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Association of genetic polymorphisms in *TERT-CLPTM1L* with lung cancer in a Chinese population

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ABSTRACT. Genome-wide association studies in several ethnic groups have reported that polymorphisms of the telomerase reverse transcriptase (*TERT*) and cleft lip and palate transmembrane 1-like (*CLPTM1L*) genes, located on 5p15.33, are associated with susceptibility to lung cancer. However, whether genetic variants of *TERT-CLPTM1L* are associated with an increased risk of lung cancer in the Chinese Han population is unknown. This study examined associations between five single nucleotide polymorphisms (SNPs) of *TERT-CLPTM1L* (rs402710, rs401681, rs465498, rs4975616, and rs2736100) and lung cancer in a Chinese Han population in the Hubei Province. The five SNPs were detected using the Sequenom MassArray® iPLEX System in 304 lung cancer patients and 319 controls. Of the five SNPs, rs4975616 did not conform to Hardy-Weinberg equilibrium in the controls. Only rs2736100 was significantly (P = 0.034) associated with an increased risk of lung cancer. In the linkage disequilibrium analyses, a block of strong linkage disequilibrium was observed between rs401681 and

rs465498 (D′ = 0.986; r^2 = 0.546). No linkage disequilibrium between rs2736100 and the other three SNPs was found. In the haplotype analyses, the frequencies of the TTCT haplotype in rs402710, rs401681, rs465498, and rs2736100 differed significantly between case and control subjects (odds ratio = 0.56; 95% confidence interval, 0.36-0.88; P = 0.012). The results of this study suggested that rs2736100 on *TERT-CLPTM1L* indicates a poor prognosis for lung cancer in the Chinese Han population.

Key words: Lung cancer; *TERT-CLPTM1L*; Polymorphism; Association study

INTRODUCTION

Lung cancer is among the most common cancers worldwide. In China, it is the leading cause of death among people with malignant tumors, and the registered lung cancer mortality rate has soared during the past three decades (She et al., 2013). Environmental factors such as smoking and pollution reportedly increase the risk of lung cancer. However, molecular research on the etiology of lung cancer strongly suggests that genetic factors play a vital role in the incidence of lung carcinoma (Lichtenstein et al., 2000; Lissowska et al., 2010; Li et al., 2013). Many genetic variants associated with the pathogenesis of lung cancer have been identified in genome-wide association studies (GWAS), meta-analyses, and case-control studies.

Telomerase reverse transcriptase (TERT) is the reverse transcriptase component of telomerase that is essential for the maintenance of telomeres and telomerase production. The cleft lip and palate transmembrane 1-like gene (*CLPTM1L*) encodes a protein linked to cisplatin resistance and is conserved and expressed in various normal and malignant tissues such as skin, lung, breast, ovarian, and cervical tissues (Yamamoto et al., 2001). *CLPTM1L* resides in a 62-kb region of the 5p15.33 locus, which encompasses the 5'-end of *TERT*. Studies have reported that *TERT* and *CLPTM1L* are associated with increased risks for many types of cancer. *TERT* is considered a more plausible candidate at 5p15.33 in terms of its association with lung cancer risk (Weinrich et al., 1997). James et al. (2012) identified CLPTM1L as an important factor in lung cancer in terms of inducing the apoptosis of lung cells exposed to genotoxic agents such as tobacco carcinogens.

The genetic loci rs402710, rs401681, rs465498, rs4975616, and rs2736100 are located on *TERT-CLPTM1L*. Recently, GWAS have shown that these single nucleotide polymorphisms (SNPs) are significantly associated with lung cancer risk in Europeans and Asians. In individuals of European descent, rs4975616, rs2736100, and rs402710 are associated with the risk of lung cancer (Landi et al., 2009; Law et al., 2012). In a Chinese population, rs465498 and rs2736100 are reportedly linked to susceptibility to lung cancer, whereas rs401681 and rs2736100 are associated with lung cancer in Koreans (Yoon et al., 2010; Hu et al., 2011).

Although these five SNPs are reportedly associated with the development of lung cancer, the association between these genetic variants and lung cancer in the Chinese population needs confirmation. Considering the heterogeneity among ethnic groups, it is essential to investigate genetic associations with lung cancer in the Chinese population. The present study was a case-control association study in a Chinese Han population of Hubei Province with the aim of evaluating the relationship between susceptibility to lung cancer and genetic variants of *TERT-CLPTM1L*.

MATERIAL AND METHODS

A total of 304 lung cancer patients with pathology-based diagnoses at Zhongshan Hospital, Hubei, Wuhan, China were enrolled in the study; 319 control subjects were selected from healthy individuals from the same geographic region who were free of illness or any type or cancer. The mean ages of the case and control subjects were 59.6 and 43.1 years, respectively. The details of the subjects are summarized in Table 1. This study was approved by the Zhongshan Hospital of Hubei Ethics Committee, and written informed consent was obtained from all participants.

Peripheral blood samples (5 mL) were collected and stored frozen. Genomic DNA was extracted from the peripheral blood samples of all subjects using a Genomic DNA Purification Kit (Omega Bio-Tek Inc., Norcross, GA, USA). The five SNPs (rs402710, rs401681, rs465498, rs4975616, and rs2736100) were genotyped using a Sequenom MassARRAY matrix-assisted laser desorption ionization-time of flight spectrometry platform (Sequenom Inc., San Diego, CA, USA). Primers were designed using Assay Design 3.1 (Sequenom) and are listed in Table 2. The call rate for each assay was set at >90%.

Table 1. Characteristics of lung cancer case and control subjects.

Characteristic	Cases (N = 304)	Controls (N = 319)	P**
Gender (male)	48.36%	49.22%	0.548
Age (years)	59.63 (10.82)	43.06 (15.02)	<0.001
Histology			
Squamous cell cancer	26.00%	-*	-
Small cell cancer	4.00%	-	-
Adenocarcinoma	67.00%	-	-
Unspecified	3.00%	-	-
Lymph node metastasis (yes)	41.45%	-	-

Data are reported as means ± standard deviation or percentage as indicated. *Data unavailable. **P values were calculated using an unpaired *t*-test for age and the chi-square test for other categorical characteristics.

Table 2. Sequences of primers of polymerase chain reaction products.

SNP	Primer sequence
rs402710	F: ACGTTGGATGAAAGCCGTCATTCCGTTCAG
	R: ACGTTGGATGTCTGGTCTACCTGTACCAGC
	U: GTGAGCAACGGCCGAGCATACGCAGC
rs401681	F: ACGTTGGATGAGGTCTGCTATCCAGACAAC
	R: ACGTTGGATGGCTCTCCAAAGTTGTCGTAG
	U: CCAGACAACTTCAGAGTC
rs465498	F: ACGTTGGATGTCTGCAGATGGCCATCTTAC
	R: ACGTTGGATGAGTTGTAATGGCTGAACCCC
	U: AGCAGATGGCCATCTTACTCTTTGC
rs2736100	F: ACGTTGGATGTGACACCCCCACAAGCTAAG
	R: ACGTTGGATGACAAAGGAGGAAAAGCAGGG
	U: TTGTTTTCCGTGTTGAGTGTTTCT
rs4975616	F: ACGTTGGATGTGAGATGGATCTGCATGAGG
	R: ACGTTGGATGTCTGACAGTCTGACTTCTGC
	U: CATCTGCATGAGGCTCAGTCCTCTCTT

SNP = single nucleotide polymorphism.

The characteristics of the patients and controls were compared using an unpaired Student *t*-test for continuous variables and the chi-square test for categorical variables. Hardy-

Weinberg equilibrium was tested for each of the SNPs using Haploview (ver. 4.2; Daly Lab, USA). The association between each SNP and lung cancer was evaluated by computing the odds ratio (OR) and 95% confidence interval (95%CI) from binary logistic regression analyses adjusted for age and gender. Haplotypes and their association with lung cancer were analyzed using the web-based tool SNPStats (http://bioinfo.iconcologia.net/SNPstats). Pairwise linkage disequilibrium (LD) graphs and LD coefficients for the five SNPs were also calculated using Haploview. Statistical analyses were carried out using the SPSS software (ver. 17.0; SPSS; Chicago, IL, USA). A P-value of <0.05 was considered to be statistically significant.

RESULTS

The characteristics of the 304 lung cancer patients and 319 healthy controls, all Han Chinese, are summarized in Table 1. The percentages of male cases and control subjects were 48.36 and 49.22%, respectively; the difference in gender was not significant. No significant difference was found in the median age of the case and control subjects (59.63 *vs* 43.06 years). Of the lung cancer patients, 67% had adenocarcinoma, 26% squamous cell cancer, 4% small cell cancer, and 3% unspecified lung cancer; 41.45% of these patients had lymph node metastasis.

Before analyzing the associations between the *TERT-CLPTM1L* polymorphisms and lung cancer, we tested the Hardy-Weinberg equilibrium of the candidate SNPs in both the case and the control groups. Of the five SNPs, rs4975616 did not conform to Hardy-Weinberg proportions in the control group (P < 0.001; data not shown) and was excluded from further analysis. The genotype distributions of the other four SNPs (rs402710, rs401681, rs465498, and rs2736100) were analyzed after adjusting for age and gender (Table 3). A significant association between rs2736100 and lung cancer risk was identified (P = 0.034). Compared with the G/T and T/T genotypes of rs2736100 in *TERT-CLPTM1L*, G/G showed an increased risk of lung cancer (OR = 1.96; 95%CI, 1.13-3.41). For the other three SNPs (rs402710, rs401681, and rs465498), no significant differences in genotype frequencies were found between the cases and controls for the dominant or codominant models (P = 0.63, P = 0.36, and P = 0.59, respectively).

Table 3. Summary results for *TERT-CLPTM1L* single-nucleotide polymorphisms in lung cancer case-control groups.

rs-ID	Model	Genotype	Controls	Cases	OR* (95%CI)	P**
rs402710	Codominant	C/C	144 (45.3%)	161 (55.9%)	1	0.63
		C/T	140 (44%)	101 (35.1%)	0.84 (0.56-1.25)	
		T/T	34 (10.7%)	26 (9%)	0.82 (0.42-1.58)	
rs401681	Codominant	C/C	138 (43.3%)	157 (53.8%)	1	0.36
		C/T	146 (45.8%)	112 (38.4%)	0.89 (0.60-1.33)	
		T/T	35 (11%)	23 (7.9%)	0.61 (0.31-1.21)	
rs465498	Codominant	T/T	198 (62.1%)	202 (68.7%)	1	0.59
		T/C	105 (32.9%)	78 (26.5%)	0.90 (0.59-1.37)	
		C/C	16 (5%)	14 (4.8%)	0.65 (0.27-1.57)	
rs2736100	Codominant	T/T	92 (29%)	72 (25%)	1	0.034
		G/T	173 (54.6%)	139 (48.3%)	1.11 (0.70-1.75)	
		G/G	52 (16.4%)	77 (26.7%)	1.96 (1.13-3.41)	

*OR = odds ratio adjusted by gender and age. 95%CI = 95% confidence interval. **P < 0.05 was considered to be statistically significant.

Pairwise LD and haplotype analyses were performed for the four examined SNPs in *TERT-CLPTM1L*. The LD structure of these SNPs is shown in Figure 1. Strong LD was ob-

served between rs401681 and rs465498, with a D' value of 0.986 and an r^2 value of 0.546, and between rs402710 and rs465498, with a D' value of 0.971 and an r^2 value of 0.91 (see Figure 1; Table 4). No LD was found between rs2736100 and the other three SNPs.

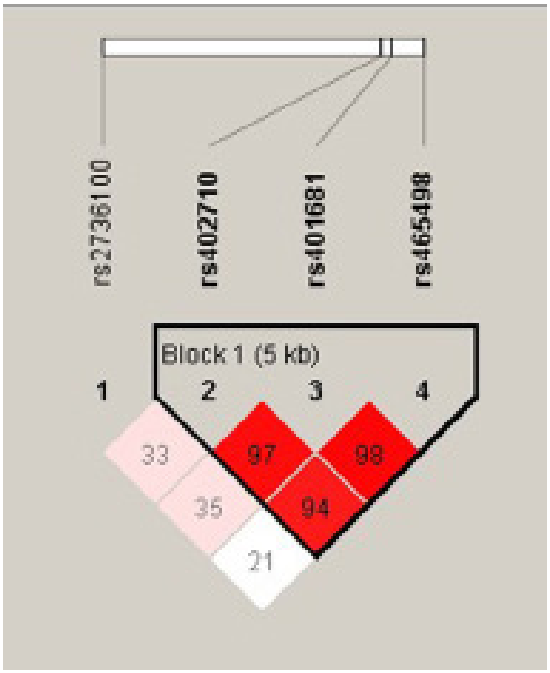

Figure 1. Linkage disequilibrium structure of the *CLPTM1L* single nucleotide polymorphisms.

Table 4. Results of linkage disequilibrium analysis of four SNPs in *TERT-CLPTM1L*.

SNP 1	SNP 2	D'[a]	LOD[b]	r^2	CIlow[c]	CIhi[d]
rs402710	rs401681	0.971	198.95	0.91	0.94	0.99
rs402710	rs465498	0.94	92.85	0.515	0.89	0.97
rs402710	rs2736100	0.332	5.67	0.042	0.22	0.43
rs401681	rs465498	0.986	103.19	0.546	0.95	1
rs401681	rs2736100	0.353	6.4	0.049	0.24	0.46
rs465498	rs2736100	0.212	1.32	0.01	0.06	0.35

SNP = single nucleotide polymorphism. D'[a] > 0.9 was considered linkage disequilibrium; LOD[b] = log of the likelihood odds ratio, a measure of confidence in the value of D'; CIlow[c] = the 95% confidence lower bound on D'; CIhi[d] = the 95% confidence upper bound on D'.

When the haplotypes in rs402710, rs401681, rs465498, and rs2736100 were reconstructed, six haplotypes were observed in the samples. All of these haplotypes were present at frequencies of >0.001. As shown in Table 5, GCCAT occurred most frequently (0.3712). The frequency of the TTCT haplotype differed significantly between case and control subjects (OR = 0.56; 95%CI, 0.36-0.88; P = 0.012).

Table 5. Haplotype association of four SNPs in *TERT-CLPTM1L*.

	rs402710	rs401681	rs465498	rs2736100	Freq	OR (95%CI)	P
1	C	C	T	G	0.3712	1	-
2	C	C	T	T	0.3134	0.79 (0.55-1.14)	0.21
3	T	T	C	T	0.1222	0.56 (0.36-0.88)	0.012
4	T	T	T	T	0.0815	0.90 (0.51-1.60)	0.73
5	T	T	C	G	0.0676	1.30 (0.67-2.52)	0.44
6	T	T	T	G	0.0237	0.60 (0.18-1.97)	0.4
Rare	*	*	*	*	0.0204	0.92 (0.32-2.68)	0.88

95%CI = 95% confidence interval; OR = odds ratio. *Means none.

DISCUSSION

Lung cancer, a disease of complex origin, is the result of many genetic and environmental factors. The candidate-gene approach is a robust method for investigating the potentially pathogenic multi-gene variants of carcinoma susceptibility. GWAS identify such variants by testing the association between thousands of SNPs and a large number of individuals. In recent years, however, GWAS have attracted criticism for the low effect sizes of SNPs in contrast to their highly significant P values. Therefore, in addition to discovering new variants, a necessary step has become the confirmation of GWAS-derived SNPs through case-control studies in various populations. Therefore, this study examined the association between *TERT-CLPTM1L* gene polymorphisms and lung cancer susceptibility in a Chinese Han population. Four SNPs (rs402710, rs401681, rs465498, and rs2736100) located in *TERT-CLPTM1L* were evaluated in 304 lung cancer patients and 319 controls. A significant association between rs2736100 and the occurrence of lung cancer was revealed, suggesting that *TERT-CLPTM1L* plays an important role in the pathogenesis of lung cancer.

The SNP marker rs2736100 lies in intron 2 of *TERT*, a telomerase gene that has been described as a risk factor for the carcinogenesis of most cancers, including lung cancer (Wu et al., 2003; Garcia et al., 2007). Lan et al. (2013) found an association between greater telomere length and increased risk of lung cancer, and rs2736100 is significantly associated with greater telomere length. Consequently, rs2736100 may be linked to lung cancer susceptibility. Another GWAS in a Han Chinese population reported an association between this SNP and the development of lung cancer (Hu et al., 2011), and our study validated that finding. Individuals carrying the rs2736100 G allele might have a higher risk of lung carcinogenesis (data not shown). However, we did not find LD between rs2736100 and the other three SNPs. According to previous reports and our finding that *TERT-CLPTM1L* variants are implicated in the development of lung cancer, genetic variants of rs2736100 in the regulatory region of *TERT-CLPTM1L* may have effects on lung tumorigenesis.

The rs402710 SNP is located in a region of high LD that includes the proximal and putative promoter regions of *TERT* as well as the entire coding region of *CLPTM1L*. Zienolddiny et al. (2009) found that rs402710 is associated with increased formation of DNA adducts in the lungs, a possible precursor to lung carcinogenesis. However, we failed to detect an association between lung cancer risk and rs402710 in Chinese Han individuals. Ke et al. (2013) replicated the significant association of rs401681 (in intron 13 of *CLPTM1L*) with lung cancer risk in a Chinese population; however, Li et al. (2013) reported no association with lung cancer risk in a nonsmoking Chinese population. Our data are consistent with those of Li et al. (2013) in

which rs401681 was not associated with lung cancer risk in the Chinese Han population of Hubei Province. The dissimilarities in outcomes among studies might be due to differences in the geographical locations of the populations studied. Although Hu et al. (2011) found that rs465498, which is localized to intron 10 of *CLPTM1L*, is associated with non-small-cell lung cancer in the Han Chinese, our findings suggested no association with lung cancer risk. According to the HapMap SNP database, rs402710 is in LD with rs465498 in Han Chinese (r^2 = 0.687), and rs401681 is in low LD with rs465498 (r^2 = 0.002) (Hu et al., 2011). We also found that rs401681 and rs465498 were linked (r^2 = 0.546) in our subjects, albeit with an LD lower than that between rs402710 and rs465498 (r^2 = 091).

In conclusion, this association study identified four SNPs located in *TERT-CLPTM1L* as genetic susceptibility factors for lung cancer in a Chinese Han population. Of the four SNPs, rs2736100 located in intron 2 of *TERT* is significantly associated with lung cancer. This result is in line with those of previous reports. The association of rs2736100 with lung cancer susceptibility has also been reported in geographically separated cohorts and in populations that have smoked and never smoked (McKay et al., 2008; Hsiung et al., 2010). Therefore, we hypothesize that rs2736100 is an important locus in the relationship between *TERT-CLPTM1L* and lung cancer risk. The presence of this SNP is an indicator of poor prognosis and may be a test site for the early diagnosis of lung cancer. Further studies are required to confirm this hypothesis.

Conflicts of interest

The authors declare no conflict of interest.

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