**RANK rs1805034 T>C Polymorphism Is Associated with Susceptibility of Esophageal Cancer in a Chinese Population**

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**Abstract**

Esophageal cancer remains the sixth leading cause of cancer associated death and eighth most common cancer worldwide. Genetic factors, such as single nucleotide polymorphisms (SNPs), may contribute to the carcinogenesis of esophageal cancer. Here, we conducted a hospital based case-control study to evaluate the genetic susceptibility of functional SNPs on the development of esophageal cancer. A total of 629 esophageal squamous cell carcinoma (ESCC) cases and 686 controls were enrolled for this study. The OPG rs3102735 T>C, rs2073618 G>C, RANK rs1805034 T>C, RANKL rs9533156 T>C and rs2277438 A>G were determined by ligation detection reaction method. Our findings suggested that RANK rs1805034 T>C is associated with the susceptibility of ESCC, which is more evident in male and elder (≥63) patients. Our study provides the first evidence that functional polymorphisms RANK rs1805034 T>C may be an indicator for individual susceptibility to ESCC. However, further larger studies among different ethnic populations are warranted to verify our conclusion.

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**Introduction**

Despite recent considerable medical advances, esophageal cancer remains a refractory disease with high morbidity and mortality. Essentially, esophageal cancer is the 6th leading cause of cancer-related mortality and the 8th most common cancer worldwide [1]. There are more than 450,000 patients diagnosed as esophageal cancer worldwide and the incidence is still rising rapidly. Meanwhile, its startling overall 5-year survival rate ranges from 15–25% [2]. In China, more strikingly, esophageal cancer ranks the 5th most common diagnosed cancer and 4th leading cause of cancer related mortality [3]. Esophageal squamous cell carcinoma (ESCC) is the predominant histological type of esophageal cancer [1]. Although multidisciplinary therapeutic strategy has been recommended, the prognosis is still poor. Tobacco use [4,5], alcohol consumption [4,6], low socioeconomic status, poor oral hygiene and nutritional deficiencies [2,7–9] have been identified as risk factors for esophageal cancer. Yet, only a subset of individuals exposed to these risk factors eventually develop esophageal cancer, indicating a pivotal role of genetic factors, such as single nucleotide polymorphisms (SNPs), in the esophageal carcinogenesis.

Recently, the osteoprotegerin (OPG), its binding protein—the receptor activator of NF-κB (RANK) and RANK ligand (RANKL) have been implicated with the pathogenesis of breast cancer [10]. OPG was initially identified from a fetal rat intestine cDNA library [11], which is unique for it only exists as a secreted molecule in contrast to the other membrane-bound cell surface members of tumor necrosis factor receptor (TNF-R) family. RANKL is the OPG binding protein (also named OPG ligand, OPGL) [12,13], while RANK constitutes the cell surface receptor which responses to OPGL. In numerous rodent models of tumor, RANKL signal is increased through diverse mechanisms [14]. OPG neutralizes RANKL, which leads to a reduced RANKL-RANK interaction [12]. RANKL expression was verified in various tumor types and inflammatory cells associated with tumor [15–17]. Elevation in stromal RANKL has been detected at local sites of bone metastasis or multiple myeloma [18,19], causing enhanced osteoclast activity and bone destruction. In experimental models, RANKL inhibitors reduced tumor-induced osteolysis in various types of cancer [14], reduced bone destruction, skeletal tumor progression, as well as tumor burden [17,20,21]. In addition, RANKL-RANK pathway may contribute to the primary tumorigenesis and metastasis independently of its effects on tumor-related osteolysis. Regulated by factors including prolactin and progesterone, RANKL could drive the primary mitogenic response of mammary epithelium and the expansion of mammary stem cells via RANK activation [22–24], which may therefore induce mammary cancer by offering a more transformation-susceptible target pool. RANKL may regulate spontaneous mammary tumor formation and metastasis.
driven by the potent oncogene Neu (ERBB2). RANKL blockade effectively attenuated the formation of mammary tumors and pulmonary metastasis in the MMTV-Neu transgenic mouse model [25,26]. Interestingly, OPG may serve as a positive regulator of microvessel formation and may promote neovascularization [27] that is important for tumor progression. OPG overexpression by breast cancer cells enhances orthotopic and osseous tumor growth [28]. In light of all these findings, RANKL/RANK/OPG signaling pathway has emerged as a promising therapeutic target [28]. In a hospital-based case-control study, we performed genotyping analyses of the five miRNA SNPs in 629 ESCC cases and 686 controls in a Chinese population.

Methods

Ethics Statement

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

Study populations

A total of 1,315 participants consisting of 629 esophageal cancer patients and 686 non-cancer controls frequency-matched to the cases with regard to age (±5 years) and sex were enrolled in this study (Table 1). All patients and controls 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 histologically. Patients who had cancer history/metastasized cancer or had received chemotherapy/radiotherapy were excluded for the current study.

Each subject was personally questioned by experienced interviewers using a questionnaire to obtain information on demographic data (e.g., age, sex) and related risk factors (including tobacco use and alcohol consumption). After the interview, 2-mL samples of venous blood were collected from each subject. “Smokers” subgroup included individuals who smoked one cigarette per day for >1 year. Subjects who consumed ≥5 alcoholic drinks a week for >6 months were subdivided into “alcohol drinkers” category.

Genomic DNA extraction, SNP selection and Genotyping

Genomic DNA was isolated from peripheral blood using QIAamp DNA Blood Mini Kit (Qiagen, Berlin, Germany) as reported previously [33]. Sample DNA were amplified by PCR according to the manufacturer’s protocol. The samples were genotyped using the ligation detection reaction (LDR) method [34] (technical support from the Biowing Applied Biotechnol-
No association was detected among OPG rs3102735 C>T, OPG rs2073618 G>C, RANKL rs9533156 T>C, RANKL rs2277438 A>G polymorphisms and the risk of ECSS (Table 3).

Stratification analyses of RANK rs1805034 T>C genotype and risk of ESCC

To evaluate the effects of RANK rs1805034 T>C genotype on ESCC risk according to different age, sex, smoking and alcohol consumption; we performed the stratification analyses (Table 4). A significantly increased risk of ESCC associated with the RANK rs1805034 T>C polymorphism was evident among male patients (CC vs. TT: adjusted OR = 1.89, 95% CI = 1.16–3.08, p = 0.011) (TC/CC vs. TT, adjusted OR = 1.38, 95% CI = 1.05–1.81, p = 0.022) (CC vs. TT/TC, adjusted OR = 1.68, 95% CI = 1.05–2.69, p = 0.031). Likewise, in elder patients (≥63 years old), RANK rs1805034 T>C polymorphism was also associated with a significantly increased risk of ESCC (CC vs. TT, adjusted OR = 1.84, 95% CI = 1.02–3.31, p = 0.041) (Table 4).

### 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 |
| ≥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 |

*pTwo-sided χ² test and student t test; Bold values are statistically significant (p<0.05).
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### Table 2. Primary information for OPG rs3102735 T>C, rs2073618 G>C, RANK rs1805034 T>C, RANKL rs9533156 T>C and rs2277438 A>G polymorphisms.

| Genotyped SNPs | OPG rs3102735 C>T | OPG rs2073618 G>C | RANK rs1805034 T>C | RANKL rs9533156 T>C | RANKL rs2277438 A>G |
|----------------|------------------|-------------------|-------------------|-------------------|-------------------|
| Chromosome | 8 | 8 | 18 | 13 | 13 |
| Gene Official Symbol | TNFRSF11B | TNFRSF11B | TNFRSF11A | TNFSF11 | TNFSF11 |
| Function | nearGene-5 | missense | missense | missense | intron region |
| Chr Pos (Genome Build 36.3) | 120034251 | 120033233 | 58178221 | 42045671 | 42053168 |
| Regulome DB Scorea | 5 | 4 | 5 | 5 | No Data |
| TFBSb | Y | — | — | — | — |
| Splicing (ESE or ESS) | — | Y | Y | — | — |
| miRNA (miRanda) | — | — | — | — | — |
| miRNA (Sanger) | — | — | — | — | — |
| MAFc for Chinese in database | 0.134 | 0.308 | 0.300 | 0.439 | 0.300 |
| MAF in our controls (n = 686) | 0.164 | 0.263 | 0.286 | 0.464 | 0.314 |
| p value for HWEd test in our controls | 0.191 | 0.371 | 0.531 | 0.488 | 0.700 |
| Genotyping methodd | LDR | LDR | LDR | LDR | LDR |
| % Genotyping value | 95.13% | 96.35% | 96.43% | 96.43% | 96.81% |

*ahttp://www.regulomedb.org/; bTFBS: Transcription Factor Binding Site (http://snpinfo.niehs.nih.gov/snpinfo/snpfunc.htm); cMAF: minor allele frequency, OPG rs2073618 G>C MAF is in CHB+JPT population; dHWE: Hardy–Weinberg equilibrium; eLDR: Ligation Detection Reaction.
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Table 3. Logistic regression analyses of associations between OPG rs3102735 T>C, rs2073618 G>C, RANK rs1805034 T>C, RANKL rs9533156 T>C and rs2277438 A>G polymorphisms and risk of ESCC.

| Genotype | Cases (n = 629) | Controls (n = 686) | Crude OR (95% CI) | p | Adjusted OR * (95% CI) | p |
|----------|-----------------|--------------------|-------------------|---|------------------------|---|
|          | n               | %                 | n                 | %  |                        |    |
| OPG rs3102735 T>C | | | | | | |
| TT       | 442             | 73.7              | 450               | 69.1 | 1.00                   | 1.00 |
| TC       | 146             | 24.3              | 188               | 28.9 | 0.79 (0.61–1.02)       | 0.069 |
| CC       | 12              | 2.0               | 13                | 2.0  | 0.94 (0.42–2.08)       | 0.878 |
| T+C      | 158             | 26.3              | 201               | 30.9 | 0.80 (0.63–1.02)       | 0.076 |
| TT+TC    | 588             | 98.0              | 638               | 98.0 | 1.00                   | 1.00 |
| CC       | 12              | 2.0               | 13                | 2.0  | 1.00 (0.45–2.21)       | 0.997 |
| T allele | 1030            | 85.8              | 1088              | 83.6 | 1.00                   | 1.00 |
| C allele | 170             | 14.2              | 214               | 16.4 | 0.84 (0.67–1.04)       | 0.116 |
| OPG rs2073618 G>C | | | | | | |
| GG       | 345             | 56.6              | 361               | 54.9 | 1.00                   | 1.00 |
| GC       | 222             | 36.4              | 246               | 37.4 | 0.94 (0.75–1.19)       | 0.631 |
| CC       | 43              | 7.0               | 50                | 7.6  | 0.90 (0.58–1.39)       | 0.634 |
| G+C      | 265             | 43.4              | 296               | 45.1 | 0.94 (0.75–1.17)       | 0.564 |
| GG+GC    | 567             | 93.0              | 607               | 92.4 | 1.00                   | 1.00 |
| CC       | 43              | 7.0               | 50                | 7.6  | 0.92 (0.60–1.41)       | 0.703 |
| G allele | 912             | 74.8              | 968               | 73.7 | 1.00                   | 1.00 |
| C allele | 308             | 25.2              | 346               | 26.3 | 0.95 (0.79–1.13)       | 0.533 |
| RANK rs1805034 T>C | | | | | | |
| TT       | 282             | 45.9              | 330               | 50.5 | 1.00                   | 1.00 |
| TC       | 264             | 42.9              | 273               | 41.8 | 1.13 (0.90–1.43)       | 0.296 |
| CC       | 69              | 11.2              | 50                | 7.7  | **1.62 (1.09–2.40)**   | **0.018** |
| T+C      | 333             | 54.1              | 323               | 49.5 | 1.21 (0.97–1.50)       | 0.096 |
| TT+TC    | 546             | 88.8              | 603               | 92.3 | 1.00                   | 1.00 |
| CC       | 69              | 11.2              | 50                | 7.7  | **1.52 (1.04–2.23)**   | **0.031** |
| T allele | 828             | 67.3              | 933               | 71.4 | 1.00                   | 1.00 |
| C allele | 402             | 32.7              | 373               | 28.6 | **1.21 (1.03–1.44)**   | **0.024** |
| RANKL rs9533156 T>C | | | | | | |
| TT       | 175             | 28.5              | 192               | 29.4 | 1.00                   | 1.00 |
| TC       | 305             | 49.6              | 316               | 48.4 | 1.04 (0.78–1.38)       | 0.803 |
| CC       | 135             | 22.0              | 145               | 22.2 | 0.98 (0.72–1.34)       | 0.894 |
| T+C      | 440             | 71.5              | 461               | 70.6 | 1.02 (0.78–1.32)       | 0.913 |
| TT+TC    | 480             | 78.0              | 508               | 77.8 | 1.00                   | 1.00 |
Table 3. Cont.

| Genotype | Cases (n = 629) | Controls (n = 686) | Crude OR (95% CI) | Adjusted ORa (95% CI) |
|----------|----------------|-------------------|------------------|----------------------|
|          | n | % | n | % | p |          | n | % | n | % | p |
| **RANKL** |    |    |    |    |    |          |    |    |    |    |    |
| rs2277438 |    |    |    |    |    |          |    |    |    |    |    |
| A | 277 | 46.2 | 315 | 46.8 | 0.981 | 0.981 | 1.00 | 1.00 | 0.981 | 0.981 |
| G | 352 | 53.8 | 371 | 53.2 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| AG | 259 | 43.2 | 294 | 43.7 | 0.988 | 0.988 | 1.00 | 1.00 | 0.988 | 0.988 |
| AA | 98 | 15.8 | 111 | 16.4 | 0.999 | 0.999 | 1.00 | 1.00 | 0.999 | 0.999 |
| **RANK** |    |    |    |    |    |          |    |    |    |    |    |
| rs1805034 |    |    |    |    |    |          |    |    |    |    |    |
| A | 813 | 67.8 | 924 | 68.6 | 1.00 | 1.00 | 0.993 | 0.993 | 0.993 | 0.993 |
| C | 406 | 32.2 | 402 | 31.4 | 1.00 | 1.00 | 1.04 | 1.04 | 1.04 | 1.04 |
| GC | 381 | 60.6 | 416 | 60.8 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| CC | 232 | 36.8 | 268 | 38.8 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |

aAdjusted for age, sex, smoking status and alcohol consumption.

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Discussion

In this hospital-based case-control study of ESCC, we investigated the association of OPG rs3102735 C>T, OPG rs2073618 G>C, RANK rs1805034 T>C, RANKL rs9533156 T>C and RANKL rs2277438 A>G polymorphisms with risk of ESCC in a Chinese population. Our multivariable logistic analyses demonstrated that RANK rs1805034 T>C genotype has an increased risk of ESCC. Significant association with increased risk of ESCC was noticed among male patients and elder patients (=63 years old). To our knowledge, this is the first study demonstrating a significant association between the RANK rs1805034 T>C genotype with the susceptibility of ESCC.

OPG was initially derived from an expressed sequence tag of a fetal rat intestine cDNA library encoding a 401-amino-acid polypeptide [11]. Subsequently, a physiological role of OPG in the maintenance of normal bone mass was underscored by several studies [11,35,36]. The later finding in murine myelomonocytic cell line 32D led to the identification of OPG binding protein or OPGL, which has identical sequence as RANKL and was further implicated with the osteoclast development [12]. Direct sequencing of a human bone marrow-derived myeloid dendritic cell cDNA library identified RANK as a novel TNFR homologue [13]. Subsequently, RANKL was identified from murine thymoma cell line EL40.5 [13] as well as in T cells [37]. RANKL exists as a homotrimer and induces receptor clustering upon engaging RANK on the cell surface, consequently causes receptor clustering. Activation events within the cell are initiated through TNFR-associated factors following sufficient RANK clustering. Genetic variants in the OPG locus have previously been implicated with osteoporotic fracture [38], bone mineral density [40], osteonecrosis [41], diabetic neuroarthropathy [42] as well as ankylosing spondylitis [43]. Alterations at the RANK locus and/or functionally related genes, such as RANKL, have also been reported to be associated with rheumatoid arthritis [30], aortic calcification [44], bone mineral density [39] and Paget’s disease of bone [31]. Recently, emerging evidence has indicated an association between OPG/RANK/ RANKL gene polymorphisms with carcinogenesis. Several studies demonstrated additional loci to be associated with breast cancer including the chromosomal region 8q24 for OPG gene [45,46]. SNP rs3102735 of the OPG gene has been reported to be associated with the susceptibility of breast cancer in Caucasian population [10]. Similarly, a genetic variant near the 5’-end of RANK (rs7226991) was associated with a breast cancer risk [47]. The mechanism underlying the association remains obscure so far. Yet, vast majority of the association on chromosome 8q24 lies at approximately 128 Mb and is related to various tumor entities in addition to breast cancer, including prostate [48] and colon cancer [49].

Among different ethnic cohorts, the frequencies of genetic polymorphisms vary drastically. Our study demonstrated that the frequency of RANK rs1805034 C was 0.286 among 686 control subjects in Chinese population, which is lower than that of European (0.438) and African American (0.478), but similar with the Japanese population (0.311). However, interestingly, another study reported the frequency of RANK rs1805034 C was 0.476 in Han population from North China, which differs our finding in cohort from East China, suggesting the ethnical impact could also be interfered with regional environmental factors [http://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs = 1805034]. Using Power and Sample Size Calculation (PS, version 3.0.43, 2009, http://biostat.mc.vanderbilt.edu/twiki/bin/view/Main/PowerSampleSize), considering RANK rs1805034 T>C mutant alleles, the power of our analysis
Table 4. Stratified analyses between RANK rs1805034 T>C polymorphism and ESCC risk by sex, age, smoking status and alcohol consumption.

| Variable          | RANK rs1805034 T>C (case/control) * | Adjusted OR b (95% CI); p<sub>h</sub><sup>c</sup> | CC | TC+CC | CC vs. (TT+TC) |
|-------------------|-------------------------------------|---------------------------------------------|----|-------|----------------|
|                   | TT | TC | CC | TC+CC | TT | TC | CC | TC+CC | TT | TC | CC | TC+CC |
| Sex               |    |    |    |       |    |    |    |       |    |    |    |       |
| Male              | 193/227 | 189/178 | 52/33 | 241/211 | 100 | 1.28 (0.86–1.71); p: 0.090; p<sub>h</sub>: 0.221 | 1.89 (1.16–3.08); p: 0.011; p<sub>h</sub>: 0.155 | 1.38 (1.05–1.81); p: 0.022; p<sub>h</sub>: 0.155 | 1.68 (1.05–2.69); p: 0.031; p<sub>h</sub>: 0.448 |
| Female            | 89/103 | 75/95 | 17/17 | 92/112 | 100 | 0.93 (0.62–1.42); p: 0.747; p<sub>h</sub>: 0.155 | 1.22 (0.59–2.55); p: 0.594; p<sub>h</sub>: 0.290 | 0.98 (0.66–1.46); p: 0.906; p<sub>h</sub>: 0.290 | 1.26 (0.62–2.56); p: 0.520; p<sub>h</sub>: 0.448 |
| Age               |    |    |    |       |    |    |    |       |    |    |    |       |
| <63               | 131/161 | 136/152 | 34/28 | 170/180 | 100 | 1.11 (0.79–1.55); p: 0.563; p<sub>h</sub>: 0.664 | 1.48 (0.83–2.61); p: 0.183; p<sub>h</sub>: 0.664 | 1.16 (0.84–1.61); p: 0.360; p<sub>h</sub>: 0.664 | 1.40 (0.81–2.42); p: 0.224; p<sub>h</sub>: 0.702 |
| ≥63               | 151/169 | 128/121 | 35/22 | 163/143 | 100 | 1.18 (0.84–1.65); p: 0.338; p<sub>h</sub>: 0.664 | 1.84 (1.02–3.31); p: 0.041; p<sub>h</sub>: 0.664 | 1.28 (0.93–1.76); p: 0.130; p<sub>h</sub>: 0.664 | 1.71 (0.97–3.03); p: 0.063; p<sub>h</sub>: 0.702 |
| Smoking status    |    |    |    |       |    |    |    |       |    |    |    |       |
| Never             | 159/234 | 149/204 | 36/37 | 185/241 | 100 | 1.06 (0.79–1.43); p: 0.689; p<sub>h</sub>: 0.451 | 1.37 (0.82–2.30); p: 0.228; p<sub>h</sub>: 0.457 | 1.11 (0.84–1.47); p: 0.471; p<sub>h</sub>: 0.358 | 1.33 (0.81–2.19); p: 0.255; p<sub>h</sub>: 0.568 |
| Ever              | 123/96 | 115/69 | 33/13 | 148/82 | 100 | 1.22 (0.81–1.84); p: 0.344; p<sub>h</sub>: 0.451 | 1.89 (0.93–3.84); p: 0.080; p<sub>h</sub>: 0.457 | 1.32 (0.90–1.95); p: 0.157; p<sub>h</sub>: 0.358 | 1.73 (0.87–3.43); p: 0.019; p<sub>h</sub>: 0.568 |
| Alcohol consumption |    |    |    |       |    |    |    |       |    |    |    |       |
| Never             | 190/248 | 180/215 | 45/38 | 225/253 | 100 | 1.09 (0.82–1.49); p: 0.551; p<sub>h</sub>: 0.354 | 1.52 (0.93–2.48); p: 0.095; p<sub>h</sub>: 0.753 | 1.16 (0.88–1.51); p: 0.298; p<sub>h</sub>: 0.504 | 1.46 (0.91–2.33); p: 0.117; p<sub>h</sub>: 0.871 |
| Ever              | 92/82 | 84/58 | 24/12 | 108/70 | 100 | 1.16 (0.73–1.84); p: 0.530; p<sub>h</sub>: 0.753 | 1.65 (0.76–3.60); p: 0.210; p<sub>h</sub>: 0.753 | 1.24 (0.80–1.92); p: 0.332; p<sub>h</sub>: 0.753 | 1.54 (0.73–3.28); p: 0.259; p<sub>h</sub>: 0.871 |

*The genotyping was successful in 615 (97.8%) ESCC cases, and 653 (95.2%) controls for RANK rs1805034 T>C;

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

cp<sub>h</sub> for heterogeneity.

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(\(\alpha = 0.05\)) was 0.946 in 629 ESCC cases and 686 control subjects with adjusted OR 1.52. In male subgroup, the power of our analysis was 0.995 among 434 cases and 438 control subjects, with the adjusted OR 1.89. In elder cohort (\(\geq 65\)), the power of analysis (\(\alpha = 0.05\)) was 0.962 among 314 cases and 312 controls with adjusted OR 1.8. The current study has highlighted the increased risk of ESCC among male patients and elder patients (\(\geq 63\) years old), which was in consistent with the previous report. In a retrospective study involving 74,854 ESCC patients from North China, the prevalence among males was higher than that among females, similar to our findings. Moreover, this study demonstrated that although the prevalence significantly declined, the median age-of-onset of ESCC postponed \([50]\), verifying our notion that elder population has higher risk.

In conclusion, our study provides with the evidence that functional polymorphism of \(\text{RANK}\) rs1805034 \(\text{T}\rightarrow\text{C}\) is associated with the susceptibility of ESCC. We acknowledge there are several limitations in this study that need to be addressed. First of all, the study subjects were all recruited from several local medical centers within same area, which may not completely represent the general Chinese population, especially when diverse regional environmental factors exist. Secondly, the detailed information regarding cancer metastasis and survival were not provided as the follow-up study is still ongoing, which hinders further analyses of the impact of these SNP polymorphisms on the ESCC progression and prognosis. Lastly, as the epidemiologic complexities of esophageal cancer are vast, rendering screening and prevention limited at best. The association between nutrition factors, exposure to fungal toxins or N-nitroso-compound in food and risk of ESCC is not studied. Further studies among different regions or ethnic populations with diverse nutrition conditions, and supplemented with functional analyses, are warranted to verify our findings.

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**Author Contributions**

Conceived and designed the experiments: JY SC HG. Performed the experiments: LW WT XW LL AS YS. Analyzed the data: JY WT GD HG. Contributed reagents/materials/analysis tools: GD SC HG. Wrote the paper: JY LW HG.

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