Association of Esophageal Squamous Cell Carcinoma With the Interaction Between Poor Oral Health and Single Nucleotide Polymorphisms in Regulating Cell Cycles and Angiogenesis: A Case-Control Study in High-Incidence Chinese

Renjia Zhao¹,²,* Xiaoxiao Li³,⁴,* Xiaorong Yang⁵, Tiejun Zhang¹,², Ming Lu⁴,⁵, Weimin Ye⁵,⁶, Li Jin³,⁴,⁷, Chen Suo¹,²,⁴,* and Xingdong Chen³,⁴,⁷,*

Abstract

Introduction: Oral health and genetic factors can independently influence the risk of developing esophageal squamous cell carcinoma (ESCC).

Objectives: The primary objective of this study was to investigate the interactive effects of oral health and genetic factors on ESCC risk.

Methods: This was a matched case-control study with 927 ESCC patients and 1701 matched controls. We selected 101 candidate single nucleotide polymorphisms (SNPs) from 59 genes that were associated with ESCC. Oral health was assessed based on tooth-brushing frequency, tooth loss, and age at the time of first tooth loss. An unconditional logistic regression model was employed in which SNP–oral health interactions were assessed as risk factors for ESCC, after adjusting for age and sex. A genetic risk score (GRS) analysis was conducted.

Results: The association between GRS and ESCC and the synergistic effect of GRS and oral health on ESCC were examined. Daily frequency of tooth-brushing was found to interact with 5 SNPs, rs3765524, rs753724, rs994771, rs3781264, and rs11187842, to increase the risk of ESCC. In particular, individuals with genotype TT of rs3765524 who brushed their teeth less than twice a day had a 5.13-times higher risk of ESCC than those with genotype CC who brushed their teeth at least twice a day. Furthermore, tooth loss interacted with two SNPs: rs1159918 from ADH1B and rs3813867 from CYP2E1.

¹Department of Epidemiology, School of Public Health, Fudan University, Shanghai, China
²Key Laboratory of Public Health Safety, Ministry of Education, Fudan University, Shanghai, China
³State Key Laboratory of Genetic Engineering and Collaborative Innovation Center for Genetics and Development, School of Life Sciences, Fudan University, Shanghai, China
⁴Institute of Health Sciences, Fudan University Taizhou, Taizhou, China
⁵Clinical Epidemiology Unit, Qilu Hospital, Shandong University, Jinan, China
⁶Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden
⁷Human Phenome Institute, Fudan University, Shanghai, China

*Contributed equally to this work.

Corresponding Authors:
Chen Suo, Department of Epidemiology, School of Public Health, Fudan University, Shanghai 200433, China.
Email: suochen@fudan.edu.cn

Xingdong Chen, State Key Laboratory of Genetic Engineering and Collaborative Innovation Center for Genetics and Development, School of Life Sciences, Fudan University, Shanghai 200433, China.
Email: xingdongchen@fudan.edu.cn

Creative Commons Non Commercial CC BY-NC: This article is distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 License (https://creativecommons.org/licenses/by-nc/4.0/) which permits non-commercial use, reproduction and distribution of the work without further permission provided the original work is attributed as specified on the SAGE and Open Access pages (https://us.sagepub.com/en-us/nam/open-access-at-sage).
Conclusion: Oral health may interact with genetic factors increasing ESCC risk, which provides new insights into the relationship between ESCC and gene–lifestyle interactions which can be used for disease prevention.

Keywords
esophageal squamous cell carcinoma, single nucleotide polymorphism, interaction effect, oral hygiene, genetic risk score

Introduction
According to Global Cancer Statistics 2018, there were 572,000 new esophageal cancer cases worldwide that year, ranking seventh among all cancers. China is among the top 5 countries with the highest incidence of esophageal cancer. In particular, Linxian, Henan Province, and Cixian and Shexian, Hebei Province has the highest incidence rates in the world. Furthermore, in Taixing, Jiangsu Province, esophageal cancer has the highest morbidity rate among all cancers. More than 90% of the esophageal cancers that are detected are diagnosed as esophageal squamous cell carcinoma (ESCC). Currently, the risk factors associated with ESCC include single nucleotide polymorphisms (SNPs) and lifestyle factors such as alcohol consumption, smoking/tobacco consumption and poor oral health care.

Poor oral health is an established risk factor for ESCC. Furthermore, problems associated with poor oral health, including tooth decay and missing and filled teeth may contribute to ESCC. Chen et al found that the loss of more than 6 teeth significantly increased the risk of ESCC compared with no tooth loss (more than 6 teeth lost vs. none, OR = 1.48, 95% CI 1.04–2.11). And tooth-brushing once or less per day, which represents poor oral hygiene to some extent, compared with tooth-brushing twice or more per day, was associated with a 1.81-fold increase in the risk of ESCC. Regular care at home, such as teeth and tongue cleaning, can reduce the growth of specific microbes in the oral cavity, thereby reducing the risk of esophageal cancer.

ESCC has been found to have genetic basis. For example, SNPs in the PCLE1 gene have been significantly associated with esophageal cancer in Asian populations. Research has also shown that alcohol interacts with functional genetic polymorphisms of aldehyde dehydrogenase (ALDH2) and alcohol dehydrogenase (ADH) and increases the risk of ESCC. With regard to head and neck cancer, the interplay between gene polymorphisms and oral health has been found to increase cancer risk. However, with regard to ESCC, the interaction between SNPs and oral health and their effect on its occurrence remain unclear.

Taixing has one of the highest incidence rates of esophageal cancer in China. A large population case-control study was conducted in Taiwan, in which biological samples and data on specific lifestyle habits, such as oral health, smoking, and drinking, were obtained from ESCC patients and controls. These findings indicate that poor oral health increases the risk of ESCC. In this study, we intend to explore whether interactions between genetic variations and oral health may influence ESCC risk.

Materials and Methods
Study Design
We performed a case-control study in Taixing, China and over 90% of the esophageal cancer cases were from four major hospitals.

We attempted to enroll all individuals with incident esophageal cancer (i.e., patients with esophageal cancer that was newly diagnosed between October 2010 and September 2013). A total of 1681 patients were recruited for the study, including 1401 from the four hospitals and 280 additional patients from the Taixing Cancer Registry. After biopsy samples and pathological reports were assessed by pathologists, 1499 patients with esophageal cancer were identified. This group was further screened according to the following inclusion criteria: age between 40 and 85 years, residence in Taixing for at least 5 years, availability of blood samples, call rate ≥ 0.9, willingness to complete the questionnaire and undergo clinical examination, and identification of the pathological type of ESCC. Based on these criteria, 927 patients with esophageal cancer were selected for this study. The patient selection process is illustrated in Figure 1.

A total of 3501 healthy controls were randomly selected from the same study region as the ESCC patients and were frequency-matched with the patient group based on sex and age (spanning 5 years). In order to improve the response rate and participation rate, the local government called on people to take an active part in the research, and we provided a basic physical examinations and certain rewards like umbrellas for participants. We ensured that the controls represented every town of Taixing. Excluding deaths and immigrant populations, 2858 controls remained. Excluding the lost follow-up population, 2011 controls remained. Excluding the population older than 85 years, 1992 people remained. Excluding those whose call rate was less than 0.9, 1701 individuals were finally included in the control group.

All recruited individuals gave their written informed consent and volunteer for scientific research. And we have identified all patient details.
Single Nucleotide Polymorphism Genotyping and Screening

A total of 101 SNPs from 59 genes were selected for genotyping; they were selected on the basis of having been previously reported to associate with ESCC in candidate or GWAS studies. Finally, our primary genes of study are those in the alcohol metabolism pathways, N-Acetyltransferase2 (NAT2), Phospholipase C Epsilon 1 (PLCE1), X-Ray Repair Complementing 1 (XRCC1) and Flagellum Associated Coiled-Coil Domains 1 (FLACC1). The detailed SNP information was in Supplementary Table 1.18 The SNPs were genotyped using a three-round multiplex polymerase chain reaction procedure with next-generation sequencing method, specific primers were designed for the sites to be detected, and multiple PCR amplification was performed in a single tube. Different samples were differentiated by different Barcode primers. After mixing the samples, high-throughput amplicon sequencing was performed on Illumina platform.20,21 We use the All SNPs had a call rate of at least 0.90 in all samples and had Hardy-Weinberg equilibrium according to chi-square tests, with p values of 0.05 or higher in the controls. To ensure genotyping accuracy, we also implemented quality control procedures, such as by including negative controls. In addition, a randomly selected 8% of total samples were genotyped twice and the consistency was higher than 98%. The average sequencing depth was 1225x. All SNPs had a minor allele frequency of 0.1 or more in both the case patient and control samples, rendering adequate statistical power. Among the 101 SNPs, 4 SNPs were monozygotic, 14 did not reach Hardy–Weinberg equilibrium, and 5 had a missing rate of >10%. The remaining 78 SNPs were included in the subsequent analysis. We established four genetic models: the codominant model, over-dominant model, dominant model, and recessive model. All SNPs was corrected by Bonferroni test. Each of the 78 SNPs were entered into the most significant model among the four models for analyzing its relationship with ESCC.

Oral Health Assessment and Quality Control

Participants were interviewed face-to-face with structured questionnaires, and information on basic characteristics was
Table 1. Basic Characteristics of the Case Patients with ESCC and Controls in a Population-Based Case-Control Study in Taixing, People’s Republic of China (n = 2628).

| Characteristic, n (%) | Control (n = 1701) | Case (n = 927) | P-Value |
|-----------------------|--------------------|----------------|---------|
| Age (mean (SD))       | 66.28 (8.80)       | 66.70 (8.62)   | .239    |
| Age                   |                     |                | .169    |
| 40–49                 | 70 (4.1)           | 29 (3.1)       |         |
| 50–59                 | 310 (18.2)         | 156 (16.8)     |         |
| 60–69                 | 676 (39.7)         | 396 (42.7)     |         |
| 70–79                 | 554 (32.6)         | 283 (30.5)     |         |
| 80–85                 | 91 (5.3)           | 63 (6.8)       |         |
| Sex = Female          | 524 (30.8)         | 305 (32.9)     | .289    |
| Smoking               |                     |                | < .001  |
| Never                 | 762 (44.8)         | 390 (42.1)     |         |
| Quitted               | 138 (8.1)          | 45 (4.9)       |         |
| Smoking               | 793 (46.6)         | 477 (51.5)     |         |
| Missing               | 8 (0.5)            | 15 (1.6)       |         |
| Alcohol consumption   |                     |                | < .001  |
| Never                 | 983 (57.8)         | 420 (45.3)     |         |
| Quitted               | 68 (4.0)           | 22 (2.4)       |         |
| Drinking              | 640 (37.6)         | 468 (50.5)     |         |
| Missing               | 10 (0.6)           | 17 (1.8)       |         |
| Education level       |                     |                | < .001  |
| Illiteracy            | 465 (27.3)         | 338 (36.5)     |         |
| Primary school        | 647 (38.0)         | 342 (36.9)     |         |
| Junior school         | 449 (26.4)         | 191 (20.6)     |         |
| High school and above | 140 (8.2)          | 56 (6.0)       |         |
| Marriage status       |                     |                | .06     |
| Unmarried             | 58 (3.4)           | 37 (4.0)       |         |
| Married               | 1361 (80.0)        | 705 (76.1)     |         |
| Divorce/Widow         | 282 (16.6)         | 185 (20.0)     |         |
| Wealth score          |                     |                | < .001  |
| Q1                    | 339 (19.9)         | 280 (30.2)     |         |
| Q2                    | 312 (18.3)         | 162 (17.5)     |         |
| Q3                    | 363 (21.3)         | 222 (23.9)     |         |
| Q4                    | 375 (22.0)         | 177 (19.1)     |         |
| Q5                    | 312 (18.3)         | 86 (9.3)       |         |
| Family history of ESCC|                     |                | < .001  |
| No                    | 1393 (81.9)        | 620 (66.9)     |         |
| Yes                   | 305 (17.9)         | 300 (32.4)     |         |
| Missing               | 3 (0.2)            | 7 (0.8)        |         |
| Tea drinking          |                     |                | < .001  |
| No                    | 1229 (72.3)        | 616 (66.5)     |         |
| Yes                   | 462 (27.2)         | 294 (31.7)     |         |
| Missing               | 10 (0.6)           | 17 (1.8)       |         |
| Fruit taking (g)      |                     |                | .106    |
| <25                   | 819 (48.1)         | 478 (51.6)     |         |
| ≥25                   | 835 (49.1)         | 417 (45.0)     |         |
| Missing               | 47 (2.8)           | 32 (3.5)       |         |
| No teeth loss         | 443 (26.0)         | 192 (20.7)     | .003    |
| Age of first occurred tooth loss (mean (SD)) | 50.48 (12.98) | 52.50 (13.07) | .001    |
| Times of brushing teeth daily | | | < .001 |
| <2                    | 504 (29.6)         | 148 (16.0)     |         |
| ≥2                    | 1083 (63.7)        | 738 (79.6)     |         |
| Missing               | 114 (6.7)          | 41 (4.4)       |         |
collected, including age, sex, smoking, alcohol consumption status, education, wealth, marital status, family history of esophageal cancer, hot tea consumption (According to the time between placing tea leaves mixed with boiling water and tea drinking, the temperature of tea drinking was classified as hot/yes (1–5 minutes) and warm/no (more than 5 minutes)), fruit consumption, and oral health information which included how many times a day a person brushes their teeth, tooth loss, and the age at which the first tooth loss occurred. The whole process was recorded by audio to facilitate quality control. In addition, we used the double-entry method by also entering the questionnaire into the electronic system and comparing the input results, and we also checked the questionnaires for logical mistakes.

Figure 2. The $-\log P$ values of the 78 SNPs of the ESCC correlation test in four genetic models in which the “daily toothbrushing times” were adjusted by adding covariates as oral health indicators. Red line: Bonferroni correction line; Pink line: .05 $P$ value line.
Statistical Analysis

Logistic regression models were established by adjusting for age, sex, smoking status, alcohol consumption, wealth score (Family wealth score was calculated based on the ownership of valuable home items using a multiple correspondence analysis. These scores were categorized as quintiles according to the observed coordinates among control participants, like cars, vacuum cleaner, washing machine et al. family history, tea consumption, and daily consumption of fresh fruits. Using “daily frequency of tooth brushing” as an example, we build two models as follows:

\[ y_0 = \beta_{00} + \beta_{01} \cdot brush\_times + \beta_{02} \cdot SNP + \epsilon_0 \]  

(1)
The likelihood statistic chi-square from the two regression models was used to determine whether the interaction term was significant. The models for the addition and multiplication are separately justified. The odds ratio (OR) and 95% confidence interval (CI) was calculated for each subgroup, defined by toothbrushing frequency and genotype.

We conducted a genetic risk score (GRS) analysis to summarize the cumulative effects of SNPs on ESCC. The genotypes were coded as 0, 1, and 2: 0 = homozygous for the wild allele, 1 = heterozygous, and 2 = homozygous for the mutant allele. The following formula was used to calculate GRS

\[
GRS = \sum_{i=1}^{I} \omega_{ORi} \cdot G_i
\]

where, \(i\) is the SNP, \(\omega_{ORi}\) = weight for SNP \(i\), and \(G_i\) = number of risk alleles (0, 1 or 2). GRS represents the comprehensive genetic effect for each subject. We stratiﬁed the participants into 4 groups based on tooth loss status and daily frequency of tooth-brushing. Group one represented people who brushed their teeth at least twice daily and have not experienced tooth loss; group 2 represented people who brushed their teeth at least twice daily but with tooth loss experience; group 3 represented people who brushed their teeth at most once daily but without tooth loss experience, and group 4 represented people who brushed their teeth at most once daily and with tooth loss experience. Subsequently, based on the GRS value, the participants within each of the 4 groups were further categorized into low (GRS ≤ 2), medium (2 < GRS ≤ 5), and high (GRS > 5) risk groups for convenient interpretation.

The reporting of this study conforms to STROBE guidelines.

**Results**

There were significant differences between the cases and controls with regard to several factors, including smoking, alcohol consumption, education level, wealth index, family history of esophageal cancer, and tea consumption. These factors were adjusted for confounders in the multivariable models. Notably, oral health-related indicators, such as tooth loss, age at the time of ﬁrst tooth loss, and daily frequency of tooth-brushing, varied signiﬁcantly between cases and controls (Table 1).

We analyzed the relationship between the 78 SNPs and ESCC in the four inheritance models: the co-dominant, dominant, recessive, and over-dominant models, which were adjusted for confounders. The ﬁndings showed that 45 SNPs were statistically signiﬁcant; therefore, they were selected for further analysis. Figures 2 and 3 and showed the distribution of SNPs’ model in different covariates.

| Oral hygiene | SNP loci | Genotype | Cases, n | Controls, n | OR (95% CI) | P-value for trend | aOR (95% CI) | P-value for trend |
|--------------|----------|----------|----------|-------------|-------------|------------------|--------------|-----------------|
| Good status  | rs11187842 | CC       | 85       | 374         | 1.00        | 1.00             | 4.75 < .001   | 3.31            |
| Poor status  |          | CC       | 474      | 765         | 2.72 (2.11–3.56) | 2.55 (1.93–3.83) |
| Good status  |          | CT/TT    | 62       | 127         | 2.14 (1.46–3.15) | 2.15 (1.43–3.22) |
| Poor status  |          | CT/TT    | 261      | 308         | 3.73 (2.81–4.99) | < .001         | 3.45 (2.55–4.70) | < .001 |
| Good status  | rs3781264 | TT       | 85       | 359         | 1.00        | 1.00             | 4.70 < .001   | 3.23            |
| Poor status  |          | TT       | 465      | 748         | 2.63 (2.03–3.43) | 2.43 (1.84–3.23) |
| Good status  |          | CT/CC    | 63       | 126         | 2.11 (2.02–3.43) | 2.10 (1.40–3.14) |
| Poor status  |          | CT/CC    | 263      | 316         | 3.51 (2.64–4.70) | < .001         | 3.21 (2.37–4.38) | < .001 |
| Good status  | rs994771 | CC/TT    | 80       | 290         | 1.00        | 1.00             | 4.38 < .001   | 3.15            |
| Poor status  |          | CC/TT    | 450      | 581         | 2.81 (2.14–3.72) | 2.60 (1.95–3.51) |
| Good status  |          | CT       | 68       | 209         | 1.17 (0.81–1.70) | 1.16 (0.78–1.71) |
| Poor status  |          | CT       | 268      | 490         | 2.12 (1.59–2.83) | < .001         | 1.91 (1.41–2.61) | < .001 |
| Good status  | rs3765524 | CC       | 67       | 324         | 1.00        | 1.00             | 4.70 < .001   | 3.83            |
| Poor status  |          | CC       | 389      | 664         | 2.83 (2.13–3.82) | 2.75 (2.03–3.78) |
| Good status  |          | CT       | 68       | 164         | 2.00 (1.36–2.95) | 2.13 (1.42–3.20) |
| Poor status  |          | CT       | 289      | 357         | 3.91 (2.89–5.34) | 3.72 (2.70–5.17) |
| Good status  |          | TT       | 12       | 11          | 5.27 (3.21–8.24) | 5.66 (2.31–13.98) |
| Poor status  |          | TT       | 52       | 49          | 5.13 (3.21–8.25) | < .001         | 4.88 (2.95–8.13) | < .001 |
| Good status  | rs753724 | GG       | 84       | 372         | 1.00        | 1.00             | 4.70 < .001   | 3.83            |
| Poor status  |          | GG       | 472      | 755         | 2.76 (2.14–3.62) | 2.58 (1.96–3.43) |
| Good status  |          | GT/TT    | 63       | 128         | 2.18 (1.48–3.20) | 2.21 (1.47–3.31) |
| Poor status  |          | GT/TT    | 255      | 302         | 3.74 (2.80–5.01) | < .001         | 3.48 (2.56–4.75) | < .001 |
Both tooth loss status and daily frequency of tooth brushing exhibit significant interactions with specific SNPs that affect ESCC risk. Detailed results are presented in Table 2. When the daily tooth brushing frequency was used as an indicator of oral health, it was found to interact with 5 SNPs, rs3765524, rs753724, rs3781264, and rs11187842 from the PLCE1 gene on chromosome 10, as well as rs994771 from the LOC102723576 gene on chromosome 4. The linkage disequilibrium value for the four SNPs on PLCE1 was below 0.4. Additionally, tooth loss was found to interact with two SNPs to affect ESCC risk: rs1159918 in the ADH1B gene of chromosome 4 and rs3813867 in the CYP2E1 gene of chromosome 10.

The findings revealed that a total of seven SNPs interacted with oral health to increase the risk of ESCC. The individuals who brushed their teeth at least twice daily and carried the ancestral allele were regarded as the reference group. In the individuals who carried the CC genotype for rs11187842 and brushed their teeth once a day at the most, the risk of ESCC was more than double (OR = 2.55, 95% CI: 1.93–3.83) than those who carried the CC genotype and brushed their teeth at least twice a day. For those with the CT/TT genotype for rs11187842, those who brushed their teeth once a day at most had a more than 3-fold risk of ESCC (OR = 3.45, 95% CI: 2.55–4.70) than those who brushed their teeth at least twice a day; and for those with the CT/TT genotype for rs3781264, those who brushed their teeth once a day at most had a more than 3-fold risk of ESCC (OR = 2.55, 95% CI: 1.93–3.83) than those who carried the TT genotype and brushed their teeth at least twice a day. Among the individuals with the GG genotype for rs753724, the ESCC risk was more than double (OR = 2.58, 95% CI: 1.96–3.43) in the individuals who brushed their teeth once a day at most than in those who brushed at least twice a day. Individuals with the GT/TT genotype for rs753724 who brushed their teeth once a day at most had a more than 3-times greater risk of ESCC (OR = 3.48, 95% CI: 2.56–4.75) than those who brushed at least twice a day. In tooth lost, we found that rs1159918 TT with tooth lost could increase 2.15-fold risk on ESCC (OR = 2.15, 95% CI: 1.26–3.86). Moreover, People who had tooth lost in rs3813867 GC had nearly 1.10-fold risk on ESCC than CC/GG with no tooth lost (OR = 1.09, 95% CI: 1.04–1.33).

Figure 4. Genetic risk score (GRS) analysis on the seven SNPs (5 SNPs interacting with daily tooth-brushing frequency and 2 SNPs interacting with tooth loss). Group 1 represented people who brushed their teeth at least twice daily and without tooth loss experience, group 2 represented people who brushed their teeth at least twice daily but with tooth loss experience, group 3 represented people who brushed their teeth at most once daily but without tooth loss experience, and group 4 represented people who brushed their teeth at most once daily and with tooth loss experience. Individuals with higher GRS, brushing teeth at most once a day or suffering from tooth loss had a higher risk of ESCC.
GRS values were calculated to summarize the effect of the seven SNPs for further analysis. The association of GRS and oral health conditions with the risk of ESCC is shown in Figure 4. Participants with GRS ≤ 1 were considered as the reference group for all comparisons. Overall, individuals with higher GRS had a higher risk of ESCC among the four groups that were defined by daily toothbrushing frequency and tooth loss status. Overall, the values indicate that brushing teeth at least twice a day and not having a tooth loss experience are associated with limited ESCC risk, even in individuals with high GRS. In contrast, among individuals with high GRS, those who brush their teeth less than twice a day and have tooth loss have more than 4-fold risk of ESCC.

Discussion

We analyzed all the collected oral health indicators and firstly found that two indicators interact with SNPs to increases ESCC risk, namely, daily frequency of tooth brushing and tooth loss. The age of tooth loss showed no significant interaction effect, so we removed it from analysis. Additionally, we identified five SNPs that interact with daily tooth brushing frequency to increase the ESCC risk. Four of the five SNPs are located in the same protein-coding gene PLCE1, which belongs to one of the phospholipase families of enzymes that regulate cell growth, differentiation, apoptosis, and angiogenesis.26 A previous study found that the epigenetically upregulated oncoprotein PLCE1 drives esophageal carcinoma angiogenesis and proliferation via activating the PI-PLC-κB signaling pathway and VEGF-C/Bcl-2 expression.27 Moreover, putatively functional PLCE1 variants and their associated susceptibility might contribute to the risk of ESCC.28 The other SNP, rs994771, is from the LOC102723576 gene on chromosome 10. This is the first study to report an interaction between oral health and LOC102723576 in relation to the risk of esophageal cancer.

We also conducted a GRS analysis on the seven SNPs to explore their cumulative interaction with oral health. We found that individuals with higher GRS have higher ESCC risk, and this association is especially prominent in individuals who brush their teeth less than twice a day and have experienced tooth loss. These findings indicate an obvious interaction between SNPs and oral hygiene that increases the risk of ESCC.

Possible mechanisms with the interaction between those SNPs and oral hygiene on ESCC is still unclear. Because of the fact that oral problems, including tooth decay and missing and filled teeth,13 may be caused by lack of oral care,9 which may lead to undesirable changes in the oral microbiota and subsequently contribute to ESCC.12 And we speculate that the possible mechanisms may involve inflammation, carcinogenic microbial metabolites, or oral microbes, which have yet to be explored.12,29-31

This is the first relevant report on the association between the LOC102723576 SNP rs994771 and esophageal cancer. However, further research is needed to explore the possible relationships between the two and the underlying mechanisms. Our study highlights the synergistic effect of gene and lifestyle factors on ESCC risk, and provides new insights into the prevention and control of ESCC. The findings indicate the importance of practicing good dental hygiene, especially in populations with a high genetic risk. This study also has some limitations. All the oral health indicators are categorical variables, and more detailed results cannot be obtained on the basis of quantitative analysis. Another potential limitation is that decayed and missing teeth, as well as periodontal health were not assessed. This study was a case-control study, which cause lower validation power in cause of disease compared with prospective cohort studies, and this conclusion needs to be verified in further research. Case-control studies are retrospective studies with a certain recall bias in data collection. To control recall bias, we tried to select new patients and controls with high compliance. And objective indexes and scientific methods were used to reduce recall deviation when collecting information.

Conclusion

In this study, we verified that tooth loss and tooth brushing were independent risk factors for ESCC. More importantly, we first identified seven SNPs that may interact with these two oral health indicators to increase ESCC risk, providing an important basis for the accurate prevention and control of ESCC, especially in populations with high-risk genetic variants. Further prospective studies are needed to verify this conclusion and explore the possible mechanisms.

Acknowledgments

We would like to thank the interviewers and technicians of Fudan-Taizhou Institute of Health Sciences for their invaluable contribution to the data collection and sample preparation, the staff of Taixing Center for Disease Control and Prevention for their help in the fieldwork, and the staff of Taixing People’s Hospital for their assistance with sample collection. We would also like to thank Editage (www.editage.com) for English language editing.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by the National Natural Science Foundation of China [grant numbers 91846302, 81502870]; the Innovation Grant from the Science and Technology Commission of Shanghai Municipality, China [grant number 20ZR1405600], and the Three-Year Action Plan for Strengthening Public Health System in Shanghai [grant number GWV-10.2-YQ32].
**Ethical Approval**

All procedures in this study were conducted in accordance with the Ethics Committee, School of Life Sciences, Fudan University approved protocols (approval number: KYLL-2018(KS)-204).

**Statement of Human and Animal Rights**

All procedures in this study were conducted in accordance with the Ethics Committee, School of Life Sciences, Fudan University approved protocols.

**Statement of Informed Consent**

Verbal informed consent was obtained from a legally authorized representative(s) for anonymized patient information to be published in this article.

**ORCID iD**

Chen Suo 🐣 https://orcid.org/0000-0002-5274-4584

**Supplemental Material**

Supplemental material for this article is available online.

**References**

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: a cancer journal for clinicians*. 2018;116(6):394-424. doi:10.3322/caac.21492.
2. Lin Y, Totsuka Y, Shan B, et al. Esophageal cancer in high-risk areas of China: Research progress and challenges. *Annals of Epidemiology*. 2017;27(3):215-221. doi:10.1016/j.annepidem.2016.11.004.
3. Zhou YC, Chen TT, Chen XD, et al. Trend Analysis in Incidence Rates of Digestive System Cancers in Taixing, 2006-2010. *Chinese Journal of Prevention & Control of Chronic Diseases* 2012;20(06):635-637. doi:10.16386/j.cjpcdc.issn.1004-6194.2012.06.044.
4. Enzinger PC, Mayer RJ. Esophageal cancer. *N Engl J Med*. 2003;349(23):2241-2252. doi:10.1056/NEJMra035010.
5. Burki TK. Mutational subgroups for oesophageal adenocarcinoma. *Lancet Oncol*. 2016;17(10):e426. doi:10.1016/S1470-2045(16)30449-1.
6. Yang X, Chen X, Zhuang M, et al. Smoking and alcohol drinking in relation to the risk of esophageal squamous cell carcinoma: A population-based case-control study in China. *Sci Rep*. 2017;7(1):17249. doi:10.1038/s41598-017-17617-2.
7. Najafi Farid. Tobacco Smoking and Alcohol Drinking: Two Clinically Significant Risk Factors for Esophageal Squamous Cell Carcinoma. *Gastroenterology*. 2019;157(3):897. doi:10.1053/j.gastro.2019.04.054.
8. Yaegashi Y, Onoda T, Morioka S, et al. Joint effects of smoking and alcohol drinking on esophageal cancer mortality in Japanese men: findings from the Japan collaborative cohort study. *Asian Pac J Cancer Prev* 2014;15(2):1023-1029.
9. Chen X, Yuan Z, Lu M, Zhang Y, Jin L, Ye W. Poor oral health is associated with an increased risk of esophageal squamous cell carcinoma - a population-based case-control study in China. *Int J Cancer*. 2017;140(3):2626-635. doi:10.1002/ijc.30484.
10. Sepehr A, Kamangar F, Fahimi S, Saidi F, Abnet CC, Dawsey SM. Poor oral health as a risk factor for esophageal squamous dysplasia in northeastern Iran. *Anticancer Res*. 2005;25(1B):543-546.
11. Abnet CC, Kamangar F, Islami F, et al. Tooth loss and lack of regular oral hygiene are associated with higher risk of esophageal squamous cell carcinoma. *Cancer Epidemiol Biomarkers Prev*. 2008;17(11):3062-3068. doi:10.1158/1055-9965.EPI-08-0558.
12. Chen X, Winckler B, Lu M, et al. Oral microbiota and risk for esophageal squamous cell carcinoma in a high-risk area of China. *PLoS One*. 2015;10(12):e0143603. doi:10.1371/journal.pone.0143603.
13. Abnet Christian C, Kamangar Farin, Islami Farhad, Nasrollahzadeh Dariush, Brennan Paul, Aghcheli Karim, Merat Shahn, Poursams Akram, Marjani Haj Amin, Ebadati Abdolhakim, Sotoudeh Masoud, Boffetta Paolo, Malezkadeh Reza, Dawsrey Sanford M. Tooth loss and lack of regular oral hygiene are associated with higher risk of esophageal squamous cell carcinoma. *Cancer Epidemiol Biomarkers Prev*. 2008;17(11):3062-8. doi:10.1158/1055-9965.EPI-08-0558.
14. Hiratsuka VY, Robinson JM, Greenlee R, Refaat A. Oral health beliefs and oral hygiene behaviours among parents of urban Alaska Native children. *Int J Circumpolar Health*. 2019;78(1):1586274. doi:10.1080/22423982.2019.1586274.
15. Tanda N, Washio J, Kamei T, Akazawa K, Takahashi N, Koseki T. Professional oral care reduces carcinogenic acetaldehyde levels in mouth air of perioperative esophageal cancer patients: A prospective comparative study. *Tohoku J Exp Med*. 2019;249(1):75-83. doi:10.1620/tjem.249.75.
16. Yu C, Chen K, Zheng H, et al. Overexpression of astrocyte elevated gene-1 (AEG-1) is associated with esophageal squamous cell carcinoma (ESCC) progression and pathogenesis. *Carcinogenesis*. 2009;30(5):894-901. doi:10.1093/carcin/bgp064.
17. Guo L-Y, Yang N, Hu D, et al. PLCE1 rs2274223 polymorphism and susceptibility to esophageal cancer: a meta-analysis. *Asian Pac J Cancer Prev*. 2014;15(21):9107-9112.
18. Suo C, Yang Y, Yuan Z, et al. Alcohol Intake Interacts with Functional Genetic Polymorphisms of Aldehyde Dehydrogenase (ALDH2) and Alcohol Dehydrogenase (ADH) to Increase Esophageal Squamous Cell Carcinoma Risk. *Cancer Res*. 2014;74(20):5572-5579. doi:10.1158/0008-5472.CAN-14-0955.
19. Tsai ST, Wong TY, Ou CY, et al. The interplay between alcohol consumption, oral hygiene, ALDH2 and ADH1B in the risk of head and neck cancer. *Int J Cancer*. 2014;135(10):2424-2436. doi:10.1002/ijc.28885.
20. Chen Z-J, Zhao H, He L, et al. Genome-wide association study identifies susceptibility loci for polycystic ovary syndrome on chromosome 2p16.3, 2p21 and 9q33.3. *Nat Genet*. 2011;43(1):55-59. doi:10.1038/ng.732.
21. Chen Ke, Zhou Yu-Xun, Li Kai, Qi Li-Xin, Zhang Qi-Fei, Wang Mao-Chun, Xiao Jun-Hua. A novel three-round multiplex PCR for SNP genotyping with next generation sequencing. *Anal
Yang X, Ni Y, Yuan Z, et al. Very hot tea drinking increases esophageal squamous cell carcinoma risk in a high-risk area of China: a population-based case-control study. *Clin Epidemiol* 2018;10:1307-1320. doi:10.2147/CLEP.S171615.

23. Humphries SE, Yiannakouris N, Talmud PJ. Cardiovascular disease risk prediction using genetic information (gene scores): is it really informative? *Curr Opin Lipidol*. 2008;19(2):128-132. doi:10.1097/MOL.0b013e3282f5283e.

24. De Jager PL, Chibnik LB, Cui J, et al. Integration of genetic risk factors into a clinical algorithm for multiple sclerosis susceptibility: A weighted genetic risk score. *Lancet Neurol*. 2009;8(12):1111-1119. doi:10.1016/S1474-4422(09)70275-3.

25. von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. *Ann Intern Med*. 2007;147(8):573-577.

26. Wang L-D, Zhou F-Y, Li X-M, et al. Genome-wide association study of esophageal squamous cell carcinoma in Chinese subjects identifies susceptibility loci at PLCE1 and C20orf54. *Nat Genet*. 2010;42(9):759-763. doi:10.1038/ng.648.

27. Chen Y, Wang D, Peng H, et al. Epigenetically upregulated oncoprotein PLCE1 drives esophageal carcinoma angiogenesis and proliferation via activating the PI-PLC-ε-NF-κB signaling pathway and VEGF-C/Bcl-2 expression. *Mol Cancer*. 2019;18(1):1. doi:10.1186/s12943-018-0930-x.

28. Hu H, Yang J, Sun Y, et al. Putatively functional PLCE1 variants and susceptibility to esophageal squamous cell carcinoma (ESCC): A case-control study in eastern Chinese populations. *Ann Surg Oncol*. 2012;19(7):2403-2410. doi:10.1245/s10434-011-1260-y.

29. Li X, Zhu S, Zhang T, Chen X. Association between oral microflora and gastrointestinal tumors (Review). *Oncol Rep*. 2021;46(2) doi:10.3892/or.2021.8111

30. Wang Q, Rao Y, Guo X, et al. Oral Microbiome in Patients with Oesophageal Squamous Cell Carcinoma. *Scientific reports* 2019;9(1):19055. doi:10.1038/s41598-019-55667-w.

31. Peters BA, Wu J, Pei Z, et al. Oral Microbiome Composition Reflects Prospective Risk for Esophageal Cancers. *Cancer Res* 2017;77(23):6777-6787. doi:10.1158/0008-5472.CAN-17-1296.

### Appendix

### Abbreviations

| Abbreviation | Description |
|--------------|-------------|
| ESCC         | esophageal squamous cell carcinoma |
| SNPs         | single nucleotide polymorphisms |
| GRS          | genetic risk score |
| ALDH2        | aldehyde dehydrogenase |
| ADH          | alcohol dehydrogenase |
| OR           | CI odds ratio confidence interval |