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Impact of RECK gene polymorphisms and environmental factors on oral cancer susceptibility and clinicopathologic characteristics in Taiwan

Tsung-Te Chung[1,2], Min-Shing Pan[3], Chien-Long Kuo[4], Ruey-Hong Wong[5], Chiao-Wen Lin[1], Mu-Kuan Chen[1,6] and Shun-Fa Yang[1,7,*]

[1]Institute of Medicine, Chung Shan Medical University, No. 110, Section 1, Chien-Kuo North Road, Taichung 402, Taiwan, [2]Department of Otolaryngology-Head and Neck Surgery, Show Chwan Memorial Hospital, Changhua 500, Taiwan, [3]Department of Otolaryngology, Chang-Bin Show Chwan Memorial Hospital, Changhua 505, Taiwan, [4]Department of Pathology, Chwan Memorial Hospital, Changhua 500, Taiwan, [5]Department of Public Health, Chung Shan Medical University, Taichung 402, Taiwan, [6]Department of Otorhinolaryngology-Head and Neck Surgery, Changhua Christian Hospital, Changhua 500, Taiwan and [7]Department of Medical Research, Chung Shan Medical University Hospital, Taichung 402, Taiwan

*To whom correspondence should be addressed. Tel: +886 4 24739595 ext. 34253; Fax: +886 4 24723229;
Email: ysf@csmu.edu.tw

Oral cancer is the fourth common male cancer and causally associated with environmental carcinogens in Taiwan. The reversion-inducing-cysteine-rich protein with Kazal motifs (RECK) has a significant effect on tumorigenesis by limiting angiogenesis and invasion of tumors through the extracellular matrix. RECK downregulation has been confirmed in many human cancers and associated with lymph node metastasis clinically. In the present hospital-based case-controlled study, the demographic, RECK genotype and clinicopathologic data from 341 male oral cancer patients and 415 cancer-free controls were investigated. We found that RECK rs10814325, rs16932912, rs11788747 or rs10972727 polymorphisms were not associated with oral cancer susceptibility. Among 488 smokers, RECK polymorphisms carriers with betel quid chewing have a 7.62-fold [95% confidence interval (CI), 2.96–19.64] to 25.33-fold (95% CI, 9.57–67.02) risk to have oral cancer compared with RECK wild-type carrier without betel quid chewing. Among 352 betel quid chewers, RECK polymorphisms carriers with smoking have a 6.68-fold (95% CI, 1.21–36.93) to 18.57-fold (95% CI, 3.80–90.80) risk to have oral cancer compared with those who carried wild-type without smoking. In 263 betel quid chewing oral cancer patients, RECK rs10814325 polymorphism have a 2.26-fold (95% CI, 1.19–4.29) risk to have neck lymph node metastasis compared with RECK wild-type carrier. These results support that gene–environment interactions between the RECK polymorphisms, smoking and betel quid may alter oral cancer susceptibility and metastasis.

Introduction

The reversion-inducing-cysteine-rich protein with Kazal motifs (RECK) gene was isolated as a novel transformation suppressor gene against activated *ras* oncogenes, and it induced flat reversion in v-K-ras-transformed NIH/3T3 cells (1,2). RECK gene encodes a membrane-anchored glycoprotein that can negatively regulates matrix metalloproteinases (MMPs) and inhibits tumor invasion, angiogenesis and metastasis (3,4). RECK-expressing tumors showed significant reduction in microvessel density and branching and result in tumor tissue death in mice (4). RECK downregulation or promoter hypermethylation had been confirmed in many human cancers including pancreatic cancer, breast cancer, lung cancer, colorectal cancer, cholangiocarcinoma, gastric cancer, prostate cancer, oral cancer, esophageal cancer and osteosarcoma and correlated with tumor metastasis or poor prognosis (5–15).

Abbreviations: CI, confidence interval; MMP, matrix metalloproteinase; PCR, polymerase chain reaction; SNP, single-nucleotide polymorphism.

In Taiwan, oral cancer is the fourth most common cancer among men since 2003. Crude mortality rate of oral cancer was 10.1 per 100 000 in 2007 and ranked as the sixth cause of cancer death (16). Betel quid chewing, tobacco smoking and alcohol consumption have been documented as risk factors for oral cancer development (17,18). Single-nucleotide polymorphisms (SNPs) in genes encoding for cancer susceptibility factors have been documented to influence gene expression, protein function and disease susceptibility in certain individuals (19). It also reflects the individual differences of response to drug and environmental factors. Several gene polymorphisms combined with betel quid or tobacco environmental carcinogens to increase oral cancer susceptibility had been documented (20–25).

The impact of RECK on human cancers metastasis or prognosis have been well documented, but RECK gene SNPs and the environmental carcinogens in oral cancer susceptibility and clinical features remains poorly investigated. In this study, a case–control study was performed for four SNPs, which located in the promoter or exon region of RECK gene (Figure 1) to analyze the associations between RECK gene SNPs, environment risk factors and oral cancer clinicopathologic characteristics.

Materials and methods

Study subjects and specimen collection

The present hospital-based case–control study comprised 341 male patients who were diagnosed as oral and oropharyngeal squamous cell carcinoma with histopathological confirmation. Subjects were recruited between April 2007–10 from Show Chwan Memorial Hospital, Changhua Christian Hospital (Changhua County) and Chung Shan Medical University Hospital (Taichung city). Changhua County and Taichung city are two neighboring area at mid-Taiwan. Meanwhile, controls were enrolled from the physical examination during those three hospitals, which are also the facilities that cases were collected from. At the end of recruitment, a total of 415 male participants that had neither self-reported history of cancer of any sites were included. In addition, subjects with oral precancerous disease such as oral submucous fibrosis, leukoplakia, erythroplakia, verrucous hyperplasia etc. were excluded from control group. The participation rate was ~89% for cases and 76% for controls.

As for cases and controls, exposure information, including betel quid chewing (ever- versus never-user), tobacco use (smoker versus non-smoker) and alcohol consumption (current heavy drinker, defined by Centers for Disease Control and Prevention as consuming an average of more than two drinks per day versus not current heavy drinker), were all obtained from questionnaires. While medical information of cases, including tumor node metastasis clinical staging, primary tumor size, lymph node involvement and histologic grade, were obtained from medical records. Oral cancer patients were staged clinically at the time of diagnosis according to the tumor node metastasis staging system of the American Joint Committee on Cancer (AJCC) (2002). Tumor differentiation examined by pathologist according to AJCC classification. This study has been reviewed and approved by Institutional Review Board and informed written consent was obtained from each individual.

Selection of RECK polymorphisms

In dbSNP database, a total of 13 SNPs has been documented in the 21 exons region of the RECK gene, including four SNPs located in the coding sequences of the gene (Exons 1, 9, 13 and 15). To obtain adequate power for evaluating the potential association, we investigated rs16932912 (V275L in exon 9), rs11788747 (P520P in exon 13) and rs10972727 (R625R in exon 15), those with minor allele frequencies ≥5% (26). Furthermore, another SNP of RECK gene in the promoter region (rs10814325; −402 C/T) was selected in this study since this SNP was predicted to be able to change transcription factor-binding sites by TRANSFAC.

Genomic DNA extraction

Genomic DNA was extracted by QIAamp DNA blood mini kits (Qiagen, Valencia, CA) according to the instructions of manufacturer. DNA was dissolved in TE buffer [10 mM Tris (pH 7.8), 1 mM ethylenediaminetetraacetic acid] and then quantified by a measurement of OD260. Final preparation was stored at −20°C and used as templates for polymerase chain reaction (PCR).

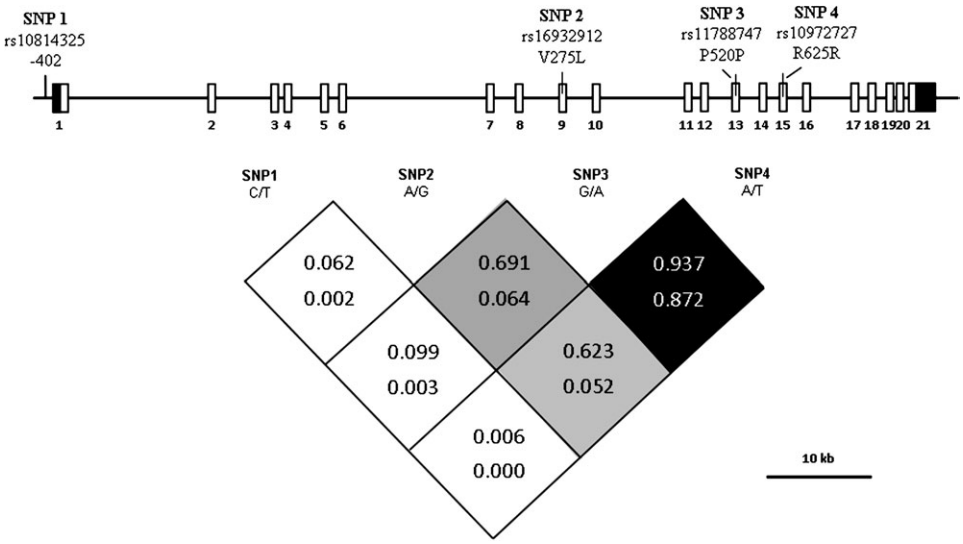

Fig. 1. The location of human RECK gene SNPs, functional amino acid and their pairwise linkage disequilibrium patterns. Schematic presentation of the RECK, indicating the locations of the SNP polymorphism and the pairwise linkage disequilibrium measures D′ (above) and r^2 (below). The measure of D′ of SNP is shown graphically according to a grey scale, where white represents low D′ and dark represents high D′.

PCR–restriction fragment length polymorphism

The RECK rs16932912, rs11788747 and rs10972727 gene polymorphisms were determined by polymerase chain reaction-restriction fragment length polymorphism (PCR–restriction fragment length polymorphism) assay. The forward and reverse primers used for analysis of RECK gene polymorphisms were designed as 5′-TGGAGATTGTTGATGGTCTC-3′ and 5′-CGGTACACAATGCTCAATAC-3′ for rs16932912 SNP at exon 9 (353 bp), 5′-GTAGAAGAAGTGACTCATCC-3′ and 5′-ATCTCACTCCGAAGATAACC-3′ for rs11788747 SNP at exon 13 (242 bp), 5′-TTCTGTCAGGTCATGGAACA-3′ and 5′-TGCAGTTAAGACTGGAGAAG-3 for rs10972727 SNP at exon 15 (224 bp). The PCR was performed in a 10 ml volume containing 100 ng DNA template, 1.0 ml of 10 × PCR buffer (Invitrogen, Carslbad, CA), 0.25 U of *Taq* DNA polymerase (Invitrogen), 0.2 mM deoxynucleoside triphosphates (Promega, Madison, WI) and 200 nM of each primer (MDBio, Taipei, Taiwan). The PCR cycling conditions were 5 min at 94°C followed by 35 cycles of 1 min at 94°C, 1 min at 60°C and 2 min at 72°C, with a final step at 72°C for 20 min. Ten microliters aliquot of PCR product was subjected to digestion at 37°C for 4 h in a 15 μl reaction buffer containing 1.5 μl 10 × buffer (New England Biolabs, Beverly, MA) and 5 U of *TfiI*, *RsaI* and *HpyCH4IV* (New England Biolabs) for RECK rs16932912, rs11788747 and rs10972727, respectively. Digested products were separated on a 3% agarose gel and then stained with ethidium bromide. For rs16932912, G allele yielded 353 bp products, whereas A alleles yielded a 250 and 103 bp product; for rs11788747, G allele yielded 102 and 140 bp products, whereas A alleles yielded a 242 bp product; for rs10972727, T allele yielded a 224 bp products, whereas A alleles yielded 105 and 119 bp products. To validate results from PCR–restriction fragment length polymorphism, ∼10% of assays were repeated and several cases of each genotype were confirmed by the DNA sequence analysis.

Real-time PCR

The allelic discrimination of the RECK rs10814325 gene polymorphisms was assessed with the ABI StepOne™ Real-Time PCR System (Applied Biosystems, Foster City, CA) and analyzed using SDS v3.0 software (Applied Biosystems), using the TaqMan assay (assay IDs: C_27084758_10). The final volume for each reaction was 5 μl, containing 2.5 μl TaqMan Genotyping Master Mix, 0.125 μl TaqMan probes mix and 10 ng genomic DNA. The real-time PCR reaction included an initial denaturation step at 95°C for 10 min, followed by 40 cycles, each consisting of 95°C for 15 s and 60°C for 1 min.

Statistical analysis

Hardy–Weinberg equilibrium was assessed using a chi-square goodness-of-fit test for biallelic markers. A Mann–Whitney *U*-test and a Fisher's exact test were used to compare the differences of age as well as demographic characteristics distributions between controls and oral cancer patients. The adjusted odds ratio with their 95% confidence intervals (CIs) of the association between genotype frequencies and oral cancer risk as well as clinical pathological characteristics were estimated by multiple logistic regression models after controlling for other covariates, such as age (year), betel nut chewing (ever-versus never-user), alcohol (current heavy drinker versus not current heavy drinker) and tobacco consumption (smoker versus non-smoker). A P value <0.05 was considered significant. The pairwise linkage disequilibrium between individual SNPs was evaluated with Haploview software. The data were analyzed on SAS statistical software (Version 9.1, 2005; SAS Institute, Cary, NC).

Results

The distributions of demographic characteristics are shown in Table I. There were significantly difference distribution of betel quid chewing ($P < 0.001$), alcohol consumption ($P < 0.001$) and tobacco consumption ($P < 0.001$) between oral cancer patients and controls.

In our recruited control group, the frequencies of RECK genes were in Hardy–Weinberg equilibrium ($P > 0.05$). The reconstructed linkage disequilibrium plot for the four SNPs was shown in Figure 1. We found that rs11788747 and rs10972727 show a high degree of D′ in our study. The genotype distributions as well as the association between oral cancer and gene polymorphisms of RECK are shown in Table II. The alleles with the highest distribution frequency for rs10814325, rs16932912, rs11788747 and rs10972727 genes of RECK in both of our recruited oral cancer patients and healthy control were heterozygous T/C, homozygous G/G, homozygous A/A and homozygous T/T, respectively. After adjusting variables, there was no significant difference to have oral cancer in individuals with rs10814325, rs16932912, rs11788747 and rs10972727 genes of RECK polymorphism compared with wild-type individuals.

The combined effects of environmental factors and RECK gene SNPs on the risk of oral cancer are shown in Tables III and IV. Among 488 smokers, these subjects with rs10814325 polymorphism and betel quid chewing habit had a risk of 7.62-folds (95% CI = 2.960–19.64) to have oral cancer comparing wild-type without betel quid chewing (Table III). For rs16932912, rs11788747 and rs10972727 gene of RECK, the smokers with polymorphic genes and betel quid chewing habit had a corresponding risk of 25.33-folds (95% CI = 9.57–67.02), 9.37-folds (95% CI =3.92–22.39) and 10.65-folds (95% CI = 4.35–26.06) to have oral cancer compared with smoker with wild-type gene without betel quid chewing habit subjects.

Among 352 betel quid consumers, these subjects with RECK polymorphic rs10814325, rs16932912, rs11788747 or rs10972727 genes and smoking habit had a corresponding risk of 6.68-folds (95% CI = 1.21–36.93), 18.57-folds (95% CI = 3.80–90.80), 6.87

Table I. The distributions of demographical characteristics in 415 controls and 341 patients with oral cancer

Variable	Controls ($N = 415$)	Patients ($N = 341$)	P value
Age (years)	Mean ± SD 52.75 ± 13.80	Mean ± SD 53.30 ± 11.09	$P = 0.556$.
Betel quid chewing			
Never-user	326 (78.6%)	78 (22.9%)	
Ever-user	89 (21.4%)	263 (77.1%)	$P < 0.001$*
Cigarette smoking			
Non-smoker	217 (52.3%)	51 (15.0%)	
Smoker	198 (47.7%)	290 (85.0%)	$P < 0.001$*
Alcohol drinking			
Not current heavy drinker	231 (55.7%)	118 (34.6%)	
Current heavy drinker	184 (44.3%)	223 (65.4%)	$P < 0.001$*

Mann–Whitney U-test or Fisher's exact test was used between controls and patients with oral cancer.
*P value <0.05 as statistically significant.

folds (95% CI = 1.57–30.05) and 11.52-folds (95% CI = 2.31–57.56) to have oral cancer as compared with betel quid chewer with wild-type gene without smoking habit (Table IV). We further evaluated the gene–environment statistical interaction between the RECK polymorphisms, smoking and betel quid on oral cancer (Tables III and IV). Statistical significance was found for the interaction between all RECK polymorphisms, smoking and betel quid on oral cancer development ($P < 0.001$), whereas the interaction of rs10814325 in smoker group nearly reached significant ($P = 0.0518$). These results suggested that RECK gene polymorphisms have a strong impact on oral cancer susceptibility in betel quid and/or smoking consumers.

To clarify the role of RECK gene polymorphisms on oral cancer clinicopathologic statuses, such as tumor node metastasis clinical staging, primary tumor size, lymph node involvement and histologic grade, the distribution frequency of clinical statuses and RECK genotypes frequencies in oral cancer patients were estimated. No significant association between rs16932912, rs11788747 and rs10972727 gene polymorphisms and the clinicopathologic statuses were observed. However, among 263 male oral cancer patients with betel quid consumption, who have polymorphic rs10814325 gene had a higher risk of neck lymph node metastasis (adjusted OR = 2.26; 95% CI = 1.19–4.29) as compared with the patients with rs10814325 wild-type, but there is no difference in early or advanced clinical stage, primary tumor size or histologic grade (Table V).

Discussion

In this study, we provide novel information of SNPs of RECK on the oral cancer susceptibility, interactions with environmental risk factors and clinicopathologic statuses association.

Rs10814325, rs16932912, rs11788747 and rs10972727 SNPs were located at promoter, exon 9, 13 and 15 region of RECK gene, respectively. Valine change to Lysine in rs16932912 SNP, but no amino acid changes happen in rs11788747 and rs10972727 SNPs (Figure 1). Rs10814325 promoter SNPs are predicted to change transcription factor-binding sites by TRANSFAC, although the functional importance of these SNPs has not been tested experimentally. Since carcinogen exposure and possible genetic predisposition vary between different geographic areas. In a Taiwan's cohort study (27) and a case–control study in Changhua county (18) show that betel quid chewing and smoking habits are a risk factor to developing oral cancer. In this study, higher ratios of individuals with betel quid chewing and smoking habits in oral cancer patients (77.1 and 85.0%) than that in control (21.4 and 47.7%) were found, which indicates betel quid chewing and tobacco smoking habit are highly associated to the increased risk of oral cancer.

Table II. Adjusted odds ratio (AOR) and 95% CI of oral cancer associated with RECK genotypic frequencies

Variable	Controls ($N = 415$) n (%)	Patients ($N = 341$) n (%)	AOR (95% CI)
rs10814325			
TT	120 (28.9%)	95 (27.9%)	1.00
TC	189 (45.5%)	143 (41.9%)	0.80 (0.49–1.31)
CC	106 (25.5%)	103 (30.2%)	1.13 (0.65–1.99)
TC + CC	295 (71.1%)	246 (72.1%)	0.89 (0.57–1.38)
rs16932912			
GG	214 (51.6%)	155 (45.5%)	1.00
GA	169 (40.7%)	155 (45.5%)	1.27 (0.84–1.93)
AA	32 (7.7%)	31 (9.1%)	0.97 (0.44–2.13)
GA + AA	201 (48.4%)	186 (54.5%)	1.21 (0.82–1.80)
rs11788747			
AA	237 (57.1%)	208 (61.0%)	1.00
AG	153 (36.9%)	106 (31.1%)	0.79 (0.51–1.23)
GG	25 (6.0%)	27 (7.9%)	1.10 (0.49–2.46)
AG + GG	178 (42.9%)	133 (39.0%)	0.82 (0.55–1.22)
rs10972727			
TT	236 (56.9%)	208 (61.0%)	1.00
TA	152 (36.6%)	111 (32.6%)	0.83 (0.54–1.28)
AA	27 (6.5%)	22 (6.5%)	0.82 (0.35–1.92)
TA + AA	179 (43.1%)	133 (39.0%)	0.81 (0.54–1.21)

The adjusted odds ratio (AOR) with their 95% CIs were estimated by multiple logistic regression models after controlling for age (years), betel nut chewing (ever- versus never-user), alcohol (current heavy drinker versus not current heavy drinker) and tobacco consumption (smoker versus non-smoker).

The betel quid used in Taiwan contains areca nut, lime and Piper betel inflorescence or leaf (28). Hydroxychavicol, a phenolic component of betel leaf, has the capacity to modulate cigarette carcinogen benzo[a]pyrene-mediated toxic effects by induction of dihydrodiol dehydrogenase and hypoxanthine phosporibisyltransferase gene mutation (29). Evidence showed that alkaline saliva generated by chewing betel quid may play a role in cigarette-related nicotine-induced DNA damage and reactive oxygen species may be involved in generating this DNA damage (30). These findings are a possible molecular explanation for synergistic effect of betel quid chewing and smoking in oral cancer development.

Ki-ras protooncogene activation and ras p21 overexpression may play an important role in the initiation and progression of oral cancer in betel quid chewer (31,32). Ras facilitates phosphorylation Sp1/Sp3 factors which increases RECK promoter Sp1 site affinity, thus reducing RECK expression (33). In human sample and cell culture studies, the arecoline (betel quid component) can increases protein and messenger RNA levels of MMP-2 (34) and betel quid chewing increases MMP-9 protein level (35).

In literatures, the MMP-9 promoter -1562 C > T polymorphism is associated with oral cancer in younger betel quid chewer (21) and urokinase plasminogen activator gene polymorphism with betel quid chewing and smoking increase oral cancer development (24). Vascular endothelial growth factor gene −406 C/T polymorphism also has a higher risk to have oral cancer than normal control (36). MMPs are a diverse family of enzymes capable of degrading extracellular matrix (ECM) components. Early expression of MMPs in tumor or adjacent tissues helps to remodel the ECM and release ECM and/or membrane-bound growth factors, which provides a favorable microenvironment for the development the primary tumor (37). The urokinase plasminogen activator system is also involved in ECM degradation. The main function of RECK is to suppress MMPs and angiogenesis in animal study (4). In tumor tissue study, an inverse relationship between RECK and both vascular endothelial growth factor and microvessel density was also identified in colorectal cancer (8) and non-small cell lung cancer (7). In our study, RECK gene SNPs alone is not contribute to oral cancer susceptibility (Table II). The

Table III. Adjusted odds ratio (AOR) and 95% CI of oral cancer associated with RECK genotypic frequencies and betel quid chewing among 488 smokers

Variable	Controls (n = 198) (%)	Patients (n = 290) (%)	AOR (95% CI)
rs10814325			
[a]TT genotype without betel quid chewing	36 (18.2%)	15 (5.2%)	1.00
[b]TC or CC genotype or betel quid chewing	115 (58.1%)	94 (32.4%)	1.44 (0.56–3.49)
[c]TC or CC genotype and betel quid chewing	47 (23.7%)	181 (62.4%)	**7.62 (2.96–19.64)**
Test for interaction	χ^2 = 37.42 (1 d.f.), $P < 0.0001$		
rs16932912			
[a]GG genotype without betel quid chewing	70 (35.3%)	20 (6.9%)	1.00
[b]GA or AA genotype or betel quid chewing	94 (47.5%)	133 (45.9%)	**8.55 (3.88–18.86)**
[c]GA or AA genotype and betel quid chewing	34 (17.2%)	137 (47.2%)	**25.33 (9.57–67.02)**
Test for interaction	χ^2 = 98.41 (1 d.f.), $P < 0.0001$		
rs11788747			
[a]AA genotype without betel quid chewing	73 (36.9%)	30 (10.3%)	1.00
[b]AG or GG genotype or betel quid chewing.	93 (47.0%)	167 (57.6%)	**5.49 (2.96–10.18)**
[c]AG or GG genotype and betel quid chewing	32 (16.1%)	93 (32.1%)	**9.37 (3.92–22.39)**
Test for interaction	χ^2 = 78.88 (1 d.f.), $P < 0.0001$		
rs10972727			
[a]TT genotype without betel quid chewing	72 (36.4%)	30 (10.3%)	1.00
[b]AT or AA genotype or betel quid chewing	96 (48.5%)	165 (56.9%)	**5.20 (2.80–9.65)**
[c]AT or AA genotype and betel quid chewing.	30 (15.2%)	95 (32.8%)	**10.65 (4.35–26.06)**
Test for interaction	χ^2 = 77.09 (1 d.f.), $P < 0.0001$		

The adjusted odds ratio (AOR) with their 95% CIs were estimated by multiple logistic regression models after controlling for age (year), betel nut chewing (ever- versus never-user), alcohol (current heavy drinker versus not current heavy drinker) and tobacco consumption (smoker versus non-smoker). Bold values as statistically significant. $P < 0.05$.
[a]Individual with wild genotype but without betel quid chewing.
[b]Individual with either at least one mutated genotype or betel quid chewing.
[c]Individual with both at least one mutated genotype and betel quid chewing.

Table IV. Adjusted odds ratio (AOR) and 95% CI of oral cancer associated with RECK genotypic frequencies and smokers among 352 betel quid chewer

Variable	Controls (n = 89) (%)	Patients (n = 263) (%)	AOR (95% CI)
rs10814325			
TT genotype and non-smoker[a]	8 (9.0%)	5 (1.9%)	1.00
TC or CC genotype or smoker[b]	34 (38.2%)	77 (29.3%)	6.57 (0.99–43.79)
TC or CC genotype and smoker[c]	47 (52.8%)	181 (68.8%)	**6.68 (1.21–36.93)**
Test for interaction	χ^2 = 3.78 (1 d.f.), P = 0.0518.		
rs16932912			
GG genotype and non-smoking[a]	12 (13.5%)	9 (3.4%)	1.00
GA or AA genotype or smoking[b]	43 (48.3%)	117 (44.5%)	**4.49 (1.31–15.35)**
GA or AA genotype with smoking[c]	34 (38.2%)	137 (52.1%)	**18.57 (3.80–90.80)**
Test for interaction	χ^2 = 7.72 (1 df), P = 0.0055		
rs11788747			
AA genotype and non-smoking[a]	12 (13.5%)	8 (3.0%)	1.00
AG or GG genotype or smoking[b]	45 (50.6%)	162 (61.6%)	**4.28 (1.26–14.53)**
AG or GG genotype and smoking[c]	32 (35.9%)	93 (35.4%)	**6.87 (1.57–30.05)**
Test for interaction	χ^2 = 7.88 (1 d.f.), P = 0.0049		
rs10972727			
TT genotype and non-smoking[a]	13 (14.6%)	8 (3.0%)	1.00
AT or AA genotype or smoking[b]	46 (51.7%)	160 (60.8%)	**4.91 (1.45–16.66)**
AT or AA genotype and smoking[c]	30 (33.7%)	95 (36.1%)	**11.52 (2.31–57.56)**
Test for interaction	χ^2 = 9.04 (1 d.f.), P = 0.0026		

The adjusted odds ratio (AOR) with their 95% CIs were estimated by multiple logistic regression models after controlling for age (years), betel nut chewing (ever- versus never-user), alcohol (current heavy drinker versus not current heavy drinker) and tobacco consumption (smoker versus non-smoker).
[a]Individual with wild genotype but without smoking.
[b]Individual with either at least one mutated genotype or smoking.
[c]Individual with both at least one mutated genotype and smoking.

synergistic effect of environmental factor (betel quid and smoking) and RECK gene polymorphisms on the risk of oral cancer (Tables III and IV) are well demonstrated. Betel quid and tobacco carcinogens influence ras expression, then alter RECK promoter affinity or down-regulate RECK function. Consequently, it may upregulate MMPs to develop oral cancer.

It have been reported that low expression of RECK in colorectal cancer was more frequent lymph node metastasis (8) and RECK promoter methylation have higher incidence of lymph node metastasis in non-small cell lung cancer (38). In breast cancer patients, RECK promoter −402 T/C SNP carriers had a better survival compared with T/T wild-type carriers (16). In the present study, betel quid chewing oral cancer patients with RECK gene promoter −402 C/T mutation type (rs10814325) has a higher risk to lymph node metastasis than wild-type (38.7% versus 21.7%). This result also implies betel quid carcinogens affinity in RECK

Table V. Adjusted odds ratio (AOR) and 95% CI of clinical statuses and RECK rs10814325 genotype frequencies in oral cancer among 263 betel quid chewers

Variable	Genotypic frequencies		
	n (%)	*n* (%)	AOR (95% CI)
TNM clinical staging	TT ($N = 69$)	TC + CC ($N = 194$)	
Stage I/II	32 (46.4%)	86 (44.3%)	1.00
Stage III/IV	37 (53.6%)	108 (55.7%)	0.98 (0.49–1.96)
Primary tumor size (T)			
≤T2	39 (56.5%)	126 (64.9%)	1.00
>T2	30 (43.5%)	68 (35.1%)	0.67 (0.34–1.35)
Lymph node involvement (*N*)			
No	54 (78.3%)	119 (61.5%)	1.00
Yes	15 (21.7%)	75 (38.7%)	**2.26 (1.19–4.29)**
Histologic grade			
Well	11 (15.9%)	38 (19.6%)	1.00
Moderate/poor	58 (84.1%)	156 (80.4%)	0.95 (0.37–2.43)

The adjusted odds ratio (AOR) with their 95% CIs were estimated by multiple logistic regression models after controlling for age (year), betel nut chewing (ever- versus never-user), alcohol (current heavy drinker versus not current heavy drinker) and tobacco consumption (smoker versus non-smoker). >T2, tumor size >2 cm in the greatest dimension. Bold values as statistically significant. $P < 0.05$. TNM, tumor node metastasis.

function and its expression and then oral cancer go to more easily metastasis. However, the mechanism should be elucidated in laboratory and clinically.

One of the limitations of our study is that information on alcohol, betel nut and tobacco use is dichotomized into 'ever-user' versus 'never-user'. As the result, more detailed analysis based on amount, length and past history of betel nut, alcohol and tobacco consumption were not able to be performed. Data collection relied on self reports, for which some individuals may be reluctant to report their habitual use of such substances. Hence, there may be residual confounding effect from betel nut, alcohol and tobacco use misclassification.

In conclusion, our results suggest that gene–environment interactions between the RECK polymorphism and betel quid with smoking may alter the susceptibility for oral cancer development. The betel quid chewing oral cancer patients with RECK promoter −402 T/C polymorphism have a higher risk to have neck lymph node metastasis than wild-type carriers.

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