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Distinct role of the *Fas* rs1800682 and *FasL* rs763110 polymorphisms in determining the risk of breast cancer among Han Chinese females

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Meng Wang[1,*]
Zheng Wang[2,*]
Xi-Jing Wang[1]
Tian-Bo Jin[3]
Zhi-Ming Dai[4]
Hua-Feng Kang[1]
Hai-Tao Guan[1]
Xiao-Bin Ma[1]
Xing-Han Liu[1]
Zhi-Jun Dai[1]

[1]Department of Oncology, Second Affiliated Hospital of Xi'an Jiaotong University, [2]Department of Medical Oncology, Xi'an Central Hospital, [3]National Engineering Research Center for Miniaturized Detection Systems, School of Life Sciences, Northwest University, [4]Department of Anesthesia, Second Affiliated Hospital of Xi'an Jiaotong University, Xi'an, People's Republic of China

*These authors contributed equally to this work

Background: In recent years, studies have demonstrated that polymorphisms in the promoters of *Fas* and *FasL* are significantly associated with breast cancer risk. However, the results of these studies were inconsistent. This case–control study was performed to explore the associations between *Fas* rs1800682 and *FasL* rs763110 polymorphisms and breast cancer.

Materials and methods: A hospital-based case–control study of 560 Han Chinese females with breast cancer (583 controls) was conducted. The MassARRAY system was used to search for a possible association between the disease risk and the two single nucleotide polymorphisms, *Fas* rs1800682 and *FasL* rs763110. Statistical analyses were performed using SNPStats software to conduct Pearson's chi-square tests in five different genetic models. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated after adjustment to age and body mass index. PHASE v2.1 software was used to reconstruct all common haplotypes.

Results: A statistically significant association was found between *Fas* rs1800682 and increased breast cancer risk (AG vs AA: OR =1.37, 95% CI =1.06–1.78; AA+AG vs GG: OR =1.32, 95% CI =1.04–1.66), and also it was found that the *FasL* rs763110 polymorphism may decrease the risk. Stratified analyses demonstrated that the rs763110 polymorphism was associated with lower breast cancer risk among postmenopausal females (heterozygote model: OR =0.69, 95% CI =0.49–0.97; dominant model: OR =0.70, 95% CI =0.51–0.96). The T allele of rs763110 was also associated with a decreased risk of lymph node metastasis (allele model: OR =0.75, 95% CI =0.57–0.97) and an increased risk of the breast cancer being human epidermal growth factor receptor 2 positive (allele model: OR =1.37, 95% CI =1.03–1.18). Moreover, haplotype analysis showed that $A_{rs1800682}T_{rs763110}$ was associated to a statistically significant degree with lower risk of breast cancer (OR =0.70, 95% CI =0.53–0.91).

Conclusion: These data suggest that the presence of *Fas* rs1800683 is an important risk factor for breast cancer, whereas *FasL* rs763110 may exert a protective effect against the onset of breast cancer.

Keywords: *Fas*, *FasL*, single nucleotide polymorphism, breast cancer, risk

Introduction

Despite the existence of a comprehensive management strategy against breast cancer, both the incidence and the mortality rates of the disease are increasing. In the United States alone, 246,660 new breast cancer cases are expected in 2016, which account for 29% of all estimated new cancer diagnoses in females.[1] Although the morbidity rate of breast cancer is lower in People's Republic of China compared with that in developed countries, since the 1990s, the incidence rate has been rising more than twice as fast as the worldwide average, resulting in its becoming the most common cancer in

Correspondence: Zhi-Jun Dai
Department of Oncology, Second Affiliated Hospital of Xi'an Jiaotong University, 157 West 5th Road, Xi'an 710004, People's Republic of China
Email dzj0911@126.com

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Chinese females.[2] As is also the case for other malignancies, the development and progression of breast cancer comprise a complex process where genetic predisposition, environment, and lifestyle all play a role.[3] For the past few years, the study of single nucleotide polymorphisms (SNPs) has been a focus area of oncological research into genetic factors, with respect to susceptibility, diagnosis, treatment, and prognosis of malignant tumors. Recent genome-wide association and large-scale replication studies have identified common variants in more than 70 loci associated with breast cancer.[4]

Apoptosis, or programmed cell death, is a vital physiological process subjected to gene regulation. Aberrant regulation of apoptosis contributes to the development and progression, but also the treatment, of multiple diseases, including cancer.[5,6] Fas, a cell surface protein also known as TNFSF6/CD95/APO-1, and Fas Ligand (*FasL*), a type II membrane protein, belong to the tumor necrosis factor receptor superfamily[7,8] and play an important role in regulating apoptosis, as their crosslinking initiates the signal cascade of programmed cell death.[7] Therefore, mutation in the *Fas* and *FasL* genes may affect the apoptotic procedure and, thus, the development and progression of tumors. Several pharmacogenetic studies revealed the association of *Fas/FasL* SNPs with the response of cancer chemotherapy, showing mutation of *Fas/FasL* may be an indicator of cancer treatment.[9,10]

A large number of studies revealed that *Fas/FasL* gene polymorphisms are associated with susceptibility to various types of cancer, including cervical,[11–14] pharyngeal,[15–17] digestive,[18–22] and breast cancer.[23–28] Further studies demonstrated that circulating, soluble *Fas* (s*Fas*) can inhibit *Fas*-mediated apoptosis by neutralizing the *FasL* or the anti-*Fas* antibodies,[29] while increased levels of s*Fas* have been observed in serum from patients with breast cancer.[30] The most extensively investigated *Fas/FasL* polymorphisms are rs1800682 (−670A>G) in the promoter region of *Fas* and rs763110 (−844C>T) in the promoter region of *FasL*. However, the association of *Fas* and *FasL* gene polymorphisms with breast cancer has not been irrefutably established as different studies often produce conflicting results. For example, the study performed by Xu et al[28] showed that the *Fas* rs1800682 and *FasL* rs763110 polymorphisms may reduce the risk of breast cancer, whereas Hashemi et al[27] reported that the same SNPs were significantly associated with an increased risk of breast cancer (odds ratio, OR =3.18, *P*=0.019; OR =2.40, *P*=0.024, respectively), while Crew et al[23] found no significant association between these two genetic polymorphisms and breast cancer risk. Thus, this case–control study was conducted to explore the role of these two polymorphisms (*Fas* rs1800682 and *FasL* rs763110) in breast cancer risk, in a Chinese population.

Materials and methods

Study population

Patients who had breast cancer and were being treated at the Department of Oncology, the Second Affiliated Hospital, Xi'an Jiaotong University, were enrolled from January 2013 to October 2014. All the cases were verified using pathology and detailed immunohistochemical analysis, as described in our previous studies.[31–33] Patients with prior cancers or lacking a detailed personal and clinical background were excluded. Ultimately, 560 breast cancer cases were enrolled in this study; 583 healthy individuals who, during the same period, had gone for a checkup to the medical examination center of the same hospital, were included as controls. All the subjects in this study were Han Chinese females, and the controls were matched according to age (±5 years) and menopausal status.

Ethics statement

The study was approved by the Human Research Committee of Xi'an Jiaotong University. The demographic and personal information of patients and controls was collected using standard epidemiological questionnaires. Clinical information was collected from the patients' medical and pathological reports. All the participants were informed of the purpose and the experimental procedures of this study, and each subject signed a consent form.

Genotyping assay

Peripheral blood samples were collected in ethylenediaminetetraacetic acid (EDTA)-coated tubes and were preserved at −80°C.[31] Genomic DNA was extracted from whole blood samples using the Universal Genomic DNA Extraction Kit (version 3.0; TaKaRa Bio Inc., Kusatsu, Japan) according to the manufacturer's instructions. DNA concentration was determined using the DU530 UV/VIS spectrophotometer (Beckman Instruments, Fullerton, CA, USA).[32,33] Data from the HapMap database were used to create a list of potentially functional *Fas* and *FasL* SNPs discovered in Chinese subjects. Only SNPs with a minor allele frequency of >0.01 were considered, in order to ensure a statistical power of at least 50%. In the end, two SNPs were chosen to be included in this study, *Fas* rs1800682 and *FasL*. A multiplexed SNP MassEXTEND assay was designed using the MassARRAY Assay Design 3.0 Software (Agena Bioscience, San Diego, CA, USA). All the SNPs were genotyped by the Sequenom

Table 1 Primers used for this study

SNP_ID	First-PCRP	Second-PCRP	UEP-SEQ
rs1800682	ACGTTGGATGTTGTGGCTGCAACATGAGAG	ACGTTGGATGCTTTTCAGAGCCCTATGGCG	tGTTAACTGTCCATTCCAG
rs763110	ACGTTGGATGCTGGGCAAACAATGAAAATG	ACGTTGGATGAGGCTGCAAACCAGTGGAAC	tcaaAGAGCTGCTTTGTATTTC

Abbreviations: PCRP, polymerase chain reaction primer; SNP, single nucleotide polymorphism; UEP-SEQ, unextension primer sequence.

MassARRAY RS1000 system (Agena Bioscience), according to the manufacturer's instructions. The primers used for the two SNPs are listed in Table 1. Data were analyzed using the Typer 3.0 Software (Agena Bioscience).

Statistical analysis

We used Microsoft Excel for data management and SPSS software (version 21.0, IBM Corporation, Armonk, NY, USA) for statistical analysis. Hardy–Weinberg equilibrium was examined using Fisher's exact test. Two-tailed Pearson's chi-square tests were used to evaluate the differences in allelic frequencies for each SNP between patients and controls, with *P*-values <0.05 considered statistically significant. Five different genetic models were used to evaluate the association between SNPs and breast cancer risk ("A" and "a" are used to symbolize the major and the minor alleles, respectively): the allele model (a vs A); the codominant model (homozygote model: aa vs AA; heterozygote model: Aa vs AA); the recessive model (aa vs AA+Aa); the dominant model (AA vs Aa+aa); and the overdominant model (AA+aa vs Aa). SPSS software was used to estimate ORs and 95% confidence intervals (CIs) for each model. Stratification analysis was used to adjust for possible cofounders. Power and Sample Size (PS) Calculation software (http://biostat.mc.vanderbilt.edu/wiki/Main/PowerSampleSize) was used to calculate the power of the significant difference.[34] Haplotype analysis was performed using PHASE v 2.1 software, while ORs and 95% CIs for all the haplotypes were determined using SPSS.[35,36]

Results

Characteristics of patients and controls

As shown in Table 2, a total of 1,143 subjects (560 cancer cases and 583 healthy controls) were included in this study. Sporadic breast cancer patients were recruited from the Department of Oncology of The Second Affiliated Hospital of Xi'an Jiaotong University. Their average age was 49.09±11.02 years, and the diagnosis was confirmed by the examination of the pathology of surgical specimens. The controls we recruited were matched according to age and menopausal status. As a result, there was no significant difference between cases and controls with regard to general characteristics ($P>0.05$). However, patients and controls significantly differed in the body mass index (kg/m^2) value ($P=0.038$), which indicates that body mass index may be a confounding variable. Therefore, all the results were adjusted to body mass index.

Associations between *Fas*/*FasL* SNPs and the risk of breast cancer

The genotype frequency distribution of *Fas* rs1800682 and *FasL* rs763110 is shown in Table 3. The genotype distributions in the control group were all in Hardy–Weinberg equilibrium (*P*-values of 0.163 and 0.817, respectively). Further analysis showed that significant differences exist among rs1800682 and rs763110 alleles with respect to breast cancer risk. The *Fas*

Table 2 The characteristics of breast cancer cases and cancer-free controls

Characteristics	Cases	Controls	*P*-value
Number	560	583	
Age (years, mean ± SD)	49.09±11.02	48.80±8.28	0.612
<49	294	311	
≥49	266	272	
Menopausal status			
Premenopausal	264	281	
Postmenopausal	296	302	0.716
Number of pregnancies and live births			
<2	289	291	0.594
≥2	271	292	
Body mass index (kg/m^2) (mean ± SD)	22.52±2.84	22.95±3.21	**0.038**
Tumor size			
<2 cm	188		
≥2 cm	372		
LN metastasis			
Negative	236		
Positive	324		
ER			
Negative	247		
Positive	313		
PR			
Negative	255		
Positive	305		
HER2			
Negative	389		
Positive	171		
Ki67			
<14%	195		
≥14%	365		

Note: The bold value indicates that the difference was significant.
Abbreviations: ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; LN, lymph node; PR, progesterone receptor; SD, standard deviation.

Table 3 Genotype frequencies of *Fas*/*FasL* polymorphisms in cases and controls

Model	Genotype	Cases (n, %)	Controls (n, %)	*P*-value*	OR (95% CI)	Study power
***Fas* rs1800682**	**HWE: *P*=0.163**					
Codominant	AA	182 (32.5)	226 (38.8)	1.00		
Heterozygote	AG	289 (51.6)	261 (44.8)	**0.015**	**1.37 (1.06–1.78)**	**0.964**
Homozygote	GG	89 (15.9)	96 (16.4)	0.428	1.15 (0.81–1.63)	
Dominant	AA	226 (38.8)	182 (32.5)	1.00		
	AG+GG	357 (61.2)	378 (67.5)	**0.027**	**1.32 (1.03–1.68)**	**0.856**
Recessive	AA+AG	487 (83.6)	471 (84.1)	1.00		
	GG	96 (16.4)	89 (15.9)	0.792	0.96 (0.70–1.31)	
Overdominant	AA+GG	322 (55.2)	271 (48.4)	1.00		
	AG	261 (44.8)	289 (51.6)	**0.021**	**1.32 (1.04–1.66)**	**0.910**
Allele	A	713 (61.1)	653 (58.3)	1.00		
	G	453 (38.9)	467 (41.7)	0.165	1.13 (0.95–1.33)	
***FasL* rs763110**	**HWE: *P*=0.817**					
Codominant	CC	303 (54.1)	276 (47.3)	1.00		
Heterozygote	CT	219 (39.1)	252 (43.2)	0.060	0.79 (0.62–1.01)	
Homozygote	TT	38 (6.8)	55 (9.5)	**0.040**	**0.63 (0.40–0.98)**	**0.834**
Dominant	CC	303 (54.1)	276 (47.3)	1.00		
	CT+TT	257 (45.9)	307 (52.7)	**0.022**	**0.76 (0.60–0.96)**	**0.906**
Recessive	CC+CT	522 (93.2)	528 (90.5)	1.00		
	TT	38 (6.8)	55 (9.5)	0.102	0.70 (0.45–1.08)	
Overdominant	CC+TT	341 (60.9)	331 (56.8)	1.00		
	CT	219 (39.1)	252 (43.2)	0.157	0.84 (0.67–1.07)	
Allele	C	825 (73.7)	804 (69.0)	1.00		
	T	295 (26.3)	362 (31.0)	**0.013**	**0.79 (0.66–0.95)**	**0.719**

Notes: *Adjusted for age and body mass index. The bold values indicate that the differences were significant.
Abbreviations: CI, confidence interval; HWE, Hardy–Weinberg equilibrium; OR, odds ratio.

rs1800682 G allele associated with high risk of breast cancer in the heterozygote, dominant, and overdominant models (AG vs AA: OR =1.37, 95% CI =1.06–1.78, *P*=0.015; AG+GG vs AA: OR =1.32, 95% CI =1.03–1.68, *P*=0.027; AA+AG vs GG: OR =1.32, 95% CI =1.04–1.66, *P*=0.021), with power values of 0.964, 0.856, and 0.910, respectively. In contrast, the rs763110 polymorphism associated with reduced risk of breast cancer (homozygote model: OR =0.63, 95% CI =0.40–0.98, *P*=0.040; dominant model: OR =0.76, 95% CI =0.60–0.96, *P*=0.022; allele model: OR =0.79, 95% CI =0.66–0.95, *P*=0.013). Moreover, power calculations confirm that the sample size was large enough to discover the differences among cases and controls in rs763110 (power =0.834, 0.906, and 0.719, respectively).

Stratification analysis of the association of *Fas*/*FasL* SNPs with breast cancer risk by age and menopausal status

The subgroup analyses were conducted in order to reveal the influence of age and menopausal status on breast cancer risk. A stratified analysis by age revealed significant associations between the two SNPs and the risk of breast cancer in females >49 years of age in the allele model (rs1800682: OR =1.29, 95% CI =1.01–1.66, *P*=0.041; rs763110: OR =0.76, 95% CI =0.59–0.99, *P*=0.040) (Table 4). Subgroup analyses by menopausal status found no association between rs1800682 and breast cancer risk in any genetic model (all, *P*>0.05) (Table 5). However, it was discovered that the rs763110 polymorphism was associated with lower breast cancer risk among postmenopausal females (heterozygote model: OR =0.69, 95%CI =0.49–0.97, *P*=0.030; dominant model: OR =0.70, 95%CI =0.51–0.96, *P*=0.028).

Associations between *Fas*/*FasL* SNPs and the clinicopathological features of breast cancer

We also explored the association between the two SNPs and the clinicopathological features of breast cancer, namely tumor size, lymph node metastasis, and the expression of the estrogen receptor, the progesterone receptor, the human epidermal growth factor receptor 2 (HER2) and Ki67. No associations were found between rs1800682 and breast cancer risk with respect to any of the aforementioned clinicopathological features, as in all the cases *P*-values were >0.05 (Table 6). In contrast, it was found that the T allele of rs763110 was associated with a decrease in the incidence

Table 4 Association between *Fas*/*FasL* SNPs and age of breast cancer patients

Age (years)	(AA/Aa/aa)		Codominant		Dominant		Recessive		Allele	
	Case	Control	*P*-value	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value	OR (95% CI)
	rs1800682									
<49	81/166/47	101/156/54	0.128[m]	1.33 (0.92–1.91)[m]	0.187	1.27 (0.89–1.79)	0.650	0.91 (0.59–1.39)	0.534	1.08 (0.86–1.35)
			0.742[n]	1.09 (0.67–1.77)[n]						
≥49	101/113/52	125/105/42	0.132[m]	1.33 (0.92–1.94)[m]	0.061	1.39 (0.99–1.96)	0.210	1.33 (0.85–2.08)	**0.041**	**1.29 (1.01–1.66)**
			0.083[n]	1.53 (0.95–2.49)[n]						
	rs763110									
<49	169/106/19	161/124/26	0.233[m]	0.81 (0.58–1.14)[m]	0.158	0.79 (0.58–1.09)	0.374	0.76 (0.41–1.40)	0.134	0.82 (0.64–1.06)
			0.258[n]	0.70 (0.37–1.31)[n]						
≥49	134/113/19	115/128/29	0.125[m]	0.76 (0.53–1.08)[m]	0.060	0.72 (0.51–1.04)	0.152	0.65 (0.35–1.18)	**0.040**	**0.76 (0.59–0.99)**
			0.071[n]	0.56 (0.30–1.06)[n]						

Notes: A represents major allele; a represents minor allele; [m] represents heterozygote model; [n] represents homozygote model. The bold values indicate that the differences were significant.
Abbreviations: CI, confidence interval; OR, odds ratio; SNP, single nucleotide polymorphism.

of lymph node metastasis among breast cancer patients (allele model: OR =0.75, 95% CI =0.57–0.97, *P*=0.031), and higher incidence of the HER2-positive type of breast cancer among patients (allele model: OR =1.37, 95% CI =1.03–1.18, *P*=0.028) (Table 6).

Association between *Fas*/*FasL* haplotypes and breast cancer risk

Haplotype analysis was conducted to evaluate the interaction of these two genes. As shown in Table 7, it was found that the haplotype $A_{rs1800682}T_{rs763110}$ was significantly associated with a lower risk of breast cancer (OR =0.70, 95% CI =0.53–0.91, *P*=0.009). However, no relation was found between the other two haplotypes ($G_{rs1800682}C_{rs763110}$ and $G_{rs1800682}T_{rs763110}$) and breast cancer risk.

Discussion

Apoptosis plays an important role in the genesis and development of tumors. The *Fas/FasL* genes are key effectors in the regulation of apoptotic cell death, and malfunction of this system has been proved to be important in cancer cell immune evasion and tumorigenesis.[37] Decreased expression of *Fas* promotes malignant transformation and progression, while lower levels of *FasL* expression have the opposite effect.[38–40] Moreover, SNPs of the *Fas/FasL* genes were reported to be associated with many types of cancer susceptibility. However, the conclusions of these studies were inconsistent and the detailed subgroup analyses were limited. This case–control study including 560 breast cancer cases and 583 controls in Han Chinese females was performed in the hope of providing further evidence regarding the association between *Fas/FasL* SNPs and cancer risk.

The *Fas* rs1800682 polymorphism is located in a STAT1 binding element on the *Fas* promoter, which has been linked to the downregulation of Fas expression.[22] The *FasL* gene rs763110 polymorphism lies within a putative binding motif for the CAAT/enhancer-binding protein β-transcription factor.[41] Our results suggest that the *Fas* rs1800682 SNP

Table 5 Association between *Fas*/*FasL* SNPs and menopausal status of breast cancer patients

Menopausal status	(AA/Aa/aa)		Codominant		Dominant		Recessive		Allele	
	Case	Control	*P*-value	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value	OR (95% CI)
	rs1800682									
Premenopausal	87/128/49	114/121/46	0.086[m]	1.39 (0.9–2.01)[m]	0.066	1.39	0.501	1.16	0.099	1.23
			0.181[n]	1.40 (0.86–2.28)[n]		(0.98–1.97)		(0.75–1.81)		(0.96–1.56)
Postmenopausal	95/161/40	112/140/50	0.093[m]	1.36 (0.95–1.93)[m]	0.200	1.25	0.298	0.79	0.731	1.04
			0.818[n]	0.94 (0.57–1.55)[n]		(0.89–1.75)		(0.50–1.24)		(0.83–1.31)
	rs763110									
Premenopausal	147/99/18	144/105/32	0.664[m]	0.92 (0.65–1.32)[m]	0.299	0.84	0.065	0.57	0.098	0.80
			0.058[n]	0.55 (0.30–1.03)[n]		(0.60–1.17)		(0.31–1.04)		(0.61–1.04)
Postmenopausal	156/120/20	132/147/23	**0.030**[m]	**0.69 (0.49–0.97)**[m]	**0.028**	**0.70**	0.684	0.88	0.062	0.79
			0.348[n]	0.74 (0.39–1.40)[n]		**(0.51–0.96)**		(0.47–1.64)		(0.62–1.01)

Notes: A represents major allele; a represents minor allele; [m] represents heterozygote model; [n] represents homozygote model. The bold values indicate that the differences were significant.
Abbreviations: CI, confidence interval; OR, odds ratio; SNP, single nucleotide polymorphism.

Table 6 Association between the *Fas*/*FasL* SNPs and clinical characteristics of breast cancer patients

Variables	AA	Aa	aa	Codominant		Dominant		Recessive		Allele	
				P-value	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value	OR (95% CI)
rs1800682											
Tumor size											
<2 cm	67	96	25	1.00 (reference)							
≥2 cm	115	193	64	0.425[m]	1.17 (0.79–1.73)[m]	0.218	0.26 (0.87–1.83)	0.232	1.36 (0.82–2.23)	0.167	1.20 (0.93–1.54)
				0.154[n]	1.49 (0.86–2.59)[n]						
LN metastasis											
Negative	84	121	31	1.00 (reference)							
Positive	98	168	58	0.361[m]	1.19 (0.82–1.73)[m]	0.182	1.27 (0.89–1.82)	0.128	1.44 (0.90–2.31)	0.090	1.23 (0.97–1.57)
				0.077[n]	1.60 (0.95–2.71)[n]						
ER											
Negative	90	119	38	1.00 (reference)							
Positive	92	170	51	0.078[m]	1.40 (0.96–2.03)[m]	0.077	1.38 (0.97–1.97)	0.770	1.07 (0.68–1.69)	0.180	1.18 (0.93–1.50)
				0.296[n]	1.31 (0.79–2.19)[n]						
PR											
Negative	89	122	44	1.00 (reference)							
Positive	93	167	45	0.155[m]	1.31 (0.90–1.90)[m]	0.267	1.22 (0.86–1.74)	0.420	0.83 (0.53–1.31)	0.747	1.04 (0.82–1.32)
				0.934[n]	0.98 (0.59–1.63)[n]						
HER2											
Negative	134	193	62	1.00 (reference)							
Positive	48	96	27	0.116[m]	1.39 (0.92–2.09)[m]	0.138	1.35 (0.91–2.00)	0.965	0.99 (0.60–1.62)	0.330	1.14 (0.88–1.47)
				0.493[n]	1.22 (0.70–2.13)[n]						
KI67											
<14%	61	108	26	1.00 (reference)							
≥14%	121	181	63	0.396[m]	0.85 (0.57–1.25)[m]	0.653	0.92 (0.63–1.33)	0.226	1.36 (0.83–2.22)	0.739	1.04 (0.81–1.34)
				0.476[n]	1.22 (0.70–2.12)[n]						
rs763110											
Tumor size											
<2 cm	98	79	11	1.00 (reference)							
≥2 cm	205	140	27	0.374[m]	0.85 (0.59–1.22)[m]	0.504	0.89 (0.62–1.26)	0.532	1.26 (0.61–2.60)	0.778	0.96 (0.73–1.27)
				0.672[n]	1.17 (0.56–2.46)[n]						
LN metastasis											
Negative	117	98	21	1.00 (reference)							
Positive	186	121	17	0.160[m]	0.78 (0.55–1.11)[m]	0.066	0.73 (0.52–1.02)	0.090	0.57 (0.29–1.10)	**0.031**	**0.75 (0.57–0.97)**
				0.049[n]	0.51 (0.26–1.01)[n]						
ER											
Negative	121	109	17	1.00 (reference)							
Positive	182	200	21	0.234[m]	1.22 (0.88–1.69)[m]	0.342	1.17 (0.85–1.60)	0.378	0.74 (0.38–1.44)	0.680	1.05 (0.82–1.35)
				0.570[n]	0.82 (0.42–1.62)[n]						
PR											
Negative	144	88	23	1.00 (reference)							
Positive	159	131	15	0.096[m]	1.35 (0.95–1.92)[m]	0.305	1.19 (0.85–1.66)	0.055	0.52 (0.27–1.02)	0.964	1.01 (0.77–1.31)
				0.131[n]	0.59 (0.30–1.18)[n]						
HER2											
Negative	221	146	22	1.00 (reference)							
Positive	82	73	16	0.122[m]	1.35 (0.92–1.97)[m]	0.053	1.43 (1.00–2.05)	0.109	1.72 (0.88–3.37)	**0.028**	**1.37 (1.03–1.18)**
				0.053[n]	1.43 (1.00–2.05)[n]						
KI67											
<14%	103	74	18	1.00 (reference)							
≥14%	200	145	20	0.961[m]	1.01 (0.70–1.46)[m]	0.655	0.92 (0.65–1.31)	0.975	1.01 (0.72–1.40)	0.300	0.86 (0.66–1.14)
				0.104[n]	0.57 (0.29–1.13)[n]						

Notes: A represents major allele; a represents minor allele; [m] represents heterozygote model; [n] represents homozygote model. The bold values indicate that the differences were significant.

Abbreviations: CI, confidence interval; ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; LN, lymph node; OR, odds ratio; PR, progesterone receptor; SNP, single nucleotide polymorphism.

Table 7 The haplotype frequencies of *Fas*/*FasL* SNPs and breast cancer risk

Haplotypes		Cases (N=1,120) n, %	Controls (N=1,166) n, %	OR (95% CI)	*P*-value
rs1800682	rs763110				
A	C	544 (48.57)	556 (47.68)	1.00 (reference)	
A	T	109 (9.73)	157 (13.46)	**0.70 (0.53–0.91)**	**0.009**
G	C	281 (25.09)	248 (21.27)	1.14 (0.92–1.40)	0.224
G	T	186 (16.61)	205 (17.58)	0.91 (0.72–1.15)	0.426

Note: The bold value indicates that the difference was significant.
Abbreviations: CI, confidence interval; OR, odds ratio; SNP, single nucleotide polymorphism.

may be associated with an increased risk of breast cancer, while the *FasL* rs763110 polymorphism may decrease breast cancer risk. Furthermore, the associations between these two SNPs and age, menopausal status, and different clinical characteristics of breast cancer were evaluated. Statistically significant associations were found between the two SNPs and the risk of breast cancer in the allele model among patients >49 years of age. Moreover, the rs763110 polymorphism was more likely to decrease breast cancer risk among postmenopausal females. In 2012, a study of the Tunisian population including 438 breast cancer cases and 332 controls found that rs763110 had a marginally significant association with lymph node-negative status (OR =0.53, *P*=0.06; OR =0.73, *P*=0.07, respectively).[25] However, it is demonstrated that the C allele of the rs763110 SNP may be associated with a lower risk of lymph node metastasis and a higher occurrence of HER2-positive breast cancers. The hormonal status is important to breast cancer patients, guiding treatment and prognosis. It has been demonstrated that upregulation of *Fas* and *FasL* in breast cancer cells induces T-cell apoptosis in Fas bearing T cells.[42] However, we did not find any associations between *Fas/FasL* SNPs and hormone-related factors, which is in agreement with the data presented by Crew et al.[23] Haplotypes are considered more meaningful than any SNP analysis during the genetic study. Xu et al[28] reported that the $G_{rs2234767}A_{rs1800682}$ haplotype was associated with an increased breast cancer risk as compared with other haplotypes, whereas the $A_{rs2234767}A_{rs1800682}$ haplotype was associated with a reduced breast cancer risk. However, they did not evaluate the interaction of the *Fas* and *FasL* genes. The present study demonstrated that $A_{rs1800682}T_{rs763110}$ was significantly associated with low risk of breast cancer in comparison with $A_{rs1800682}C_{rs763110}$. Compared with the previous reports of breast cancer, this study offered more detailed information on these two SNPs and clinical characteristics of breast cancer, which may provide insights into the associations between *Fas/FasL* polymorphisms and the occurrence and development of breast cancer. Functional studies revealed that rs2234767 and rs1800682 polymorphisms were able to alter the SP1 and STAT1 binding site, leading to the abnormal expression of *Fas*,[43] and C allele of rs763110 could increase basal *FasL* expression, affecting the *FasL*-mediated signaling.[41] It was found that rs1800682 SNP had a high risk of breast cancer, and the mutation of rs763110 C allele may decrease the breast cancer risk. Thus, it was supposed that G allele of rs1800682 may downregulate *Fas* transcription via SP1/STAT1 way and the mutation of rs763110 may decrease the *FasL* expression by regulating *FasL*-mediated apoptotic signaling. However, the mechanisms still need more functional studies to testify.

Limitations

The study had certain limitations: First, all the subjects were recruited from the same hospital, and therefore, selection bias is inevitable. Second, the sample size was not large enough to support the stratified analyses. Third, the effects of other important risk factors, such as polycyclic aromatic hydrocarbons, exposure of the chest area to high-dose radiation, and alcohol consumption, as well as benign breast lesions or environmental exposures, were not analyzed because of lack of relative data. Fourth, most patients included in this study were invasive ductal carcinoma cases; hence, stratification analyses were not performed for histopathological types of breast cancers. Fifth, only the differences of gene distribution in the subjects were analyzed. Functional studies are needed to reveal the molecular mechanism. Thus, a study with more patients and controls, containing both gene distribution and functional data, would allow us to both confirm and mechanistically interpret the results presented in the current study.

Conclusion

In conclusion, this case–control study indicates that the *Fas* rs1800683 SNP is associated with a statistically significant degree of increased breast cancer risk, especially in females >49 years of age. Conversely, the *FasL* rs763110

polymorphism was shown to be associated with decreased risk of breast cancer, especially in females >49 years of age and after menopause. Moreover, stratified analyses demonstrated that the *FasL* rs763110 SNP was related to reducing the incidence of lymph node metastasis and increased chance for the breast cancer to be HER2-positive. Furthermore, the $A_{rs1800682}T_{rs763110}$ haplotype may play a protective role against breast cancer.

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Disclosure

The authors report no conflicts of interest in this work.

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