted for 13.0%.

3.2. Genotype distribution and lung cancer susceptibility

As shown in Table 2, genotyping results showed the relationship between two SNPs of NEAT1 and the risk of lung cancer and non-small cell lung cancer. The findings demonstrated that there was no significant difference between the two SNPs polymorphisms and the risk of the whole lung cancer and non-small cell lung cancer. Similarly, Table 3 showed that rs2239895 was association between with the risk of lung squamous cell carcinoma. The GC genotype had a 1.805-fold increased risk of developing lung squamous cell carcinoma than GG genotype (adjusted OR = 1.805, 95% CI = 1.168–2.789, $P = 0.008$). In dominant model, members of case group significantly increased the risk of lung squamous cell carcinoma with CC and GC genotypes (adjusted OR = 1.668, 95%CI = 1.093–2.545, $P = 0.018$). However, the relationship between rs512715 polymorphisms and susceptibility of lung adenocarcinoma and lung squamous cell carcinoma was not remarkable in this research.

This study conducted stratified analyses based on age, gender, and cigarette smoking. In the age stratification analysis, homozygous rs2239895 CC could increase the risk of non-small-cell lung cancer (NSCLC) compared with GG genotype in Table 4, adjusted OR = 1.562, 95% CI = 1.029–2.371, $P = 0.036$ (> 59 years old). In the stratified

Table 2
The association of the two SNPs with lung cancer risk and non-small cell lung cancer.

Genotyping	Controls(%)	Lung cancer			Non-small-cell lung cancer		
		Cases(%)	OR[a](95%CI)	P^a value	Cases(%)	OR[a](95%CI)	P^a value
	n = 559	n=462			n = 394		
rs512715							
GG	308(55.1)	244(52.8)	1.00(Ref)		205(52.0)	1.00(Ref)	
GC	208(37.2)	189(40.9)	1.145(0.848-1.547)	0.375	163(41.4)	1.258(0.943-1.677)	0.118
CC	43(7.7)	29(6.3)	0.713(0.300-1.692)	0.443	26(6.6)	1.008(0.579-1.756)	0.977
CC + GC vs. GG			1.100(0.822-1.470)	0.194		1.217(0.924-1.602)	0.163
CC vs. GG + GC			0.688(0.291-1.625)	0.393		0.916(0.533-1.572)	0.749
G allele	824(73.7)	677(73.3)	1.00(Ref)		573(72.7)	1.00(Ref)	
C allele	294(26.3)	247(26.7)	1.038(0.852-1.265)	0.712	215(27.3)	1.052(0.856-1.292)	0.631
rs2239895							
GG	398(71.2)	327(70.8)	1.00(Ref)		272(69.0)	1.00(Ref)	
GC	143(25.6)	125(27.1)	1.236(0.937-1.630)	0.133	112(28.4)	1.214(0.891-1.655)	0.220
CC	18(3.2)	10(2.1)	0.965(0.564-1.652)	0.897	10(2.5)	0.845(0.357-1.999)	0.701
CC + GC vs. GG			1.192(0.915-1.552)	0.194		1.175(0.871-1.585)	0.292
CC vs. GG + GC			0.883(0.523-1.492)	0.642		0.801(0.340-1.887)	0.612
G allele	939(84.0)	779(84.3)	1.00(Ref)		656(83.2)	1.00(Ref)	
C allele	179(16.0)	145(15.7)	0.976(0.769-1.240)	0.845	132(16.8)	1.056(0.825-1.350)	0.667

[a] Adjusted for age, gender, cigarette smoking. OR, odds ratio; CI, confidence interval.

analysis of gender and cigarette smoking, negative associations between the two SNPs polymorphisms and the risk of lung cancer were determined (The data are available, but the table is not attached). Further, the risks of the combination of two SNPs alleles were assessed in lung cancer and non-small cell lung cancer in Tables 5 and 6. We observed that the genotype risks of the two SNPs did not increase in a similar dose-dependent manner with increasing risk of lung cancer and non-small cell lung cancer.

3.3. Interactions between SNPs and cigarette smoking

Our study investigated the interaction between the two SNPs and cigarette smoking to lung cancer and its pathological types. In Tables 7 and 8, qualitative analysis results display the ORs of the two SNPs were basically increased in each lung cancer, non-small cell lung cancer, lung adenocarcinoma and lung squamous cell carcinoma. However, quantitative research results showed different results. In the additive model (Table 9), our results did not support the association between the four kinds of lung cancer classification and the two SNPs. In the different lung cancer classifications of the two SNPs, 95% CI of each RERI contains 1. In the multiplicative model (Table 10), the interactions between the two SNPs and cigarette smoking were not statistically significant with P values greater than 0.05.

4. Discussion

This hospital-based case-control study showed that polymorphisms rs2239895 in NEAT1 were associated with susceptibility to lung squamous cell carcinoma. Meanwhile, the risk of non-small cell lung cancer increased 1.56-fold(age > 59 years old), compared with the control group.

LncRNA NEAT1 produces two transcripts by RNA polymerase II: NEAT1_1 and NEAT1_2. Although many studies suggested that molecule NEAT1 plays a role in cancer progression, few studies had directly linked paraspeckles or NEAT1_2 to cancer. Paraspeckles are specific RNA–protein nuclear bodies of mammals that regulate gene expression,

Table 3
The association of the two SNPs with lung adenocarcinoma risk and lung squamous cell carcinoma risk.

Genotyping	Controls(%)	Lung adenocarcinoma			Lung squamous cell carcinoma		
		Cases(%)	OR[a](95%CI)	P^a value	Cases(%)	OR[a](95%CI)	P^a value
	n = 559	n = 235			n = 159		
rs512715							
GG	308(55.1)	125(53.2)	1.00(Ref)		80(50.3)		
GC	208(37.2)	95(40.4)	1.156(0.830-1.610)	0.391	68(42.8)	1.447(0.959-2.184)	0.078
CC	43(7.7)	15(6.4)	0.886(0.462-1.697)	0.715	11(6.9)	1.255(0.570-2.761)	0.572
CC + GC vs. GG			1.110(0.809-1.524)	0.517		1.417(0.955-2.102)	0.083
CC vs. GG + GC			0.834(0.442-1.575)	0.576		1.071(0.498-2.303)	0.860
G allele	824(73.7)	345(73.4)	1.00(Ref)		228(71.7)	1.00(Ref)	
C allele	294(26.3)	125(26.6)	1.156(0.830-1.610)	0.391	90(28.3)	1.106(0.838-1.461)	0.476
rs2239895							
GG	398(71.2)	169(71.9)	1.00(Ref)		103(64.8)		
GC	143(25.6)	59(25.1)	0.981(0.681-1.413)	0.916	53(33.3)	**1.805(1.168-2.789)**	**0.008**
CC	18(3.2)	7(3.0)	0.906(0.349-2.352)	0.839	3(1.9)	0.697(0.181-2.679)	0.599
CC + GC vs. GG			0.973(0.685-1.381)	0.877		**1.668(1.093-2.545)**	**0.018**
CC vs. GG + GC			0.911(0.352-2.353)	0.847		0.590(0.155-2.243)	0.438
G allele	939(84.0)	397(84.5)	1.00(Ref)		259(81.4)	1.00(Ref)	
C allele	179(16.0)	73(15.5)	0.965(0.717-1.297)	0.812	59(18.6)	1.195(0.864-1.654)	0.282

[a] Adjusted for age, gender, cigarette smoking. OR, odds ratio; CI, confidence interval.

Table 4
Stratified analyses of the two SNPs with lung cancer risk and NSCLC by age.

Genotyping and age		Lung cancer			Non-small-cell lung cancer		
	Controls (%) n = 559	Cases (%) n = 462	OR[a] (95%CI)	P^a value	Cases (%) n = 394	OR[a](95%CI)	P^a value
rs512715							
≤59							
GG	127(52.3)	115(53.5)	1.00(ref)		87(51.8)	1.00(ref)	
GC	96(39.5)	90(41.9)	1.134(0.741-1.736)	0.562	73(43.5)	1.178(0.748-1.854)	0.480
CC	20(8.2)	10(4.7)	0.563(0.232-1.365)	0.203	8(4.8)	0.568(0.219-1.475)	0.245
CC + GC vs. GG			1.035(0.688-1.557)	0.870		1.071(0.692-1.657)	0.759
CC vs. GG + GC			0.533(0.224-1.268)	0.155		0.529(0.208-1.344)	0.181
>59							
GG	181(57.3)	129(52.2)	1.00(ref)		118(52.2)	1.00(ref)	
GC	112(35.4)	99(40.1)	1.317(0.900-1.928)	0.157	90(39.8)	1.300(0.881-1.920)	0.186
CC	23(7.3)	19(7.7)	1.318(0.652-2.663)	0.442	18(8.0)	1.321(0.648-2.695)	0.444
CC + GC vs. GG			1.317(0.917-1.893)	0.136		1.304(0.901-1.888)	0.160
CC vs. GG + GC			1.177(0.594-2.335)	0.640		1.187(0.593-2.377)	0.628
rs2239895							
≤59							
GG	167(68.7)	159(74.0)	1.00(ref)		122(72.6)	1.00(ref)	
GC	69(28.4)	52(24.2)	0.839(0.528-1.333)	0.457	42(25.0)	0.852(0.518-1.399)	0.526
CC	7(2.9)	4(1.9)	0.599(0.137-2.622)	0.497	4(2.4)	0.806(0.182-3.566)	0.776
CC + GC vs. GG			0.819(0.521-1.286)	0.386		0.848(0.524-1.373)	0.502
CC vs. GG + GC			0.627(0.144-2.732)	0.535		0.840(0.191-3.697)	0.818
>59							
GG	231(73.1)	168(68.0)	1.00(ref)		150(66.4)	1.00(ref)	
GC	74(23.4)	73(29.6)	1.492(0.988-2.253)	0.057	70(31.0)	**1.562(1.029-2.371)**	**0.036**
CC	11(3.5)	6(2.4)	0753(0.250-2.269)	0.614	6(2.7)	0.836(0.278-2.514)	0.750
CC + GC vs. GG			0.718(0.483-1.067)	0.101		1.466(0.982-2.190)	0.062
CC vs. GG + GC			0.677(0.226-2.023)	0.485		0.740(0.248-2.205)	0.589

[a] Adjusted for age, gender, cigarette smoking. OR, odds ratio; CI, confidence interval, NSCLC, Non-small-cell lung cancer.

Table 5
Cumulative effects of rs512715-C and rs2239895-C on lung cancer and non-small cell lung cancer susceptibility.

Controls (%)		Lung Cancer			Non-small-cell lung cancer		
	n = 559	Cases(%) n = 462	OR[a](95%CI)	P^a value	Cases(%) n = 394	OR[a](95%CI)	P^a value
0	308(55.1)	242(52.4)	1.00(ref)		204(51.8)	1.00(ref)	
1	83(14.8)	85(18.4)	1.376(0.951-1.991)	0.091	67(17.0)	1.296(0.878-1.915)	0.192
2	132(23.6)	108(23.4)	1.133(0.818-1.570)	0.453	99(25.1)	1.210(0.865-1.691)	0.265
3	18(3.2)	17(3.7)	1.401(0.678-2.896)	0.363	14(3.6)	1.355(0.631-2.910)	0.435
4	18(3.2)	10(2.2)	0.756(0.317-1.804)	0.529	10(2.5)	0.885(0.372-2.106)	0.783
Trend			P^b = 0.386			P^b = 0.311	
0	308(55.1)	242(52.4)	1.00(ref)		204(51.8)	1.00(ref)	
1-4	251(44.9)	220(47.6)	1.208(0.927-1.574)	0.161	190(48.2)	1.227(0.932-1.615)	0.145

OR odds ratio, CI confident interval.
OR[a] was adjusted by age, gender and cigarette smoking.
P^b value was calculated by linear-by-linear association of χ^2 test.

Table 6
Cumulative effects of rs512715-C and rs2239895-C on lung adenocarcinoma and lung squamous cell carcinoma susceptibility.

	Controls (%)	Lung adenocarcinoma			Lung squamous cell carcinoma		
	n = 559	Cases(%) n = 235	OR[a](95%CI)	P^a value	Cases(%) n = 159	OR[a](95%CI)	P^a value
0	308(55.1)	124(52.8)	1.00(ref)		80(50.3)	1.00(ref)	
1	83(14.8)	44(18.7)	1.365(0.882-2.111)	0.163	23(14.5)	1.088(0.610-1.943)	0.775
2	132(23.6)	54(23.0)	1.042(0.704-1.543)	0.837	45(28.3)	**1.645(1.026-2.638)**	**0.039**
3	18(3.2)	6(2.6)	0.852(0.323-2.243)	0.745	8(5.0)	2.458(0.899-6.719)	0.080
4	18(3.2)	7(3.0)	0.965(0.369-2.520)	0.942	3(1.9)	0.701(0.181-2.711)	0.606
Trend			P^b = 0.547			P^b = 0.286	
0	308(55.1)	124(52.8)	1.00(ref)		80(50.3)	1.00(ref)	
1-4	251(44.9)	111(47.2)	1.129(0.823-1.550)	0.451	79(49.7)	1.417(0.955-2.102)	0.083

OR odds ratio, CI confident interval.
OR[a] was adjusted by age, gender and cigarette smoking.
P^b value was calculated by linear-by-linear association of χ^2 test.

Table 7
The interaction between the two SNPs and cigarette smoking with lung cancer risk and NSCLC.

	Controls(%)	cigarette smoking	Lung Cancer			Non-small-cell lung cancer		
	n = 559		Cases(%) *n* = 462	OR[a] (95%CI)	P^a value	Cases(%) *n* = 394	OR[a](95%CI)	P^a value
rs512715								
CC	33(5.9)	Never	15(3.2)	1.00(ref)		13(3.3)	1.00(ref)	
GG + GC	389(69.6)	Never	208(45.0)	1.056(0.547-2.036)	0.872	187(47.5)	1.079(0.541-2.153)	1.079
CC	10(1.8)	Ever	14(3.0)	6.469(2.222-18.832)	0.001*	13(3.3)	6.856(2.277-20.643)	0.001*
GG + GC	127(22.7)	Ever	225(48.7)	8.235(4.012-16.903)	0.000*	181(45.9)	7.632(3.585-16.247)	0.000*
rs2239895								
CC	13(2.3)	Never	5(1.1)	1.00(ref)		5(1.3)		
GG + GC	409(73.2)	Never	218(47.2)	1.119(0.377-3.325)	0.839	195(49.5)	1.008(0.338-3.007)	0.989
CC	5(0.9)	Ever	5(1.1)	4.251(0.795-22.735)	0.091	5(1.3)	4.346(0.808-23.359)	0.087
GG + GC	132(23.6)	Ever	234(50.6)	8.807(2.878-26.953)	0.000*	189(48.0)	7.255(2.351-22.383)	0.001*

NSCLC, Non-small-cell lung cancer.
[a] Adjusted for age, gender, smoking. OR, odds ratio; CI, confidence interval, *Indicates statistical significance ($P < 0.05$).

and it is involved in cell proliferation, migration and carcinogenic process. NEAT1 usually plays an up-regulation role in the pathogenesis and expression of various malignancies in previous studies. And a great number of evidences revealed that paraspeckles may have correlations with cancer [33]. In accordance with this possibility of paraspeckles, it provides a reliable basis for studying the mechanism of NEAT1. Only one article has reported the study of NEAT1 polymorphisms and cervical cancer (CC) susceptibility in Xinjiang Uygur population of China. However, previous studies had not showed the investigations into lung cancer susceptibility and the two SNPs.

Currently, our research is first to study the risk relationship between lncRNA NEAT1 polymorphisms and the risk of lung cancer. Our research found a positive relationship between rs2239895 polymorphisms and lung squamous cell carcinoma. The previous study showed that rs512715 polymorphisms had risks with cervical cancer in allele, codominant, dominant, overdominant and log-additive models [29]. It is clear to find that the results of cervical cancer in rs512715 of NEAT1 are different from those in lung cancer. And the difference in the mechanism or pathway may be the cause of this result.

Our research studied the relationship between NEAT1 polymorphisms and lung cancer susceptibility, and the findings in lung squamous cell carcinoma may have a positive effect. More people will study the relationship between NEAT1 and other malignant tumors susceptibility in the future, such as liver cancer, glioma, ovarian cancer, breast cancer, prostate cancer and colorectal cancer and so on. The interactions between these two SNPs and lung cancer were not obvious, which was partially similar to other findings in our previous studies. However, other researchers found the relationship between cigarette smoking and gene interactions [34–37]. The results may indicate that gene-environment interactions are difficult to detect. It also may be caused by different analytical methods.

Obviously, there are a few deficiencies in this study. Although a statistical association was found in lung squamous cell carcinoma, the sample size was not very large. The results may be limited by statistical power because of the sample size, especially in stratified and interaction analysis. The subjects are all from the Han population of Northeast China, the samples do not fully represent the overall population in the region. Therefore, the present study may have selection bias. And the samples will be better represented if a multicenter case-control study is used in the region. Simultaneously, more studies are needed to verify our findings with a larger sample size, different ethnicities and regions. As predicted by HaploReg4.1 [38], rs2239895 can bind to the 4 proteins POL2, WHIP, POL2S2 and CTCF. Therefore, we can learn from previous studies [14,17,39,40] and further investigate the function and mechanism to verify the relationship between them.

5. Conclusion

The polymorphisms rs2239895 in NEAT1 was associated with the risk of lung squamous cell carcinoma in a Chinese Northeast Han population. The interaction between the two SNPs and the cigarette smoking was not significant.

Table 8
The interaction between the two SNPs and cigarette smoking with AD risk and SQ risk.

	Controls(%)	cigarette smoking	Lung adenocarcinoma			Lung squamous cell carcinoma		
	n = 559		Cases(%) *n* = 235	OR[a] (95%CI)	P^a value	Cases(%) *n* = 159	OR[a](95%CI)	P^a value
rs512715								
CC	33(5.9)	Never	8(3.4)	1.00(ref)		5(3.1)	1.00(ref)	
GG + GC	389(69.6)	Never	146(62.1)	1.430(0.629-3.249)	0.393	41(25.8)	0.605(0.219-1.670)	0.332
CC	10(1.8)	Ever	7(3.0)	7.201(1.927-26.909)	0.003*	6(3.8)	6.959(1.598-30.310)	0.010*
GG + GC	127(22.7)	Ever	74(31.5)	6.386(2.544-16.028)	0.000*	107(67.3)	9.678(3.292-28.458)	0.000*
rs2239895								
CC	13(2.3)	Never	4(1.7)	1.00(ref)		1(0.6)	1.00(ref)	
GG + GC	409(73.2)	Never	150(63.8)	0.971(0.295-3.193)	0.962	45(28.3)	1.207(0.150-9.686)	0.860
CC	5(0.9)	Ever	8(1.0)	3.418(0.498-23.464)	0.211	2(1.3)	8.872(0.615-128.075)	0.109
GG + GC	132(23.6)	Ever	210(26.4)	4.571(1.312-15.930)	0.017*	111(69.8)	18.086(2.192-149.244)	0.007*

AD: lung adenocarcinoma, AQ: lung squamous cell carcinoma.
[a] Adjusted for age, gender, smoking. OR, odds ratio; CI, confidence interval, *Indicates statistical significance ($P < 0.05$).

Table 9
Interaction measures between the two SNPs and cigarette smoking on lung cancer, NSCLC, AD and SQ.

	Lung cancer		NSCLC		Lung adenocarcinoma		lung squamous cell carcinoma	
Measure	Estimate	95%CI	Estimate	95%CI	Estimate	95%CI	Estimate	95%CI
rs512715								
RERI	1.710	−3.940-7.360	0.697	−5.300-6.694	−1.245	−8.860-6.370	3.114	−4.730-10.957
AP	0.208	−0.465-0.880	0.091	−0.692-0.875	−0.195	−1.377-0.987	0.322	−0.439-1.082
S	1.309	0.480-3.571	1.117	0.408-3.062	0.812	0.260-2.538	1.560	0.412-5.900
rs2239895								
RERI	4.437	−2.516-11.391	2.902	−3.312-9.115	1.182	−4.212-6.576	9.007	−13.751-31.766
AP	0.504	−0.134-1.141	0.400	−0.392-1.192	0.259	−0.924-1.441	0.498	−0.328-1.324
S	2.317	0.422–12.722	1.865	0.327–10.640	1.495	0.151–14.817	2.115	0.326–13.718

NSCLC, non-small cell lung cancer; AD: lung adenocarcinoma, AQ: lung squamous cell carcinoma;
RERI, relative excess risk due to interaction; AP, attributable proportion due to interaction; S, synergy index;
95 % CI, 95% confidence interval.

Table 10
Multiplicative interaction between the two SNPs risk genotypes and cigarette smoking.

		Lung Cancer		Non-small-cell lung cancer	
SNPs	Variables	OR[a] (95% CI)	P^a value	OR[a] (95% CI)	P^a value
rs512715	Cigarette smoking	6.469(2.222-18.832)	0.001	6.856(2.277-20.643)	0.001
	GG + GC	1.056(0.547-2.036)	0.872	1.079(0.541-2.153)	0.829
	Interaction	1.206(0.412-3.527)	0.733	1.032(0.341-3.122)	0.956
rs2239895	Cigarette smoking	4.251(0.795-22.735)	0.091	4.346(0.808-23.359)	0.087
	GG + GC	1.119(0.377-3.325)	0.839	1.008(0.338-3.007)	0.989
	Interaction	1.851(0.341-10.055)	0.476	1.657(0.303-9.053)	0.560

CI confidence interval, OR odds ratio.
OR[a] was adjusted by age, gender.

Ethics approval and consent to participate

All participants in the study signed informed consent forms before participating in the study. And the Institutional Review Board of China Medical University approved the study.

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Declaration of Competing Interest

The authors declared no conflicts of interest.

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