Functional Variants in Cell Death Pathway Genes and Risk of Pancreatic Cancer

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Abstract

Purpose: Fas-Fas ligand (FasL)–mediated death pathway is important in the life and death of immune cells and, therefore, influences immune surveillance of carcinogenesis. This study examined the association between functional variants of *Fas* (-1377G→A and -670A→G), *FasL* (-844T→C), and caspase-8 (*CASP8*) six-nucleotide deletion polymorphism (-652 6N ins→del) and risk of pancreatic cancer.

Experimental Design: Genotypes were determined in 397 cases with pancreatic cancer and 907 controls. Odds ratios (OR) and 95% confidence intervals (95% CI) were estimated by logistic regression, and all statistical tests were two sided.

Results: We found a significant decrease in risk of pancreatic cancer associated with *FasL* and *CASP8* but not *Fas* polymorphisms. Compared with noncarriers, the ORs of developing pancreatic cancer for *FasL* -844CT and TT carriers were 0.73 (95% CI, 0.57-0.94) and 0.35 (95% CI, 0.19-0.63), and for *CASP8* -652 6N ins/del and del/del carriers were 0.65 (95% CI, 0.50-0.85) and 0.56 (95% CI, 0.33-0.98), respectively. Gene-gene interaction between the *FasL* and *CASP8* variants further reduced the cancer risk in a multiplicative manner (OR for the presence of both *FasL* -844TT and *CASP8* -652 6N del/del genotype, 0.10; 95% CI, 0.01-0.75). On the other hand, a multiplicative joint effect between the *FasL* -844CC or *CASP8* -652 6N ins/ins genotype and smoking or diabetes mellitus in intensifying risk of pancreatic cancer was also evident.

Conclusions: These results suggest that genetic variations in the death pathway genes *FasL* and *CASP8* are involved in susceptibility to developing pancreatic cancer.

Antitumor T lymphocytes play a pivotal role in immunosurveillance of cancer cells. However, T lymphocytes can also be triggered into an apoptosis process known as activation-induced cell death after activation by tumor-associated or tumor-specific antigens (1). Accumulating evidence shows that activation-induced cell death is one of the important mechanisms responsible for the increased apoptosis rate among tumor-infiltrating lymphocytes; this process may lead to transformed cells escaping elimination by antitumor immunosurveillance and, therefore, contributes to carcinogenesis and cancer progression (2, 3). Activation-induced cell death of T lymphocytes is caused mainly by death receptor–induced apoptotic signaling (4, 5), which is initiated by Fas and Fas ligand (FasL) interaction and consequential activation of caspase-8 (CASP8), an apical caspase in caspase cascade (6, 7). Antigenic stimulation of cancer within the tumor microenvironment can induce tumor-infiltrating lymphocytes to overexpress FasL, and this may lead to suicide and/or fratricide of tumor-infiltrating lymphocytes via Fas-FasL interaction–triggered apoptosis (8). On the other hand, a wide variety of cancers acquire mutations in genes encoding Fas, FasL, and CASP8 (9–12) or reduced expression of Fas and CASP8 but enhanced expression of FasL (13–15). The aberration of this death pathway leads to cancer cells not only resisting the killing of anticancer T lymphocytes but also counterattacking T lymphocytes by expressing FasL. Thus, the Fas-FasL death pathway may play an important role in the development of cancer.

Genetic polymorphisms in the promoter regions of *Fas, FasL*, and *CASP8* have been associated with differential expression levels of these three genes. The *Fas* -1377G→A and -670A→G polymorphisms are located within the consensus sequences of binding sites for transcriptional factors stimulatory protein 1 (Sp1) and signal transducers and activators of transcription 1 (STAT1), respectively. These genetic polymorphisms have been shown to decrease *Fas* expression, probably due to the destruction of binding elements for the transcription factors (16, 17). The *FasL* -844T→C change is located in a binding

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motif for transcription factor CAAT/enhancer-binding protein β, and a considerably higher basal expression of *FasL* is associated with the C allele compared with the T allele (18). For the *CASP8* gene, a six-nucleotide deletion polymorphism (-652 6N del) has been identified in the promoter that abolishes an Sp1 binding site and is associated with decreased RNA expression in lymphocytes and lower CASP8 activity and activation-induced cell death of T lymphocytes (19). We and other investigators have shown that these polymorphisms in *Fas*, *FasL*, and *CASP8* are associated with susceptibility to certain cancers (19–28).

Pancreatic cancer is one of the leading causes of cancer-related death in the world (29, 30). Smoking, diabetes mellitus history, and, perhaps, alcohol drinking are risk factors for pancreatic carcinogenesis (31–34). However, only a part of exposed individuals develops pancreatic cancer in their life span, suggesting that genetic susceptibility factors also play a role in pancreatic carcinogenesis. It has been shown that pancreatic cancer cells often present nonfunctional Fas and aberrant expression of FasL, and this mechanism may contribute to the malignant and often rapid course of the disease (35–37). In view of the importance of Fas-FasL–mediated death pathway in pancreatic cancer, we hypothesized that functional polymorphisms in genes involved in this death pathway might confer susceptibility to the cancer. In this study, we did a case-control study in a Chinese population to evaluate the associations of the aforementioned *Fas*, *FasL*, and *CASP8* variants, alone and in combination, with risk of developing pancreatic cancer. We also analyzed gene-environment interaction between these genetic variants and smoking, drinking, or diabetes mellitus history.

Materials and Methods

Study subjects. This study consisted of 397 incident patients with pancreatic cancer and 907 controls. All subjects were Han Chinese. Patients were recruited between June 2001 and May 2007 at the Peking Union Hospital and Cancer Hospital, Chinese Academy of Medical Sciences, Beijing. All patients with confirmed pancreatic ductal adenocarcinoma were enrolled with a response rate of 94%. The detailed diagnosis of patients was described previously (38). Controls were cancer-free individuals selected from a community cancer-screening program for early detection of cancer conducted in the same regions during the same time period the patients were collected. These controls were randomly selected from a pool of 2,800 individuals on the basis of physical examination. The selection criteria included no individual history of cancer and frequency matching to cases by sex and age (±5 y). The characteristics of part of controls (n = 337) were described previously (38). In the present study, we selected 570 more controls from the same database matched to cases described above, for a total of 907 controls, to increase the statistical power. At recruitment, informed consent was obtained from each subject and each participant was then interviewed to collect detailed information on demographic characteristics, such as sex and age, and related risk factors, such as cigarette smoking, diabetes mellitus history, and alcohol drinking. This study was approved by the institutional review board.

Polymorphism genotyping. Genotypes of *Fas* -1377G→A, *Fas* -670A→G, *FasL* -844T→C, and *CASP8* -652 6N indel polymorphisms were determined by PCR-based RFLP assays as described previously (19, 20). All subjects were successfully genotyped. To ensure quality control, genotyping was done without knowledge of case/control status of the subjects, and a 15% random sample of cases and controls was genotyped twice by different persons; the reproducibility was 100%.

Statistical analysis. A χ^2 test was used to examine the differences in demographic variables, smoking status, drinking status, diabetes mellitus history, and genotype distributions of *Fas* -1377G→A, *Fas* -670A→G, *FasL* -844T→C, and *CASP8* -652 6N indel polymorphisms between patients and controls. Associations between genotypes and risk of developing pancreatic cancer were estimated by odds ratios (OR) and their 95% confident intervals (95% CI) computed using the logistic regression model. Smokers were considered current smokers if they smoked up to 1 y before the date of cancer diagnosis or if they smoked up to 1 y 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 <1 y 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 and heavy smokers were categorized by using the 50th percentile pack-year value of the controls as the cut points (i.e., ≤20 and >20 pack-years). Participants were classified as drinkers if they drank at least twice a week and continuously for at least 1 y during their lifetime; otherwise, they were defined as nondrinkers. All ORs were adjusted for age, sex, smoking, drinking, and diabetes mellitus history, where it was appropriate. We tested the null hypotheses of multiplicative gene-gene, gene-smoking, gene-drinking, and gene-diabetes mellitus history interactions and evaluated departures from multiplicative interaction models (39) by including main-effect variables and their product terms in the logistic regression model. All analyses were done with computer programs from Statistical Analysis System (version 6.12; SAS Institute).

Results

Subject characteristics. No statistically significant differences were found between patients and controls in terms of median age, sex distribution, and drinking status, suggesting that the frequency matching was adequate (Table 1). However, smokers were overrepresented in patients compared with controls (36.8% versus 26.4%; $P < 0.001$) although light or heavy smokers who smoked ≤20 or >20 pack-years were not significantly different ($P = 0.671$). Moreover, 16.9% of pancreatic cancer patients had diabetes mellitus that was significantly higher than that in controls (7.5%; $P < 0.001$).

Allelic frequencies and genotype distributions of Fas, FasL, and CASP8 variants. Allele frequencies and genotype distributions of *Fas*, *FasL*, and *CASP8* in patients and controls are shown in Table 2. The respective allele frequencies for *Fas* -1377A, *Fas* -670G, *FasL* -844C, and *CASP8* -652 6N del were 0.33, 0.38, 0.30, and 0.25 in controls and 0.32, 0.37, 0.22, and 0.19 in patients. All observed genotype frequencies in both controls and patients conform to Hardy-Weinberg equilibrium. Frequencies of *FasL* -844CC, CT, and TT genotypes among patients differed significantly from those among controls (χ^2 = 16.60; $P < 0.001$; df, 2), with the frequency of TT homozygote being significantly higher among controls than among patients (8.7% versus 3.8%; $P < 0.001$). Similarly, frequencies of the *CASP8* -652 6N ins/ins, ins/del, and del/del genotypes were significantly different among patients and controls (χ^2 = 12.07; $P = 0.002$; df, 2), with the frequency of the del/del genotype being higher in controls than in patients (7.0% versus 4.5%; $P < 0.001$). However, the distributions of *Fas* -1377 and *Fas* -670 genotypes were not significantly different between patients and controls (χ^2 = 0.75; $P = 0.688$ and χ^2 = 0.02; $P = 0.989$).

Table 1. Selected characteristics of patients with pancreatic cancer and controls

Variable	Patients (n = 397), n (%)	Controls (n = 907), n (%)	P*
Age (y)			0.681
≤50	96 (24.2)	198 (21.8)	
51-60	108 (27.2)	270 (29.8)	
61-70	121 (30.5)	267 (29.4)	
>70	72 (18.1)	172 (19.0)	
Sex			0.163
Male	256 (66.8)	562 (62.0)	
Female	132 (33.2)	346 (38.0)	
Smoking status			<0.001
Nonsmoker	251 (63.2)	668 (73.6)	
Smoker	146 (36.8)	239 (26.4)	
Pack-years smoked			0.671
≤20	78 (53.4)	133 (55.6)	
>20	68 (46.6)	106 (44.4)	
Alcohol drinking			0.541
No	277 (69.8)	648 (71.4)	
Yes	120 (30.2)	259 (28.6)	
Diabetes mellitus history			<0.001
No	330 (83.1)	839 (92.5)	
Yes	67 (16.9)	68 (7.5)	

*Two-sided χ^2 test.

Association between individual polymorphism and pancreatic cancer risk. Unconditional logistic regression analysis was used to estimate associations between genotypes of *FasL* and *CASP8*, and risk of pancreatic cancer (Table 2). The *FasL* -844T allele was shown to be protective allele; subjects having the -844TT or -844CT genotype had an OR of 0.35 (95% CI, 0.19-0.63) or 0.73 (95% CI, 0.57-0.94) for developing pancreatic cancer, respectively, compared with subjects having the -844CC genotype, suggesting that this genetic polymorphism acts in an allele dose–dependent manner (trend test; $P < 0.0001$). Similarly, the odds of having the *CASP8* -652 6N del/del or -652 6N ins/del genotype in patients was 0.56 (95% CI, 0.33-0.98) or 0.65 (95% CI, 0.50-0.85) compared with the -652 6N ins/ins genotype, suggesting that the effect of this polymorphism on protecting against pancreatic cancer is dominant (trend test; $P = 0.0007$). Adjustment for sex, age, smoking, alcohol drinking, and diabetes mellitus history did not significantly change the respective ORs.

Interaction between FasL and CASP8 polymorphisms. Because *FasL* or *CASP8* polymorphism alone was respectively associated with decreased risk of pancreatic cancer, we further investigated whether there was a statistical interaction between *FasL* and *CASP8* genotypes in reducing the risk (Table 3). We found that patients that carried the *FasL* -844TT genotype were also less likely to carry the *CASP8* -652 6N del/del genotype than controls (0.3% versus 1.9%; $P < 0.001$). The presence of the *FasL* -844TT genotype or *CASP8* -652 6N del/del genotype alone was associated with a decreased risk of pancreatic cancer (OR, 0.51; 95% CI, 0.25-1.05 or OR, 0.76; 95% CI, 0.34-1.68, respectively) compared with the absence of such a genotype. However, the presence of both *FasL* -844TT and *CASP8* -652 6N del/del genotypes was associated with an even lower risk of pancreatic cancer (OR, 0.10; 95% CI, 0.01-0.75; $P < 0.05$, test for homogeneity) compared with the lack of both genotypes. These results clearly indicate a supermultiplicative interaction

Table 2. *Fas, FasL*, and *CASP8* allelic and genotype frequencies among patients and controls and their association with pancreatic cancer risk

Genotype	Patients (n = 397), n (%)	Controls (n = 907), n (%)	OR* (95% CI)	P
Fas -1377G→A				
GG	186 (46.9)	420 (46.3)	1.00 (Reference)	
AG	169 (42.6)	376 (41.5)	1.06 (0.82-1.37)	0.642
AA	42 (10.5)	111 (12.2)	0.86 (0.58-1.28)	0.447
A allele frequency	0.32	0.33		
P_{trend} †			0.590	
Fas -670A→G				
AA	158 (39.8)	357 (39.4)	1.00 (Reference)	
AG	182 (45.8)	419 (46.2)	0.98 (0.75-1.27)	0.858
GG	57 (14.4)	131 (14.4)	0.99 (0.69-1.43)	0.971
G allele frequency	0.37	0.38		
P_{trend} †			0.890	
FasL -844C→T				
CC	238 (59.9)	451 (49.7)	1.00 (Reference)	
CT	144 (36.3)	377 (41.6)	0.73 (0.57-0.94)	0.015
TT	15 (3.8)	79 (8.7)	0.35 (0.19-0.63)	<0.001
T allele frequency	0.22	0.30		
P_{trend} †			<0.0001	
CASP8 -652 6N ins→del				
ins/ins	268 (67.5)	521 (57.4)	1.00 (Reference)	
ins/del	111 (28.0)	323 (35.6)	0.65 (0.50-0.85)	0.002
del/del	18 (4.5)	63 (7.0)	0.56 (0.33-0.98)	0.041
del allele frequency	0.19	0.25		
P_{trend} †			0.0007	

*Data were calculated by logistic regression, adjusted for age, sex, smoking, drinking, and diabetes mellitus history.
†Tests for trend of odds were two sided and based on likelihood ratio tests assuming a multiplicative model.

Table 3. Risk of pancreatic cancer associated with *FasL* genotypes by *CASP8* genotypes

Genotypes		Patients, *n* (%)	Controls, *n* (%)	OR* (95% CI)	*P*
***FasL* -844C→T**	***CASP8* -652 6N ins→del**				
CC	ins/ins	153 (38.5)	270 (29.7)	1.00 (Reference)	
CC	ins/del	74 (18.6)	163 (18.0)	0.77 (0.54-1.09)	0.133
CC	del/del	11 (2.8)	18 (2.0)	0.76 (0.34-1.68)	0.491
CT	ins/ins	104 (26.2)	213 (23.5)	0.87 (0.63-1.18)	0.375
CT	ins/del	34 (8.5)	136 (15.0)	0.46 (0.30-0.70)	0.001
CT	del/del	6 (1.5)	28 (3.1)	0.32 (0.13-0.82)	0.017
TT	ins/ins	11 (2.8)	38 (4.2)	0.51 (0.25-1.05)	0.066
TT	ins/del	3 (0.8)	24 (2.6)	0.17 (0.05-0.62)	0.007
TT	del/del	1 (0.3)	17 (1.9)	0.10 (0.01-0.75)	0.025

*Data were calculated by logistic regression, adjusted for sex, age, smoking, drinking, and diabetes mellitus history.

(39) between the *FasL* -844TT and *CASP8* -652 6N del/del genotypes in reducing pancreatic cancer risk.

Interaction between genetic polymorphisms and environmental risk factors. We examined whether there exist interactions between *FasL* or *CASP8* genotypes and smoking or drinking, two lifestyle factors known to be associated with pancreatic cancer risk (31, 32). As shown in Table 4, although the *FasL* -844CC genotype was associated with increased risk of pancreatic cancer among nonsmokers compared with the -844TT genotype, smokers having this genotype had highest risk, with the OR being 11.85 (95% CI, 4.44-31.57), which is 5-fold greater than the product of the OR for nonsmokers with the -844CC genotype and the OR for smokers with the -844TT genotype (i.e., 2.17 × 1.03 = 2.24). A similar result was also seen for the *FasL* -844CT genotype, with the joint OR of 3.92 (95% CI, 1.54-9.97) being 2-fold greater than the product of the OR for nonsmokers with the -844CT genotype and the OR for smokers with the -844TT genotype (i.e., 1.63 × 1.03 = 1.68). These results suggest a multiplicative joint effect between the *FasL* -844 polymorphism and smoking in intensifying pancreatic cancer risk. However, no significant interaction was observed between the *CASP8* -652 6N indel polymorphism and smoking although the higher ORs were seen among subjects who smoked and carried the -652 6N ins/ins or ins/del genotype (all $P > 0.05$ for homogeneity test). No significant effect of ethanol drinking on risk of the cancer related to the genetic polymorphisms was observed (data not shown).

Diabetes mellitus is another risk factor for pancreatic cancer (33, 34). Therefore, we also investigated whether it has an effect on the risk attributed to the *FasL* or *CASP8* polymorphism (Table 5). Multivariate logistic regression analysis showed that subjects who had diabetes mellitus history and carried the *FasL* -844CC genotype had an OR of 4.62 (95% CI, 2.80-7.64), which is 1.7-fold greater than the product of the OR for subjects without diabetes mellitus history but with the -844CC genotype and the OR for subjects with the -844TT or CT genotype but with diabetes mellitus history. Similar results were evident for the *CASP8* -652 polymorphism, where the highest risk was seen among subjects with both diabetes mellitus history and the ins/ins genotype (OR, 4.74; 95% CI, 2.83-7.94). These results show a multiplicative joint effect between *FasL* and *CASP8* variants and diabetes mellitus in susceptibility to the development of pancreatic cancer.

Discussion

In this study, we examined whether genetic polymorphisms in three death-receptor pathway genes, *Fas*, *FasL*, and *CASP8*, alone and in combination, are associated with risk of developing pancreatic cancer. On the basis of the analysis of 397 patients and 907 controls, we showed that the functional polymorphisms in the promoters of the *FasL* (-844T→C) and *CASP8* (-652 6N indel) genes have a significant effect on risk of the cancer. These genetic variants display the modifier effects

Table 4. Risk of pancreatic cancer associated with *FasL* or *CASP8* genotypes by smoking

Genotype	Nonsmokers		Smokers	
	Cases/controls	OR* (95% CI)	Cases/controls	OR* (95% CI)
FasL -844C→T				
TT	11/56	1.00 (Reference)	4/23	1.03 (0.21-4.92)
CT	91/279	1.63 (0.81-3.27)	53/98	3.92 (1.54-9.97) †
CC	149/333	2.17 (1.08-4.35)	89/118	11.85 (4.44-31.57) ‡
CASP8 -652 6N ins→del				
del/del	12/45	1.00 (Reference)	6/18	1.05 (0.27-4.12)
ins/del	68/249	1.00 (0.49-2.06)	43/74	4.33 (1.59-11.74)
ins/ins	171/374	1.69 (0.86-3.35)	97/147	5.04 (2.19-11.63)

*Data were calculated by logistic regression, adjusted for sex, age, drinking, and diabetes mellitus history.
†$P < 0.05$, test for homogeneity between smoking related ORs among the CT and TT genotypes.
‡$P < 0.01$, test for homogeneity between smoking-related ORs among the CC and TT genotypes.

Table 5. Risk of pancreatic cancer associated with *FASL* or *CASP8* genotypes by diabetes history

Genotypes	Without diabetes mellitus history		With diabetes mellitus history	
	Cases/controls	OR* (95% CI)	Cases/controls	OR* (95% CI)
FasL -844C→T				
TT + CT	137/420	1.00 (Reference)	22/36	1.93 (1.08-3.45)
CC	193/419	1.42 (1.10-1.84)	45/32	4.62 (2.80-7.64) †
CASP8 -652 6N ins→del				
del/del + ins/del	107/351	1.00 (Reference)	22/35	2.25 (1.25-4.06)
ins/ins	223/488	1.49 (1.14-1.95)	45/33	4.74 (2.83-7.94) †

*Data were calculated by logistic regression, adjusted for sex, age, smoking, and drinking.
†$P < 0.05$, test for homogeneity between diabetes mellitus history–related ORs among different *FasL* or *CASP8* genotypes.

on the risk not only by themselves but also by gene-gene or gene-environment interaction manner. To summarize, both *FasL* -844T and *CASP8* -652 6N del alleles are associated with significantly lower risk for developing pancreatic cancer compared with the *FasL* -844C and *CASP8* -652 6N ins alleles. In addition, the presence of both *FasL* -844TT and *CASP8* -652 6N del/del genotypes showed a multiplicative joint effect in attenuating susceptibility to pancreatic cancer. On the other hand, *FasL* -844CC and *CASP8* -652 6N ins/ins genotypes seem to have joint effects with smoking or diabetes mellitus history on intensifying pancreatic cancer risk.

The tested polymorphisms in the *FasL* and *CASP8* promoters were selected because they functionally affect transcription factor–binding sites and promoter activity. The *FasL* -844T→C affects a binding motif for transcription factor CAAT/enhancer–binding protein β, and a considerably higher basal expression of *FasL* is associated with the C allele compared with the T allele (18). The *CASP8* -652 6N ins→del polymorphism abolishes an Sp1 binding site and is associated with decreased RNA expression and lower CASP8 activity in T lymphocytes (19). The observations in the present study are, therefore, biologically plausible. Activation-induced cell death of T lymphocytes, which is mediated mainly by the death receptor pathway, may help malignant cells to escape from CTL killing and, therefore, may contribute to cancer development (1–5). We have previously established that the *FasL* -844C→T and *CASP8* -652 6N ins→del changes strongly reduce the expression of FasL and CASP, respectively, and consequentially reduce activation-induced cell death of tumor-specific T lymphocytes in response to malignant cells, which may be an underlying mechanism contributing to more powerful immune surveillance and attenuated susceptibility to the development of multiple types of common cancers among individuals carrying the *FasL* -844T and/or *CASP8* -652 6N del alleles (19, 22, 23). The present study has extended our findings to pancreatic cancer and further supports our hypothesis that functional polymorphisms in the death receptor pathway genes, which may influence an individual's immune status, modify susceptibility to cancer.

Our results in the present study show a multiplicative interaction between *FasL* -844T and *CASP8* -652 6N del variants in attenuating pancreatic cancer risk. It is well known that *CASP8*-induced caspase cascade resulting in apoptosis of T lymphocytes is triggered upon receipt of signaling from interaction between Fas and FasL (40). If individuals carry both *FasL* -844T and *CASP8* -652 6N del alleles that have reduced FasL and CASP8 expression, it would be expected that their T lymphocytes are much less subject to activation-induced cell death upon stimulation with tumor antigens compared with those carrying only *FasL* -844T allele or *CASP8* -652 6N del allele alone and, therefore, are at the lowest risk for developing pancreatic cancer. This finding is consistent with the proposed model that multiplicative interaction of two alleles often indicates that they act in the same causal pathway (39).

We also observed a greater than multiplicative gene-environment interaction between *FasL* -844C allele and smoking. Because smoking is an established risk factor for pancreatic cancer (29, 31, 32) and has a destructive effect on human immune responses (41–43), such an interaction is expected. It has been documented that chronic smoking enhances Fas and FasL expression in peripheral blood lymphocytes, which is believed to play a role in the immune impairment in smokers (44, 45). In addition to higher constitutive expression resulting from the *FasL* -844C allele, smoking may induce a higher level of FasL expression from the *FasL* -844C allele than from the *FasL* -844T allele. Consequently, smoking and carrying the *FasL* -844C allele may have lower immune response to malignant cells and higher risk for developing pancreatic cancer. Alternatively, a higher risk of pancreatic cancer associated with smoking and the *FasL* -844C allele may be attributed to many preinvasive or transformed pancreatic cells resulting from exposure to tobacco carcinogens, which, in turn, increase the possibility that one of these cells will evade tumor-infiltrating lymphocyte killing to become malignant because of high expression of FasL (36, 37). The gene-environment interaction between *FasL* -844C allele and smoking observed in the present study is consistent with our previous studies on esophageal cancer and lung cancer among Chinese populations (20, 21).

Another interesting finding in the present study is that we detected a significant interaction between the *FasL* -844C or *CASP8* -652 6N ins allele and diabetes mellitus history in intensifying risk of pancreatic cancer. Although diabetes mellitus is a risk factor for pancreatic cancer (32–34), the reason why there is interaction between *FasL* or *CASP8* polymorphism and diabetes mellitus in risk of pancreatic cancer is not immediately evident. However, it is well established that the death-receptor pathway also plays a vital role in the pathogenesis of diabetes (46–49). Interestingly, it has been shown in a study using bone marrow chimeras and adoptive transfer analysis that FasL expressed on hematopoietic and nonhematopoietic compartments plays nonredundant

roles in the pathogenesis of autoimmune diabetes. Mutation of *FasL* in either compartment interferes with the autoimmune process and prevents the onset of diabetes, but FasL expressed in the hematopoietic compartment is the dominant regulator of T-cell homeostasis (49). The results indicate that pathogenesis of diabetes is dependent on normal FasL expression, whereas only minimal FasL function is required to maintain T-cell homeostasis, which shares the same mechanism with activation-induced cell death of T cells. In addition, a significant association between type II diabetes and a microsatellite in the 3′untranslated region of *FasL* was reported (48). These findings suggest that genetic variants of *FasL* and *CASP*8 may be the common susceptibility factors for both pancreatic cancer and diabetes mellitus and, thus, diabetes mellitus may be a strong modifier for the risk of developing pancreatic cancer associated with the *FasL* -844C or *CASP8* -652 6N ins allele.

In summary, our study shows for the first time that functional *FasL* and *CASP8* polymorphisms are associated with risk of pancreatic cancer. The association displays a manner of multiplicative gene-gene interaction between *FasL* and *CASP8* polymorphisms and gene-environment interaction between these polymorphisms and smoking or diabetes mellitus history. These results are consistent with our initial findings in the previous studies, further supporting the hypothesis that naturally occurring variants in death pathway genes modify cancer susceptibility.

Disclosure of Potential Conflicts of Interest

The authors declare that they have no competing financial interests.

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