



# *p21* rs3176352 G>C and *p73* rs1801173 C>T Polymorphisms Are Associated with an Increased Risk of Esophageal Cancer in a Chinese Population

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## Abstract

**Objective:** Esophageal cancer was the fifth most commonly diagnosed cancer and the fourth leading cause of cancer-related death in China in 2009. Genetic factors might play an important role in esophageal squamous cell carcinoma (ESCC) carcinogenesis.

**Designs and Methods:** To evaluate the effect *p21*, *p53*, *TP53BP1* and *p73* single nucleotide polymorphisms (SNPs) on the risk of ESCC, we conducted a hospital based case-control study. A total of 629 ESCC cases and 686 controls were recruited. Their genotypes were determined using ligation detection reaction (LDR) method.

**Results:** When the *p21* rs3176352 GG homozygote genotype was used as the reference group, the CC genotype was associated with a significantly increased risk of ESCC. When the *p73* rs1801173 CC homozygote genotype was used as the reference group, the CT genotype was associated with a significantly increased risk of ESCC. After Bonferroni correction, for *p21* rs3176352 G>C, the  $p_{correct}$  was still significant. For the other six SNPs, in all comparison models, no association between the polymorphisms and ESCC risk was observed.

**Conclusions:** *p21* rs3176352 G>C and *p73* rs1801173 C>T SNPs are associated with increased risk of ESCC. To confirm the current findings, additional, larger studies and tissue-specific biological characterization are required.

**Citation:** Zheng L, Tang W, Shi Y, Chen S, Wang X, et al. (2014) *p21* rs3176352 G>C and *p73* rs1801173 C>T Polymorphisms Are Associated with an Increased Risk of Esophageal Cancer in a Chinese Population. PLoS ONE 9(5): e96958. doi:10.1371/journal.pone.0096958

**Editor:** Ralf Krahe, University of Texas MD Anderson Cancer Center, United States of America

**Received:** January 13, 2014; **Accepted:** April 12, 2014; **Published:** May 12, 2014

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**Funding:** This study was supported in part by National Natural Science Foundation of China (81370001, 81371927, 81101889, 81000028), Jiangsu Province Natural Science Foundation (BK2010333, BK2011481), Social Development Foundation of Zhenjiang (SH2010017) and Changzhou Young Talents and Science-Technology Foundation of Health Bureau (QN201102) and Affiliated People's Hospital of Jiangsu University fund (Y200913). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

**Competing Interests:** The authors have declared that no competing interests exist.

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## Introduction

As the fourth leading cause of cancer deaths and the fifth most common diagnosed cancer in China in 2009 [1], the 5-year-survival rate of esophageal cancer is very poor and accounts only 12.3% in 23 European countries [2]. More than 90% esophageal cancers are esophageal squamous cell carcinoma (ESCC). In addition to environmental risk factors such as smoking and heavy drinking, single nucleotide polymorphisms (SNPs) as genetic factors might play an important role in ESCC carcinogenesis [3].

Tumor suppressor protein p53 is frequently mutated in diverse types of cancers and is implicated in cell proliferation and tumor progression [4]. The *p53* gene is on chromosome 17p13.1. A well-studied *p53* polymorphism, Arg72Pro (rs1042522 C/G; R/P) has been reported to have functional significance [5,6]. Compared with Arg wild-type protein, the Pro variant allele encoded protein

is more efficient in inducing nuclear DNA repair genes expression [7]. Polymorphism *p53* rs1042522 G>C has been associated with risk of numerous kinds of cancers [8].

*p21* (Waf1/Cip1/CDKN1A), a non-specific cdk inhibitor and a key mediator of G0-G1 cell cycle arrest, is upregulated by wild-type p53. *p21* functions during gene repair and angiogenesis [9]. Cell cycle arrest at the G1-S phase restriction point is mediated through *p21* up-regulation induced by p53, and the associated G1 cyclins-cdk2 complexes inhibition [10]. In p53-deficient cells, *p21* interacts with proliferating cell nuclear antigen (PCNA) and causes both G1 and G2 cell cycle arrest [11]. By inhibiting PCNA-dependent DNA replication, *p21* expression can suppress tumor growth and mismatch repair in vitro [12]. *p21* encodes a 21-kDa protein, is located on chromosome 6p21.2 and consists of three exons and two introns [13].

Tumor protein 53-binding protein 1 (TP53BP1) interacts specifically with p53 and participates in both DNA repair and cell cycle control. By cooperating with damage sensors and signal transducers, TP53BP1 helps mediate the DNA damage checkpoint [14].

p73 shares structural and functional similarities to p53. p73 is located at 1p36.33, mapping to a region that is often deleted in cancers [15]. p73 activates transcription of p21- and p53-responsive genes, which participate in cell cycle control, DNA repair, apoptosis and inhibits cell growth in a p53-like manner by inducing apoptosis or G1 cell cycle arrest [16,17]. This suggests that p73 has tumor-suppressor functions. Otherwise, the p73 gene has some significant differences from p53. In contrast to p53-deficient mice, those lacking p73 show no increased susceptibility to spontaneous tumorigenesis [18].

Genetic variations in the p53 pathway genes, such as p21, p53, TP53BP1 and p73, may contribute to the development of ESCC. In a hospital-based case-control study, we performed genotyping analyses of eight functional p21, p53, TP53BP1 and p73 SNPs in 629 ESCC cases and 686 controls in a Chinese population.

## Materials and Methods

### Ethical approval of the study protocol

The data has been deposited in Supporting Information files. Regarding ethical conduct of research involving human subjects and/or animals, we complied with the World Medical Association Declaration of Helsinki. The review Board of Jiangsu University (Zhenjiang, China) approved this hospital-based case-control study. Written informed consent was provided by all subjects in the study.

### Patients and controls

Between October 2008 and December 2010, from the Affiliated People's Hospital of Jiangsu University and Affiliated Hospital of Jiangsu University (Zhenjiang, China), 629 subjects with esophageal cancer were recruited consecutively. By pathological means, all cases of esophageal cancer were diagnosed as ESCC. Exclusion criteria were: patients who previously had cancer; any metastasized cancer and radiotherapy or chemotherapy. 686 patients without cancer were matched to the cases with regard to age ( $\pm 5$  years) and sex, as controls. The controls were recruited from the above-mentioned two hospitals at the same time period. Most of the controls were being treated for trauma (including 612 trauma patients, 45 infectious disease patients and 29 hypertension patients).

Using a pre-tested questionnaire, trained interviewers questioned each subject personally and obtained demographic data information (e.g., age, sex) and related risk factors (such as tobacco smoking and alcohol consumption). Venous blood samples (2-mL) were collected after the interview from each subject. The definition of "smokers" was smoking one cigarette per day for  $>1$  year. The definition of "alcohol drinkers" was consumption  $\geq 3$  alcoholic drinks a week for  $>6$  months.

### Isolation of DNA, SNPs selection and genotyping by ligation detection reaction (LDR)

From whole blood, genomic DNA was isolated [19]. The 8 SNPs selection was based on previous published articles with functional consideration [20,21,22,23]. With technical support from the Shanghai Biowing Applied Biotechnology Company, the samples were genotyped using the LDR method [24]. In 160 (12.17%) randomly selected samples with high DNA quality, repeated analyses were done for quality control.

## Statistical analyses

Using the  $\chi^2$  test, between the cases and controls, the distributions of demographic characteristics, selected variables, and genotypes of the p21, p53, TP53BP1 and p73 variants differences were evaluated. Using logistic regression analyses, the associations between the eight SNPs and risk of ESCC were estimated for crude ORs and adjusted ORs when adjusting for age, sex, smoking and drinking status. Because of the number of comparisons, the Bonferroni correction procedure was applied. By a goodness-of-fit  $\chi^2$  test, the Hardy-Weinberg equilibrium (HWE) was tested to compare the observed genotype frequencies to the expected ones among the control subjects. With SAS 9.1.3 (SAS Institute, Cary, NC, USA), all statistical analyses were performed.

## Results

### Characteristics of the study population

Cases and controls' characteristics are summarized in Table 1. By the  $\chi^2$  tests, the cases and controls are adequately matched on age and sex. Between the cases and the controls, significant difference was detected on smoking and drinking status, which is shown in Table 1. The primary information for eight genotyped SNPs was in Table 2. The concordance rates of repeated analyses were 100% except p21 rs3176352 G>C (158/160, 98.75%). For all SNPs, minor allele frequency (MAF) in our controls was similar to MAF for Chinese in database. In the controls, for these eight polymorphisms, the observed genotype frequencies were all in HWE (Table 2).

### Associations between p21, p53, TP53BP1 and p73 polymorphisms and risk of ESCC and genotype combination analysis

When the p21 rs3176352 GG homozygote genotype was used as the reference group, the GC genotype was not associated with the risk for ESCC; the CC genotype was associated with a significantly increased risk for ESCC (CC vs. GG: adjusted OR = 1.61, 95% CI = 1.18–2.20,  $p = 0.0030$ ). In the dominant model, the p21 rs3176352 GC/CC variants were not associated with the risk of ESCC, compared with the p21 rs3176352 GG genotype. In the recessive model, when the p21 rs3176352 GG/GC genotypes were used as the reference group, the CC homozygote genotype was associated with a 63% increased risk of ESCC (CC vs. GG/GC: adjusted OR = 1.63, 95% CI = 1.23–2.15,  $p = 0.0006$ ) (Table 3).

When the p73 rs1801173 CC homozygote genotype was used as the reference group, the CT genotype was associated with a significantly increased risk for ESCC (CT vs. CC: adjusted OR = 1.39, 95% CI = 1.10–1.76,  $p = 0.006$ ); the TT genotype was not associated with the risk of ESCC. In the dominant model, the p73 rs1801173 CT/TT variants were associated with a significantly increased risk for ESCC (CT/TT vs. CC: adjusted OR = 1.37, 95% CI = 1.10–1.72,  $p = 0.006$ ), compared with the p73 rs1801173 CC genotype. In the recessive model, when the p73 rs1801173 CC/CT genotypes were used as the reference group, the TT homozygote genotype was not associated with the risk of ESCC (Table 3).

When the p21 rs1801270 CC homozygote genotype was used as the reference group, the AA genotype was associated with a significantly decreased risk for ESCC. When the TP53BP1 rs560191 GG/GC genotypes were used as the reference group, the CC genotype was associated with a significantly decreased risk for ESCC (Table 3). Logistic regression analyses revealed that the p21 rs2395655 G>A, p21 rs1059234 C>T, p21 rs762623 C>A and p53 rs1042522 G>C polymorphisms were not associated with the risk of ESCC (Table 2). After the Bonferroni correction

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

| Variable              | Cases (n = 629) |       | Controls (n = 686) |       | p <sup>a</sup> |
|-----------------------|-----------------|-------|--------------------|-------|----------------|
|                       | n               | %     | n                  | %     |                |
| 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 |                |
| ≥63                   | 319             | 50.72 | 321                | 46.79 |                |
| Sex                   |                 |       |                    |       | 0.185          |
| 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 |                |

<sup>a</sup>Two-sided  $\chi^2$  test and student t test; Bold values are statistically significant ( $p < 0.05$ ).  
doi:10.1371/journal.pone.0096958.t001

(number of multiple test = 32), for p21 rs3176352 G>C, the adjusted  $p = 0.096$  for CC vs. GG, adjusted  $p = 0.0192$  for CC vs. GG/GC. For p73 rs1801173 C>T, the  $p_{\text{correct}} = 0.202$  for CT vs. CC after adjusted for age et al.,  $p_{\text{correct}} = 0.195$  for CT/TT vs. CC. For the rest 6 SNPs, in all comparison models,  $p > 0.05$ .

When the p21 rs3176352 CC genotype and p73 rs1801173 CT/TT genotypes were considered as risk variant genotypes. When the no risk variant genotype carrier group was used as the reference group, the either one risk variant genotype carrier group (adjusted OR = 1.42, 95% CI = 1.12–1.80,  $p = 0.0035$ ) and both risk variant genotypes carrier group (adjusted OR = 2.47, 95% CI = 1.60–3.82,  $p < 0.0001$ ) were associated with a significantly increased risk for ESCC.

#### Stratification analyses on the p21 rs3176352 G>C and p73 rs1801173 C>T polymorphism and the risk of ESCC

To evaluate the effects of p21 rs3176352 G>C genotypes on ESCC risk according to different age, sex, smoking and alcohol drinking status; we performed the stratification analyses. A significantly increased risk of ESCC associated with the p21 rs3176352 G>C polymorphism was evident among all subgroups except in female patients after stratification (Table S1). A significantly decreased risk of ESCC associated with the p73 rs1801173 C>T polymorphism was evident among older patients, female patients and patients who never drinking or smoking (Table S2).

#### Discussion

In this hospital-based case-control study of ESCC, we found that the p21 rs3176352 CC and p73 rs1801173 CT/TT genotypes were associated with increased risk of ESCC; positive results were also observed in genotype combination analysis. To the best of our knowledge, this is the first positive association of p21 rs3176352 G/C and p73 rs1801173 C/T polymorphisms with ESCC risk.

P21 is a cyclin-dependent kinase inhibitor. It has been observed that in a wide variety of cancers, p21 expression is altered. At the G1 phase, the p21 protein disrupts cell cycle progression [25,26].

Binding of the tumor suppressor protein p53 to the p21 promoter inducing p21 expression [27].

P21 rs3176352 G/C (IVS2+16 G>C) is located in intron 2 of p21, 16 bp downstream from the splicing site. This C-to-G transition is predicted to affect the p21 messenger RNA splicing [28]. Choi *et al.* demonstrated that p21 rs3176352 G/C polymorphism appeared to be in linkage disequilibrium with Ser31Arg in a Korean population. Analysis of this haplotype for lung cancer susceptibility demonstrated a protective effect that was dependent on the number of variant alleles. In a previous study involving 80 esophageal cancer patients and 200 cancer-free controls from Ningxia Region of China, the p21 rs3176352 G/C polymorphism was not associated with esophageal cancer risk [29]. A case-control study from northeastern Iran, with 126 cases and 100 controls, was carried out to detect associations of p21 polymorphisms (rs1801270 and rs1059234) with ESCC risk [30]. The data suggested that these two p21 polymorphisms, both alone and in combination, are not ESCC genetic susceptibility biomarkers, which agrees with our results.

P73, a p53 homolog, has some p53-like activities and plays an important role in modulating the cell cycle, apoptosis and DNA repair. In a high incidence region of China, p73 polymorphisms were not associated with ESCC susceptibility [31]. However, our results are more reliable because of the higher numbers of cases and controls. p73 rs1801173 C/T polymorphism merits further functional study to elucidate the etiology of this SNP and ESCC.

The frequencies of genetic polymorphisms often vary between ethnic groups. In the present Chinese study, the allele frequency of p21 rs3176352 C was 0.410 in 686 control subjects, which is consistent with the values reported in the SNP database for the Chinese Han (0.422) and Japanese populations (0.455), higher than that of the Sub-Saharan African (0.233) population and African American population (0.250), and but lower than that of the European population (0.758). The allele frequency of p73 rs1801173 T was 0.230 in 686 control subjects, which is consistent with the values reported in the SNP database for the CHB+JPT (Chinese Han+Japanese) populations (0.267), higher than that of the Sub-Saharan African (0.102) population and European population (0.150).

**Table 2** Primary information for p21, p53, TP53BP1 and p73 polymorphisms.

| Genotyped SNPs                                    | p21 rs2395655<br>G>A | p21 rs1059234<br>C>T | p21 rs3176352<br>G>C | p21 rs1801270<br>C>A | p21 rs762623<br>C>A | p53 rs1042522<br>G>C | TP53BP1 rs560191<br>G>C | p73 rs1801173<br>C>T |
|---|----------------------|----------------------|----------------------|----------------------|---------------------|----------------------|-------------------------|----------------------|
| Chromosome  | 6                    | 6                    | 6                    | 6                    | 6                   | 17                   | 15                      | 1                    |
| Gene Official Symbol                              | CDKN1A               | CDKN1A               | CDKN1A               | CDKN1A               | CDKN1A              | TP53                 | TP53BP1                 | TP73                 |
| Function  | 5-UTR                | 3-UTR                | intron region        | missense             | intron region       | missense             | missense                | 5-UTR                |
| Chr Pos (Genome Build 36.3)                       | 36753674             | 36761575             | 36760317             | 36759949             | 36753444            | 7520197              | 41555066                | 3588770              |
| Regulome DB Score <sup>a</sup>                    | 1f                   | 4                    | 4                    | 3a                   | 1b                  | 5                    | 1f                      | 5                    |
| TFBS <sup>b</sup>                                 | Y                    | —                    | —                    | —                    | Y                   | —                    | —                       | —                    |
| Splicing (ESE or ESS)                             | —                    | Y                    | —                    | —                    | —                   | —                    | Y                       | Y                    |
| miRNA (miRanda)                                   | —                    | Y                    | —                    | —                    | —                   | —                    | —                       | —                    |
| miRNA (Sanger)                                    | —                    | Y                    | —                    | —                    | —                   | —                    | —                       | —                    |
| MAF <sup>c</sup> for Chinese in database          | 0.465                | 0.453                | 0.422                | 0.465                | 0.111               | 0.489                | 0.444                   | 0.267                |
| MAF in our controls (n = 686)                     | 0.473                | 0.478                | 0.410                | 0.479                | 0.112               | 0.436                | 0.447                   | 0.230                |
| p value for HWE <sup>d</sup> test in our controls | 0.606                | 0.234                | 0.443                | 0.639                | 0.852               | 0.372                | <b>0.009</b>            | 0.739                |
| Genotyping method <sup>e</sup>                    | LDR                  | LDR                  | LDR                  | LDR                  | LDR                 | LDR                  | LDR                     | LDR                  |
| % Genotyping value                                | 95.13%               | 95.13%               | 96.81%               | 98.63%               | 98.63%              | 96.35%               | 96.43%                  | 96.81%               |

<sup>a</sup><http://www.regulomedb.org/>;  
<sup>b</sup>TFBS: Transcription Factor Binding Site (<http://snpinfo.nih.gov/snpinfo/snpfunc.htm>);  
<sup>c</sup>MAF: minor allele frequency, p73 rs1801173 C>T MAF is in CHB+JPT population;  
<sup>d</sup>HWE: Hardy-Weinberg equilibrium;  
<sup>e</sup>LDR: ligation detection reaction; Bold values are statistically significant (p<0.05).  
 doi:10.1371/journal.pone.0096958.t002

**Table 3.** Logistic regression analyses of associations between p21, p53, TP53BP1 and p73 polymorphisms and risk of ESCC.

| Genotype                    | Cases (n = 629) |      | Controls (n = 686) |      | Crude OR (95%CI)        | p             | Adjusted OR <sup>a</sup> (95%CI) | p             |
|-----------------------------|-----------------|------|--------------------|------|-------------------------|---------------|----------------------------------|---------------|
|                             | n               | %    | n                  | %    |                         |               |                                  |               |
| <i>p21</i> rs2395655 G>A    |                 |      |                    |      |                         |               |                                  |               |
| GG                          | 148             | 24.7 | 184                | 28.3 | 1.00                    |               | 1.00                             |               |
| GA                          | 327             | 54.5 | 318                | 48.8 | 1.28 (0.98–1.67)        | 0.070         | 1.23 (0.94–1.62)                 | 0.128         |
| AA                          | 125             | 20.8 | 149                | 22.9 | 1.04 (0.76–1.44)        | 0.798         | 1.00 (0.72–1.39)                 | 0.982         |
| GA+AA                       | 452             | 75.3 | 467                | 71.7 | 1.20 (0.94–1.55)        | 0.150         | 1.16 (0.90–1.50)                 | 0.256         |
| GG+GA                       | 475             | 79.2 | 502                | 77.1 | 1.00                    |               | 1.00                             |               |
| AA                          | 125             | 20.8 | 149                | 22.9 | 0.89 (0.68–1.16)        | 0.381         | 0.87 (0.66–1.15)                 | 0.332         |
| <i>p21</i> rs1059234 C>T    |                 |      |                    |      |                         |               |                                  |               |
| CC                          | 172             | 28.7 | 170                | 26.1 | 1.00                    |               | 1.00                             |               |
| CT                          | 321             | 53.5 | 340                | 52.2 | 0.93 (0.72–1.21)        | 0.604         | 0.88 (0.67–1.15)                 | 0.334         |
| TT                          | 107             | 17.8 | 141                | 21.7 | 0.75 (0.54–1.04)        | 0.086         | 0.72 (0.51–1.00)                 | 0.050         |
| CT+TT                       | 428             | 71.3 | 481                | 73.9 | 0.88 (0.69–1.13)        | 0.311         | 0.83 (0.64–1.07)                 | 0.149         |
| CC+CT                       | 493             | 82.2 | 510                | 78.3 | 1.00                    |               | 1.00                             |               |
| TT                          | 107             | 17.8 | 141                | 21.7 | 0.79 (0.59–1.04)        | 0.090         | 0.78 (0.59–1.04)                 | 0.089         |
| <i>p21</i> rs3176352 G>C    |                 |      |                    |      |                         |               |                                  |               |
| GG                          | 191             | 31.8 | 239                | 35.5 | 1.00                    |               | 1.00                             |               |
| GC                          | 258             | 43.0 | 316                | 47.0 | 1.02 (0.79–1.31)        | 0.868         | 0.98 (0.76–1.27)                 | 0.866         |
| CC                          | 151             | 25.2 | 118                | 17.5 | <b>1.60 (1.18–2.18)</b> | <b>0.0026</b> | <b>1.61 (1.18–2.20)</b>          | <b>0.0030</b> |
| GC+CC                       | 409             | 68.2 | 434                | 64.5 | 1.18 (0.93–1.49)        | 0.166         | 1.15 (0.91–1.46)                 | 0.255         |
| GG+GC                       | 449             | 74.8 | 555                | 82.5 | 1.00                    |               | 1.00                             |               |
| CC                          | 151             | 25.2 | 118                | 17.5 | <b>1.58 (1.21–2.07)</b> | <b>0.0009</b> | <b>1.63 (1.23–2.15)</b>          | <b>0.0006</b> |
| <i>p21</i> rs1801270 C>A    |                 |      |                    |      |                         |               |                                  |               |
| CC                          | 179             | 29.1 | 182                | 26.7 | 1.00                    |               | 1.00                             |               |
| CA                          | 322             | 52.3 | 346                | 50.8 | 0.95 (0.73–1.22)        | 0.672         | 0.89 (0.68–1.15)                 | 0.373         |
| AA                          | 115             | 18.7 | 153                | 22.5 | 0.76 (0.56–1.05)        | 0.097         | <b>0.72 (0.52–0.99)</b>          | <b>0.044</b>  |
| CA+AA                       | 437             | 70.9 | 499                | 73.3 | 0.89 (0.70–1.14)        | 0.349         | 0.84 (0.65–1.07)                 | 0.154         |
| CC+CA                       | 501             | 81.3 | 528                | 77.5 | 1.00                    |               | 1.00                             |               |
| AA                          | 115             | 18.7 | 153                | 22.5 | 0.79 (0.60–1.04)        | 0.092         | 0.77 (0.59–1.02)                 | 0.070         |
| <i>p21</i> rs762623 G>A     |                 |      |                    |      |                         |               |                                  |               |
| GG                          | 480             | 77.9 | 537                | 78.9 | 1.00                    |               | 1.00                             |               |
| GA                          | 129             | 20.9 | 136                | 20.0 | 1.06 (0.81–1.39)        | 0.667         | 1.06 (0.81–1.40)                 | 0.675         |
| AA                          | 7               | 1.1  | 8                  | 1.2  | 0.98 (0.35–2.72)        | 0.967         | 0.95 (0.33–2.70)                 | 0.923         |
| GA+AA                       | 136             | 22.1 | 144                | 21.1 | 1.06 (0.81–1.38)        | 0.683         | 1.06 (0.81–1.38)                 | 0.700         |
| GG+GA                       | 609             | 98.9 | 673                | 98.8 | 1.00                    |               | 1.00                             |               |
| AA                          | 7               | 1.1  | 8                  | 1.2  | 0.97 (0.35–2.68)        | 0.949         | 0.94 (0.33–2.67)                 | 0.905         |
| <i>p53</i> rs1042522 G>C    |                 |      |                    |      |                         |               |                                  |               |
| GG                          | 177             | 28.8 | 213                | 32.6 | 1.00                    |               | 1.00                             |               |
| GC                          | 321             | 52.3 | 310                | 47.5 | 1.25 (0.97–1.61)        | 0.089         | 1.19 (0.92–1.54)                 | 0.186         |
| CC                          | 116             | 18.9 | 130                | 19.9 | 1.07 (0.78–1.48)        | 0.663         | 1.04 (0.75–1.44)                 | 0.810         |
| GC+CC                       | 437             | 71.2 | 440                | 67.4 | 1.20 (0.94–1.52)        | 0.144         | 1.15 (0.90–1.46)                 | 0.273         |
| GG+GC                       | 498             | 81.1 | 523                | 80.1 | 1.00                    |               | 1.00                             |               |
| CC                          | 116             | 18.9 | 130                | 19.9 | 0.94 (0.71–1.24)        | 0.648         | 0.93 (0.70–1.24)                 | 0.639         |
| <i>TP53BP1</i> rs560191 G>C |                 |      |                    |      |                         |               |                                  |               |
| GG                          | 213             | 34.6 | 216                | 33.1 | 1.00                    |               | 1.00                             |               |
| GC                          | 291             | 47.3 | 290                | 44.4 | 1.02 (0.79–1.31)        | 0.891         | 1.01 (0.78–1.30)                 | 0.959         |
| CC                          | 111             | 18.0 | 147                | 22.5 | 0.77 (0.56–1.05)        | 0.092         | 0.76 (0.56–1.05)                 | 0.093         |
| GC+CC                       | 402             | 65.4 | 437                | 66.9 | 0.93 (0.74–1.18)        | 0.558         | 0.92 (0.73–1.17)                 | 0.515         |
| GG+GC                       | 504             | 82.0 | 506                | 77.5 | 1.00                    |               | 1.00                             |               |

**Table 3. Cont.**

| Genotype   | Cases (n = 629) |      | Controls (n = 686) |      | Crude OR (95%CI)        | p                 | Adjusted OR <sup>a</sup> (95%CI) | p                 |
|--|-----------------|------|--------------------|------|-------------------------|-------------------|----------------------------------|-------------------|
|  | n               | %    | n                  | %    |                         |                   |                                  |                   |
| CC   | 111             | 18.0 | 147                | 22.5 | <b>0.76 (0.58–1.00)</b> | <b>0.049</b>      | 0.76 (0.57–1.01)                 | 0.055             |
| <i>p73</i> rs1801173 C>T   |                 |      |                    |      |                         |                   |                                  |                   |
| CC   | 311             | 51.8 | 401                | 59.6 | 1.00                    |                   | 1.00                             |                   |
| CT   | 251             | 41.8 | 235                | 34.9 | <b>1.38 (1.09–1.74)</b> | <b>0.007</b>      | <b>1.39 (1.10–1.76)</b>          | <b>0.006</b>      |
| TT   | 38              | 6.3  | 37                 | 5.5  | 1.32 (0.82–2.13)        | 0.248             | 1.27 (0.78–2.07)                 | 0.337             |
| CT+TT  | 289             | 48.2 | 272                | 40.4 | <b>1.37 (1.10–1.71)</b> | <b>0.006</b>      | <b>1.37 (1.10–1.72)</b>          | <b>0.006</b>      |
| CC+CT  | 562             | 93.7 | 636                | 94.5 | 1.00                    |                   | 1.00                             |                   |
| TT   | 38              | 6.3  | 37                 | 5.5  | 1.16 (0.73–1.85)        | 0.527             | 1.11 (0.69–1.79)                 | 0.667             |
| <i>p21</i> rs3176352 G>C and <i>p73</i> rs1801173 C>T combinations |                 |      |                    |      |                         |                   |                                  |                   |
| No risk variant genotype <sup>b</sup>                              | 226             | 37.7 | 323                | 48.0 | 1.00                    |                   | 1.00                             |                   |
| Either one risk variant genotype                                   | 308             | 51.3 | 310                | 46.1 | <b>1.42 (1.13–1.79)</b> | <b>0.0030</b>     | <b>1.42 (1.12–1.80)</b>          | <b>0.0035</b>     |
| Both risk variant genotypes  | 66              | 11.0 | 40                 | 5.9  | <b>2.36 (1.54–3.62)</b> | <b>&lt;0.0001</b> | <b>2.47 (1.60–3.82)</b>          | <b>&lt;0.0001</b> |

<sup>a</sup>Adjusted for age, sex, smoking status and alcohol consumption;

<sup>b</sup>Risk variant genotype means *p21* rs3176352 CC or *p73* rs1801173 CT/TT; *p21* rs3176352 C vs. G OR = 1.26, 95% CI = 1.08–1.47, *p* = 0.0041; *p73* rs1801173 T vs. C OR = 1.26, 95% CI = 1.05–1.50, *p* = 0.0126. Bonferroni correction (number of multiple test = 32) was performed to correct the *p* value (*p*<sub>correct</sub>); for *p21* rs3176352 G>C, the *p*<sub>correct</sub> = 0.096 for CC vs. GG after adjusted for age et al., *p*<sub>correct</sub> = 0.0192 for CC vs. GG/GC. For *p73* rs1801173 C>T, the *p*<sub>correct</sub> = 0.202 for CT vs. CC after adjusted for age et al., *p*<sub>correct</sub> = 0.195 for CT/TT vs. CC. For the rest 6 SNPs, *p*<sub>correct</sub> > 0.05 in all comparison models; Bold values are statistically significant (*p* < 0.05). doi:10.1371/journal.pone.0096958.t003

This case-control study had several limitations. First, because the patients and controls were enrolled from hospitals, inherent bias may have resulted in spurious findings. Second, the polymorphisms we studied may not provide a comprehensive view of *p21*, *p53*, *TP53BP1* and *p73* genetic variability. Fine-mapping studies are required. Third, because of the moderate sample size and absence of a validation cohort, the statistical power was limited. Finally, the viral infections and immune parameters information was not available, thus the power of our analyses was restricted.

In conclusion, our study provides strong evidence that *p21* rs3176352 G/C and *p73* rs1801173 C/T polymorphisms may contribute to ESCC risk. Tissue-specific biological characterization and replication studies with larger populations are required to confirm our findings.

## Supporting Information

**Table S1** Stratified analyses between *p21* rs3176352 G>C polymorphism and ESCC risk by sex, age, smoking status and alcohol consumption. (DOCX)

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**Table S2** Stratified analyses between *p73* rs1801173 C>T polymorphism and ESCC risk by sex, age, smoking status and alcohol consumption. (DOC)

**Data S1** Data of *p21*, *p53*, *TP53BP1* and *p73* polymorphisms. (SAV)

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

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