Original Research Article

Cancer Control
Volume 29: 1–11

DOI: 10.1177/10732748221075811
journals.sagepub.com/home/ccx
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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

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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.

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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.[1] In particular, Linxian, Henan Province, and Cixian and Shexian, Hebei Province has the highest incidence rates in the world.[2] Furthermore, in Taixing, Jiangsu Province, esophageal cancer has the highest morbidity rate among all cancers.[3] More than 90% of the esophageal cancers that are detected are diagnosed as esophageal squamous cell carcinoma (ESCC).[4] Currently, the risk factors associated with ESCC include single nucleotide polymorphisms (SNPs)[5] and lifestyle factors such as alcohol consumption[6], smoking/tobacco consumption[7,8] and poor oral health care.[9]

Poor oral health is an established risk factor for ESCC.[10] Furthermore, problems associated with poor oral health, including tooth decay and missing and filled teeth[11] may contribute to ESCC.[12,13] Chen et.al[9] 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,[14] compared with tooth-brushing twice or more per day, was associated with a 1.81-fold increase in the risk of ESCC.[9] 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.[15]

ESCC has been found to have genetic basis.[16] For example, SNPs in the *PCLE1* gene have been significantly associated with esophageal cancer in Asian populations.[17] 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.[18] With regard to head and neck cancer, the interplay between gene polymorphisms and oral health has been found to increase cancer risk.[19] 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.[3] 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,[9] 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.

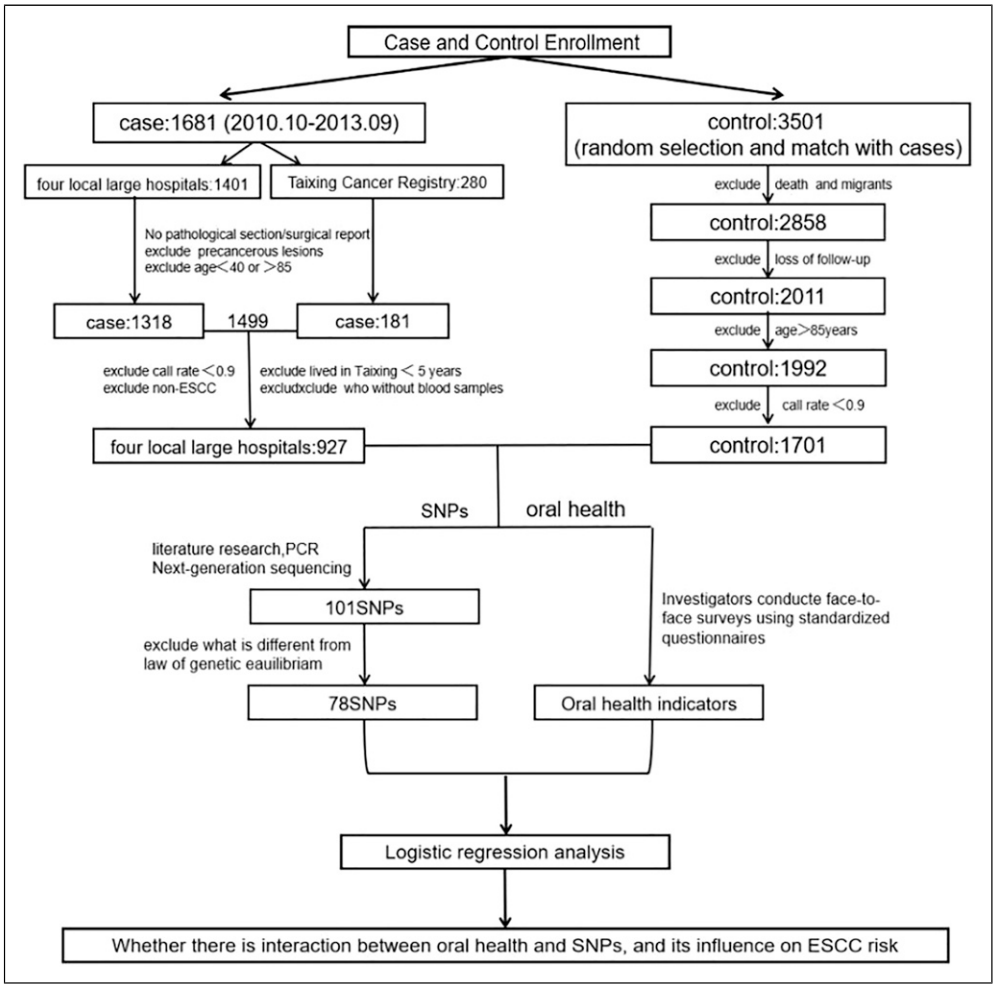

Figure 1. Flow chart for the recruitment of esophageal squamous cell carcinoma cases and controls.

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 had 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 Cross Complementing 1(XRCC1) and Flagellum Associated Containing 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)	

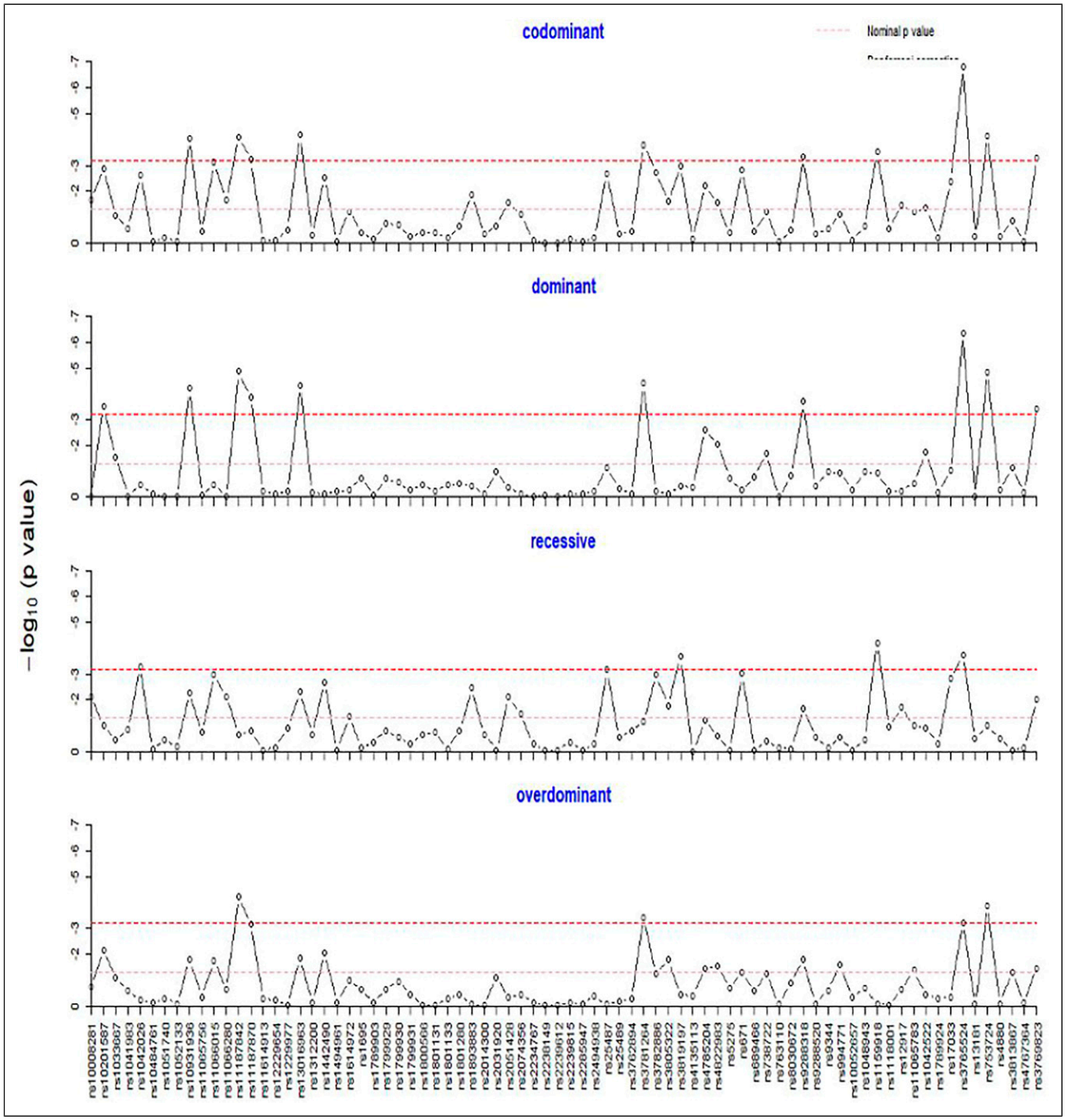

Figure 2. The −log *P* values of the 78 SNPs of the ESCC correlation test in four genetic models in which the "daily toothbrushing times" were adjusted by adding covariates as oral health indicators. Red line: Bonferroni correction line; Pink line: .05 *P* value line.

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.

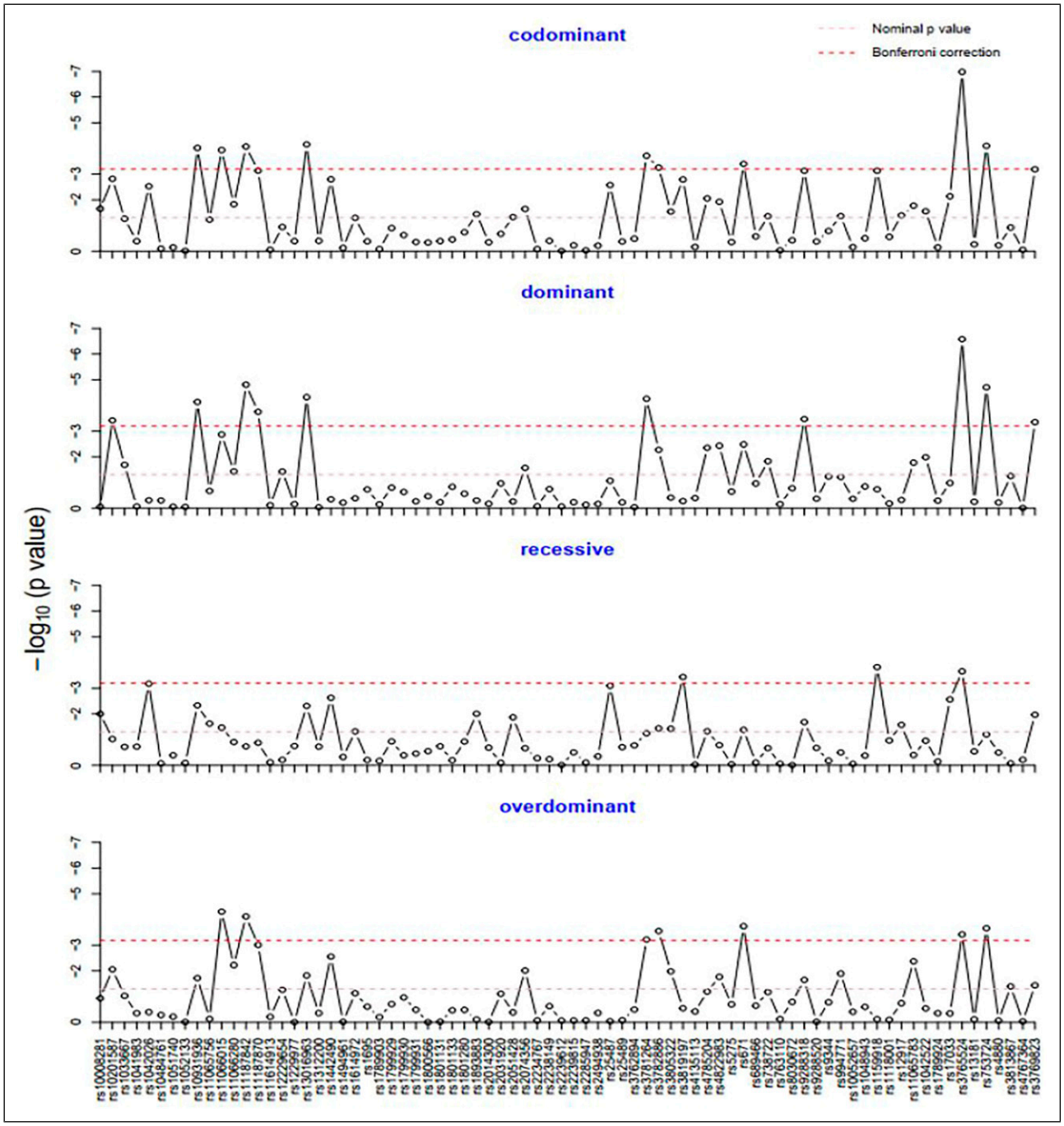

Figure 3. The −log P values of the 78 SNPs of the ESCC correlation test in four genetic models, where "tooth loss" was adjusted by adding covariates as oral health indicators. Red line: Bonferroni correction line; Pink line: .05 *P* value line.

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),[9] family history, tea consumption,[22] and daily consumption of fresh fruits. Using "daily frequency of tooth brushing" as an example, we build two models as follows:

$$y_0 = \beta_{00} + \beta_{01} \cdot brush\_times + \beta_{02} \cdot SNP + \varepsilon_0 \qquad (1)$$

$$y_1 = \beta_{10} + \beta_{11} \cdot brush\_times + \beta_{12} \cdot SNP + \beta_{13} \cdot bursh\_times \cdot SNP + \varepsilon_1 \quad (2)$$

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)[23] 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

$$GRS = \sum_{i=1}^{I} \varpi_{ORi} \cdot G_i \quad (3)$$

where, i is the SNP, ϖ_{ORi} = weight for SNP i, and G_i = number of risk alleles (0, 1 or 2).[24] 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.[25]

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.

Table 2. Interaction Effect Between Oral Health and SNPs on the Incidence of ESCC. Good Oral Hygiene Means that Individuals Brush their Teeth At Least Twice a Day, and Poor Means Individuals Brush their Teeth At Most Once a Day.

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	
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	
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	
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	
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	
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) that of the individuals with the same genotype who brushed their teeth at least twice a day. Among the individuals with the CT/TT genotype for rs11187842, those who brushed their teeth once a day at the most had a risk of ESCC more than three times greater (OR = 3.45, 95% CI: 2.55–4.70) than those who carried the CC genotype and brushed their teeth at least twice a day. For individuals with the rs3781264 TT genotype, those who brushed their teeth once a day at most had more than double the risk of ESCC (OR = 2.43, 95% CI: 1.84–3.23) 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 = 3.21, 95% CI: 2.37–4.38) 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).

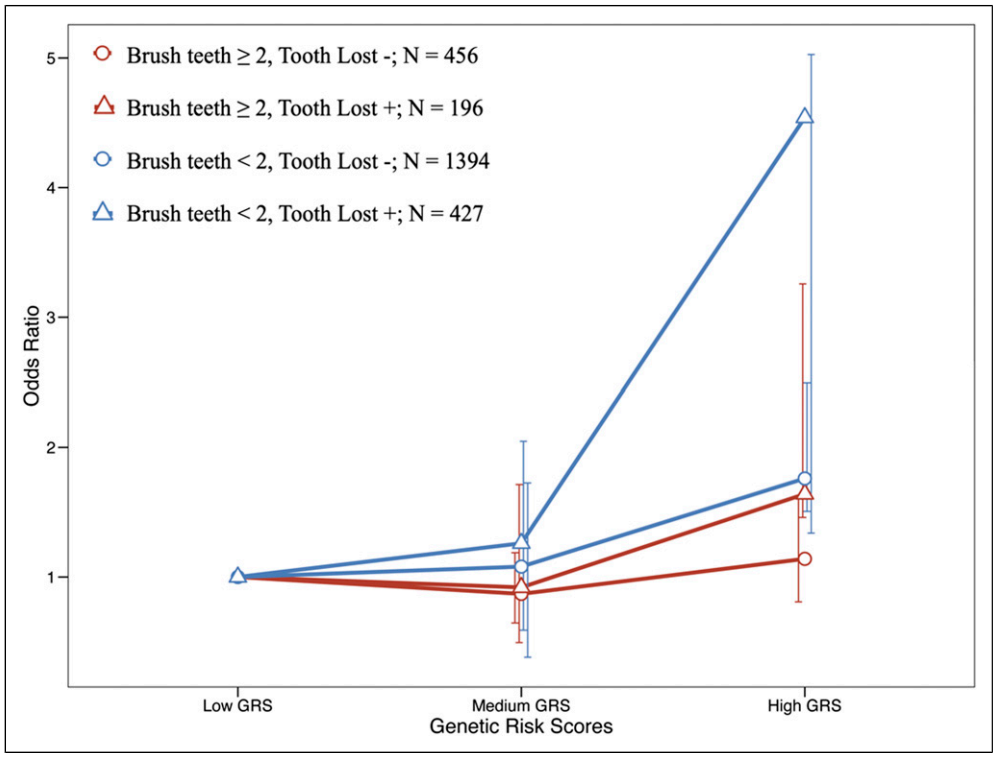

Figure 4. Genetic risk score (GRS) analysis on the seven SNPs (5 SNPs interacting with daily tooth-brushing frequency and 2 SNPs interacting with tooth loss). Group 1 represented people who brushed their teeth at least twice daily and without tooth loss experience, group 2 represented people who brushed 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-PLCε-NF-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.

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Supplemental Material

Supplemental material for this article is available online.

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Appendix

Abbreviations

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