

FAS and FASLG Genetic Variants and Risk for Second Primary Malignancy in Patients with Squamous Cell Carcinoma of the Head and Neck

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Abstract

Background: Single-nucleotide polymorphisms in the promoter region of the *FAS* and *FASLG* may alter the transcriptional activity of these genes. We therefore investigated the association between the *FAS* and *FASLG* polymorphisms and risk for second primary malignancy (SPM) after index squamous cell carcinoma of the head and neck (SCCHN).

Methods: We used log-rank test and Cox proportional hazard models to assess the association of the four single-nucleotide polymorphisms (*FAS* -1377 G > A, *FAS* -670 A > G, *FASLG* -844 C > T, and *FASLG* -124 A > G) with the SPM-free survival and SPM risk among 1,286 incident SCCHN patients.

Results: Compared with patients having the *FAS* -670 AA or the *FASLG* -844 CC genotypes, the patients having variant genotypes of *FAS* -670 AG/GG or *FASLG* -844 CT/TT genotypes had significantly increased risk for SPM, respectively. A trend for significantly increased SPM risk with increasing number of risk genotypes of the four polymorphisms was observed in a dose-response manner. Moreover, the patients with three or four combined risk genotypes had an ~1.8- or 2.5-fold increased risk for developing SPM compared with patients with zero or one risk genotypes, respectively.

Conclusions: Our results suggest a modestly increased risk for SPM after index SCCHN with *FAS* -670 A > G and *FASLG* -844 C > T polymorphisms and an even greater risk for SPM with multiple combined *FAS* and *FASLG* risk genotypes.

Impact: The *FAS* and *FASLG* polymorphisms may serve as a susceptible marker for SCCHN patients at high SPM risk. *Cancer Epidemiol Biomarkers Prev*; 19(6); 1484–91. ©2010 AACR.

Introduction

The incidence of squamous cell carcinoma of the head and neck (SCCHN) in the United States has been in decline over the past two decades, largely because of a decline in the prevalence of smoking (1). The poor prognosis for SCCHN patients has not significantly improved, partly because of the high frequency of second primary malignancies (SPM), which occurs in ~15% of SCCHN patients (2-4), although the diagnostic and therapeutic approaches for SCCHN patients have been improved.

Although previous and continued exposures to smoking and alcohol use are associated with risk for develop-

ing SPMs (5-7), only a small proportion of exposed individuals develops SPM, suggesting that genetic factors may contribute to the interindividual variation in susceptibility to SPMs (8-10). We and others (11-16) have reported that genetic predisposition involved in several molecular pathways, such as carcinogen metabolism, DNA repair, and cell cycle control, is associated with the risk for SPM after primary SCCHN.

Apoptosis is the physiologic mechanism of programmed cell death that plays an important role in diverse biological processes such as development, homeostasis of tissues, and elimination of cancer cells (17, 18). The acquired ability to resist apoptotic stimuli is one of the primary characteristics of a malignant cell, and abnormal regulation of apoptosis is a key mechanism in the development of cancer (19). FAS is a cell surface receptor that can interact with the FAS ligand (FASLG) to trigger apoptosis (20-22). Therefore, the FAS/FASLG pathway plays an important role in regulation of apoptosis and maintenance of cellular homeostasis, and genetic alteration of the FAS/FASLG signaling pathway may result in immune escape and, thus, tumorigenesis, including SPM.

Existing data suggest that polymorphisms of *FAS*/*FASLG* have been associated with increased susceptibility to a variety of cancers, including SCCHN (23-30).

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Single-nucleotide polymorphisms are the most common form of human genetic variation, and the functional single-nucleotide polymorphisms in the promoters of *FAS* and *FASLG* genes have been identified to be related to the differential expression of these two genes (31-33), which may affect risk for SPM after index SCCHN. For example, the *FAS* -1377 G > A and -670 A > G polymorphisms have been shown to interfere with the specificity protein 1 and signal transducers and activators of transcription 1 transcription factor binding sites, respectively, hence decreasing promoter activity and, in turn, *FAS* gene expression (31, 32), whereas the C allele of the *FASLG* -844 C > T polymorphism creates a binding site for the CAAT/enhancer binding protein β transcription factor, resulting in higher basal expression of the *FASLG* gene (33). However, there is no report on the functional relevance of the *FASLG* -124 A > G polymorphism. Our previous study showed that the *FAS* -670 A > G and -1377 G > A polymorphisms were associated with an increased risk for SCCHN (30), but no risk for SCCHN was associated with the *FASLG* -844 C > T and -124 A > G polymorphisms. To date, the association between the *FAS* and *FASLG* polymorphisms and risk for SPM after index SCCHN has not been reported.

Given the role of the *FAS* and *FASLG* genes in regulating cell death and abnormal expression of *FAS* and/or *FASLG* in various types of tumors, including SCCHN, we hypothesized that *FAS* and *FASLG* polymorphisms contribute to genetic susceptibility to SPMs after index SCCHN, and these polymorphisms may be genetic markers to identify high-risk subgroups of SCCHN patients who might benefit from management of alternative treatment and predictable patient outcome. To test the hypothesis, we compared the SPM-free survival and the risk for SPM between the different genotyping groups in a cohort of 1,286 incident SCCHN patients.

Materials and Methods

Study subjects

Between May 1995 and January 2007, 1,667 patients with incident SCCHN were consecutively recruited at the University of Texas M.D. Anderson Cancer Center as part of an ongoing molecular epidemiologic study on SCCHN. These patients were newly diagnosed, histopathologically confirmed, and untreated squamous cell carcinomas of the oral cavity, oropharynx, hypopharynx, or larynx. All patients completed an Institutional Review Board–approved informed consent, without the restriction of age, sex, ethnicity, or clinical stage. Approximately 95% of contacted patients consented to enrollment in the study. The exclusion criteria included any previous cancer history excepting nonmelanoma skin cancer, distant metastases at presentation, primary sinonasal tumors, salivary gland tumors, cervical metastases of unknown origin, and tumors outside the upper aerodigestive tract. In addition, blood samples for genotyping data were not available for some patients recruited early in the study,

and these patients were excluded from this analysis, as were patients without follow-up and patients who underwent only palliative treatment. Therefore, there are a total of 1,286 patients available for the final analysis of this study.

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 and Gates (34). 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 considered SPM. The second lesion was classified as a local recurrence rather than a SPM if there was discrepancy or differing opinion about the origin of the tumor. Pulmonary lesions were considered SPM if they had a nonsquamous 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 (e.g., SCCHN or cancers of the esophagus, lung, or bladder) and non-tobacco-associated SPM.

At presentation, all patients provided epidemiologic data, including alcohol and smoking status. Those subjects who had smoked at least 100 cigarettes in their lifetime were defined as ever smokers; otherwise, they were considered never smokers. Subjects who had drunk at least one alcoholic beverage per day for at least 1 year during their lifetime were defined as ever drinkers, and those who never had such a pattern of drinking were defined as never drinkers. Clinical data were obtained at initial presentation and through follow-up examinations and included overall stage at presentation of index tumor, site of index tumor, and treatment. Index cancer stage was then dichotomized into the early stage (including I and II clinical stage) and late stage (III and IV). We also grouped treatment into four categories: surgery only, surgery with radiotherapy and/or chemotherapy, radiotherapy, and radiotherapy plus chemotherapy.

Genotyping of the *FAS* and *FASLG* polymorphisms

DNA was extracted from 1 mL of blood sample with the Qiagen DNA Blood Mini Kit (Qiagen) according to manufacturer's instructions. We genotyped the four single-nucleotide polymorphisms of the *FAS* and *FASLG* gene, *FAS* -1377 G > A, *FAS* -670 A > G, *FASLG* -844 C > T, and *FASLG* -124 A > G, by the PCR-RFLP assay (30). The primers, PCR and restriction enzymes for these polymorphisms have been described previously (30). Approximately 10% of samples have been reassayed, showing 100% concordance.

Statistical analysis

For all analyses in this study, 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 do all statistical analyses. SPM

occurrence was considered as the primary endpoint of the study. Student's *t* test was used to compare the mean age and follow-up time of the patients who developed a SPM and those who did not. The differences in ethnicity, sex, smoking and alcohol status, index tumor site, index tumor stage, treatment, and genotype distributions between the two groups were evaluated using the χ^2 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 epidemiologic risk factors; clinical characteristics, including index tumor site, index tumor stage, and treatment variables; and time to the occurrence of 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.

In the univariate analysis, we evaluated epidemiologic variables, assessed at the time of diagnosis (such as age in years, ethnicity, sex, and smoking and alcohol status), and clinical characteristics (such as index tumor site, index 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 ($P < 0.25$). Because of epidemiologic 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 and their 95% confidence intervals (95% CI) for an SPM development. The final fully adjusted Cox regression models included age, sex, ethnicity, and smoking and alcohol status.

Results

Patient characteristics

Table 1 shows the demographics, risk exposure, and clinical variables for the 1,286 patients, which included 1,166 patients who did not develop SPM and 120 (9.3%) patients who developed SPM. The overall median follow-up time was 29.7 months (range, 0-142.4 mo).

Of the 120 patients with SPM, 81 patients developed SPMs at tobacco-associated sites, including 44 (36.7%) SCCHN and 37 (30.8%) other tobacco-associated cancers (34, 28.3% lung cancer; 2, 1.7% esophageal cancer; 1, 0.83% bladder cancer); 35 (29.2%) developed SPMs at other sites (10, 8.3% prostate cancer; 8, 6.7% papillary thyroid carcinoma; 4, 3.3% colon adenocarcinoma; 3,

2.5% lymphoma; 3, 2.5% hepatic adenocarcinoma; 2, 1.7% breast cancer; 1, 0.83% each for the remainder, including sarcoma, renal cell carcinoma, endometrial carcinoma, leukemia, and maxillary sinus adenocarcinoma); and 4 (3.3%) developed SPMs at both sites (2, 1.7% patients with both SCCHN and prostate cancer; 2, 1.7% patients with both SCCHN and papillary thyroid carcinoma). 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 oropharyngeal cancers, one had bilateral hypopharyngeal cancers, and the remainder had simultaneous cancers of more than one head and neck subsite.

The mean age at diagnosis for the total patients was 57.5 years (range, 18-94 y; median, 57 y), 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 versus 57.1 y, respectively; $P < 0.0001$). Compared with the SPM-free group, patients who developed SPM were more likely older ($P < 0.0001$) and non-Hispanic Whites ($P = 0.050$). However, no significant differences were observed between patients who did not develop SPM and patients who developed SPM with regard to sex ($P = 0.525$), smoking ($P = 0.129$), alcohol drinking ($P = 0.352$), index cancer site ($P = 0.322$), index cancer stage ($P = 0.681$), and treatment ($P = 0.889$).

Association between the *FAS* and *FASLG* polymorphisms and risk for SPM

As shown in Table 2, *FAS* -670 AG + GG genotypes were more frequent in the patients who developed SPM (83.3%) than in the patients who did not develop SPM (73.2%) and were associated with a significantly increased risk for SPM compared with the *FAS* -670 AA genotype (odds ratio, 1.57; 95% CI, 1.00-2.54). Compared with the *FASLG* -844 CC genotype, the *FASLG* -844 CT + TT genotypes were also more frequent in the patients who developed SPM (70.8%) than in the patients who did not develop SPM (59.2%) and were associated with a significantly increased risk for SPM (odds ratio, 1.71; 95% CI, 1.15-2.54). However, the differences between the variant genotypes (*FAS* -1377 GA + AA or *FASLG* -124 AG + GG) and the wild-type homozygous genotypes (*FAS* -1377 GG or *FASLG* -124 AA) for *FAS* -1377 G > A or *FASLG* -124 A > G polymorphism were not statistically significant ($P = 0.879$ and 0.458, respectively). For these two polymorphisms, no significant SPM risks were observed between the patients who developed SPM and who did not develop SPM (odds ratio, 0.87; 95% CI, 0.56-1.36 for *FAS* -1377 G > A; odds ratio, 1.15; 95% CI, 0.75-1.77 for *FASLG* -124 A > G).

Association between the combined genotypes of the *FAS* and *FASLG* polymorphisms and SPM risk

Because any of the four single-nucleotide polymorphisms of the *FAS* and *FASLG* genes in the apoptotic

Table 1. Distribution of selected characteristics of the patient cohort (N = 1,286)

Variable	Total		SPM-free		SPM		P*
	n	%	n	%	n	%	
Total patients	1,286	100	1,166	90.7	120	9.3	
Age							
≤Median (57 y)	665	51.7	626	53.7	39	32.5	<.0001
>Median (57 y)	621	48.3	540	46.3	81	67.5	
Sex							
Male	977	76.0	883	75.7	94	78.3	0.525
Female	309	24.0	283	24.3	26	21.7	
Ethnicity							
Non-Hispanic White	1,087	84.5	993	85.2	94	78.3	0.050
Other	199	15.5	173	14.8	26	21.7	
Smoking							
Never	343	26.7	318	27.3	25	20.8	0.129
Ever	943	73.3	848	72.7	95	79.2	
Alcohol							
Never	335	26.1	308	26.4	27	22.5	0.352
Ever	951	73.9	858	73.6	93	77.5	
Index cancer site							
Oral cavity	417	32.4	379	32.5	38	31.7	0.322
Oropharynx	573	44.6	525	45.0	48	40.0	
Larynx/hypopharynx	296	23.0	262	22.5	34	28.3	
Index cancer stage							
I or II	323	25.1	291	25.0	32	26.7	0.681
III or IV	963	74.9	875	75.0	88	73.3	
Treatment							
Surgery only	229	17.8	208	17.8	21	17.5	0.889
Surgery + adjuvant Tx†	320	24.9	287	24.6	33	27.5	
XRT	329	25.6	301	25.8	28	23.3	
XRT + chemotherapy	408	31.7	370	31.7	38	31.7	

Abbreviations: Tx, treatment; XRT, radiotherapy.

*P values were calculated from χ^2 test.

†Adjuvant treatment: adjuvant radiotherapy and/or chemotherapy.

pathway seemed to have a minor effect on risk for SPM, we then did a combined analysis of all four single-nucleotide polymorphisms to focus on potentially modifying effect of the combined genotypes on risk for SPM (Table 3). In the 1,286 patients who had data available on all four single-nucleotide polymorphisms, we categorized all putative risk (odds ratio, >1.0) genotypes of each single-nucleotide polymorphism into a new variable according to the number of risk genotypes (for the protective genotype, e.g., *FAS* -1377 G > A, we reversed the reference group). For the combined analysis, we found that the patients with 0 to 2 risk genotypes of the four polymorphisms experienced a significantly reduced SPM-free survival compared with patients with 3 to 4 risk genotypes (log-rank, $P = 0.0143$; Fig. 1). There was a trend for increased SPM risk with increasing number of risk genotypes, and this trend in risk was statistically significant in a dose-response manner ($P = 0.004$ for trend).

Specifically, the patients with 3 or 4 risk genotypes had an ~1.8- (hazard ratio, 1.83; 95% CI, 1.00-3.36) or 2.5-fold (hazard ratio, 2.53; 95% CI, 1.26-5.06) increased risk for developing SPM compared with patients with 0 to 1 risk genotypes, respectively.

Discussion

In this study, we investigated the association between the *FAS* -1377 G > A and -670A > G and the *FASLG* -844 C > T and *FASLG* -124 A > G polymorphisms on the risk for SPM after index SCCHN. We found that *FAS* -670 A > G and *FASLG* -844 C > T polymorphisms were associated with a significantly increased risk for SPM in patients with SCCHN. Although we did not observe any significant association of *FAS* -1377 G > A or *FASLG* -124 A > G polymorphism with risk for SPM, we did observe an effect of the combined risk genotypes of the four polymorphisms

Table 2. SPM risk associated with *FAS* and *FASLG* polymorphisms after index SCCHN

Genotypes	Total (N = 1,286)		SPM-free (n = 1,166)		SPM (n = 120)		P*	HR (95% CI) [†]
	n	%	n	%	n	%		
<i>FAS</i> -1377 G > A								
GG (ref)	1,023	79.6	927	79.5	96	80.0	0.879	0.87 (0.56-1.36)
GA + AA	263	20.4	239	20.5	24	20.0		
<i>FAS</i> -670 A > G								
AA (ref)	333	25.9	313	26.8	20	16.7	0.014	1.57 (1.00-2.54)
AG + GG	953	74.1	853	73.2	100	83.3		
<i>FASLG</i> -844 C > T								
CC (ref)	511	39.7	476	40.8	35	29.2	0.038	1.71 (1.15-2.54)
CT + TT	775	60.3	690	59.2	85	70.8		
<i>FASLG</i> -124 A > G								
AA (ref)	981	76.3	889	76.2	92	76.7	0.458	1.15 (0.75-1.77)
AG + GG	305	23.7	277	23.8	28	23.3		

Abbreviations: HR, hazard ratio; ref, reference group.

* χ^2 Test for differences in the distribution of *FAS* and *FASLG* genotypes between the patients who developed SPM and the patients who did not.

[†]Adjusted for age, sex, ethnicity, tobacco smoking, and alcohol drinking in a Cox model.

on SPM risk in patients with primary SCCHN, and the trend in risk was statistically significant in a dose-response manner. In addition, the patients with 3 or 4 risk genotypes had almost 1.8- or 2.5-fold increased risk for developing SPM compared with patients with 0 or 1 risk genotypes. To the best of our knowledge, there have been no previous studies examining the combined effects of genetic variants in the apoptotic pathway on risk for SPM after index SCCHN.

It has been shown that downregulation of *FAS* may protect tumor cells from elimination by antitumor immune responses, whereas upregulation of *FASLG* may increase the ability of tumor cells to counterattack the immune system by inducing apoptosis of *FAS*-sensitive

lymphocytes (35-38). Alteration of *FAS* and *FASLG* expression decrease the apoptotic capacity of cells, and many tumor cells might evade or suppress the immune system. Most studies indicated that decreasing the expression of *FAS* and/or increasing the expression of *FASLG* is a common feature of malignant transformation and an early event associated with the development of most human cancers, including SCCHN (23-27, 30, 39-41). Given the important roles of *FAS* and *FASLG* in apoptosis process, it is biologically plausible that alteration of *FAS* and *FASLG* genes, such as genetic polymorphisms, may affect risk for cancer, including SPM.

The *FAS* -1377 G > A polymorphism has been reported to be associated with increased risk for developing lung

Table 3. SPM risk associated with *FAS* and *FASLG* polymorphisms after index SCCHN

No. risk genotypes	Total (N = 1,286)		SPM-free (n = 1,166)		SPM (n = 120)		P*	HR (95% CI) [†]
	n	%	n	%	n	%		
0-1 (ref)	235	18.3	221	19.0	14	11.7	0.103	1.00 (Ref)
2	480	37.3	438	37.6	42	35.0		1.43 (0.78-2.63)
3	420	32.7	375	32.1	45	37.5		1.83 (1.00-3.36)
4	151	11.7	132	11.3	19	15.8		2.53 (1.26-5.06)
Trend								P = 0.004

* χ^2 test for differences in the distribution of combined genotypes between the patients who developed SPM and the patients who did not.

[†]Adjusted for age, sex, ethnicity, tobacco smoking and alcohol drinking in a Cox model.

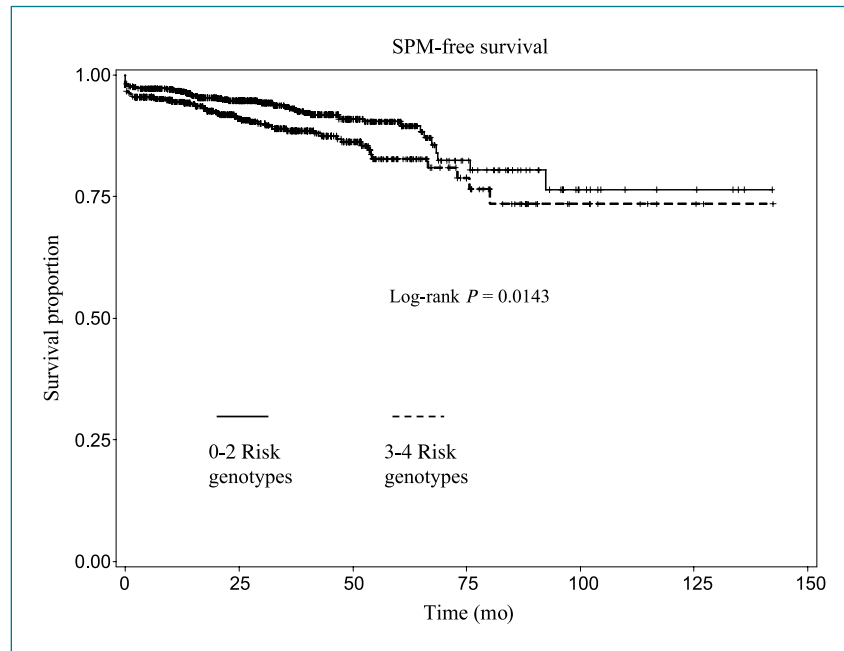


Figure 1. Kaplan-Meier SPM-free survival curve stratified by combined *FAS*/*FASLG* risk genotypes.

cancer (24), breast cancer (25, 39), esophageal squamous cell cancer (26), colorectal cancer (27), SCCHN (30), and acute myeloid leukemia (32). *FAS* -670 A > G polymorphism was found to be associated with increased risk for esophageal squamous cell cancer (26), SCCHN (30), and gynecologic cancer (40). In the current study, we observed the significant association of *FAS* -670 A > G but not *FAS* -1377 G > A polymorphism with risk for SPM after index SCCHN. Although the exact mechanism of how the polymorphism affect SPM development is unclear, Sibley et al. (32) reported that the *FAS* -670 G allele had a greatly reduced ability to bind transcription factor signal transducers and activators of transcription 1 and less expressed on *ex vivo*-stimulated T cells (41). Decreased *FAS* expression resulting from a *FAS* promoter polymorphism may help the transformed cells evade *FAS*-mediated cell death, subsequently affecting risk for cancer, including SPM.

The *FASLG* -844 T/C polymorphism is also located in the promoter region of the gene, and basal *FASLG* expression is higher in cells carrying the C allele than in cells carrying the T allele as measured in a luciferase reporter assay and when expressed in peripheral blood fibrocytes (33). Sun et al. (41) found that *FASLG* -844 C allele is associated with increased activation-induced T cell apoptosis *in vitro*, which is consistent with the findings in current study (25). Transformed cells with the *FASLG* -844 CC genotype that express a high level of *FASLG* may create an immunoprivileged site by killing cytotoxic immune cells and thus escape host immunosurveillance. The association between the *FASLG* -844 C > T polymorphism and increased risk for some cancers has been reported in previous studies (24-27, 33, 41). In this

study, we found that the *FASLG* -844 variant genotypes (CT + TT) were associated with a significantly increased risk for SPM in patients after index SCCHN compared with the *FASLG* -844 CC genotype, although our previous case-control study indicated that no risk for SCCHN was associated with any of the *FASLG* genotypes (30). The exact mechanism for these conflicting results remains unknown. It might be possible that the effect of this *FASLG* -844 C > T polymorphism in normal epithelium of the head and neck differs from those in SCCHN tumor tissues, which have numerous somatic changes. It also might be that this *FASLG* -844 C > T polymorphism may function differently in etiology (case-control study) and prognosis (case only study) because the normal epithelium of the head and neck and SCCHN tumor tissues have significant differences in genetic profiles such as somatic genetic changes. Moreover, this polymorphism of *FASLG* -844 C > T may have different roles in etiology and prognosis through the interaction of this *FASLG* -844 C > T variant with the normal genes in normal tissues, genetically altered genes in SCCHN tissues, smoking behavior, human papillomavirus (HPV), and other environmental risk factors. Several studies have also suggested that genetic factors and previous treatments, within the context of previous or continued exposure to risk factors, may affect the risk for SPM after index SCCHN (42-44). Therefore, all these factors may affect functionality of this *FASLG* -844 C > T polymorphism in the development of SPM and SCCHN. However, these hypotheses need to be tested in future studies.

Although this was a large and well-characterized cohort in SCCHN patients by Head and Neck Center at M.D. Anderson Cancer Center, there were several

inherent limitations in our study. Firstly, multiple ethnicities were included in this cohort, in which 84.5% of patients were non-Hispanic Whites. Secondly, the demographics, exposure, and clinical data for the cohort were collected prospectively, whereas clinical outcomes, including SPM, were collected retrospectively without a strictly defined screening or follow-up regimen. Furthermore, the follow-up time to the development of SPM in this study may have been limited by patients with stage III and IV index cancer who were lost to follow-up. These patients may not have had as much opportunity to develop SPM because of being recruited lately or dying relatively soon after diagnosis. It is also possible that a screening bias in the detection of SPMs exists, such that tobacco-associated SPMs (that is, SCCHN, esophageal, or lung cancers) were detected more readily than non-tobacco-associated cancers. However, such a bias should be nondifferential (that is, not different between groups having different genotypes). In addition, the low SPM rate may be due to our high prevalence of never smokers (26.7%) and our strict criteria in defining SPM. Finally, the absence of HPV status did not allow us to evaluate its potential influence on the development of SPMs in patients with index SCCHN. With the information available, we will take HPV and smoking status into account as confounders in our future studies when we

analyze the associations between this and/or other genetic polymorphisms and risk for SPM. Despite these limitations, the current investigation supports a significant role of *FAS* and *FASLG* polymorphisms in individual variation in susceptibility to SPM after index SCCHN.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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