Polymorphisms of Death Pathway Genes FAS and FASL in Esophageal Squamous-Cell Carcinoma

Tong Sun, Xiaoping Miao, Xuemei Zhang, Wen Tan, Ping Xiong, Dongxin Lin

**Background:** The FAS receptor–ligand system is a key regulator of apoptotic cell death, and loss of FAS ligand (FASL) expression and gain of FAS ligand (FASL) expression play important roles in the development and progression of cancer. Single-nucleotide polymorphisms in the promoter region of the FAS (G or A at position –1377 [FAS –1377G/A] and A or G at position –670 [FAS –670A/G]) and FASL (T or C at position –844 [FASL –844T/C]) genes alter the transcriptional activity of these genes. We examined the association between these polymorphisms and risk of the development and metastasis of esophageal squamous-cell carcinoma. **Methods:** Genotypes of 588 case patients with esophageal squamous-cell carcinoma and 648 control subjects were determined by polymerase chain reaction–based restriction fragment length polymorphism. Associations with the risk of esophageal squamous-cell carcinoma were estimated by logistic regression. All statistical tests were two-sided. **Results:** We observed a statistically significantly increased risk of esophageal squamous-cell carcinoma associated with the FAS –1377AA (odds ratio [OR] = 1.79, 95% confidence interval [CI] = 1.29 to 2.48; \(P < .001\)) or FAS –670GG (OR = 1.72, 95% CI = 1.26 to 2.34; \(P < .001\)) genotype, which are in strong linkage disequilibrium compared with the FAS –1377GA or GG or the FAS –670AG or AA genotype, respectively. An increased risk of esophageal squamous-cell carcinoma was also associated with the FASL –844CC genotype (OR = 2.06, 95% CI = 1.64 to 2.59; \(P < .001\)) compared with the FASL –844CT or TT genotype. Gene–gene interactions of FAS and FASL polymorphisms increased the risk of esophageal squamous-cell carcinoma in a multiplicative manner (OR for the presence of both FAS –1377AA and FASL –844CC genotypes = 4.55, 95% CI = 2.75 to 7.48; \(P = .001\), test for homogeneity). Statistically significant interactions were found between these polymorphisms in FAS and FASL and tobacco smoking. None of the polymorphisms was associated with risk of differentiation or metastasis of esophageal squamous-cell carcinoma at diagnosis. **Conclusion:** Genetic polymorphisms in the death pathway genes FAS and FASL appear to be associated with an increased risk of developing esophageal squamous-cell carcinoma. [J Natl Cancer Inst 2004;96:1030–6]

Apoptosis plays an important role in sculpting the developing organism and eliminating unwanted or potentially dangerous cells throughout life. Abnormal regulation of apoptosis is associated with a variety of diseases. Cells that should die but do not can cause cancer and autoimmune diseases, whereas cells that should not die but do can cause stroke and neurodegenerative disorders [for a review, see (1)]. The acquired ability to resist apoptotic stimuli is shared by almost all types of malignant diseases, and mutation in the components of apoptotic pathways is a pivotal mechanism in the development of cancer [for reviews, see (2,3)].

FAS (also known as TNFSF6, CD95, or APO-1) is a cell surface receptor that plays a central role in apoptotic signaling in many cell types (4–6). This receptor interacts with its natural ligand FASL (also known as CD95L), a member of the tumor necrosis factor superfamily, to initiate the death signal cascade, which results in apoptotic cell death (7). An immuno-privileged status for tumors is established via the FAS-mediated apoptosis of tumor-specific lymphocytes (8,9). Decreased expression of FAS and/or increased expression of FASL favors malignant transformation and progression [for a review, see (10)]. In addition, functional germline and somatic mutations in the FAS gene and perhaps also in the FASL gene that impair apoptotic signal transduction are associated with a high risk of cancer (11–13). Thus, the FAS/FASL system appears to have a role in the development and progression of cancer.

In human esophageal squamous-cell carcinomas, expression of FAS is lower and expression of FASL is higher than in the corresponding normal tissue, indicating an association between the aberrant expression of FAS and FASL and esophageal squamous-cell carcinoma (16–19). In addition, aberrant expression of FAS and FASL occurs early in dysplasia and in carcinoma in situ (17,18) and has been associated with differentiation, invasiveness, and metastasis of cancer cells and with patients’ survival (16,19). These findings suggest that FAS and FASL are likely to be involved in the initiation and development of esophageal squamous-cell carcinoma. It has been proposed that decreased expression of FAS also decreases the ability of esophageal squamous-cell carcinoma cells to undergo apoptosis, whereas increased expression of FASL may increase the ability of esophageal squamous-cell carcinoma cells to counterattack the immune system by killing FAS-sensitive lymphocytes (16,17).

Single-nucleotide polymorphisms have been identified in the promoter region of the FAS gene (20,21)–G or A at position –1377 (FAS –1377G/A) and A or G at position –670 (FAS –670A/G). The FAS –1377A allele and the FAS –670G allele disrupt Sp1 and STAT1 transcription factor binding sites, respectively, and thus diminish promoter activity and decrease FAS gene expression (20–22). The promoter of FASL also has a functional single-nucleotide polymorphism—a T or C at position –844(FASL –844T/C)—that is located in a binding motif for another transcription factor, CAAT/enhancer-binding protein.
\( \beta \) (23). Higher basal expression of FASL is statistically significantly associated more with the FASL \( \sim \) 844C allele than with the FASL \( \sim \) 844T allele (23).

Because of the role that FAS and FASL play in cancer development and progression and because of their aberrant expression in various types of cancer, we hypothesized that these functional polymorphisms in FAS and FASL are associated with an increased risk of cancer attributable to the reduced expression of FAS and/or the elevated expression of FASL. In this study, we recruited 588 case patients with incident esophageal squamous-cell carcinoma and 648 healthy population control subjects, and we genotyped FAS and FASL for these polymorphisms to test the hypothesis that they are associated with the risk of esophageal squamous-cell carcinoma. In addition, we also evaluated whether the FAS and FASL polymorphisms were associated with the risk of metastasis.

**MATERIALS AND METHODS**

**Study Subjects**

This case–control study consisted of 588 patients with esophageal squamous-cell carcinoma and 648 population control subjects. All subjects were unrelated ethnic Han Chinese and residents in Beijing and the surrounding regions. Patients were recruited between July 20, 1999, and December 20, 2001, at the Cancer Hospital, Chinese Academy of Medical Sciences (Beijing, China). All patients with histologically confirmed esophageal squamous-cell carcinoma were enrolled. There was no sex and age restriction. The response rate for case patients was 94%. Exclusion criteria included previous cancer and previous chemotherapy or radiotherapy. Two hundred forty of the patients who underwent esophagectomy and had detailed metastatic data. The presence or absence of lymph node metastasis was evaluated according to the postoperative histopathologic examination of esophageal tumor specimens. Control subjects were cancer-free individuals who were recruited between July 20, 1999, and December 20, 2001. The response rate for control subjects was 89%. The characteristics of 360 control subjects were described previously (24). The selection criteria for the control subjects included no individual history of cancer, and control subjects were frequency matched to case patients on the basis of sex and age (\( \pm 5 \) years). In this study, we also selected 288 more control subjects from the same database matched to case patients as described above, for a total of 648 control subjects. At recruitment, written informed consent was obtained from each subject, and personal data from each participant regarding demographic characteristics such as sex and age and related risk factors including tobacco smoking were collected via questionnaire. This study was approved by the institutional review board of the Chinese Academy of Medical Sciences Cancer Institute.

**Polymorphism Analysis**

Genomic DNA was extracted from blood samples of all control subjects and most case patients. Twenty-eight percent of DNA samples from case patients were isolated from surgically resected normal tissues adjacent to the esophageal tumors. Genotypes were determined by polymerase chain reaction–based restriction fragment length polymorphism (PCR–RFLP) as described below, which was performed in a blinded manner (i.e., the case–control status of participants was unknown to those performing this test). For quality control, a 15% masked, random sample of DNAs prepared from case patients and control subjects was tested twice by different people, and the results were concordant for all of the masked duplicate sets. Genotypes identified by PCR–RFLP were confirmed with DNA sequencing.

PCR primers for amplification of the FAS promoter region containing the FAS \( \sim \) 1377G/A polymorphism were FasIF 5’-TGTGTGCACAGGCTGGGC-3’ and FasIR 5’-TGATCCTGTACTGACCTACCA-3’, which produce a 122-base-pair (bp) fragment. To introduce a restriction endonuclease site, we changed the 3’ end of primer FasIF from CAC to CGC, which created a BstUI site. Primers for the FAS \( \sim \) 844T/G polymorphism were FasIF 5’-ATAGCTGGGGCTATGCG ATT-3’ and FasIR 5’-CATTGACTGGCGTGCAT-3’, which produce a 193-bp fragment. PCR primers for amplification of the FASL promoter region containing the FASL \( \sim \) 844T/C polymorphism were FaslL 5’-CATTTGACTGGGCTGTCCAT-3’, which produce a 401-bp fragment. These fragments were amplified separately under the following conditions: a 25-\( \mu \)L reaction mixture containing approximately 100 ng of template DNA, 0.5 \( \mu \)M of each primer, all four deoxyribonucleoside 5’ triphosphates (each at 0.2 \( \mu \)M), 2.0 \( \mu \)M MgCl\(_2\), and 1.0 U of Taq DNA polymerase in 1× reaction buffer (Promega, Madison, WI). The reaction was carried out with an initial melting step of 2 minutes at 94°C; followed by 35 cycles of 30 seconds at 94°C, 30 seconds at 62°C, and 45 seconds at 72°C; and a final elongation step of 7 minutes at 72°C.

The restriction endonucleases BstUI, ScrFI, and BsrDI (New England Biolabs, Beverly, MA) were used to distinguish the FAS \( \sim \) 1377G/A, FAS \( \sim \) 844T/G, and FASL \( \sim \) 844T/C polymorphisms, respectively. The restriction endonuclease products were separated on agarose gels containing ethidium bromide. The RFLPs of the three polymorphisms were readily distinguished. BstUI digestion generated the following fragments: FAS \( \sim \) 1377G allele, fragments of 104 bp and 18 bp; FAS \( \sim \) 1377A allele, a single fragment of 122 bp; FAS \( \sim \) 844T allele, a single fragment of 193 bp; and FASL \( \sim \) 844C allele, fragments of 136 bp and 57 bp (because anScrFI site was gained). The FASL \( \sim \) 844C allele had a BsrDI restriction endonuclease site that resulted in two fragments of 233 bp and 168 bp, and the T allele lacked this site and so resulted in a single 401-bp fragment.

**Statistical Analysis**

Pearson’s chi-square test was used to examine differences in demographic variables, smoking status, and the genotype distribution of FAS \( \sim \) 1377GA, FAS \( \sim \) 844T/G, and FASL \( \sim \) 844T/C polymorphisms between case patients and control subjects and between metastatic and nonmetastatic case patients. Associations between polymorphisms and risk of the development and
metastasis of esophageal squamous-cell carcinoma were estimated by use of unconditional logistic regression. Smokers were considered current smokers if they smoked up to 1 year before the date of cancer diagnosis or if they smoked up to 1 year before the date of the interview for control subjects. Information was collected on the number of cigarettes smoked per day, the age at which the subjects started smoking, and the age at which ex-smokers stopped smoking. Subjects who never smoked or smoked less than 1 year before the date of cancer diagnosis for case patients or the date of interview for control subjects were defined as nonsmokers. The number of pack-years smoked was determined as an indication of the cumulative cigarette dose level [pack-years = (cigarettes per day)/20] × (years smoked). Light, moderate, and heavy smokers were categorized by using the 25th and 75th percentile pack-year values of the control subjects as the cut points (i.e., ≤17 pack-years, 18–32 pack-years, and >32 pack-years). Because only 16 case patients and 18 control subjects were ex-smokers, they were combined with current smokers for analysis. All odds ratios (ORs) were adjusted for age, sex, and smoking status or pack-years, where it was appropriate. A P value of less than .05 was used as the criterion of statistical significance, and all statistical tests were two-sided. We tested the null hypotheses of additive and multiplicative gene–gene and gene–smoking interactions and evaluated departures from additive and multiplicative interaction models (26) by including main effect variables and their product terms in the logistic regression model. All analyses were performed with computer programs from Statistical Analysis System (version 6.12; SAS Institute, Cary, NC).

RESULTS

This study included 588 case patients with esophageal squamous-cell carcinoma and 648 control subjects. Characteristics of the subjects are summarized in Table 1. No statistically significant differences were found between case patients and control subjects in terms of median age, sex distribution, and smoking status, suggesting that the frequency matching was adequate. Metastasis was not detected in 291 (49.5%) of the 588 case patients but was detected in the other 297 (50.5%) at the time of diagnosis. Among the 588 case patients, 156 (26.5%) had well-differentiated tumors (grade I), 319 (54.3%) had poorly differentiated tumors (grade II), and 113 (19.2%) had undifferentiated tumors (grade III).

Allele frequencies and genotype distributions of FAS and FASL in case patients and control subjects are shown in Table 2. The allele frequencies for FAS –1377A, FAS –670G, and FASL 844C, respectively, were 0.34, 0.37, and 0.68 in control subjects and 0.38, 0.41, and 0.77 in case patients. Among control subjects, the observed genotype frequencies of both FAS –1377G/A and FAS –670A/G polymorphisms did not deviate statistically significantly from those expected from the Hardy–Weinberg equilibrium (P = .686 and .561, respectively). Distributions of these FAS genotypes were then compared among case patients and control subjects. Frequencies of FAS –1377GG, -GA, and -AA genotypes among case patients differed statistically significantly from those among control subjects (χ² = 14.81; P<.001; df = 2), with the frequency of the AA homozygotes being statistically significantly higher among case patients than that among control subjects (17.7% versus 10.6%; P<.001). Similarly, frequencies of the FAS –670AA, -AG, and -GG genotypes were statistically significantly different among case patients and control subjects (χ² = 14.34; P<.001; df = 2), with the frequency of the rare homozygous GG genotype being higher in case patients than in control subjects (19.9% versus 12.5%; P<.001). The two FAS polymorphisms were also in strong linkage disequilibrium (D’ = 0.99; P<.001) in our study population. Distributions of FASL genotypes in both control subjects and case patients conformed to the Hardy–Weinberg equilibrium (P = .414 and .158, respectively). However, frequencies of the FASL –844TT, -TC, and -CC genotypes differed statistically significantly among case patients and control subjects (χ² = 13.22; P<.001; df = 2), with the frequency of the TT genotype being statistically significantly higher among case patients than that among control subjects (24.8% versus 12.6%; P<.001). Similarly, frequencies of the FASL –844TT, -TC, and -CC genotypes differed statistically significantly among case patients and control subjects (χ² = 13.22; P<.001; df = 2), with the frequency of the TT genotype being statistically significantly higher among case patients than that among control subjects (24.8% versus 12.6%; P<.001). Similarly, frequencies of the FASL –844TT, -TC, and -CC genotypes differed statistically significantly among case patients and control subjects (χ² = 13.22; P<.001; df = 2), with the frequency of the TT genotype being statistically significantly higher among case patients than that among control subjects (24.8% versus 12.6%; P<.001). Similarly, frequencies of the FASL –844TT, -TC, and -CC genotypes differed statistically significantly among case patients and control subjects (χ² = 13.22; P<.001; df = 2), with the frequency of the TT genotype being statistically significantly higher among case patients than that among control subjects (24.8% versus 12.6%; P<.001). Similarly, frequencies of the FASL –844TT, -TC, and -CC genotypes differed statistically significantly among case patients and control subjects (χ² = 13.22; P<.001; df = 2), with the frequency of the TT genotype being statistically significantly higher among case patients than that among control subjects (24.8% versus 12.6%; P<.001). Similarly, frequencies of the FASL –844TT, -TC, and -CC genotypes differed statistically significantly among case patients and control subjects (χ² = 13.22; P<.001; df = 2), with the frequency of the TT genotype being statistically significantly higher among case patients than that among control subjects (24.8% versus 12.6%; P<.001). Similarly, frequencies of the FASL –844TT, -TC, and -CC genotypes differed statistically significantly among case patients and control subjects (χ² = 13.22; P<.001; df = 2), with the frequency of the TT genotype being statistically significantly higher among case patients than that among control subjects (24.8% versus 12.6%; P<.001). Similarly, frequencies of the FASL –844TT, -TC, and -CC genotypes differed statistically significantly among case patients and control subjects (χ² = 13.22; P<.001; df = 2), with the frequency of the TT genotype being statistically significantly higher among case patients than that among control subjects (24.8% versus 12.6%; P<.001). Similar
differed statistically significantly between case patients and control subjects, with the frequency of the CC genotype being higher in case patients than in control subjects (61.9% versus 44.3%; \( P < .001 \)).

Unconditional logistic regression analysis was used to estimate associations between the genotypes and risk of esophageal squamous-cell carcinoma (Table 2). The FAS –1377AA genotype was associated with an increased risk for the development of esophageal squamous-cell carcinoma (OR = 1.62, 95% confidence interval [CI] = 1.14 to 2.30) compared with the GG genotype. Similarly, the homozygous FAS –670GG genotype was associated with an increased risk of esophageal squamous-cell carcinoma (OR = 1.57, 95% CI = 1.12 to 2.20) compared with the AA genotype. Because of tight linkage, the haplotype of FAS –1377GG/A and FAS –670AA/G did not further increase the risk of the cancer (data not shown). The FASL –844CC genotype was associated with an increased risk of esophageal squamous-cell carcinoma (OR = 1.72, 95% CI = 1.12 to 2.64) compared with the TT genotype. Heterozygous genotypes for all three polymorphisms (FAS –1377GA, FAS –670AG, or FASL –844CT) were not associated with risk of esophageal squamous-cell carcinoma, suggesting a possible recessive effect of the polymorphisms in these two genes. Because of this observation, we further analyzed combinations of heterozygous genotypes by using the corresponding wild-type genotype (FAS –1377GG, FAS –670AA, or FASL –844TT) as the reference group and found that the increased risks of esophageal squamous-cell carcinoma associated with the FAS –1377AA genotype (OR = 1.79, 95% CI = 1.29 to 2.48), FAS –670GG (OR = 1.72, 95% CI = 1.26 to 2.34), and FASL –844CC (OR = 2.06, 95% CI = 1.64 to 2.59) genotypes appeared to be more pronounced (for all comparisons, \( P < .001 \)).

We next investigated whether there was a statistical interaction between the FAS and FASL genotypes that was associated with the risk of esophageal squamous-cell carcinoma (Table 3). Because the two polymorphisms in FAS were tightly linked, only the FAS –1377GA polymorphism was analyzed. Case patients with the FAS –1377AA genotype were also more likely to carry the FASL –844CC genotype than control subjects with the FAS –1377AA genotype (11.1% versus 3.9%; \( P < .001 \)). The presence of one FAS –1377AA genotype or one FASL –844CC genotype was associated with an increased risk for esophageal squamous-cell carcinoma (OR = 1.49, 95% CI = 0.93 to 2.39, or OR = 1.97, 95% CI = 1.54 to 2.52, respectively), compared with the lack of such a genotype. However, the presence of both FASL –844CC and FAS –1377AA genotypes was associated with an even higher risk for esophageal squamous-cell carcinoma increase (OR = 4.55, 95% CI = 2.75 to 7.48) \( (P = .001; \text{test for homogeneity}) \) compared with those who lacked both genotypes. These results indicate that a more than multiplicative interaction exists between the FASL –844CC and FAS –1377AA genotype that is associated with the risk of developing esophageal squamous-cell carcinoma (26).

The risk of esophageal squamous-cell carcinoma associated with the FAS and FASL genotypes was further examined by stratifying for age, sex, smoking status, and smoking level (Table 4). The increased risk of esophageal squamous-cell carcinoma associated with the FAS –1377AA genotype compared with the GG or GA genotype was higher for the group aged 55 years or younger at diagnosis (adjusted OR = 2.32, 95% CI = 1.53 to 3.87) than for the group aged older than 55 years at diagnosis (adjusted OR = 1.51, 95% CI = 0.94 to 2.47). The increased risk of esophageal squamous-cell carcinoma associated with the FAS –670GG genotype compared with the AA or AG genotype was also higher for the group aged 55 years or younger (data not shown). However, the increased risk in younger subjects was not associated with the FASL –844T/C polymorphism. Because smoking is a risk factor for esophageal squamous-cell carcinoma and alters FAS and FASL expression (27–29), we investigated whether an interaction existed between the examined genetic polymorphisms and smoking status (Table 4). The FAS –1377AA genotype compared with the GG or GA genotype was not associated with the risk of esophageal squamous-cell carcinoma among nonsmokers (OR = 1.52, 95% CI = 0.86 to 2.69); however, this genotype was associated with an increased risk of esophageal squamous-cell carcinoma among smokers (OR = 1.89, 95% CI = 1.26 to 2.84). When smoking was then stratified by the number of pack-years, the FAS –1377AA genotype was associated with an increased risk among light smokers but not among heavy smokers. Similar results were also observed for the tightly linked FAS –670AA genotype (data not shown). In contrast, although an increased risk of esophageal squamous-cell carcinoma was associated with the FASL –844CC genotype in both smokers and nonsmokers, the risk was increased consistently with cumulative smoking dose among smokers (OR for \( \leq 17 \) pack-years = 1.60, 95% CI = 0.97 to 2.64; OR for 18–32 pack-years = 2.05, 95% CI = 1.33 to 3.15; OR for \( > 32 \) pack-years = 2.87, 95% CI = 1.60 to 5.14; \( P_{\text{trend}} = .184 \)) compared with that for the TT or TC genotype within the corresponding strata (Table 4). Thus, there appears to be an interaction between the FASL polymorphism and tobacco smoking that contributes to the risk for esophageal squamous-cell carcinoma.

Because FAS and FASL affect the differentiation, invasiveness, and metastasis of cancer cells (10,16,19), the association between FAS and FASL genotypes and these pathologic characteristics of esophageal squamous-cell carcinoma was also analyzed. Distributions of the FAS –1377GA, FAS –670AA, and FASL –844T/C genotypes were not statistically significantly different between the subgroup with lymph node metastases and

**Table 3.** Risk of esophageal squamous-cell carcinoma associated with FAS genotypes by FASL genotypes among 588 case patients and 648 control subjects

<table>
<thead>
<tr>
<th>Genotype(s)</th>
<th>FASL position –844</th>
<th>FASL position –1377</th>
<th>Case patients, No. (%)</th>
<th>Control subjects, No. (%)</th>
<th>OR* (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TT + TC</td>
<td>GG + GA</td>
<td>185 (31.5)</td>
<td>317 (48.9)</td>
<td>1.00 (referent)</td>
<td></td>
</tr>
<tr>
<td>TT + TC</td>
<td>AA</td>
<td>39 (6.6)</td>
<td>44 (6.8)</td>
<td>1.49 (0.93 to 2.39)</td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>GG + GA</td>
<td>299 (50.8)</td>
<td>262 (40.4)</td>
<td>1.97 (1.54 to 2.52)</td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>AA</td>
<td>65 (11.1)</td>
<td>25 (3.9)</td>
<td>4.55 (2.75 to 7.48)</td>
<td></td>
</tr>
</tbody>
</table>

*Data were calculated by unconditional logistic regression, adjusting for sex, age, and smoking status. OR = odds ratio; CI = confidence interval.

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the subgroup without lymph node metastases (OR = 1.12, 95% CI = 0.72 to 1.75 for the FAS –1377AA genotype compared with the GG or GA genotype; and OR = 0.87, 95% CI = 0.61 to 1.23 for the FASL –844CC genotype compared with the TT or TC genotype). No statistically significant difference with respect to the genotype distribution was observed among differentiation subgroups (data not shown).

**DISCUSSION**

This molecular epidemiologic study examined whether genetic polymorphisms in death pathway genes FAS and FASL, alone or in combination, were associated with risk of the development and metastasis of esophageal squamous-cell carcinoma. Our analysis of 588 case patients and 648 frequency-matched control subjects demonstrated that polymorphisms in the promoter region of the death receptor FAS and its ligand FASL were associated with an increased risk of the development, but not the metastasis, of esophageal squamous-cell carcinoma in a Chinese population. Subjects carrying the variant FAS –1377AA, FAS –670GG, or FASL –844CC genotype were at an increased risk for developing esophageal squamous-cell carcinoma compared with the FAS –1377GG or -GA, –670AA or -GA, or FASL –844TT or -TC genotype, respectively. In addition, we observed a more than multiplicative interaction between individual polymorphisms of FAS or FASL, and polymorphisms in both FAS and FASL are associated with an increased risk of esophageal squamous-cell carcinoma. Moreover, these data indicate an interaction between the polymorphisms and tobacco smoking. To the best of our knowledge, this is the first study to investigate whether the FASL polymorphism was associated with the risk of developing cancer and whether the associated FAS and FASL polymorphisms were associated with the risk of esophageal squamous-cell carcinoma.

Our results demonstrating an association between the variant FAS and FASL genotypes and the risk of developing esophageal squamous-cell carcinoma are biologically plausible. First, many studies have shown that increasing the expression of FASL and/or decreasing the expression of FAS is a common feature of malignant transformation and an early event associated with the development of most human cancers, including esophageal squamous-cell carcinoma (10,13,14,16–19). Second, the investigated polymorphisms in the FAS and FASL genes are functionally important. The FAS –1377G/A and –670A/G polymorphisms occur in the promoter region within the Sp1 and STAT1 transcription factor binding sites, respectively (20–22). Because Sp1 is an important transcriptional activator (30) and its ability to bind to the FAS –1377A allele is reduced, decreased expression of FAS in cells carrying the FAS –1377AA genotype was expected (20–22). The FASL –844TT/G polymorphism is also located in the promoter region of the gene, and basal FASL 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 (23). Given the role of FAS and FASL in the development of cancer, one might expect individuals who carry the FAS –1377AA and/or FASL –844CC genotype, and thus have decreased expression of FAS and/or increased expression of FASL over a lifetime, to be at a higher risk for developing cancer. Finally, an association has been reported between the FAS polymorphisms and risk of lymphoproliferative diseases and some cancers. For example, increased risks for acute myeloid leukemia (21), lung cancer (31), and cervical cancer (32) have been associated with FAS –1377G/A or FASL –670A/G polymorphism. Although no study has reported whether the FASL polymorphism is associated with the risk of cancer, the FASL –844CC genotype has been linked to systemic lupus erythematosus (23), an autoimmune disease characterized by accelerated FAS-mediated apoptosis of lymphocytes and monocytes. Thus, these data strongly support our molecular epidemiologic findings that functional polymorphisms influencing the expression of FAS and/or FASL are associated with an increased risk of cancer.

In this study, we also found a greater than multiplicative gene–gene interaction between the FAS and FASL polymorphisms. This statistical interaction suggests that these two polymorphisms increase the risk of esophageal squamous-cell carcinoma and are likely to be active in the same causal pathway (33). This speculation is biologically reasonable because FAS and FASL are a receptor–ligand system whose induction of apopto-

### Table 4. Risk of esophageal squamous-cell carcinoma associated with FAS and FASL genotypes by age, sex, and smoking status

<table>
<thead>
<tr>
<th>Category</th>
<th>FAS –1377 genotype</th>
<th>FASL –844 genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AA*</td>
<td>GG + GA*</td>
</tr>
<tr>
<td>Age, y</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤55</td>
<td>47/28</td>
<td>201/264</td>
</tr>
<tr>
<td>&gt;55</td>
<td>57/41</td>
<td>283/315</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>86/57</td>
<td>387/460</td>
</tr>
<tr>
<td>Female</td>
<td>18/12</td>
<td>97/119</td>
</tr>
<tr>
<td>Smoking status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonsmoker</td>
<td>32/24</td>
<td>167/187</td>
</tr>
<tr>
<td>Smoker</td>
<td>72/45</td>
<td>317/392</td>
</tr>
<tr>
<td>Smoking level, pack-years</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤17</td>
<td>27/10</td>
<td>101/139</td>
</tr>
<tr>
<td>18–32</td>
<td>33/22</td>
<td>134/160</td>
</tr>
<tr>
<td>&gt;32</td>
<td>12/13</td>
<td>82/93</td>
</tr>
</tbody>
</table>

*Number of case patients with genotype/number of control subjects with genotype.
†Data were calculated by unconditional logistic regression with the FAS –1377 GG + GA or FASL –844TT + TC genotype as reference group and adjusted for sex, age, smoking status, and pack-years within the strata. OR = odds ratio; CI = confidence interval.
‡Two-sided chi-square test.
sis requires both FAS and FASL (34). If a cell carries functional polymorphisms in both genes that affect their level of expression, then a greater than additive effect is to be expected. Transformed cells with the FASL–844CC genotype that express a high level of FASL may create an immuno-privileged site by killing cytotoxic immune cells and thus escape host immuno-surveillance; in contrast, decreased FAS expression resulting from a FAS promoter polymorphism may help the transformed cells evade FAS-mediated cell death. Consequently, the presence of both FAS and FASL polymorphisms would be associated with a greater increase of esophageal carcinogenesis than that associated with the presence of either FAS or FASL polymorphism alone.

Our results indicate that FAS and FASL polymorphisms interacted statistically with tobacco smoking. FAS polymorphisms modulated the risk of esophageal squamous-cell carcinoma among smokers but not among nonsmokers, suggesting a gene–environment interaction. Because smoking is a risk factor for esophageal squamous-cell carcinoma (27,35), such an interaction is not surprising. A higher risk of esophageal squamous-cell carcinoma associated with smoking and a variant FAS genotype (–1377AA or –670GG) may be attributed to many preinvasive or transformed esophageal cells resulting from exposure to tobacco carcinogens, which in turn increase the possibility that one of these cells will evade immuno-surveillance to become malignant because of low FAS expression. We found an increased risk associated with the FAS polymorphism in light and moderate smokers but not in heavy smokers (>32 pack-years in this study). This observation may reflect the fact that the genetic effect can be overwhelmed by the environmental effect. In contrast to FAS polymorphisms, the risk for esophageal squamous-cell carcinoma associated with the FASL polymorphism was statistically significantly increased in both smokers and nonsmokers, and the increased risk among smokers depended on the number of pack-years of smoking. A higher risk associated with the FASL polymorphism in nonsmokers than in smokers who smoked for ≤17 pack years might be attributed to the exposure of nonsmokers to high levels of secondhand smoke. This exposure is very possible because smoking is prevalent and is not restricted in public places in China. The explanation proposed for the interaction between FAS polymorphisms and smoking may also explain the interaction observed between the FASL polymorphism and smoking. Furthermore, because FASL expression can be induced by tobacco smoking (28,29), another hypothesis for the interaction is that, in addition to higher constitutive expression resulting from the FASL –844TC polymorphism, smoking may induce a higher level of expression from the FASL C allele than from the FASL T allele. Consequently, smoking and carrying the FASL –844CC genotype increase the risk of developing esophageal squamous-cell carcinoma.

In addition to tobacco smoke, other factors such as alcohol consumption, nutritional deficiency, and exposure to certain chemical carcinogens have been associated with risk of esophageal squamous-cell carcinoma in China [for a review, see (36)]. These factors could interact with FAS and/or FASL genotypes or act as potential confounders in our analysis. Unfortunately, our case–control study did not collect information on these factors. However, because ethanol and its metabolite acetaldehyde increase the expression of FASL (37,38), an association between the risk of esophageal squamous-cell carcinoma and the interaction between excess alcohol consumption and FAS and/or FASL should be investigated.

Although the loss of FAS expression and the gain of FASL expression has been associated with differentiation, invasiveness, and metastasis of most cancers, including esophageal squamous-cell carcinoma (10,16,19), we did not find a statistically significant association between the FAS and FASL polymorphisms and the histologic differentiation or lymph node metastasis of esophageal squamous-cell carcinoma. These results indicate that the FAS and FASL polymorphisms studied may not have an important role in metastatic disease. However, our data on metastasis are preliminary and are limited because they were obtained at the time of diagnosis and were essentially restricted to lymph node metastasis. Additional studies investigating more detailed clinicopathologic features and clinic outcomes, especially patient survival data, may be required to resolve this issue.

The allele and genotype frequencies of FAS and FASL vary with ethnicity. In this study with 648 healthy control subjects, we found that the FAS–1377A allele frequency was 0.34 and that AA homozygotes accounted for 10.6% of the Han Chinese population studied, compared with around 0.13 and 2%, respectively, among Caucasians from the United Kingdom, Australia, and Italy (21,39,40). In a Japanese study of 104 participants, however, the frequency of the –1377A allele and the percentage of participants with the AA genotype were 0.46 and 24%, respectively (22). A statistically significant difference was also observed for the FAS –670 polymorphism, with the GG genotype frequency being generally higher in Caucasians (21,40–42) than in Asians ([43,44] and this study). We also observed strong linkage disequilibrium between the FAS –1377 and –670 polymorphisms in our study population. Less strong linkage disequilibrium was also suggested in a Japanese population (22) but not in a Caucasian population (21). Distribution of FASL –844T/C polymorphism was reported to be different among African Americans and among American Caucasians, with –844C allele frequencies of 0.18 and 0.64, respectively (23), the latter being similar to that obtained in this study (i.e., 0.68). Ethnic variation in the FAS/FASL genotype distribution warrants additional comparative studies with more participants to confirm our results.

In conclusion, our study provides, to our knowledge, the first evidence that polymorphisms in the promoter region of FAS and FASL are associated with the risk of developing esophageal squamous-cell carcinoma in a Chinese population, although no association with the risk of metastasis was found. The association of FAS and FASL polymorphisms with the risk of esophageal squamous-cell carcinoma displayed a multiplicative gene–gene interaction and appeared to be influenced by tobacco smoking. These results may also support the hypothesis that the FAS- and FASL-triggered apoptosis pathway plays an important role in human carcinogenesis.

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NOTES
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