Original Paper

Effects of FGFR1 Gene Polymorphisms on the Risk of Breast Cancer and FGFR1 Protein Expression

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Key Words

FGFR1 • Gene polymorphisms • Breast cancer • Susceptibility • Protein expression

Abstract

Background/Aims: Fibroblast growth factor receptor 1 (FGFR1) is widely considered to play an important role in mammary carcinogenesis. Some common variants in *FGFR1* might be associated with its expression, and further affect breast cancer risk. The aim of this study was to investigate effects of single-nucleotide polymorphisms (SNPs) in *FGFR1* on breast cancer susceptibility and FGFR1 protein expression. **Methods:** SNPs rs17182023, rs17175624 and rs10958704 in *FGFR1* were genotyped in 747 breast cancer cases and 716 healthy controls by SNaPshot method. The associations between SNPs and breast cancer were examined by logistic regression. Immunohistochemistry(IHC) was used to detect FGFR1 protein expression, and the association of *FGFR1* polymorphisms with its protein expression was analyzed by Pearson's chi-square test. Additionally, Cox regression and Kaplan-Meier analysis was used to evaluate the association between FGFR1 protein expression and breast cancer prognosis.

Results: The minor allele of rs17182023 in *FGFR1* was significantly associated with reduced breast cancer risk, with an odds ratio of 0.800 (95%CI = 0.684-0.935). No significant associations were detected between other SNPs and breast cancer. Moreover, rs17182023 was correlated to FGFR1 protein expression ($P = 0.006$), and patients with high FGFR1 protein expression tended to have poor outcomes. **Conclusions:** SNP rs17182023 was correlated to reduced breast cancer risk, and was associated with FGFR1 protein expression. High FGFR1 protein expression was an independent risk factor of breast cancer, and resulted in poor prognosis.

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Introduction

Breast cancer is considered to be one of the most common malignancies in women [1]. It is widely recognized that breast cancer is a complex, systemic disease which associated with multiple genetic and environmental factors. According to a large number of studies, activation of tyrosine kinase growth factor receptors is proved to be a pervasive oncogenic event in breast cancer. The fibroblast growth factor receptors (FGFRs) belong to receptor tyrosine kinase (RTK) superfamily, and the amino-acid sequences of FGFRs are highly conserved. They function in a wide range of biological process, such as development controlling, angiogenesis, wound repair and endocrine functions [2-4]. Moreover, pre-clinical evidences have demonstrated that activation of FGFR signalling pathways, which may be caused by point mutations, amplifications and translocations, play significant roles in tumor development and progression [5, 6].

Multiple genetic alterations in *FGFRs* have been found in breast cancer. An intronic variant in *FGFR2* was one of the first SNPs being identified [7, 8], and was recognized as a breast cancer susceptibility locus, especially in estrogen receptor (ER)-positive disease. Other variants in *FGFRs* and the downstream genes in the FGF pathway may also be associated with breast carcinogenesis. For instance, amplification of *FGFR1* is identified in up to 10% of all breast cancer and 16% to 27% of luminal type B breast cancer cases, and it is correlated with endocrine therapy resistance and poor prognosis [9, 10]. Studies from breast cancer cell lines and mouse models also support a role for *FGFR1* amplification in mammary oncogenesis [11, 12]. The aberrant activation of FGFR1 in mouse or human mammary cell lines resulted in increased cell proliferation, survival and invasion, confirming the carcinogenic nature of FGFR1 signalling.

Currently, as the carcinogenic effect of *FGFR1* is widely accepted, an increasing number of studies have been working on it, since *FGFR1* could be a potential diagnostic and (or) prognostic biomarker. To date, several studies about *FGFR1* genetic polymorphisms have been carried out, trying to find SNP loci which could be a predictor for breast cancer risk. However, according to the previous studies, few loci were confirmed to be significantly correlated with breast cancer susceptibility [13]. Thus, we carried out this case-control study. Three SNPs in *FGFR1* gene: rs17182023, rs17175624 and rs10958704, were picked out from the SNP databases, and all of them were intronic variants near the 5' end of *FGFR1*. According to the analyses, These variants were potentially functional SNPs, which might alter the expression of *FGFR1*. Therefore, we genotyped the three SNPs in breast cancer cases and healthy controls, trying to identify their effect on FGFR1 protein expression, revealing the associations between these common genetic variants and breast cancer risk, and moreover, confirming the impacts of FGFR1 protein expression on disease characters and prognosis of breast cancer.

Materials and Methods

Subjects

There were 747 breast cancer cases and 716 healthy controls included in this study. The cases were patients with sporadic breast cancer from the Department of Breast Surgery at the Third Affiliated Hospital of Harbin Medical University from September 2008 to December 2011. The patients were diagnosed based on their surgical and pathological evaluation, and the information of their disease was obtained from the medical files (Table 1).

Table 1. The demographic characteristics of 747 breast cancer cases and 716 controls. Note: Data presented as the mean $\pm$ standard deviation or number (% of total number)

Characteristics	Case (n = 747)	Controls (n = 716)	P
Age	49.91 $\pm$ 10.28	49.55 $\pm$ 10.61	0.508
Body mass index, BMI	24.03 $\pm$ 3.44	23.32 $\pm$ 3.13	<0.001
Age at menarche	15.37 $\pm$ 1.80	15.61 $\pm$ 1.94	0.015
Age at first birth	23.79 $\pm$ 6.11	24.00 $\pm$ 7.41	0.556
Breastfeeding duration	16.59 $\pm$ 13.77	11.43 $\pm$ 7.48	<0.001
Menopausal status			
Pre-menopausal	415(55.6)	457(63.8)	0.001
Post-menopausal	332(44.4)	259(36.2)	
Family history			
No	622(83.3)	607(84.8)	0.431
Yes	125(16.7)	109(15.2)	

The control group consisted of women of Han origin living in Harbin, in northeastern China. The individuals who were selected had no history of cancer, and their age and ethnicity is matched with the cancer patients. Furthermore, the participants were interviewed respectively to collect information on demographic characteristics, and each of them provided 5 ml of venous blood. The study was approved by the ethics committee of Harbin Medical University.

SNP selection and genotyping

In this study, a combined analysis of functional significance and Tag SNP strategies was utilized to select three potentially functional SNPs in the *FGFR1* gene from the dbSNP and HapMap databases. The minor allele frequencies (MAF) of these SNPs were greater than 5%, and the pair-wise r^2 values were greater than 0.8. Genomic DNA was isolated from EDTA anti-coagulated whole blood using the AxyPrep Blood Genomic DNA Miniprep Kit (Axygen Biotechnology, Tewksbury, MA, USA). We performed the SNaPshot SNP assay to checkout the polymorphisms at the three SNPs. The resulting data were analyzed with GeneMapper™ 4.0 software (Applied Biosystems, Foster City, CA, USA).

IHC

IHC was used to detect FGFR1 protein expression of 348 breast cancer tissues which were randomly selected from the 747 breast cancer cases. IHC staining was performed using a Benchmark XT auto-stainer (Ventana Medical Systems Inc., Tucson, AZ, USA), and the FGFR1 antibody (ab10646, Abcam, Cambridge, UK) was diluted to 1:2000. IHC was proceeded as follows. Briefly, 4-micron thick tissue sections were cut in microtome, deparaffinized in xylene, rehydrated through graded ethanol (100% and 95%), and rinsed in water. Subsequently, the sections were subjected to heat-induced antigen retrieval, and finally loaded onto the Benchmark auto-stainer and the detection was performed using the I-View detection kit (Ventana Medical Systems Inc., Tucson, AZ, USA). IHC of ER, PR, HER2, Ki67 and P53 (ZSBG-BIO: ZM-0104, ZM-0215, ZM-0065, ZM-0165, ZM-0405) was performed similarly to the description above. Protein expression of FGFR1 was quantified as a percentage (range 0-100%) of positive cells present among all tumour cells presented. A cut-off value of 10% was selected for FGFR1 protein expression. Above 10% was defined as “high expression” and below as “low expression” [14] (Fig. 1). IHC staining for ER and PR was considered positive if $\geq 1\%$ of tumour cell nuclei were stained [15]. Expression of HER2 was evaluated with the HercepTest kit (Dako) and scored as 0, 1+, 2+ and 3+. Scores of 0 and 1+ were determined as negative, and 3+ was determined as positive [16]. The intrinsic subtypes were determined using the molecular typing method [17]. Each of the sections was scored by two observers, who were blinded to patients’ medical information, and disagreement between the observers was solved by discussion.

Statistical analysis

The genotype frequencies were tested for Hardy-Weinberg equilibrium using the chi-square test. Differences between the cases and controls in demographic characteristics were evaluated by Pearson chi-square test (for categorical variables) or Student’s t-test (for continuous variables). Associations between genotypes and breast cancer risk were estimated by computing odds ratios (ORs) and 95% confidence intervals (CIs) from logistic regression. To eliminate the effect of confounding factors, we corrected for multivariate analysis and adjusted for age, body mass index (BMI), breastfeeding duration, age at menarche and menopausal status. Associations between disease characteristics and patient genotypes were evaluated by Pearson chi-square test. Differences in protein expression and genotypes and associations between FGFR1 protein expression and clinicopathological variables were analyzed with a Pearson chi-square test. Spearman rank correlation analysis was employed to test the linear correlations. Cox proportional hazards model was performed to evaluate prognostic

Fig. 1. Representative sections of FGFR1 protein expression in breast cancer tissue and adjacent normal tissue.

factors of breast cancer by measuring risk ratios and CIs. Kaplan-Meier analysis was used to analyze the overall survival (OS). The value of $P < 0.05$ was considered to be of statistical significance. All the statistical analysis was computed by SPSS 13.0 (SPSS Inc., Chicago, IL).

Results

Subject characteristics

The 747 breast cancer cases and 716 healthy controls were similar in age at interview, age at first birth and family history (Table 1), but otherwise show significant differences. In contrast to the controls, the cases tended to have higher body mass index (BMI), earlier age at menarche, longer breastfeeding duration, and higher proportion of post-menopausal individuals ($P < 0.05$).

Correlations between SNPs and breast cancer susceptibility

This study involved three SNPs in *FGFR1* gene: rs17182023, rs17175624 and rs10958704, and the distributions of alleles and genotypes were exhibited in Table 2. The genotype frequencies of the SNPs conform to Hardy-Weinberg equilibrium. According to the table, the cases tend to carry less minor allele of rs17182023 compared with controls. But for the other two loci, there were no significant differences in allele and genotype distributions between the cases and controls.

We performed a logistic linear regression to investigate the correlation between the SNPs and breast cancer susceptibility. Results showed that the minor allele of rs17182023 in the *FGFR1* gene had a great tendency to reduce the risk of breast cancer, with an OR of 0.785 (95%CI = 0.676-0.911, $P = 0.001$). We further analyzed the effect of genotypes under two genetic models (Table

Table 2. Genotype distributions, odds ratios (OR) and 95% confidence intervals (CI) for the association between breast cancer susceptibility loci of *FGFR1* in 747 breast cancer cases and 716 controls. Note: ^aAdjusted for age, BMI, breastfeeding duration, age at menarche and menopausal status

Genotype	Case (n=747)	Control (n=716)	OR (95%CI)		OR (95%CI) ^a
rs17182023					
GG	317(42.4)	248(34.6)	1.000		1.000
GA	333(44.6)	349(48.7)	0.746(0.597-0.934)	0.010	0.755(0.598-0.953)
AA	97(13.0)	119(16.6)	0.638(0.465-0.874)	0.005	0.665(0.478-0.924)
A allele	527(35.3)	587(41.0)	0.785(0.676-0.911)	0.001	0.800(0.684-0.935)
GA+AA	430(57.6)	468(65.4)	0.719(0.582-0.888)	0.002	0.733(0.588-0.913)
rs17175624					
CC	346(46.3)	344(48.0)	1.000		1.000
CA	313(41.9)	301(42.0)	1.034(0.832-1.285)	0.764	1.072(0.854-1.344)
AA	88(11.8)	71(9.9)	1.232(0.871-1.742)	0.237	1.224(0.853-1.757)
A allele	489(32.7)	443(30.9)	1.086(0.930-1.269)	0.298	1.097(0.933-1.291)
CA+AA	401(53.7)	372(52.0)	1.072(0.873-1.316)	0.509	1.101(0.889-1.364)
rs10958704					
GG	342(45.8)	343(47.9)	1.000		1.000
GA	318(42.6)	300(41.9)	1.063(0.855-1.321)	0.581	1.097(0.874-1.376)
AA	87(11.6)	73(10.2)	1.195(0.846-1.688)	0.311	1.192(0.831-1.710)
A allele	492(32.9)	446(31.1)	1.086(0.929-1.268)	0.301	1.096(0.932-1.289)
GA+AA	405(54.2)	373(52.1)	1.089(0.887-1.337)	0.416	1.115(0.901-1.382)

Table 3. The association between SNP locus rs17182023 and clinico-pathological characteristics of breast cancer. Note: ^aUnder dominate model, ^bunder co-dominate model. LNM: lymph node metastasis, ER: estrogen receptor, PR:

Clinico-pathological characteristics	Cases	rs17182023			P ^a	P ^b
		GG(%)	GA(%)	AA(%)		
TNM stage						
I, II	532	225(42.3)	238(44.7)	69(13.0)	0.705	0.906
III	143	63(44.1)	63(44.1)	17(11.9)		
Tumor size(cm)						
≤2	583	253(43.4)	255(43.7)	75(12.9)	0.297	0.428
>2	88	33(37.5)	45(51.1)	10(11.4)		
Pathological stage						
I, II	446	188(42.2)	200(44.8)	58(13.0)	0.924	0.993
III	120	50(41.7)	54(45.0)	16(13.3)		
LNM						
Negative	425	178(41.9)	195(45.9)	52(12.2)	0.795	0.701
Positive	294	126(42.9)	127(43.2)	41(13.9)		
ER status						
Negative	244	103(42.2)	111(45.5)	30(12.3)	0.896	0.838
Positive	447	191(42.7)	195(43.6)	61(13.6)		
PR status						
Negative	319	144(45.1)	138(43.3)	37(11.6)	0.202	0.335
Positive	372	150(40.3)	168(45.2)	54(14.5)		
HER2 status						
Negative	492	206(41.9)	224(45.5)	62(12.6)	0.717	0.478
Positive	119	52(43.7)	48(40.3)	19(16.0)		
Ki67 status						
Negative	258	116(45.0)	109(42.2)	33(12.8)	0.296	0.571
Positive	428	175(40.9)	196(45.8)	57(13.3)		
P53 status						
Negative	499	215(43.1)	214(42.9)	70(14.0)	0.550	0.320
Positive	185	75(40.5)	90(48.6)	20(10.8)		
Intrinsic subtypes						
Luminal A	149	64(43.0)	67(45.0)	18(12.1)	0.489	0.758
Luminal B	273	110(40.3)	122(44.7)	41(15.0)		
HER2-positive	162	69(42.6)	74(45.7)	19(11.7)		
Triple-negative	27	15(55.6)	9(33.3)	3(11.1)		

2). For rs17182023, the GA and AA genotypes significantly lower the risk of breast cancer compared to GG genotype under co-dominate model with ORs of 0.746 (95%CI = 0.597-0.934, $P = 0.010$) and 0.638 (95%CI = 0.465-0.874, $P = 0.005$) respectively, and the result under dominate model was also of statistical significance (OR = 0.719, 95%CI = 0.582-0.888, $P = 0.002$). However, no significant associations were found between the other two SNPs and breast cancer susceptibility.

After adjusting for age, BMI, breastfeeding duration, age at menarche and menopausal status, the results were similar to the above. The minor allele of rs17182023 was associated with reduced breast cancer risk (OR = 0.800 95%CI = 0.684-0.935, $P = 0.005$), and there were also significant correlations between the GA and AA genotypes and lower risk of breast cancer compared with the GG genotype under co-dominate model (OR = 0.755, 95%CI = 0.598-0.953, $P = 0.018$; OR = 0.665, 95%CI = 0.478-0.924, $P = 0.015$, respectively) and dominate model (OR = 0.733, 95%CI = 0.588-0.913, $P = 0.006$).

Correlations between SNPs and clinicopathological characteristics

Next, we analyzed the effect of SNP rs17182023, rs17175624 and rs10958704 on a series of clinico-pathological characteristics in the 747 breast cancer cases, including lymph node metastasis, tumor size, tumor grade, clinic stage, and the status of estrogen receptor (ER) or progesterone

Table 4. The association between SNP locus rs17175624 and clinico-pathological characteristics of breast cancer. Note: ^aUnder dominate model, ^bunder co-dominate model

Clinico-pathological characteristics	Cases	CC(%)	rs17175624 CA(%)	AA(%)	P ^a	P ^b
TNM stage						
I, II	532	244(45.9)	225(42.3)	63(11.8)	0.162	0.375
III	143	75(52.4)	53(37.1)	15(10.5)		
Tumor size(cm)						
≤2	583	272(46.7)	245(42.0)	66(11.3)	0.433	0.603
>2	88	45(51.1)	32(36.4)	11(12.5)		
Pathological stage						
I, II	446	216(48.4)	177(39.7)	53(11.9)	0.731	0.564
III	120	56(46.7)	53(44.2)	11(9.2)		
LNM						
Negative	425	189(44.5)	189(44.5)	47(11.1)	0.311	0.301
Positive	294	142(48.3)	114(38.8)	38(12.9)		
ER status						
Negative	244	112(45.9)	103(42.2)	29(11.9)	0.963	0.981
Positive	447	206(46.1)	186(41.6)	55(12.3)		
PR status						
Negative	319	145(45.5)	133(41.7)	41(12.9)	0.782	0.869
Positive	372	173(46.5)	156(41.9)	43(11.6)		
HER2 status						
Negative	492	226(45.9)	207(42.1)	59(12.0)	0.441	0.581
Positive	119	50(42.0)	51(42.9)	18(15.1)		
Ki67 status						
Negative	258	105(40.7)	115(44.6)	38(14.7)	0.029	0.058
Positive	428	211(49.3)	172(40.2)	45(10.5)		
P53 status						
Negative	499	229(45.9)	210(42.1)	60(12.0)	0.890	0.970
Positive	185	86(46.5)	76(41.1)	23(12.4)		
Intrinsic subtypes						
Luminal A	149	64(43.0)	64(43.0)	21(14.1)	0.683	0.595
Luminal B	273	129(47.3)	110(40.3)	34(12.5)		
HER2-positive	162	73(45.1)	73(45.1)	16(9.9)		
Triple-negative	27	10(37.0)	11(40.7)	6(22.2)		

Table 5. The association between SNP locus rs10958704 and clinico-pathological characteristics of breast cancer. Note: ^aUnder dominate model, ^bunder co-dominate model

Clinico-pathological characteristics	Cases	GG(%)	rs10958704 GA(%)	AA(%)	P ^a	P ^b
TNM stage						
I, II	532	242(45.5)	227(42.7)	63(11.8)	0.237	0.474
III	143	73(51.0)	56(39.2)	14(39.2)		
Tumor size(cm)						
≤2	583	268(46.0)	250(42.9)	65(11.1)	0.365	0.513
>2	88	45(51.1)	32(36.4)	11(12.5)		
Pathological stage						
I, II	446	214(48.0)	179(40.1)	53(11.9)	0.676	0.535
III	120	55(45.8)	54(45.0)	11(9.2)		
LNM						
Negative	425	187(44.0)	192(45.2)	46(10.8)	0.338	0.289
Positive	294	140(47.6)	116(39.5)	38(12.9)		
ER status						
Negative	244	111(45.5)	105(43.0)	28(11.5)	0.984	0.946
Positive	447	203(45.4)	189(42.3)	55(12.3)		
PR status						
Negative	319	144(45.1)	136(42.6)	39(12.2)	0.883	0.982
Positive	372	170(45.7)	158(42.5)	44(11.8)		
HER2 status						
Negative	492	223(45.3)	212(43.1)	57(11.6)	0.515	0.545
Positive	119	50(42.0)	51(42.9)	18(15.1)		
Ki67 status						
Negative	258	103(39.9)	118(45.7)	37(14.3)	0.023	0.056
Positive	428	209(48.8)	174(40.7)	45(10.5)		
P53 status						
Negative	499	226(45.3)	214(42.9)	59(11.8)	0.878	0.949
Positive	185	85(45.9)	77(41.6)	23(12.4)		
Intrinsic subtypes						
Luminal A	149	63(42.3)	65(43.6)	21(14.1)	0.676	0.755
Luminal B	273	128(46.9)	112(41.0)	33(12.1)		
HER2-positive	162	72(44.4)	74(45.7)	16(9.9)		
Triple-negative	27	10(37.0)	12(44.4)	5(18.5)		

receptor (PR), HER2, P53, Ki67, and intrinsic subtypes (Luminal A, Luminal B, HER2-positive, and Triple-negative) (Table 3-5).

According to the results, for SNP rs17175624 and rs10958704, patients of mutant heterozygotes and homozygotes were more likely to have Ki67-negative tumors under dominate model ($P = 0.029$; $P = 0.023$, respectively). However, no correlations were found for the two SNPs with other disease characteristics, including lymph node metastasis, tumor size, tumor grade, clinic stage, and the status of ER or PR, HER2, P53, and intrinsic subtypes. In addition, we did not find associations between rs17182023 and all disease characteristics.

Association of FGFR1 genetic polymorphisms with FGFR1 protein expression

A total of 348 patients were randomly selected from the 747 breast cancer cases for the detection of FGFR1 protein expression levels. The association between the three SNP loci and FGFR1 protein expression was shown in Table 6. For rs17182023, the GA and AA genotypes conferred a significantly lower FGFR1 expression compared to the GG genotype under both dominant and co-dominant model ($P = 0.010$; $P = 0.006$, respectively). Furthermore, we observed a dose-dependent effect of the A allele; each additional copy increased the proportion of FGFR1 low expression patients. The negative correlation between the A allele and FGFR1 protein expression was confirmed by a Spearman rank correlation analysis ($r = -0.162$, $P = 0.002$). Nevertheless, no significant correlations were found between the other two SNP loci and the expression of FGFR1 protein.

Association of FGFR1 expression with disease characteristics and prognosis of breast cancer

In this part, we analyzed the effect of FGFR1 expression on disease characteristics and prognosis of breast cancer. Firstly, the association between clinico-pathological features and FGFR1 level was exhibited in Table 7. According to the results, patients with higher FGFR1 expression were more likely to have ER and HER2 positive breast cancer ($P = 0.022$; $P = 0.024$, respectively), and were prone to lymph node metastasis ($P = 0.028$). Besides, the results of intrinsic subtypes were also of statistical significance, that patients with higher level of FGFR1 tended to have luminal type breast cancer ($P = 0.047$). There were no significant differences between FGFR1 protein expression and other disease characteristics.

Table 6. The correlations between FGFR1 genetic polymorphisms and FGFR1 protein expression. Note: Data presented as the number (% of total number)

SNP	Genotype	N	FGFR1 protein expression		χ^2	P
			Low expression(%)	High expression(%)		
rs17182023	GG	153	47(34.8)	106(49.8)		
	GA	146	62(45.9)	84(39.4)		
	AA	49	26(19.3)	23(10.8)	9.231	0.010
	GA+AA vs GG	195	88(65.2)	107(50.2)	7.497	0.006
rs17175624	CC	169	73(54.1)	96(45.1)		
	CA	138	50(37.0)	88(41.3)		
	AA	41	12(8.9)	29(13.6)	3.327	0.189
	CA+AA vs CC	179	62(45.9)	117(54.9)	2.682	0.102
rs10958704	GG	169	72(53.3)	97(45.5)		
	GA	138	51(37.8)	87(40.8)		
	AA	41	12(8.9)	29(13.6)	2.796	0.247
	GA+AA vs GG	179	63(46.7)	116(54.5)	2.009	0.156

Table 7. The correlations between FGFR1 protein expression and clinico-pathological characteristics of breast cancer

Clinico-pathological characteristics	Cases	FGFR1 protein expression		P
		Low expression(%)	High expression(%)	
TNM stage				
I, II	259	106(79.1)	153(72.9)	0.190
III	85	28(20.9)	57(27.1)	
Tumor size(cm)				
≤2	305	120(90.2)	185(88.1)	0.540
>2	38	13(9.8)	25(11.9)	
Pathological stage				
I, II	187	67(60.9)	120(67.4)	0.261
III	101	43(39.1)	58(32.6)	
LNM				
Negative	182	80(61.5)	102(49.3)	0.028
Positive	155	50(38.5)	105(50.7)	
ER status				
Negative	126	58(48.7)	68(35.6)	0.022
Positive	184	61(51.3)	123(64.4)	
PR status				
Negative	154	64(53.8)	90(47.1)	0.254
Positive	156	55(46.2)	101(52.9)	
HER2 status				
Negative	252	104(94.5)	148(86.0)	0.024
Positive	30	6(5.5)	24(14.0)	
Ki67 status				
Negative	96	36(30.5)	60(31.7)	0.820
Positive	211	82(69.5)	129(68.3)	
P53 status				
Negative	214	79(66.9)	135(71.8)	0.367
Positive	92	39(33.1)	53(28.2)	
Intrinsic Subtypes				
Luminal A	52	17(32.7)	35(67.3)	0.047
Luminal B	137	47(34.3)	90(65.7)	
HER2-positive	79	37(46.8)	42(53.2)	
Triple-negative	14	9(64.3)	5(35.7)	

Next, the Cox proportional hazards model was employed to analyze the impact of specific factors on breast cancer prognosis, including lymph node metastasis, tumor size, tumor grade, clinic stage, and the status of ER or PR, HER2, P53, Ki67, and FGFR1 protein expression (Table 8). The results showed that TNM stage, lymph node metastasis and FGFR1 expression were strongly correlated with the outcomes of breast cancer under univariate analysis, with risk ratios of 1.812 (95% CI = 1.210-2.713, $P = 0.004$), 1.693 (95% CI = 1.219-2.351, $P = 0.002$) and 1.587 (95% CI = 1.055-2.386, $P = 0.027$), respectively. After that, a multivariate analysis was performed, the three prognostic factors which showed statistical significance above were involved. It was found that lymph node metastasis and FGFR1 expression were independent prognostic factors of breast cancer (OR = 1.834, 95%CI = 1.243-2.705, $P = 0.002$; OR = 1.555, 95%CI = 1.027-2.356, $P = 0.037$, respectively), while TNM stage did not show direct correlations to breast cancer prognosis. Besides, the overall survival curve are shown in Fig. 2. Patients with high FGFR1 protein expression were more likely to have poor outcomes ($P = 0.025$).

Discussion

In this study, we focused on three SNP loci in *FGFR1* gene, rs17182023, rs17175624 and rs10958704, and investigated their correlation with susceptibility of breast cancer, and their impact on FGFR1 protein expression. It was found that SNP rs17182023 was significantly associated with reduced risk of breast cancer, and had a certain connection with lower FGFR1 expression. Besides, we also confirmed that high FGFR1 expression was significantly correlated with poor prognosis.

FGFR1 is located at chromosome 8p12, and multiple studies have demonstrated that amplifications at this chromosomal region are found in up to 10% breast carcinomas, and are correlated to *FGFR1* overexpression [18-21]. Likewise, the Cancer Genome Atlas (TCGA) showed that copy number gain / amplification of *FGFR1* was around 10% in breast cancer [17]. The aberrant high expression of *FGFR1* results in excessive activation of MAPK and PI3K-AKT signalling pathways [22, 23], which are strongly associated with oncogenesis and tumor progression. Recent studies have confirmed the correlations between *FGFR1* amplification and breast cancer, and have provided important insights into the relationship between FGFR1 expression, histological subtype and survival. Turner et al. suggested that the amplification and overexpression of *FGFR1* contributed to poor prognosis in luminal-type breast cancers, and drive anchorage-independent proliferation and endocrine therapy resistance [10]. Another research carried out by Elsheikh et al. also concluded that *FGFR1* amplification was an independent predictor of breast cancer prognosis [9]. Our study showed that high FGFR1 protein expression was significantly associated with poor outcome

Table 8. Prognostic factors of breast cancer in Cox proportional hazards model. Note: ^aUnder univariate analysis, ^bunder multivariate analysis. LNM: lymph node metastasis, ER: estrogen receptor, PR: progesterone receptor, HER2: human epidermal growth factor receptor 2

Variables	Risk ratio ^a (95%CI)	P ^a	Risk ratio ^b (95%CI)	P ^b
TNM stage I, II / III	1.812(1.210-2.713)	0.004	1.436(0.859-2.400)	0.168
Tumor size(cm) ≤2 / >2	1.211(0.704-2.082)	0.490		
Pathological stage I, II / III	1.002(0.665-1.512)	0.991		
LNM				
Negative / Positive	1.693(1.219-2.351)	0.002	1.834(1.243-2.705)	0.002
ER status				
Negative / Positive	0.707(0.656-1.331)	0.707		
PR status				
Negative / Positive	0.672(0.657-1.311)	0.672		
HER2 status				
Negative / Positive	1.384(0.701-2.733)	0.350		
Ki67 status				
Negative / Positive	1.193(0.824-1.726)	0.350		
P53 status				
Negative / Positive	1.116(0.744-1.674)	0.595		
FGFR1 protein expression				
Low expression / High expression	1.587(1.055-2.386)	0.027	1.555(1.027-2.356)	0.037

Fig. 2. Kaplan-Meier analysis for overall survival (OS) in breast cancer patients, based on FGFR1 protein expression.

in breast cancer patients, which was consistent with the previous studies. Based on these findings, further studies are warranted to determine the utility of FGFR1 expression analysis as a prognostic marker for breast cancer treatment.

FGFR1 amplification is frequent in luminal-type breast cancers [9, 10, 24, 25], and a number of studies have demonstrated that ER+ / FGFR1-amplified breast cancer cells and tumors retain proliferation and ERα genomic activity despite estrogen deprivation therapy. It is reported that one third of FGFR1-amplified tumors also harbor amplification of *CCND1*, *FGF3*, *FGF4*, and *FGF19* [26]. The *FGF3/4/19* genes are located on chromosome 11q13, a region which also harbors *CCND1*, and is amplified in up to 15% of breast cancers [17]. This co-amplification has also been associated with resistance to estrogen deprivation in ER+ breast cancer and poor patient outcome. Interestingly, Hanker et al. found a copy number gains of *FGF3/4/19* gene and evidence of increased FGFR signaling in HER2-amplified cell lines [27]. Preclinical and clinical evidence indicated that ER+ tumors with HER2 amplification responded poorly to endocrine therapy, particularly tamoxifen, probably due to the crosstalk between ER and HER2 signaling pathways [28, 29]. With the activation of HER2 signalling, tamoxifen may lose its estrogen antagonist activity and may acquire more agonist-like activity, resulting in tumor growth stimulation [30]. In this study, we observed that patients with higher FGFR1 protein expression were more likely to have ER+ / HER2+ breast cancers, and this result showed the links between FGFR1 overexpression and HER2 amplification. Combining with the above, this might explain why FGFR1 overexpression contributes to endocrine therapy resistance to some extent.

SNP rs17182023 is located in the intronic region, near the 5' end of *FGFR1* gene. To date, the biological function of this locus remains unclear, and there's few reports about its influence on breast cancer. Our study revealed that SNP rs17182023 in *FGFR1* gene was strongly associated with breast cancer susceptibility, and interestingly, it showed a tendency of reducing the risk. Furthermore, we also found a correlation between the SNP locus and FGFR1 protein expression, that the patients with mutant genotypes had a lower FGFR1 level. It had been reported that some intronic variants could effect the expression of coding genes, via exon deletion, activation of the recessive splice sites and (or) the change of shear effect [31]. Since rs17182023 was located in intron region of *FGFR1*, it might effect FGFR1 protein expression in these ways. Therefore, we proposed a possibility that SNP rs17182023 could act as a protective factor on reducing breast cancer risk, via declining FGFR1 protein expression. This might provide a new sight to understand the association of genetic polymorphisms with breast tumorigenesis, but further studies are still required to illuminate the mechanisms. Additionally, we did not find associations of SNP rs17175624 and rs10958704 with the risk of breast cancer. Although patients with mutant genotypes of these two SNPs were more likely to have Ki67-negative tumors under dominate model according to the results, this could hardly be considered as a result of significance.

In conclusion, high FGFR1 protein expression was an independent risk factor of breast cancer, and implicated to poor prognosis. We evaluated three SNP loci, rs17182023, rs17175624 and rs10958704 in *FGFR1* gene, and revealed the association between rs17182023 and the reduction of breast cancer risk. Moreover, SNP rs17182023 also correlated to FGFR1 protein expression, and its negative effect on FGFR1 expression may be a protective factor on breast cancer. Although this study might provide new insights to understand the relationships between breast carcinogenesis and FGFR1, and might contributes to the prognostic evaluation for breast cancer patients, these results still await further confirmation by ethnicity-matched larger studies.

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Disclosure Statement

The authors declare that they have no conflict of interests. The manuscript has been read and approved by all named authors.

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