Polymorphisms in the \textit{ERCC5} Gene and Risk of Esophageal Squamous Cell Carcinoma (ESCC) in Eastern Chinese Populations

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Abstract

\textbf{Background:} Excision repair cross complementing group 5 (\textit{ERCC5} or \textit{XPG}) plays an important role in regulating DNA excision repair; its functional single nucleotide polymorphisms (SNPs) may alter DNA repair capacity and thus contribute to cancer risk.

\textbf{Methodology/Principal Findings:} In a hospital-based case-control study of 1115 esophageal squamous cell carcinoma (ESCC) cases and 1117 cancer-free controls, we genotyped three potentially functional SNPs of \textit{ERCC5} (SNPs, rs2296147T>C, rs2094258C>T and rs873601G>A) and estimated crude and adjusted odds ratios (ORs) and 95\% confidence intervals (CIs) for their associations with risk of ESCC using unconditional logistic regression models. We also calculated false-positive report probabilities (FPPRs) for significant findings. We found that compared with the TT genotype, \textit{ERCC5} rs2296147 C variant genotypes were associated with a significantly lower ESCC risk (CT: adjusted OR = 0.76, 95\% CI = 0.63–0.93, CT/CC: adjusted OR = 0.80, 95\% CI = 0.67–0.96); however, this risk was not observed for the other two SNPs (rs2094258C>T and rs873601 G>A), nor in further stratification and haplotype analysis.

\textbf{Conclusions/Significances:} These findings suggested that \textit{ERCC5} polymorphisms may contribute to risk of ESCC in Eastern Chinese populations, but the effect was weak and needs further validation by larger population-based case-control studies.

Introduction

Esophageal cancer is the sixth leading cause of cancer-related death worldwide. In 2008, there were 482,300 new cases, and 406,800 patients died of this disease around the world. In the highest incidence areas that extend from northern Iran and through the central Asian republics to north-central China, esophageal squamous cell carcinomas (ESCC) account for 90\% of the cases [1]. Although cigarette smoking, alcohol intake, nutritional deficiencies and dietary carcigen exposure may contribute to the etiology of ESCC [2], only a small fraction of exposed individuals develop ESCC, suggesting that genetic susceptibility may also play a vital role in the etiology of ESCC [3,4]. Recently, genetic polymorphisms in genes involved in multiple biological pathways, including DNA repair, have been identified as potential risk factors for ESCC [5,6].

DNA repair is essential for maintaining genomic stability in response to the insults of environmental carcinogens that causes DNA damage; if left unrepaired, such DNA damage can lead to mutation fixation and initiation of carcinogenesis. To date, there are at least five known major DNA repair pathways, including more than 150 human DNA repair genes, among which the nucleotide-excision repair (NER) pathway is the most versatile and particularly important in association with cancer risk. This is because NER repairs several kinds of bulky and helix-distorting lesions in DNA, involving coordinated actions of damage detection and recognition by helicase and nuclease proteins [7]. More importantly, NER defects can lead to severe diseases, such as

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as well as suggested that variations in ERCC5 transcript and protein levels nonsynonymous SNP (rs17655) and esophagus cancer in Caucasians and bladder [25,26], but there is only one published study of a polymorphism in ERCC5, which shares similar risk factors with ESCC. Therefore, we hypothesized that potential functional ERCC5 SNPs are associated with ESCC risk, and we tested this hypothesis in an Eastern Chinese population.

Materials and Methods

Study Subjects

This hospital-based case-control study included 1115 cases of ESCC and 1117 cancer-free controls. All subjects were genetically unrelated ethnic Han Chinese from Eastern China, including Shanghai, Jiangsu province and the surrounding regions. Patients newly diagnosed with histopathologically confirmed primary ESCC were recruited from Fudan University Shanghai Cancer Center (Shanghai, China) between March 2009 and September 2011. Blood samples of all patients were collected and processed by the Institutional Tissue Bank at Shanghai Cancer Center as a daily routine practice. Patients who had primary tumors outside the esophagus, tumors of an unknown origin, or any histologic diagnosis other than ESCC were excluded. Cancer-free controls were all recruited from a large prospective cohort - the Taizhou Longitudinal Study (TZL) in Taizhou, Jiangsu province, China, during the same period for the recruitment of cases [31]. Control subjects were frequency matched to the cases by age (±5 years) and sex. The response rates for cases and controls were approximately 93.0 and 90.0%, respectively. Each participant was asked to sign a written informed consent or next of kin or guardians consented on the behalf of participants whose capacity to consent was reduced, before we interviewed each eligible participant to obtain data on demographic characteristics and environmental exposure history, such as age, sex, ethnicity, body mass index (BMI), smoking and alcohol consumption. Individual who smoked <100 cigarettes in their lifetime were considered “non-smokers”, and the others were considered “smokers”. Similarly, subjects who drank alcoholic beverages at least once a week for ≥1 year were defined as “drinkers”, and the others were “non-drinkers”. Additionally, BMI was divided into two groups of “<24.0 and ≥24.0 kg/m²” according to the recommendations of the Working Group on Obesity in China, in which the BMI cut-off point for Chinese is 24 kg/m² for overweight [32]. At the end of the interview, a 10-mL venous blood sample was collected from each subject. The research protocol was approved by the Institutional Review Board of the Shanghai Cancer Center, Fudan University.

SNP Selection

Because the published genome-wide association studies (GWASs) of ESCC included all tagging SNPs of DNA repair genes but did not report any of such SNPs to be positively associated with cancer risk, our strategy in the present study was to include functional SNPs of ERCC5, a candidate gene rarely investigated for ESCC, that were not included in previously published GWASs. We searched the NCBI dbSNP database (http://www.ncbi.nlm.nih.gov/) and SNPInfo (http://snpinfo.niehs.nih.gov/) for putatively functional SNPs of ERCC5 that are located in the regulatory region (i.e., the 5’ near gene, 3’ near gene, 5’ untranslated regions (UTR) and 3’ UTR) with a minor allele frequency (MAF) ≥5% in Chinese Han, Beijing (CHB) descendants. As a result, five SNPs (rs2094258, rs2016073, rs751402, rs2296147, and rs873601) were selected as previously described [33]. Although both rs2094258 and rs2016073 are predicted to have similar functions, rs2094258 has a higher allele frequency for CHB; therefore, we genotyped rs2094258 only but not rs2016073.

Table 1. Distributions of selected variables in ESCC cases and cancer-free controls.

| Variables                                | Cases No. (%) | Controls No. (%) | P     |
|------------------------------------------|---------------|------------------|-------|
| All subjects                             | 1115 (100)    | 1117 (100)       |       |
| Age, yr (Mean ± SD)                      | 60.40±8.31    | 60.76±10.26      | 0.110 |
| ≤50                                      | 137 (12.28)   | 155 (13.88)      |       |
| >50–≤60                                  | 417 (37.40)   | 402 (35.99)      |       |
| >60–≤70                                  | 425 (38.12)   | 392 (35.09)      |       |
| >70                                      | 136 (12.20)   | 168 (15.04)      |       |
| Sex                                       |               |                  | 0.100 |
| Males                                    | 901 (80.81)   | 871 (77.98)      |       |
| Females                                  | 214 (19.19)   | 246 (22.02)      |       |
| Smoking status                           |               |                  | 0.0004|
| Yes                                      | 687 (61.61)   | 605 (54.16)      |       |
| No                                       | 428 (38.39)   | 512 (45.84)      |       |
| Drinking status                          |               |                  | <0.0001|
| Yes                                      | 494 (44.30)   | 364 (32.59)      |       |
| No                                       | 621 (55.70)   | 753 (67.41)      |       |
| pack-years                               |               |                  | <0.0001|
| 0                                        | 424 (38.03)   | 512 (45.84)      |       |
| ≤16 (mean)                               | 151 (13.54)   | 244 (21.84)      |       |
| >16 (mean)                               | 540 (48.43)   | 361 (32.32)      |       |
| BMI                                      |               |                  | <0.0001|
| ≤24.0                                    | 633 (56.77)   | 433 (38.76)      |       |
| >24.0                                    | 482 (43.23)   | 684 (61.24)      |       |

ESCC = esophageal squamous cell carcinoma; SD = standard deviation; BMI = body mass index.

*pTwo-sided χ² test for distributions between cases and controls.

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transcription factor binding sites (TFBS) activity, and the 3' UTR SNP may have an impact on splicing and miRNA binding sites. These three selected SNPs also captured other 20 untyped SNPs in the nearby genes [33], including one non-synonymous SNP (rs17655) that is the only one common SNP with MAF > 5% in CHB (LD for rs873601 and rs17655; \( r^2 = 0.910 \)).

Genotyping

Genomic DNA was extracted from the buffy-coat fraction of the blood samples using the Qiagen Blood DNA Mini Kit (Qiagen Inc., Valencia, CA). All the three SNPs were genotyped using the Taqman real-time PCR method with a 7900 HT sequence detector system (Applied Biosystems, Foster City, CA). To ensure high genotyping accuracy, strict quality control procedures were implemented, and four duplicated positive controls and four negative controls (no DNA) were used in each of 384-well plates. Approximately 5% of the samples were repeatedly genotyped, and the results were 100% concordant.

Statistical Methods

Differences in frequency distributions of the selected demographic variables, risk factors and each of alleles and genotypes of the selected SNPs between patients with ESCC and control subjects were evaluated using the \( \chi^2 \) test. Univariate and multivariate logistic regression analyses were used to calculate crude and adjusted odds ratios (ORs) to estimate the risk of ESCC and 95% confidence intervals (95% CIs). Multivariate adjustments were made, where appropriate, for age, sex, BMI, and smoking and drinking status, in further stratification analysis of genotype data. The heterogeneity of associations between subgroups was estimated using the Chi-square-based \( Q \) test. The Hardy–Weinberg equilibrium for genotype distribution in controls was tested by a goodness-of-fit \( \chi^2 \) test. Haplotype frequencies and individual haplotypes were estimated using unphased genotype data. The haplotype of the highest frequency was used as the reference group to calculate ORs for haplotype associated with ESCC risk in logistic regression analysis.

We also calculated the false-positive report probability (FPRP) to detect the false-positive association findings. For all the significant results, a prior probability of 0.01 was assigned to detect an OR of 1.56 (for a risk effect) or 0.67 (for a protective effect) for an association with genotypes and alleles of each SNP. Only significant results with an FPRP value less than 0.20 were considered a noteworthy association. Statistical significance was established at a \( P<0.05 \), and all tests were two-sided and performed with the SAS software (version 9.1; SAS Institute, Cary, NC).

Results

Population Characteristics

The final analysis included 1115 cases of confirmed ESCC and 1117 healthy controls, whose characteristics are summarized in Table 1. By the study design, there were no statistical differences in the distributions of age and sex between the cases (mean age of 60.40±8.31 years with 80.81% males) and controls (mean age of 60.76±10.26 with 77.98% males), indicating the frequency matching on age and sex was adequate (\( P=0.110 \) and 0.100, respectively). However, the cases were more likely to be smokers (81.61% vs. 54.16%, \( P=0.0004 \)) and drinkers (44.30% vs. 28.53%, \( P=0.0015 \)).

| Variants | Genotypes/Alleles | Cases | Controls | \( p \) | Crude OR | 95% CI | \( p \) | Adjusted OR | 95% CI | \( p \) |
|----------|-------------------|-------|----------|-------|---------|-------|-------|-----------|-------|-------|
| rs2296147 | TT | 757 (67.89) | 699 (62.58) | 0.016 | 0.77 (0.64–0.92) | 0.004 | 0.76 (0.63–0.93) | 0.006 |
| CT | 305 (27.35) | 368 (32.95) | 0.956 | 1.00 | 1.00 |
| CC | 53 (4.75) | 50 (4.48) | 0.96 (0.88–1.07) | 0.016 | 0.96 (0.88–1.07) | 0.016 |
| CT/CC | 358 (32.11) | 418 (37.42) | 0.008 | 0.79 (0.66–0.94) | 0.008 | 0.80 (0.67–0.96) | 0.016 |
| T | 1819 (0.82) | 1766 (0.79) | 0.602 | 1.03 (0.92–1.16) | 0.602 | 1.02 (0.90–1.16) | 0.664 |
| C | 411 (0.18) | 468 (0.21) | 0.025 | 0.85 (0.73–0.98) | 0.026 | 0.87 (0.74–1.01) | 0.066 |
| rs2094258 | CC | 414 (37.13) | 424 (37.96) | 0.838 | 1.00 | 1.00 |
| CT | 524 (47.00) | 525 (47.00) | 1.02 | 0.85 (0.75–0.95) | 0.813 | 0.86 (0.76–0.95) | 0.722 |
| TT | 177 (15.87) | 168 (15.04) | 0.98 | 0.88 (0.78–0.99) | 0.552 | 0.98 (0.88–1.09) | 0.481 |
| TT/CT | 701 (62.87) | 693 (62.04) | 0.686 | 1.04 | 0.87 | 0.686 | 0.713 |
| C | 1352 (0.63) | 1373 (0.62) | 0.965 | 1.00 | 1.00 |
| T | 878 (0.39) | 861 (0.38) | 0.602 | 1.03 | 0.92 | 0.602 | 0.771 |
| rs873601 | GG | 314 (28.16) | 311 (27.84) | 0.956 | 1.00 | 1.00 |
| AG | 566 (50.76) | 565 (50.58) | 0.99 | 1.02 | 0.937 | 1.00 | 0.98 | 0.988 |
| AA | 235 (21.08) | 241 (21.58) | 0.97 | 1.00 | 0.75 | 0.95 | 0.664 |
| AG/AA | 801 (71.84) | 806 (72.16) | 0.867 | 0.98 | 0.98 | 0.867 | 0.876 |
| G | 1194 (0.54) | 1187 (0.53) | 1.00 | 1.00 |
| A | 1036 (0.46) | 1047 (0.47) | 0.812 | 0.98 | 0.98 | 0.740 | 0.670 |

*a* Chi-square test for genotype distributions between cases and controls.

*b* Adjusted for age, sex, body mass index (BMI), smoking status, and drinking status in logistic regress models.

*c* for dominant genetic models.

For Table 2, logistic regression analysis of associations between the ERCC5 variant genotypes and ESCC risk.

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32.59%, \(P<0.0001\) than the controls. In addition, the cases exhibited a lower proportion of BMI \(\geq 24.0\) than the controls (43.23% vs. 61.24%, \(P<0.0001\)). Therefore, these variables were further adjusted for in subsequent multivariate logistic regression analyses.

**ERCC5 Allele and Genotype Distributions and Association with ESCC Risk**

The allele and genotype frequencies of the three selected SNPs in cases and controls are summarized in Table 2. All the observed genotype frequencies agreed with the Hardy–Weinberg equilibrium in the controls (\(P=0.860\) for rs2296147, \(P=0.793\) for rs2094258, and \(P=0.601\) for rs873601). The rs2296147 C allele and rs873601 A allele were less frequent among cases than among controls (0.18 vs. 0.21, \(P=0.025\) and 0.46 vs. 0.47, \(P=0.812\), respectively), whereas the rs2094258 T allele was more frequent among cases than among controls, though the difference was not statistically significant (0.39 vs. 0.38, \(P=0.602\)). In the single-locus analyses, only genotype frequency of rs2296147 was significantly different between the cases and the controls (\(P=0.016\)). After adjustment for age, sex, BMI and smoking, and drinking status in multivariate logistic regression analysis, rs2296147 variant C genotypes were associated with a significantly decreased risk of ESCC (adjusted OR = 0.76, 95% CI = 0.63–0.93, \(P=0.006\) for CT vs. TT and adjusted OR = 0.80, 95% CI = 0.67–0.96, \(P=0.016\) for CT/CC vs. TT). In addition, the rs2296147 variant C allele was borderline significantly associated with a lower risk of ESCC (adjusted OR = 0.87, 95% CI = 0.74–1.01, \(P=0.066\)). However, there was no evidence of an association between other two SNPs (rs2094258 and rs873601) and overall ESCC risk.

**Stratification Analysis of ESCC Risk by ERCC5 Polymorphisms**

We further evaluated the association between ERCC SNPs and ESCC risk stratified by selected variables including age, sex, BMI, smoking status and drinking status. As shown in Table 3, the stratification analysis indicated that the protective effect of rs2296147 CT/CC genotypes was more evident in older subjects (adjusted OR = 0.75, 95% CI = 0.57–0.97, \(P=0.016\)), never-smokers and alcoholics (adjusted OR = 0.75, 95% CI = 0.59–0.95), BMI < 24.0 (adjusted OR = 0.77, 95% CI = 0.59–1.00), compared with the TT genotype. However, further homogeneity tests did not show statistical evidence for any differences (\(P>0.05\)) between every two strata, suggesting no risk modification by the variables under investigation. Additionally, no associations were observed between the genotypes of other two SNPs (rs2094258 and rs873601) and risk of ESCC in the stratification analysis, nor was there any statistical evidence for gene-environment interactions between the variant genotypes and these variables on risk of ESCC (data not shown).

**ERCC5 Haplotypes and ESCC Risk**

We found that the LD among these three SNPs of ERCC5 was incomplete in all control subjects (rs2094258 and rs2296147: \(r^2 = 0.103\); rs2094258 and rs873601: \(r^2 = 0.438\); rs873601 and rs2296147: \(r^2 = 0.102\)), suggesting that variant haplotypes may play a role in cancer risk. We inferred eight ERCC5 haplotypes, four of which had a frequency less than 0.05 and were merged into one group of “minor haplotypes” for further analysis. However, no ERCC5 haplotype in this study population was associated with ESCC risk, when the most common haplotype T-T-G was used as the reference (Table 4).

Finally, the FPRP values were calculated at different prior probability levels for all significant findings. For a prior probability of 0.01, assuming the OR for specific genotype or allele was 0.67, with statistical power of 0.918, 0.963 and 0.998, the FPRP values were 0.301, 0.451 and 0.721, respectively, for an association of the CT, CT/CC genotypes and C allele with a reduced risk of ESCC in all individuals (Table 5).

**Discussion**

In the present case-control study, we investigated the associations between three common, putatively functional SNPs of the ERCC5 gene and the risk of ESCC in an Eastern Chinese population. When we evaluated each SNP separately, only the rs2296147 variant CT/CC genotypes were associated with significantly reduced ESCC risk, but this risk was not observed for the other two rs2094258C>T and rs873601G>A SNPs. To the best of our knowledge, this is the first study that investigated the association of these three ERCC5 SNPs with the ESCC risk.

Given the role of the ERCC5 gene in the NER pathway, it is biologically plausible that functional ERCC5 SNPs may contribute to ESCC susceptibility. To date, only three reported studies have investigated the association between rs2296147 and risk of cancers other than ESCC, of which one was conducted for SCCHN with 1059 white patients and 1066 healthy controls [30]; another included mixed populations with 722 cases of endometrial cancer and 726 cancer-free controls [34]; and the third was performed in a Chinese population with 1125 gastric cancer cases and 1196 healthy controls [33]. Although the rs2296147 C allele was associated with a non-significantly altered risk of cancers in these three reported studies of other cancers, the rs873601 A variant genotypes were found to be associated with an increased risk of gastric cancer [33]. For the rs2094258C>T SNP, only one study investigated its association with risk of SCCHN, but the result was null, which is in agreement with the finding in the present study of ESCC. The possible discrepancy of the results among different studies may be due to different genetic backgrounds and etiologies. For example, the frequency of the rs2296147 C allele in non-Hispanic White populations was 0.47 in the study by Ma et al. [30] but was 0.20 in the present study (in a Chinese population). Another possibility may be related to different etiologies of the primary tumors. Although some studies have shown that some sequence variants in the region of chromosome 5p15.33 and 8q24 are associated with risk of different cancer types [35–39], most published studies reported that the same SNP may be cancer-specific. It is likely that our present study still had a limited statistical power to detect a weak effect. For instance, for the sample size in the present study, we had only a 54.9% power to detect \(OR = 1.23\) for rs873601 under a recessive model as reported by He et al. [33]. Meanwhile, greater FPRP values were observed for the significant associations between rs2296147 C variant genotypes and ESCC risk, which further suggests a relatively small sample size in the present study. Therefore, whether or not an association between ERCC5 SNPs with cancer risk is cancer-specific needs to be validated in additional large studies of different cancer types, either in additional groups of homogenous ethnicity or in more ethnically diverse groups.

Moreover, in the published GWASs for cancers, including ESCC, based on common tagging SNPs, none of the SNPs in NER genes has been identified as susceptibility locus. This poses a challenge to the common variants and common diseases theory [40]. Because NER genes are well-known susceptibility genes, it is plausible that individuals carrying NER variants may be prone to exposures that instigate damage to DNA and thus to cancer. Consequently, without comprehensive information about such exposures for additional adjustment or stratification, the actual...
Table 3. Stratification analysis for associations between ERCC5 variant genotypes and ESCC risk.

| Variables | rs2296147 (cases/controls) | Adjusted OR* (95% CI) | p  | rs2094258 (cases/controls) | Adjusted OR* (95% CI) | p  | rs873601 (cases/controls) | Adjusted OR* (95% CI) | p  |
|-----------|-----------------------------|-----------------------|----|-----------------------------|-----------------------|----|-----------------------------|-----------------------|----|
|           | TT                          | CT/CC                 |    | CC                          | TT/CT                 |    | GG                          | AG/AA                 |    |
| Age, yr   | ≤ 60 (median)               | 361/344               | 193/213 | 0.87 (0.67–1.13)  | 0.31 | 211/221 | 343/336 | 1.06 (0.82–1.37)  | 0.71 | 149/147 | 405/410 | 1.06 (0.80–1.41)  | 0.92 |
|           | > 60 (median)               | 396/355               | 165/205 | 0.75 (0.57–0.97)  | 0.24 | 203/203 | 358/357 | 0.98 (0.76–1.26)  | 0.71 | 165/164 | 396/396 | 0.95 (0.72–1.24)  | 0.79 |
| Sex       | Males                       | 608/545               | 293/326 | 0.83 (0.68–1.02)  | 0.64 | 338/336 | 563/535 | 1.04 (0.85–1.28)  | 0.88 | 252/243 | 649/628 | 1.00 (0.81–1.25)  | 0.79 |
|           | Females                     | 149/154               | 65/92  | 0.66 (0.43–1.01)  | 1.13 | 76/88  | 138/158 | 1.06 (0.80–1.41)  | 0.92 | 62/68  | 152/178 | 0.93 (0.60–1.44)  | 0.79 |
| Smoking status | 0                           | 287/319               | 137/193 | 0.81 (0.61–1.07)  | 0.95 | 161/193 | 263/319 | 1.02 (0.77–1.34)  | 0.76 | 114/136 | 310/376 | 0.96 (0.71–1.30)  | 0.87 |
|           | ≤ 16 (mean)                 | 110/148               | 41/96  | 0.59 (0.37–0.94)  | 1.09 | 56/98  | 95/146  | 1.09 (0.71–1.69)  | 0.76 | 48/66  | 103/178 | 0.77 (0.49–1.23)  | 0.79 |
|           | > 16 (mean)                 | 360/232               | 180/129 | 0.84 (0.62–1.14)  | 1.05 | 197/133 | 343/228 | 1.05 (0.78–1.41)  | 0.88 | 152/109 | 388/252 | 1.15 (0.83–1.58)  | 0.88 |
| Drinking status | Never                       | 429/469               | 192/284 | 0.75 (0.59–0.95)  | 0.38 | 225/288 | 396/465 | 1.10 (0.88–1.38)  | 0.49 | 176/197 | 445/556 | 0.88 (0.69–1.13)  | 0.16 |
|           | Ever                        | 328/230               | 166/134 | 0.83 (0.61–1.13)  | 0.97 | 189/136 | 305/228 | 0.97 (0.72–1.31)  | 1.38 | 138/114 | 356/250 | 1.18 (0.86–1.61)  | 0.51 |
| BMI       | < 24.0                      | 434/273               | 199/160 | 0.77 (0.59–1.00)  | 1.07 | 236/161 | 407/272 | 1.07 (0.83–1.39)  | 0.61 | 172/120 | 461/313 | 1.04 (0.79–1.38)  | 0.51 |
|           | ≥ 24.0                      | 323/426               | 159/258 | 0.82 (0.64–1.06)  | 0.97 | 188/263 | 294/421 | 0.97 (0.76–1.24)  | 0.94 | 142/191 | 340/493 | 0.94 (0.73–1.24)  | 0.94 |

*a Obtained in logistic regression models with adjustment for age, sex, BMI, smoking status and drinking status.

*b P for heterogeneity test using the Chi-square-based Q test.

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associations may be either biased or masked, as likely to be in the published GWASs. XP patients, for instance, can greatly decrease their risk of developing skin cancer, if they can effectively avoid sun exposure in their lifetime. Alternatively, common variants are unlikely to have a significant biological effect or when their effects are weak, sufficient study power is needed, even in the GWASs. For example, when 500 000 SNPs were analyzed in a GWAS, multiple comparisons demand a \( P \)-value less than \( 10^{-7} \) or \( 10^{-8} \) to avoid finding false significant associations, which markedly decreases the power to detect SNPs associated with the disease phenotype [41]. This is simply because common variants may moderately contribute to risk of cancer due to their weak penetrance. In that case, the identification of additional rare but functional variants requires a single large GWAS or combined analysis of several published GWASs in the future.

There is some biological plausibility that functional genetic variants, like those of \( ERCC5 \), may contribute to risk of carcinogenesis initiated by carcinogen-induced DNA damage. Under such circumstances, a candidate approach, rather than a GWAS, may be more appropriate and feasible. Acting as a structure-specific endonuclease and also a 5'-3' exonuclease, the \( ERCC5 \) protein is required for two sub-pathways in NER. One is transcription-coupled DNA repair (TCR), which preferentially removes DNA damage in the transcribed DNA strand of active genes; the other is global genomic repair (GGR), which removes lesions throughout the genome [42,43].

For example, when 500 000 SNPs were analyzed in a GWAS, multiple comparisons demand a \( P \)-value less than \( 10^{-7} \) or \( 10^{-8} \) to avoid finding false significant associations, which markedly decreases the power to detect SNPs associated with the disease phenotype [41]. This is simply because common variants may moderately contribute to risk of cancer due to their weak penetrance. In that case, the identification of additional rare but functional variants requires a single large GWAS or combined analysis of several published GWASs in the future.

### Table 4. Haplotype analysis for genotypes of \( ERCC5 \) and ESCC risk.

| Haplotype | Haplotype frequencies | Crude OR (95% CI) | \( P \) | Adjusted OR (95% CI)* | \( P \) |
|-----------|-----------------------|-------------------|------|-----------------------|------|
|           | Cases (N = 2230)      | Controls (N = 2234) |      |                       |      |
| T-T-G     | 835                   | 37.44             | 824  | 36.88                 | 1.00 | 1.00                  |
| C-C-A     | 361                   | 16.19             | 397  | 17.77                 | 0.90 | (0.76–1.07)            | 0.217| (0.90–0.75–1.08)      | 0.263|
| T-C-A     | 634                   | 28.43             | 613  | 27.44                 | 1.02 | (0.88–1.18)            | 0.785| (1.02–0.88–1.19)      | 0.760|
| T-C-G     | 309                   | 13.86             | 293  | 13.12                 | 1.04 | (0.86–1.25)            | 0.675| (1.06–0.87–1.29)      | 0.577|
| All minor haplotypes*| 91               | 4.08              | 107  | 4.78                  | 0.84 | (0.63–1.13)            | 0.246| (0.88–0.65–1.20)      | 0.414|

*Obtained in logistic regression models with adjustment for age, sex, BMI, smoking status and drinking status.

### Table 5. False-Positive Report Probability Values for Associations between ESCC risk and the Frequency of Genotypes and Allele of \( ERCC5 \) rs2296147.

| Genotype/Allele | OR* (95% CI) | \( P \) valueb | Statistical Power* | Prior probability |
|-----------------|-------------|---------------|------------------|------------------|
|                 |             |               |                  | 0.2500           | 0.1000          | 0.0100          | 0.0010          | 0.0001          |
| \( ERCC5 \) rs2296147 |            |               |                  |                  |                |                |                |                |
| CT versus TT    | 0.77        | (0.64–0.92)   | 0.004            | 0.918            | 0.013           | 0.038           | 0.301           | 0.813           | 0.978           |
| CT/CC versus TT | 0.79        | (0.66–0.94)   | 0.008            | 0.963            | 0.024           | 0.070           | 0.451           | 0.892           | 0.988           |
| C versus T      | 0.85        | (0.73–0.98)   | 0.026            | 0.998            | 0.072           | 0.190           | 0.721           | 0.963           | 0.996           |

*The crude OR reported in Table 2.

The omnibus chi-square test of the genotype and allele frequency distributions reported in Table 2.

Statistical power was calculated using the number of observations in the study and the OR and \( P \) values in this table.

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Additionally, rs2296147 in the 5’UTR may have some effects on regulating posttranscriptional processing, because the 5’UTR length of ERCC5 may be critical for its recruitment to the polyribosomal cellular fraction that could increase translation in response to the protein kinase C activity as a result of DNA damage response [32]. However, it would be more conclusive, if we had data on ERCC5 mRNA/protein expression by genotypes. Unfortunately, the target tissues were not made available to us at the time the study initiated. Taken together, because we do not have directly biological evidence, it may be premature to conclude that rs2296147 is a causal SNP.

There are several potential limitations in the present study that should be addressed. First, although our study was relatively large, the small sample size in subgroup analysis may have limited statistical power to detect significant associations for each of the strata, let alone the power to assess gene-gene or gene-environment interactions adequately. Second, we used common, potentially functional SNPs instead of tagging SNPs, which may miss some important genetic variations within the gene. Third, because it was a hospital-based case-control study, there may be inherent biases for selection of the nonrepresentative population and retrospective collection of exposure data. Finally, because of our tissue access constraint, this study did not include the detection of the influence of the ERCC5 variants on its protein expression in the target tissue. These limitations can only be overcome in larger, well designed prospective studies in the future.

In summary, our findings suggested that ERCC5 polymorphisms may contribute to risk of ESCC among Eastern Chinese populations, but the effect was weak and needs to be further validated in larger studies in the future.

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Author Contributions

Conceived and designed the experiments: QYW JQX. Performed the experiments: MLZ JH HCH. Analyzed the data: MLZ TYS MYW. Contributed reagents/materials/analysis tools: LJ YJY JCW MHS HC. KLZ ZZ HQC. Wrote the paper: MLZ QYW.

References

1. Jemal A, Bray F, Center MM, Ferlay J, Ward E, et al. (2011) Global cancer statistics. CA Cancer J Clin 61: 69–90.
2. Vaughan TL, Davis S, Kristal A, Thomas DB (1995) Obesity, alcohol, and tobacco as risk factors for cancers of the esophagus and gastric cardia. Adenocarcinoma versus squamous cell carcinoma. Cancer Epidemiology Biomarkers and Prevention 4: 85–92.
3. Li XY, Su M, Huang JJ, Li H, Tian DP, et al. (2007) mRNA evidence. Genetic background associated with related populations at high risk for esophageal cancer between Chaoshan and Taihang Mountain areas in China. Gut 56: 491–498.
4. Chang-Claude J, Becher H, Blettner M, Qiu SL, Yang GR, et al. (1997) Familial aggregation of oesophageal cancer in a high incidence area in China. International Journal of Epidemiology 26: 1159–1165.
5. Hiyama T, Yoshihara M, Tanaka S, Chayama K (2007) Genetic polymorphisms and esophageal cancer risk. International Journal of Cancer 121: 1643–1650.
6. Xing DY, Tan W, Lin DX (2003) Genetic polymorphisms and susceptibility to esophageal cancer among Chinese population (Review). Oncology Reports 10: 1615–1625.
7. Rouillon C, White MF (2011) The evolution and mechanisms of nucleotide excision repair proteins. Res Microbiol 162: 19–26.
8. Cleaver JE, Lam ET, Revert I (2009) Disorders of nucleotide excision repair: the molecular basis for different disease states caused by mutations in TFIIH and XPG. DNA Repair 7: 339–365.
9. Kraemer KH, Lee MM, Andrews AD, Lambert WC (1994) The role of sunlight and DNA repair in melanoma and nonmelanoma skin cancer. The xeroderma pigmentosum paradigm. Arch Dermatol 130: 1018–1021.
10. Scherly D, Nouspikel T, Corlet J, Ucla C, Bairoch A, et al. (1993) Complementation of the DNA repair defect in xeroderma pigmentosum group G cells by a human αDNA related to yeast RAD2. Nature 363: 182–185.
11. Ito S, Kuraoka I, Chymkowitch P, Compe E, Takedachi A, et al. (2007) XPG nucleotide excision repair gene polymorphisms and risk of cutaneous melanoma. Eur J Hum Genet 15: 235–237.
12. Li C, Hu Z, Liu Z, Wang LE, Strom SS, et al. (2006) Polymorphisms in the DNA repair genes XPC, XPD, and XPG and risk of cutaneous melanoma: a case-control analysis. Cancer Epidemiol Biomarkers Prev 15: 2326–2332.
13. Provey JE, Darakshidian F, Robertson K, Bisset Y, Melsky M, et al. (2007) DNA repair gene polymorphisms and genetic predisposition to cutaneous melanoma. Carcinogenesis 28: 1087–1093.
14. Sanyle S, Festa F, Sakano S, Zhang Z, Steineck G, et al. (2004) Polymorphisms in DNA repair and metabolic genes in bladder cancer. Carcinogenesis 25: 729–734.
15. Garcia-Closas M, Malats N, Real FX, Welch R, Kogevinas M, et al. (2006) Genetic variation in the nucleotide excision repair pathway and bladder cancer risk. Cancer Epidemiol Biomarkers Prev 15: 536–543.
16. Pan J, Liu J, Izzo JG, Liu Y, Xing J, et al. (2009) Genetic susceptibility to esophageal cancer: The role of the nucleotide excision repair pathway. Carcinogenesis 30: 785–792.
17. Cheng L, Spitz MR, Hong WK, Wei Q (2000) Reduced expression levels of nucleotide excision repair genes in lung cancer: A case-control analysis. Cancer Epidemiology Biomarkers Prev 19: 1527–1530.
18. Cheng L, Sturgis EM, Eicher SA, Spitz MR, Wei Q (2002) Expression of nucleotide excision repair genes and ddxd risk for squamous cell carcinoma of the head and neck. Cancer 94: 393–397.
19. Blankenburg S, Konig IR, Moessner R, Laspe P, Thoms KM, et al. (2005) No association between three xeroderma pigmentosum group C and one group G gene polymorphisms and risk of cutaneous melanoma. Eur J Hum Genet 13: 235–237.
20. Knip M, Apajalahti J, Makela T, Ranta E, Koskinen P, et al. (2005) Improved results in the serodiagnosis of type 1 diabetes mellitus. J Clin Endocrinol Metab 90: 2512–2519.
21. Chang JS, Wrensch MR, Hansen HM, Sison JD, Aldrich MC, et al. (2008) Host cell reactivation. Stabilizes TFIIH, Allowing Transactivation of Nuclear Receptors: Implications for Cockayne Syndrome in XPG/ERCC5 and risk of squamous cell carcinoma of the head and neck. Cancer 94: 393–397.
22. Blankenburg S, Konig IR, Moessner R, Laspe P, Thoms KM, et al. (2005) No association between three xeroderma pigmentosum group C and one group G gene polymorphisms and risk of cutaneous melanoma. Eur J Hum Genet 13: 235–237.
23. Li C, Hu Z, Liu Z, Wang LE, Strom SS, et al. (2006) Polymorphisms in the DNA repair genes XPC, XPD, and XPG and risk of cutaneous melanoma: a case-control analysis. Cancer Epidemiol Biomarkers Prev 15: 2326–2332.
24. Provey JE, Darakshidian F, Robertson K, Bisset Y, Melsky M, et al. (2007) DNA repair gene polymorphisms and genetic predisposition to cutaneous melanoma. Carcinogenesis 28: 1087–1093.
25. Sanyle S, Festa F, Sakano S, Zhang Z, Steineck G, et al. (2004) Polymorphisms in DNA repair and metabolic genes in bladder cancer. Carcinogenesis 25: 729–734.
26. Garcia-Closas M, Malats N, Real FX, Welch R, Kogevinas M, et al. (2006) Genetic variation in the nucleotide excision repair pathway and bladder cancer risk. Cancer Epidemiol Biomarkers Prev 15: 536–543.
27. Pan J, Liu J, Izzo JG, Liu Y, Xing J, et al. (2009) Genetic susceptibility to esophageal cancer: The role of the nucleotide excision repair pathway. Carcinogenesis 30: 785–792.
28. Cheng L, Spitz MR, Hong WK, Wei Q (2000) Reduced expression levels of nucleotide excision repair genes in lung cancer: A case-control analysis. Cancer Epidemiology Biomarkers Prev 19: 1527–1530.
29. Cheng L, Sturgis EM, Eicher SA, Spitz MR, Wei Q (2002) Expression of nucleotide excision repair genes and ddxd risk for squamous cell carcinoma of the head and neck. Cancer 94: 393–397.
30. Ma HX, Yu HP, Liu ZS, Wang LE, Sturgis EM, et al. (2011) Polymorphisms of XPG/ERCC5 and risk of squamous cell carcinoma of the head and neck. Pharmacogenetics and Genomics 22: 50–57.
31. Wang X, Lu M, Qian J, Yang Y, Li S, et al. (2009) Rationales, design and recruitment of the Taizhou Longitudinal Study. BMC Public Health 9: 223.
32. Blankenburg S, Konig IR, Moessner R, Laspe P, Thoms KM, et al. (2005) No association between three xeroderma pigmentosum group C and one group G gene polymorphisms and risk of cutaneous melanoma. Eur J Hum Genet 13: 235–237.
33. Li C, Hu Z, Liu Z, Wang LE, Strom SS, et al. (2006) Polymorphisms in the DNA repair genes XPC, XPD, and XPG and risk of cutaneous melanoma: a case-control analysis. Cancer Epidemiol Biomarkers Prev 15: 2326–2332.
34. Doherty JA, Weiss NS, Fish S, Fan WH, Loomis MM, et al. (2011) Polymorphisms in the DNA nucleotide excision repair genes and the risk for squamous cell carcinoma of the head and neck. Cancer 94: 393–397.
35. Wang X, Lu M, Qian J, Yang Y, Li S, et al. (2009) Rationales, design and recruitment of the Taizhou Longitudinal Study. BMC Public Health 9: 223.
35. Gudmundsson J, Sulem P, Manolescu A, Amundadottir LT, Gudbjartsson D, et al. (2007) Genome-wide association study identifies a second prostate cancer susceptibility variant at 8q24. Nature genetics 39: 631–637.

36. Yeager M, Orr N, Hayes RB, Jacobs KB, Kraft P, et al. (2007) Genome-wide association study of prostate cancer identifies a second risk locus at 8q24. Nature genetics 39: 645–649.

37. Haiman CA, Patterson N, Freedman ML, Myers SR, Pike MC, et al. (2007) Multiple regions within 8q24 independently affect risk for prostate cancer. Nature genetics 39: 638–644.

38. Tomlinson I, Webb E, Carvajal-Carmona L, Broderick P, Kemp Z, et al. (2007) A genome-wide association scan of tag SNPs identifies a susceptibility variant for colorectal cancer at 8q24.21. Nature genetics 39: 645–649.

39. Kiemeney LA, Thorlacius S, Sulem P, Geller F, Aben KKH, et al. (2008) Sequence variant on 8q24 confers susceptibility to urinary bladder cancer. Nature genetics 40: 1307–1312.

40. Bodmer W, Bonilla C (2008) Common and rare variants in multifactorial susceptibility to common diseases. Nat Genet 40: 695–701.

41. Riancho JA (2012) Genome-wide association studies (GWAS) in complex diseases: Advantages and limitations. Reumatologia Clinica 8: 56–57.

42. Hanawalt PC (2001) Controlling the efficiency of excision repair. Mutat Res 485: 3–13.

43. Emmert S, Slor H, Busch DB, Batko S, Albert RB, et al. (2002) Relationship of neurologic degeneration to genotype in three xeroderma pigmentosum group G patients. J Invest Dermatol 118: 972–982.

44. Lomonaco SL, Xu XS, Wang G (2009) The role of Bcl-x(L) protein in nucleotide excision repair-facilitated cell protection against cisplatin-induced apoptosis. DNA Cell Biol 28: 265–294.

45. Schmutz KM, Schmitt N, Hoffmann-Rohrer U, Schafer A, Grummt I, et al. (2009) TAF12 recruits Gadd45a and the nucleotide excision repair complex to the promoter of rRNA genes leading to active DNA demethylation. Mol Cell 33: 344–353.

46. Barreto G, Schafer A, Marhold J, Stach D, Swaminathan SK, et al. (2007) Gadd45a promotes epigenetic gene activation by repair-mediated DNA demethylation. Nature 445: 671–675.

47. Noupikel TP, Hyka-Noupikel N, Hanawalt PC (2006) Transcription domain-associated repair in human cells. Mol Cell Biol 26: 8722–8730.

48. Cartharius K, Frech K, Grote K, Kloeck R, Hallmeier M, et al. (2005) MalInspector and beyond: Promoter analysis based on transcription factor binding sites. Bioinformatics 21: 2933–2942.

49. Mohrenweiser H (2007) Survey of polymorphic sequence variation in the immediate 5’ region of human DNA repair genes. Mutat Res 616: 221–226.

50. Pittman AM, Naranjo S, Jalava SE, Tswe P, Ma Y, et al. (2010) Allelic Variation at the 8q23.3 Colorectal Cancer Risk Locus Functions as a Cis-Acting Regulator of E2F3. PLoS Genetics 6.

51. Bloomquist TM, Crawford EL, Willey JC (2010) Cis-acting genetic variation at an E2F1/YY1 response site and putative p53 site is associated with altered allele-specific expression of ERCC5 (XPG) transcript in normal human bronchial epithelium. Carcinogenesis 31: 1242–1250.

52. Powley IR, Kondrashov A, Young LA, Dobryn HC, Hill K, et al. (2009) Translational reprogramming following UVB irradiation is mediated by DNA-PKcs and allows selective recruitment to the polysomes of mRNAs encoding DNA repair enzymes. Genes and Development 23: 1207–1220.