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ORIGINAL RESEARCH

Association of FGFR2 and PI3KCA genetic variants with the risk of breast cancer in a Chinese population

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Purpose: Genome-wide association studies have found plenty of single nucleotide polymorphisms (SNPs) which are associated with breast cancer risk. SNPs in FGFR2 are mostly identified. However, the association between PI3KCA SNP and breast cancer risk remains largely unknown. The aim of this study was to investigate the significance of FGFR2 and PI3KCA genetic variants in breast cancer and their association with prognosis.

Methods: We performed genotyping of 328 breast cancer patients and 389 healthy controls. Then, we evaluated the associations of FGFR2 rs1219648 and PI3KCA rs6443624 with the susceptibility and clinicopathological features of breast cancer. Kaplan-Meier curve with log-rank test was performed to determine the prognostic values of FGFR2 rs1219648 and PI3KCA rs6443624.

Results: The results indicated that genotype frequencies of rs1219648 and rs6443624 were significantly different between breast cancer patients and healthy controls. Furthermore, PI3KCA rs6443624 A carriers and FGFR2 rs1219648 G carriers more frequently had advanced stages and shorter survival times.

Conclusion: The SNPs of FGFR2 rs1219648 and PI3KCA rs6443624 may contribute to the identification of breast cancer patients at risk of more aggressive disease and may be potential prognostic factors in breast cancer in a Chinese population.

Keywords: rs1219648, rs6443624, breast cancer, prognosis

Introduction

Breast cancer (BC) is the most common malignancy and the first leading cause of cancer-related death among women worldwide.[1] In China, over 272,400 new cases were diagnosed and there were 70,700 deaths in 2015, and the incidence has increased annually.[2] Many BC risk factors have been identified, such as serum hormone levels, dietary and family history.[3–5] It was reported that genes (BRCA1/2, ATM et al) inherited from family significantly increased susceptibility to BC, and make up a large proportion of BC cases.[6]

Among different risk factors, RAS/MAPK and PI3K/AKT pathways through FGFs are widely recognized to be involved in the initiation and progression of BC.[7] FGFR2 is widely overexpressed in BC cell lines and tumor samples.[8] Genome-wide association studies (GWAS) have suggested that a variety of FGFR2 single nucleotide polymorphisms (SNPs) were associated with BC risk.[9,10] For instance, Liang et al performed genotyping and found that FGFR2 rs2981582, rs1219648, and rs2420946 were significantly associated with increased BC risk.[11] Indeed, a variety of FGFR2 SNPs were found recently and were associated with elevated BC risk. However, considering the

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complex mechanisms of cancer development, we may need to combine several genes together to identify the cancer risk.

PI3KCA has been widely linked to cancer development.[12,13] PI3KCA was reported to be frequently highly mutated in many types of cancers, such as esophageal cancer, colorectal cancer, and BC.[14,15] Recently, PI3KCA genomic variants were identified as being associated with increased cancer risk.[16] Park et al[17] reported that PI3KCA rs2699905 and rs7640662 were significantly associated with melanoma patients' survival. Wang et al[18] reported that PIK3CA rs2699887 showed notable associations with survival of endometrial cancer patients. However, the association between PI3KCA rs6443624 and BC risk and patient survival remains elusive.

In the current study, we focused on the combination of two reported cancer-related SNPs, FGFR2 rs1219648 and PI3KCA rs6443624. We investigated the correlations between these genes and BC risk and patient survival among female patients in China.

Materials and methods

Study population

The study was approved by the Shandong Cancer Hospital Affiliated to Shandong University and written informed consent was obtained from all participants. A total of 328 BC patients (aged 28–80 years, mean age 56 years) were analyzed retrospectively for the study from June 2010 to April 2011. The histology and TNM stages were classified according to the 7th edition of the American Joint Committee on Cancer staging. Detailed clinicopathological features are presented in Table 1. The follow-up of each BC patient was performed at 3-month intervals in the first year and thereafter at 6-month intervals. The latest follow-up data in the study were obtained in December 2016. Additionally, 389 age-matched, healthy women were recruited as controls for the present study.

Table 1 Clinicopathologic parameters of breast cancer patients

Parameters	Case number
Overall	328
Age (years)	
≤ 50	88
> 50	240
Tumor size (cm)	
≤ 2	72
> 2	256
Stage	
I+II	234
III	94
Grade	
I+II	138
III	190
Lymph node invasion	
No	157
Yes	171
ER status	
Negative	146
Positive	182
PR status	
Negative	139
Positive	189
HER2 status	
Negative	242
Positive	86

Extraction of genomic DNA and genotyping

An amount of 3 mL peripheral blood was obtained from patients and healthy controls for DNA extraction. The blood samples were treated with EDTA-K2 and stored at -80° until analysis. Genomic DNA were extracted with DNA Extraction Kits (Tiangen, Beijing, China) according to the instructions of the manufacturer.

Genotyping of rs1219648 and rs6443624 was carried out using pre-designed TaqMan SNP Genotyping assays (C_2917314_20, and C_2917314_20) as described previously.[19] In brief, 10 μL reaction solution containing 5 ng genomic DNA and TaqMan Master Mix was performed Genotyping assay. PCR was performed with an ABI7500 (Thermo Fisher Scientific, Waltham, MA, USA). As quality control, approximately 10% of the samples were randomly used to repeat the reaction, and the results were 100% concordant.

Statistical analysis

The Hardy-Weinberg equilibrium was assessed in controls using asymptotic χ^2 test. Fisher's exact test was performed to test the association between FGFR2 and PI3KCA genotypes in cases and controls. The strength of associations in FGFR2 and PI3KCA genotypes in BC risk was measured by binary logistic regression analysis and ORs and 95% CIs. The overall survival was depicted by Kaplan-Meier method followed by the log-rank test. Multivariate Cox regression analyses were performed to assess the clinicopathological features in evaluating BC prognosis. All statistical analyses were performed with SPSS 21.0. A *p*-value less than 0.05 was considered statistically significant.

Results

The association between FGFR2 rs1219648 and PI3KCA rs6443624 and BC risk

We performed the TaqMan allelic discrimination assay (details in Supplementary materials) and firstly, we found no

deviations from Hardy-Weinberg equilibrium in both controls and cases; data shown in Table 2 ($p>0.05$).

Then, the allele and genotype distribution of FGFR2 rs1219648 and PI3KCA rs6443624 in controls and BC cases revealed that the FGFR2 rs1219648 GG allele was significantly associated with BC risk by logistic regression analysis compared to AA genotype (OR: 2.40, 95% CI: 1.32–4.36, $p=0.004$). Meanwhile, compared to patients carrying the CC allele for PI3KCA rs6443624, those with AC and AA had an OR of 1.33 (95% CI: 0.90–1.96, $p=0.154$) and 1.95 (95% CI: 1.28–2.96, $p=0.002$), respectively, suggesting that PI3KCA rs6443624 AA allele was significantly associated with the risk of BC. Moreover, we determined the dominant models of FGFR2 rs1219648 and PI3KCA rs6443624 and found that PI3KCA rs6443624 AA/AC genotype was significantly correlated with BC risk (Table 2).

Association between FGFR2 rs1219648 or PI3KCA rs6443624 and BC clinicopathological features

To further evaluate the significance of FGFR2 rs1219648 or PI3KCA rs6443624 in BC, we assessed the association between these two SNPs and clinicopathological features, including age, tumor size, clinical stage, grade, lymph node invasion status, ER, PR, and HER2 status. The results indicated that PI3KCA rs6443624 AC allele was significantly correlated with advanced stage while AA allele was correlated with high grade. Also, the PI3KCA rs6443624 dominant model was notably correlated with advanced stage (Table 3).

For FGFR2 rs1219648, the AG allele had a significantly higher distribution in BC patients with advanced stage. Moreover, the FGFR2 rs1219648 dominant model also showed significantly higher risk of advanced stage (Table 4).

FGFR2 rs1219648 predicts poor prognosis of BC patients

To evaluate the prognostic significance of FGFR2 rs1219648 or PI3KCA rs6443624 in BC patients, we performed Kaplan-Meier analysis with log-rank test to measure the patients' survival with different alleles. Intriguingly, we noticed that relative to patients with FGFR2 rs1219648 AA allele, those with AG and GG had shorter survival times ($p=0.006$, Figure 1). However, patients with PI3KCA rs6443624 showed no difference between each allele ($p=0.275$, Figure 2). Moreover, we performed multivariate analysis with stepwise Cox regression model and found that clinical stage, ER and PR status could be significant prognosticators (Table 5).

Discussion

In the present study, we evaluated the impact of FGFR2 and PI3KCA polymorphisms on BC patients. Recently, GWAS have identified a variety of genetic variants which were associated with BC risk.[20,21] FGFR2 belongs to the FGF receptor family. The ligands, FGFs, could initiate the downstream signals and then modulate cell migration, angiogenesis, and growth.[22] Among these, FGFR2 rs1219648 was reported to be related with BC development.[23] Mechanically, it was demonstrated that rs1219648 altered the transcription activity of FGFR2. Since this SNP was presented in the promoter region of FGFR2 gene, we thought it might up-regulate the expression of FGFR2, and high expression of FGFR2 promoted BC progression.[24,25] Previous studies have revealed that FGFR2 rs1219648 was highly associated with BC lymph node metastasis, but showed no significant difference between healthy individuals and BC patients.[26] However, our data suggested that FGFR2 rs1219648 was notably related to high risk of BC. Moreover, the AG/GG alleles were significantly associated

Table 2 Genotype frequencies of FGFR2 rs1219648 and PI3KCA rs6443624 single nucleotide polymorphisms in patients and healthy controls

Genotype	Controls (%) n=389	Cases (%) n=328	OR (95% CI)	*p*-value
FGFR2 rs1219648				
AA	224 (57.6)	167 (50.9)	1.00	
AG	146 (37.5)	127 (38.7)	1.17 (0.86–1.59)	0.331
GG	19 (4.9)	34 (10.4)	2.40 (1.32–4.36)	0.004
AG+GG	165 (42.4)	161 (49.1)	1.31 (0.98–1.76)	0.074
PI3KCA rs6443624				
CC	97 (24.9)	58 (17.7)	1.00	
AC	189 (48.6)	150 (45.7)	1.33 (0.90–1.96)	0.154
AA	103 (26.5)	120 (36.6)	1.95 (1.28–2.96)	0.002
AA+AC	292 (75.1)	270 (82.3)	1.55 (1.07–2.23)	0.019

Table 3 Association between PI3KCA rs6443624 and clinicopathological features of 328 breast cancer patients

Parameters	PI3KCA rs6443624			
	CC	AC	AA	AC+AA
Age (years)				
≤50/>50	14/44	42/108	32/88	74/196
OR (95% CI)	1.00	0.82 (0.41–1.65)	0.88 (0.42–1.81)	0.84 (0.44–1.63)
R^2 value		1.52E-3	7.29E-4	7.84E-4
p-value		0.573	0.718	0.610
Tumor size (cm)				
≤2/>2	10/48	40/110	22/98	62/208
OR (95% CI)	1.00	0.57 (0.27–1.24)	0.93 (0.41–2.11)	0.79 (0.33–1.46)
R^2 value		9.80E-3	1.69E-4	2.81E-3
p-value		0.154	0.859	0.340
Stage				
I+II/III+IV	49/9	106/44	87/33	193/77
OR (95% CI)	1.00	2.26 (1.02–4.99)	2.07 (0.91–4.67)	2.17 (1.05–4.49)
R^2 value		2.02E-2	1.74E-2	1.37E-2
p-value		0.040	0.078	0.034*
Grade				
I+II/III	31/27	63/87	44/76	107/163
OR (95% CI)	1.00	1.59 (0.86–2.92)	1.98 (1.05–3.75)	1.75 (0.99–3.10)
R^2 value		1.06E-2	2.53E-2	1.14E-2
p-value		0.137	0.034	0.053
Lymph node invasion				
No/yes	32/26	76/74	51/69	126/143
OR (95% CI)	1.00	1.20 (0.65–2.20)	1.67 (0.89–3.13)	1.55 (0.88–2.75)
R^2 value		1.60E-3	1.42E-2	7.06E-3
p-value		0.560	0.112	0.129
ER status				
Negative/positive	24/34	61/89	61/59	122/148
OR (95% CI)	1.00	1.03 (0.56–1.91)	0.68 (0.36–1.29)	0.86 (0.48–1.52)
R^2 value		4.90E-5	7.92E-3	8.41E-4
p-value		0.925	0.237	0.597
PR status				
Negative/positive	22/36	60/90	57/63	117/153
OR (95% CI)	1.00	0.92 (0.49–1.71)	0.68 (0.36–1.28)	0.80 (0.45–1.43)
R^2 value		3.61E-4	8.10E-3	1.76E-3
p-value		0.784	0.228	0.450
HER2 status				
Negative/positive	42/16	119/31	81/39	200/70
OR (95% CI)	1.00	0.68 (0.34–1.38)	1.26 (0.63–2.52)	0.92 (0.49–1.74)
R^2 value		5.48E-3	2.50E-3	1.96E-4
p-value		0.285	0.506	0.794

Note: *$p<0.05$, as compared to CC genotype.

with advanced stage and poor prognosis, which has not been reported previously. These results might be attributable to the different study population.

PI3KCA/Akt signaling is critical in many types of cancers, such as colorectal cancer, BC, and osteosarcoma.[27,28] Also, somatic mutations in PI3KCA have been commonly identified in many types of cancers.[29,30] A previous study reported that a majority of mutations happened in exon 20, and some missense mutations and silent mutations were found in BC patients.[31] PI3KCA rs17849079 was more frequently found in BC patients compared with disease-free controls. However, Stevens et al[32] indicated that common variations in PI3KCA did not have a strong influence on BC as the patients were mostly Caucasian, and rs1607237 was associated with a decreased risk. The rs6443624 SNP located in the intron of PI3KCA, might affect the binding of transcription factors and alter the splicing patterns or transcription of the PIK3CA gene.[18] In our study on a Chinese population, we suggested that PI3KCA rs6443624 was significantly associated with the risk of BC, and AC/

Table 4 Association between FGFR2 rs1219648 and clinicopathological features of 328 breast cancer patients

Parameters	FGFR2 rs1219648			
	AA	AG	GG	AG+GG
Age (years)				
≤50/>50	39/128	35/92	14/20	49/112
OR (95% CI)	1.00	0.80 (0.47–1.36)	0.44 (0.20–0.94)	0.70 (0.43–1.12)
R^2 value		2.30E-3	2.31E-2	6.40E-3
p-value		0.410	0.032	0.148
Tumor size (cm)				
≤2/>2	42/125	20/107	10/24	30/131
OR (95% CI)	1.00	1.80 (1.00–3.25)	0.81 (0.37–1.82)	1.47 (0.87–2.49)
R^2 value		1.30E-2	1.29E-3	6.24E-3
p-value		0.05	0.61	0.154
Stage				
I+II/III+IV	129/38	84/43	21/13	105/56
OR (95% CI)	1.00	1.74 (1.04–2.91)	2.10 (0.96–4.59)	1.82 (1.11–2.94)
R^2 value		1.51E-2	1.77E-2	1.77E-2
p value		0.035*	0.059	0.016*
Grade				
I+II/III	62/105	70/57	6/28	76/85
OR (95% CI)	1.00	0.48 (0.30–0.77)	2.76 (1.08–7.03)	0.66 (0.43–1.03)
R^2 value		3.20E-2	2.37E-2	1.04E-2
p-value		0.002*	0.029*	0.065
Lymph node invasion				
No/Yes	87/80	58/69	12/22	70/91
OR (95% CI)	1.00	1.29 (0.82–2.06)	1.99 (0.93–4.29)	1.41 (0.92–2.18)
R^2 value		4.10E-3	1.59E-2	7.40E-3
p-value		0.275	0.074	0.118
ER status				
Negative/positive	74/93	55/72	17/17	72/89
OR (95% CI)	1.00	1.04 (0.65–1.66)	0.80 (0.38–1.67)	0.98 (0.64–1.52)
R^2 value		1.00E-4	1.85E-3	1.60E-5
p-value		0.864	0.544	0.941
PR status				
Negative/positive	65/102	58/69	16/18	74/87
OR (95% CI)	1.00	0.76 (0.48–1.21)	0.72 (0.34–1.51)	0.75 (0.48–1.16)
R^2 value		4.62E-3	3.84E-3	5.04E-3
p-value		0.245	0.378	0.197
HER2 status				
Negative/positive	118/49	94/33	30/4	124/37
OR (95% CI)	1.00	0.85 (0.50–1.42)	0.32 (0.11–0.96)	0.72 (0.44–1.18)
R^2 value		1.37E-3	2.25E-2	5.18E-3
p-value		0.525	0.034*	0.190

Note: *$p<0.05$, as compared to AA genotype.

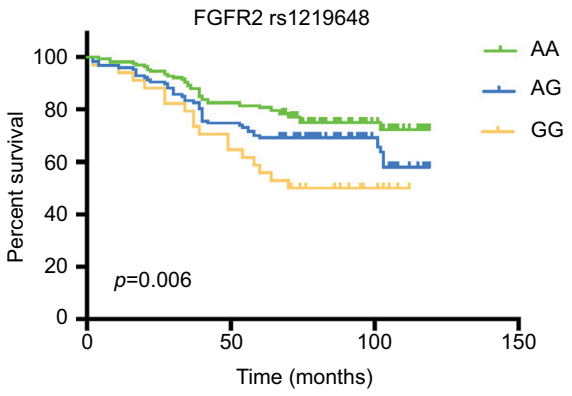

Figure 1 The Kaplan-Meier curve illustrates overall survival based on FGFR2 allele status.

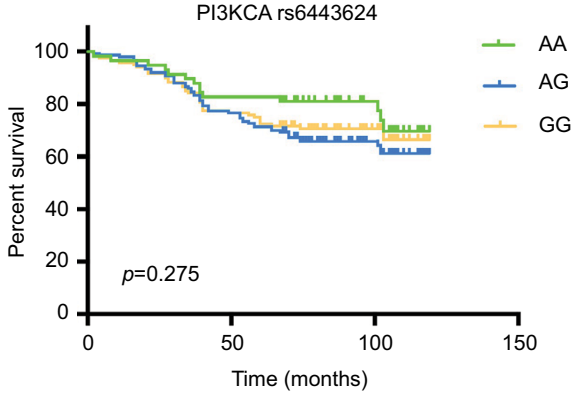

Figure 2 The Kaplan-Meier curve illustrates overall survival based on PI3KCA allele status.

Table 5 Multivariate analysis for overall survival (stepwise Cox regression analysis)

Parameters	HR	95% CI	*p*
Age	1.259	0.475–3.335	0.643
Tumor size	0.856	0.549–1.337	0.495
Stage	1.847	1.195–2.855	0.006*
Grade	1.093	0.728–1.640	0.670
Lymph node invasion	1.023	0.663–1.580	0.917
ER status	0.638	0.421–0.968	0.034*
PR status	0.317	0.121–0.833	0.020*
HER2 status	1.083	0.573–2.068	0.781
rs1219648 (AA vs. AG+GG)	1.449	0.968–2.168	0.071
rs6443624 (CC vs. AC+AA)	1.273	0.720–2.250	0.406

Note: *$p<0.05$.

AA alleles were associated with advanced stage, implying their biological functions, which need further investigation.

Moreover, we noticed that FGFR2 rs1219648 was significantly associated with poor BC prognosis, which has not been reported previously. However, no such association was found between PI3KCA rs6443624 and short survival time. A previous report showed that rs6443624 could be an independent predictor and prognostic factor in renal cell carcinoma patients.[33] So, further study will be performed to confirm and validate this finding.

In conclusion, our results obtained from a cohort of Chinese BC patients, indicated that FGFR2 rs1219648 and PI3KCA rs6443624 were significantly associated with BC risk and FGFR2 rs1219648 AG/GG alleles were associated with shorter survival. Therefore, detection of the SNPs might help to identify patients and predict prognosis.

Disclosure

The authors report no conflicts of interest in this work.

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