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Gene–environment interaction involved in oral carcinogenesis: molecular epidemiological study for metabolic and DNA repair gene polymorphisms

Tomotaka Sugimura[1,2], Hiroshi Kumimoto[1], Iwai Tohnai[3], Takafumi Fukui[2], Keitaro Matsuo[4], Shinichi Tsurusako[5], Kenji Mitsudo[2], Minoru Ueda[2], Kazuo Tajima[4], Kanji Ishizaki[1]

[1]*Central Laboratory and Radiation Biology, Aichi Cancer Center Research Institute, Nagoya;* [2]*Department of Oral and Maxillofacial Surgery, Nagoya University Graduate School of Medicine, Nagoya;* [3]*Department of Cell Therapy, Nagoya University School of Medicine, Nagoya;* [4]*Division of Epidemiology and Prevention, Aichi Cancer Center Research Institute, Nagoya;* [5]*Department of Oral and Maxillofacial Surgery, Holy Spirit Hospital, Nagoya, Japan*

BACKGROUND: Exposure to environmental carcinogens leads to oral squamous cell carcinoma (OSCC); however, the impact of genetic variations in carcinogen metabolisms and DNA repair on OSCC risk considering environmental exposures has not been clearly elucidated.
METHODS: We conducted a case–control study with 122 cases and 241 controls. The risk of OSCC was evaluated in 10 genetic polymorphisms of nine genes, such as *CYP1A1*, *CYP2E1*, *GSTM1*, *GSTT1*, *XPA*, *XPC*, *XPC*, *XPF* and *ERCC1*. Gene–environment interaction was also evaluated.
RESULTS: We found that *CYP2E1* and *XPA* polymorphisms significantly affected the OSCC risk. Gene–environment interactions with smoking were significant for *CYP2E1* and *ERCC1* polymorphisms. Odds ratios for gene–environment interaction were 7.98 ($P = 0.036$), 9.67 ($P = 0.017$) and 8.49 ($P = 0.031$) for *CYP2E1 Rsa*I, *Dra*I and *ERCC1* polymorphisms, respectively. No interaction was observed with heavy drinking and any polymorphisms.
CONCLUSION: *CYP2E1*, *XPA* and *ERCC1* polymorphisms may affect the risk of OSCC.
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Keywords: cancer risk; gene–environmental interaction; oral; polymorphism

Introduction

Synthetic or natural chemical compounds in the environment may cause genetic changes that result in many human cancers. Among them, oral squamous cell carcinoma (OSCC) is one of the cancers strongly affected by environmental factors, such as tobacco smoking and alcohol drinking (1). Many chemical compounds in environmental factors need metabolic conversion into active carcinogens so that they can damage DNA in cells, and most carcinogens are also detoxified by drug-metabolizing enzymes. For example, xenobiotics such as polycyclic aromatic hydrocarbon and *N*-nitrosamines can be bio-activated into ultimate carcinogens by phase I enzymes, such as CYP1A1 and CYP2E1, and subsequently detoxified by phase II enzymes, such as GSTM1 and GSTT1. As most of phase I and II enzymes have polymorphic sites, which may affect their activity, the strength and balance of activities must differ according to the individual genotype of these metabolic genes (2, 3).

Repair of DNA damage is the most important factor-protecting cells against carcinogenesis due to the environment (4). Nucleotide excision repair (NER) is one of the most important pathways, especially removing bulky adducts induced by environmental carcinogens (5, 6). Common polymorphisms in DNA repair genes within this pathway such as *XPA*, *XPC*, *XPF*, *XPG* and *ERCC1* may alter their functions so that their capacity to repair DNA damage may be affected. Individuals with low repair capacity may have genetic instability and an increased risk of developing various cancers (6–8).

Numerous studies around the world have investigated the possible association between OSCC risks and polymorphisms of loci involved in metabolic pathways of environmental agents (9–12) and DNA repair genes (5, 13), yet only a few such studies involving Japanese patients have been reported to date (14–17). In addition, little is known about the gene–environment interaction between smoking/alcohol exposures and genetic polymorphisms involved in their metabolism and DNA

Correspondence: Tomotaka Sugimura, Central Laboratory and Radiation Biology, Aichi Cancer Center Research Institute, 1-1 Kanokoden, Chikusa-ku, Nagoya 466-8681, Japan. Tel: +81-52-763-6111 (ext 7013). Fax: +81-52-763-5233. E-mail: sugitomo@aichi-cc.jp
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repair. Therefore, we carried out an epidemiological study to examine whether genetic polymorphisms of tobacco- and alcohol-related metabolic genes and DNA repair genes are associated with risks of OSCC, and to elucidate the possible interaction between these polymorphisms and cancer risks by smoking or heavy drinking.

Materials and methods

Subjects

Cases ($n = 122$) were those who had been diagnosed as having OSCC from 1988 to 2004 at Nagoya University Hospital in Nagoya city. Non-cancer controls ($n = 241$) were gastroscopy examinees enrolled in a *Helicobacter pylori* eradication study at Aichi Cancer Center Hospital (ACCH) as described elsewhere (18). Both hospitals are closely cooperated in many aspects, such as clinical treatments and studies, and located in the same area of Nagoya city. Those with pre-cancerous lesion of upper aerodigestive tract and previous malignancy were excluded from non-cancer controls. We have been demonstrated that genotype distributions in many genes among our non-cancer controls are similar to other Japanese population (19, 20). Habitual smoking and drinking were also similar between source population of this control and general population living in Nagoya city (21). Therefore, it is considered to be feasible to use non-cancer outpatients in ACCH as controls in our epidemiological study enrolling OSCC cases in Nagoya city. All the subjects participated after giving their written informed consent. This study was approved by the ethical committees at both institutions.

Genotyping of genomic DNA

Genomic DNA was isolated from peripheral blood cells using the QIAamp DNA Blood Mini Kit (Qiagen Inc., Hilden, Germany), and its concentration was adjusted to 50 ng/μl with water. Genotypes of *CYP1A1* exon 7 (Ile462Val, A/G), *CYP2E1* 5′-untranslated region (UTR; *Rsa*I site), intron 6 (*Dra*I site), *XPA* 5′-UTR (A23G) and *XPG* exon 15 (Asp1104His, C/G) were determined using the polymerase chain reaction (PCR) restriction fragment length polymorphism technique according to methods described previously (22–26). The genotype of *XPC* was determined by electrophoresis of PCR product described previously (27). Multiplex PCR with primer sets for *GSTM1*, *GSTT1* and albumin was performed to detect the null genotypes of the *GSTM1* and *GSTT1* genes (28). To analyse the genetic polymorphisms in *XPF* 5′-UTR (T2063A) and *ERCC1* 3′-UTR (C8092A), *Pst*I and *Pvu*II sites were created by substituting nucleotide G to T at three bases and T to A at four bases prior to these polymorphic sites with mismatched primers, respectively (Table 1). PCR was performed with 25 ng of genomic DNA in a total volume of 25 μl which included PCR buffer, dNTPs, 20 pmol each primer, 4% dimethyl sulphoxide (DMSO) and 0.5 unit of AmpliTaq DNA polymerase (Applied Biosystems Inc., Foster City, CA, USA). PCR products were digested with the appropriate restriction endonucleases (Table 1) and then electrophoresed on 2–3% agarose gel. Accuracy of genotyping for *XPF* and *ERCC1* was confirmed by direct sequencing analysis of PCR product amplified by primers located at flanking region of genotyping primers (Table 1). Direct sequencing was performed to several subjects who showed each genotype in novel genotyping method.

Questionnaire and data collection

Information about age, sex, smoking status and alcohol consumption were obtained from all the subjects by self-administered questionnaire. Subjects were classified into two smoking status categories: never smokers and ever-smokers, ever-smokers were defined as combination of former smokers and current smokers. Heavy drinkers were defined as those who drink alcohol for 5 days or more per week with more 50 ml ethanol/day.

Statistical analysis and risk assessment for lifestyles

We estimated odds ratios (ORs) to assess the strength of association between each polymorphism on risk of OSCC. Unconditional logistic regression models were applied to estimate ORs and their 95% confidence intervals (CIs). In the model, age using four indicator variables according to age categories (< 45, 45–54, 55–64 and > 65), sex, smoking status and alcohol consumption were adjusted as confounders. We aimed to simplify the model to obtain easier interpretation by dichotomizing genotype for each polymorphism. We explored dominant model (variant/variant + variant/common vs. common/common) and recessive model (variant/variant vs. variant/common + common/common) in addition to the model comparing two mutant allele-positive geno-

Table 1 PCR primers and endonucleases used for genotyping *XPF* and *ERCC1* polymorphisms

Polymorphism	*Primer sequence*	*Annealing temperature (°C)*	*Endonuclease*
XPF (5′-UTR)	F: 5′-CTAGGAGTCGGCTTCCTTCT[a] GC-3′ R: 5′-ACCAGCTGTCGCTCGTACTCC-3′	62	*Pst*I
ERCC1 (3′-UTR)	F: 5′-GATGCCAGAGACAGTGCCCCAAG-3′ R: 5′-ACTACACAGGCTGCTGCTGCA[b] GC-3′	66	*Pvu*II

[a]A base was changed from G to T to create *Pst*I site.
[b]A base was changed from T to A to create *Pvu*II site.
PCR, polymerase chain reaction; UTR, untranslated region.

types separately (variant/variant vs. common/common and variant/common vs. common/common: reference model). Then we chose one dichotomizing model accordant with the reference model. If the ORs for variant/variant and variant/common genotypes in the reference model showed same direction of association, we chose dominant model. Otherwise, we chose recessive model. Gene–environmental interactions between each gene and lifestyle factors (smoking and alcohol drinking) were assessed as case-only designs under the assumption that exposure (smoking and alcohol drinking) and genotypes of each polymorphism are mutually independent (29). In that design using the unconditional logistic model, a measure of interaction was estimated as ORs. Smoking status was treated as a binary variable (ever-smoker and never smoker). Alcohol consumption was also treated as a binary variable (heavy drinker and non-heavy drinker). Statistical package STATA version 8.0 (Stata Corp., College Station, TX, USA) was used for all analyses.

Results

Subject characteristics

As shown in Table 2, cases were older than controls. Male was dominant among cases while male and female were almost equal among controls. Therefore, we decided to adjust age and sex in the following analyses. Regarding smoking status, ever-smoker was dominant among cases. The age- and sex-adjusted OR for ever-smoking in this population was 2.45 (95% CI: 1.33–4.52). Habitual drinking was more common among cases with adjusted OR (2.79, 95% CI: 1.47–5.28).

Table 2 Sex, age, smokers and heavy drinkers among cases and controls

Parameters	*Patients (OSCC; n = 122)*	*Healthy controls (n = 241)*	*OR (95% CI)*[c]
Gender			
Male, *n* (%)	68 (55.7)	118 (49.0)	
Female, *n* (%)	54 (44.3)	123 (51.0)	
Age (mean ± SD)	60.4 ± 12.2	56.8 ± 7.9	
Smoking			
Never smoker, *n* (%)	52 (42.6)	140 (58.1)	1.00
Male	5	34	
Female	47	106	
Ever-smoker[a], *n* (%)	70 (57.4)	101 (41.9)	2.45 (1.33–4.52)*
Male	63	84	
Female	7	17	
Alcohol consumption			
Non-heavy drinker, *n* (%)	92 (65.6)	215 (81.3)	1.00
Male	40	93	
Female	52	122	
Heavy drinker[b], *n* (%)	30 (34.4)	26 (18.7)	2.79 (1.47–5.28)**
Male	28	25	
Female	2	1	

[a]Current + former smokers.
[b]Those who drink alcohol for 5 days or more per week with more 50 ml ethanol/day.
[c]Age- and sex-adjusted.
*$P = 0.004$; **$P = 0.002$.
OSCC, oral squamous cell carcinoma; OR, odds ratio; CI, confidence interval.

Distribution of genotypes in cases and controls

Genotypes of 10 polymorphisms in nine different genes including the *CYP1A1*, two sites of *CYP2E1*, *XPA*, *XPC*, *XPF*, *XPG*, *ERCC1*, *GSTM1* and *GSTT1* in OSCC cases and controls are presented in Table 3. The genotype distributions of all the genetic polymorphisms studied among controls were in accordance with the Hardy–Weinberg equilibrium.

Adjusted ORs for each polymorphism are also presented in Table 3. A significant association was found in c2/c2 (*CYP2E1*5B/*5B*) genotypes of *CYP2E1* 5′-UTR (*Rsa*I site) relative to the c1/c1 and c1/c2 (OR = 3.13, 95% CI: 1.15–8.52). *CYP2E1* intron 6 polymorphism (*Dra*I site) also showed a significant association. Subjects with CC (*CYP2E1*6/*6*) genotype showed two times higher risk of OSCC compared with DD and DC subjects (OR = 2.36, 95% CI: 1.14–4.86). In addition, AG and GG genotypes of *XPA* 5′-UTR polymorphism showed significantly increased risk relative to AA (OR = 2.04, 95% CI: 1.18–3.55). Marginal association was observed with *CYP1A1* exon 7 polymorphism. The subjects with the GG (*CYP1A1*2C/*2C*) and AG genotypes showed reduced risk of OSCC relative to those with the AA (OR = 0.65, 95% CI: 0.40–1.04). Also for *ERCC1* 3′-UTR polymorphism, marginal association was observed. Subjects with AA genotype showed two times higher risk of OSCC compared with CC and CA subjects (OR = 1.95, 95% CI: 0.93–4.09). We did not find any association with other polymorphisms. We also analysed the impact of combined genotypes between *GSTM1* and *GSTT1*. However, no significant combined effect was observed (data not shown).

Interaction between polymorphisms and risks by smoking and alcohol drinking for OSCC

Table 4 shows the ORs for ever-smoking relative to never smoking stratified by genotypes for each polymorphism. Interactions between ever-smoking and dichotomized genotypes by case-only design were also presented. We found statistically significant interactions between ever-smoking and two of *CYP2E1* polymorphisms. The impact of ever-smoking was approximately eight times higher in those with *CYP2E1* 5′-UTR c1/c1 and c1/c2 subjects compared with c2/c2 subjects ($P = 0.036$). Similarly, the impact of ever-smoking was nine times higher in those with DD and DC genotypes in *CYP2E1* intron 6 polymorphisms relative to those with CC genotype ($P = 0.017$). In addition, *ERCC1* polymorphism showed a significant interaction with ever-smoking. The impact of smoking is eight times higher in those with CC and CA genotypes compared to those with the AA genotype ($P = 0.031$). No other polymorphism showed any statistical significance with ever-smoking for the risk of OSCC. As shown in Table 5, no obvious interaction was evident between heavy drinking and any polymorphism examined.

Table 3 Genotype distributions in OSCC cases and controls

Polymorphism	*Genotype*	*Cases, n (%)*	*Controls, n (%)*	*OR (95% CI)*[a,b]	*P-value*[a]
CYP1A1 (exon 7)	AA	81 (66.3)	138 (57.3)	1.00	
	AG	33 (27.1)	88 (36.5)	0.61 (0.37–1.01)	0.053
	GG	8 (6.6)	15 (6.2)	0.97 (0.38–2.46)	0.950
	AG + GG	41 (33.6)	103 (42.7)	0.65 (0.40–1.04)	0.073
CYP2E1 (5′-UTR)	c1/c1	72 (59.0)	164 (68.1)	1.00	
	c1/c2	39 (32.0)	70 (29.1)	1.26 (0.76–2.07)	0.366
	c2/c2	11 (9.0)	7 (2.9)	3.38 (1.22–9.36)	0.019
	c1/c1 + c1/c2	111 (91.0)	234 (97.1)	1.00	
	c2/c2	11 (9.0)	7 (2.9)	3.13 (1.15–8.52)	0.025
CYP2E1 (intron 6)	DD	59 (48.3)	126 (52.3)	1.00	
	DC	45 (36.9)	97 (40.3)	0.97 (0.59–1.58)	0.889
	CC	18 (14.8)	18 (7.5)	2.28 (1.06–4.91)	0.034
	DD + DC	104 (85.3)	223 (92.5)	1.00	
	CC	18 (14.8)	18 (7.5)	2.36 (1.14–4.86)	0.020
GSTM1	Positive	63 (51.6)	115 (47.7)	1.00	
	Null	59 (48.3)	126 (52.3)	0.87 (0.55–1.37)	0.547
GSTT1	Positive	76 (62.3)	136 (56.4)	1.00	
	Null	46 (37.7)	105 (43.6)	0.78 (0.49–1.23)	0.282
XPA (5′-UTR)	AA	23 (18.9)	74 (30.7)	1.00	
	AG	65 (53.3)	105 (43.6)	2.15 (1.19–3.90)	0.011
	GG	34 (27.9)	62 (25.7)	1.88 (0.97–3.62)	0.060
	AG + GG	99 (81.2)	167 (69.3)	2.04 (1.18–3.55)	0.011
XPC (intron 9)	−/−	42 (34.4)	78 (32.4)	1.00	
	−/+	63 (51.6)	128 (53.1)	0.86 (0.52–1.42)	0.556
	+/+	17 (13.9)	35 (14.5)	0.75 (0.36–1.55)	0.434
	−/+ + +/+	80 (65.6)	163 (67.6)	0.83 (0.51–1.34)	0.436
XPF (5′-UTR)	TT	66 (54.1)	119 (49.4)	1.00	
	TA	47 (38.5)	101 (41.9)	0.86 (0.53–1.38)	0.529
	AA	9 (7.4)	21 (8.7)	0.69 (0.28–1.69)	0.416
	TA + AA	56 (45.9)	122 (50.6)	0.84 (0.53–1.32)	0.437
XPG (exon 15)	CC	43 (35.3)	77 (32.0)	1.00	
	CG	59 (48.4)	112 (46.5)	1.01 (0.61–1.69)	0.962
	GG	20 (16.4)	52 (21.6)	0.81 (0.42–1.58)	0.538
	CC + CG	102 (83.6)	189 (78.4)	1.00	
	GG	20 (16.4)	52 (21.6)	0.79 (0.44–1.42)	0.428
ERCC1 (3′-UTR)	CC	75 (61.5)	130 (53.9)	1.00	
	CA	30 (24.6)	94 (39.0)	0.56 (0.33–0.93)	0.025
	AA	17 (13.9)	17 (7.1)	1.56 (0.72–3.36)	0.256
	CC + CA	105 (86.1)	224 (93.0)	1.00	
	AA	17 (13.9)	17 (7.1)	1.95 (0.93–4.09)	0.079

[a]Age and sex smoking status alcohol consumption adjusted.
[b]ORs and 95% CIs for genotypes.
OR, odds ratio; CI, confidence interval; OSCC, oral squamous cell carcinoma; UTR, untranslated region.

Discussion

In this study, we found that (i) *CYP2E1*, *XPA* and *ERCC1* polymorphisms affect the risk of OSCC, (ii) *CYP2E1* and *ERCC1* polymorphisms had significant interactions with smoking and (iii) none of the gene polymorphisms we examined had any interaction with heavy drinking. We evaluated the susceptibility of each genotype of nine different genes to smoking or heavy drinking. Although several studies have investigated the possible association between OSCC risk of smoking or heavy drinking and polymorphisms of several genes involving metabolism of environmental agents (9–12), or DNA repair (5, 13), no investigations to date have been conducted on so many genes with the same samples, especially among Japanese patients (14–17).

As to the polymorphism of *CYP2E1* intron 6 (*Dra*I site), the risk by smoking for OSCC was higher in DD and DC genotypes than in CC genotypes, while the total risk for OSCC in each genotype was higher in CC genotypes than in DD and DC. Also, the results of *CYP2E1* 5′-UTR (*Rsa*I site) exhibited similar trends as intron 6, because of a linkage disequilibrium of these two polymorphisms. Lucas et al. (30) showed that the common D allele homozygotes of *CYP2E1* intron 6 had higher enzyme activity after ethanol induction than the C allele carriers but not at the basal level in *in vivo* studies. The common D allele might have a high CYP2E1 enzyme inducibility against exposing environmental factors while a basal expression level of the CYP2E1 may be lower in the D allele than in C. However, it was also reported that c2/c2 genotype of CYP2E1 5′-UTR had higher protein expression than the common c1/c1 genotype in *in vitro* experiments (23). To clarify this contradiction, further study is necessary to know the functional implication of these polymorphisms in *CYP2E1* gene.

Table 4 Age- and sex-adjusted ORs and 95% CIs by smoking according to genotypes

Polymorphism	*Genotype*	*Cases*[a]	*Controls*[a]	*OR (95% CI)*[b] *for smoking*	*P*[b] *for smoking*	*OR (95% CI)*[b,c] *for interaction*	*P-value*[b,c] *for interaction*
Total		70/52	101/140	2.45 (1.33–4.52)	0.004		
CYP1A1 (exon 7)	AA	45/36	58/80	2.71 (1.28–5.71)	0.009		
	AG	21/12	36/52	1.53 (0.43–5.49)	0.512		
	GG	4/4	7/8	–	–		
	AG +GG	25/16	43/60	1.88 (0.61–5.79)	0.269	1.34 (0.37–4.84)	0.659
CYP2E1 (5′-UTR)	c1/c1	36/36	69/95	2.16 (0.99–4.69)	0.052		
	c1/c2	29/10	28/42	4.92 (1.48–16.30)	0.009		
	c2/c2	5/6	4/3	0.33 (0.18–6.21)	0.463		
	c1/c1 + c1/c2	65/46	97/137	2.81 (1.48–5.36)	0.002	7.98 (1.14–55.68)	0.036
	c2/c2	5/6	4/3	0.33 (0.18–6.21)	0.463		
CYP2E1 (intron 6)	DD	31/28	50/76	2.01 (0.84–4.83)	0.118		
	DC	30/15	41/56	5.93 (1.91–18.41)	0.002		
	CC	9/9	10/8	0.66 (0.13–3.51)	0.629		
	DD + DC	61/43	91/132	3.13 (1.59–6.16)	0.001	9.67 (1.51–61.96)	0.017
	CC	9/9	10/8	0.66 (0.13–3.51)	0.629		
GSTM1	Positive	40/23	51/64	2.47 (1.06–5.74)	0.035		
	Null	30/29	50/76	2.42 (0.99–5.93)	0.053	0.76 (0.23–2.53)	0.655
GSTT1	Positive	42/34	55/81	2.94 (1.27–6.80)	0.012		
	Null	28/18	46/59	2.06 (0.82–5.17)	0.124	0.63 (0.17–2.31)	0.486
XPA (5′-UTR)	AA	13/10	36/38	2.45 (0.67–8.92)	0.175		
	AG	35/30	43/62	3.20 (1.21–8.47)	0.019		
	GG	22/12	22/40	1.91 (0.60–6.04)	0.271		
	AG +GG	57/42	65/102	2.57 (1.27–5.22)	0.009	0.48 (0.11–2.16)	0.336
XPC (intron 9)	−/−	24/18	33/45	3.31 (1.14–9.59)	0.028		
	−/+	39/24	50/78	2.45 (1.04–5.76)	0.040		
	+/+	7/10	18/17	1.21 (0.19–7.62)	0.838		
	−/+ + +/+	46/34	68/95	2.05 (0.95–4.39)	0.066	0.48 (0.13–1.87)	0.291
XPF (5′-UTR)	TT	37/29	53/66	2.20 (0.94–5.18)	0.070		
	TA	28/19	41/60	2.04 (0.81–5.15)	0.181		
	AA	5/4	7/14	–	–		
	TA + AA	33/23	48/74	2.65 (1.10–6.36)	0.030	0.60 (0.17–2.12)	0.452
XPG (exon 15)	CC	26/17	35/42	3.23 (1.11–9.41)	0.031		
	CG	35/24	45/67	2.03 (0.85–4.86)	0.111		
	GG	9/11	21/31	2.10 (0.43–10.33)	0.363		
	CC + GG	61/41	80/109	2.50 (1.29–4.87)	0.007		
	GG	9/11	21/31	2.10 (0.43–10.33)	0.363	0.33 (0.06–1.74)	0.192
ERCC1 (3′-UTR)	CC	39/36	52/78	2.03 (0.89–4.63)	0.093		
	CA	17/13	43/51	1.80 (0.60–5.42)	0.294		
	AA	14/3	6/11	14.01 (1.69–116.15)	0.014		
	CC + CA	56/49	95/129	1.98 (1.03–3.79)	0.040		
	AA	14/3	6/11	14.01 (1.69–116.15)	0.014	8.49 (1.22–59.31)	0.031

[a]Ever/never smoker.
[b]Age- and sex-adjusted.
[c]Interaction between smoking and genotype by case-only study.
OR, odds ratio; CI, confidence interval; UTR, untranslated region.

To our knowledge, this is the first study examining the association of OSCC and a polymorphism in *XPA*. Our results showed that AG and GG genotypes had a significantly increased risk of OSCC. However, in a few studies for lung cancer, it was reported that GG or AG/GG genotypes had a significantly decreased risk (25, 31). It was also reported that AG and GG genotypes had a higher DNA repair capacity than AA genotypes in lymphocytes of healthy controls (31). Inconsistent results might be due to a different role of *XPA* in different cell types or tissues. Moreover, linkage disequilibrium between *XPA* polymorphism and other polymorphisms located close to *XPA* might be a possible explanation for this inconsistency. Loss of heterozygosity at 9q13-qter where *XPA* is located has been found in 35% of head and neck squamous cell carcinoma (32), and tumour suppressor genes may be expected in this region. Association with the *XPA* polymorphism requires further evaluation.

We found a significant risk change with the *ERCC1* 3′-UTR polymorphism. In addition, we observed a significant gene–environment interaction with smoking in connection with this polymorphism. Although little evidence is available on the function of the *ERCC1* polymorphism, our results might suggest a decreased repair capacity in the AA genotype. Interestingly, this polymorphism overlapped the 3′-end of *ASE-1/CAST* gene whose direction was opposite to *ERCC1* on the genome (33, 34), accompanied with amino acid substitution of lysine or glutamine in the lysine/arginine basic repeat region of *ASE-1/CAST* (35). While low mRNA expression of *ERCC1* gene among head and neck SCC patients was reported (8), the risk of OSCC might be influenced by not only the *ERCC1* gene but also the

Table 5 Age- and sex-adjusted ORs and 95% CIs by heavy drinking according to genotypes

Polymorphism	*Genotype*	*Cases*[a]	*Controls*[a]	*OR (95% CI)*[b] *for heavy drinking*	*P-value*[b] *for heavy drinking*	*OR (95% CI)*[b,c] *for interaction*	*P-value*[b,c] *for interaction*
Total		30/92	26/215	2.79 (1.47–5.28)	0.002		
CYP1A1 (exon 7)	AA	22/59	13/125	4.67 (2.04–10.72)	< 0.001		
	AG	7/26	11/77	1.12 (0.34–3.67)	0.858		
	GG	1/7	2/13	1.60 (0.97–29.28)	0.720		
	AG + GG	8/33	13/90	1.24 (0.42–3.67)	0.694	0.54 (0.20–1.47)	0.226
CYP2E1 (5′-UTR)	c1/c1	15/57	21/143	2.15 (0.92–4.99)	0.076		
	c1/c2	12/27	5/65	4.93 (1.48–16.44)	0.009		
	c2/c2	3/8	0/7	–	–		
	c1/c1 + c1/c2	65/46	97/137	2.67 (1.38–5.17)	0.004	1.00 (0.22–4.67)	0.997
	c2/c2	3/8	0/7	–	–		
CYP2E1 (intron 6)	DD	15/44	14/112	2.88 (1.13–7.33)	0.026		
	DC	12/33	12/85	2.81 (1.05–7.55)	0.040		
	CC	3/15	0/18	–	–		
	DD + DC	61/43	91/132	2.87 (1.46–5.64)	0.002	2.54 (0.63–10.21)	0.190
	CC	3/15	0/18	–	–		
GSTM1	Positive	15/48	13/102	2.26 (0.94–5.42)	0.069		
	Null	15/44	13/113	3.70 (1.43–9.54)	0.007	1.56 (0.61–3.95)	0.353
GSTT1	Positive	18/58	11/125	3.94 (1.63–9.54)	0.002		
	Null	12/34	15/90	1.88 (0.73–4.88)	0.193	0.88 (0.34–2.23)	0.781
XPA (5′-UTR)	AA	7/16	10/64	3.78 (1.06–13.48)	0.041		
	AG	12/53	10/95	2.38 (0.88–6.45)	0.087		
	GG	11/23	6/56	2.59 (0.75–8.93)	0.131		
	AG + GG	23/76	16/151	2.69 (1.26–5.78)	0.011	0.47 (0.14–1.54)	0.212
XPC (intron 9)	−/−	12/30	6/72	7.41 (2.21–24.88)	0.001		
	−/+	15/48	13/115	2.03 (0.83–4.97)	0.121		
	+/+	3/14	7/28	1.28 (0.23–7.23)	0.782		
	−/+ + +/+	18/62	20/143	1.73 (0.79–3.77)	0.167	0.52 (0.19–1.42)	0.202
XPF (5′-UTR)	TT	19/47	12/107	4.29 (1.76–10.45)	0.001		
	TA	8/39	10/91	1.50 (0.51–4.38)	0.459		
	AA	3/6	4/17	6.32 (0.41–96.99)	0.186		
	TA + AA	11/35	14/108	1.63 (0.64–4.20)	0.305	0.42 (0.16–1.10)	0.079
XPG (exon 15)	CC	13/30	12/65	2.80 (1.01–7.74)	0.047		
	CG	12/47	7/105	3.04 (1.05–8.82)	0.041		
	GG	5/15	7/45	3.50 (0.77–15.88)	0.105		
	CC + CG	25/77	19/170	2.76 (1.35–5.63)	0.005		
	GG	5/15	7/45	3.50 (0.77–15.88)	0.105	1.23 (0.35–4.34)	0.745
ERCC1 (3′-UTR)	CC	17/58	13/117	2.61 (1.11–6.12)	0.027		
	CA	7/23	12/82	2.45 (0.75–7.97)	0.138		
	AA	6/11	1/16	7.14 (0.58–87.18)	0.124		
	CC + CA	24/81	25/199	2.52 (1.28–4.96)	0.007		
	AA	6/11	1/16	7.14 (0.58–87.18)	0.124	1.42 (0.42–4.80)	0.568

[a]Heavy drinker/non-heavy drinker.
[b]Age- and sex-adjusted.
[c]Interaction between heavy drinking and genotype by case-only study.
OR, odds ratio; CI, confidence interval; UTR, untranslated region.

ASE-1/*CAST* gene with the modulation of this polymorphism. Further molecular investigation to determine the functions of this polymorphism on such two genes must be encouraged.

For these 10 genetic polymorphisms, we found some interactions with smoking but none with heavy drinking. Our results suggest a possible difference between DNA damage and its repair system induced by smoking and that by alcohol drinking. Generally speaking, bulky adducts which is one of the smoking-induced DNA damages are considered to be repaired by NER (6). Because only polymorphisms in NER-related genes were examined in this study, we might be able to find interactions with smoking but without drinking. On the contrary, oxidative DNA damage produced by alcohol (36, 37) and tobacco (38) is expected to be repaired by base excision repair (39). As little evidence is available on the actual impact of the polymorphisms we evaluated in alcohol-induced DNA damage, biological studies specific to this issue are required. Furthermore, molecular epidemiological studies evaluating this issue are also warranted.

Except for three polymorphisms, *CYP2E1* 5′-UTR, intron 6 and *ERCC1* 3′-UTR, we could not find any significant interaction between genetic polymorphisms and environmental factors such as smoking and heavy drinking. Because our study size was large compared with previous studies for OSCC but too small to provide enough statistical power, a significant interaction between genetic polymorphisms and environmental factors may be found by a study with a larger sample. In other words, the significant interaction we found in case-only design, which is free from the selection bias of controls, was one of the strengths of this study. If the

genotypes enhancing the effects of smoking and/or heavy drinking are identified, more individualized recommendations to quit smoking and/or reduce alcohol intake will become possible with a beneficial impact on cancer prevention.

In conclusion, we found an increased risk of OSCC with *CYP2E1*, *XPA* and *ERCC1* polymorphisms. Significant gene–environment interactions between *CYP2E1* and *ERCC1* polymorphisms were also detected with smoking but not with alcohol drinking. Further biological and epidemiological studies are thus warranted.

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