Article

Genetic Polymorphisms of ACE1 rs4646994 Associated with Lung Cancer in Patients with Pulmonary Nodules: A Case Control Study

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Abstract: Background: Currently, many detection methods have high sensitivity to the diagnosis of lung cancer. However, some postoperative patients with pulmonary nodule were eventually diagnosed as benign nodules. The ideal evaluation of an individual with a pulmonary nodule would expedite therapy for a malignant nodule and minimize testing for those with a benign nodule.**Methods:** This case-control study is designed to explore the relationship between *ACE1* rs4646994 polymorphism and the risk of lung cancer in patients with pulmonary nodules, 400 individuals with lung cancer and benign pulmonary nodules were included. A DNA extraction kit was used to extract plasm DNA from peripheral blood. The relationship between *ACE1* rs4646994 and the risk of lung cancer in patients with pulmonary nodules was determined by chi-square test, logistic regression analysis and cross analysis. **Results:** The results showed that the DD genotype of *ACE1* rs4646994 may increase the risk of lung cancer in patients with pulmonary nodules, and this correlation was more significant in the female subgroup. In the age stratification analysis, it was found that the risk of lung cancer was significantly increased in the DD genotype of *ACE1* rs4646994 in the older subgroup (> 45 years). In addition, the possibility of EGFR mutation in lung adenocarcinoma patients with *ACE1* rs4646994 DD genotype was lower than that of II or ID genotype carriers. **Conclusions:** Our study indicated that *ACE1* rs4646994 polymorphism increases the risk of lung cancer in patients with pulmonary nodules from China.

Keywords: genetic polymorphism; *ACE1* rs4646994; lung cancer; pulmonary nodules

1. Introduction

Lung cancer is a primary cancer type with high incidence and mortality, and the number of new cases of lung cancer in China has continued to rise [1]. The overall 5-year survival rate of lung cancer patients is less than 15%, but the 5-year survival rate of early lung cancer can be increased to more than 80% after reasonable treatment [2]. Lung cancer is classified as small cell lung cancer (SCLC) (15% of total diagnoses) and non-small cell lung cancer (NSCLC) (85% of total diagnoses) [3]. In the classification of NSCLC, adenocarcinoma (LUAD) is the most common subtype of lung cancer, followed by squamous cell carcinoma (SCC) [4].

A pulmonary nodule is an abnormal area in the lung that is less than 3cm in size, and most pulmonary nodules are benign (not caused by cancer) [5]. The risk of cancer increases with pulmonary nodules that are larger in size. Risk factors for increasing the likelihood of lung nodule carcinogenesis: current or previous smoking, older, personal cancer history, family history of lung cancer, emphysema, exposure to asbestos or radon and EGFR mutation [5–8]. This suggests that the development of pulmonary nodules into

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lung cancer is a complex and gradual process [9]. In recent years, several studies have reported the correlation between single nucleotide polymorphisms (SNPs) of genes related to metabolism, DNA damage repair, cell cycle regulation and lung diseases [9–12]. The treatment of patients with pulmonary nodules should be guided by the probability of malignant nodules, the safety of detection, and other additional tests [13].

Angiotensin-converting enzyme (ACE) encodes an enzyme involved in blood pressure regulation and electrolyte balance that converts of angiotensin I into a physiologically active peptide angiotensin II, and degrades bradykinin [14]. ACE plasma levels depend on the 287 bp insertion / deletion (I/D) polymorphism of the ACE gene on chromosome 17q23 [15]. According to its variation, there are three different genotypes: II, ID and DD [15]. It has been reported that the D allele is associated with increased ACE expression, and it has been observed that D allele heterozygous carriers have higher levels of systemic ACE protein than I allele carriers [16–18]. A large number of studies have reported that *ACE1* rs4646994 polymorphism is associated with a variety of diseases, including cardiovascular disease, psoriasis, kidney disease, stroke and Alzheimer 's disease [14,19,20]. A few studies have reported that *ACE1* rs4646994 polymorphism may be a possible risk factor for lung cancer [12]. On basis of previous relevant studies, the aim of this study was to investigate the possible correlation between *ACE1* rs4646994 polymorphism and lung cancer in patients with pulmonary nodules, providing evidence for molecular markers and etiological study of lung cancer.

2. Materials and Methods

2.1. Ethical Conformity

This pilot study was approved by the Research Ethics Committees of the Shanghai Jiao Tong University, under protocol KS1407, in the city of Shanghai, China. All participants signed informed consent.

2.2. Case and Control

Data were prospectively collected from October 2020 to September 2022 for a cohort of 460 subjects enrolled in Shanghai Chest Hospital under an active institutional review board, and written informed consents were acquired. All the cases were reviewed by a multidisciplinary lung nodule board, and CT images were collected at 3-12 months intervals until they were determined to be malignant or benign. For our study, IRB approval and a waiver of written informed consent were obtained. Inclusion criteria were as follows: (1) Only the patients with at least one pulmonary nodule that was at least 1 mm in diameter, when using lung parenchymal CT display thresholds, on the baseline CT image were included [21], (2) For patients with malignant pulmonary nodules, only subjects with nodules that were determined to be primary lung cancer (LC) by histopathology before the consent date of May 2022 were included, (3) For benign nodules, only those confirmed by histopathologic examination of tissue obtained via surgical resection, or the lesion was found to be stable radiographically for at least 2 years of follow-up, or resolved under CT surveillance, were included. Finally, subjects with lung cancer (n= 300) were included as the case group and matched with subjects with benign nodules (n= 100) as the control group. Both groups were investigated for clinical demographic data, including age, gender, and histological type.

2.3. Genotyping

TaqMan®-MGB probe assays were used to genotype the polymorphisms in 384-well plates on LightCycler® 480 system (Roche Ltd., Basel, CH). The primers and probes of TaqMan® assays were designed using Primer Express Oligo Design soft-ware v3.0 (Applied Biosystems, Foster city, CA, USA). PCR reactions were performed in a 6 μL reaction mixture containing 1 μL DNA, 3 μL TaqMan Genotyping Master Mix (Thermo Fisher, Waltham, MA, USA), 0.015 μL each primer, 0.012 μL FAM- and HEX-labelled TaqMan-MGB probes and 1 μL DNA. The program of amplification contained 10 min heat preservation at 95°C

followed by 50 cycles of 15s at 95°C and annealing at 60°C for 1 min. The normalized intensities of the 2 reporter dyes in each sample were plotted on an allelic discrimination plot and were algorithmically clustered. Genotype calls were assigned according to the sample position on the allelic discrimination plot.

2.4. Statistical analysis

All data were analysed using SPSS 20.0 statistical software (IBM, Armonk, NY, USA). The χ^2-test was used to verify whether the allele frequency of polymorphism met the Hardy–Weinberg (H-W) equilibrium. Pearson 's Chi-square and Mann-Whitney tests were used to compare the demographic and clinical variables between the study groups. Pearson's Chi-square was used to compare the frequency distribution of genotypes and alleles among groups. The odds ratios (OR) and their 95% confidence intervals (CI) of lung cancer incidence in patients with pulmonary nodule and genotype distribution was dealt with statistically by unconditional logistic regression model. A significance level of $p < 0.05$ was considered for all statistical analyses.

3. Results

3.1. Baseline characteristic of study subjects

In the results of demographic and clinical analyses, it can be observed that the groups differed in terms of age. No significant variation in gender was found between lung cancer patients and lung benign ($p > 0.05$). Most cases of lung cancer were LUAD, followed by SCLC and SCC. Among the 400 patients with pulmonary nodules included in the study, a total of 146 patients were tested for EGFR gene mutation. There was no significant difference in EGFR gene mutation between lung cancer patients and benign lung disease patients ($p = 1.000$). It is noteworthy that EGFR mutations exist in patients with benign nodules (Table 1).

Table 1. Demographic and clinical characteristics of the investigated groups.

Characteristics	Case (n=300)	Control (n=100)	*p*-Value
Gender, n (%)			0.862 [a]
Male	135 (45%)	44 (44%)	
Female	165 (55%)	56 (56%)	
Age (years)			
Median (p25-p75%)	57.00 (49.00-64.00)	55.00 (48.25-61.00)	0.038 [b*]
Histology			
Squamous Cell Carcinoma	14 (5.3%)	-	
Adencarcinoma	282 (93.4%)	-	NA
Small Cell Carcinoma	2 (0.6%)	-	
Malt	2 (0.6%)	-	
EGFR Mutation-positive			1.000 [c]
Yes	75 (52.1%)	1 (50%)	
No	69 (47.9%)	1 (50%)	

NA, not applicable; -, no data. [a] Chi-square Test; [b] Mann-Whitney; [c] Chi-square Test with Yates' continuity correction. * *p*-Value < 0.05

3.2. Correlations of allele and genotype frequencies of ACE1 rs4646994 polymorphism with risk of lung cancer in pulmonary nodules

The distribution of *ACE1* rs4646994 genotype and allele in the case and control groups is shown in Table 2. The results found that the ID genotype of *ACE1* rs4646994 was predominate in both groups. The DD genotype in case group (OR=2.654, 95% CI, 1.057–6.664, p=0.038) were higher than that in the control group, suggesting the DD genotype was closely associated with the risk of lung cancer. Compared with the I carrier (II+ID) genotype, DD genotype significantly increased the onset risk of lung cancer (OR=2.910, 95% CI, 1.205–7.031, p=0.018).

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Table 2. Genotypes and allele frequencies of *ACE1* rs4646994 polymorphisms in patients with lung cancer and benign pulmonary nodules.

Genotype/allele	Case(n=300)	Control(n=100)	*p*-Value	OR	95% CI
II	121	41		Reference	
ID	132	53	0.485	0.844	0.524-1.359
DD	47	6	0.038	2.654	1.057-6.664
DD *vs.* ID+II			0.018	2.91	1.205-7.031
I allele	374	135		Reference	
D allele	226	65	0.189	1.255	0.894-1.761

CI, confidence interval; OR, odds ratio. Case group versus control group.

3.3. Correlations of allele and genotype frequencies of ACE1 rs4646994 polymorphism with histological types of SCC and LUAD

The distribution of genotypic frequencies of *ACE1* rs4646994 in the SCC, LUAD, and control group are listed in Table 3. Patients with pulmonary nodules carrying the DD genotype are about 2.7 times as likely to develop lung adenocarcinoma (OR=2.685, 95% CI, 1.065–6.770, p=0.036). Compared with the I genotype (II+ID) carriers, patients with DD genotype were about 3 times more likely to develop LUAD than patients with benign nodules (OR=2.896, 95% CI, 1.194–7.023, p=0.019). The comparison on *ACE1* rs4646994 between SCC group and control group manifested no statistical significance (p> 0.05). In addition, there was no significant difference between lung squamous cell carcinoma and lung adenocarcinoma in the polymorphism of *ACE1* rs4646994 (p> 0.05).

As shown in Table 4, genotype with DD was more frequent in lung adenocarcinoma patients without EGFR mutation (22.6%). The risk of lung adenocarcinoma in patients with EGFR mutation-positive pulmonary nodules carrying genotype DD (OR=0.295, 95% CI, 0.098–0.885, p=0.029) was lower than that in patients carrying genotype II. The DD genotype decreased the onset risk EGFR mutation when compared with the I carrier (II+ID) genotype (OR=0.298, 95% CI, 0.107–0.831, p=0.021), and D allele was a protect factor contributing to the incidence of EGFR mutation (OR=0.606, 95% CI, 0.372–0.990, p=0.045).

Table 4. Correlations of *ACE1* rs4646994 genotype polymorphism with EGFR mutation in lung adenocarcinoma.

Genotype/allele	EGFR+ (n=75)	EGFR- (n=62)	*p*-Value	OR	95% CI
II	32 (42.7%)	22 (35.5%)		Reference	
ID	37 (49.3%)	26 (41.9%)	0.954	0.978	0.467-2.049
DD	6 (8.0%)	14 (22.6%)	0.029	0.295	0.098-0.885
DD *vs.* ID+II	69 (57.3%)	48 (64.5%)	0.021	0.298	0.107-0.831
I allele	101 (67.3%)	70 (56.5%)		Reference	
D allele	49 (32.7%)	54 (43.5%)	0.045	0.606	0.372-0.990

CI, confidence interval; OR, odds ratio. Case group versus control group.

3.4. ACE1 rs4646994 polymorphism and lung cancer risk in patients with pulmonary nodules by gender

The gender stratification analysis of the correlation between lung cancer risk and *ACE1* rs4646994 polymorphism in patients with pulmonary nodules is shown in Table 5. No statistical association was found between *ACE1* rs4646994 polymorphism and the risk of lung cancer in male patients with pulmonary nodules (p> 0.05). In the female subgroup, patients with pulmonary nodules carrying DD genotype have an increased risk of lung cancer (OR=3.652, 95% CI, 1.025–13.019, p=0.046). Compared with the I carrier (II+ID) genotype, DD genotype (OR=3.457, 95% CI, 1.006–11.875, p=0.049) increased the onset risk of lung cancer with pulmonary nodules. As shown in Table 6, there was no significant

Table 3. Correlations of genotype polymorphism in *ACE1* rs4646994 with histological types of lung squamous carcinoma and adenocarcinoma.

Genotype/ allele	SCC (n=14)	LUAD (n=282)	Control (n=100)	p-Value †	OR †	95%CI †	p-Value ‡	OR ‡	95%CI ‡	p-Value §	OR §	95%CI §
II	7 (50%)	112 (39.7%)	41 (41%)		Reference			Reference			Reference	
ID	6 (42.9%)	126 (44.7%)	53 (53%)	0.489	0.663	0.207-2.124	0.571	0.87	0.538-1.407	0.634	1.312	0.428-4.022
DD	1 (7.1%)	44 (14.6%)	6 (6%)	0.983	0.976	0.101-9.389	0.036	2.685	1.065-6.770	0.351	2.75	0.329-23.005
DD *vs.* ID+II				0.868	1.205	0.134-10.822	0.019	2.896	1.194-7.023	0.404	2.403	0.307-18.842
I allele	20 (71.4%)	350 (62.1%)	135		Reference			Reference			Reference	
D allele	8 (28.6%)	214 (37.9%)	65	0.922	1.038	0.489-2.206	0.157	1.279	0.910-1.798	0.31	1.543	0.668-3.564

CI, confidence interval; OR, odds ratio. † SCC group versus control group; ‡ LUAD group versus control group; § LUAD group versus SCC group.

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difference between SCC group and LUAD group in the polymorphism of *ACE1* rs4646994 by gender stratification analysis (p> 0.05). No evidence shown there was relationship between *ACE1* rs4646994 gene polymorphism and EGFR mutation in male (p> 0.05) and female (p> 0.05) lung adenocarcinoma patients (Table 7).

Table 5. Correlations of genotype polymorphism in *ACE1* rs4646994 and lung cancer risk in patients with pulmonary nodules according to gender.

Gender	Genotype /allele	Case (n=300)	Control (n=100)	*p*-Value	OR	95% CI
Male		135	44			
	II	52 (38.5%)	13 (29.5%)		Reference	
	ID	63 (46.7%)	28 (63.6%)	0.134	0.562	0.265-1.195
	DD	20 (14.8%)	3 (6.8%)	0.461	1.667	0.429-6.475
	DD *vs*.ID+II			0.18	2.377	0.671-8.419
	I allele	167 (61.9%)	54 (61.4%)		Reference	
	D allele	103 (38.1%)	34 (38.6%)	0.935	0.98	0.597-1.606
Female		165	56			
	II	69 (41.8%)	28 (50%)		Reference	
	ID	69 (41.8%)	25 (44.6%)	0.726	1.12	0.594-2.112
	DD	27 (16.4%)	3 (5.4%)	0.046	3.652	1.025-13.019
	DD *vs*.ID+II			0.049	3.457	1.006-11.875
	I allele	207 (62.7%)	81 (72.3%)		Reference	
	D allele	123 (37.3%)	31 (27.7%)	0.067	1.553	0.970-2.485

CI, confidence interval; OR, odds ratio. Case group versus control group.

Table 6. Correlations of genotype polymorphism in *ACE1* rs4646994 with histological types of SCC and LUAD according to gender.

Gender	Genotype /allele	LUAD (n=282)	SCC (n=14)	*p*-Value	OR	95% CI
Male		118	14			
	II	43 (36.4%)	7 (50%)		Reference	
	ID	57 (48.3%)	6 (42.9%)	0.461	1.547	0.485-4.934
	DD	18 (15.3%)	1 (7.1%)	0.331	2.93	0.336-25.570
	DD*vs*.ID+II			0.426	2.34	0.288-19.012
	I allele	143 (60.6%)	20 (71.4%)		reference	
	D allele	93 (39.4%)	8 (28.6%)	0.268	1.626	0.688-3.844
Female		164	0			
	II	69 (42.1%)	0		Reference	
	ID	69 (42.1%)	0	-	-	-
	DD	26 (15.9)	0	-	-	-
	DD*vs*.ID+II			-	-	-
	I allele	207 (63.1%)	0		Reference	
	D allele	121 (36.9%)	0	-	-	-

CI, confidence interval; OR, odds ratio. LUAD group versus SCC group.

Table 7. Correlations of ACE1 rs4646994 genotype polymorphism with EGFR mutation in lung adenocarcinoma according to gender.

Gender	Genotype /allele	EGFR+ (n=75)	EGFR- (n=62)	*p*-Value	OR	95% CI
Male		29	32			
	II	10 (34.5%)	11 (34.4%)		Reference	
	ID	18 (62.1%)	14 (43.8%)	0.539	1.414	0.468-4.270
	DD	1 (3.4%)	7 (21.9%)	0.109	0.157	0.016-1.511
	DD*vs*.ID+II			0.062	0.128	0.015-1.110
	I allele	38 (65.5%)	36 (56.2%)		Reference	
	D allele	20 (34.5%)	28 (43.8%)	0.232	0.65	0.321-1.316
Female		46	30			
	II	22 (47.8%)	11 (36.7%)		Reference	
	ID	19 (41.3%)	12 (40%)	0.654	0.792	0.285-2.202
	DD	5 (10.9%)	7 (23.3z%)	0.137	0.357	0.092-1.387
	DD*vs*.ID+II			0.154	0.401	0.114-1.407
	I allele	63 (68.5%)	34 (56.7%)		Reference	
	D allele	29 (31.5%)	26 (43.3%)	0.14	0.602	0.307-1.181

CI, confidence interval; OR, odds ratio. EGFR+ group versus EGFR- group.

3.5. ACE1 rs4646994 polymorphism and lung cancer risk in patients with pulmonary nodules by age

Stratification analysis was carried out on the rs4646994 polymorphism in *ACE1* by taking age in case group into consideration. The results shown in Table 8 illustrated that DD genotype could increase the risk of lung cancer in older (> 45 years) patients with pulmonary nodules (OR=2.693, 95% CI, 0.983–7.377, p=0.054). Moreover, the probability of lung cancer in patients with pulmonary nodules carrying the DD genotype is about 3 times that of patients carrying the I genotype (II+ID) (OR=2.943, 95% CI, 1.121–7.728, p=0.028). Table 9 showed that there was no correlation between *ACE1* rs4646994 gene polymorphism and different histological types of lung cancer at different ages (both p> 0.05). As shown in Table 10, *ACE1* rs4646994 gene polymorphism was not found to be associated with EGFR mutation in patients with LUAD by age stratification analysis (p> 0.05).

Table 8. Correlations of genotype polymorphism in *ACE1* rs4646994 and lung cancer risk in patients with pulmonary nodules according to age.

Age	Genotype /allele	Case (n=300)	Control (n=100)	*p*-Value	OR	95% CI
≤ 45		48	17			
	II	20 (41.7%)	7 (41.2%)		Reference	
	ID	21 (43.8)	9 (52.9%)	0.733	0.817	0.255-2.611
		7 (14.6)	1 (5.9%)	0.438	2.45	0.254-23.601
	DD *vs*.ID+II			0.365	2.732	0.311-24.009
	I allele	201 (63.8%)	50 (61.0%)		Reference	
	D allele	114 (36.2%)	32 (39.0%)	0.636	0.886	0.538-1.461
>45		252	83			
	II	101 (40.1%)	34 (41.0%)		Reference	
	ID	111 (44.0%)	44 (53.0%)	0.54	0.849	0.504-1.432
	DD	40 (15.9%)	5 (6%)	0.054	2.693	0.983-7.377
	DD *vs*.ID+II			0.028	2.943	1.121-7.728
	I allele	313 (62.1%)	112 (67.5%)		Reference	
	D allele	191 (37.9%)	54 (32.5%)	0.214	1.266	0.873-1.835

CI, confidence interval; OR, odds ratio. Case group versus control group.

Table 9. Correlations of genotype polymorphism in *ACE1* rs4646994 with histological types of SCC and LUAU according to age.

Age	Genotype /allele	LUAD (n=282)	SCC (n=14)	*p*-Value	OR	95% CI
≤ **45**		48	0			
	II	20 (41.7%)	0		Reference	
	ID	21 (43.8%)	0	-	-	-
	DD	7 (14.6%)	0	-	-	-
	DD *vs*.ID+II	-	-	-	-	-
	I allele	61 (63.5%)	0		Reference	
	D allele	35 (36.5%)	0	-	-	-
>45		234	14			
	II	92 (39.3%)	7 (50%)		Reference	
	ID	105 (44.9%)	6 (42.9%)	0.618	1.332	0.432-4.105
	DD	37 (15.8%)	1 (7.1%)	0.341	2.815	0.335-23.684
	DD *vs*.ID+II			0.397	2.442	0.310-19.235
	I allele	289 (61.8%)	20 (71.4%)		Reference	
	D allele	179 (38.2%)	8 (28.6%)	0.308	1.548	0.668-3.590

CI, confidence interval; OR, odds ratio.LUAD group versus SCC group.

Table 10. Correlations of *ACE1* rs4646994 genotype polymorphism with EGFR mutation in lung adenocarcinoma according to age.

Age	Genotype /allele	EGFR+ (n=75)	EGFR- (n=62)	*p*-Value	OR	95% CI
≤ **45**		9	10			
	II	4 (44.4%)	3 (30%)		Reference	
	ID	5 (55.6%)	4 (40%)	0.949	0.938	0.128-6.875
	DD	0	3 (30%)	0.999	0	0
	DD *vs*.ID+II			0.999	0	0
	I allele	13 (72.2%)	10 (50%)		Reference	
	D allele	5 (27.8%)	10 (50%)	0.166	0.385	0.099-1.489
>45		66	52			
	II	28 (42.4%)	19 (36.5%)		Reference	
	ID	32 (48.5%)	22 (42.3%)	0.974	0.987	0.445-2.188
	DD	6 (9.1%)	11 (21.2%)	0.091	0.37	0.117-1.172
	DD *vs*.ID+II			0.071	0.373	0.128-1.088
	I allele	88 (66.7%)	60 (57.7%)		Reference	
	D allele	44 (33.3%)	44 (42.3%)	0.158	0.682	0.401-1.160

CI, confidence interval; OR, odds ratio. EGFR+ group versus EGFR- group.

4. Discussion

Angiotensin-converting enzyme (ACE) gene polymorphism is one of the most studied genetic systems in recent years, including cardiovascular, metabolic, immune, cancer, aging, neurodegenerative diseases and mental illness [22–26]. This case-control study aimed to investigate the potential association between *ACE1* rs4646994 polymorphism and lung cancer risk in Chinese patients with pulmonary nodules, and whether this association is related to lung cancer histology and EGFR mutation. We found that the DD genotype of *ACE1* rs4646994 may be associated with lung cancer in patients with pulmonary nodules (OR=2.654, 95% CI, 1.057–6.664, p=0.038). The results of a previous meta-analysis did not show any significant association between *ACE1* rs4646994 polymorphism and lung cancer risk [12]. The inconsistency between the results of this study and our results may be affected by race and the number of samples. We did not observe strong evidence that

increased risk was associated with specific histological types of lung cancer (OR=2.750, 95% CI, 0.329–23.005, p=0.351). More interestingly, we found that patients with pulmonary nodules carrying the DD genotype had an increased risk of adenocarcinoma and a lower probability of EGFR gene mutation (OR=0.606, 95% CI, 0.372–0.990, p=0.045).

In the gender stratification analysis, we found that DD genotype carriers of *ACE1* rs4646994 had a significantly increased risk of lung cancer in female patients with pulmonary nodules (OR=3.652, 95% CI, 1.025–13.019, p=0.046). No statistically significant association between ACE1 gene polymorphism and lung cancer risk was found in male patients with pulmonary nodules (OR=1.667, 95% CI, 0.429–6.475, p=0.461). However, it is certain that in both male and female subgroups, we observed that the DD genotype of *ACE1* was positively correlated with lung cancer susceptibility in patients with pulmonary nodules. Male patients with pulmonary nodules carrying DD genotype of *ACE1* rs4646994 is more strongly associated with lung adenocarcinoma than lung squamous cell carcinoma (OR=2.930, 95% CI, 0.336–25.570, p=0.331). Due to the limited number of samples, there was no lung squamous cell carcinoma in female patients diagnosed with lung cancer. There was no significant correlation between *ACE1* polymorphism and EGFR mutation after gender stratification. However, we observed that patients with DD genotype of *ACE1* rs4646994 were less likely to have EGFR mutations in male and female subgroups of lung adenocarcinoma patients, which was consistent with the results of no gender stratification analysis. It is noteworthy that in this study, we observed that patients with benign nodules also had EGFR mutations, suggesting that patients with benign nodules should also be screened for EGFR mutations.

In patients with pulmonary nodules under 45 years old, no statistical association was found between ACE1 rs4646994 and lung cancer risk, while in older patients (> 45), the risk of lung cancer was significantly increased in patients with DD genotype (DD *vs.* ID+II: OR=2.943, 95% CI, 1.121–7.728, p=0.028). It was reported that advanced age is risk factor for lung cancer and pulmonary nodule [27–29]. In the age stratification analysis, no significant correlation was found between *ACE1* rs4646994 polymorphism and different pathological types of lung cancer and EGFR mutation in patients with lung adenocarcinoma.

As far as we know, this is first study exploring the association between *ACE1* rs4646994 polymorphism and lung cancer risk in patients with pulmonary nodules. And this study is one of the few that has investigated the association between gene polymorphism and the risk of SCC and LUAD. However, there are some limitations in our research. It is difficult to avoid selection bias and information bias in the whole research process. Although the number of samples included in this study can meet the experimental requirements, the number of respondents in the stratified analysis is not sufficient. In order to further understand the role of *ACE1* gene in the development of lung cancer and find out the complex relationship between *ACE1* polymorphism and gene-environment interaction, larger sample size and various expression studies will be needed. In addition, as with all association studies, it is certainly necessary to reproduce our findings in independent studies and in different ethnic populations.

5. Conclusions

In conclusion, our study indicates that the DD genotype of *ACE1* rs4646994 may contribute to an increased risk of lung cancer in patients with pulmonary nodules. Furthermore, the possibility of EGFR mutation in lung adenocarcinoma patients with *ACE1* rs4646994 DD genotype was lower than that of II or ID genotype carriers.

Author Contributions:

Rong Qiao: Ideas, Conduction a research and investigation process, Data collection, Data analysis, Writing original draft.
Siyao Sang: Writing original draft, Methodology, Visualization.
Jiajun Teng: Writing review and editing, Visualization.
Hua Zhong: Supervision, Writing review and editing.
Hui Li: Funding acquisition, Writing review and editing.

Baohui Han: Supervision, Writing review and editing, Data collection.

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Abbreviations

ACE	Angiotensin-converting enzyme
CI	confidence intervals
LUAD	adenocarcinoma
NSCLC	non-small cell lung cancer
OR	odds ratios
SCC	squamous cell carcinoma
SCLC	small cell lung cancer
SNPs	single nucleotide polymorphisms

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