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# Combined Effects of *ICAM-1* Single-Nucleotide Polymorphisms and Environmental Carcinogens on Oral Cancer Susceptibility and Clinicopathologic Development

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## Abstract

***Background:*** In Taiwan, oral cancer has causally been associated with environmental carcinogens. Intercellular adhesion molecule (ICAM)-1, a cell adhesion molecule with a key role in inflammation and immunosurveillance, was implicated in carcinogenesis by facilitating instability in the tumor environment. The current study explored the combined effect of *ICAM-1* gene polymorphisms and exposure to environmental carcinogens on the susceptibility of developing oral squamous cell carcinoma (OSCC) and the clinicopathological characteristics of the tumors.

***Methodology and Principal Findings:*** Four single-nucleotide polymorphisms (SNPs) of the *ICAM-1* gene from 595 patients with oral cancer and 561 non-cancer controls were analyzed by a real-time PCR. We found that the *ICAM-1* rs5498 polymorphism and the TAGG or TACG haplotype of 4 *ICAM-1* SNPs (rs3093030, rs5491, rs281432, and rs5498) combined were associated with oral-cancer susceptibility. Among 727 smokers, *ICAM-1* polymorphisms carriers with the betel-nut chewing habit had a 27.49–36.23-fold greater risk of having oral cancer compared to *ICAM-1* wild-type (WT) carriers without the betel-nut chewing habit. Among 549 betel-nut chewers, *ICAM-1* polymorphisms carriers who smoked had a 9.93–14.27-fold greater risk of having oral cancer compared to those who carried the WT but did not smoke. Finally, patients with oral cancer who had at least 1 T allele of *ICAM-1* rs5491 or 1 G allele of rs281432 were at lower risk of developing an advanced clinical stage (III/IV) ($p<0.05$), compared to those patients with AA or CC homozygotes.

***Conclusions:*** Our results suggest that the *ICAM-1* rs5498 SNP and either of 2 haplotypes of 4 SNPs combined have potential predictive significance in oral carcinogenesis. Gene-environment interactions of *ICAM-1* polymorphisms, smoking, and betel-nut chewing might alter oral-cancer susceptibility. *ICAM-1* rs5491 and rs281432 may be applied as factors to predict the clinical stage in OSCC patients.

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## Introduction

Oral cancers can originate in any tissues of the mouth, but approximately 90% are squamous cell carcinomas (SCCs) [1]. Such cancers are known worldwide for their poor prognosis and major oncologic problems. In Taiwanese males, oral cancer is ranked as the fourth most common type of cancer, with a peak at 55–59 years old, and is the leading type of cancer causing death in the 40–50-year-old age group [2].

Oral SCC (OSCC) development is a multistep process requiring the accumulation of multiple genetic alterations, influenced by a patient's genetic predisposition and by environmental factors, which include alcohol and tobacco consumption, betel-nut chewing, chronic inflammation, and viral infection [3–6]. Among genetic factors, single-nucleotide polymorphisms (SNPs) are the most common type of DNA sequence variation which influences the occurrence and progression of gene-related diseases. Previous

reports showed that SNPs may possibly predict the risk of oral cancer [7–9]. Moreover, combinations of environmental carcinogens and certain gene polymorphisms might also increase a person's susceptibility to oral cancer [7–9]. Thus, to elucidate the complex process of carcinogenesis and improve the scientific basis for preventive interventions, the identification of major genes influencing a patient's susceptibility to OSCC should be prioritized.

Intercellular adhesion molecule (ICAM)-1, also known as CD54, is a transmembrane glycoprotein in the immunoglobulin (Ig) superfamily containing five extracellular Ig-like domains, a transmembrane domain, and a short cytoplasmic tail [10–14]. Recently, it was shown that ICAM-1 possibly contributes to tumorigenesis and metastasis including oral cancer [15–17]. Binding of tumor cells to endothelial ICAM-1 [18] leads to auto-upregulation of tumor ICAM-1 and more importantly to chemotaxis of tumor-associated macrophages and neutrophils that eventually facilitate loosening of adhesive contacts and the breaking down of endovascular barriers, permitting tumor cell migration, neoangiogenesis, and ultimately instability of the tumor environment [15–17]. This mechanism is supported by studies showing that patients with increased ICAM-1 expression in tumors have more-advanced stages of the disease [15,19].

ICAM-1 also exists in a soluble form (sICAM-1) which proteolytically cleaves the full-length ICAM-1 near its transmembrane region. sICAM-1 is partially detectable in the serum of healthy subjects, but its level is elevated with inflammatory and malignant disorders [20–22]. The positive correlation of the sICAM-1 serum level and clinical tumor size/lymph node involvement/metastasis staging of some human malignancies was reported [23–25]. The main cause of sICAM-1 release in human malignancies is not well defined; but in recent studies, matrix metalloproteinase (MMP)-9 and human leukocyte elastase were implicated in this process [22,26,27].

Prior research reported that polymorphic variations in exon (rs5498 and rs5491) or intron (rs281432) regions of the *ICAM-1* gene and in the region (rs3093030) between the *ICAM-1* and *ICAM-4* genes were associated with risks for prostate cancer, gastric cancer, breast cancer, type 1 diabetes, metabolic syndrome, and systemic lupus erythematosus [28–33]. Until now, to the best of our knowledge, there has been no documented report studying these polymorphisms in oral cancer. The current study investigated relationships of SNPs (rs3093030, rs5498, rs5491, and rs281432) of the *ICAM-1* gene with the risk of oral cancer. The influences of these SNPs combined with betel-nut and tobacco consumption, leading to susceptibility to oral cancer, were evaluated. We also investigated the relationship between genetic influences and the clinicopathological characteristics of oral cancer.

## Materials and Methods

### Subjects and Specimen Collection

In 2007–2012, we recruited 595 patients (573 males and 22 females with a mean age of 54.36±11.31 years) at Chung Shan Medical University Hospital in Taichung and Changhua Christian Hospital and Show Chwan Memorial Hospital in Changhua, Taiwan as the case group. 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 561 non-cancer 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. For both cases and controls, we used a questionnaire to obtain exposure information about betel-nut chewing, tobacco use, and alcohol consumption. Medical information of the cases, including TNM clinical staging, the primary tumor size, lymph node involvement, and histologic grade, was obtained from their medical records. Oral-cancer patients were clinically staged at the time of their diagnosis according to the TNM staging system of the American Joint Committee on Cancer (AJCC) Staging Manual (7th ed.). Tumor differentiation was examined by a pathologist according to the AJCC classification. Whole-blood specimens collected from controls and OSCC patients were placed in tubes containing ethylenediaminetetraacetic acid (EDTA), were immediately centrifuged, and then stored at −80°C. This study was approved by the Institutional Review Boards of Show Chwan Memorial Hospital, and informed written consent to participate in the study was obtained from each individual.

### Selection of ICAM-1 Polymorphisms

In the dbSNP database, over 20 SNPs have been documented in the 7-exon region of the ICAM-1 gene. We included the non-synonymous SNPs rs5491 (K56M in exon 2) and rs5498 (E469K in exon 6) in the coding sequences of the gene. To obtain adequate power to evaluate the potential association, we investigated rs281432 (C/G in intron 2) with minor allelic frequencies of >5%. Furthermore, another SNP between the ICAM-1 and ICAM-4 genes (rs3093030) was selected in this study since this SNP was found to affect the production of sICAM-1 in a Chinese population [34].

### Genomic DNA Extraction

Genomic DNA was extracted using QIAamp DNA blood mini kits (Qiagen, Valencia, CA, USA) following the manufacturer's instructions [35]. We dissolved DNA in TE buffer (10 mM Tris and 1 mM EDTA; pH 7.8) and then quantified it by measuring the optical density at 260 nm. The final preparation was stored at −20°C and used to create templates for the polymerase chain reaction (PCR) [36].

### Real-time PCR

Allelic discrimination of the rs3093030, rs5498, rs5491, and rs281432 polymorphisms of the *ICAM-1* gene was assessed with the ABI StepOne$^{TM}$ Real-Time PCR System (Applied Biosystems, Foster City, CA, USA), and analyzed with SDS vers. 3.0 software (Applied Biosystems) using the TaqMan assay [37]. The primer sequences and probes for analysis of the *ICAM-1* gene polymorphisms are described in Table 1. The final volume for each reaction was 5 µL, containing 2.5 µL TaqMan Genotyping Master Mix, 0.125 µL TaqMan probe mix, and 10 ng genomic DNA. The real-time PCR included an initial denaturation step at 95°C for 10 min, followed by 40 cycles at of 95°C for 15 s and then at 60°C for 1 min. For each assay, appropriate controls (nontemplate and known genotype) were included in each typing run to monitor reagent contamination and as a quality control. To validate results from real-time PCR, around 5% of assays were repeated and several cases of each genotype were confirmed by the DNA sequence analysis.

### Statistical Analysis

Differences between the 2 groups were considered significant if $p$ values were $<0.05$. Hardy-Weinberg equilibrium (HWE) was assessed using a goodness-of-fit $X^2$-test for biallelic markers. The Mann-Whitney $U$-test and Fisher's exact test were used to compare differences in the distributions of patient demographic

**Table 1.** TaqMan primer sets for *ICAM-1* genotyped SNPs.

| SNP | Probe |
|---|---|
| *ICAM-1* rs3093030 | VIC-5′- TTGTGGGTTGATGGCCATACC |
| | FAM-5′- ATTGTGGGTTGATGGTCATACC |
| *ICAM-1* rs5491 | VIC-5′- TCCTGTGACCAGCCCAAGTTGT |
| | FAM-5′- CCTGTGACCAGCCCATGTTGT |
| *ICAM-1* rs281432 | VIC-5′- TGGAGGGTTTCTGAGCAGG |
| | FAM-5′- TGGAGGGTTTGTGAGCAGG |
| *ICAM-1* rs5498 | VIC-5′- AGGTCACCCGCAAGGTGAC |
| | FAM-5′- AGGTCACCCGCGAGGTGAC |

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characteristics between the non-cancer (control) and oral-cancer groups. The adjusted odds ratios (ORs) and 95% confidence intervals (CIs) of the association between genotype frequencies and risk plus clinicopathological characteristics were estimated using multiple logistic regression models, after controlling for other covariates. We analyzed all data with Statistical Analytic System (SAS Institute, Cary, NC, USA) software (vers. 9.1, 2005) for Windows.

## Results

Results of the statistical analysis of demographic characteristics are shown in Table 2. We found significantly different distributions of age ($p = 0.001$), gender ($p<0.0001$), betel-nut chewing ($p<0.0001$), alcohol consumption ($p<0.0001$), and tobacco use ($p<0.0001$) between control participants and OSCC patients. To diminish the possible interference of environmental factors, adjusted ORs (AORs) with 95% CIs were estimated by multiple logistic regression models after controlling for other covariates in each comparison.

In our recruited control group, frequencies of *ICAM-1* genes were in HWE ($p>0.05$). Genotype distributions and associations between oral cancer and *ICAM-1* gene polymorphisms are shown in Table 3. Alleles with the highest distribution frequency for the rs3093030, rs5491, rs281432, and rs5498 genes of *ICAM-1* in both of our recruited oral-cancer patients and healthy controls were respectively homozygous for C/C, homozygous for A/A, homozygous for C/C, and homozygous for A/A. After adjusting for several variables, there were no significant differences in the incidences of oral cancer in individuals with the rs3093030, rs5491, and rs281432 polymorphisms of the *ICAM-1* gene compared to wild-type (WT) individuals. However, subjects with the *ICAM-1* polymorphic rs5498 AG, GG, and the combination of AG and GG genotypes respectively exhibited significantly ($p<0.05$) higher risks of 1.377- (95% CI: 1.006–1.968), 1.202- (95% CI: 1.029–4.712), and 1.465-fold (95% CI: 1.041–2.063) of having OSCC compared to their corresponding WT homozygotes.

Interactive effects between environmental risk factors and genetic polymorphisms of *ICAM-1* are shown in Tables 4 and 5. Among 727 smokers, subjects with at least 1 T allele of rs3093030 or rs5491, 1 G allele of rs281432 or rs5498, and the betel-nut chewing habit respectively had 35.47- (95% CI: 16.497–76.242), 32.49- (95% CI: 8.325–126.775), 36.233- (95% CI: 17.596–74.610), and 27.49-fold (95% CI: 13.856–54.553) higher risks of having oral cancer. Individuals with either at least 1 T allele of rs3093030 or rs5491, 1 G allele of rs281432 or rs5498, or who chewed betel nut had respective 7.51- (95% CI: 4.522–12.486), 18.43- (95% CI: 11.093–30.620), 11.35- (95% CI: 6.842–19.861), and 7.49-fold (95% CI: 4.403–12.756) higher risks of having oral cancer compared to individuals with WT homozygotes who did not chew betel- nut (Table 4).

Among betel-nut consumers in our cohort, subjects with *ICAM-1* polymorphic rs3093030, rs5491, rs281432, or rs5498 genes and who smoked had corresponding 11.99- (95% CI: 4.134–34.785), 14.27- (95% CI: 2.851–71.452), 13.77- (95% CI: 4.586–41.318), and 9.93-fold (95% CI: 3.516–28.058) higher risks of having oral cancer compared to betel-nut chewers with the WT gene who did not smoke (Table 5). Moreover, people who were either polymorphic for *ICAM-1* in 4 loci (rs3093030, rs5491, rs281432,

**Table 2.** The distributions of demographical characteristics in 561 controls and 595 patients with oral cancer.

| Variable | Controls (N = 561) | Patients (N = 595) | OR (95% CI) | *p* value |
|---|---|---|---|---|
| **Age (yrs)** | **Mean ± S.D.** | **Mean ± S.D.** | | |
| | 51.81±14.71 | 54.36±11.31 | | $p = 0.001$* |
| **Gender** | **n (%)** | **n (%)** | | |
| Male | 457 (81.5%) | 573 (96.3%) | | |
| Female | 104 (18.5%) | 22 (3.7%) | | $p<0.0001$* |
| **Betel nut chewing** | | | | |
| No | 468 (83.4%) | 139 (23.4%) | Reference | |
| Yes | 93 (16.6%) | 456 (76.6%) | 16.509 (12.322–22.119) | $p<0.0001$* |
| **Alcohol consumption** | | | | |
| No | 347 (61.9%) | 243 (40.8%) | Reference | |
| Yes | 214 (38.1%) | 352 (59.2%) | 2.349 (1.855–2.974) | $p<0.0001$* |
| **Tobacco consumption** | | | | |
| No | 341 (60.8%) | 88 (14.8%) | Reference | |
| Yes | 220 (39.2%) | 507 (85.2%) | 8.930 (6.731–11.848) | $p<0.0001$* |

Mann-Whitney U test or Fisher's exact test was used between healthy controls and patients with oral cancer.
*$p$ value$<0.05$ as statistically significant.
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**Table 3.** Distribution frequency of *ICAM-1* genotypes in 561 healthy controls and 595 oral cancer patients.

| Variable | Controls (N = 561) n (%) | Patients (N = 595) n (%) | OR (95% CI) | AOR (95% CI) |
|---|---|---|---|---|
| **rs3093030** | | | | |
| CC | 365 (65.1%) | 384 (64.5%) | 1.00 | 1.00 |
| CT | 179 (31.9%) | 183 (30.8%) | 0.972 (0.756–1.249) | 1.126 (0.784–1.617) |
| TT | 17 (3.0%) | 28 (4.7%) | 1.566 (0.843–2.909) | 1.149 (0.468–2.822) |
| CT+TT | 196 (34.9%) | 211 (35.5%) | 1.023 (0.804–1.303) | 1.129 (0.797–1.599) |
| **rs5491** | | | | |
| AA | 514 (91.6%) | 537 (90.3%) | 1.00 | 1.00 |
| AT | 47 (8.4%) | 55 (9.2%) | 1.120 (0.745–1.684) | 1.523 (0.826–2.809) |
| TT | 0 (0%) | 3 (0.5%) | — | — |
| AT+TT | 47 (8.4%) | 58 (9.7%) | 1.181 (0.789–1.768) | 1.618 (0.887–2.953) |
| **rs281432** | | | | |
| CC | 324 (57.8%) | 332 (55.8%) | 1.00 | 1.00 |
| CG | 200 (35.7%) | 218 (36.6%) | 1.064 (0.832–1.360) | 1.211 (0.849–1.728) |
| GG | 37 (6.6%) | 45 (7.6%) | 1.187 (0.748–1.882) | 1.199 (0.604–2.379) |
| CG+GG | 237 (42.2%) | 263 (44.2%) | 1.083 (0.858–1.367) | 1.209 (0.863–1.696) |
| **rs5498** | | | | |
| AA | 350 (62.4%) | 329 (55.3%) | 1.00 | 1.00 |
| AG | 182 (32.4%) | 220 (37.0%) | **1.286 (1.004–1.647)*** | **1.377 (1.006–1.968)*** |
| GG | 29 (5.2%) | 46 (7.7%) | **1.687 (1.035–2.750)*** | **1.202 (1.029–4.712)*** |
| AG+GG | 211 (37.6%) | 266 (44.7%) | **1.341 (1.060–1.697)*** | **1.465 (1.041–2.063)*** |

The odds ratios (ORs) and with their 95% confidence intervals (CIs) were estimated by logistic regression models. The adjusted odds ratios (AORs) with their 95% confidence intervals (CIs) were estimated by multiple logistic regression models after controlling for age, gender, betel nut chewing, tobacco and alcohol consumption.
*$P$ value$<0.05$ as statistically significant.
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**Table 4.** Adjusted odds ratio (AOR) and 95% confidence interval (CI) of oral cancer associated with *ICAM-1* genotypic frequencies and betel nut chewing among 727 smokers.

| Variable | Controls (n = 220) (%) | Patients (n = 507) (%) | OR (95% CI) | AOR (95% CI) |
|---|---|---|---|---|
| **rs3093030** | | | | |
| [a]CC genotype & non-betel nut chewing | 98 (44.5%) | 54 (10.7%) | 1.00 | 1.00 |
| [b]CT or TT genotype or betel nut chewing | 105 (47.7%) | 300 (59.2%) | **5.185 (3.477–7.733)** | **7.514 (4.522–12.486)** |
| [c]CT or TT genotype with betel nut chewing | 17 (7.8%) | 153 (30.1%) | **16.333 (8.953–29.796)** | **35.465 (16.497–76.242)** |
| **rs5491** | | | | |
| [a]AA genotype & non-betel nut chewing | 141 (64.1%) | 67 (13.2%) | 1.00 | 1.00 |
| [b]AT or TT genotype or betel nut chewing | 76 (34.5%) | 403 (79.5%) | **11.159 (7.629–16.324)** | **18.430 (11.093–30.620)** |
| [c]AT or TT genotype with betel nut chewing | 3 (1.4%) | 37 (7.3%) | **29.955 (7.724–87.213)** | **32.487 (8.325–126.775)** |
| **rs281432** | | | | |
| [a]CC genotype & non-betel nut chewing | 99 (45.0%) | 42 (8.3%) | 1.00 | 1.00 |
| [b]CG or GG genotype or betel nut chewing | 99 (45.0%) | 281 (55.4%) | **6.690 (4.363–10.259)** | **11.346 (6.482–19.861)** |
| [c]CG or GG genotype with betel nut chewing | 22 (10.0%) | 184 (36.3%) | **19.714 (11.141–34.886)** | **36.233 (17.596–74.610)** |
| **rs5498** | | | | |
| [a]AA genotype & non-betel nut chewing | 92 (41.8%) | 44 (8.7%) | 1.00 | 1.00 |
| [b]AG or GG genotype or betel nut chewing | 103 (46.8%) | 274 (54.0%) | **5.562 (3.637–8.505)** | **7.493 (4.403–12.756)** |
| [c]AG or GG genotype with betel nut chewing | 25 (11.4%) | 189 (37.3%) | **15.807 (9.115–27.412)** | **27.493 (13.856–54.553)** |

The odds ratios (ORs) with their 95% confidence intervals were estimated by logistic regression models.
The adjusted odds ratios (AORs) with their 95% confidence intervals were estimated by multiple logistic regression models after controlling for age, gender and alcohol consumption.
[a]Individual with wild genotype but without betel nut chewing.
[b]Individual with either at least one mutated genotype or betel nut chewing.
[c]Individual with both at least one mutated genotype and betel nut chewing.
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**Table 5.** Adjusted odds ratio (AOR) and 95% confidence interval (CI) of oral cancer associated with *ICAM-1* genotypic frequencies and smokers among 549 betel nut consumers.

| Variable | Controls (n = 93) (%) | Patients (n = 456) (%) | OR (95% CI) | AOR (95% CI) |
|---|---|---|---|---|
| **rs3093030** | | | | |
| [a]CC genotype & non-smoker | 15 (16.1%) | 17 (3.7%) | 1.00 | 1.00 |
| [b]CT or TT genotype or smoker | 61 (65.6%) | 286 (62.7%) | **4.137 (1.959–8.734)** | **4.173 (1.672–10.416)** |
| [c]CT or TT genotype with smoking | 17 (18.3%) | 153 (33.6%) | **7.941 (3.373–18.696)** | **11.992 (4.134–34.785)** |
| **rs5491** | | | | |
| [a]AA genotype & non-smoker | 19 (20.4%) | 21 (4.6%) | 1.00 | 1.00 |
| [b]AT or TT genotype or smoker | 71 (76.3%) | 398 (87.3%) | **5.072 (2.595–9.911)** | **8.016 (3.303–19.454)** |
| [c]AT or TT genotype with smoking | 3 (3.2%) | 37 (8.1%) | **11.159 (2.951–42.200)** | **14.273 (2.851–71.452)** |
| **rs281432** | | | | |
| [a]CC genotype & non-smoker | 15 (16.1%) | 13 (2.9%) | 1.00 | 1.00 |
| [b]CG or GG genotype or smoker | 56 (60.2%) | 259 (56.8%) | **5.337 (2.405–11.840)** | **8.063 (2.901–22.413)** |
| [c]CG or GG genotype with smoking | 22 (23.7%) | 184 (40.4%) | **9.650 (4.066–22.905)** | **13.765 (4.586–41.318)** |
| **rs5498** | | | | |
| [a]AA genotype & non-smoker | 15 (16.1%) | 12 (2.6%) | 1.00 | 1.00 |
| [b]AG or GG genotype or smoker | 53 (57.0%) | 255 (55.9%) | **6.014 (2.663–13.583)** | **5.723 (2.150–15.232)** |
| [c]AG or GG genotype with smoking | 25 (26.9%) | 189 (41.4%) | **9.450 (3.974–22.469)** | **9.933 (3.516–28.058)** |

The odds ratios (ORs) with their 95% confidence intervals were estimated by logistic regression models.
The adjusted odds ratios (AORs) with their 95% confidence intervals were estimated by multiple logistic regression models after controlling for age, gender and alcohol consumption.
[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.
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and rs5498) or who smoked were at a 4.17~8.06-fold risk ($p<0.05$) of developing oral cancer, compared to people with the WT gene who did not smoke (Table 5). In light of the above results, we suggest that *ICAM-1* gene polymorphisms have strong impacts on oral-cancer susceptibility in betel-nut and/or smoking consumers.

To explore the effects of polymorphic genotypes of *ICAM-1* on the clinical status of OSCC, we classified OSCC patients into 2 subgroups. In the first subgroup, patients had homozygous WT alleles; in the other subgroup they had at least 1 polymorphic allele. For the genotypic frequencies of the SNPs, only *ICAM-1* rs5491 and rs281432 showed significant associations with clinical pathological variables in OSCC patients. Compared to the WT genotype, patients with at least 1 polymorphic T allele of *ICAM-1* rs5491 or 1 polymorphic G allele of *ICAM-1* rs281432 showed a significant lower risk ($p<0.05$) for being at an advanced clinical stage (III/IV) (Table 6).

We further explored the haplotypes to evaluate the combined effect of the 4 polymorphisms on oral-cancer susceptibility. The distribution frequencies of the *ICAM-1* rs3093030, rs5491, rs281432, and rs5498 haplotypes in our recruited individuals were analyzed. Three haplotypes had frequencies of >5% among all cases; the most common haplotype in the control was CACA (71.6%); and it was therefore chosen as a reference. Compared to the reference, 2 *ICAM-1* haplotypes, TAGG and TACG, significantly ($p<0.05$) respectively increased the risks for OSCC by 1.69- (95% CI: 1.256–2.283) and 1.45-fold (95% CI: 1.028–2.043) (Table 7).

## Discussion

ICAM-1 is believed to play an important role in several malignancies. In breast, gastric, and colorectal cancers, increased ICAM-1 expression in cancer cells was correlated with a more-favorable prognosis, suggesting a role of ICAM-1 in enhancement of immune surveillance [38–40]. Conversely, the potential involvement of ICAM-1 expression in cancer invasion and metastasis was reported in melanomas, and pancreatic, lung, and oral cancers [15,17,41]. Thus, the biological significance of ICAM-1 expression in cancer remains controversial. In this study, we first investigated whether polymorphisms within the *ICAM-1* gene likely played a significant role in the susceptibility to and development of oral cancer. Four SNPs were selected for inclusion in this hypothesis-generating study, two of which (rs5491 and rs5498) encode amino acid substitutions in the expressed ICAM-1 molecule and all of which are thought to affect production of sICAM-1 in Chinese populations [31,34]. Our data showed that individuals with the *ICAM-1* rs5498-G allele had a higher risk for OSCC compared to the WT genotype. Similar to our results, the *ICAM-1* rs5498 SNP showed a statistical difference in susceptibility to prostate cancer [28], breast cancer [33], and grade II astrocytomas [42].

The rs5498 polymorphism entailed a glutamic acid (rs5498 A/G or G/G) to lysine (rs5498 A/A) substitution within coding exon 6 that was thought to lead to decreased integrin receptor binding and increased sICAM-1 production in a German pediatric asthma case-control study [43]. The amino-acid exchange between glutamic acid (negative polar) and lysine (positive polar) is located in the fifth Ig-like domain of ICAM-1. This region seems to be particularly important for the dimerization of ICAM-1. Compared to ICAM-1 monomers, ICAM-1 dimers exhibit enhanced binding to lymphocyte function-associated protein-1 [44]. Therefore, the amino-acid exchange might diminish ICAM-1 dimerization and in turn lead to decreased integrin receptor binding. Moreover, sICAM-1 lacks transmembrane and intracellular domains, which

**Table 6.** Clinical status and *ICAM-1* genotypic frequencies in 595 oral cancer patients.

| Variable | rs3093030 | | | rs5491 | | | rs281432 | | | rs5498 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | CC (N = 384) | CT+TT (N = 211) | *p* value | AA (N = 537) | AT+TT (N = 58) | *p* value | CC (N = 332) | CG+GG (N = 263) | *p* value | AA (N = 329) | AG+GG (N = 266) | *p* value |
| **Clinical Stage** | | | | | | | | | | | | |
| Stage I/II | 168 (43.8%) | 93 (44.1%) | 0.939 | 226 (42.1%) | 35 (60.3%) | **0.008*** | 131 (39.5%) | 130 (49.4%) | **0.015*** | 136 (41.3%) | 125 (47.0%) | 0.167 |
| Stage III/IV | 216 (56.2%) | 118 (55.9%) | | 311 (57.9%) | 23 (39.7%) | | 201 (60.5%) | 133 (50.6%) | | 193 (58.7%) | 141 (53.0%) | |
| **Tumor size** | | | | | | | | | | | | |
| ≤T2 | 239 (62.2%) | 124 (58.8%) | 0.406 | 322 (60.0%) | 41 (70.7%) | 0.112 | 193 (58.1%) | 170 (64.6%) | 0.106 | 195 (59.3%) | 168 (63.2%) | 0.334 |
| >T2 | 145 (37.8%) | 87 (41.2%) | | 215 (40.0%) | 17 (29.3%) | | 139 (41.9%) | 93 (35.4%) | | 134 (40.7%) | 98 (36.8%) | |
| **Lymph node metastasis** | | | | | | | | | | | | |
| No | 242 (63.0%) | 137 (64.9%) | 0.643 | 337 (62.8%) | 42 (72.4%) | 0.146 | 208 (62.7%) | 171 (65.0%) | 0.551 | 206 (62.6%) | 173 (65.0%) | 0.541 |
| Yes | 142 (37.0%) | 74 (35.1%) | | 200 (37.2%) | 16 (27.6%) | | 124 (37.3%) | 92 (35.0%) | | 123 (37.4%) | 93 (35.0%) | |
| **Distant metastasis** | | | | | | | | | | | | |
| No | 378 (98.7%) | 209 (98.5%) | 0.533 | 529 (98.5%) | 58 (100.0%) | 0.349 | 325 (97.9%) | 262 (99.6%) | 0.069 | 322 (97.9%) | 35 (99.6%) | 0.065 |
| Yes | 6 (1.6%) | 2 (0.9%) | | 8 (1.5%) | 0 (0%) | | 7 (2.1%) | 1 (0.4%) | | 7 (2.1%) | 1 (0.4%) | |

T2: tumor size >2 cm in the greatest dimension.
*P value<0.05 as statistically significant.
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**Table 7.** Distribution frequency of *ICAM-1* haplotype in controls and oral cancer patients.

| Variable | | | | Controls (N = 1122) n (%) | Patients (N = 1190) n (%) | OR (95% CI) | *P* value |
|---|---|---|---|---|---|---|---|
| rs3093030 | rs5491 | rs281432 | rs5498 | | | | |
| C/T | A/T | C/G | A/G | | | | |
| C | A | C | A | 803 (71.6%) | 772 (64.9%) | Reference | |
| T | A | G | G | 78 (7.0%) | 127 (10.7%) | **1.694 (1.256–2.283)** | **<0.001** |
| T | A | C | G | 61 (5.4%) | 85 (7.1%) | **1.449 (1.028–2.043)** | **0.033** |
| Other# | | | | 180 (16.0%) | 206 (17.3%) | 1.190 (0.952–1.488) | 0.125 |

#Others: CAGA (54),CACG (53), CTGG (26), TAGA(23), CAGG(19), CTGA(3), TACA (2).

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are crucial for efficient transendothelial adhesion [45] and migration of lymphocytes [46]. As it is soluble, it may compete with membrane ICAM-1 for β2-integrin binding, hence further inhibiting leukocyte recruitment trafficking [47]. Taken together, it appears that within an rs5498 AG or GG genetic environment, the increased incidence of OSCC might be due to defects in leukocyte functions, which can ultimately lead to defective immunosurveillance, shortened cancer dormancy, stimulation of angiogenesis, and growth of tumor cells. In contrast to rs5498, we also found that oral-cancer patients with 1 T allele of *ICAM-1* rs5491 or 1 G allele of *ICAM-1* rs281432 showed a significant lower risk for being at an advanced clinical stage. Thus, these 2 SNPs might confer protection against the progression of OSCC, but the underlying mechanisms of polymorphic rs5491 and rs281432 on OSCC progression are still unknown. Further specifically designed studies are needed to verify the effects and underlying mechanism of polymorphic rs5498, rs5491, and rs281432 on OSCC progression. For example, we should first determine the correlation between sICAM-1 levels and rs5498, rs5491, or rs281432 SNPs in OSCC patients.

Alcohol consumption, tobacco smoking, and betel-nut chewing are the main known etiologic factors for oral cancer. In this study, higher ratios were observed of individuals who had chewed betel nut and consumed alcohol and tobacco in the group of OSCC patients (76.6%, 59.2%, and 85.2%, respectively) than control subjects (16.6%, 38.1%, and 39.2%, respectively), which indicates that these 3 environmental carcinogens may be associated with risks for oral cancer. Betel-nut and tobacco consumption appeared to be particularly strongly associated with oral cancer, a finding that is congruent with prior research [6]. Betel-nut chewing was found to stimulate the protein level of matrix metalloproteinase (MMP)-9 in the saliva of healthy people [48]. Lime-piper betel nut may also increase protein levels of the c-fos and c-jun proto-oncogenes [49]. Consumption of tobacco may significantly induce the expression of nuclear hypoxia-inducible factor (HIF)-1α, which is an unfavorable prognostic factor in oral cancer [50]. Moreover, cigarette smoke condensate was also found to induce MMP-9 expression in oral keratinocytes [51]. These lines of evidence suggest that environmental carcinogen exposure is involved in the formation or pathogenesis of oral cancer.

Exposure to environmental carcinogens might partially involve the formation or pathogenesis of oral cancer, but increasing evidence indicates that genomic changes progressively alter cellular phenotypes and might more significantly lead cells to evolve from the preneoplastic stage into cancer [52]. In our study, only *ICAM-1* rs5498 SNPs alone contributed to oral-cancer susceptibility (Table 3). The synergistic effects of environmental factors (betel-nut and smoking) and 4 *ICAM-1* gene SNPs (rs3093030, rs5491, rs281432, and rs5498) on the risk of oral cancer (Tables 4 and 5) are well demonstrated. Betel-nut and tobacco carcinogens might enhance MMP-9 expression, and then alter the proteolytic cleavage of the full-length ICAM-1 [27]. Consequently, it may upregulate sICAM-1 to affect immunosurveillance and then promote oral cancer formation.

A variety of SNPs might be silent, that is to say, with no direct effect on gene products. However, by virtue of linkage disequilibrium (LD) that exists across the human genome, they can still be used as genetic markers to locate adjacent functional variants that contribute to disease. When each SNP constructing haplotype has a true contribution to the susceptibility of disease, even though unapparent, haplotype analyses can provide a greater statistical power and are sometimes advantageous over analysis of an individual SNP for detecting an association between alleles and a disease phenotype [53]. We analyzed contributions of different haplotype combinations of 4 *ICAM-1* SNPs (rs3093030, rs5491, rs281432, and rs5498) to the risk of oral cancer and eventually found that the *TAGG* or *TACG* haplotype showed a high risk for OSCC (Table 7). It is possible that the *TAGG* or *TACG* haplotype of *ICAM-1* is in LD with other functional polymorphisms that are responsible for the susceptibility to OSCC.

In conclusion, our results suggest that *ICAM-1* polymorphic rs5498 might be correlated with susceptibility to oral cancer, and combined effects of *ICAM-1* gene polymorphisms with environmental carcinogens significantly increase the risk of developing oral cancer. The *TAGG* or *TACG* haplotype of the 4 *ICAM-1* SNPs (rs3093030, rs5491, rs281432, and rs5498) combined also showed a high-risk association with OSCC. Patients with oral cancer who carry at least 1 T allele of *ICAM-1* rs5491 or 1 G allele of *ICAM-1* rs281432 have a lower risk of developing an advanced clinical stage compared to patients carrying A/A or C/C homozygotes.

## Author Contributions

Conceived and designed the experiments: CWL MHC. Performed the experiments: CYC LML WJL. Analyzed the data: JLC CHT JMC. Contributed reagents/materials/analysis tools: CWL MHC. Wrote the paper: MHC SFY.

## References


1. Jemal A, Siegel R, Ward E, Hao Y, Xu J, et al. (2008) Cancer statistics, 2008. CA Cancer J Clin 58: 71–96.
2. Liu SY, Lu CL, Chiou CT, Yen CY, Liaw GA, et al. (2010) Surgical outcomes and prognostic factors of oral cancer associated with betel quid chewing and tobacco smoking in taiwan. Oral Oncol 46: 276–282.
3. Biolchini F, Pollastri G, Figurelli S, Chiarini L (2005) Carcinogen metabolism, DNA damage repair and oral head and neck squamocellular carcinoma (hnscc). A review. Minerva Stomatol 54: 405–414.
4. Califano J, van der Riet P, Westra W, Nawroz H, Clayman G, et al. (1996) Genetic progression model for head and neck cancer: Implications for field cancerization. Cancer Res 56: 2488–2492.
5. Nagaraj NS, Beckers S, Mensah JK, Waigel S, Vigneswaran N, et al. (2006) Cigarette smoke condensate induces cytochromes p450 and aldo-keto reductases in oral cancer cells. Toxicol Lett 165: 182–194.
6. Yen CY, Liu SY, Chen CH, Tseng HF, Chuang LY, et al. (2008) Combinational polymorphisms of four DNA repair genes xrcc1, xrcc2, xrcc3, and xrcc4 and their association with oral cancer in taiwan. J Oral Pathol Med 37: 271–277.
7. Chu YH, Tzeng SL, Lin CW, Chien MH, Chen MK, et al. (2012) Impacts of microrna gene polymorphisms on the susceptibility of environmental factors leading to carcinogenesis in oral cancer. PLoS One 7: e39777.
8. Weng CJ, Lin CW, Chung TT, Tsai CM, Chen MK, et al. (2011) Impact of upa system gene polymorphisms on the susceptibility of environmental factors to carcinogenesis and the development of clinicopathology of oral cancer. Ann Surg Oncol 18: 805–812.
9. Chung TT, Pan MS, Kuo CL, Wong RH, Lin CW, et al. (2011) Impact of reck gene polymorphisms and environmental factors on oral cancer susceptibility and clinicopathologic characteristics in taiwan. Carcinogenesis 32: 1063–1068.
10. Dietrich JB (2002) The adhesion molecule icam-1 and its regulation in relation with the blood-brain barrier. J Neuroimmunol 128: 58–68.
11. Hopkins AM, Baird AW, Nusrat A (2004) Icam-1: Targeted docking for exogenous as well as endogenous ligands. Adv Drug Deliv Rev 56: 763–778.
12. van de Stolpe A, van der Saag PT (1996) Intercellular adhesion molecule-1. J Mol Med (Berl) 74: 13–33.
13. Roebuck KA, Finnegan A (1999) Regulation of intercellular adhesion molecule-1 (cd54) gene expression. J Leukoc Biol 66: 876–888.
14. Springer TA (1990) Adhesion receptors of the immune system. Nature 346: 425–434.
15. Lin YC, Shun CT, Wu MS, Chen CC (2006) A novel anticancer effect of thalidomide: Inhibition of intercellular adhesion molecule-1-mediated cell invasion and metastasis through suppression of nuclear factor-kappab. Clin Cancer Res 12: 7165–7173.
16. Roland CL, Harken AH, Sarr MG, Barnett CC Jr (2007) Icam-1 expression determines malignant potential of cancer. Surgery 141: 705–707.
17. Usami Y, Ishida K, Sato S, Kishino M, Kiryu M, et al. (2013) Intercellular adhesion molecule-1 (ICAM-1) expression correlates with oral cancer progression and induces macrophage/cancer cell adhesion. Int J Cancer 133: 568–578.
18. Rosette C, Roth RB, Oeth P, Braun A, Kammerer S, et al. (2005) Role of icam1 in invasion of human breast cancer cells. Carcinogenesis 26: 943–950.
19. Hayes SH, Seigel GM (2009) Immunoreactivity of icam-1 in human tumors, metastases and normal tissues. Int J Clin Exp Pathol 2:553–560.
20. Gho YS, Kim PN, Li HC, Elkin M, Kleinman HK (2001) Stimulation of tumor growth by human soluble intercellular adhesion molecule-1. Cancer Res 61: 4253–4257.
21. Jun CD, Carman CV, Redick SD, Shimaoka M, Erickson HP, et al. (2001) Ultrastructure and function of dimeric, soluble intercellular adhesion molecule-1 (icam-1). J Biol Chem 276: 29019–29027.
22. Melis M, Pace E, Siena L, Spatafora M, Tipa A, et al. (2003) Biologically active intercellular adhesion molecule-1 is shed as dimers by a regulated mechanism in the inflamed pleural space. Am J Respir Crit Care Med 167: 1131–1138.
23. Kostler WJ, Tomek S, Brodowicz T, Budinsky AC, Flamm M, et al. (2001) Soluble icam-1 in breast cancer: Clinical significance and biological implications. Cancer Immunol Immunother 50: 483–490.
24. Maruo Y, Gochi A, Kaihara A, Shimamura H, Yamada T, et al. (2002) Icam-1 expression and the soluble icam-1 level for evaluating the metastatic potential of gastric cancer. Int J Cancer 100: 486–490.
25. Sun JJ, Zhou XD, Liu YK, Tang ZY, Feng JX, et al. (1999) Invasion and metastasis of liver cancer: Expression of intercellular adhesion molecule 1. J Cancer Res Clin Oncol 125: 28–34.
26. Champagne B, Tremblay P, Cantin A, St Pierre Y (1998) Proteolytic cleavage of icam-1 by human neutrophil elastase. J Immunol 161: 6398–6405.
27. Fiore E, Fusco C, Romero P, Stamenkovic I (2002) Matrix metalloproteinase 9 (mmp-9/gelatinase b) proteolytically cleaves icam-1 and participates in tumor cell resistance to natural killer cell-mediated cytotoxicity. Oncogene 21: 5213–5223.
28. Chen H, Hernandez W, Shriver MD, Ahaghotu CA, Kittles RA (2006) Icam gene cluster snps and prostate cancer risk in african americans. Hum Genet 120: 69–76.
29. Tian MM, Sun Y, Li ZW, Wu Y, Zhao AL, et al. (2012) Polymorphisms of icam-1 are associated with gastric cancer risk and prognosis. World J Gastroenterol 18: 368–374.
30. Ma J, Mollsten A, Prazny M, Falhammar H, Brismar K, et al. (2006) Genetic influences of the intercellular adhesion molecule 1 (icam-1) gene polymorphisms in development of type 1 diabetes and diabetic nephropathy. Diabet Med 23: 1093–1099.
31. Hsu LA, Chang CJ, Wu S, Teng MS, Chou HH, et al. (2010) Association between functional variants of the icam1 and crp genes and metabolic syndrome in taiwanese subjects. Metabolism 59: 1710–1716.
32. Kim K, Brown EE, Choi CB, Alarcon-Riquelme ME, Kelly JA, et al. (2012) Variation in the icam1-icam4-icam5 locus is associated with systemic lupus erythematosus susceptibility in multiple ancestries. Ann Rheum Dis 71: 1809–1814.
33. Kammerer S, Roth RB, Reneland R, Marnellos G, Hoyal CR, et al. (2004) Large-scale association study identifies icam gene region as breast and prostate cancer susceptibility locus. Cancer Res 64: 8906–8910.
34. Ogawa Y, Hirakawa K, Nakata B, Fujihara T, Sawada T, et al. (1998) Expression of intercellular adhesion molecule-1 in invasive breast cancer reflects low growth potential, negative lymph node involvement, and good prognosis. Clin Cancer Res 4: 31–36.
35. Wu CS, Yen CJ, Chou RH, Li ST, Huang WC, et al. (2012) Cancer-associated carbohydrate antigens as potential biomarkers for hepatocellular carcinoma. PLoS One 7:e39466.
36. Yu YL, Su KJ, Chen CJ, Wei CW, Lin CJ, et al. (2012) Synergistic anti-tumor activity of isochaihulactone and paclitaxel on human lung cancer cells. J Cell Physiol 227: 213–222.
37. Yu YL, Chou RH, Wu CH, Wang YN, Chang WJ, et al. (2012) Nuclear EGFR suppresses ribonuclease activity of polynucleotide phosphorylase through DNAPK-mediated phosphorylation at serine 776. J Biol Chem 287: 31015–31026.
38. Fujihara T, Yashiro M, Inoue T, Sawada T, Kato Y, et al. (1999) Decrease in icam-1 expression on gastric cancer cells is correlated with lymph node metastasis. Gastric Cancer 2: 221–225.
39. Tachimori A, Yamada N, Sakate Y, Yashiro M, Maeda K, et al. (2005) Up regulation of icam-1 gene expression inhibits tumour growth and liver metastasis in colorectal carcinoma. Eur J Cancer 41:1802–1810.
40. Johnson JP, Stade BG, Holzmann B, Schwable W, Riethmuller G (1989) De novo expression of intercellular-adhesion molecule 1 in melanoma correlates with increased risk of metastasis. Proc Natl Acad Sci U S A 86: 641–644.
41. Bielinski SJ, Pankow JS, Li N, Hsu FC, Adar SD, et al. (2008) Icam1 and vcam1 polymorphisms, coronary artery calcium, and circulating levels of soluble icam-1: The multi-ethnic study of atherosclerosis (mesa). Atherosclerosis 201: 339–344.
42. Burim RV, Teixeira SA, Colli BO, Peria FM, Tirapelli LF, et al. (2009) Icam-1 (lys469glu) and pecam-1 (leu125val) polymorphisms in diffuse astrocytomas. Clin Exp Med 9: 157–163.
43. Puthothu B, Krueger M, Bernhardt M, Heinzmann A (2006) Icam1 amino-acid variant k469e is associated with paediatric bronchial asthma and elevated sicam1 levels. Genes Immun 7: 322–326.
44. Reilly PL, Woska JR Jr, Jeanfavre DD, McNally E, Rothlein R, et al. (1995) The native structure of intercellular adhesion molecule-1 (icam-1) is a dimer. Correlation with binding to lfa-1. J Immunol 155: 529–532.
45. Oh HM, Lee S, Na BR, Wee H, Kim SH, et al. (2007) Rkikk motif in the intracellular domain is critical for spatial and dynamic organization of icam-1: Functional implication for the leukocyte adhesion and transmigration. Mol Biol Cell 18: 2322–2335.
46. Greenwood J, Amos CL, Walters CE, Couraud PO, Lyck R, et al. (2003) Intracellular domain of brain endothelial intercellular adhesion molecule-1 is essential for t lymphocyte-mediated signaling and migration. J Immunol 171: 2099–2108.
47. Mendez MP, Morris SB, Wilcoxen S, Du M, Monroy YK, et al. (2008) Disparate mechanisms of sicam-1 production in the peripheral lung: Contrast between alveolar epithelial cells and pulmonary microvascular endothelial cells. Am J Physiol Lung Cell Mol Physiol 294: L807–814.
48. Liu SY, Lin MH, Yang SC, Huang GC, Chang L, et al. (2005) Areca quid chewing enhances the expression of salivary matrix metalloproteinase-9. J Formos Med Assoc 104: 113–119.
49. Lin MH, Wang CJ, Huang HP, Chou MY, Chou FP (2004) The tumorigenic characteristics of lime-piper betel quid-transformed jb6 cells. Arch Toxicol 78: 167–173.
50. Lin PY, Yu CH, Wang JT, Chen HH, Cheng SJ, et al. (2008) Expression of hypoxia-inducible factor-1 alpha is significantly associated with the progression and prognosis of oral squamous cell carcinomas in taiwan. J Oral Pathol Med 37: 18–25.
51. Wuertz B, Ondrey F (2010) MMP-9 analysis in carcinogen-treated oral keratinocytes. Otolaryng Head Neck Surg 143: 190
52. Thorgeirsson SS, Grisham JW (2002) Molecular pathogenesis of human hepatocellular carcinoma. Nat Genet 31: 339–346.
53. Shifman S, Bronstein M, Sternfeld M, Pisante-Shalom A, Lev-Lehman E, et al. (2002) A highly significant association between a comt haplotype and schizophrenia. Am J Hum Genet 71: 1296–1302.