**TERT-CLPTM1L Rs401681 C>T Polymorphism Was Associated with a Decreased Risk of Esophageal Cancer in a Chinese Population**

Jun Yin1*, Liming Wang2*, Liang Zheng3*, Xu Wang1, Yijun Shi1, Aizhong Shao1, Guowen Ding1, Chao Liu1, Suocheng Chen1, Weifeng Tang1*, Haiyong Gu1*

1 Department of Cardiothoracic Surgery, Affiliated People's Hospital of Jiangsu University, Zhenjiang, Jiangsu Province, China, 2 Cancer institute, Department of chemotherapy, People’s Hospital Affiliated to Jiangsu University, Zhenjiang, Jiangsu Province, China, 3 Department of Cardiothoracic Surgery, The First People's Hospital of Changzhou and The Third Affiliated Hospital of Suzhou University, Changzhou, Jiangsu Province, China

**Abstract**

**Background:** Esophageal cancer was the fifth most commonly diagnosed cancer and the fourth leading cause of cancer-related death in China in 2009. Esophageal squamous cell carcinoma (ESCC) accounts for more than 90 percent of esophageal cancers. Genetic factors probably play an important role in the ESCC carcinogenesis.

**Methods:** We conducted a hospital based case-control study to evaluate functional hTERT rs2736098 G>A and TERT-CLPTM1L rs401681 C>T single nucleotide polymorphisms (SNPs) on the risk of ESCC. Six hundred and twenty-nine ESCC cases and 686 controls were recruited. Their genotypes were determined using the ligation detection reaction (LDR) method.

**Results:** When the TERT-CLPTM1L rs401681 CC homozygote genotype was used as the reference group, the CT genotype was associated with a significantly decreased risk of ESCC (adjusted OR = 0.74, 95% CI = 0.58–0.94, p = 0.012); the CT/TT variants were associated with a 26% decreased risk of ESCC (adjusted OR = 0.74, 95% CI = 0.59–0.93, P = 0.009). The significantly decreased risk of ESCC associated with the TERT-CLPTM1L rs401681 C>T polymorphism was associated with male sex, young age (<63 years in our study) and alcohol consumption. No association between the hTERT rs2736098 G>A polymorphism and ESCC risk was observed.

**Conclusion:** TERT-CLPTM1L rs401681 CT and CT/TT genotypes were associated with decreased risk of ESCC, particularly among men, young patients and those reported to be drinkers. However, our results are preliminary conclusions. Larger studies with more rigorous study designs are required to confirm the current findings.

Citation: Yin J, Wang L, Zheng L, Wang X, Shi Y, et al. (2014) TERT-CLPTM1L Rs401681 C>T Polymorphism Was Associated with a Decreased Risk of Esophageal Cancer in a Chinese Population. PLoS ONE 9(7): e100667. doi:10.1371/journal.pone.0100667

**Introduction**

Esophageal cancer was the fifth most commonly diagnosed cancer and the fourth leading cause of cancer-related death in China in 2009 [1]. Esophageal cancer is very aggressive and is associated with a poor prognosis [2]. Esophageal squamous cell carcinoma (ESCC) accounts for more than 90 percent of esophageal cancers [3]. Smoking and heavy drinking are major environmental risk factors for ESCC [4]. However, only a subset of individuals exposed to these environmental risk factors develop ESCC, suggesting that genetic factors, such as single nucleotide polymorphisms (SNPs), may also contribute to ESCC carcinogenesis.

Recently, several genome-wide association studies (GWAS) reported that common polymorphisms of Telomerase reverse transcriptase-cleft lip and palate transmembrane 1 like, CLPTM1L (TERT-CLPTM1L), which is located at locus 5p15.33, were associated with the risk of many types of cancer [5,6]. The 5p15.33 locus, which is associated with telomerase function, contains two key genes: the TERT gene and the CLPTM1L gene. The TERT-CLPTM1L SNP, rs401681 (C>T, located in intron 13 of CLPTM1L, 27 kb from the TERT gene), is one of the most extensively studied SNPs. Two variants in 5p15 (rs401681 and rs2736098) are significantly associated with bladder cancer in individuals of European ancestry. These variants are in linkage disequilibrium (LD) with CLPTM1L and TERT, and both variants are also associated with basal cell carcinoma [6], lung
controls in a Chinese population.
rs401681 C
CLPTM1L
genotyping analyses of
susceptibility in a hospital-based case-control study. We performed
and
overexpressed in lung cancer cells [15]. The
CLPTM1L
gene may play a role in the apoptotic response. Overexpression of
CLPTM1L
mRNA has been observed in many cancer types including non-
clinal melanoma skin cancers [13]. Although the function of the
CLPTM1L
gene is largely unknown, studies have demonstrated that it may induce apoptosis. For example,
CLPTM1L, as a predicted transmembrane protein, is upregulated in cisplatin-resistant ovarian cancer cell lines, and may be involved in the apoptotic response of cells to cisplatin-induced genotoxic stress.
CLPTM1L
variants are hypothesized to enhance the metabolic activation of reactive metabolites and/or the formation and persistence of DNA adducts [6]. Jiang et al. found that
TERT-
CLPTM1L
rs401681 was a genetic variant associated with the risk of lung cancer [16].

The role of CLPTM1L was initially described in ovarian cancer cells, where overexpression of the gene induced apoptosis in cisplatin-sensitive cells [14]. CLPTM1L is also involved in mitochondrial apoptosis in normal cells, and was reported to be overexpressed in lung cancer cells [15]. The
CLPTM1L
gene may play a role in the apoptotic response. Overexpression of
CLPTM1L
mRNA has been observed in many cancer types including non-melanoma skin cancers [13]. Although the function of the
CLPTM1L
gene is largely unknown, studies have demonstrated that it may induce apoptosis. For example, CLPTM1L, as a predicted transmembrane protein, is upregulated in cisplatin-resistant ovarian cancer cell lines, and may be involved in the apoptotic response of cells to cisplatin-induced genotoxic stress.
CLPTM1L
variants are hypothesized to enhance the metabolic activation of reactive metabolites and/or the formation and persistence of DNA adducts [6]. Jiang et al. found that
TERT-
CLPTM1L
rs401681 was a genetic variant associated with the risk of lung cancer [16].

The biological and pathological significance of hTERT and TERT-CLPTM1L suggests that functional genetic variations in the
hTERT
and
TERT-CLPTM1L
genes may contribute to the development of ESCC. Thus, the objective of this investigation was to evaluate the association between
hTERT
rs2736098 G>A and
TERT-CLPTM1L
rs401681 C>T polymorphisms and ESCC susceptibility in a hospital-based case-control study. We performed genotyping analyses of
hTERT
rs2736098 G>A and
TERT-CLPTM1L
rs401681 C>T SNPs in 629 ESCC cases and 686 controls in a Chinese population.

Patients and Methods

Ethical approval of the study protocol
This hospital-based case-control study was approved by the Review Board of Jiangsu University (Zhenjiang, China). We have complied with the World Medical Association Declaration of Helsinki regarding ethical conduct of research involving human subjects and/or animals [17]. All subjects provided written informed consent to be included in the study.

Study subjects
629 subjects with esophageal cancer were consecutively recruited from the Affiliated People’s Hospital of Jiangsu University and Affiliated Hospital of Jiangsu University (Zhenjiang, China) between October 2008 and December 2010. All cases of esophageal cancer were diagnosed as ESCC by pathologic means. The exclusion criteria were patients who previously had: cancer; any metastasized cancer; radiotherapy or chemotherapy. The 686 controls were patients without cancer frequency-matched to the cases with regard to age (±5 years) and sex recruited from the two hospitals mentioned above during the same time period. Most of the controls were admitted to the hospitals for the treatment of trauma.

Each subject was personally questioned by trained interviewers using a pre-tested questionnaire to obtain information on demographic data (e.g., age, sex) and related risk factors (including tobacco smoking and alcohol consumption). After the interview, 2-

Statistical Analyses
Differences in the distributions of demographic characteristics, selected variables, and genotypes of the
hTERT
rs2736098 G>A and
TERT-CLPTM1L
rs401681 C>T variants between the cases and controls were evaluated using the
χ2
test. The associations between
hTERT
rs2736098 G>A and
TERT-CLPTM1L
rs401681 C>T genotypes and risk of ESCC were estimated by computing the ORs and their 95% CIs using logistic regression analyses for crude ORs and adjusted ORs when adjusting for age, sex, smoking and drinking status. The Hardy-Weinberg equilibrium (HWE) was tested by a goodness-of-fit
χ2
test to compare the observed genotype frequencies to the expected ones among the control subjects. All statistical analyses were performed with SAS 9.1.3 (SAS Institute, Cary, NC, USA).

Results
Characteristics of the study population
Characteristics of cases and controls included in the study are summarized in Table 1. The cases and controls appeared to be adequately matched on age and sex as suggested by the
χ2
tests ($P=0.541$ and
P=0.185,
respectively). As shown in Table 1, significant difference was detected on smoking status between the cases and the controls ($P<0.001$), and drinking rate was higher in ESCC patients than in control subjects ($P<0.001$). The primary information for two genotyped SNPs was in Table S1. For the
hTERT rs2736098 G>A, the genotyping was successful in 600 (95.39%) ESCC cases and 651 (94.90%) controls in all 1315 samples, and for TERT-CLPTM1L rs401681 C>T, the genotyping was successful in 604 (96.06%) ESCC cases and 664 (96.78%) controls. The concordance rates of repeated analyses were 100%. Minor allele frequency (MAF) in our controls was similar to MAF for Chinese in database for all two SNPs (Table S1). The observed genotype frequencies for these two polymorphisms in the controls were all in HWE (Table S1).

Associations between two polymorphisms and risk of ESCC

The genotype distributions of hTERT rs2736098 G>A and TERT-CLPTM1L rs401681 C>T in the cases and the controls are shown in Table 2. In the single locus analyses, the genotype frequencies of TERT-CLPTM1L rs401681 C>T were 47.68% (CC), 41.72% (CT), and 10.60% (TT) in the case patients and 40.06% (CC), 47.74% (CT), and 12.20% (TT) in the control subjects, and the difference was statistically significant (P = 0.024). When the TERT-CLPTM1L rs401681 C allele was used as the reference group, the T allele was associated with a significantly decreased risk for ESCC (T vs C: adjusted OR = 0.81, 95% CI = 0.69–0.96, P = 0.014). When the TERT-CLPTM1L rs401681 CC homozygote genotype was used as the reference group, the CT genotype was associated with a significantly decreased risk for ESCC (CT vs CC: adjusted OR = 0.74, 95% CI = 0.58–0.94, P = 0.012). When the TERT-CLPTM1L rs401681 CC homozygote genotype was used as the reference group, the TT genotype was not associated with the risk for ESCC (TT vs CC: adjusted OR = 0.75, 95% CI = 0.51–1.09, P = 0.126). In the dominant model, the TERT-CLPTM1L rs401681 CT/TT variants were associated with a 26% decreased risk of ESCC, compared with the TERT-CLPTM1L rs401681 CC genotype (adjusted OR = 0.74, 95% CI = 0.59–0.93, P = 0.009). In the recessive model, when the TERT-CLPTM1L rs401681 CC/CT genotypes were used as the reference group, the TT homozygote genotype was not associated with the risk for ESCC (adjusted OR = 0.87, 95% CI = 0.61–1.24, P = 0.447) (Table 2).

hTERT rs2736098 G>A was not showed a significant difference in the genotype distributions between cases and controls (P = 0.727). Logistic regression analyses revealed that the hTERT rs2736098 G>A polymorphism was not associated with the risk of ESCC (Table 2).

Using Power and Sample Size Calculation (PS, version 3.0, http://biostat.mc.vanderbilt.edu/twiki/bin/view/Main/PowerSampleSize) and considering TERT-CLPTM1L rs401681 C>T mutant alleles in the control group, ORs, ESCC samples and control samples, the power of our analysis (α = 0.05) was 0.708 in 604 ESCC cases and 664 controls, with an OR of 0.74.

Stratification analyses on the TERT-CLPTM1L rs401681 C>T polymorphisms and the risk of ESCC

To evaluate the effects of TERT-CLPTM1L rs401681 C>T genotypes on ESCC risk according to different age, sex, smoking and alcohol drinking status; we performed the stratification analyses (Table 3). A significantly decreased risk of ESCC associated with the TERT-CLPTM1L rs401681 C>T polymorphism was evident among male patients (CT vs CC: P = 0.0003; CT/TT vs CC: P = 0.0002), younger patients (<63 years in our study) (CT/TT vs CC: P = 0.049) and patients who were in drinking status (CT vs CC: P = 0.012; CT/TT vs CC: P = 0.016) (Table 3).

Discussion

In this hospital-based case-control study, we investigated the association of the hTERT rs2736098 G>A and TERT-CLPTM1L rs401681 C>T SNPs with the risk of ESCC in a Chinese population. Multivariate logistic analysis revealed that the TERT-CLPTM1L rs401681 C>T polymorphisms, particularly among men, young patients and those reported to be drinkers.

Table 1. Distribution of selected demographic variables and risk factors in ESCC cases and controls.

| Variable               | Cases (n = 629) | Controls (n = 686) | P*       |
|------------------------|----------------|-------------------|----------|
| Age (years) mean ± SD  | 62.85 (±8.13)  | 62.58 (±7.89)     | 0.541    |
| Age (years)            |                |                   | 0.155    |
| < 63                   | 310 (49.28)    | 365 (53.21)       | 0.315    |
| ≥ 63                   | 319 (50.72)    | 321 (46.79)       | 0.185    |
| Sex                    |                |                   | <0.001   |
| Male                   | 444 (70.59)    | 461 (67.20)       |         |
| Female                 | 185 (29.41)    | 225 (32.80)       |         |
| Tobacco use            |                |                   | <0.001   |
| Never                  | 355 (56.44)    | 499 (72.74)       |         |
| Ever                   | 274 (43.56)    | 187 (27.26)       |         |
| Alcohol use            |                |                   | <0.001   |
| Never                  | 428 (68.04)    | 526 (76.68)       |         |
| Ever                   | 201 (31.96)    | 160 (23.32)       |         |

*Two-sided χ² test and student t test; Bold values are statistically significant (P<0.05).

doi:10.1371/journal.pone.0100667.t001
Table 2. Logistic regression analyses of associations between hTERT rs2736098 G>A and TERT-CLPTM1L rs401681 C>T polymorphisms and risk of ESCC.

| Genotype                        | Cases (n = 629) |         | Controls (n = 686) |         | Crude OR (95%CI) | P     | Adjusted OR * (95%CI) | P     |
|---------------------------------|-----------------|---------|--------------------|---------|------------------|-------|-----------------------|-------|
|                                 | n (%)           | n (%)   |                    | n (%)   |                  |       |                       |       |
| hTERT rs2736098 G>A             |                 |         |                    |         |                  |       |                       |       |
| GG                              | 245 (40.83)     | 270 (41.47) | 1.00 (reference value) | 1.00 (reference value) |       | 1.00 (reference value) | 1.00 (reference value) |
| GA                              | 277 (46.17)     | 306 (47.00) | 1.00 (0.79–1.27)   | 0.984   | 1.01 (0.79–1.28) | 0.970 |
| AA                              | 78 (13.00)      | 75 (11.52)  | 1.15 (0.80–1.65)   | 0.459   | 1.18 (0.82–1.71) | 0.372 |
| AA vs. GA vs. GG                 |                 |         |                    |         | 0.727            |       |                       |       |
| GA/AA                           | 355 (59.17)     | 381 (58.53) | 1.03 (0.82–1.29)   | 0.818   | 1.04 (0.83–1.31) | 0.742 |
| GG/GA                           | 522 (87.00)     | 576 (88.48) | 1.00 (reference value) | 1.00 (reference value) |       | 1.00 (reference value) | 1.00 (reference value) |
| AA                              | 78 (13.00)      | 75 (11.52)  | 1.15 (0.82–1.61)   | 0.425   | 1.18 (0.84–1.67) | 0.348 |
| G allele                        | 767 (63.92)     | 846 (64.98) | 1.00 (reference value) | —       | —                |       |
| A allele                        | 433 (36.08)     | 456 (35.02) | 1.05 (0.89–1.23)   | 0.580   | —                |       |
| TERT-CLPTM1L rs401681 C>T       |                 |         |                    |         |                  |       |                       |       |
| CC                              | 288 (47.68)     | 266 (40.06) | 1.00 (reference value) | 1.00 (reference value) |       | 1.00 (reference value) | 1.00 (reference value) |
| CT                              | 252 (41.72)     | 317 (47.74) | 0.73 (0.58-0.93)   | 0.010   | 0.74 (0.58-0.94) | 0.012 |
| TT                              | 64 (10.60)      | 81 (12.20)  | 0.73 (0.51–1.05)   | 0.093   | 0.75 (0.51–1.09) | 0.126 |
| TT vs. CT vs. CC                |                 |         |                    |         | 0.024            |       |                       |       |
| CT/TT                           | 316 (52.32)     | 398 (59.94) | 0.73 (0.59-0.92)   | 0.006   | 0.74 (0.59-0.93) | 0.009 |
| CC/CT                           | 540 (89.40)     | 583 (87.80) | 1.00 (reference value) | 1.00 (reference value) |       | 1.00 (reference value) | 1.00 (reference value) |
| TT                              | 64 (10.60)      | 81 (12.20)  | 0.85 (0.60–1.21)   | 0.371   | 0.87 (0.61–1.24) | 0.447 |
| C allele                        | 828 (68.54)     | 849 (63.93) | 1.00 (reference value) | —       | —                |       |
| T allele                        | 380 (31.46)     | 479 (36.07) | 0.81 (0.69-0.96)   | 0.014   | —                |       |

*Adjusted for age, sex, smoking and drinking status; Bold values are statistically significant (P<0.05).

doi:10.1371/journal.pone.0100667.t002
### Table 3. Stratified analyses between TERT-CLPTM1L rs401681 C>T polymorphism and ESCC risk by sex, age, smoking status and alcohol consumption.

| Variable                  | TERT-CLPTM1L rs401681 C>T (case/control) | Adjusted OR \(^b\) (95% CI); \(P\); \(P\)_h \(^c\) | CC     | CT   | TT   | CT/TT  |
|---------------------------|------------------------------------------|-----------------------------------------------|--------|------|------|--------|
| Sex                       |                                          |                                               |        |      |      |        |
| Male                      | 219/172                                  | 1.00 (reference value)                        | 0.59 (0.44–0.78); \(P\): 0.0003; \(P_h\):0.007 | 0.64 (0.41–1.00); \(P\): 0.052; \(P_h\):0.227 | 0.60 (0.45–0.79); \(P\): 0.0002; \(P_h\):0.007 | 0.03 (0.54–1.27); \(P\): 0.397; \(P_h\):0.719 |
| Female                    | 69/94                                    | 1.00 (reference value)                        | 1.24 (0.81–1.89); \(P\): 0.330; \(P_h\):0.007 | 1.11 (0.56–2.20); \(P\): 0.757; \(P_h\):0.227 | 1.21 (0.81–1.82); \(P\): 0.355; \(P_h\):0.007 | 0.99 (0.52–1.88); \(P\): 0.979; \(P_h\):0.719 |
| Age                       |                                          |                                               |        |      |      |        |
| <63                       | 147/142                                  | 1.00 (reference value)                        | 0.75 (0.53–1.06); \(P\): 0.099; \(P_h\):0.963 | 0.64 (0.38–1.08); \(P\): 0.091; \(P_h\):0.297 | 0.72 (0.52–1.00); \(P\): 0.049; \(P_h\):0.699 | 0.73 (0.45–1.21); \(P\): 0.221; \(P_h\):0.264 |
| ≥63                       | 141/124                                  | 1.00 (reference value)                        | 0.73 (0.52–1.03); \(P\): 0.071; \(P_h\):0.963 | 0.93 (0.54–1.60); \(P\): 0.790; \(P_h\):0.297 | 0.77 (0.56–1.06); \(P\): 0.103; \(P_h\):0.699 | 1.09 (0.65–1.83); \(P\): 0.739; \(P_h\):0.264 |
| Smoking status            |                                          |                                               |        |      |      |        |
| Never                     | 158/194                                  | 1.00 (reference value)                        | 0.79 (0.59–1.07); \(P\): 0.124; \(P_h\):0.414 | 0.73 (0.46–1.17); \(P\): 0.189; \(P_h\):0.739 | 0.78 (0.59–1.03); \(P\): 0.083; \(P_h\):0.562 | 0.82 (0.53–1.28); \(P\): 0.391; \(P_h\):0.540 |
| Ever                      | 130/72                                   | 1.00 (reference value)                        | 0.68 (0.45–1.03); \(P\): 0.069; \(P_h\):0.414 | 0.82 (0.42–1.59); \(P\): 0.556; \(P_h\):0.739 | 0.71 (0.48–1.05); \(P\): 0.082; \(P_h\):0.562 | 0.99 (0.53–1.87); \(P\): 0.980; \(P_h\):0.540 |
| Alcohol consumption       |                                          |                                               |        |      |      |        |
| Never                     | 189/209                                  | 1.00 (reference value)                        | 0.84 (0.63–1.12); \(P\): 0.231; \(P_h\):0.118 | 0.78 (0.50–1.21); \(P\): 0.262; \(P_h\):0.928 | 0.83 (0.63–1.08); \(P\): 0.167; \(P_h\):0.175 | 0.85 (0.56–1.29); \(P\): 0.439; \(P_h\):0.665 |
| Ever                      | 99/57                                    | 1.00 (reference value)                        | 0.55 (0.35–0.88); \(P\): 0.012; \(P_h\):0.118 | 0.74 (0.35–1.56); \(P\): 0.421; \(P_h\):0.928 | 0.58 (0.37–0.90); \(P\): 0.016; \(P_h\):0.175 | 1.00 (0.49–2.03); \(P\): 0.996; \(P_h\):0.665 |

\(^a\)The genotyping was successful in 600 (95.4%) ESCC cases, and 651 (94.9%) controls for TERT-CLPTM1L rs401681 C>T;

\(^b\)Adjusted for age, sex, smoking status and alcohol consumption (besides stratified factors accordingly) in a logistic regression model;

\(^c\)\(P_h\) for heterogeneity; Bold values are statistically significant (\(P\) or \(P_h\)<0.05).

doi:10.1371/journal.pone.0100667.t003
The function of CLPTM1L and its role in tumorigenesis is largely unknown. However, a recent study reported that CLPTM1L was a commonly overexpressed anti-apoptotic factor in lung cancer [15]. This suggested an inhibitory role in genotoxic stress-induced apoptosis, and identified CLPTM1L as an important factor affecting the survival of DNA-damaged tumor cells and potentially lung cancer susceptibility [20].

The CLPTM1L gene is upregulated in cisplatin-resistant cell lines, and is linked to cisplatin-induced apoptosis; furthermore, over-expression of CLPTM1L mRNA has been observed in many cancers [6,14,21,22]. Variants at this locus are hypothesized to regulate telomere length and be associated with multiple malignancies, including cancers of the lung, prostate, urinary bladder, cervix and pancreas. Rs401681 is located in intron 13 of CLPTM1L at 5p15.33, and it is one of the most studied SNPs. Although little is known about the function of this SNP, our bioinformatics analysis indicated that it might affect transcription regulation and further affect the expression of the gene. To show that these alterations can indeed contribute to cancer properties, in vitro validation studies with specific in vitro cell lines of ESCC that harbor these genetic alterations are warranted. Such as cell cultures, transient transfections, luciferase assay, electrophoretic mobility shift assays, Western blot analysis, reverse transcriptase PCR, chromatin immunoprecipitation assays and quantitative Real-Time PCR.

Several studies addressing the association between the CLPTM1L rs401681 polymorphism and cancer have been published, with inconsistent results [6,15,19,23,24,25]. An association study that included 2,396 lung cancer cases and 3,001 controls showed that the CLPTM1L T allele was associated with a significantly decreased risk of lung cancer [15]. Nan and collaborators observed a suggestive positive relationship between the rs401681 C allele and shorter relative telomere length [7]. Rafnar et al. suggested that the rs401681 C allele might be associated with the acceleration of the gradual shortening of telomeres with age [6]. Possible links between shorter telomeres and decreased risk of melanoma have been reported. This could be attributed to the shorter replicative lifespan of melanocytes conferred by a shorter telomere length, which provides a more stringent barrier to unlimited cell division. A decreased risk of melanoma might also be associated with the reduction of nevi size and count in individuals with shorter telomeres. Rs401681 is also associated with the risk of pancreatic cancer, as shown by the presence of chromosome ends lacking telomeric repeat sequences in this cancer [26]. Jiang et al. found that TERT-CLPTM1L rs401681 T allele was associated with decreased risk of lung cancer [15]. And in ESCC cohort, the trend of TERT-CLPTM1L rs401681 T allele is protective but not reach significant (OR = 0.935, 95% CI = 0.800-1.093 in additive model) [16], indicating necessary for replications in other cohorts.

The frequencies of genetic polymorphisms often vary between ethnic groups. In the present Chinese study, the allele frequency of rs401681 T was 0.361 in 686 control subjects, which is consistent with the values reported in the SNP database for the Chinese Han (0.305) and Japanese populations (0.343); however, the frequency was lower than that of a Sub-Saharan African (0.642) population [http://www.ncbi.nlm.nih.gov/SNP].

This case-control study had several limitations. First, the patients and controls were enrolled from hospitals and may therefore not be representative of the general population; the information familial cancer history of the cases and controls was not obtained, this inherent bias may have resulted in spurious findings. Second, the polymorphisms investigated in our study were based on functional considerations and may not provide a comprehensive view of the genetic variability of TERT-CLPTM1L, such as rs402710 and rs2736100 et al. Further studies are needed to clarify the genetic mechanism of esophageal carcinogenesis by fine-mapping the susceptibility region of the variants. Third, the statistical power of our study was limited because of the moderate sample size and absence of a validation cohort. Larger, well-designed studies are warranted to confirm the associations observed in the present study. Finally, we did not obtain detailed information on cancer metastasis and survival, which further restricted the analysis of the roles of the hTERT rs2736098 G>A and TERT-CLPTM1L rs401681 C>T polymorphisms in ESCC progression and prognosis.

In conclusion, our study provides strong evidence that the functional TERT-CLPTM1L rs401681 C>T polymorphism may contribute to the risk of ESCC. However, the exact functional relevance of the CLPTM1L rs401681 SNP remains unclear. It may be in strong LD with other potential functional or causal SNPs, contributing to the risk of ESCC. Additional, larger studies and in vitro or tissue-specific biological characterization are required to confirm the current preliminary findings.

Supporting Information

Table S1 Primary information for hTERT rs2736098 G>A and TERT-CLPTM1L rs401681 C>T polymorphisms.

Data S1 Data of hTERT rs2736098 G>A and TERT-CLPTM1L rs401681 C>T polymorphisms.

Acknowledgments

We appreciate all patients who participated in this study. We wish to thank Dr. Yiqun Chen (Bowing Applied Biotechnology Company, Shanghai, China) for technical support.

Author Contributions

Conceived and designed the experiments: JY XW WT SC HG. Performed the experiments: AS LW. Analyzed the data: HG JY SC. Contributed reagents/materials/analysis tools: XW GD CL. Wrote the paper: JY WT SC HG. Critical review of manuscript: YS LZ SC HG.

References

1. Chen W, He Y, Zheng R, Zhang S, Zeng H, et al. (2013) Esophageal cancer incidence and mortality in China, 2009. J Thorac Dis 5: 19–26.
2. Jemal A, Siegel R, Ward E, Hao Y, Xu J, et al. (2008) Cancer statistics, 2008. CA Cancer J Clin 58: 71–96.
3. Macfarlane GJ, Boyle P (1994) The epidemiology of oesophageal cancer in the UK and other European countries. J R Soc Med 87: 334–337.
4. Morita M, Kuwano H, Ohno S, Sugimachi K, Sano Y, et al. (1994) Multiple occurrence of carcinoma in the upper aero digestive tract associated with esophageal cancer: reference to smoking, drinking and family history. Int J Cancer 58: 207–210.
5. Prescott J, Wentzensen NM, Savage SA, De Vivo I (2012) Epidemiologic evidence for a role of telomere dysfunction in cancer etiology. Mutat Res 730: 75–84.
6. Rafnar T, Salem P, Stacey SN, Geller F, Godsmundsson J, et al. (2009) Sequence variants at the TERT-CLPTM1L locus associate with many cancer types. Nat Genet 41: 221–227.
7. McKay JD, Hung RJ, Gaborieau V, Boffetta P, Chabrier A, et al. (2008) Lung cancer susceptibility locus at 5p15.33. Nat Genet 40: 1404–1406.
8. Shete S, Hosking FJ, Robertson LB, Dobkins SE, Samson M, et al. (2009) Genome-wide association study identifies five susceptibility loci for glioma. Nat Genet 41: 899–904.
9. Nan H, Qureshi AA, Prescott J, De Vivo I, Han J (2011) Genetic variants in telomere-maintaining genes and skin cancer risk. Hum Genet 129: 247–253.
10. Wyatt HD, West SC, Beattie TL (2010) InTERTpreting telomerase structure and function. Nucleic Acids Res 38: 5609–5622.
11. Young NS (2010) Telomere biology and telomere diseases: implications for practice and research. Hematology Am Soc Hematol Educ Program 2010: 30–35.
12. Rodier F, Kim SH, Niijiar Y, Yaswen P, Campini J (2003) Cancer and aging: the importance of telomeres in genome maintenance. Int J Biochem Cell Biol 37: 977–990.
13. Baird DM (2010) Variation at the TERT locus and predisposition for cancer. Expert Rev Mol Med 12: e16.
14. Yamamoto K, Okamoto A, Iouishi S, Ochiai K, Ohtake Y (2001) A novel gene, CRR9, which was up-regulated in CDDP-resistant ovarian tumor cell line, was associated with apoptosis. Biochem Biophys Res Commun 280: 1148–1154.
15. James MA, Wen W, Wang Y, Byers LA, Heymach JV, et al. (2012) Functional characterization of CLPTM1L as a lung cancer risk candidate gene in the 5p15.33 locus. PLoS One 7: e36116.
16. Jiang M, Wu H, Qin C (2013) Genetic Variant rs401681 at 5p15.33 Modifies Susceptibility to Lung Cancer but Not Esophageal Squamous Cell Carcinoma. PLoS One 8: e69427.
17. World Medical Association (2013) World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. JAMA 310: 2191–2194.
18. Gu H, Ding G, Zhang W, Liu C, Chen Y, et al. (2012) Replication study of PLCE1 and C20orf54 polymorphism and risk of esophageal cancer in a Chinese population. Mol Biol Rep 39: 9105–9111.
19. Chen ZJ, Zhao H, He L, Shi Y, Qin Y, et al. (2011) Genome-wide association study identifies susceptibility loci for polycystic ovary syndrome on chromosome 2p16.3, 2p21 and 9q33.3. Nat Genet 43: 55–59.
20. Zienolddiny S, Skang V, Landvik NE, Ryberg D, Phillips DH, et al. (2009) The TERT-CLPTM1L lung cancer susceptibility variant associates with higher DNA adduct formation in the lung. Carcinogenesis 30: 1368–1371.
21. Liu ZS, Li GJ, Wei S, Xia J, Wang LE, et al. (2010) Genetic variations in TERT–CLPTM1L genes and risk of squamous cell carcinoma of the head and neck. Carcinogenesis 31: 1977–1981.
22. Colombo J, Fachel AA, De Freitas Calmon M, Cury PM, Fukuyama EF, et al. (2009) Gene expression profiling reveals molecular marker candidates of laryngeal squamous cell carcinoma. Oncol Rep 21: 649–663.
23. Myneni AA, Chang SC, Niu RG, Liu L, Ochs-Balcom HM, et al. (2013) Genetic polymorphisms of TERT and CLPTM1L and risk of lung cancer—A case-control study in a Chinese population. Lung Cancer 80: 131–133.
24. Law MH, Montgomery GW, Brown KM, Martin NG, Mann GJ, et al. (2012) Meta-analysis combining new and existing data sets confirms that the TERT-CLPTM1L locus influences melanoma risk. J Invest Dermatol 132: 485–487.
25. Pooley KA, Tyrer J, Shah M, et al. (2010) No association between TERT-CLPTM1L single nucleotide polymorphism rs401681 and mean telomere length or cancer risk. Cancer Epidemi Biomarkers Prev 19: 1862–1863.
26. Petersen GM, Aamundadottir L, Fuchs CS, Kraft P, Steutenberg-Solomon RZ, et al. (2010) A genome-wide association study identifies pancreatic cancer susceptibility loci on chromosomes 13q22.1, 1q32.1 and 5p15.33. Nature Genetics 42: 224–230.