Genetic polymorphisms of p21 and risk of second primary malignancy in patients with index squamous cell carcinoma of the head and neck

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p21 plays an important role in modulating cell cycle control, inducing apoptosis, and inhibiting cell growth, subsequently affecting cancer risk. We investigated the association between two putatively functional single-nucleotide polymorphisms (SNPs) of p21 (p21 C98A and p21 C70T) among 1282 patients diagnosed with index squamous cell carcinoma of the head and neck (SCCHN) and risk of second primary malignancy (SPM) in an ongoing molecular epidemiology study. We used Log-rank test and Cox proportional hazard models to assess the association of these two SNPs with SPM-free survival and SPM risk. We found that patients with either p21 variant genotypes of the two polymorphisms had a significantly reduced SPM-free survival compared with patients with either p21 wild-type homozygous genotypes (Log-rank test, \( P = 0.0016 \)). Compared with patients having the p21 98 CC and p21 70 CC genotypes, the patients having p21 98 CA/AA and p21 70 CT/TT variant genotypes had a significantly greater risk of developing SPM, respectively, [hazard ratio (HR) = 1.80, 95% CI = 1.14–2.82 for p21 C98A and HR = 1.82, 95% confidence interval (CI) = 1.16–2.85 for p21 C70T]. Moreover, after combining the variant genotypes of two SNPs, patients with variant genotypes had a significantly moderately increased risk for SPM compared with patients with no variant genotypes (HR = 2.00, 95% CI = 1.26–3.00), and the risk was particularly pronounced in several subgroups. Our results support an increased risk of SPM after index SCCHN with both p21 polymorphisms individually and in combination.

Introduction

Squamous cell carcinoma of the head and neck (SCCHN) including the oral cavity, oropharynx, hypopharynx and larynx is the fifth most common malignancy; SCCHN, squamous cell carcinoma of the head and neck.

Abbreviations: CI, confidence interval; CDK, cyclin-dependent kinase; HR, hazard ratio; SNP, single-nucleotide polymorphism; SPM, second primary malignancy; SCCHN, squamous cell carcinoma of the head and neck.

Materials and methods

Study subjects

A cohort of 1167 patients with incident SCCHN, who were newly diagnosed, histopathologically confirmed, and untreated squamous cell carcinomas of the oral cavity, oropharynx, hypopharynx or larynx were consecutively recruited from May 1995 to January 2007 at The University of Texas M. D. Anderson Cancer Center as part of an ongoing molecular epidemiologic study, which has been described previously elsewhere (39,40). All patients completed an institutional review boards-approved informed consent and were recruited without discrimination regarding age, sex, ethnicity or clinical stage (except associated with the risk of developing a SPM after an index SCCHN (12–17).

Alterations in genes involved in cell cycle control frequently result in dysregulated cellular proliferation; specifically, genes associated with the regulation of the G1 checkpoint in the cell cycle are frequently altered in cancer cells (18). 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 (19–21). The p21 (also known as Waf1/Cip1/CDKN1A) protein is one of CDK inhibitors that are essential for cellular growth, differentiation and apoptosis (22). p21, a putative tumor suppressor gene, is located on chromosome 6p21.2 and encodes a 21 kDa protein (23) that belongs to the CDK-interacting proteins/kinase inhibitor proteins family including p27 (22) and p57 (24,25). Overexpression of p21 inhibits proliferation in mammalian cells and has been found to inhibit all cyclin–CDK complexes, indicating that it is a universal cyclin–CDK inhibitor (22). The CDK-interacting proteins/kinase inhibitor proteins share some common sequence motifs that mediate interaction between CDK inhibitors and cyclin–CDK complexes (26,27). p21 is known to be directly regulated by the tumor suppressor p53 and is considered to be one of the most important and potent effector molecules of p53. The p53 protein directly activates p21 expression by binding its promoter (28). Hence, in human cancers, inactivation of p53 will also lead to decreased levels of p21.

Since p21 expression is one of the most prominent markers for the functional activity of p53, many studies have analyzed p21 expression in different types of human cancer but the results are conflicting. In some reports, the lack of p21 was indeed shown to correlate with tumor progression and negative prognosis, as reported, for example, for small-cell lung (29), colorectal (30), cervical (31) and head and neck cancers (32). In some cases, however, p21 expression was concluded to have no prognostic value (33–37). Given the functional importance of p21 in critical p53 pathway, genetic alteration of p21 could be associated with prognosis in SCCHN patients.

We previously reported that each or in combination of two putatively functional polymorphisms of p21 were significantly associated with risk of SCCHN (38). One is a common single-nucleotide polymorphism, p21 C98A in exon 2, which causes a non-synonymous serine-to-arginine substitution at codon 31. Another is the p21 C70T within the 3’ untranslated region, which causes a single C-to-T substitution 20 nt downstream of the stop codon at exon 3. However, the role of p21 polymorphisms in the etiology of SPM after index SCCHN has not been investigated. Because both common single-nucleotide polymorphisms are thought to alter p21 function, the two polymorphisms could also contribute to the risk of SPM among SCCHN patients.

To test whether each or in combination of the two p21 polymorphisms is associated with the risk of SPM within a cohort of 1282 patients initially recruited with incident index SCCHN, we hypothesize that each and, more probably, in combination of the two p21 polymorphisms confer an increased risk of SPM for SCCHN patients.
patients having distant metastases on presentation were not recruited. Patients
with any prior cancer history excepting non-melanoma skin cancer were not
recruited. Also, patients with primary sinonasal tumors, salivary gland tumors,
cervical metastases of unknown origin or tumors outside the upper aerodiges-
tive tract were not recruited. Approximately 95% of contacted patients con-
sented to enrollment in the study. Blood samples for p21 genotyping data were
not available for some patients recruited early in the study, and these patients
were excluded from this genotype and SPM analysis, as were patients without
follow-up or who underwent only palliative treatment. Consequently, 1282
patients were available for this study of SPM risk.

Data collection
Patients were monitored through their treatment and post-treatment course
with regularly scheduled clinical and radiographic examinations. SPMs were
distinguished from local recurrences based on modified criteria of Warren et al.
(41). Second lesions with different histopathologic type and/or occurring >5
years following treatment for the primary tumor and/or clearly separated by
normal epithelium based on clinical and radiographic assessment were con-
sidered as SPM. If there was discrepancy or differing opinion regarding
the origin of the tumor, the second lesion was classified as a local recurrence rather
than an SPM. Pulmonary lesions were considered as SPM if they had a non-
squamous histology or if they were isolated squamous lesions >5 years from
initial SCCHN and felt to be SPM by the thoracic oncologist and thoracic
surgeon. SPMs were then classified as tobacco-associated sites (oral cavity,
oropharynx, larynx, hypopharynx, esophagus, lung or bladder) and non-
tobacco-associated sites.

Clinical data were obtained at initial presentation and through follow-up
examinations and included overall stage at presentation of the index tumor,
site of the index tumor and treatment. Index cancer stage was then dichoto-
mized into the early stage (I/II) and late stage (III/IV). We also grouped
treatment into those with DNA damaging agents (radiation and/or chemother-
apy) and those without (surgery only). All patients completed at presentation an epidemiological questionnaire including data on
alcohol and smoking status. Drinking status was categorized as ‘ever-drinkers’
(those who had drunk at least one alcoholic beverage per day for at least 1 year
during their lifetime) and ‘never-drinkers’ (those who had never had such a pattern
of drinking). Smoking status was categorized as ‘ever-smokers’ (those who had
smoked at least 100 cigarettes in their lifetime) and ‘never-smokers’ (those
who had smoked <100 cigarettes in their lifetime).

p21 genotyping
We extracted genomic DNA from the buffy-coat fraction of the blood samples
using a DNA Blood Mini Kit (QUAGEN, Valencia, CA) according to the
manufacturer’s instructions for genotyping p21 polymorphisms. The methods
for genotyping of the polymorphisms have been described previously (38). We
performed the polymerase chain reactions and evaluated the results without
knowing the subjects with SPM or without SPM. At least 10% of the random
samples were retested, and the results were 100% concordant.

Statistical analysis
SPM occurrence was the primary endpoint of the study. The Student’s t-test
was used to compare the mean age and follow-up time of the patients who
developed a second primary cancer and those who did not. The differences in
ethnicity, sex, smoking and alcohol status, tumor site, tumor stage, treatment,
genotype distributions and allele frequencies between the two groups were
evaluated using the chi-squared test. Time-to-event was calculated from the
date of diagnosis of the index SCCHN to the date of SPM occurrence. Patients
who were not known to have an event at the date of last contact or who died
were censored. The associations between individual epidemiological risk fac-
tors, clinical characteristics, including tumor site, staging and treatment vari-
able, and time to the occurrence of the SPMs were initially assessed using
univariate Cox proportional hazards regression models. The data were consist-
ent with the assumptions of the Cox proportional hazards regression model
from the examination of Kaplan–Meier survival curves and log-minus-log
survival plots.

In the univariate analysis, we evaluated epidemiological variables, assessed
at the time of diagnosis, such as age in years, ethnicity, sex and smoking and
alcohol status, and clinical characteristics, such as tumor site, tumor stage
and treatment. We did not incorporate any interaction terms in the first step in
building a multivariable model for time to SPM occurrence. A multivariable
proportional hazards model was built using the variables that had prognostic
potential suggested by the univariate analysis (P < 0.25). Due to epidemi-
ological and clinical considerations in building the model, age, sex and ethnicity
were always retained in the main-effects and final multivariable model. We
used a stepwise search strategy to build the multivariable models, for which
a threshold level of 0.25 for the likelihood ratio test was used as a cutoff to
determine whether a variable could be entered into, or removed from, the
regression model. We assessed associations using hazard ratios (HRs) and their
95% confidence intervals (CIs) for a SPM development. The final fully ad-
justed Cox regression models included age, sex, ethnicity and smoking and
alcohol status. For all analyses, statistical significance was set at P < 0.05, and
all tests were two sided. The Statistical Analysis System software (version
9.1.3, SAS Institute, Cary, NC) was used to perform all statistical analyses.

Results

Patient characteristics
The demographics, risk exposure and clinical variables for the 1282
patients were shown in Table I. We followed up these 1282 patients
with a median follow-up time of 34.1 months (range 0–142.4 months).
Overall, 1162 patients did not develop SPM, whereas 120 (9.4%)
patients developed SPM. Of the 120 patients with SPM, 81 patients
developed SPMs at tobacco-associated sites (44 SCCHN), 35 devel-
oped SPMs at other sites and four developed SPMs at both sites. Of
the 44 patients with second SCCHN, 24 (55%) were synchronous
SCCHN primaries. Of these 24 patients with synchronous SCCHN,
two patients had bilateral oral cavity cancers, three had bilateral or-
opharyngeal cancers, one had bilateral hypopharyngeal cancers and the
remainder had simultaneous cancers of more than one head and neck
subsite.

In this cohort of 1282 patients, the mean age at diagnosis for the
index SCCHN patients was 57.4 years (range: 18–94 years and me-
dian: 57 years), and the mean age of patients at index SCCHN who
developed SPM was significantly older compared with the mean age
of patients who did not develop SPM (60.8 years versus 57.1 years,
respectively). The differences in age at presentation of index cancer
between patients who developed SPM and who did not were statistically
significant (P < 0.005). Over 90% of patients had a known smoking
status (976, 76.1%versus 366, 31.7%), and 75% of the patients
developed SPM were ever-smokers (940, 73.3% versus 334, 26.0%).

Table I. Distribution of selected characteristics of the patient cohort
(n = 1282)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total patients</th>
<th>SPM-Free patients</th>
<th>SPM patients</th>
<th>P-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total patients</td>
<td>1282 (100)</td>
<td>1162 (90.6)</td>
<td>120 (9.4)</td>
<td></td>
</tr>
<tr>
<td>Age at presentation of index cancer</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; median (57 years)</td>
<td>663 (51.7)</td>
<td>624 (53.7)</td>
<td>39 (32.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>≥ median (57 years)</td>
<td>619 (48.3)</td>
<td>538 (46.3)</td>
<td>81 (67.5)</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>976 (76.1)</td>
<td>882 (75.9)</td>
<td>94 (78.3)</td>
<td>0.552</td>
</tr>
<tr>
<td>Female</td>
<td>306 (23.9)</td>
<td>280 (24.1)</td>
<td>26 (21.7)</td>
<td></td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-Hispanic white</td>
<td>1083 (84.5)</td>
<td>989 (85.1)</td>
<td>94 (78.3)</td>
<td>0.015</td>
</tr>
<tr>
<td>Other</td>
<td>199 (15.5)</td>
<td>173 (14.9)</td>
<td>26 (21.7)</td>
<td></td>
</tr>
<tr>
<td>Smoking status at presentation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>342 (26.7)</td>
<td>317 (27.3)</td>
<td>25 (20.8)</td>
<td>0.128</td>
</tr>
<tr>
<td>Ever</td>
<td>940 (73.3)</td>
<td>845 (72.7)</td>
<td>95 (79.2)</td>
<td>0.352</td>
</tr>
<tr>
<td>Alcohol status at presentation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>334 (26.0)</td>
<td>307 (26.4)</td>
<td>27 (22.5)</td>
<td>0.308</td>
</tr>
<tr>
<td>Ever</td>
<td>948 (74.0)</td>
<td>855 (73.6)</td>
<td>93 (77.5)</td>
<td></td>
</tr>
<tr>
<td>Index cancer site</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oral cavity</td>
<td>415 (32.4)</td>
<td>377 (32.4)</td>
<td>38 (31.7)</td>
<td></td>
</tr>
<tr>
<td>Oropharynx</td>
<td>573 (44.7)</td>
<td>525 (45.2)</td>
<td>48 (40.0)</td>
<td></td>
</tr>
<tr>
<td>Larynx/hypopharynx</td>
<td>294 (22.9)</td>
<td>260 (22.4)</td>
<td>34 (28.3)</td>
<td></td>
</tr>
<tr>
<td>Index cancer stage</td>
<td></td>
<td></td>
<td></td>
<td>0.697</td>
</tr>
<tr>
<td>1 or 2</td>
<td>323 (25.5)</td>
<td>291 (25.0)</td>
<td>32 (26.7)</td>
<td></td>
</tr>
<tr>
<td>3 or 4</td>
<td>959 (74.8)</td>
<td>871 (75.0)</td>
<td>88 (73.3)</td>
<td></td>
</tr>
<tr>
<td>Index cancer treatment</td>
<td></td>
<td></td>
<td></td>
<td>0.887</td>
</tr>
<tr>
<td>Surgery only</td>
<td>226 (17.6)</td>
<td>205 (17.6)</td>
<td>21 (17.5)</td>
<td></td>
</tr>
<tr>
<td>Surgery + adjuvant treatment</td>
<td>320 (25.0)</td>
<td>287 (24.7)</td>
<td>33 (27.5)</td>
<td></td>
</tr>
<tr>
<td>XRT</td>
<td>330 (25.7)</td>
<td>302 (26.0)</td>
<td>28 (23.3)</td>
<td></td>
</tr>
<tr>
<td>XRT + chemotherapy</td>
<td>406 (31.7)</td>
<td>368 (31.7)</td>
<td>38 (31.7)</td>
<td></td>
</tr>
</tbody>
</table>

*P-values were calculated from chi-square test.

bAdjuvant treatment: adjuvant radiotherapy and/or chemotherapy.

XRT: radiotherapy.
respective; \( P < 0.01 \)). Although the participants in this study were predominantly male (76.1%), sex was not associated with SPM development (\( P = 0.552 \)). Compared with the SPM-free group, patients who developed SPM had similar characteristics regarding index smoking (\( P = 0.128 \)), alcohol drinking (\( P = 0.352 \)), cancer site (\( P = 0.308 \)), cancer stage (\( P = 0.697 \)) and treatment (\( P = 0.887 \)). However, the patients who developed SPM were more probably to be older (\( P < 0.001 \)) and non-Hispanic whites (\( P = 0.051 \)) than the patients who did not develop SPM.

Association of \( p21 \) polymorphisms with risk of SPM after index SCCHN

Table II showed distributions of \( p21 \) C98A and \( p21 \) C70T genotypes between the patients who developed SPM and the patients who did not and the associations with risk of SPM development. For the two \( p21 \) polymorphisms, the distribution of \( p21 \) C98A and \( p21 \) C70T genotypes was significantly different between patients who developed SPM and those who did not (\( P = 0.010 \) for \( p21 \) C98A and \( P = 0.0050 \) for \( p21 \) C70T). Additionally, patients who possessed 

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Total (( N = 1282 ))</th>
<th>SPM-free (( N = 1162 ))</th>
<th>SPM (( N = 120 ))</th>
<th>( P^a )</th>
<th>HR (95% CI)(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( p21 ) C98A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC (Ref)</td>
<td>1095 (85.4)</td>
<td>1002 (86.2)</td>
<td>93 (77.5)</td>
<td>0.029</td>
<td>1.00</td>
</tr>
<tr>
<td>CA</td>
<td>168 (13.1)</td>
<td>143 (12.3)</td>
<td>25 (20.8)</td>
<td>1.81</td>
<td>(1.14–2.87)</td>
</tr>
<tr>
<td>AA</td>
<td>19 (1.5)</td>
<td>17 (1.5)</td>
<td>2 (1.7)</td>
<td>1.71</td>
<td>(0.41–7.03)</td>
</tr>
<tr>
<td>CA + AA</td>
<td>187 (14.6)</td>
<td>160 (13.8)</td>
<td>27 (22.5)</td>
<td>0.010</td>
<td>1.80              (1.14–2.82)</td>
</tr>
<tr>
<td>( p21 ) C70T</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC (Ref)</td>
<td>1102 (86.0)</td>
<td>1009 (86.8)</td>
<td>93 (77.5)</td>
<td>0.020</td>
<td>1.00</td>
</tr>
<tr>
<td>CT</td>
<td>164 (12.8)</td>
<td>139 (11.9)</td>
<td>25 (20.8)</td>
<td>1.92</td>
<td>(1.21–3.05)</td>
</tr>
<tr>
<td>TT</td>
<td>16 (1.2)</td>
<td>14 (1.3)</td>
<td>2 (1.7)</td>
<td>1.06</td>
<td>(0.26–4.42)</td>
</tr>
<tr>
<td>CT + TT</td>
<td>180 (14.0)</td>
<td>153 (13.2)</td>
<td>27 (22.5)</td>
<td>0.0050</td>
<td>1.82              (1.16–2.85)</td>
</tr>
</tbody>
</table>

Combined risk genotypes

<table>
<thead>
<tr>
<th>Genotype</th>
<th>N (%)</th>
<th>HR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No variant</td>
<td>1081 (84.3)</td>
<td>1.00</td>
</tr>
<tr>
<td>Variants</td>
<td>201 (15.7)</td>
<td>2.00 (1.26–3.00)</td>
</tr>
</tbody>
</table>

\( a \)Chi-squared test for differences in the distribution of \( p21 \) genotypes between the patients who developed SPM and the patients who did not.

\( b \)Adjusted for age, sex, ethnicity, tobacco smoking and alcohol drinking in a Cox model.

Stratification analysis of the combined \( p21 \) genotypes with risk of SPM

Table III showed the association between the combined \( p21 \) genotypes and risk of SPM in each subgroup further stratified by age, sex, ethnicity, smoking/drinking status, index cancer site, index cancer stage, index cancer treatment and SPM type. When we used the combined no variant genotype group (\( p21 \) 98 CC and \( p21 \) 70 CC) as the reference, there was a >2-fold significantly increased SPM risk for those with any \( p21 \) variant genotypes among older patients, males, patients with early stage cancers and patients with cancers of non-oropharyngeal sites (Table III). Additionally, a significantly elevated SPM risk associated with any \( p21 \) variant genotypes was found among non-Hispanic whites, smokers, drinkers and for those who received some DNA-damaging agent during the treatment of their index cancer (Table III). Additionally, the \( p21 \) variant genotypes were associated with significantly increased risk of SPM, whether at tobacco-associated SPM sites (HR = 1.71, 95% CI = 1.02–2.87) or other SPM sites (HR = 2.32, 95% CI = 1.07–5.05). After we performed the tests of heterogeneity of the stratified estimates, we found that there was a significant interaction effect between the combined variant genotypes of \( p21 \) polymorphisms and age, sex, ethnicity, smoking/alcohol status, treatment and index cancer site on risk of SPM, and the

\[ \text{HR} = 1.26–2.87 \]

Fig. 1. Kaplan–Meier SPM-free survival curve stratified by combined \( p21 \) wild-type genotypes and variant genotypes.
interaction effect of the combined variant genotypes of p21 polymorphisms with index cancer stage on risk of SPM was only borderline statistically significant (Table III).

Discussion

It is plausible that p21 polymorphisms might affect cancer risk, as p21 can be induced by p53 in response to DNA damage, to arrest cellular growth allowing for DNA repair (42). Previous studies have evaluated the association of these p21 polymorphisms and risk of or survival from several types of cancer (38,43–46), but none of these evaluated the association of these p21 polymorphisms and risk of SPM after index SCCHN. In the current study of 1282 patients with SCCHN, we found that patients with p21 98 CA/AA and p21 70 CT/TT variant genotypes had a significantly greater risk of developing SPM compared with patients with the p21 98 CC and p21 70 CC wild-type homozygous genotype, respectively, and the combined variant genotypes of the two polymorphisms had a moderately increased risk of SPM compared with the combined wild-type homozygous genotypes after multivariable adjustment. Additionally, the SPM risk associated with the combined variant genotypes was for SPM at both \( P = 0.001 \).

Like other key cell cycle regulators, p21 plays an important role in regulating critical cellular activities, such as cell cycle control, DNA repair and apoptosis, and consequently could influence the efficiency of response to DNA damage and tumor development. To date, there is no known functional relevance of the p21 polymorphisms, but our study and others did suggest that the p21 C98A and p21 C70T polymorphisms have functional significance and are probably to contribute to genetic susceptibility to cancer (38,47,48). The p21 C98A polymorphism causes a serine-to-arginine substitution in its zinc-finger motif, which could alter the p21 protein’s function (49). Because p21 C70T polymorphism at exon 3 lies within the 3’ untranslated region 20 nt downstream of the stop codon, it is more probably that the p21 C70T polymorphism may increase cancer risk by altering messenger RNA stability, thereby affecting intracellular levels of p21 protein. However, all these hypotheses remain to be tested in future studies.

One published SCCHN study examined the association between p21 C98A and p21 C70T and SCCHN risk in 42 patients and 110 controls (50) and found p21 polymorphisms in 9.1% of controls (10 of 110) and 21.4% of SCCHN patients (9 of 42), but the difference was not statistically significant, most probably due to the study with a small sample size. We have also previously reported that these p21 polymorphisms were associated with a significantly increased risk of SCCHN (38). In the current study, we observed a nearly 1.8-fold increased risk of SPM for each of the two polymorphisms and an ~2-fold increased risk of SPM for having either variant genotype.

A greater risk associated with the p21 variant genotypes in older (>57 years) subjects supports the implication of age as a risk factor for the development of SPM after index SCCHN \( (P < 0.001) \). We also found that the SPM risk associated with the combined variant genotypes reached the statistically significant level in men but not in women. This finding may be because that the men are more probably ever-smokers and less probably never-smokers than women in this study \( (P = 0.002) \) and men with the combined p21 variant genotypes may be more sensitive to tobacco carcinogens. The finding of a greater risk in ever-smokers and ever-drinkers also suggests that the combined p21 variant genotypes may have an impact on cell cycle control that was induced by DNA damage caused by carcinogens in tobacco smoke and alcohol use. However, we found that the risk of SPM associated with the p21 variant genotypes was for SPM at both tobacco-associated sites and other sites.

Previous studies have reported that there is no significant association of SPM occurrence with clinical characteristics or treatment but, rather, with previous and continued exposure to tobacco or alcohol.
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References

p21 genetic polymorphisms and second malignancies


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