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Apoptotic variants as predictors of risk of oropharyngeal cancer recurrence after definitive radiotherapy

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Single nucleotide polymorphisms (SNPs) in the promoter region of *FAS* and *FASLG* may alter their transcriptional activity. Thus, we determined the associations between four *FAS* and *FASLG* promoter variants (*FAS*1377G>A, rs2234767; 670A>G, rs1800682; *FASLG*844T>C, rs763110 and 124A>G, rs5030772) and the risk of recurrence of squamous cell carcinoma of the oropharynx (SCCOP). We evaluated the associations between *FAS* and *FASLG* genetic variants and the risk of recurrence in a cohort of 1,008 patients. The log-rank test and multivariate Cox models were used to evaluate the associations. Compared with patients with common homozygous genotypes of *FAS*670 and *FASLG*844 polymorphisms, patients with variant genotypes had lower disease-free survival rates (log-rank $p < 0.0001$ and $p < 0.0001$, respectively) and an approximately threefold higher risk of SCCOP recurrence (HR, 3.2;95% CI, 2.2–4.6; and HR, 3.1; 95% CI, 2.2–4.4, respectively) after multivariate adjustment. Furthermore, among patients with HPV16-positive tumors, those with variant genotypes of these two polymorphisms had lower disease-free survival rates (log-rank, $p < 0.0001$ and $p < 0.0001$, respectively) and a higher recurrence risk than did patients with common homozygous genotypes (HR, 12.9; 95% CI, 3.8–43.6; and HR, 8.1; 95% CI, 3.6–18.6, respectively), whereas no significant associations were found for *FAS*1377 and *FASLG*124 polymorphisms. Our findings suggest that *FAS*670 and *FASLG*844 polymorphisms modulate the risk of recurrence of SCCOP, particularly in patients with HPV16-positive tumors. Larger studies are needed to validate these results.

Epidemiology

Despite declining smoking rates in the United States, the incidence of squamous cell carcinoma of the oropharynx (SCCOP), a subset of squamous cell carcinomas of the head and neck (SCCHN), is increasing.[1] This increase can be mainly attributed to the increasing prevalence of infection with high-risk types of human papillomavirus (HPV), particularly HPV16. Such infections have been established as risk factors for SCCOP, in addition to smoking and alcohol use.[2–4] Surgery, radiotherapy, and chemotherapy are successful in the treatment of SCCOP, but the long-term survival rates have improved only moderately. SCCOP has a high rate of recurrence (approximately 15–30% of cases).[5] The overall 5-year relative survival rate is approximately 50%, while patients who develop disease recurrence after multimodal treatment have a 5-year survival rate of <5%.[6]

Although clinical (*e.g.,* index tumor site and disease stage), lifestyle (*e.g.,* history of smoking and alcohol consumption), and other risk factors are used to assess the risk of recurrence of SCCOP, they do not account for all recurrences; genetic factors may also contribute to recurrent SCCOP. SCCOP recurrence rates differ among patients with similar clinical characteristics (*e.g.,* tumor grade, stage and therapeutic approach); thus, understanding and modifying patients' genetic risk factors for recurrence may help us identify

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What's new?

Recurrence of squamous cell carcinoma of the oropharynx (SCCOP) may be influenced by genetic factors, the identification of which could lead to the discovery of predictive markers with the potential to improve patient outcomes. In this study, the risk of SCCOP recurrence was linked to apoptotic variants in the promoter regions of the *FAS* and *FASLG* genes. The variants were associated with reduced disease-free survival, particularly in patients who were positive for human papillomavirus. Though the mechanistic basis remains unclear, the variants may affect *FAS* and *FASLG* expression, thereby altering apoptotic responses to treatments such as radiotherapy.

predictive markers on the basis of individual patients' germline genetic variations, leading to improved survival and better quality of life.

Apoptosis plays an important role in tumorigenesis and cancer treatment.[7,8] 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 factor in the development and prognosis of cancer.[9] FAS is a cell surface receptor that can interact with the FAS ligand (FASLG) to trigger apoptosis.[10–12] Therefore, the FAS/FASLG pathway plays an important role in regulating apoptosis and maintaining cellular homeostasis. Genetic alterations in the FAS/FASLG signaling pathway may affect these genes' expression and apoptotic efficacy, affecting cancer development and prognosis. Furthermore, since apoptosis-related genes are implicated in the apoptotic cascade, genetic variants of apoptotic variants may affect response to radiotherapy in SCCOP patients, particularly tumor HPV-positive SCCOP cases with p53-induced apoptotic pathways and fewer somatic mutations.

Functional single nucleotide polymorphisms (SNPs) in the promoters of *FAS* and *FASLG* genes have been found to affect the differential expression of the two genes[13–15]; such alterations of expression may affect the risk of SCCOP recurrence. For example, the *FAS*1377 and *FAS*670 polymorphisms have been shown to interfere with the SP1 and STAT1 transcription factor binding sites, respectively, decreasing promoter activity and in turn, *FAS* gene expression[13,14]; the C allele of the *FASLG*844 polymorphism creates a binding site for the CAAT/enhancer binding protein β transcription factor, resulting in higher basal expression of the *FASLG* gene.[15]

The results of previous studies suggest that polymorphisms of *FAS/FASLG* are associated with increased susceptibility to a variety of cancers.[16–23] In our previous study, we showed that the *FAS*670, *FAS*1377 and *FASLG*844 polymorphisms were significantly associated with the risk of primary SCCHN or second primary tumors after index SCCHN,[23,24] but no such risk was associated with *FASLG*124 polymorphisms. To date, no associations have been reported between *FAS* and *FASLG* polymorphisms and the risk of SCCOP recurrence. Given the roles of the *FAS* and *FASLG* genes in regulating cell death and the abnormal expression of FAS and FASLG in various types of tumors, we hypothesized that *FAS* and *FASLG* polymorphisms affect their transcription levels, cause interindividual variations in apoptotic efficacy, and lead to different treatment responses. In the current study, we tested the hypothesis that the variant genotypes of these apoptotic promoter variants were predictive of an increased risk of recurrence, particularly in HPV16-positive [HPV16(+)] tumors, in a cohort of 1,008 SCCOP patients.

Patients and Methods

Study subjects

Patients with SCCOP were consecutively enrolled from May 1995 through April 2010, as described previously.[25] In brief, all patients had newly diagnosed, histopathologically confirmed, untreated SCCOP; patients of all ages, sexes, ethnicities, and clinical stages were recruited. Patients with distant metastases at presentation were excluded. Approximately 95% of contacted patients consented to enrollment in the study. Patients were excluded if they (*i*) had known distant metastases; (*ii*) had any prior cancers, except nonmelanoma skin cancer; (*iii*) had a primary sinonasal tumor, a salivary gland tumor, cervical metastases of unknown origin, or a tumor outside the upper aerodigestive tract; (*iv*) had no blood samples available for genotyping; (*v*) had undergone treatment outside of our institution; or (*vi*) had undergone only palliative treatment. All subjects signed an informed consent form that had been approved by the institutional review board of The University of Texas MD Anderson Cancer Center (Houston, Texas).

Patients were monitored during and after treatment with scheduled regular clinical and radiographic examinations. Patients were considered disease free if absence of disease was documented at the date of the last visit with the head and neck surgeon, head and neck radiation oncologist, or head and neck medical oncologist. There were no universal standards for imaging. Typically, patients underwent either routine serial imaging or follow-up imaging on the basis of symptoms or physical examination findings.

Patients were considered to have recurrent disease if they developed a new lesion of the same histologic type, as verified by biopsy (incisional, excisional or needle), and any lesions that had disappeared. Clinical data, such as stage at presentation of the index tumor, treatment, comorbidity, and recurrence, were obtained from a review of the medical records. The sixth edition of the American Joint Committee on Cancer TNM staging system was used to determine disease stage at the time of presentation for all study patients. Definitive radiotherapy was defined as radiotherapy with or without other therapeutic modalities. Medical comorbidities

were classified according to a modification of the Kaplan–Feinstein comorbidity index (Adult Comorbidity Evaluation 27), which categorizes related comorbidities as none to mild, moderate, or severe.[26] The ACE-27 grades specific diseases and conditions as 1 of 3 levels of comorbidity: Grade 1 (mild), Grade 2 (moderate) or Grade 3 (severe), according to the severity of individual organ decompensation and the prognostic effect. Once the patient's individual diseases or comorbid conditions have been classified, an overall comorbidity score (none, mild, moderate or severe) is assigned on the basis of the highest ranked single ailment. In cases in which two or more moderate ailments occur in different organ systems or disease groups, the overall comorbidity score is designated as severe.

"Ever drinkers" were defined as patients who had drunk at least one alcoholic beverage per week for at least 1 year during their lifetime; patients who had no such pattern of drinking were considered "never drinkers." Patients who had smoked at least 100 cigarettes in their lifetime were defined as "ever smokers," and patients who had smoked fewer than 100 cigarettes in their lifetime were categorized as "never smokers."

Genotyping

DNA was extracted from 1 ml of blood with the Qiagen DNA Blood Mini Kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. We genotyped the four SNPs of *FAS* and *FASLG* genes: *FAS*1377, *FAS* 670, *FASLG*844 and *FASLG*124 using the polymerase chain reaction-restriction fragment length polymorphism (PCR) assay.[23] In brief, the following primers were used to amplify the target fragments containing these four polymorphisms (mismatch bases are underlined): 5′-TGTGTGCACAAGGCTGGCGC-3′ (forward) and 5′-TGCATCTGTCACTGCACTTACCACCA-3′ (reverse) for *FAS* 1377 G>A, 5′-ATAGCTGGGGCTATGCGATT-3′ (forward) and 5′-CATTTGACTGGGCTGTCCAT-3′ (reverse) for *FAS* 670 A>G, 5′-CAATGAAAATGAACACATTG-3′(forward) and 5′-CCCACTTTAGAAATTAGATC-3′(reverse) for *FASLG* 844 C>T, and 5′-GCAGTTCAGACC TACATGATTAGGAT-3′(forward) and 5′-CCAGATAC AGACCTGTTAAATGGGC-3 for *FASLG* 124 A>G. The amplified PCR products were 122, 193, 85 and 230 bp for 1,377 G>A, 670 A>G, 844 C>T, and 124 A>G polymorphisms, respectively. The *Bst*UI, *Scr*FI, *Dra*III, and *Fok*I restriction enzymes (New England Biolabs, Beverly, MA) were used to distinguish 1,377 G>A, 670 A>G, 844 C>T, and 124 A>G polymorphisms, respectively, which resulted in 104- and 18-bp fragments in the 1,377 G allele; 136- and 57-bp fragments in the 670 G allele; 66- and 19-bp fragments in the 844 T allele; and 180- and 50-bp fragments in the 124 G allele. Approximately 10% of samples were retested, demonstrating 100% concordance.

Determination of tumor *HPV16* status

Paraffin-embedded tissue biopsies or specimens from study patients with tissues available were used to extract DNA for tumor HPV16 detection using PCR and *in situ* hybridization methods, described previously.[27] For quality control, a subset of samples was re-assayed for tumor HPV16 status. The results of the re-run samples were 100% concordant with the original results.

Statistical analysis

For all analyses, statistical significance was set at $p < 0.05$, and all tests were two-sided. SAS software (version 9.2.3; SAS Institute) was used to perform all statistical analyses. The primary endpoint of the study was recurrence. The time to event was calculated from the date of diagnosis of the index SCCOP to the date of clinically detectable recurrence (local, regional, or distant). Patients who were not known to have had an event at the date of last contact and patients who were lost to follow-up or died of other or unknown causes were censored. We first used Student's *t test* to compare the mean age and follow-up time between patients with and without recurrence. The associations between individual epidemiologic risk factors, clinical characteristics (including stage, comorbidity, and treatment variables), and time to recurrence were initially assessed using univariate Cox proportional hazards regression models. An examination of Kaplan–Meier survival curves and log-minus-log survival plots indicated that the data were consistent with the assumptions of the Cox proportional hazard regression models. The associations between variables and disease-free survival (DFS) were evaluated using the log-rank test. We assessed the associations between individual epidemiologic risk factors, clinical characteristics (including stage, comorbidity and treatment variables), and time to recurrence using both univariate and multivariate Cox proportional hazards regression models. Associations between genotypes and risk of recurrence were quantified by calculating the hazard ratios (HRs) and their 95% CIs. The Cox model included adjustment for potential confounders, including age, sex, ethnicity, smoking status, alcohol use status, tumor stage, comorbidity and treatment.

Results

From May 1995 to April 2010, 1,226 patients with SCCOP were enrolled in this study. Of these patients, 218 were excluded because insufficient information was available about follow-up and treatment or no blood samples were available for genotyping. Therefore, our final analysis included 1,008 patients with previously untreated incidental SCCOP. These patients were followed up from May 1995 to October 2013, with an overall median follow-up time of 44.7 months (range, 1.7 to 170.9 months); 181 patients experienced disease recurrence. The median follow-up durations for patients without and with recurrence were 50.9 and 11.6 months, respectively. Of the 181 patients with recurrence, 70 (38.7%) had distant recurrence, 49 (27.1%) had local recurrence, 20 (11.0%) had regional recurrence, and 42 (23.2%) had recurrence of more than one type.

Table 1. Characteristics of patients with SCCOP ($N = 1{,}008$)

Variable	No. (%) of patients	No. of patients with recurrence	5-year recurrence rate (%)	p^1 value
No. of patients	1,008 (100)	181	0.20	
Age				
$\leq$ 57 years	621 (61.6)	85	0.15	< 0.0001
$>$ 57 years	387 (38.4)	96	0.27	
Sex				
Male	872 (86.5)	161	0.20	0.3110
Female	136 (13.5)	20	0.19	
Ethnicity				
Non-Hispanic white	913 (90.6)	146	0.17	< 0.0001
Other	95 (9.4)	35	0.41	
Smoking				
Never	388 (38.5)	51	0.14	0.0004
Ever	620 (61.5)	130	0.23	
Alcohol use				
Never	247 (24.5)	26	0.10	0.0005
Ever	761 (75.5)	155	0.23	
Comorbidity				
None or mild	913 (90.6)	157	0.19	0.0370
Moderate to severe	95 (9.4)	24	0.27	
Index cancer stage				
1 or 2	72 (7.1)	11	0.19	0.5280
3 or 4	936 (92.9)	170	0.20	
Treatment				
X/XC/XS/S	947 (93.9)	166	0.19	0.0030
SXC	61 (6.1)	15	0.32	

X, radiotherapy; C, chemotherapy; and S, surgery.
[1] p: Log-rank test for DFS between the two groups.

The mean ages at diagnosis for the overall cohort, patients who developed recurrence, and patients without recurrence were 55.8, 58.6 and 55.2 years, respectively. Table 1 shows patients' demographic, risk, and clinical factors and the corresponding 5-year actuarial recurrence rates. Patients in the overall group were predominantly male (86.5%) and non-Hispanic white (90.6%). The univariate Kaplan–Meier analyses showed that age, ethnicity, smoking, alcohol use, comorbidity, and treatment were significantly associated with DFS (all $p < 0.05$), whereas significant associations were not found for sex and index cancer stage (all $p > 0.05$).

The DFS was significantly poorer in patients with SCCOP with the *FAS670* AG+GG and *FASLG*844 CT+TT variant genotypes than in patients with the corresponding common homozygous genotypes, respectively (log-rank, $p < 0.0001$ and $p < 0.0001$; Fig. 1), whereas no significant differences in DFS were observed between different genotypes of the *FAS1377* (log-rank $p = 0.211$) or *FASLG124* polymorphism (log-rank $p = 0.121$). To evaluate the associations between the four SNPs and recurrence risk in patients with SCCOP, we performed a multivariate Cox proportional hazards regression analysis with adjustment for several major confounders, including age, sex, ethnicity, smoking status, alcohol status, comorbidity, stage and treatment. As shown in Table 2, compared with the *FAS*670 AA and *FASLG*844 CC common homozygous genotypes, patients with the *FAS*670 AG+GG variant and *FASLG*844 CT+TT variant genotypes had an approximately threefold significantly higher risk of disease recurrence (HR, 3.2; 95% CI, 2.2–4.6; and HR, 3.1; 95% CI, 2.2–4.4, respectively); no such significant associations were observed in patients with *FAS*1377 and *FASLG*124 polymorphisms.

We further explored the associations between the four polymorphisms and risk of recurrence among 233 HPV16(+) patients with SCCOP since both HPV and apoptosis play important roles in SCCOP carcinogenesis and prognosis. As shown in Figure 2, a significantly better DFS was found in patients with the *FAS670* AA and *FASLG*844

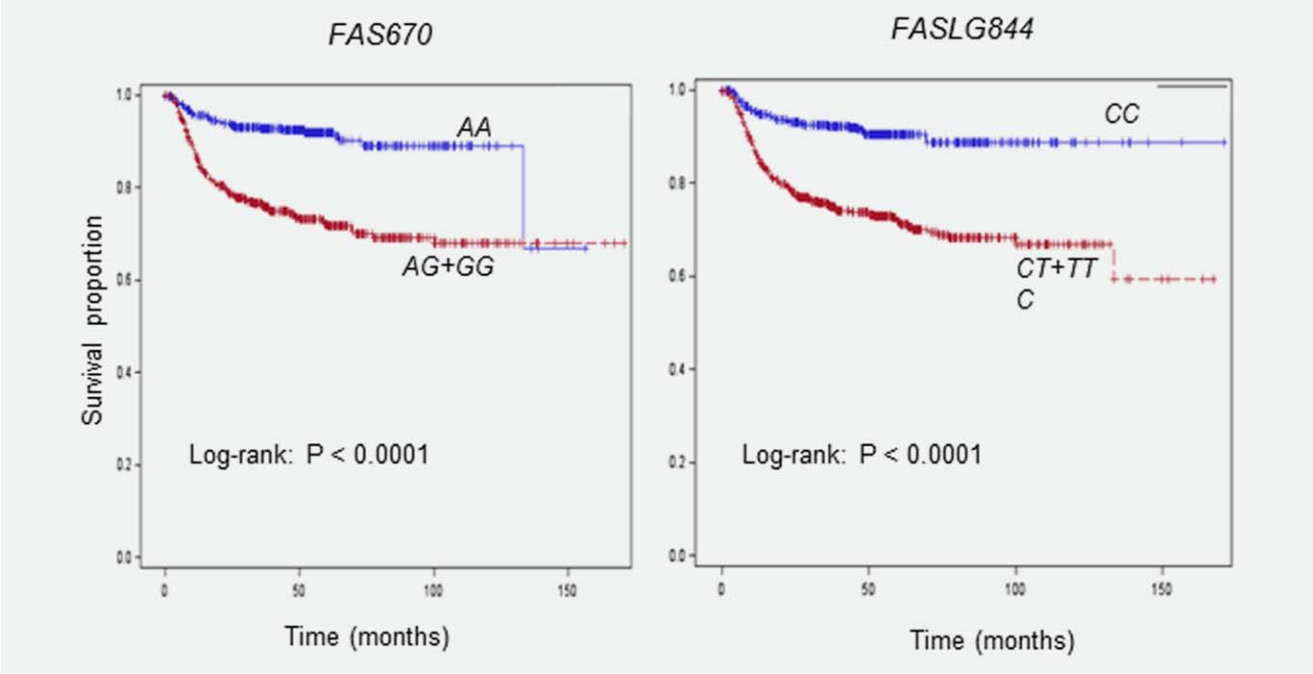

Figure 1. Kaplan–Meier estimates of the cumulative recurrence rates in all patients according to the *FAS670* and *FASLG844* genotypes ($N = 1{,}008$). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

CC genotypes than in those with the corresponding AG+GG (log-rank $p < 0.0001$) and CT+TT variant genotypes (log-rank $p < 0.0001$), respectively. However, no significant differences in DFS were observed for the *FAS*1377 and *FASLG*124 polymorphisms. Furthermore, after adjustment for several major confounders, patients with the *FAS*670 AG+GG and *FASLG*844 CT+TT variant genotypes had approximately 13- and eightfold significantly increased risks of recurrence compared with those with the *FAS*670 AA and *FASLG*844 CC common homozygous genotypes, respectively (HR, 12.9; 95% CI, 3.8–43.6 for *FAS*670; and HR, 8.1; 95% CI, 3.6–18.6 for *FASLG*844; Table 3); no significant associations were observed for the *FAS*1377 or *FASLG*124 polymorphisms. In addition, we observed no similar associations between the four polymorphisms and recurrence risk among patients with HPV16(−) SCCOP; these polymorphisms might not affect the risk of recurrence in HPV16(−) SCCOP, or it is possible that we did not have enough samples or outcome events in this study (only 78 patients had HPV16(−) SCCOP).

Discussion

In this large cohort study, we found that *FAS*670 and *FASLG*844 polymorphisms modified the recurrence risk of SCCOP, particularly in patients with HPV16(+) tumors. The SNPs were reported to alter the expression of *FAS* and *FASLG* genes,[19] resulting in nonphysiological levels of *FAS*/*FASL*, which may disrupt cellular homeostasis. However, the underlying mechanisms that govern the effects of FAS and FALG on cell death are not understood. It has been shown that downregulation of FAS and FASLG may protect tumor cells from elimination by inducing a reduced apoptotic response; upregulation of FAS/FASLG has the opposite effect.[28–31] The results of most studies indicate that low expression of FAS/FASLG is a common feature of malignant transformation and an early event, that is, associated with the development of most human cancers.[16–20,23,32–34] Given the important roles of FAS and FASLG in apoptosis, it is biologically plausible that genetic variants in the promoters of the *FAS* and *FASLG* genes affect the expression levels of these genes, thus affecting the regulation of apoptotic efficacy and the responses of cancer patients to treatment, such as radiotherapy. Therefore, *FAS* and *FASLG* polymorphisms may serve as predictive biomarkers of clinical outcome in SCCOP patients and help physicians individualize treatment, leading to an improved prognosis and better quality of life for patients with this disease.

The associations between *FAS/FASLG* polymorphisms and the risk of developing many types of human cancer have been previously reported.[14,17–20,23,24,32,33] However, few studies have investigated the associations between these polymorphisms and recurrence risk in SCCOP and HPV-associated SCCOP. For example, the association between the *FASLG*844 polymorphism and the risk of some cancers has been reported in previous studies,[15,17–20,34] but not for *FASLG*124. Previously, we also reported that both *FAS*670 AG+GG and *FASLG*844 CT+TT variant genotypes were associated with a significantly higher risk of second primary malignancies in patients with index SCCHN compared with the corresponding *FAS*670 AA and *FASLG*844 CC genotype,[24] although the results of our previous case-control study indicated that these polymorphisms were not significantly associated with risk of SCCHN.[23] In the current study, however, we observed an

Table 2. Association between *FAS* and *FASLG* genotypes and SCCOP recurrence in patients with SCCOP ($N = 1{,}008$)

Genotype	No. of recurrences/no. of patients	5-year recurrence rate	Log-rank *p* values	aHR[2], 95% CI
FAS1377G>A				
GG (ref.)[1]	152/872	0.19	0.323	1.0
GA	28/128	0.22		1.1 (0.4–1.4)
AA	1/8	0.18		0.9 (0.3–1.2)
p_{trend}				0.662
GG (ref.)	152/872	0.19	0.211	1.0
GA+AA	29/136	0.22		1.1 (0.8–2.0)
FAS670A>G				
AA (ref.)[1]	36/449	0.10	0.0021	1.0
AG	106/430	0.28		2.7 (2.1–9.2)
GG	39/129	0.21		4.2 (0.8–10.3)
p_{trend}				0.024
AA (ref.)	36/449	0.10	< 0.0001	1.0
AG+GG	145/559	0.28		3.2 (2.2–4.6)
FASLG844C>T				
CC (ref.)[1]	42/491	0.11	0.0011	1.0
CT	111/420	0.27		3.3 (1.7–9.8)
TT	28/97	0.29		3.8 (0.7–11.4)
p_{trend}				0.132
CC (ref.)	42/491	0.11	< 0.0001	1.0
CT+TT	139/517	0.28		3.1 (2.2–4.4)
FASLG124A>G				
AA (ref.)[1]	119/715	0.19	0.226	1.0
AG	56/265	0.23		1.5 (0.9–2.4)
GG	6/28	0.20		1.1 (0.4–2.8)
p_{trend}				0.743
AA (ref.)	119/715	0.19	0.121	1.0
AG+GG	62/293	0.22		1.4 (0.9–2.0)

HR, hazard ratio.
[1]The observed genotype frequencies were in agreement with the Hardy-Weinberg equilibrium ($p^2 + 2pq + q^2 = 1$). (HWE by χ^2 tests: $p = 0.175$ for *FAS*1377, $p = 0.104$ for *FAS*670, $p = 0.603$ for *FASLG*844, and $p = 0.565$ for *FASLG*124, respectively).
[2]Adjusted for age, sex, ethnicity, smoking status, alcohol use status, stage, comorbidity, and treatment.

association between the *FAS* 670 AG+GG and *FASLG*844 CT+TT variant genotypes and a high risk of recurrence compared with that of their corresponding common homozygous genotypes; no such significant associations were found for the *FAS*1377 and *FASLG*124 polymorphisms. Although the exact mechanisms by which these polymorphisms affect disease recurrence are unclear, it is expected that *FAS*670 and *FASLG*844 in the promoters of these genes affect FAS and FASLG expression, which subsequently affects treatment response and cancer prognosis. *In vitro* studies have shown that the *FAS*670 G allele and *FASLG*844 T allele result in reduced promoter activity, leading to lower expression of *FAS/FASLG* genes.[13–15] The low expression of both genes might result in a lower apoptotic capacity in cancer cells, leading to an increased risk of disease recurrence after radiotherapy. In the current study, we observed significant associations between the variant genotypes (AG+GG for *FAS*670 and CT+TT for *FASLG*844) and an increased risk of recurrence in SCCOP patients, which is consistent with our previous findings that patients with the *FAS*670 and *FASLG*844 variant genotypes had a significantly increased risk of second primary tumors after index SCCHN.[24] Nevertheless, future studies of the underlying mechanisms are needed.

Although many factors, such as genetic, clinical, and lifestyle factors, affect recurrence risk in patients with SCCOP, this risk can also be influenced by tumor HPV status. Since HPV(+) and HPV(−) SCCOP are distinct groups with different molecular, pathologic, and clinical characteristics, HPV(+) SCCOP patients experience a better response to radiotherapy and have a better prognosis than do HPV(−)

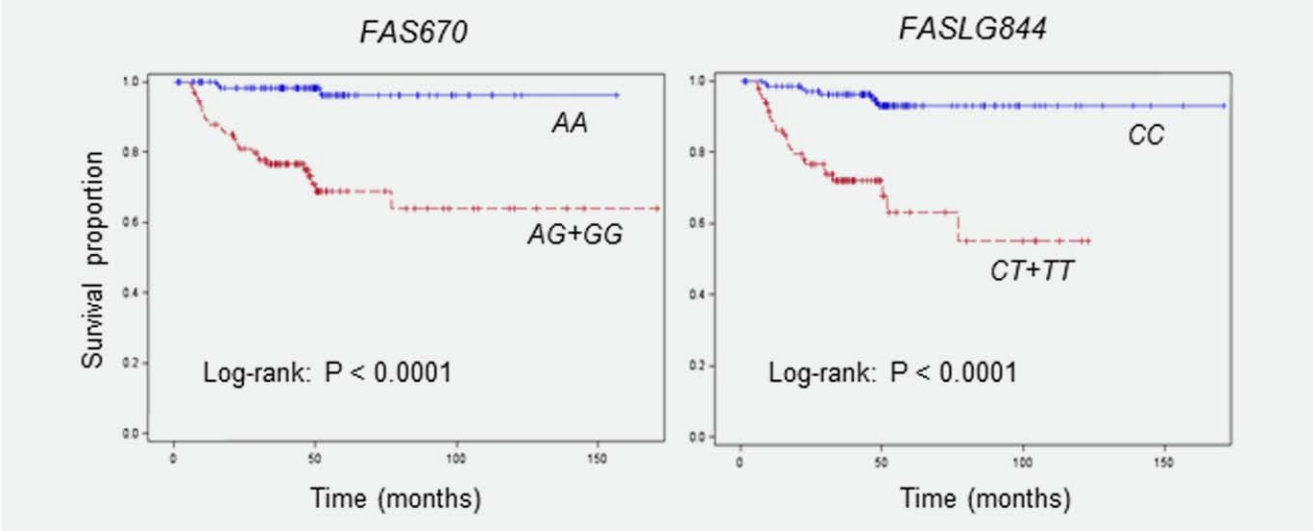

Figure 2. Kaplan–Meier estimates of the cumulative recurrence rates in HPV-positive SCCOP patients according to the *FAS670* and *FASLG844* genotypes ($N = 233$). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Table 3. Association between *FAS* and *FASLG* genotypes and HPV-positive SCCOP recurrence in patients with SCCOP

Genotype	No. of recurrences/no. of patients	5-year recurrence rate	Log-rank *p* value	aHR[1], 95% CI
FAS1377G>A			0.662	
GG (ref.)	30/213	0.17		1.0
GA+AA	2/20	0.19		0.8 (0.2–3.3)
FAS670A>G			< 0.0001	
AA (ref.)	3/125	0.10		1.0
AG+GG	29/108	0.36		12.9 (3.8–43.6)
FASLG844C>T			< 0.0001	
CC (ref.)	8/151	0.12		1.0
CT+TT	24/82	0.40		8.1 (3.6–18.6)
FASLG124A>G			0.100	
AA (ref.)	21/185	0.17		1.0
AG+GG	11/48	0.21		1.6 (0.8–3.3)

HR, hazard ratio.
[1]Adjusted for age, sex, ethnicity, smoking status, alcohol use, stage, comorbidity and treatment.
[2]Reference group.

SCCOP patients. HPV(+) SCCOP patients generally lack somatic genetic changes (*e.g.,* intact p53), whereas patients with HPV(−) SCCOP, which is mostly driven by smoking, have the most common p53 mutations. Such p53 mutations seem to be correlated with a poor response to radiotherapy, partially due to inactivation of the p53-mediated apoptotic pathway. Therefore, HPV status is highly relevant to SCCOP prognosis, suggesting that a stratified analysis by tumor HPV status should be considered in future studies of the prognosis of SCCOP patients, such as studies of recurrence.

We further explored the roles of *FAS/FASLG* polymorphisms in recurrence risk in SCCOP patients stratified by tumor HPV16 status. The association between *FAS*670 and *FASLG*844 variant genotypes and recurrence risk of SCCOP was higher in patients with HPV16(+) tumors, suggesting that the modifying effect of *FAS*670 and *FASLG*844 variant genotypes on the risk of SCCOP recurrence was more pronounced in these patients. The mechanism behind these results in patients with SCCOP is not fully understood. When HPV-positive SCCOP patients undergo radiotherapy or chemoradiotherapy, tumor cells harboring intact p53 may induce apoptosis. We expect that HPV-positive SCCOP patients with variant genotypes will have lower apoptotic efficacy than will patients with the corresponding common homozygous genotypes; thus, these patients are at higher risk of disease recurrence or progression through apoptotic escape. Genetic variants of these genes may lead to interindividual differences in apoptotic response, resulting in different

susceptibilities to the genotoxic effects of radiation and different clinical outcomes. However, these hypotheses need to be verified in future studies.

Our study has limitations. First, we did not collect information on the exact dosage and duration of radiotherapy for each patient. Therefore, well-designed studies with detailed information on radiotherapy and a uniform treatment plan are needed. Second, due to the relatively small sample size and small number of outcome events in patients with HPV16(+) tumors, our findings could be due to chance. Third, our cases were recruited at a single cancer center and the population was primarily non-Hispanic whites; thus, our results are not generalizable to other racial and ethnic groups. Finally, the disease recurrence outcomes were collected retrospectively, with no strictly defined screening or follow-up regimen. Despite these limitations, the current investigation supports a significant role for *FAS*670 and *FASLG*844 polymorphisms in individual variation in disease recurrence susceptibility after definitive radiotherapy in SCCOP patients. Large and prospective studies are needed to validate our results and further explore the molecular mechanisms that underlie the observed associations, which may have future utility as clinical prognostic biomarkers.

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