BJMG 19 (2), 2016 • 61-40
DOI: 10.1515/bjmg-2016-0037

ORIGINAL ARTICLE

SUSCEPTIBILITY TO ORAL SQUAMOUS CELL CARCINOMA: CORRELATION WITH VARIANTS OF *CYP1A1-Msp*I, *GSTT1*, *GSTM1*, *ALDH2*, *EC-SOD* AND LIFESTYLE FACTORS

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ABSTRACT

In order to investigate the association between polymorphisms in genes encoding metabolizing enzymes (*CYP1A1-Msp*I, *EC-SOD* (*extracellular superoxide dismutase*), *GSTT1*, *GSTM1*, *ALDH2)*, cigarette and alcohol consumption, and the risk of oral squamous cell carcinoma, we conducted a prospective case-control study comprised of 750 individuals with oral squamous cell carcinoma (OSCC) and 750 healthy individuals. Data about smoking and drinking habits were collected along with other demographic and clinical information. Peripheral blood samples were collected for DNA extraction, and polymerase chain reaction (PCR) and PCR-RFLP (restriction fragment length polymorphism) were used to determine genotypes of *CYP1A1*, *EC-SOD, GSTT1*, *GSTM1*, *ALDH2*. The results showed that smoking and alcohol consumption were significantly more common among patients than controls (p <0.05). There were significant differences in the genotype distribution for each locus between groups, with the *CYP1A1* (m2/m2), *EC-SOD* (C/G), *GSTT1* [–], *GSTM1* [–] and *ALDH2* (non G/G) genotypes being more common among patients (p <0.05). Furthermore, the majority of patients had at least two or more variant genotypes, while controls had one or no variant genotype (p <0.05). Finally, multiple variant genotypes combined with smoking, drinking, or both smoking and drinking significantly increased the risk of OSCC, with greater increase for heavier smoking/drinking. In brief, genetic polymorphism of *CYP1A1*, *EC-SOD*, *GSTT1*, *GSTM1*, and *ALDH2* and smoking and drinking history are closely associated with susceptibility to OSCC.

Keywords: Drinking; genetic polymorphism; oral squamous cell carcinoma (OSCC); smoking.

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INTRODUCTION

Head and neck squamous cell carcinoma (HNSCC) is a common malignancy that ranks sixth in incidence of all cancers. The HNSCC tumors display dysregulation of cell differentiation, cell cycle control, epithelial and stromal interactions, apoptosis, angiogenesis and their associated pathways [1,2]. Although its exact cause remains unclear, like most malignancies, HNSCC pathogenesis is affected by both genetic and environmental factors [1].

Of the approximately 500,000 new cases of HNSCC each year, many occur in the oral cavity, pharynx, and larynx. Oral squamous cell carcinoma (OSCC) is the most common type of HNSCC, and China has one of the highest incidences of this cancer [2]. Importantly, OSCC is nearly asymptomatic, which makes early diagnosis very difficult; to date, there are no accurate predictors of OSCC onset and/or progression. Therefore, identification of risk factors and high-risk populations for OSCC would enable advancements in the primary and secondary prevention of OSCC.

Cigarette smoking and alcohol consumption are known environmental risk factors for OSCC [3,4]. Cigarette smoke contains polycyclic aromatic hydrocarbons, heterocyclic amines, and nitrosamines that are all carcinogenic. Long-term alcohol consumption can lead to combined overdose of reactive oxygen species (ROS) and acetaldehyde, inducing carcinogenesis. Certain enzymes have been shown to be responsible for the biotransformation of chemical carcinogens, either for activation or excretion. For example, cytochrome P4501A1, encoded by CYP1A1, is a catabolite-activating enzyme involved in the biotransformation of both tobacco and alcohol. P4501A1-mediated metabolism of tobacco combustion products, mainly polycyclic aromatic hydrocarbons, can lead to

the formation of DNA adducts that contribute to tumor formation, specifically HNSCC [5]. Other metabolizing enzymes, with variations in their respective genes, have also been previously implicated in cancer susceptibility. These include, but are not limited to, glutathione S-transferase (GST), superoxide dismutate (SOD), proteins of the SOD family, and acetaldehyde dehydrogenase (ALDH). Glutathione S-transferase plays a role in metabolizing benzo[a]pyrene (a tobacco-specific carcinogen), as well as other carcinogenic compounds. Superoxide dismutase, an endogenous antioxidant enzyme, has certain polymorphisms implicated in cancer susceptibility. Acetaldehyde dehydrogenase, along with alcohol dehydrogenase (ADH), metabolizes ethanol by breaking apart the molecule in order to eliminate it from the body. Genetic polymorphism in ALDH2 has been previously investigated and shown to be associated with specific cancer types [5]. However, genes encoding these enzymes have multiple functional variants, blurring their role in OSCC susceptibility. At least one recent study found that expression of CYP1A1 and ALDH2 proteins did not affect OSCC prognosis [6]. Thus, the specific contribution of polymorphisms in genes encoding enzymes involved in biotransformation of alcohol and tobacco components remains unclear; specifically, the role in promoting OSCC requires further study. This study reports the investigation of the association between genetic polymorphism of *CYP1A1*, *EC-SOD* (*extracellular SOD*), *GSTT1*, *GSTM1*, *ALDH2*, smoking and alcohol consumption, and susceptibility to oral squamous cell carcinoma.

PARTICIPANTS AND METHODS

Participants. This prospective study included 750 patients who were admitted to our hospital from June 2011 to May 2015. Another 750 participants who received physical examinations during the same time period were selected as healthy controls; the physical examination showed no cancer or hereditary diseases. There was no statistically significant difference in age, gender, place of origin or nationality, and the subjects were unrelated. Participant demographic data, smoking history, alcohol drinking history, occupational history and family tumor history were collected. The smoking status was evaluated using the smoking index (SI) that was the product of the daily number of cigarettes multiplied by number of years of smoking. Based on SI values, the participants were divided into the following categories: non smokers, individuals with SI ≤400, and individuals with SI 400. Alcohol consumption was evaluated with the drinking index (DI) that was the product of the daily amount of drinking (in grams) multiplied by the number of years of drinking. Based on DI values, the participants were classified as non drinkers, those with DI ≤3000, and those with DI 3000. The study was approved by the General Hospital of Daqing Oil Field, Daqing, Heilongjiang Province, People's Republic of China (PRC). Informed written consent was obtained from all participants.

Genotyping. From each participant, 3 mL blood was collected in vacutainers with EDTA as anticoagulant. The QIAmpDNA extraction kit (Qiagen GmbH, Hilden, Germany) was used to extract DNA from white blood cells. Extracted DNA was stored at –30 °C. Polymerase chain reaction (PCR) was used to amplify the DNA to the levels required for restriction fragment length polymorphism (RFLP) analysis. For all reactions, a total volume of 25 μL comprised 2.5 μL 10 × buffer, 2.5 μL dNTP, 20 pmol upstream primers, 20 pmol downstream primers (see below), 0.75 μL Taq DNA polymerase (all PCR reagents from Promega, Madison, WI, USA), and 100 ng template DNA. Reactions were performed on PE480 thermocycler (Perkin Elmer?, Norwalk, CT, USA), as follows: initial denaturation at 94 °C for 4 min., and 35 cycles of denaturation at 94 °C for 40 seconds, annealing at 55 °C for 40 seconds, and extension at 72 °C for 50 seconds, and final extension at 72 °C for 5 min. The PCR products were digested with restriction endonucleases as appropriate (described below). A total reaction volume of 20 μL comprised 1 ng PCR product, 2 μL 10 × NEB (New England Biolabs, Ipswich, MA, USA) reaction buffer, and 10 U endonuclease; reactions were performed at 37 °C for 3 hours. Digestion products were separated by 100V electrophoresis on a 3.0% agarose gel, for 1 hour. After 30 min. in ethidium bromide, bands were detected by ultraviolet light.

The *CYP1A1-Msp*I polymorphism was detected using the following primer sequences: upstream primer (5'-CAG TGA AGA GGT GTA GCC GCT-3'), downstream primer (5'-TAG GAG TCT TGT CTC ATG CCT-3' (synthesized by TaKaRa, Dalian, China). Restriction digestion with *Msp*I produced three genotypes: wild-type (m1/m1) with a band at 340 bp, heterozygous (m1/m2) with bands at 340, 200 and 140 bp, and homozygous mutant (m2/m2) with bands at 200 and 140 bp.

The *EC-SOD* polymorphism was detected using primer sequences as reported in a previous study [7]: upstream primer (5'-GCA ACC AGG CCA GCG TGG AGA ACG GGA A-3'), and downstream primer 5'-CCA GAG GAG AAG CTC AAA GGC AGA-3'). The PCR product was digested with restriction endonuclease *Pau*I. Two genotypes were produced: homozygous C/C with bands

at 111 and 109 bp; and heterozygous C/G with bands at 220, 111 and 109 bp.

The *GSTT1* polymorphism was detected using primer sequences according to Wilson *et al.* [8]: upstream primer (5'-TCT CCT TAC TGG TCC TCA CAT CTC-3'), and downstream primer (5'-TCA CCG GAT CAT GGC CAG CA-3'). *GSTT1* positive [+] was indicated by the presence of a 480 bp fragment after PCR amplification; *GSTT1* negative [–] meant lack of PCR product. The control β-globin gene was detected with upstream primer (5'-CAA GAG CCA ACC ACA GGT AC-3') and downstream primer (5'-GAA GAG CCA AGG ACA GGT AC-3').

The *GSTM1* polymorphism was detected using primer sequences selected according to Nguyen *et al.* [9]: upstream primer (5'-GAA CTC CCT GAA AAG CTA AAG C-3'), and downstream primer (5'-GTT GGG CTC AAA TAT ACG GTG G-3'). *GSTM1* positive [+] indicated the presence of bands at 230 and 219 bp; *GSTM1* negative [–] indicated the presence of only one 219 bp band. The control β-globin gene was detected with upstream primer (5'-CAA GAG CCA ACC ACA GGT AC-3'), and downstream primer (5'-GAA GAG CCA AGG ACA GGT AC-3').

The *ALDH2* polymorphism was detected using primer sequences selected according to Ishibashi *et al.* [10]: upstream primer (5'-CCC TTT GGT GGC TAG AAG ATG-3'), and downstream primer (5'-CCA CAC TCA CAG TTT TCT CTT-3'). The PCR products were digested with the restriction endonuclease *Mbo*I. The amplified fragment was 91 bp and three genotypes could be seen after digestion: homozygous G/G with a band at 55 bp, heterozygous G/L with bands at 65 and 55 bp, and homozygous L/L with a band at 65 bp. The ALDH2 (G/L) and ALDH2 (L/L) were combined and marked as ALDH2 (non G/G) for co-analysis.

Statistical Methods. The difference in genotype distributions between the patient and control groups was determined using the χ^2 test. A non conditional logistic regression model was used to analyze the adjusted odds ratio (OR) for risk of oral squamous cell carcinoma with different genotypes, as well as the combined effects between smoking, drinking, and genotype. The 95% confidence interval (CI) is also reported. The Statistical Package for the Social Sciences (SPSS Inc., Chicago, IL, USA) version 11.0 was used for statistical analysis. Statistical significance was accepted at a p value of <0.05.

RESULTS

Oral Squamous Cell Carcinoma and Lifestyle. General demographic and clinical information for participants in both groups are provided in Table 1. There was no statistically significant difference in gender or age between case and control groups (p >0.05). However, smoking and drinking were significantly more common in individuals with OSCC than in controls (p <0.01).

Genotype Distribution. For each of the enzyme encoding genes we investigated, the distribution of genotypes, significantly differed between cases and controls (all p <0.01). *CYP1A1-Msp*I (m2/m2), *EC-SOD* (C/G), *GSTT1* [–], *GSTM1* [–] and *ALDH2* (non G/G) genotypes were significantly more common in individuals with OSCC than in control individuals (p <0.01) (Table 2).

Multiple Genotype Variants Distribution. Because there was a significant shift of the genotype frequencies observed in individuals with OSCC compared to controls, we also examined whether OSCC patients were more likely to carry multiple variant genotypes than healthy subjects. The combined distribution of *CYP1A1-Msp*I (m2/ m2), *EC-*

Table 1. General characteristics of healthy controls and patients with oral squamous cell carcinoma.

Characteristics		Control Group (n = 750)	Patient Group (n = 750)	OR Value	95% CI	p Value
Sex n (%)	males females	498 (66.40%) 252 (33.60%)	504 (67.20%) 246 (32.80%)	0.97	0.78-1.20	>0.05
Age	years; mean±SD	55.51±4.42	55.49±4.33	–	–	>0.05
Smoking status n (%)	non-smoker smoker	451 (60.13%) 299 (39.87%)	265 (35.33%) 485 (64.67%)	2.76	2.24-3.40	<0.01
	SI ≤400 SI 400	202 (26.93%) 97 (12.93%)	120 (16.00%) 365 (48.67%)	6.33	4.61-8.71	<0.01
Drinking status n (%)	did not drink did drink alcohol	468 (62.40%) 282 (37.60%)	256 (34.13%) 494 (65.87%)	3.20	2.59-3.96	<0.01
	DI ≤3000 DI 3000	172 (22.93%) 110 (14.67%)	114 (15.20%) 380 (50.67%)	5.21	5.79-7.16	<0.01

OR: odds ratio; 95% CI: 95% confidence interval; SD: standard deviation; SI: smoking index; DI: drinking index.

Table 2. Distribution of *CYP1A1-Msp*I, *EC-SOD*, *GSTT1*, *GSTM1,* and *ALHD2* genotypes in the control and patient groups.

Genotype	Control Group		Patient Group		OR Value	95% CI	*p* Value
	n	%	*n*	%			
*CYP1A1-Msp*I:							
m1/m1+m1/m2	593	79.00	463	61.73	1.00	1.86-2.95	<0.01
m2/m2	157	20.93	287	38.37	2.34		
EC-SOD:							
C/C	605	80.67	380	50.67	1.00	3.23-5.12	<0.01
C/G	145	19.33	370	49.27	4.06		
GSTT1:							
[+]	598	79.73	395	52.67	1.00	2.81-43.44	<0.01
[–]	152	20.27	355	47.33	3.54		
GSTM1:							
[+]	419	55.87	231	30.80	1.00	1.95-4.47	<0.01
[–]	331	44.13	519	69.20	2.83		
ALHD2:							
G/G	444	55.60	212	30.53	1.00	2.97-4.57	<0.01
non G/G	306	44.40	538	69.47	3.68		

OR: odds ratio; 95% CI: 95% confidence interval.

Table 3. The combined distribution of *CYP1A1-Msp*I (m2/m2), *EC-SOD* (C/G), *GSTT1*[–], *GSTM1* [–] and *ALHD2* (non G/G) variant genotypes in the control and patient groups.

Number of Genotypes	Control Group		Patient Group		OR Value	95% CI	*p* Value
	n	%	*n*	%			
0	97	12.93	8	1.07	1.00	–	<0.01
1	290	38.67	64	8.53	2.68	1.23-5.78	–
2	210	28.00	230	30.67	13.28	6.31-27.97	–
3	90	12.00	278	37.07	37.45	17.53-80.02	–
4	56	7.47	156	20.80	33.78	15.44-73.90	–
5	7	0.93	14	1.86	24.25	7.61-77.27	–

OR: odds ratio; 95% CI: 95% confidence interval.

Table 4. Combined analysis of smoking status, and variant genotype distribution in relation to oral squamous cell carcinoma susceptibility.

Number of Genotypes	Smoking Status	Control Group		Patient Group		OR Value	95% CI	*p* Value
		n	%	*n*	%			
0	[–]	92	12.27	31	4.13	1.00	–	–
1	[–]	118	15.73	43	5.73	1.04	0.64-1.70	0.86
2	[–]	116	15.47	90	12.00	2.22	1.44-3.44	<0.01
3	[–]	71	9.47	53	7.07	2.14	1.31-3.50	<0.01
4	[–]	40	5.33	46	6.13	3.30	1.92-5.67	<0.01
5	[–]	14	1.87	2	0.27	0.41	0.09-1.87	0.25
0	[+]	37	4.93	14	1.87	0.89	0.43-1.86	0.76
1	[+]	100	13.33	92	12.27	2.52	1.61-3.96	<0.01
2	[+]	78	10.40	147	19.60	2.43	1.65-3.58	<0.01
3	[+]	35	4.67	128	17.07	4.90	2.92-8.21	<0.01
4	[+]	44	5.87	81	10.80	1.60	0.91-2.80	0.10
5	[+]	5	0.67	23	3.07	32.20	5.49-118-91	<0.01

OR: odds ratio; 95% CI: 95% confidence interval.

SOD (C/G), *GSTT1* [–], *GSTM1* [–] and *ALHD2* (non G/G) genotypes in both groups are provided in Table 3. Within the patient group, the majority of individuals (90.4%) had at least two of the variants. Namely, 1.9% of individuals were positive for five of the variant genotypes, 20.8% for four genotypes, 37.1% for three genotypes, 30.7% for two genotypes, 8.5% for only one variant genotype, and only 1.1% did not have any of the variant genotypes. In contrast, in the control group, the majority of individuals had one or no variants (51.6%). Thus, the presence of two or more variants in the OSCC population was significantly more common than in controls (p <0.01).

Smoking Status and Gene Polymorphisms. Table 4 depicts the results of the analysis of *CYP1A1-Msp*I (m2/m2), *EC-SOD* (C/G), *GSTT1* [–], *GSTM1* [–] and *ALHD2* (non G/G) variant genotype presence and smoking status. In individuals with OSCC, multiple variants and smoking were significantly more common compared to the control group; smoking in combination with multiple variant genotypes had a synergistic effect on cancer likelihood.

Table 5. Combined analysis of smoking index, and variant genotype distribution in relation to oral squamous cell carcinoma susceptibility.

Number of Genotypes	Smoking Status	Control Group		Patient Group		OR Value	95% CI	*p* Value
		n	%	*n*	%			
0	≤400	45	6.00	2	0.27	1.00	–	–
1	≤400	54	7.20	16	2.13	0.22	0.05-1.00	0.07
2	≤400	45	6.00	23	3.07	0.38	0.08-1.86	0.23
3	≤400	33	4.40	44	5.87	1.00	1.00-0.21	1.00
4	≤400	23	3.07	32	4.27	1.04	0.21-5.12	0.96
5	≤400	2	0.27	3	0.40	1.12	0.11-11.60	0.92
0	400	3	0.40	4	0.53	1.82	0.51-2.85	0.49
1	400	45	6.00	24	3.20	1.80	0.85-3.80	0.12
2	400	28	3.73	123	16.40	8.60	4.50-16.44	<0.01
3	400	11	1.47	153	20.40	10.43	4.88-22.31	<0.01
4	400	9	1.20	57	7.60	4.55	1.88-11.02	<0.01
5	400	1	0.13	4	0.53	2.67	0.16-45.14	0.50

OR: odds ratio; 95% CI: 95% confidence interval.

Table 6. Combined analysis of drinking status, and variant genotype distribution in relation to oral squamous cell carcinoma susceptibility.

Number of Genotypes	Drinking Status	Control Group		Patient Group		OR Value	95% CI	*p* Value
		n	%	*n*	%			
0	[–]	100	13.33	27	3.60	1.00	–	–
1	[–]	166	22.13	42	5.60	0.41	0.21-0.80	0.01
2	[–]	105	14.00	77	10.27	1.18	0.61-2.28	0.62
3	[–]	51	6.80	65	8.67	2.05	1.03-4.11	0.04
4	[–]	43	5.73	43	5.73	1.61	0.78-3.32	0.20
5	[–]	3	0.40	2	0.27	1.07	0.16-7.06	0.94
0	[+]	29	3.87	18	2.40	0.44	0.21-0.90	0.03
1	[+]	52	6.93	93	12.40	7.07	4.38-11.42	<0.01
2	[+]	89	11.87	160	21.33	2.45	1.66-3.63	<0.01
3	[+]	55	7.33	116	15.47	1.66	1.02-2.69	0.04
4	[+]	41	5.47	84	11.20	2.05	1.67-3.60	0.01
5	[+]	16	2.13	23	3.07	2.16	0.32-14.41	0.43

OR: odds ratio; 95% CI: 95% confidence interval.

Table 7. Combined analysis of drinking index, and variant genotype distribution in relation to oral squamous cell carcinoma susceptibility.

Number of Genotypes	Drinking Index	Control Group		Patient Group		OR Value	95% CI	*p* Value
		n	%	*n*	%			
0	≤3000	21	2.80	3	0.40	1.00	–	–
1	≤3000	47	6.27	15	2.00	0.80	0.01-077	0.03
2	≤3000	49	6.53	26	3.47	0.13	0.01-1.25	0.08
3	≤3000	36	4.80	38	5.07	0.26	0.03-2.47	3.79
4	≤3000	16	2.13	28	3.73	0.44	0.05-4.26	0.48
5	≤3000	3	0.40	4	0.53	0.33	0.02-4.74	0.42
0	3000	1	0.13	4	0.53	2.96	0.55-6.28	0.08
1	3000	24	3.20	44	5.87	5.74	2.67-12.35	<0.01
2	3000	39	5.20	122	16.27	5.90	3.27-10.71	<0.01
3	3000	30	4.00	138	18.40	4.36	2.38-7.97	<0.01
4	3000	14	1.87	54	7.20	2.20	0.94-5.16	0.07
5	3000	2	0.27	18	2.40	6.75	0.83-54.66	0.07

OR: odds ratio; 95% CI: 95% confidence interval.

Table 8. Combined analysis of smoking and drinking status, and variant genotype distribution in relation to oral squamous cell carcinoma susceptibility.

Number of Genotypes	Smoking Status	Drinking Status	Controls		Patients		OR Value	95% CI	*p* Value
			n	%	*n*	%			
0	[–]	[–]	68	9.07	15	2.00	1.00	–	–
1	[–]	[–]	82	10.93	12	1.60	1.04	0.64-1.70	0.86
2	[–]	[–]	48	6.40	42	5.60	2.22	1.44-3.44	<0.01
3	[–]	[–]	27	3.60	21	2.80	2.14	1.31-3.50	<0.01
4	[–]	[–]	18	2.40	18	2.40	3.30	1.91-5.67	<0.01
5	[–]	[–]	1	0.13	1	0.13	0.41	0.09-1.87	0.25
0	[–]	[+]	24	3.20	16	2.13	1.91	0.31-11.81	0.48
1	[–]	[+]	36	4.80	31	4.13	7.07	4.38-11.42	<0.01
2	[–]	[+]	68	9.07	48	6.40	2.45	1.66-3.63	<0.01
3	[–]	[+]	44	5.87	32	4.27	1.66	1.02-2.69	0.04
4	[–]	[+]	22	2.93	28	3.73	2.05	1.17-3.60	0.01
5	[–]	[+]	13	1.73	1	0.13	2.16	0.32-14.40	0.43
0	[+]	[–]	32	4.27	12	1.60	0.86	0.16-4.63	0.87
1	[+]	[–]	84	11.20	30	4.00	2.53	1.51-3.96	<0.01
2	[+]	[–]	57	7.60	35	4.67	2.43	1.65-3.58	<0.01
3	[+]	[–]	24	3.20	44	5.87	4.90	2.92-8.21	<0.01
4	[+]	[–]	25	3.33	25	3.33	1.60	0.91-2.80	0.10
5	[+]	[–]	2	0.27	1	0.13	32.20	5.49-188.91	<0.01
0	[+]	[+]	5	0.67	2	0.27	3.45	1.62-5.41	<0.01
1	[+]	[+]	16	2.13	62	8.27	10.36	6.53-16.44	<0.01
2	[+]	[+]	21	2.80	112	14.93	6.20	4.12-9.33	<0.01
3	[+]	[+]	11	1.47	84	11.20	5.23	3.33-820	<0.01
4	[+]	[+]	19	2.53	56	7.47	4.19	2.67-6.58	<0.01
5	[+]	[+]	3	0.40	22	2.93	13.26	4.09-42.97	<0.01

OR: odds ratio; 95% CI: 95% confidence interval.

Smokers with five variant genotypes were 32-times more likely to have OSCC.

Furthermore, within the sub-population of smokers, SI was synergistic with multiple variant genotypes (Table 5). In the patient group, the presence of multiple variant genotypes and an SI >400 was significantly more common compared to the control group (p <0.01).

Drinking Status and Gene Polymorphisms. A similar analysis was applied to *CYP1A1-Msp*I (m2/m2), *EC-SOD* (C/G), *GSTT1* [–], *GSTM1* [–] and *ALHD2* (non G/G) variant genotypes and alcohol consumption (Table 6). The presence of multiple variants and alcohol consumption was significantly more common in patients compared to the control group; furthermore, as with smoking, the drinking behavior and combined variant genotypes had a synergistic effect on the likelihood of cancer.

The synergistic effect of the DI and combined expression of variant genotypes is depicted in Table 7. In the patient group, the presence of multiple variants and a DI >3000 was significantly more common compared to the control group (p <0.01).

Analysis of Combined Smoking/Drinking Status and Gene Polymorphisms. Table 8 displays the results of the analysis of the presence of variant genotypes combined with smoking and drinking status. In the patient group, there were significantly more patients who had multiple variants and were positive for both smoking and drinking history as compared to the control group.

DISCUSSION

The pathogenesis of OSCC is complex, involving combined action of a variety of environmental and genetic factors [11-13]. Smoking and drinking are the main risk factors for OSCC [14,15]. Our findings confirm that smoking and drinking were significantly more frequent in individuals with OSCC than in the control individuals. Heavy cigarette and alcohol consumption were also significantly higher in the OSCC group compared to the control group.

The CYP1A1 is a member of the cytochrome P450 family involved in the metabolism of exogenous materials, encoding aryl hydrocarbon hydrolase (AHH), and activating polycyclic aromatic hydrocarbon and aromatic amine [16]. Our results showed that the distribution of genotypes for *CYP1A1-Msp*I was significantly different between the patient group and the control group, and that the *CYP1A1-Msp*I (m2/m2) genotype may increase susceptibility to OSCC. This finding, aligned with prior studies, shed light on the involvement of the *CYP1A1-Msp*I polymorphism in carcinogenesis through modified enzymatic activity [16]. Our results also confirmed the results of a meta-analysis which found that *CYP1A1-Msp*I increased the odds of OSCC, specifically among Asian populations [30].

Superoxide dismutase is generally considered as the first line antioxidative defense in the body [17]. This enzyme can be highly effective against ROS to protect the cells and tissues from oxidative stress [18]. The SOD dysregulation is correlated with growth of human malignant tumors [19,20]. Further, EC-SOD appears to be important for tumor formation [21-23], and is correlated with OSCC [24]. Our study mirrored the earlier studies that indicated EC-SOD association with OSCC and showed a significant difference in distribution of EC-SOD genotypes between the patient and control groups [24].

The GST polymorphisms are also correlated with cancer susceptibility. Glutathione S-transferase can catalyze the binding of electrophilic carcinogens and glutathione, to metabolize compounds that are easily soluble in water and excrete them. *GSTM1*/*GSTT1* has been previously associated with susceptibility to oral carcinomas [25,31]. Our study confirmed that *GSTM1*[–]/GSTT1[–] genotypes are correlated with susceptibility to OSCC.

Animal studies have found that the *in vivo* metabolite of ethanol, acetaldehyde, has significant carcinogenic effects [26,27], and ALDH2 is the main enzyme to metabolize acetaldehyde in the liver. Furthermore, *ALDH2* gene polymorphism is correlated with a variety of tumors [28-30,32]. Our study confirmed the correlation between *ALDH2* (non G/G) genotypes and increased susceptibility to OSCC.

Interestingly, our study also found significant differences in the prevalence of combined *CYP1A1-Msp*I (m2/ m2), *EC-SOD* (C/G), *GSTT1* [–], *GSTM1* [–] and *ALHD2* (non G/G) variant genotypes between the patient and control groups. In the patient group, the percentage of patients with a combination of variant genotypes (more than two genotypes) was significantly higher than that of the control group; the relative risks of OSCC in such patients were significantly increased. Previous studies have shown that OSCC patients are more likely to carry multiple variants and have a history of smoking or drinking; the interaction of two or more of these factors enhances risk [3,5,29, 30,32]. Our simultaneous analyses confirmed these previous results, as individuals with both multiple variant genotypes and a smoking and drinking history exhibited a significantly higher risk of OSCC. Therefore, OSCC risk increases with the increasing amount and period of smoking and drinking.

Further investigations and studies of the effects of these gene and environmental interactions is paramount to an earlier diagnosis of OSCC. More studies of non Asian populations is another avenue of research worth undertaking.

ACKNOWLEDGMENTS

This work was supported by Natural Science Foundation of Heilongjiang Province (H201644).

Declaration of Interest. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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