Nucleotide excision repair core gene polymorphisms and risk of second primary malignancy in patients with index squamous cell carcinoma of the head and neck

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The nucleotide excision repair (NER) pathway is central in response to damage induced by environmental carcinogens. Efficiency of this pathway, probably genetically determined, may modulate individual risk of developing squamous cell carcinoma of the head and neck (SCCHN) as well as second primary malignancy (SPM) after the index tumor. We hypothesized that common non-synonymous and regulatory single-nucleotide polymorphisms (SNPs) in the NER core genes individually, and more probably collectively, associated with the risk of SPM. We genotyped for seven selected SNPs in 1376 incident SCCHN patients who were prospectively recruited between 1995 and 2006 and followed for SPM development. We found that 110 patients (8%) developed SPM; 43 (39%) second SCCHN; 38 (35%) other tobacco-associated sites and 29 (26%) other non-tobacco-associated sites. The associations of these SNPs with SPM risk were assessed assuming a recessive genetic model. We did not find any significant associations of each or in combination of the seven SNPs with SPM risk in the recessive models. However, when we explored the combined effect based on an alternatively dominant genetic model, we found that the number of observed risk genotypes was associated with a significantly increased SPM risk in a dose-response manner (P = 0.005) and patients with five to seven risk genotypes had a significantly 2.4-fold increased SPM risk compared with patients with zero to two risk genotypes. These findings suggest that a profile of NER core gene polymorphisms might collectively contribute to risk of SPM not in a recessive model but in a dominant model among patients with an index primary SCCHN. These findings need to be validated in future studies with larger sample sizes and longer follow-up time.

Introduction

Although the incidence of squamous cell carcinoma of the head and neck (SCCHN) has been declining for two decades (1), an estimated 47,560 incident diagnoses will be made in the USA in 2008 (2). While the majority of SCCHN are associated with tobacco and alcohol (3,4), most smokers and drinkers never develop SCCHN, suggesting that genetic susceptibility plays a role in SCCHN etiology. Surgery, radiotherapy and chemotherapy cure many SCCHN, suggesting that genetic susceptibility plays a role in SCCHN etiology. However, the majority of patients, even those who were/are smokers, do not develop SPMs (7,8). We and others have hypothesized that the same genetic predisposition to develop an initial SCCHN potentially plays a role in SPM risk. In fact, independent phenotypic studies of sensitivity to mutagenic agents have shown an increased risk for SPM associated with hypersensitivity (9–11).

Polymorphisms in genes regulating carcinogen detoxification, nucleotide excision repair (NER), cell cycle control and apoptosis have all been studied as potential factors involved in a genetic predisposition to SPM (12). NER proteins function synergistically to repair bulk DNA damage induced by endogenous and external environmental factors by a complex mechanism of incision of damaged DNA followed by insertion of new base pairs synthesized from the complementary DNA strand. Among the eight core genes in the NER pathway, 1098 single-nucleotide polymorphisms (SNPs) have been identified; 40 of these are non-synonymous SNPs that lead to different polypeptide sequences. However, there are only five with a minor allele frequency >0.05 in non-Hispanic whites (XPA Ala499Val, XPC Lys939Gln, XPD Asp312Asn, XPD Lys751Gln and XPG His1104Asp) (13).

Genetic variations in the NER pathway have been studied in association with many types of cancer including non-melanoma skin cancer (14), cutaneous melanoma (15), cancers of the lung (16), prostate (17), breast (18), colorectum (19), bladder (20) and esophagus (21) and soft tissue sarcomas (22). We have recently studied seven common regulatory variants in NER genes: the above-mentioned five common non-synonymous SNPs as well as two common regulatory SNPs (ERCC1 8092A and XPA G23A), which have been shown to be associated with a non-significantly increased risk of SCCHN (13,23,24). While we found a significantly increased risk of SCCHN associated with only one SNP (XPC Ala499Val), in combining all seven variants, the number of observed risk genotypes was associated with SCCHN risk in a dose-response manner (13).

Gal et al. (25) studied the association of SPMs in oral cavity and oropharynx cancer patients with four polymorphisms in the base excision repair, NER, and mismatch repair pathways. A SNP in the base excision repair X-ray repair cross-complementing enzyme (XRCC3) was associated with a significantly increased risk of SPM, but there was no association between SPM and the XPD Lys751Gln SNP of the NER pathway. Two other studies looking at SNPs in the same gene (XPD) of the NER pathway have not found an association with SCCHN SPM (26,27). To the best of our knowledge, with the exception of XPD, the influence of common genetic polymorphisms in the NER pathway has not been previously studied in association with risk of SPM after index SCCHN.

Considering genetic models of inheritance of traits associated with age at onset of these selected polymorphisms, the recessive model appeared to be the best model. The biological effects of functional variants of a tumor suppressor gene appear to follow a recessive model because the function of one normal allele may compensate the function loss of the other variant allele. For example, a recessive model typically represents inherited genetic effects for these variants in xeroderma pigmentosum (XP) (28). Therefore, in the current study, we assumed a recessive model in which an individual must have two copies of the variant allele to have an increased SPM risk. We also explored alternative genetic models, such as a dominant model, in which the allele effects will be assessed by either an allele–dose response or the combined variant genotypes.

Abbreviations: HPV, human papillomavirus; NER, nucleotide excision repair; SCCHN, squamous cell carcinoma of the head and neck; SPM, second primary malignancy; XPC, xeroderma pigmentosum complementation group C.
Others have suggested that a polygenic cancer model, in which each high-risk allele confers a slight variance in phenotype and increased susceptibility to a cancer endpoint, may account for a significant proportion of cancer diagnoses (29). In keeping with this polygenic cancer model, we hypothesized that potentially functional SNPs in the core NER genes individually, and more probably collectively, will contribute to the risk of SPM after index SCCHN in a recessive genetic model. We prospectively recruited incident SCCHN patients without prior treatment or prior malignancy as part of a molecular epidemiologic study of SCCHN, obtaining relevant clinical information and blood samples for genetic testing at the time of initial presentation to our institution. Patients were followed throughout their treatment and posttreatment course with regularly scheduled clinical and radiographic examinations. We present a prospective cohort analysis assessing the association between the previously mentioned seven potentially functional SNPs in the NER pathway and in the risk of SPM.

Materials and methods

Study subjects

Between May 1995 and December 2006, 1600 newly diagnosed and previously untreated patients with histopathologically confirmed SCCHN were consecutively recruited as part of an ongoing prospective molecular epidemiologic study at University of Texas M. D. Anderson Cancer Center which has been described previously (13,30). All subjects who completed Institutional Review Board-approved informed consent were recruited without discrimination regarding age, sex, ethnicity or cancer stage, except that patients with known distant metastases were excluded. Patients with any prior cancer history excepting non-melanoma skin cancer were not recruited. All patients with primary sinonasal tumors, salivary gland tumors, cervical metastases of unknown origin or tumors outside the upper aerodigestive tract were also not recruited. Approximately 95% of contacted patients consented to enrollment in the study. Blood samples for genotyping data were not available for some patients recruited early in the parent study, and these patients were excluded from further analysis, as were a small number of patients without follow-up. Additionally, patients who underwent only palliative treatment were excluded from the current analysis.

Patients were monitored through their treatment and posttreatment course with regularly scheduled clinical and radiographic examinations. SPMs were distinguished from local recurrences based on modified criteria of Warren (31). Second lesions with different histopathological type, or occurring >5 years following treatment for the primary tumor or clearly separated by normal recurrence rather than an SPM. Pulmonary lesions were considered 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 head and neck (oral cavity, oropharynx, hypopharynx and larynx), other tobacco associated (esophagus, lung and bladder) or other non-tobacco associated.

All patients completed at presentation an epidemiological questionnaire including data on alcohol and smoking status. Alcohol status was categorized as ‘ever drinkers’ (those who had drunk at least one alcoholic beverage/week for at least 1 year during their lifetime) and ‘never drinkers’ (those who never had such a pattern of drinking). ‘Ever smokers’ were those who had smoked <100 cigarettes in their lifetime. Clinical data were obtained from review of the medical record at initial presentation and through follow-up examinations and included overall stage at presentation of the index tumor, site and histology of primary and second primary tumor and treatment.

Genotyping

DNA was extracted from patient blood samples and used for genotyping for the following seven polymorphisms of the NER pathway: ERCC1 C8092A, XPA G23A, XPC Ala499Val and Lys939Gln, XPD Asp312Asn and Lys751Gln and XPG His1104Asp. The primers, polymerase chain reaction and restriction enzymes for these polymorphisms have been described previously (13). There was 100% concordance when 10% of the genotyping assays were repeated.

Statistical analysis

The primary endpoint of the study was SPM occurrence. 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 were lost to follow-up or who died following the initial date of diagnosis of their first primary head and neck cancer were censored. The associations between individual epidemiological risk factors, clinical characteristics including tumor site, staging, and treatment variables and time to the occurrence of the SPMs were initially assessed using univariate Cox proportional hazards regression models. The data were consistent with the assumptions of the Cox proportional hazards regression model from the examination of Kaplan–Meier survival curves and log-minus-log survival plots.

Epidemiological variables in the univariate analysis, assessed at the time of diagnosis, included age in years, sex, ethnicity and smoking and alcohol status. Clinical characteristics included tumor site, tumor stage and treatment. The first step in building a multivariable model for time to SPM occurrence did not incorporate any interaction terms. A multivariable proportional hazards model was built using the variables that had prognostic potential suggested by the univariate analysis (P < 0.25). Due to epidemiological and clinical considerations in building the model, age, sex and ethnicity were always retained in the main effects and final multivariable model. In building multivariable models, a stepwise search strategy was used. 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. Associations were quantified using hazard ratios and their 95% confidence intervals for developing a SPM.

The final fully adjusted Cox regression models included age, sex, ethnicity and smoking and alcohol status. The mean age and follow-up time for those who developed a second primary cancer and those who did not were compared using the Student’s t-test. The chi-squared test was used to evaluate differences in ethnicity, sex, smoking and alcohol status, tumor site, tumor stage, treatment, genotype distributions and allele frequencies between the two groups. We defined a risk genotype based on the results of the individual SNP analysis. To assess the combined effect of all genotypes, the number of risk genotypes was added, assuming the risk associated with each of these genotypes was simply additive. We evaluated the individual and combined variants assuming a recessive genetic model, in which we compared the variant homozygous genotype with the combined variant heterozygous and wild-type homozygous genotypes. We also explored the combined effect of these polymorphisms on risk of SPM in an alternative dominant model, in which we added the variant homozygous genotype and the variant heterozygous genotype and compared with the wild-type homozygous genotype. 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) was used to perform all statistical analyses.

Results

Based on the exclusion criteria, a prospective cohort of 1376 patients with index SCCHN were evaluated for SPM. The overall median follow-up time was 26.3 months (range 0–142.4 months), during which 110 (8.0%) patients developed SPM. Of patients with SPMs, 43 (39%) developed second SCCHN, 38 (35%) developed other tobacco-associated cancers and 29 (26%) developed other non-tobacco-associated cancers. Of the 43 patients with SPM of the head and neck, 24 (56%) were simultaneous SCCHN primaries. Of these 24 patients with simultaneous SCCHN, two had bilateral oral cavity cancers, three had bilateral oropharyngeal cancers, one had bilateral hypopharyngeal cancers and the remainder had simultaneous cancers of more than one head and neck site.

Demographics, exposure and clinical variables for the total cohort of patients, the patients who did not develop SPM and the patients who developed SPM are summarized in Table I. The mean age at diagnosis for the total patients was 57.3 years (range, 18–94 years; median, 57 years), and the mean age of patients who developed SPM was significantly older compared with the mean age of patients who did not develop a second primary tumor (60.8 years versus 57.0 years, respectively; P < 0.01). All patients were predominantly male (76.0%) and non-Hispanic whites (84.0%), but sex and ethnicity were not significantly associated with SPM development (P = 0.734 for sex and P = 0.090 for ethnicity). Compared with the SPM-free group, patients who developed SPM were more probably older (P < 0.001), smokers (P = 0.022) and drinkers (P = 0.050). However, compared with the SPM-free group, patients who developed SPM had similar characteristics with respect to index cancer site (P = 0.118), index cancer stage (P = 0.830) and treatment (P = 0.982).
The genotype distributions of the selected SNPs and their associations with risk of SPM risk are summarized in Table II. We tested the hypothesis that variant homozygotes genotypes are associated with risk of SPM, assuming a recessive genetic model for the effect of a variant allele (i.e., only considering the variant homozygous genotypes as the risk genotype). However, we did not find a significant SPM risk associated with any of the selected SNPs with or without adjustment for age, sex, ethnicity, smoking and alcohol (P > 0.05 for all SNPs) (Table II) or in stratified analysis by SPM type (Table III). Furthermore, when analyses were restricted to the 997 ever smokers, we also found no significant SPM risk associated with any NER genotype (data not shown). When SPM risk was stratified according to smoking status at the time of diagnosis of index SCCHN (never versus ever smokers), there was no significant difference in the hazards ratios among these two subgroups (data not shown).

Because any of the seven SNPs of the core genes in the NER pathway appeared to have a minor effect on risk of SPM, we then performed combined analysis of all seven SNPs to focus on potentially modifying effect of the combined genotypes on risk of SPM in both recessive and dominant models (Table IV). In the 779 patients who had data available on all seven SNPs, we categorized all putative risk (ORs > 1.0) genotypes of each SNP into a new variable according to the number of risk genotypes (for the protective genotype, we reversed the reference group). In a recessive model, there was a trend for increased SPM risk with increasing number of risk genotypes, but this trend was not statistically significant (P = 0.779), whereas in a dominant model, such a trend in risk was significant in a dose–response manner (P = 0.005 for trend). Specifically, the patients with five to seven risk genotypes had a 2.4-fold (adjusted hazards ratios, 2.4; 95% confidence interval, 1.3–4.4) increased risk for developing SPM, compared with patients with zero to two risk genotypes.

**Discussion**

The present study examined the association between the seven selected potentially functional SNPs in the NER pathway critical to the repair of tobacco-related damage and risk of SPMs. Although we did not observe any significant association in the assumption of recessive genetic model, we did observe an effect of the combined risk genotypes in a dominant genetic model. We found that a statistically significant trend in the association between combined risk genotypes of the seven NER SNPs and risk of SPM. An increasing number of risk genotypes were associated, in a dose-response manner, with an increasing risk of SPM: patients with five to seven risk genotypes had a 2.4-fold increased risk for SPM compared with patients with zero to two risk genotypes. To the best of our knowledge, there have been no previous studies examining the combined effects of genetic variants in the NER pathway on risk of developing SPM after the index SCCHN.

The NER pathway specifically exerts bulk base damage induced by environmental carcinogens such as tobacco compounds (32), and therefore, this pathway is particularly relevant to SCCHN, in which the effect of tobacco is well documented. It has been shown that a genetically inherited DNA repair capacity to remove tobacco-induced DNA damage has been shown to modulate individual susceptibility to tobacco carcinogenesis (33). In a phenotypic study measuring in vitro messenger RNA levels of the five genes in the NER pathway, Cheng et al. (34) demonstrated that individuals with reduced NER gene expression were at a >2-fold increased risk of SCCHN. Additionally, the SNPs included in the current study have been previously examined in case–control analyses for their association with risk of primary SCCHN (23,35). It was found that these seven core NER SNPs appeared to modify the risk of index SCCHN in a case–control study using a recessive risk model (13).

Few studies have examined the effects of NER polymorphisms and SPM after index SCCHN. In a USA study of 279 oral cancer patients, Gal et al. (25) did not find an association between the XPD Lys751Gln polymorphism and SPM risk. In another Polish study of 182 head and neck cancer patients, Rydzaniez et al. similarly found no association.
between XPD A35931C and C22541A polymorphisms and risk of SPM (26). While these two studies have explored the association of individual SNPs in the NER pathway and risk of SPM, they did not evaluate pathway-oriented combined effects.

The xeroderma pigmentosum complementation group C (XPC) protein forms a supramolecular complex that recognizes and binds to sites of damaged DNA and initiates the NER pathway (36). The alanyl to valine substitution at codon 499 (Ala499Val) affects the interaction of XPC with Rad23, an accessory protein that both stimulates and stabilizes the XPC protein (37). Recent literature has suggested a protective effect for this alanine to valine substitution in tobacco-related cancer, particularly for smokers. For example, Huang et al. (38) observed that smoking-associated risk for advanced colorectal adenoma was significantly diminished for patients with the XPC valine allele. Similarly, Zhou et al. (39) found that smokers with the variant XPC 499 Ala/Val genotype had significantly reduced risk of developing gastric adenocarcinoma. Zhu et al. (40) demonstrated that patients with the XCP Ala499Val polymorphism had significantly decreased benzo[a]pyrene diol epoxide and gamma radiation-induced DNA damage. Wang et al. (41) reported that oral premalignancy risk was reduced 40% among patients with the XPC Ala499Val polymorphism. Consistent with these findings, our results also demonstrated a significant protective effect for the XPC 499 Ala/Val polymorphism in a dominant model (data not shown), which contributed to the overall effect of the combined genotypes.

There is some uncertainty as to what would be the best genetic model to represent genetic effect for these variants in the NER pathway. We analyzed the data in the current study first assuming a recessive model and did not identify any significant association. In contrast, when we assumed a dominant genetic model, we found a significant association of these putatively functional polymorphisms with risk of SPM. It becomes obvious that the results could vary depending on the model used in nominal statistical significance, particularly for weak associations of individual SNPs such as the results we found in the current study. Therefore, the combination of all variants of the core genes in the NER pathway could generate more stable risk estimates than that from a single variant.

This work has several inherent limitations. First of all, although this study had a relatively large sample size, we included both non-Hispanic whites (83.9%) and other ethnicities (16.1%) in the analysis to increase the study power, though we did adjust our results for ethnicity. Additionally, while demographics, exposure and clinical data for the cohort were collected prospectively, analysis of clinical outcomes including SPM was conducted retrospectively. Therefore, there was no strictly defined screening or follow-up regimen, and consequently 224 patients who had no follow-up time after index SCCHN had to be excluded. Furthermore, the follow-up time to the development of SPM during the study period may have been limited by the large proportion of patients with a stage III or IV index cancer, with only 110 patients having developed SPM, thus limiting statistical power to detect modest associations. Furthermore, the high prevalence (28%) of never smokers and our strict definition of SPM may have limited the observed incidence of SPM in this patient cohort. Finally, the absence of human papillomavirus (HPV) status did not allow us to evaluate its potential influence on the

### Table III. Association between genotypes of selected NER SNPs and SPM risk in a recessive genetic model stratified by SPM type

<table>
<thead>
<tr>
<th></th>
<th>SCCHN SPM</th>
<th>SCCHN or other tobacco-associated SPM</th>
<th>Non-tobacco-associated SPM</th>
<th>HRa (95% CI)</th>
<th>HRa (95% CI)</th>
<th>HRa (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ERCC1 C8092A</td>
<td></td>
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<tr>
<td>CC and CA</td>
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<td></td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>AA</td>
<td>0.6 (0.1–2.5)</td>
<td>0.7 (0.3–1.9)</td>
<td>1.2 (0.4–3.9)</td>
<td>1.0</td>
<td>1.0</td>
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<tr>
<td>XPAP223</td>
<td>1.0</td>
<td></td>
<td></td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>GG and GA</td>
<td>0.8 (0.3–1.8)</td>
<td>0.6 (0.3–1.3)</td>
<td>0.7 (0.2–2.0)</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
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<tr>
<td>XPC Ala499Val</td>
<td></td>
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<tr>
<td>Ala/Ala and Ala/Val</td>
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<td></td>
<td></td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Val/Val</td>
<td>0.4 (0.1–1.3)</td>
<td>0.8 (0.4–1.6)</td>
<td>0.5 (0.2–1.8)</td>
<td>1.0</td>
<td>1.0</td>
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<tr>
<td>XPC Lys939Gln</td>
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<tr>
<td>Lys/Lys and Lys/Gln</td>
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<td></td>
<td></td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Gln/Gln</td>
<td>0.9 (0.4–2.3)</td>
<td>0.8 (0.4–1.6)</td>
<td>0.9 (0.3–2.6)</td>
<td>1.0</td>
<td>1.0</td>
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<tr>
<td>XPD Asp312Asn</td>
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<tr>
<td>Asp/Asp and Asp/Asn</td>
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<td></td>
<td>1.0</td>
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<tr>
<td>Asn/Asn</td>
<td>0.8 (0.3–2.1)</td>
<td>0.9 (0.4–1.8)</td>
<td>0.5 (0.1–1.9)</td>
<td>1.0</td>
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<tr>
<td>XPD Lys751Gln</td>
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<td>Gln/Gln</td>
<td>0.6 (0.2–1.9)</td>
<td>1.0 (0.5–1.9)</td>
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<td>His/His and His/Asp</td>
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<tr>
<td>Asp/Asp</td>
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<td>0.4 (0.0–2.6)</td>
<td>2.4 (0.8–7.1)</td>
<td>1.0</td>
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</table>

*aAdjusted for age, sex, ethnicity, smoking and alcohol.

### Table IV. Association between the combined NER risk genotypes and SPM risk in both recessive and dominant genetic models

<table>
<thead>
<tr>
<th>No. risk genotypesa</th>
<th>Recessive</th>
<th></th>
<th></th>
<th>Dominant</th>
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<tr>
<td></td>
<td>Total SPM</td>
<td>SPM free</td>
<td>HRa (95% CI)</td>
<td>Total SPM</td>
<td>SPM free</td>
<td>HRa (95% CI)</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
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<td>n</td>
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<tr>
<td>0–2</td>
<td>19</td>
<td>2.1</td>
<td>17</td>
<td>2.5</td>
<td>1.0</td>
<td>158</td>
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<tr>
<td>3</td>
<td>163</td>
<td>18.4</td>
<td>143</td>
<td>21.3</td>
<td>1.4 (0.3–6.2)</td>
<td>211</td>
</tr>
<tr>
<td>4</td>
<td>459</td>
<td>60.5</td>
<td>393</td>
<td>58.7</td>
<td>1.7 (0.4–6.8)</td>
<td>219</td>
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<tr>
<td>5</td>
<td>128</td>
<td>17.5</td>
<td>109</td>
<td>16.3</td>
<td>1.3 (0.3–5.6)</td>
<td>137</td>
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<tr>
<td>6</td>
<td>10</td>
<td>1.8</td>
<td>8</td>
<td>1.20</td>
<td>3.0 (0.4–21.6)</td>
<td>49</td>
</tr>
<tr>
<td>7</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Trend</td>
<td></td>
<td></td>
<td>0.779</td>
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*aAdjusted for age, sex, ethnicity, smoking and alcohol.

bAdjusted for age, sex, ethnicity, smoking and alcohol.
development of SPMs in patients with index SCCHN. However, never-smoking status may be in part a surrogate marker for HPV status, and we had included smoking status in the multivariable model. We will closely monitor the role of HPV in the outcomes of SCCHN patients in our future studies when a much larger patient cohort with HPV-associated tumor becomes available.

A polygenic cancer model includes not only considering individual variation in specific genes but also studying the effects of combined genetic variances in critical pathways. The current investigation demonstrates a potential modest protective effect for the genetic variances in critical pathways. The current investigation demonstrates a potential modest protective effect for the genetic variances in critical pathways. The current investigation demonstrates a potential modest protective effect for the genetic variances in critical pathways. The current investigation demonstrates a potential modest protective effect for the genetic variances in critical pathways. The current investigation demonstrates a potential modest protective effect for the genetic variances in critical pathways.

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