Polymorphisms of *FAS* and *FAS* Ligand Genes Involved in the Death Pathway and Risk and Progression of Squamous Cell Carcinoma of the Head and Neck

Zhengdong Zhang,[1] Li-E Wang,[1] Erich M. Sturgis,[1,2] Adel K. El-Naggar,[3] Waun K. Hong,[4] Christopher I. Amos,[1] Margaret R. Spitz,[1] and Qingyi Wei[1]

Abstract

Purpose: Alteration of the FAS/FAS ligand (FASLG) pathway regulating cell death may lead to cancer development, but the effects of functional promoter polymorphisms of the *FAS* and *FASLG* genes on risk of squamous cell carcinoma of the head and neck (SCCHN) are unknown.

Design: We genotyped the *FAS* −1377 G>A, *FAS* −670 A>G, *FASLG* −844 C>T, and *FASLG* IVS2nt −124 A>G polymorphisms in 721 case patients with SCCHN and 1,234 cancer-free non–Hispanic White control subjects frequency-matched by age and sex. Multivariate logistic regression was used to assess the adjusted odds ratios (OR) and 95% confidence intervals (CI).

Results: Compared with the *FAS* −1377 GG and −670 AA genotypes, the *FAS* −1377 AA and −670 (GG + AG) genotypes were associated with an increased risk of SCCHN (OR, 2.23; 95% CI, 1.07-4.64 and OR, 1.24; 95% CI, 1.01-1.52, respectively), whereas no risk of SCCHN was associated with any of the *FASLG* genotypes. When we used the combined *FAS* −1377 (GG + AG)/−670 AA genotypes as the reference, we found that the individuals carrying the *FAS* −1377 AA/−670 (GG + AG) had the highest risk (OR, 2.69; 95% CI, 1.24-5.83), whereas individuals carrying genotypes other than *FAS* −1377 (GG + AG)/−670 AA had a higher risk of SCCHN (OR, 1.24; 95% CI, 1.01-1.52). Furthermore, the elevated risk was particularly evident for pharyngeal cancer with the larger tumors without regional lymph metastasis (OR, 1.77; 95% CI, 1.07-2.94).

Conclusions: The *FAS* (but not *FASLG*) polymorphisms seem to contribute to risk of developing SCCHN, particularly the pharyngeal cancer in non–Hispanic Whites. However, potential selection bias warrants future population-based studies to verify the findings.

Squamous cell carcinoma of the head and neck (SCCHN), including those of the oral cavity, pharynx, and larynx, is the sixth most frequently occurring cancer and the seventh leading cause of cancer-related deaths worldwide (1). In the U.S., there will be an estimated 40,500 new cases and 11,170 deaths from SCCHN in 2006 (2). Although tobacco smoking and alcohol use are the major risk factors in the etiology of SCCHN (3), only a proportion of exposed individuals develops SCCHN in their life span, suggesting individual susceptibility to exposure-related carcinogenesis.

Apoptosis is a biological process that regulates physiologic cell death, varies in different tissues and organisms (4), and plays an important role in maintaining homeostasis (5). Accumulating evidence suggests that abnormal regulation of apoptosis is likely to contribute to the pathogenesis of a variety of human diseases, including cancer (6).

FAS, also known as TNFRSF6/CD95/APO-1, is a cell surface receptor that is involved in apoptotic signaling in many cell types (7). FAS ligand (FASLG), also known as TNFSF6/CD95LG, is a member of the tumor necrosis factor superfamily that can trigger the apoptotic cell–death cascade by cross-linking with its receptor, FAS (8). Therefore, the FAS/FASLG pathway plays a crucial role in regulating apoptosis and maintaining cellular homeostasis. Studies have shown that dysregulation of this pathway leads not only to reduced FAS expression but also to aberrant FASLG expression in a variety of tumors, including SCCHN (9–12). The FAS/FASLG pathway may also participate in the immunosuppression process observed in head and neck cancer (12). These data collectively suggest that the FAS/FASLG pathway may play an important role in the development and progression of SCCHN.

The human *FAS* gene (GenBank accession no. AY450925), located on chromosome 10q24.1, consists of nine exons and eight introns, and encodes 334 amino acids (13). Two polymorphisms have been identified in the *FAS* promoter region: one in the silencer region, G to A substitution at nucleotide position −1377 (*FAS* −1377 G>A, rs2234767), and

Authors' Affiliations: Departments of [1]Epidemiology, [2]Head and Neck Surgery, [3]Pathology, and [4]Thoracic and Head and Neck Medical Oncology, The University of Texas M.D. Anderson Cancer Center, Houston, Texas

Received 8/9/05; revised 5/15/06; accepted 6/29/06.

Grant support: NIH grants CA 86390 (M.R. Spitz), CA 97007 (W.K. Hong), ES 11740 and CA100264 (Q. Wei), ES 11047 and CA 16672 (The University of Texas M.D. Anderson Cancer Center).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Requests for reprints: Qingyi Wei, Department of Epidemiology, Unit 1365, The University of Texas M.D. Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, TX 77030. Phone: 713-792-3020; Fax: 713-792-0807; E-mail: qwei@mdanderson.org.

doi:10.1158/1078-0432.CCR-05-1739

the other in the enhancer region, A to G substitution at nucleotide position −670 (*FAS* −670 A>G, rs1800682). These two polymorphisms are located within the Sp1 and the signal transducers and activators of transcription 1 transcription factor binding sites, respectively (13, 14). Because these sequence variations in the *FAS* gene promoter region may influence FAS expression and dysregulate cell death signaling, they could contribute to carcinogenesis (14).

The human *FASLG* gene (GenBank accession no: Z96050) is located on chromosome 1q23, consists of four exons spanning ~8 kb, and encodes 281 amino acids (15). There are two reported polymorphisms: C to T changes at nucleotide position −844 (*FASLG* −844 C>T, rs763110) in the promoter region (16) and A to G change at nucleotide position −124 of intron 2 (*FASLG* INV2nt −124 A>G, rs5030772; ref. 17). *FASLG* −844 C>T is located in a putative binding motif for a transcription factor, CAAT/enhancer-binding protein β, and the −844 C allele may increase basal expression of FASLG compared with the −844 T allele (16), suggesting that the *FASLG* −844 C>T polymorphism may influence FASLG expression and FASLG-mediated signaling, and ultimately, the susceptibility to cancer. To date, the functional relevance of the *FASLG* IVS2nt −124 A>G polymorphism has not been reported.

The *FAS* −1377 G>A, *FAS* −670 A>G, and *FASLG* −844 C>T polymorphisms have been reported to be associated with increased risk of developing esophageal squamous cell carcinoma in a northern Chinese population (18), and the *FAS* −1377 G>A and *FASLG* −844 C>T polymorphisms were found to be associated with increased risk of lung cancer (19). Our previous study showed that the *FAS* −670 A>G polymorphism was associated with an increased risk of lung cancer (20), and a similar association was reported with cervical cancer in a Japanese population (21). Both the *FAS* −1377 G>A and *FAS* −1377 A/−670 A haplotype were also found to be associated with risk of acute myeloid leukemia (14). No association was found, however, between the *FAS* −670 A>G polymorphism and risk of non–melanoma skin cancer (22), although mutations in the *FAS* gene were reportedly involved in skin carcinogenesis (23, 24). To the best of our knowledge, there is no report on the association between the *FAS* and *FASLG* polymorphisms and risk of SCCHN.

Because of the role of the *FAS* and *FASLG* genes in regulating cell death, and because abnormal expression of FAS and/or FASLG has been observed in a variety of tumors, including SCCHN, we hypothesized that the *FAS* and *FASLG* polymorphisms contribute to genetic susceptibility to SCCHN. To test this hypothesis, we genotyped four polymorphisms of *FAS* and *FASLG* genes in our ongoing hospital-based case-control study of SCCHN and evaluated their associations with the risk of developing SCCHN.

Materials and Methods

Study subjects and data collection. The recruitment of study subjects has been described previously (25). Because this analysis was built on an ongoing DNA phenotype study, only those patients who did not have any treatment at the time of recruitment were eligible. Briefly, the study population included 721 patients with newly diagnosed and pathohistologically confirmed SCCHN and 1,234 cancer-free control subjects recruited between May 1995 and September 2003. Approximately 95% of the eligible patients contacted (among those registered with the M.D. Anderson Cancer Center) chose to participate in this study. The response rate among the eligible patients with SCCHN was 95%. Among the 721 patients with primary SCCHN who were included in the analysis, 222 (30.8%) had cancers of the oral cavity, 362 (50%) had cancers of the pharynx, and 131 (19%) had cancers of the larynx. Patients with second SCCHN primary tumors, primary tumors of the nasopharynx or sinonasal tract, primary tumors outside the upper aerodigestive tract, cervical metastases of unknown origin, or histopathologic diagnoses other than SCC were excluded.

The regional lymph node (N) involvement of pharyngeal cancer was defined from N_0 to N_3 as follows (26): N_0, no regional node metastasis; N_1, metastasis in a single ipsilateral lymph node, ≤3 cm in the greatest dimension; N_2, metastasis in a single ipsilateral lymph node, >3 cm but <6 cm in the greatest dimension; or in multiple ipsilateral lymph nodes, none >6 cm in the greatest dimension; or in bilateral or contralateral lymph nodes, <6 cm in the greatest dimension; N_3, metastasis in a lymph node >6 cm in the greatest dimension. The extent of the primary pharyngeal cancer was defined from T_1 to T_4 as follows (26): T_1, tumor ≤2 cm at the greatest dimension (oropharynx) or tumor limited to one subsite of hypopharynx (hypopharynx); T_2, tumor >2 cm but <4 cm in the greatest dimension (oropharynx) or tumor invades more than one subsite of the hypopharynx or an adjacent site, without fixation of hemilarynx (hypopharynx); T_3, tumor >4 cm in the greatest dimension (oropharynx) or the tumor invades more than one subsite of the hypopharynx or an adjacent site, with fixation of hemilarynx (hypopharynx); and T_4, tumor invades adjacent structures.

Cancer-free control subjects were recruited from persons who were not hospital patients or seeking health care, and who accompanied the case patients to the clinics. We first surveyed potential control subjects at the clinics by using a screening sheet to determine their willingness to participate in research studies and to obtain information on demographics, smoking status, and personal history of cancer. We then selected the frequency-matched eligible subjects to the cases by age (±5 years) and sex. Among the respondents for the screening, ~90% were eligible (no personal cancer history) and the response rate was ~90% in those we further contacted for recruitment. We interviewed each eligible subject to obtain data on tobacco smoking and alcohol use. After signing informed consent forms, each subject donated 30 mL of blood, of which, 1 mL was used for genomic DNA extraction with a DNA blood Mini Kit (Qiagen, Inc., Valencia, CA) according to the manufacturer's instructions. The research protocol was approved by the M.D. Anderson Institutional Review Board. It should be noted that in such a hospital-based case-control study, these cases and controls were not a random sample of a defined population and that the cases were not a random sample of all patients seen at M.D. Anderson. However, it is unlikely that the genotype of the subjects may have determined the selection of the subjects.

Genotyping. *FAS* and *FASLG* polymorphisms were identified by using the PCR-RFLP method, as previously described (27). 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 the *FAS* −670 A>G (18), 5′-CAATGAAAATGAACACATTG-3′ (forward) and 5′-CCCACTTTAGAAATTAGATC-3′ (reverse) for *FASLG* −844 C>T, and 5′-GCAGTTCAGACCTACATGATTAGGAT-3′ (forward) and 5′-CCAGATACAGACCTGTTAAATGGGC-3′ for *FASLG* IVS2nt −124 A>G (28). The amplified PCR products were 122, 193, 85, and 230 bp for −1377 G>A, −670 A>G, −844 C>T, and IVS2nt −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 the −1377 G>A, −670 A>G, −844 C>T, and IVS2nt −124 A>G polymorphisms, respectively, which resulted in 104 and 18 bp fragments in the case of the −1377 G allele; 136 and 57 bp fragments in the case of the −670 G allele; 66 and 19 bp fragments in the case of the −844 T allele; and 180 and 50 bp fragments in the case of the IVS2nt

−124 G allele. More than 10% of the samples were randomly selected for confirmation, and the results were 100% concordant.

Statistical analysis. We used the χ^2 test to evaluate differences in the frequency distributions of selected demographic variables, smoking status, alcohol use, and each allele and genotype of the four polymorphisms of the *FAS* and *FASLG* genes between the cases and controls. Unconditional univariate and multivariate logistic regression analyses were done to obtain the crude and adjusted odds ratios (OR) for risk of SCCHN and their 95% confidence intervals (CI). Multivariable adjustment was conditional on effects of age, sex, smoking status, and alcohol use. Furthermore, the 2LD software was used to calculate the D' value among the two *FAS* polymorphisms (−1377 G>A and −670 A>G) and among the two *FASLG* polymorphisms (−844 C>T and IVS2nt −124 A>G; refs. 29, 30). Considering the potential interaction of the *FAS* and *FASLG* genes on risk of SCCHN, the associations between the combined genotypes of the *FAS* or *FASLG* polymorphisms and risk of SCCHN were evaluated. The combined genotype data were further stratified by subgroups of age, sex, smoking status, alcohol use, and primary tumor site. To increase the statistical power, a priori genetic models (that is, dominant, codominant, or recessive) were assumed to collapse the genotype data. We tested the null hypotheses of additive and multiplicative gene-gene interactions and assessed departures from additive and multiplicative interaction models (31). A greater-than-multiplicative interaction was suggested when $OR_{11} > OR_{01} \times OR_{10}$, in which OR_{11} is the OR when both factors were present, OR_{01} is the OR when only factor 1 was present, OR_{10} is the OR when only factor 2 was present (31). To assess evidence for departure from a multiplicative model, we modeled interaction terms between variables using standard unconditional logistic regression. We were specifically interested in searching for interactions indicating a greater-than-multiplicative relationship (i.e., interaction terms from the logistic regression with positive coefficients) because these interactions identify subgroups of individuals who may be at particularly high risk for developing SCCHN. We were also interested in identifying departures from additive models. Empirically, a greater-than-additive interaction was indicated if $OR_{11} > OR_{10} + OR_{01} - 1$. When the test for multiplicative interaction was not rejected, further tests for additive interaction were done by a bootstrapping test of goodness of fit of the null hypothesis of an additive model with no interaction against an alternative hypothesis that allows an additive interaction. To perform the hypothesis test for additive models, we implemented bootstrapping using Stata 8.2 (Stata Corp. LP, College Station, TX). All statistical tests were two-sided, and $P < 0.05$ was considered statistically significant. We analyzed all data, except for additive models, using SAS software (version 8e; SAS Institute, Cary, NC).

Results

Characteristics of the study population. The frequency distributions of selected characteristics of the case patients and control subjects are presented in Table 1. The cases and controls seemed to be well matched on age and sex: the mean age was 57.0 years for the cases (±11.9 years; range, 18-90 years) and 57.2 years for the controls (±11.6 years; range, 20-87 years; $P = 0.277$), and 74.9% and 25.1% of the cases and 74.1% and 25.9% of the controls were men and women, respectively ($P = 0.686$). As expected, however, there were more current smokers (34.8%) and drinkers (51.2%) among the cases compared with controls (25.4% and 43.7%, respectively), and these differences were statistically significant ($P < 0.001$ for both exposures). Therefore, these variables were further adjusted for in the multivariate logistic regression analysis.

Association between the FAS and FASLG polymorphisms and risk of SCCHN. As shown in Table 2, the frequencies of minor −1377 A and −670 G alleles were higher in the cases (0.122 and 0.483, respectively) than in the controls (0.118 and 0.452, respectively) but the difference was not statistically significant ($P = 0.748$ and $P = 0.065$, respectively). The −1377 AA genotype was more frequent in the cases (2.4%) than in the controls (1.0%) and was associated with a significantly increased risk of SCCHN compared with the GG genotype (adjusted OR, 2.23; 95% CI, 1.07-4.64) compared with the −1377 GG genotype, and the risk did not change substantially under the assumption of a recessive genetic model (OR, 2.27; 95% CI, 1.09-4.72), compared with the variant genotypes (i.e., GG + AG). The −670 GG was also more frequent in the cases (23.2%) than in the controls (20.9%) and was associated with a borderline significantly increased risk of SCCHN (OR, 1.29; 95% CI, 0.99-1.68), and the risk associated with the variant genotypes (i.e., GG + AG) under the assumption of a dominant genetic model became statistically significant (OR, 1.24; 95% CI, 1.01-1.52) than in the −670 AA genotype. For the two *FASLG* polymorphisms, the frequencies of minor −844 T and IVS2nt −124 G alleles were lower in the cases (0.333 and 0.141, respectively) compared with the controls (0.354 and 0.147, respectively) but the differences were not statistically significant ($P = 0.195$ and $P = 0.640$, respectively). Although there were no statistically significant associations between these two polymorphisms and risk of SCCHN, the frequencies of the variant (i.e., −844 T and IVS2nt −124 G) genotypes were lower in the cases than in the controls. All distributions of the frequencies of observed genotypes in the controls were consistent with the Hardy-Weinberg equilibrium model (Table 2).

Association between the combined genotypes of the FAS or FASLG polymorphisms and risk of SCCHN. Further analysis suggested that there was linkage disequilibrium between the two *FAS* polymorphisms ($D' = 0.875$, $P < 0.001$ for −1377 G>A and −670 A>G) and the two *FASLG* polymorphisms ($D' = 0.892$, $P < 0.001$ for −844 C>T and IVS2nt −124 A>G). Such an incomplete linkage disequilibrium suggests, however, that a joint effect between the two *FAS* polymorphisms or the two

Table 1. Frequency distributions of selected variables in SCCHN cases and cancer-free controls

Variables	Cases (*n* = 721)	Controls (*n* = 1,234)	*P**
	n (%)	*n* (%)	
Age (y)			0.277
≤45	105 (14.6)	195 (15.8)	
46-55	227 (31.5)	345 (28.0)	
56-65	220 (30.5)	370 (30.0)	
>65	169 (23.4)	324 (26.2)	
Sex			0.686
Female	181 (25.1)	320 (25.9)	
Male	540 (74.9)	914 (74.1)	
Smoking status			<0.001
Never	188 (26.1)	370 (30.0)	
Former	282 (39.1)	550 (44.6)	
Current	251 (34.8)	314 (25.4)	
Alcohol use			<0.001
Never	158 (21.9)	373 (30.2)	
Former	194 (26.9)	322 (26.1)	
Current	369 (51.2)	539 (43.7)	

*Two-sided χ^2 test.

Table 2. Genotype and allele frequencies of the *FAS* and *FASLG* polymorphisms among the cases and controls and their associations with risk of SCCHN

Genotypes	Cases (*n* = 721)	Controls (*n* = 1,234)*	*P*†	Crude OR (95% CI)	Adjusted OR (95% CI)‡
	n (%)	*n* (%)			
FAS −1377 G>A					
GG	562 (77.9)	957 (77.6)	0.058	1.00	1.00
AG	142 (19.7)	264 (21.4)		0.92 (0.73-1.15)	0.91 (0.73-1.15)
AA	17 (2.4)	13 (1.0)		2.23 (1.07-4.62)	2.23 (1.07-4.64)
GG + AG	704 (97.6)	1,221 (99.0)	0.024	1.00	1.00
AA	17 (2.4)	13 (1.0)		2.27 (1.10-4.70)	2.27 (1.09-4.72)
A allele	0.122	0.118	0.748		
FAS −670 A>G					
AA	191 (26.5)	377 (30.6)	0.139	1.00	1.00
AG	363 (50.3)	599 (48.5)		1.20 (0.96-1.49)	1.21 (0.98-1.51)
GG	167 (23.2)	258 (20.9)		1.28 (0.98-1.66)	1.29 (0.99-1.68)
GG + AG	530 (73.5)	857 (69.4)	0.056	1.22 (0.99-1.50)	1.24 (1.01-1.52)
G allele	0.483	0.452	0.065		
FASLG −844 C>T					
CC	323 (44.8)	522 (42.3)	0.416	1.00	1.00
CT	316 (43.8)	551 (44.7)		0.93 (0.76-1.21)	0.93 (0.76-1.13)
TT	82 (11.4)	161 (13.0)		0.82 (0.61-1.11)	0.82 (0.61-1.11)
TT + CT	398 (55.2)	712 (57.7)	0.282	0.90 (0.75-1.09)	0.90 (0.75-1.09)
T allele	0.333	0.354	0.195		
FASLG IVS2nt −124 A>G					
AA	534 (74.1)	907 (73.5)	0.814	1.00	1.00
AG	170 (23.6)	292 (23.7)		0.99 (0.80-1.23)	0.97 (0.78-1.20)
GG	17 (2.3)	35 (2.8)		0.83 (0.46-1.49)	0.83 (0.46-1.50)
GG + AG	187 (25.9)	327 (26.5)	0.785	0.97 (0.79-1.20)	0.95 (0.77-1.17)
G allele	0.141	0.147	0.640		

*The observed genotype frequencies among the control subjects were in agreement with the Hardy-Weinberg equilibrium ($\chi^2 = 1.23$, $P = 0.268$ for −1377 G>A; $\chi^2 = 0.50$, $P = 0.481$ for −670 A>G; $\chi^2 = 0.68$, $P = 0.411$ for −844 C>T; and $\chi^2 = 3.69$, $P = 0.055$ for IVS2nt −124 A>G).
†Two-sided χ^2 test for either genotype distributions or allele frequencies between the cases and controls.
‡ORs were obtained from a multivariate logistic regression model with adjustment for age, sex, smoking status, and alcohol use.

FASLG polymorphisms may exist. To evaluate the genotype-genotype interaction, we dichotomized the *FAS* genotypes as either −1377 (GG + AG) or −1377 AA and −670 AA or −670 (GG + GA), and the *FASLG* genotypes as either −844 CC or −844 CT + TT, and −IVS2nt −124 AA or −IVS2nt −124 (AG + GG; Table 3). When we used the combined genotype of the −1377 (GG + AG)/−670 AA as the reference, we found that only the −1377 AA/−670 (GG + AG) genotype was associated with a significantly higher risk of SCCHN (OR, 2.69; 95% CI, 1.24-5.83). In contrast, compared with the −844 CC and -IVS2nt −124 AA genotype, none of the other genotype combinations was associated with risk of SCCHN, although they tended to be associated with a lower risk (Table 3). No statistical evidence was found for any interaction from either multiplicative or additive models between the two *FAS* polymorphisms or the two *FASLG* polymorphisms.

Association and stratification analysis of the combined genotypes of the FAS polymorphisms associated with risk of SCCHN. We then focused on the stratification analysis of the *FAS* polymorphisms because we found some association of the *FAS*, but not *FASLG*, polymorphisms with risk of SCCHN. To facilitate further analysis, we dichotomized combined genotypes of the two *FAS* polymorphisms into two groups, i.e., with −1377 (GG + AG)/−670 AA and without −1377 (GG + AG)/−670 AA [i.e., the −1377 (GG + AG)/−670 (GG + AG), −1377 AA/−670 AA, and −1377 AA/−670 (GG + AG) all combined]. As shown in Table 4, compared with the −1377 (GG + AG)/−670 AA genotype, we found that individuals carrying the combined genotypes without −1377 (GG + AG)/−670 AA had a higher risk of SCCHN (adjusted OR, 1.24; 95% CI, 1.01-1.52), and this increased risk was more pronounced among the subgroups of patients ages ≥50 years (OR, 1.32; 95% CI, 1.04-1.68), women (OR, 1.67; 95% CI, 1.09-2.56), and patients with pharyngeal cancer (OR, 1.32; 95% CI, 1.01-1.73). However, these ORs between subgroups were not statistically different, and the *P* value for the additive interaction was 0.213 and 0.066 for the age and sex subgroups, respectively.

Association between the combined genotypes of the FAS polymorphisms and progression of pharyngeal cancer. Because pharyngeal cancer was the predominant group among the cases and was associated with the combined variant genotypes of *FAS*, we further evaluated the association between the combined genotypes and progression of pharyngeal cancer. In this analysis, we found a statistically significant association between the combined genotypes and the extent of primary pharyngeal tumor (T) as well as regional lymph node metastasis at diagnosis (N; data not shown). However, when we incorporated the T and N data into three groups (i.e., $T_{1\text{-}2}N_{2\text{-}3}$, $T_{3\text{-}4}N_0$, and others), we found that the combined genotypes without −1377 (GG + AG)/−670 AA were associated with risk of $T_{3\text{-}4}N_0$ pharyngeal cancer compared with the combined genotypes with −1377 (GG + AG)/−670 AA (adjusted OR, 1.77; 95% CI, 1.07-2.94; Table 5). There was no such association observed between these combined genotypes and $T_{3\text{-}4}N_0$ oral cavity cancer and laryngeal

Table 3. Combined genotype frequencies of the *FAS* and *FASLG* polymorphisms among the case patients and control subjects and their associations with risk of SCCHN in non–Hispanic Whites

Combined genotypes		Cases (*n* = 721)	Controls (*n* = 1,234)	*P**	Crude OR (95% CI)	Adjusted OR (95% CI)†
		n (%)	*n* (%)			
FAS −1377 G>A	*FAS* −670 A>G			0.041		
GG + AG	AA	190 (26.3)	376 (30.4)		1.00	1.00
GG + AG	GG + AG	514 (71.3)	845 (68.5)		1.20 (0.98-1.48)	1.22 (0.99-1.50)
AA	AA	1 (0.1)	1 (0.1)		1.98 (0.12-31.81)	1.70 (0.11-27.45)
AA	GG + AG	16 (2.2)	12 (1.0)		2.64 (1.22-5.69)	2.69 (1.24-5.83)
Trend test					*P* = 0.007	*P* = 0.006
FASLG −844 C>T	*FASL* IVS2nt −124 A>G			0.702		
CC	AA	315 (43.7)	507 (41.1)		1.00	1.00
CC	AG + GG	8 (1.1)	15 (1.2)		0.86 (0.36-2.05)	0.82 (0.34-1.97)
CT + TT	AA	219 (30.4)	400 (32.4)		0.88 (0.71-1.09)	0.89 (0.72-1.11)
CT + TT	AG + GG	179 (24.8)	312 (25.3)		0.92 (0.73-1.16)	0.91 (0.72-1.15)
Trend test					*P* = 0.346	*P* = 0.314

*Two-sided χ^2 test for the combined genotype distributions between the cases and controls.
†ORs were obtained from a multivariate logistic regression model with adjustment for age, sex, smoking status, and alcohol use.

cancer (data not shown). Lastly, we examined the distributions of the haplotypes and combined diplotype of both genes, but we did not find any significant difference (data not shown).

Discussion

In the present study, we investigated the association between four known polymorphisms in the promoters of the *FAS* and *FASLG* genes, and risk of developing SCCHN in a hospital-based case-control study. We found that both the −1377 AA and −670 (GG + AG) genotypes were associated with an increased risk of SCCHN and a joint effect on risk, but not for the *FASLG* variant genotypes. The joint effects of the two *FAS* polymorphisms on risk of SCCHN were more pronounced among older female subjects, ever drinkers, and patients with pharyngeal cancer with larger tumors without regional lymph node metastases. To the best of our knowledge, no reported study has investigated the association between the *FAS* and *FASLG* polymorphisms and risk of SCCHN. Given the role of the FAS/FASLG pathway in carcinogenesis, it is biologically plausible that the *FAS* and *FASLG* polymorphisms may modulate risk of cancer.

There are several lines of evidence that support our findings. Cell death by the apoptotic pathway is essential for maintaining

Table 4. Stratification analyses between the combined genotypes of the *FAS* polymorphisms and risk of SCCHN

Variables	Case/control (*n*)	Combined genotypes (case/control)		*P**	Crude OR (95% CI)	Adjusted OR (95% CI)†
		With −1377 (GG + AG)/ −670 AA, *n* (%)	Without −1377 (GG + AG)/ −670 AA, *n* (%)			
Total	721/1,234	190/376 (26.3/30.5)	531/858 (73.7/69.5)	0.053	1.23 (1.00-1.50)	1.24 (1.01-1.52)
Age (y)						
<50	185/328	50/93 (27.0/28.3)	135/235 (73.0/71.7)	0.748	1.07 (0.71-1.60)	1.08 (0.72-1.63)
≥50	536/906	140/283 (26.1/31.2)	396/623 (73.9/68.8)	0.039	1.29 (1.01-1.63)	1.32 (1.04-1.68)
Sex						
Female	181/320	43/105 (23.8/32.8)	138/215 (76.2/67.2)	0.033	1.57 (1.04-2.37)	1.67 (1.09-2.56)
Male	540/914	147/271 (27.2/29.7)	393/643 (72.8/70.3)	0.323	1.13 (0.89-1.43)	1.13 (0.89-1.44)
Smoking status						
Never	188/370	44/108 (23.4/29.2)	144/262 (76.6/70.8)	0.147	1.35 (0.90-2.02)	1.35 (0.90-2.03)
Ever	533/864	146/268 (27.4/31.0)	387/596 (72.6/69.0)	0.149	1.19 (0.94-1.51)	1.21 (0.95-1.54)
Drinking status						
Never	158/373	40/106 (25.3/28.4)	118/267 (74.7/71.6)	0.464	1.17 (0.77-1.79)	1.16 (0.76-1.78)
Ever	563/861	150/270 (26.6/31.4)	413/591 (73.4/68.6)	0.056	1.26 (0.99-1.59)	1.26 (0.99-1.60)
Tumor site						
Oral cavity	222/1,234	56/376 (25.2/30.5)	166/858 (74.8/69.5)	0.115	1.30 (0.94-1.80)	1.35 (0.97-1.87)
Pharynx‡	362/1,234	90/376 (24.9/30.5)	272/858 (75.1/69.5)	0.039	1.32 (1.01-1.73)	1.32 (1.01-1.73)
Larynx	137/1,234	44/376 (32.1/30.5)	93/858 (67.9/69.5)	0.692	0.93 (0.63-1.35)	0.95 (0.65-1.40)

*Two-sided χ^2 test for the combined genotype distributions between the cases and controls.
†ORs were obtained from a multivariate logistic regression model with adjustment for age, sex, smoking status, and alcohol use.
‡Included both the oropharyngeal and hypopharyngeal cancer cases.

Table 5. Associations between the combined genotypes of the *FAS* polymorphisms and progression of pharyngeal cancer

Combined genotypes	$T_{1-2}N_{2-3}$		$T_{3-4}N_0$		Others	
	Case/control, *n* (%)	Adjusted OR (95% CI)*	Case/control, *n* (%)	Adjusted OR (95% CI)*	Case/control, *n* (%)	Adjusted OR (95% CI)*
Total	193/1,234 (53.3/100.0)		101/1,234 (27.9/100.0)		68/1,234 (18.8/100.0)	
With −1377 (GG + AG)/ −670 AA	51/376 (26.4/30.5)	1.00	20/376 (19.8/30.5)	1.00	19/376 (27.9/30.5)	1.00
Without −1377 (GG + AG)/ −670 AA	142/858 (73.6/69.5)	1.19 (0.84-1.68)	81/858 (80.2/69.5)	1.77 (1.07-2.94)	49/858 (72.1/69.5)	1.15 (0.67-2.00)

NOTE: T, extent of the primary tumor; N, involvement of regional lymph node. The rules for classification of the T and N for pharyngeal cancer were defined by the American Joint Committee on Cancer Manual for Staging of Cancer, 4th edition (26).
*ORs were obtained from multivariate logistic regression models with adjustment for age, sex, smoking status, and alcohol use.

the normal function of cells, but it can be initiated or inhibited by a variety of stimuli (32). It has been shown that alterations of FAS and FASLG expression decrease the apoptotic capacity of cells and that many tumor cells might evade or suppress the immune system (33, 34). The loss of FAS or gain of FASLG expression are common features of most human malignancies and are associated with the progression of cancers, including SCCHN (9–12). Experimental data also suggest that this FAS/FASLG pathway may participate in immunosuppression, a phenomenon that has been observed in head and neck cancers as well (12). Therefore, the variants of the *FAS* and *FASLG* genes, if functional, could be expected to have an effect on cell death, and thus, carcinogenesis.

Several association studies have reported that the *FAS* and *FASLG* polymorphisms are associated with risk of diseases including cancer and systemic lupus erythematosus in different ethnic groups (14, 16, 18–20, 35, 36), although one study reported the lack of an association between the *FAS* −670 A>G polymorphism and skin cancer (22). Our data from this much larger study further support the notion that the *FAS* −1377 G>A and −670 A>G polymorphisms (located in the Sp1 and signal transducers and activators of transcription factor 1 binding sites, respectively) are potentially implicated in cancer risk.

In the present study, we observed a significantly increased risk of SCCHN among ever drinkers, suggesting that a gene-environment interaction may be involved in the development of SCCHN. It is reported that ethanol-mediated alteration of caspase-3 activation may play a role in cell apoptosis (37, 38), which may explain the possible role of the *FAS* polymorphisms in alcohol-induced SCCHN. However, this finding may be by chance, owing to the small number of observations in the stratified analysis. More interestingly, the finding that the risk associated with the combined genotypes without *FAS* −1377 (GG + AG)/−670 AA was significantly higher for pharyngeal cancer with larger tumors without regional lymph node metastasis, suggesting that the combined *FAS* genotypes may be associated with progression of pharyngeal cancer, or alternatively, that pharyngeal carcinoma may have a different etiology in terms of genetic susceptibility. It is possible that these results may be due to selection bias which is common in hospital-based case-control studies, and it is difficult to compare with genotyping data from other studies because few studies on *FASLG* polymorphisms have been published. However, the 42.3% of *FASLG* −844 CC and 44.7% of *FASLG* −844 CT genotypes of our 1,234 Caucasian controls were similar to previously published data in one study on American Caucasians (16). Because there were other genetic variants that were not assayed in this study, this hypothesis needs to be tested in future studies with a larger number of patients with pharyngeal cancer and data from dense gene maps, such as the HapMap database (39).

In conclusion, *FAS* (but not *FASLG*) polymorphisms have a main effect on risk of developing SCCHN. The combined genotypes of the *FAS* polymorphisms were associated with a significantly increased risk of SCCHN, which was more pronounced among older women, drinkers, and patients with pharyngeal cancer. We also found that the combined *FAS* genotypes were associated with a statistically significantly increased risk of pharyngeal cancer with larger tumors, but no regional lymph metastasis. These findings suggest that the *FAS* polymorphisms may jointly contribute to risk and progression of SCCHN, particularly for pharyngeal cancer. However, because the number of observations in the subgroup analyses were limited, the findings could be due to chance. In addition, because the frequency of the genotypes associated with risk of SCCHN was very low, the attributable risk in the general population might be low as well. Additional studies which include a larger number of patients with pharyngeal cancer, more detailed data on environmental exposure, inclusion of more single nucleotide polymorphisms in one gene or genes in the same biological pathway, and survival data are required to verify these findings. However, it is likely that the low participation rate relative to all SCCHN patients seen at M.D. Anderson may have introduced potential selection bias, particularly when the genotype may be associated with disease progression. Therefore, future population-based studies are needed to verify the findings.

Acknowledgments

We thank Margaret Lung, Peggy Schuber, and Leanel Fairly for their assistance in recruiting the subjects; Zhensheng Liu for his technical support; Qiuling Shi, Xiangjun Gu, and Shenying Fang for their help on data analysis; Jianzhong He and Kejin Xu for their laboratory assistance; Betty J. Larson and Joanne Sider for manuscript preparation; and Kathryn Hale for scientific editing.

References

1. Parkin DM, Pisani P, Ferlay J. Global cancer statistics. CA Cancer J Clin 1999;49:33–64, 1.

2. Jemal A, Siegel R, Ward E, et al. Cancer statistics, 2006. CA Cancer J Clin 2006;56:106–30.

3. Blot WJ, McLaughlin JK, Winn DM, et al. Smoking and drinking in relation to oral and pharyngeal cancer. Cancer Res 1988;48:3282–7.

4. Hacker G, Vaux DL. A chronology of cell death. Apoptosis 1997;2:247–56.

5. Zornig M, Hueber A, Baum W, Evan G. Apoptosis regulators and their role in tumorigenesis. Biochim Biophys Acta 2001;1551:F1–37.

6. Thompson CB. Apoptosis in the pathogenesis and treatment of disease. Science 1995;267:1456–62.

7. Muschen M, Warskulat U, Beckmann MW. Defining CD95 as a tumor suppressor gene. J Mol Med 2000; 78:312–25.

8. Houston A, O'Connell J. The Fas signalling pathway and its role in the pathogenesis of cancer. Curr Opin Pharmacol 2004;4:321–6.

9. Loro LL, Vintermyr OK, Johannessen AC, Liavaag PG, Jonsson R. Suppression of Fas receptor and negative correlation of Fas ligand with differentiation and apoptosis in oral squamous cell carcinoma. J Oral Pathol Med 1999;28:82–7.

10. Muraki Y, Yoshioka C, Tateishi A, Fukuda J, Haneji T, Kobayashi N. Localization of Fas antigen in oral squamous cell carcinoma. Br J Oral Maxillofac Surg 1999; 37:37–40.

11. Sundelin K, Jadner M, Norberg-Spaak L, Davidsson A, Hellquist HB. Metallothionein and Fas (CD95) are expressed in squamous cell carcinoma of the tongue. Eur J Cancer 1997;33:1860–4.

12. Gastman BR, Atarshi Y, Reichert TE, et al. Fas ligand is expressed on human squamous cell carcinomas of the head and neck, and it promotes apoptosis of T lymphocytes. Cancer Res 1999;59:5356–64.

13. Huang QR, Morris D, Manolios N. Identification and characterization of polymorphisms in the promoter region of the human Apo-1/Fas (CD95) gene. Mol Immunol 1997;34:577–82.

14. Sibley K, Rollinson S, Allan JM, et al. Functional FAS promoter polymorphisms are associated with increased risk of acute myeloid leukemia. Cancer Res 2003;63:4327–30.

15. Takahashi T, Tanaka M, Inazawa J, Abe T, Suda T, Nagata S. Human Fas ligand: gene structure, chromosomal location and species specificity. Int Immunol 1994;6:1567–74.

16. Wu J, Metz C, Xu X, et al. A novel polymorphic CAAT/enhancer-binding protein β element in the FasL gene promoter alters Fas ligand expression: a candidate background gene in African American systemic lupus erythematosus patients. J Immunol 2003;170: 132–8.

17. Bolstad AI, Wargelius A, Nakken B, Haga HJ, Jonsson R. Fas and Fas ligand gene polymorphisms in primary Sjogren's syndrome. J Rheumatol 2000;27:2397–405.

18. Sun T, Miao X, Zhang X, Tan W, Xiong P, Lin D. Polymorphisms of death pathway genes FAS and FASL in esophageal squamous-cell carcinoma. J Natl Cancer Inst 2004;96:1030–6.

19. Zhang X, Miao X, Sun T, et al. Functional polymorphisms in cell death pathway genes FAS and FASL contribute to risk of lung cancer. J Med Genet 2005; 42:479–84.

20. Wang LE, Cheng L, Spitz MR, Wei Q. Fas A670G polymorphism, apoptotic capacity in lymphocyte cultures, and risk of lung cancer. Lung Cancer 2003; 42:1–8.

21. Ueda M, Hung YC, Terai Y, et al. Fas gene promoter −670 polymorphism (A/G) is associated with cervical carcinogenesis. Gynecol Oncol 2005;98:129–33.

22. Nelson HH, Kelsey KT, Bronson MH, Mott LA, Karagas MR. Fas/APO-1 promoter polymorphism is not associated with non-melanoma skin cancer. Cancer Epidemiol Biomarkers Prev 2001;10:809–10.

23. Boldrini L, Loggini B, Gisfredi S, et al. Mutations of Fas (APO-1/CD95) and p53 genes in nonmelanoma skin cancer. J Cutan Med Surg 2003;7:112–8.

24. Shin MS, Park WS, Kim SY, et al. Alterations of Fas (Apo-1/CD95) gene in cutaneous malignant melanoma. Am J Pathol 1999;154:1785–91.

25. Li G, Sturgis EM, Wang LE, et al. Association between the V109G polymorphism of the p27 gene and the risk and progression of oral squamous cell carcinoma. Clin Cancer Res 2004;10:3996–4002.

26. American Joint Committee on Cancer. Manual for staging of cancer. 4th ed. Philadelphia: J.B. Lippincott; 1988.

27. Zhang Z, Shi Q, Sturgis EM, Spitz MR, Hong WK, Wei Q. Thymidylate synthase 5′- and 3′-untranslated region polymorphisms associated with risk and progression of squamous cell carcinoma of the head and neck. Clin Cancer Res 2004;10:7903–10.

28. Stuck BJ, Pani MA, Besrour F, et al. Fas ligand gene polymorphisms are not associated with Hashimoto's thyroiditis and Graves' disease. Hum Immunol 2003; 64:285–9.

29. Zhao JH. 2LD, GENECOUNTING and HAP: Computer programs for linkage disequilibrium analysis. Bioinformatics 2004;20:1325–6.

30. Zapata C, Carollo C, Rodriguez S. Sampling variance and distribution of the D′ measure of overall gametic disequilibrium between multiallelic loci. Ann Hum Genet 2001;65:395–406.

31. Kleinbaum D, Kupper L, Morgenstern H. Epidemiologic research: principles and quantitative methods. London: Lifetime Learning Publications; 1982.

32. Krammer PH. CD95's deadly mission in the immune system. Nature 2000;407:789–95.

33. Shimonishi T, Isse K, Shibata F, et al. Up-regulation of fas ligand at early stages and down-regulation of Fas at progressed stages of intrahepatic cholangiocarcinoma reflect evasion from immune surveillance. Hepatology 2000;32:761–9.

34. Das H, Koizumi T, Sugimoto T, et al. Quantitation of Fas and Fas ligand gene expression in human ovarian, cervical and endometrial carcinomas using real-time quantitative RT-PCR. Br J Cancer 2000; 82:1682–8.

35. Lai HC, Sytwu HK, Sun CA, et al. Single nucleotide polymorphism at Fas promoter is associated with cervical carcinogenesis. Int J Cancer 2003; 103:221–5.

36. Chen JY, Wang CM, Ma CC, Chow YH, Luo SF. The −844C/T polymorphism in the Fas ligand promoter associates with Taiwanese SLE. Genes Immun 2005; 6:123–8.

37. D'Mello SR, Kuan CY, Flavell RA, Rakic P. Caspase-3 is required for apoptosis-associated DNA fragmentation but not for cell death in neurons deprived of potassium. J Neurosci Res 2000;59:24–31.

38. Han JY, Joo Y, Kim YS, et al. Ethanol induces cell death by activating caspase-3 in the rat cerebral cortex. Mol Cells 2005;20:189–95.

39. Altshuler D, Brooks LD, Chakravarti A, Collins FS, Daly MJ, Donnelly P. International HapMap Consortium. A haplotype map of the human genome. Nature 2005;437:1299–320.