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ORIGINAL RESEARCH

Association of two *FOXP3* polymorphisms with breast cancer susceptibility in Chinese Han women

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Background: Forkhead box P3 (*FOXP3*) is a key gene in the immune system which also plays a role in tumor development. This study aims to explore the association of two *FOXP3* polymorphisms (rs3761548 and rs3761549) with susceptibility to breast cancer (BC).

Method: A case–control study was conducted, involving 560 patients and 583 healthy individuals from the Chinese Han population. The genotypes of *FOXP3* polymorphisms were detected using the Sequenom MassARRAY method. The association between *FOXP3* polymorphisms and BC risk was evaluated using a χ^2 test with an odds ratio (OR) and 95% confidence intervals (95% CIs) under six genetic models. False-positive report probability was utilized to examine whether the significant findings were noteworthy.

Results: We observed that rs3761548 was associated with a higher BC risk in heterozygous, dominant, overdominant, and allele genetic models (CA vs CC: OR =1.32, *P*=0.031; CA/AA vs CC: OR =1.32, *P*=0.023; CA vs CC/AA: OR =1.29, *P*=0.042; A vs C: OR =1.26, *P*=0.029), whereas no significant association was found between rs3761549 and BC risk. In addition, CA, CA/AA genotype, and A allele of rs3761548 were related to larger tumor size, and the A allele was also correlated with a positive status of Her-2 in BC patients.

Conclusion: Our study suggests that *FOXP3* polymorphism rs3761548 is associated with BC susceptibility in the Chinese and may be involved in tumor progression. Future studies are needed to confirm the results in a larger population with more races.

Keywords: forkhead box P3, polymorphism, breast cancer, risk

Introduction

Breast cancer (BC) is the most common type of cancer and the leading cause of cancer death among females globally.[1] Hereditary factors are considered to play a key role in the genesis of breast cancer. Among all breast carcinomas, about 5%–10% are inherited, arising from genetic variants in susceptible genes.[2,3] Single-nucleotide polymorphisms (SNPs) are the most frequent variation that occur in a single nucleotide at a specific position in the genome. Numerous SNPs have been identified through sequencing, and many of them in critical genes such as *BRCA1/2*, *TP53*, *TNF*, and *VEGF* were demonstrated to be associated with cancer susceptibility.[4–6]

FOXP3 is a protein-coding gene located at human chromosome Xp11.23. Its product, forkhead box P3 (FoxP3), also known as scurfin, is specifically expressed in $CD4^+CD25^+$ regulatory T cells (Tregs) and functions mainly as a key regulator for the development and function of Tregs.[7,8] As a member of the FOX family, FoxP3 also regulates transcription and DNA repair and is involved in cell growth and differentiation

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as well as embryogenesis.[9,10] In addition to its critical function in immune response, *FOXP3* plays an important role in cancer development, although it is still a controversy whether it is an oncogene or tumor suppressor gene.[11,12]

Nevertheless, several *FOXP3* SNPs have been reported to be associated with susceptibility to multiple cancers including lung cancer, hepatocellular carcinoma (HCC), and colorectal cancer.[13–15] Among these SNPs, rs3761548 and rs3761549 were the most common polymorphisms. Studies revealed that A allele and AA/AC genotype of rs3761548 significantly increased the risk of thyroid cancer, colorectal cancer, and non-small-cell lung cancer.[13,15,16] T allele of rs3761549 was found to be associated with susceptibility to lung cancer, while C allele was related with higher risk of HCC. In addition, TT/CT genotype was linked to an increased incidence of tumor recurrence in HCC.[17,18] Here, we conducted a case–control study to explore the association of the two polymorphisms with BC risk in a Chinese Han population.

Methods

Study subjects

Cases were selected from female BC patients treated at the Department of Oncology, the Second Affiliated Hospital of Xi'an Jiaotong University (Xi'an, People's Republic of China). Healthy women who had a checkup at the hospital during the same period of time were recruited as controls. The criteria for enrollment were the same as our previous study.[19] Cases were confirmed by pathology, and controls were matched according to age and menopausal status. None of the cases had received chemotherapy or radiotherapy before surgery. Patients with other types of cancer were excluded. Finally, 560 patients and 583 healthy individuals were enrolled in our study. All of the subjects were from the Chinese Han population, had a mean age of 49 years, and were not related to each other.

Ethics statement

This study was approved by the Ethics Committee of Xi'an Jiaotong University. The purpose of this study was well informed to the subjects and written informed consent was obtained from each of them. Then, personal and clinical information of the subjects was collected from their medical records.

Genotyping assay

Peripheral blood of each subject was collected in tubes containing EDTA and stored at −80°C. Then, DNA was extracted from whole blood using the Universal Genomic DNA Extraction Kit (TaKaRa, Kyoto, Japan) according to the manufacturer's protocol, and the quantity of extracted DNA was assessed utilizing the UV/Vis spectrophotometer (DU530, Beckman Instruments, Brea, CA, USA). Two tag-SNPs (rs3761548 and rs3761549) were selected from the HapMap database, with minor allele frequency (MAF) >0.01 in Chinese Han population. Sequenom MassARRAY Assay Design Software (version 3.0, Agena Bioscience, San Diego, CA, USA) was used to design multiplexed SNP MassEXTEND assay. And SequenomMassARRAY RS1000 was used to detect SNP genotyping.[20] Primers of the two SNPs are listed in Table 1. Data were analyzed with Sequenom Typer Software (version 4.0, Sequenom, San Diego, CA, USA).

Statistical analysis

SPSS software (version 22.0, IBM Corporation, Armonk, NY, USA) was used for statistical analyses of data. The differences of basic parameters between the two groups were examined with Student *t*-test or χ^2 test. For each SNP, Hardy–Weinberg equilibrium as well as the differences in allele frequencies between patients and healthy controls were examined by χ^2 test. The association of SNPs with BC risk was evaluated by odds ratios (ORs) with 95% confidence intervals (CIs) for five different genetic models ("A" indicates the major allele and "a" indicates the minor allele): codominant model including homozygote (aa vs AA) and heterozygote (Aa vs AA), dominant model (Aa/aa vs AA), recessive model (aa vs AA/Aa), overdominant model (Aa vs AA/aa), and allele model (a vs A). *P*-values were adjusted by logistic regression analysis. All the tests were two-tailed, and *P*-value <0.05 was considered statistically significant. We further calculated false-positive report probability (FPRP) to examine whether the significant findings were noteworthy. The prior probability of 0.1 was set in our study, and 0.5 was determined as a cut-off value for FPRP.[21,22]

Table 1 Primers used in this study

SNP_ID	1st-PCRP	2nd-PCRP	UEP_SEQ
rs3761548	ACGTTGGATGTGGGTGCTGAGGGGTAAACT	ACGTTGGATGAAGCCTAG ATCTCAGGACTC	GCTCTCTCCCCAACTG
rs3761549	ACGTTGGATGACATCACCTACCACATCCAC	ACGTTGGATGACCCCACA GGTTTCGTTCC	TTTCGTTCCGAGAACT

Abbreviations: SNP, single-nucleotide polymorphism; PCRP, primer for polymerase chain reaction; UEP_SEQ, primer for single nucleotide extension.

Results

The basic characteristics of study subjects

No significant difference was observed between the two groups in regards to age, menopausal status, and procreative times. However, the mean value of body mass index (BMI) in the patient group was significantly lower compared with that in control group (P=0.038). As obesity is also associated with BC risk,[23] BMI may be a confounding factor for the analysis. Therefore, the results were adjusted for BMI. The basic parameters of cases and controls are presented in Table 2.

Table 2 The comparison of basic characteristics between cases and controls

Characteristics	Cases (560)	Controls (583)	*P*-value
Age (years, mean ± SD)	49.09±11.02	48.80±8.28	0.612
<49	294	311	
≥49	266	272	
Menopausal status			0.716
Premenopausal	264	281	
Postmenopausal	296	302	
Procreative times			0.594
<2	289	291	
≥2	271	292	
BMI (kg/m^2, mean ± SD)	22.52±2.84	22.95±3.21	**0.038**

Note: *P*-value <0.05 was shown in bold
Abbreviation: BMI, body mass index.

Association of *FOXP3* SNPs with BC susceptibility

For both of the *FOXP3* polymorphisms, the genotype distribution in controls complied with Hardy–Weinberg equilibrium (P=0.895 and 0.934 respectively). In the overall analysis, *FOXP3*-rs3761548 was associated with a higher BC risk in heterozygous, dominant, overdominant, and allele genetic models (CA vs CC: OR =1.32, 95% CI =1.03–1.69, P=0.031; CA/AA vs CC: OR =1.32, 95% CI =1.04–1.69, P=0.023; CA vs CC/AA: OR =1.29, 95% CI =1.01–1.66, P=0.042; A vs C: OR =1.26, 95% CI =1.02–1.54, P=0.029). FPRP analysis showed that the associations were noteworthy. However, *FOXP3*-rs3761549 was not related to BC susceptibility (Table 3). We then carried out stratified analysis based on age and menopausal status in five genetic models. However, no relationship was observed between the two SNPs and BC susceptibility in either of the subgroups (Table S1).

Relationship between *FOXP3* SNPs and clinical features of BC

We also explored the relationship between the two *FOXP3* SNPs and BC clinical features, including tumor size, metastasis of lymph node, status of hormone receptor (estrogen receptor and progesterone receptor) as well as Her-2, and

Table 3 Genotype frequencies of *FOXP3* polymorphism in cases and controls

Model	Genotype	Cases (n, %)	Controls (n, %)	OR (95% CI)	*P*[a]	FPRP
rs3761548 (P_{HWE}=0.895)		559	581			
Codominant	C/C	337 (60.3%)	388 (66.8%)	1.00		
Heterozygote	C/A	198 (35.4%)	173 (29.8%)	**1.32 (1.03–1.69)**	**0.031**	0.228
Homozygote	A/A	24 (4.3%)	20 (3.4%)	1.38 (0.75–2.55)	0.298	
Dominant	C/C	337 (60.3%)	388 (66.8%)	1.00		
	C/A+A/A	222 (39.7%)	193 (33.2%)	**1.32 (1.04-1.69)**	**0.023**	0.228
Recessive	C/C+C/A	535 (95.7%)	561 (96.6%)	1.00		
	A/A	24 (4.3%)	20 (3.4%)	1.26 (0.69–2.31)	0.456	
Overdominant	C/C+A/A	361 (64.6%)	408 (70.2%)	1.00		
	C/A	198 (35.4%)	173 (29.8%)	**1.29 (1.01–1.66)**	**0.042**	0.328
Allele	C	872 (78.0%)	949 (81.7%)	1.00		
	A	246 (22.0%)	213 (18.3%)	**1.26 (1.02–1.54)**	**0.029**	0.184
rs3761549 (P_{HWE}=0.934)		560	582			
Codominant	C/C	385 (68.8%)	372 (63.9%)	1.00		
Heterozygote	T/C	157 (28%)	187 (32.1%)	0.81 (0.63–1.05)	0.108	
Homozygote	T/T	18 (3.2%)	23 (4.0%)	0.76 (0.40–1.42)	0.386	
Dominant	C/C	385 (68.8%)	372 (63.9%)	1.00		
	T/C-T/T	175 (31.2%)	210 (36.1%)	0.81 (0.63–1.03)	0.084	
Recessive	C/C-T/C	542 (96.8%)	559 (96.0%)	1.00		
	T/T	18 (3.2%)	23 (4.0%)	0.81 (0.43–1.51)	0.503	
Overdominant	C/C-T/T	403 (72.0%)	395 (67.9%)	1.00		
	T/C	157 (28.0%)	187 (32.1%)	0.82 (0.64–1.06)	0.132	
Allele	C	927 (82.8%)	931 (80.0%)	1.00		
	T	193 (17.2%)	233 (20.0%)	0.83 (0.67–1.03)	0.088	

Note: [a]Adjusted for BMI. OR of significant association is presented in bold.
Abbreviations: BMI, body mass index; CI, confidence interval; FPRP, false-positive report probability; FOXP3, forkhead box P3; HWE, Hardy–Weinberg equilibrium; OR, odds ratio.

the expression level of Ki-67. We observed that CA, CA/AA genotype, and A allele of rs3761548 were related with a larger size of tumor (CA vs CC: OR =1.47, 95% CI =1.01–2.15; CA/AA vs CC: OR =1.54, 95% CI =1.07–2.23; A vs C: OR =1.48, 95% CI =1.08–2.03). In addition, the A allele was correlated with a positive status of Her-2 (A vs C: OR =1.36, 95% CI =1.01–1.85) (Table 4). As for rs3761549, there was still no significant association with BC clinicopathological features existing in any of the genetic models (Table S2).

Discussion

FOXP3 is a key gene in the immune system. It encodes a protein for a transcriptional regulator which belongs to the forkhead/winged-helix family. Defects in this gene can cause immunodeficiency syndrome.[8,24] But the role of *FOXP3* in tumorigenesis has long been controversial. *FOXP3* was found to represses some oncogenes such as *MYC*, *HER2,* and *SATB1*. Its expression was downregulated in several tumors including breast, prostate, and ovarian tumors.[11,12,25] However, it was reported that most human tumors were infiltrated by Tregs with high FoxP3 labeling, and an excess of Treg activity can prevent the immune system from destroying cancer cells.[26,27]

No matter what the exact role of this gene in tumor is, the genetic variation of *FOXP3* can indeed affect cancer susceptibility.[9,26] The two common polymorphisms rs3761549 (C>T) and rs3761548 (C>A) were located in the promoter region of the *FOXP3* gene, which is considered to affect FoxP3 production and activity.[14] They were investigated in several tumors previously and were demonstrated to be associated with cancer susceptibility.[13,15–18] However, the conclusions of the relationship between these two SNPs and BC susceptibility were controversial. Lopes et al[28] observed that the homozygote of rs3761548 was associated with triple-negative breast cancer risk in Brazilians (OR =3.78; 95% CI =1.02–14.06). Jahan et al[29] evaluated both rs3761548 and rs3761549 in an Indian population but failed to find any correlation with BC risk. However, the AA genotype of rs3761548 was significantly associated with advanced tumor stage (T3-T4) (OR =3.90; p=0.03),[29] whereas Zheng et al's[30] and Raskin et al's[31] study reported that neither rs3761548 nor rs3761549 were associated with BC. Among all these researchers, only Zheng et al[30] studied the Chinese population. However, their study evaluated just two genetic models (homozygotes and heterozygotes) and did not explore the relationship of these two SNPs with clinical parameters of BC.

Our study focused on a Chinese Han population and found that the CA, CA/AA genotype, and the A allele of rs3761548 can increase the risk of BC. Moreover, the A allele of rs3761548 was related with a larger size of tumor (≥2 cm). It was also correlated with a Her-2 positive status, indicating that patients with A allele of rs3761548 are more likely to have overexpression of *HER2*. It means that the polymorphism of this locus may be a potential biomarker for tumor subtype classification and help to guide treatment. The results were in accord with some of previous studies, suggesting that the A allele of rs3761548 is a risk factor for BC and may be involved in tumor progression. The mechanism of action is not clear, which is a potential subject for future studies. Maybe the critical location of this SNP could affect the production and activity of FoxP3, thus influencing tumorigenesis though FoxP3's function.

Some limitations could not be ignored in the study. First, selection bias is inevitable as this is a hospital-based, single-center study. Second, our sample size was insufficient to support stratified analysis for tumor subtypes. Finally, we did

Table 4 The association between rs3761548 and clinical features of BC

Variables		OR (95% CI)				
		Homozygote	Heterozygote	Dominant	Recessive	Allele
Tumor size	<2 cm	1.00				
	≥2 cm	2.51 (0.83–7.64)	**1.47 (1.01–2.15)**	**1.54 (1.07–2.23)**	2.19 (0.73–6.61)	**1.48 (1.08–2.03)**
LN metastasis	(–)	1.00				
	(+)	1.61 (0.59–4.40)	1.28 (0.90–1.83)	1.31 (0.92–1.85)	1.47 (0.55–3.99)	1.26 (0.94–1.69)
ER	(–)	1.00				
	(+)	0.59 (0.26–1.36)	0.76 (0.54–1.08)	0.74 (0.53–1.04)	0.66 (0.29–1.49)	0.77 (0.58–1.03)
PR	(–)	1.00				
	(+)	0.62 (0.30–1.27)	0.77 (0.54–1.10)	0.75 (0.53–1.05)	0.68 (0.34–1.38)	0.78 (0.59–1.02)
Her-2	(–)	1.00				
	(+)	2.34 (0.96–5.69)	1.29 (0.88–1.89)	1.37 (0.95–1.98)	2.13 (0.89–5.13)	**1.36 (1.01–1.85)**
Ki-67	<14%	1.00				
	≥14%	1.05 (0.41–2.690)	0.96 (0.67–1.38)	0.97 (0.68–1.38)	1.07 (0.43–2.70)	0.98 (0.73–1.32)

Note: OR of significant association is presented in bold.
Abbreviations: BC, breast cancer; CI, confidence interval; ER, estrogen receptor; LN, lymph node; OR, odds ratio; PR, progesterone receptor.

not analyze the impact of other risk factors such as lifestyle, radiation exposure to the chest, family history, and other benign breast lesions because of lacking relative data. Hence, population-based studies are required in the future to improve the accuracy of evaluation and to explore gene–environment interactions as well.

Conclusion

To sum up, the present study suggests that the *FOXP3* polymorphism rs3761548 is associated with BC susceptibility in the Chinese and may be involved in tumor progression. Future studies in a large population with more races are needed to confirm the results. There is also a need to explore gene–environment interactions and possible mechanisms of action for this SNP.

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Disclosure

The authors report no conflicts of interest in this work.

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Supplementary materials

Table S1 Stratified analyses on association between *FOXP3* SNPs and BC risk

SNPs	Genotype						OR (95% CI)				
	Case (n=560)			Control (n=583)			Homozygote	Heterozygote	Dominant	Recessive	Allele
rs3761548	C/C	C/A	A/A	C/C	C/A	A/A					
Age <49 (n=604)	177	104	13	207	92	11	1.38 (0.60–3.16)	1.32 (0.94–1.87)	1.33 (0.95–1.85)	1.26 (0.55–2.85)	1.26 (0.95–1.67)
Age ≥49 (n=536)	160	94	11	181	81	9	1.38 (0.56–3.42)	1.31 (0.91–1.89)	1.32 (0.93–1.88)	1.26 (0.51–3.09)	1.25 (0.93–1.69)
Premenopausal (n=544)	159	94	11	187	83	10	1.29 (0.54–3.13)	1.33 (0.93–1.92)	1.33 (0.94–1.88)	1.17 (0.49–2.81)	1.25 (0.93–1.68)
Postmenopausal (n=596)	178	104	13	201	90	10	1.47 (0.63–3.43)	1.30 (0.92–1.85)	1.32 (0.95–1.85)	1.34 (0.58–3.11)	1.26 (0.95–1.68)
rs3761548	C/C	T/C	T/T	C/C	T/C	T/T					
Age <49 (n=604)	202	82	10	198	100	12	0.82 (0.35–1.93)	0.80 (0.57–1.14)	0.81 (0.57–1.13)	0.87 (0.37–2.06)	0.85 (0.63–1.13)
Age ≥49 (n=538)	183	74	9	174	87	11	0.78 (0.31–1.92)	0.81 (0.56–1.17)	0.81 (0.56–1.15)	0.83 (0.34–2.04)	0.83 (0.61–1.13)
Premenopausal (n=544)	182	74	8	179	90	11	0.72 (0.28–1.82)	0.81 (0.56–1.17)	0.80 (0.56–1.14)	0.76 (0.30–1.93)	0.83 (0.61–1.12)
Postmenopausal (n=598)	204	83	9	193	97	12	0.71 (0.29–1.72)	0.81 (0.57–1.15)	0.80 (0.57–1.12)	0.76 (0.31–1.83)	0.82 (0.61–1.10)

Abbreviations: BC, breast cancer; CI, confidence interval; FOXP3, forkhead box P3; OR, odds ratio; SNP, single-nucleotide polymorphism.

Table S2 The association between rs3761549 and clinical features of BC

Variables		OR (95% CI)				
		Homozygote	Heterozygote	Dominant	Recessive	Allele
Tumor size	<2 cm	1.00				
	≥2 cm	0.85 (0.28–2.59)	0.84 (0.59–1.18)	0.84 (0.60–1.18)	0.91 (0.30–2.75)	0.87 (0.65–1.17)
LN metastasis	(–)	1.00				
	(+)	1.43 (0.63–3.25)	0.74 (0.52–1.05)	0.81 (0.58–1.13)	1.66 (0.74–3.71)	0.92 (0.71–1.19)
ER	(–)	1.00				
	(+)	1.02 (0.40–2.59)	1.26 (0.88–1.79)	1.24 (0.88–1.75)	0.97 (0.39–2.43)	1.15 (0.86–1.54)
PR	(–)	1.00				
	(+)	1.04 (0.35–3.07)	0.84 (0.59–1.18)	0.85 (0.60–1.19)	1.12 (0.38–3.26)	0.90 (0.67–1.19)
Her-2	(–)	1.00				
	(+)	1.12 (0.33–3.79)	0.95 (0.65–1.37)	0.95 (0.66–1.38)	1.14 (0.34–3.84)	0.98 (0.71–1.33)
Ki-67	<14%	1.00				
	≥14%	0.59 (0.26–1.36)	0.72 (0.42–1.25)	0.68 (0.43–1.10)	0.62 (0.27–1.41)	0.68 (0.45–1.01)

Abbreviations: BC, breast cancer; CI, confidence interval; ER, estrogen receptor; LN, lymph node; OR, odds ratio; PR, progesterone receptor.

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