Featured Article

Association between the V109G Polymorphism of the p27 Gene and the Risk and Progression of Oral Squamous Cell Carcinoma

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ABSTRACT

Purpose: Abnormalities in p27 may alter cell cycle delay required for DNA repair after exposure to carcinogens. A coding exon 1 polymorphism at codon 109 (T → G) in p27 was identified and thought to have an effect on the functions of its protein. We hypothesized that this p27 T109G polymorphism is associated with squamous cell carcinoma of the head and neck (SCCHN) risk.

Experimental Design: We tested this hypothesis in a hospital-based case-control study of 713 patients newly diagnosed with SCCHN and 1224 cancer-free controls frequency matched to the cases by age (±5 years), sex, and smoking status. All subjects were non-Hispanic whites. We genotyped for this p27 variant using genomic DNA from each subject.

Results: Compared with the p27 109VV variant, the p27 109GG variant was associated with a nonsignificantly increased risk of SCCHN [crude odds ratio (OR) = 1.29; 95% confidence interval (CI) = 0.88–1.90; adjusted OR = 1.20; 95% CI = 0.81–1.77], but the risk was statistically significant among men (adjusted OR = 1.55, 95% CI = 1.00–2.42), current alcohol users (adjusted OR = 1.68, 95% CI = 1.01–2.82), and patients with oral cavity cancer (adjusted OR = 1.77, 95% CI = 1.03–3.04). The p27 109GG variant was also associated with oral tumor overall stage, suggesting that it may play a role in tumor progression.

Conclusions: Our findings suggest that the p27 109GG variant genotype may not play a major role in the etiology of SCCHN but may be associated with an increased risk in at-risk subgroups or subsets of SCCHN, particularly oral cavity cancer and possibly tumor progression. Larger studies with oral squamous cell carcinoma are needed to verify these findings.

INTRODUCTION

Squamous cell carcinoma of the head and neck (SCCHN), which includes cancers of the oral cavity, pharynx, and larynx, is a common malignancy with an estimated number of >500,000 new cases worldwide (1, 2). In the United States, approximately 37,200 new SCCHN cases and 11,000 SCCHN deaths occurred in 2003 (3). Survival rates vary depending on tobacco and alcohol consumption, age, sex, ethnic background, and geographic area (3). Many factors contribute to SCCHN, including tobacco smoking (4, 5), alcohol use (5, 6), viral infection (7, 8), and genetic factors (8). Although smoking and alcohol use play a major role in the etiology of SCCHN, only a fraction of smokers and drinkers develop SCCHN, suggesting that there is inter-individual variation in genetic susceptibility to SCCHN in the general population. Identification of genetic factors that modulate the risk of SCCHN could be of great value in identifying high-risk subgroups that could benefit from primary prevention programs and management of predictable patient outcome.

Although no major susceptibility genes for SCCHN have been identified, gains and losses at several loci and altered expression of p53 and DNA repair genes suggest the involvement of altered oncogenes and tumor suppressor genes in SCCHN tumorigenesis (9, 10). Alterations in genes involved in cell cycle control frequently result in deregulated cellular proliferation; specifically, genes associated with the regulation of the G1 checkpoint in the cell cycle are frequently altered in cancer cells (11). For instance, cyclins and cyclin-dependent kinases (CDKs) form protein complexes to modulate cell proliferation through the cell cycle control, and CDK inhibitors inhibit kinase activities of the complexes and block transitions of the cell cycle (12–14). Therefore, abnormalities in genes that regulate cell proliferation may alter cell-cycle control and DNA repair activities in response to DNA damage, especially p53 response to DNA damage (15, 16). In oral cancer, the functions of p53, p27, p16, and cyclin D1 are often altered through mutation, amplification, or deactivation (17). Therefore, individuals carrying polymorphic CDK inhibitors that may affect its protein function are likely more susceptible to SCCHN development.

The p27, a CDK inhibitor belonging to the ciprofloxacin/kinase inhibitor proteins family, which includes p21 (18) and p57 (19, 20), is a putative tumor suppressor (21, 22). The p27 maps to chromosome 12p13 and encodes for a 27-kDa protein (23, 24). The p27, p21, and p57 share common sequence motifs that mediate interaction between the CDK inhibitor and cyclin-CDK complexes (10, 25, 26). Because the expression level of p27 correlates positively with cell differentiation (27), it is speculated that p27 is involved in pathways regulated by both
mitogenic and antiproliferative extrinsic signals (25) and that loss of its function contributes to tumorigenesis. The expression of p27 is frequently reduced in cancers, including gastric, breast, prostate, and non-small cell lung cancers (16), but p27 is rarely mutated in human malignancies (28). Previous studies indicate that reduced p27 expression correlates with poor clinical outcome (29, 30), invasiveness (31), poor prognosis (30, 32–38), tumor grade (32, 39, 40), and progression (32, 41) in human malignancies. However, it is unknown whether genetic variants in p27 play a role in the carcinogenesis or progression of SCCHN.

A total of 21 single nucleotide polymorphisms of p27 have been described (GenBank entry no. AF480891). Of these 21 single nucleotide polymorphisms, 11 have low allele frequency (<5%) and 9 occur within the noncoding regions of p27. Only one single nucleotide polymorphism (T→G) at codon 109 causes an amino acid substitution of glycine for valine. This p27 V109G polymorphism may have an effect on p27 degradation in vivo (42) and appears to be associated with the risk of prostate carcinoma (42), particularly advanced prostate carcinoma (43). Because no reports have been published on an association between the p27 V109G polymorphism and the risk of SCCHN thus far, we hypothesized that the p27 V109G polymorphism may contribute to the risk of SCCHN. In this study, we tested our hypothesis in a large, hospital-based case-control study of patients with incident SCCHN and cancer-free controls frequency matched by age, sex, and smoking status.

MATERIALS AND METHODS

Study Subjects. Between May 1995 and October 2003, 1043 patients with histologically confirmed SCCHN were recruited at our Head and Neck Surgery Clinic. The overall stage of SCCHN is defined from I to IV as follows (American Joint Committee for Cancer Staging and End-Results reporting, 1992): overall stage I (T1 N0 M0), overall stage II (T2 N0 M0), overall stage III (T3 N0 M0 or T1–2 N1 M0), and overall stage IV (T4 N0 M0 or any TN2–3 M0 or any T, and N, M1). T, N, and M are defined as tumor stage (T1, T2, T3, and T4), nodal status (N0, N1, N2, and N3), and distant metastases (M0 and M1), respectively (here M0 for all cases in this study). Approximately 95% of eligible incident cases agreed to participate in this study. Patients with second SCCHN, primary tumors, primary tumors of the nasopharynx or sinonasal tract, primary tumors outside the upper aerodigestive tract, cervical metastases of unknown origin, or histopathological diagnoses other than squamous cell carcinoma were excluded. Because genotype frequencies can vary between ethnic groups, only 716 non-Hispanic white patients were included in this analysis with primary tumors of the oral cavity (n = 220; 31%), oropharynx (n = 325; 45%), hypopharynx (n = 36; 5%), or larynx (n = 135; 19%). The response rate among the patients with SCCHN was 81%. We also included 1229 cancer-free control subjects in this analysis, recruited from two populations during a similar period. One group were enrollees (n = 607) in a multispecialty physician practice, the Kelsey-Seybold Foundation, which has >20 clinics throughout the Houston, Texas metropolitan area. This control population is part of an ongoing lung cancer study, which provided older men who were former and current smokers for our frequency matching purpose. The overall response rate among this hospital control group was approximately 75%. The other control population was genetically unrelated and were M. D. Anderson hospital visitors (n = 622) accompanying other cancer patients to our outpatient clinics. We had first surveyed the potential control subjects with a short questionnaire to determine their willingness to participate in research studies and to obtain information about their smoking behavior and demographic factors. The response rate for this community control group was approximately 80%.

We frequency matched the controls to the cases by age (±5 years), sex, and smoking status (i.e., current, former, and never). The purpose of frequency matching was to minimize confounding to evaluate the main effect of the p27 polymorphism. We interviewed each eligible subject to obtain data on age, sex, ethnicity, smoking status, and alcohol consumption (before the disease diagnosis for the cases and at the time of the interview for the controls). Subjects who had smoked >100 cigarettes in their lifetimes were categorized as ever smokers, and others were never smokers. Ever smokers who had quit smoking >1 year previously were categorized as former smokers, and the other smokers were categorized as current smokers. Similarly, subjects who had drunk alcoholic beverages at least once a week for >1 year previously were categorized as ever drinkers, and others were never drinkers. Ever drinkers who had quit drinking >1 year previously were categorized as former drinkers, and the other drinkers were categorized as current drinkers.

After the subjects provided their informed consent, each subject donated 30 ml of blood collected into heparinized tubes. The research protocol was approved by the M. D. Anderson Cancer Center and Kelsey-Seybold institutional review boards.

PCR Restriction Fragment Length Polymorphism Analysis. We extracted genomic DNA from the buffy-coat fraction of the blood samples by using a Qiagen DNA blood mini kit (Qiagen Inc., Valencia, CA) according to the manufacturer’s instructions. We typed for the p27 V109G genotype by PCR and restriction digestion by using published primers and a modification of a method published previously (43). We performed the PCRs with a PTC-200 DNA engine (Peltier thermal cycler; MJ Research, Inc., Waltham, MA) in 10 μl of PCR mixture. This PCR mixture included approximately 20 ng of genomic DNA, 0.1 mM each deoxynucleoside triphosphate, 1× PCR buffer (50 mM KCl, 10 mM Tris HCl, and 0.1% Triton X-100), 1.5 mM MgCl2, 0.5 units of Taq polymerase (Sigma-Aldrich Corp.; St. Louis, MO), and 2 pmol of each primer. The amplification conditions were 5 min for initial denaturation at 95°C followed by 35 cycles of 15 s at 94°C, 30 s at 60°C, 1 min at 72°C, and a final 5-min step at 72°C for final extension. PCR products (454 bp) were digested with the restriction enzyme BglII (New England BioLabs, Beverly, MA) overnight at 37°C and separated with 3% agarose gel containing ethidium bromide. The genotype of codon 109 was determined by a Glycerol allele with fragment lengths of 76, 116, and 262 bp and a Val allele with fragment lengths of 76 and 378 bp. We performed the PCRs and evaluated the results without knowing the case or control status of the subjects. At least 10% of the samples were retested, and the results were 100% concordant.

Statistical Analysis. We first evaluated the differences in the distributions of selected demographic variables, smoking,
alcohol consumption, and p27 genotype frequencies between cases and controls by using the \( \chi^2 \) test. We estimated the association between the p27 genotype and risk for SCCHN by computing the odds ratios (ORs) and their 95% confidence intervals (CIs) by both univariate and multivariate logistic regression analyses. For logistic regression analysis, the p27 genotype was also recoded as a dummy variable. We further stratified the genotype data by subgroups of age, sex, smoking, alcohol drinking, and tumor site and assessed any trend in risk in multivariate logistic regression models. All of the statistical analyses were performed with the SAS software (version 8e; SAS Institute Inc., Cary, NC).

**RESULTS**

Three DNA samples from the 716 cases and five DNA samples from the 1,229 cancer-free control subjects failed to yield genotyping data on repeated experiments. Therefore, the final data analysis included 713 cases and 1,224 cancer-free controls, as summarized in Table 1. All subjects were non-Hispanic whites. Because the cases and controls were frequency matched by age (±5 years) and sex, there were no significant differences in the distributions of age and sex between the cases and controls (\( P = 0.316 \) for age and \( P = 0.574 \) for sex). The median age was 57 years for the cases (mean = 56.9 years, range = 18–90 years) and 58 years for the controls (mean = 57.2 years, range = 20–88 years). However, the frequency matching by smoking status was imperfect. There were more current smokers and current drinkers among the cases than among the controls, and these differences were statistically significant (\( P < 0.001 \) for both smoking and drinking status). Therefore, all of these variables were further adjusted for in the multivariate regression analyses.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cases (n = 713)</th>
<th>Controls (n = 1,224)</th>
<th>( P^* )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, (mean, ±SD in yrs)</td>
<td>56.9 ± 11.84</td>
<td>57.2 ± 11.65</td>
<td>0.663</td>
</tr>
<tr>
<td>Age group by yrs, (n, %)</td>
<td></td>
<td></td>
<td>0.316</td>
</tr>
<tr>
<td>≤45</td>
<td>104</td>
<td>146</td>
<td>195</td>
</tr>
<tr>
<td>46–50</td>
<td>104</td>
<td>146</td>
<td>163</td>
</tr>
<tr>
<td>51–55</td>
<td>122</td>
<td>17.1</td>
<td>180</td>
</tr>
<tr>
<td>56–60</td>
<td>107</td>
<td>15.0</td>
<td>182</td>
</tr>
<tr>
<td>61–65</td>
<td>112</td>
<td>15.7</td>
<td>178</td>
</tr>
<tr>
<td>65–70</td>
<td>78</td>
<td>10.9</td>
<td>175</td>
</tr>
<tr>
<td>&gt;70</td>
<td>86</td>
<td>12.1</td>
<td>151</td>
</tr>
<tr>
<td>Sex (n, %)</td>
<td></td>
<td></td>
<td>0.574</td>
</tr>
<tr>
<td>Male</td>
<td>536</td>
<td>75.2</td>
<td>906</td>
</tr>
<tr>
<td>Female</td>
<td>177</td>
<td>24.8</td>
<td>318</td>
</tr>
<tr>
<td>Smoking status (n, %)</td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Current</td>
<td>247</td>
<td>34.6</td>
<td>313</td>
</tr>
<tr>
<td>Former</td>
<td>279</td>
<td>39.1</td>
<td>543</td>
</tr>
<tr>
<td>Never</td>
<td>187</td>
<td>26.2</td>
<td>368</td>
</tr>
<tr>
<td>Drinking status (n, %)</td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Current</td>
<td>364</td>
<td>51.1</td>
<td>533</td>
</tr>
<tr>
<td>Former</td>
<td>193</td>
<td>27.1</td>
<td>321</td>
</tr>
<tr>
<td>Never</td>
<td>156</td>
<td>21.8</td>
<td>370</td>
</tr>
</tbody>
</table>

*Two-sided \( \chi^2 \) and Student \( t \) tests for differences between the cases and controls.

Because the controls in this study were composed of subjects from two separate populations, we compared the genotype distributions between the 607 hospital and 617 community controls. The frequencies of the p27 VV, VG, and GG were 56.8, 36.9, and 6.26, %, respectively, in the hospital controls and 60.5, 35.0, and 4.54, %, respectively, in the community controls, and the p27 G allele frequency was 0.247 and 0.220 for the hospital and community controls, respectively; however, these differences were not statistically significant (\( \chi^2 = 2.671, P = 0.263 \)) for the genotype distribution and \( \chi^2 = 2.55, P = 0.110 \) for the allele frequency (Table 2). Therefore, to increase study power, these two groups were combined and used as one control group (\( n = 1,224 \)) in the final analysis, and the distributions of the genotype frequencies among the combined controls were in agreement with the Hardy-Weinberg disequilibrium (\( P = 0.895 \); Table 2). Although the frequencies of the p27 109G homozygous allele and variant genotype were greater among the cases (0.239 and 7.01, %, respectively) than among the controls (0.234 and 5.39, %, respectively), these differences were not statistically significant (\( P = 0.720 \) for the allele frequency and \( P = 0.274 \) for the genotype frequency). In the logistic regression analysis, we assessed the association of p27 genotypes with risk of SCCHN using hospital, community, and all combined controls, respectively (Table 2). Compared with the p27 109V genotype, the adjusted OR associated with risk of SCCHN was 0.88 (0.70–1.11), 0.95 (0.75–1.21), and 0.92 (95% CI = 0.75–1.12) for the p27 109VG genotype and 1.10 (0.69–1.69), 1.35 (0.82–2.24), and 1.20 (95% CI = 0.81–1.77) for the p27 109GG genotype for using these three control groups, respectively. None of these associations reached the level of statistical significance.

Associations between the p27 V109G polymorphism and the risk of SCCHN as stratified by the age, sex, smoking status, and alcohol use are shown in Table 3. The adjusted ORs for the p27 genotypes associated with risk of SCCHN at different sites were obtained from the comparison between all combined controls and each sub-site of SCCHN (Table 3). We observed significantly increased risks of SCCHN associated with the p27 109GG genotype among men (adjusted OR = 1.55, 95% CI = 1.00–2.42), current alcohol users (adjusted OR = 1.68, 95% CI = 1.01–2.82), and patients with oral cavity cancer (adjusted OR = 1.77, 95% CI = 1.03–3.04) but not among the other subgroups (Table 3). We also observed a nonsignificantly elevated risk associated with the p27 109GG genotype among younger subjects (age ≤ median, 58 years; adjusted OR = 1.48, 95% CI = 0.7–3.1), both current and never smokers (adjusted OR = 1.75; 95% CI = 0.9–3.4 and OR = 1.67; 95% CI = 0.76–3.64, respectively), and subjects with larynx cancer (adjusted OR = 1.65, 95% CI = 0.86–3.16). These nonsignificantly increased risks from stratified analyses could be attributed to relatively small numbers of the strata, and these findings need larger studies to be further verified. We did not find any evidence of an interaction between the p27 variant genotypes and smoking status or alcohol use on the risk of SCCHN in the multivariate logistic regression models (data not shown), probably because of insufficient statistical power.

Because the significantly elevated risk was confined to oral cavity cancer, we also evaluated the association between the p27 polymorphism and overall stage of oral squamous cell carcinoma (Table 4). The overall stage has four levels from stage I to...
Table 2  
*p27* genotype and allele frequencies among cases and controls and their association with the risk of SCCHN<sup>a</sup>  

<table>
<thead>
<tr>
<th>P27 Genotype</th>
<th>Case patients (n = 713)</th>
<th>Hospital controls&lt;sup&gt;b&lt;/sup&gt; (n = 607)</th>
<th>Community controls&lt;sup&gt;c&lt;/sup&gt; (n = 617)</th>
<th>All control subjects&lt;sup&gt;d&lt;/sup&gt; (n = 1224)</th>
<th>Adjusted OR using hospital controls (95% CI)&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Adjusted OR using community controls (95% CI)&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Adjusted OR using all combined controls (95% CI)&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>V109G&lt;sup&gt;e&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VV (ref.)</td>
<td>422</td>
<td>59.2</td>
<td>345</td>
<td>56.8</td>
<td>718</td>
<td>58.7</td>
<td>1.00</td>
</tr>
<tr>
<td>VG</td>
<td>241</td>
<td>33.8</td>
<td>224</td>
<td>36.9</td>
<td>440</td>
<td>35.9</td>
<td>0.88</td>
</tr>
<tr>
<td>GG</td>
<td>50</td>
<td>7.0</td>
<td>38</td>
<td>6.26</td>
<td>28</td>
<td>4.54</td>
<td>1.10</td>
</tr>
<tr>
<td>G allele</td>
<td></td>
<td>0.239</td>
<td>0.247</td>
<td>0.220</td>
<td>0.234</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>  SCCHN, squamous cell carcinoma of the head and neck; OR, odd ratio; CI, confidence interval; ref., reference group; ref., reference group.

<sup>b</sup>  The *χ²* tests for comparisons with the following cases: *P* = 0.476 for hospital controls, *P* = 0.159 for community controls, and *P* = 0.274 for difference in genotype distributions and *P* = 0.633 for hospital controls, *P* = 0.233 for community controls, and *P* = 0.720 for differences in allele frequencies; the observed genotype frequency in the control subjects was in agreement with Hardy-Weinberg equilibrium (p² + 2pq + q² = 1; *P* = 0.839 for hospital controls, *P* = 0.643 for community controls, and *P* = 0.895 for all combined controls).

<sup>c</sup>  Adjusted for age, sex, smoking status, and alcohol use in a logistic regression model.

DISCUSSION

In this study, we investigated the role of the V109G polymorphism in the CDK inhibitor gene *p27* in the etiology of SCCHN. We observed a nonsignificantly increased risk of SCCHN associated with the p27 variant 109GG genotype compared with the p27 109VV genotype, and the significantly increased risk was mostly limited to male subjects and current alcohol users. However, there was a significantly elevated risk increased as the overall stage of oral cavity cancer increased in a dose-effect manner (trend test: *P* = 0.025) but not for the variant VG genotype (trend test: *P* = 0.437) and the combined *p27* GG/VG genotypes (trend test: *P* = 0.164).
for oral cavity cancers associated with the p27 variant 109GG genotype, and this risk was more pronounced in the advanced stage of oral cavity cancer; there was a dose-effect relationship between risk for the overall stage of oral cavity cancer and the p27 homozygous 109GG variant genotype. To the best of our knowledge, this is the first large molecular epidemiological study investigating the association between the p27 V109G polymorphism and the risk of SCCHN.

Our findings suggest that although the p27 109G variant genotypes (i.e., VG and GG) may not play a major role in the etiology of SCCHN, it may contribute to subsets of SCCHN and possibly tumor progression. These findings are biologically plausible. p27 is involved in cell cycle control, which is critical for DNA repair in response to carcinogens, and therefore, p27 plays an important role in carcinogenesis. It is possible that this p27 V109G polymorphism may interact with and modify other risk factors for SCCHN. Alternatively, the p27 variant allele may be functionally relevant or in linkage disequilibrium with alleles at other susceptibility loci.

To date, there is no published study that provides direct evidence on potential functional relevance of this p27 polymorphism, which may cause structural change of p27 protein and potentially alter interaction with other proteins. For instance, p38 encoded by the Jab1 gene (44) interacts specifically with p27, and overexpression of p38 in mammalian cells results in the translocation of p27 from the nucleus to the cytoplasm, causing a decrease in the amount of p27 in the cell by accelerating its degradation through the ubiquitin/proteasome pathway (45). Therefore, p38 is a specifically binding and negative regulator of p27, and their interaction may explain the regulation of cell-cycle-dependent proteolytic machinery and selection of the key cell-cycle regulators for degradation. The p27 V109G polymorphism, which results in a glycine for valine substitution, lies within the binding domain of p38<sup>ab1</sup> that promotes the phosphorylation and cytoplasmic translocation of p27 for its subsequent degradation in the cytoplasm (46). Therefore, it is also possible that PGP9.5 might play a role in cancer growth by regulating the level of nuclear p38<sup>ab1</sup>, leading to increased degradation of p27 via its interaction and nuclear translocation with p38<sup>ab1</sup> (48). Another possibility is that the p27 polymorphism may be in linkage disequilibrium with other functional polymorphisms that affect either the expression or activity levels of enzymes involved in tumorigenesis of oral cavity (49). However, all these hypotheses remain to be tested in future studies.

Thus far, only one published case-control study has examined the association between the p27 V109G polymorphism and the risk of cancer (43), and no studies have examined the association between this polymorphism and the risk of SCCHN. In a case-control study of 96 cases and 106 controls in a European-American population, Kibel <i>et al.</i> (43) examined whether the p27 V109G polymorphism was associated with advanced prostate cancer and found that the p27 109VV genotype was associated with a 95% increased risk of advanced prostate carcinoma (OR = 1.95, 95% CI = 1.09–3.47). The risk was particularly increased in patients with androgen-independent disease (OR = 2.11, 95% CI = 1.05–4.22) and in those under the median age of diagnosis (OR = 2.23, 95% CI = 1.08–4.59). The same research group previously studied this polymorphism in 19 metastatic prostate carcinomas and found that the distribution of the p27 genotypes was not in Hardy-Weinberg equilibrium (84% VV homozygous, 5% VG heterozygous, and 11% GG homozygous; Ref. 50). Previously estimated frequencies of the T and G alleles were 0.73 and 0.27, respectively, from 105 controls (43). Consistently, our estimates were 0.77 and 0.23, respectively, from 1224 controls in our study. To the best of our knowledge, this is the first large molecular epidemiological study investigating the association between the p27 V109G polymorphism and the risk of SCCHN.

**Table 4** Associations between the p27 genotypes and overall stage of squamous cell carcinoma of the oral cavity

<table>
<thead>
<tr>
<th>Overall stage&lt;sup&gt;a&lt;/sup&gt;</th>
<th>VV</th>
<th>VG</th>
<th>GG</th>
<th>VG+GG</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>29 of 718</td>
<td>14 of 440</td>
<td>4 of 66</td>
<td>18 of 506</td>
</tr>
<tr>
<td>1.00 (ref.)</td>
<td>0.76 (0.40–1.47)</td>
<td>1.49 (0.50–4.41)</td>
<td>0.86 (0.47–1.56)</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>30 of 718</td>
<td>16 of 440</td>
<td>5 of 66</td>
<td>21 of 506</td>
</tr>
<tr>
<td>1.00 (ref.)</td>
<td>0.85 (0.46–1.58)</td>
<td>1.71 (0.63–4.61)</td>
<td>0.96 (0.54–1.71)</td>
<td></td>
</tr>
<tr>
<td>III-IV</td>
<td>58 of 718</td>
<td>46 of 440</td>
<td>12 of 66</td>
<td>58 of 506</td>
</tr>
<tr>
<td>1.00 (ref.)</td>
<td>1.29 (0.86–1.95)</td>
<td>1.99 (1.00–3.95)</td>
<td>1.39 (0.95–2.05)</td>
<td></td>
</tr>
<tr>
<td>Trend test &lt;i&gt;P&lt;/i&gt;</td>
<td>0.437</td>
<td>0.025</td>
<td>0.164</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Overall stage I, T<sub>N</sub>M<sub>0</sub>; overall stage II, T<sub>N</sub>M<sub>0</sub>; overall stage III, T<sub>N</sub>M<sub>0</sub> or T<sub>N</sub>M<sub>1</sub>; and overall stage IV, T<sub>N</sub>M<sub>0</sub> or any T, N, M; any T, N, M; or T<sub>N</sub>M<sub>0</sub> or any T, and N, M<sub>1</sub>; T, tumor stage (T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub>, and T<sub>4</sub>). N, nodal status (N<sub>0</sub>, N<sub>1</sub>, N<sub>2</sub>, and N<sub>3</sub>). M, distant metastases (M<sub>0</sub> and M<sub>1</sub>; here M<sub>0</sub> for all the cases in this study; American Joint Committee for Cancer Staging and End-Results reporting, 1992).

<sup>b</sup> OR, odds ratio; CI, confidence interval; ref., reference group.

<sup>c</sup> Adjusted for age, sex, smoking status, and alcohol status in a logistic regression model for the strata.
growth (51, 52), which may explain the possible role of the \textit{p27} polymorphism in alcohol-induced SCCHN, but this hypothesis needs to be further tested in future studies. It is also possible that this borderline significant finding from a subgroup analysis could be because of chance.

We found that the SCCHN risk associated with the \textit{p27} variant genotypes was significantly higher for patients with cancers of the oral cavity but not for those with cancers of the oropharynx, hypopharynx, or larynx, suggesting that oral cavity carcinoma may have a different etiology in relation not only to environmental risk factors but also to genetic susceptibility. Although the finding of the \textit{p27} variant-associated cancer risk in a tissue-selective manner is unknown, this is consistent with the elevated risk associated with the \textit{p27} variant genotype in current alcohol drinkers described above. One study has demonstrated that among SCCHN sites, oropharyngeal cancers are strongly associated with human papillomavirus type 16, oral cavity cancers are not associated with the virus, and laryngeal cancers have a variable association (7). Therefore, it appeared that the \textit{p27} variant genotype is possibly a risk factor for alcohol-induced tumors but not for human papillomavirus-initiated tumors. Other studies suggest that abnormalities of cell cycle-related genes, such as \textit{p53}, \textit{p27}, \textit{p16}, and \textit{cyclin D1}, are common in oral cavity cancer and possibly occur through mutation, amplification, or deactivation (17). The more pronounced risk in a selective tissue of oral cavity may be because of low levels of \textit{p27} protein expression caused by \textit{p27} V109G polymorphism that interferes in interactions with \textit{p38} subunits. Furthermore, tissue-specifically expressed PGPH5 may interfere with the ability of cells with the \textit{p27} variant to halt cell cycling and leading to the accumulation of genetic alterations caused by oral mucosa etiological agents including cigarette smoking and alcohol. Nevertheless, these hypotheses need to be validated in future studies.

Finally, we observed that risk associated with the \textit{p27} variant genotypes increased as the overall stage of oral cavity cancer increased from I to III-IV. Compared with the VV genotype, the variant GG genotype was significantly associated with the overall stage of oral cavity cancer. This finding could have been related to the survival of the SCCHN patients, because it is possible that patients with an adverse variant allele may have a shorter survival. Previous studies indicated that there was an inverse correlation between tumor grade and the level of \textit{p27} expression in oral-tongue squamous cell carcinoma, breast, esophageal squamous cell, and colon carcinomas, which may serve as a prognostic factor (30, 32, 39, 40, 53). The \textit{p27} V109G polymorphism likely causes low-protein expression in cancer, leading to a more aggressive tumor stage and possibly a poor prognosis. This hypothesis also needs to be further tested in larger studies of oral cancers.

In conclusion, the \textit{p27} 109G variant may not play a major role in the overall etiology of SCCHN but likely among subgroups of SCCHN, such as men, current alcohol users, and patients with oral cavity cancer. However, these nonsignificantly increased risks from stratified analysis should be considered preliminary, which may be attributable to relatively small numbers of the strata or a result of chance. Therefore, these findings need larger studies to be further verified. Additional studies are warranted involving larger numbers of patients with oral cavity cancer, quantitative measures of smoking and alcohol exposure, and an emphasis on molecular changes in the target tissue.

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