ASSOCIATION OF POLYMORPHISMS IN INTRON 2 OF *FGFR2* AND BREAST CANCER RISK IN CHINESE WOMEN

Z. PAN[1], Y. BAO[1], X. ZHENG[1], W. CAO[2], W. CHENG[1], X. XU [1]*

[1] Clinical Laboratory Department, Zhejiang Cancer Hospital, Hangzhou, China
[2] Department of Medical Oncology, Zhejiang Cancer Hospital, Hangzhou, China
E-mail: zjhzxxh@163.com

Recent genome-wide association studies (GWAS) demonstrated that genetic variation in intron 2 of fibroblast growth factor receptor 2 (FGFR2) was a novel risk for breast cancer. We investigated whether two SNPs rs1219648 and rs2981582 in intron 2 of FGFR2 were associated with the risk of breast cancer in Chinese women. A total of 340 female breast cancer patients and 400 normal age-matched controls were recruited. Two SNPs were genotyped using matrix-assisted laser desorption/ionization mass spectrometry. The two SNPs rs1219648 and rs2981582 showed no association with the risk of breast cancer. A subgroup analysis by menopausal status demonstrated that the distribution of rs2981582 T alleles, including CT and TT genotypes, was significantly higher in premenopausal patients compared with postmenopausal patients. The TT genotype in rs2981582 was more strongly associated with ER-positive than with ER-negative tumors by ER status analysis. Analysis by haplotypes showed that no haplotypes associated with breast cancer. The results showed no association between two SNPs, rs1219648 and rs2981582 and breast cancer risk, although in a stratified analysis rs2981582 strongly associated with premenopausal and ER-positive breast cancer patients in Chinese women.

***Key words**: breast cancer, FGFR2 gene, single nucleotide polymorphism, association analysis.*

Introduction. Breast cancer is one of the most common malignancies in women worldwide. It was estimated that in the United States approximately 192,000 female patients would be diagnosed with breast cancer and 40,000 would die from it in 2009 [1]. In China, breast cancer has become one of the most common cancers among women. Environment, genetics and immunological defects are major factors in the etiology of breast cancer [2].

Apart from rare high-penetrance mutations in a small number of susceptibility genes such as breast cancer 1, early onset (*BRCA1*) and *BRCA2*, more common but lower risk genetic variants also account for the increased risk of breast cancer. FGFR2 is a member of the fibroblast growth factor receptor family and plays pro-oncogenic and anti-oncogenic roles in a context-dependent manner [3]. Mutation, amplification or over-expression of *FGFR2* occurs in breast, lung and gastric cancers [4, 5]. Additionally, genetic variation of *FGFR2* is a risk factor for breast cancer. Genome-wide association studies have revealed that SNPs in intron 2 of *FGFR2* were highly associated with breast cancer, and identified as susceptibility loci. Fine-scale genetic mapping and resequencing of intron 2 of *FGFR2* identified up to eight variants in a linkage disequilibrium (LD) block that was strongly related to increased breast cancer risk [6]. Other GWAS identified a set of four SNPs (including rs1219648 and rs2981582) in intron 2 of *FGFR2* that were highly associated with breast cancer and confirmed this association in other studies [7, 8]. It was reported that the SNP rs2981582 in the second intron of *FGFR2* was significantly associated with an increased risk of breast cancer in a dose-dependent manner, with a higher risk of breast cancer in homozygous compared with heterozygous carriers. For rs2981582, the estimated odds ratios were greater for a diagnosis of breast cancer before 40 years of age [9].

Two different meta-analyses suggested that *FGFR2* polymorphisms are important markers for breast cancer susceptibility among Caucasian, Asian and mixed ethnicities [10, 11]. However, association analyses between rs1219648 and rs2981582 and breast cancer risk in Chinese women have shown varying results. Chen et al. [12] reported that the genotype frequencies of rs1219648 were not significantly different between breast cancer patients and healthy controls. Liu et al. [13] investigated four SNPs and found a statistically significant difference only in the frequency of rs2981582 between case and control groups. However, when subjects were stratified by menopausal status the SNP rs1219648 CC was significantly associated with the risk of breast cancer in postmenopausal patients.

Our case-control study investigated these two SNPs (rs1219648 and rs2981582) in 340 cases and 400 controls of the same ethnic group to investigate

the association between *FGFR2* polymorphisms and the risk of breast cancer.

Materials and methods. *Study population.* This study was approved by the Ethics Committee of Zhejiang Cancer Hospital. A total of 340 breast cancer patients (aged 27–84 years; mean age 46 years) were recruited for this study from May 2011 to September 2013. All diagnoses were confirmed based on pathological examination. Detailed clinicopathological information including tumor size, histological grade, lymph node involvement, as well as ER, progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) status were obtained from patients' medical records (Table 1). Approximately 400 cancer-free women, matched to the cases by age (± 5 years) and residential area, were randomly selected for controls.

DNA extraction and genotyping. Genomic DNA was extracted from peripheral blood samples using a blood Genomic DNA extraction kit (Xinjin Genetech Ltd., Corp., Hangzhou, China) according to the manufacturer's protocol. Genotyping for the two selected *FGFR2* SNPs (rs1219648 and rs2981582) was performed on these DNA samples with Sequenom's MassARRAY Iplex platform (San Diego, CA 92121, USA) using an allele-specific MALDI-TOF mass spectrometry assay [14]. Primers for amplification and extension reactions were designed according to the guidelines of Sequenom and performed using MassARRAY Assay Design software. All procedures were performed according to Sequenom standard protocols. The average genotype call rate for these SNPs was >99 % and concordance rate for all the SNPs was 100 %.

Statistical analysis. Deviation from Hardy-Weinberg equilibrium (HWE) was examined in the controls by the chi-squared test. The following statistical analyses were performed using SNPStats software (http://bioinfo.iconcologia.net/SNPstats) [15]. Using the logistic regression method, the case-control association of genotypes tested, and the odds ratio (OR) and 95 % confidence interval (CI) were obtained. The association analysis of haplotypes was similar to that of genotypes with logistic regression, and results are shown as OR and 95 % CI. The most frequent haplotype was automatically selected as the reference category. The significance level of these tests was 0.05. Heterogeneity between the associations of SNPs with ER-positive and ER-negative diseases was assessed using logistic regression analysis restricted to cases (cases-only analysis).

Results. The genotype frequencies of rs1219648 were 36 % AA, 47 % AG, and 17 % GG, and for

Table 1. **Clinicopathological characteristics of breast cancer patients and controls**

Characteristics	Cases (%)	Characteristics	Cases (%)
Age (years)		Lymph node status	
≤ 50	200 (59.5)	negative	116 (34.5)
controls	246 (63.1)	positive	213 (63.4)
> 50	136 (40.5)	missing	7 (2.1)
controls	144 (36.9)	ER status	
Menopausal status		negative	114 (33.9)
premenopausal	203 (60.4)	positive	211 (62.8)
postmenopausal	133 (39.6)	missing	11 (3.3)
Tumor size (cm)		PR status	
≤ 2	59 (17.6)	negative	135 (40.2)
> 2	238 (70.8)	positive	190 (56.5)
Missing	39 (11.6)	missing	11 (3.3)
Histological grade		HER2 status	
I+II	137 (40.8)	negative	201 (59.8)
III	82 (24.4)	positive	114 (33.9)
Missing	117 (34.8)	missing	21 (6.3)

Note. Missing the number (%) of cases for which the corresponding information was not available.

rs2981582 were 44 % CC, 47 % CT, 9 % TT in controls. The Hardy-Weinberg equilibrium analysis of these two SNPs showed no significant deviations from the control group (P > 0.05).

Logistic regression analysis showed that compared with the AA genotype of rs1219648, those with AG and GG genotype had a hazard ratio of 1.22 (95 % CI: 0.88–1.68) and 0.79 (95 % CI: 0.5–1.25) respectively, suggesting that the SNP rs1219648 did not correlate with the risk of breast cancer (P > 0.05). Similarly, the genotype distributions of SNP rs2981582 were not significantly different between cases and controls (P > 0.05) (Table 2).

To further characterize the significance of rs1219648 and rs2981582 in breast cancer, we analyzed their association with various clinicopathological characteristics including ER, PR, HER2 and patient menopausal status. For rs2981582, the CT and TT genotype presented a significantly higher distribution in premenopausal patients compared with postmenopausal patients with OR 1.65 (95 % CI: 1.05–2.61, P < 0.05). In the ER status analysis, the TT genotype in rs2981582 was more strongly associated with ER-positive tumors than with ER-negative tumors, although the statistical significance of this association was marginal (P = 0.05). No further significant associations were observed between these two SNPs and other clinicopathological features (Table 3).

We also evaluated the association between the haplotype of the two SNPs and breast cancer. Association analysis showed that no haplotypes was associated with breast cancer in Chinese (data not shown).

Discussion. In this study, we genotyped two SNPs, rs1219648 and rs2981582, in intron 2 of *FGFR2* that was associated with breast cancer in previous GWAS study. Two previous GWAS studies showed that SNPs in intron 2 of *FGFR2* were significantly associated with breast cancer risk in European women [7, 9]. These results were extended to include Ashkenazi and Sephardi Jews, which appeared to have a modest risk of breast cancer associated with these SNPs in Middle Eastern populations [8].

A number of studies have focused on the risk of breast cancer associated with SNPs in Chinese women. Chen et al. [12] showed that rs1219648 was not associated with breast cancer risk in women of the Chinese Han ethnic group. Liu et al. study found that rs2981582 but not rs1219648 was significantly associated with the risk of breast cancer. However, Long et al. [16] reported that both rs2981582 and rs1219648 showed significant associations with breast cancer risk in Chinese women in a large sample size study. Our results demonstrated that these two SNPs rs1219648 and rs2981582 have no association with breast cancer risk. This finding was in agreement with Chen et al. [12], which found no association between rs1219648 and the risk of breast cancer.

A previous analysis by menopausal status, showed that the two SNPs studied here were strongly associated with the risk of breast cancer in postmenopausal patients, but there was no significant association

Table 2. **Genotype frequencies of FGFR2 rs1219648 and rs2981582 in breast cancer patients and control groups**

Genotype	Controls (%)	Cases (%)	OR (95% CI)	*P* value
rs1219648				
A/A	142 (36.2)	114 (33.9)	1.00	–
A/G	184 (46.9)	180 (53.6)	1.22 (0.88–1.68)	142 (36.2)
G/G	66 (16.8)	42 (12.5)	0.79 (0.5–1.25)	0.32
A/G+G/G	250 (63.8)	222 (66.1)	1.11 (0.81–1.50)	0.52
rs2981582				
C/C	169 (44)	148 (47.3)	1.00	–
C/T	180 (46.9)	143 (45.7)	0.91 (0.66–1.24)	0.54
T/T	35 (9.1)	22 (7)	0.72 (0.40–1.28)	0.29
C/T+T/T	215 (56.2)	165 (52.7)	0.88 (0.65–1.18)	0.39

Note. Here and Table 3 OR: odds ratio; 95%CI: 95 % confidence interval.

Table 3. **Stratified analysis between *FGFR2* rs121964, rs2981582 and clinicopathological features of breast cancer patients**

Characteristics	rs1219648			
	A/A	A/G	G/G	A/G+G/G
Menopause				
post/premenopausal	49/65	71/109	13/29	84/138
OR(95%CI)	1.00	1.16(0.72−1.86)	1.68(0.79−3.57)	1.24(0.78−1.96)
P value		0.54	0.17	0.36
ER status				
Neg/pos	44/66	59/115	11/30	70/145
OR(95%CI)	1.00	1.30(0.79−2.13)	1.82(0.83−4.00)	1.38(0.86−2.22)
P value		0.29	0.13	0.18
PR status				
Neg/pos	48/62	73/101	14/27	87/128
OR(95%CI)	1.00	1.07(0.66−1.74)	1.49(0.71−3.15)	1.14(0.72−1.81)
P value		0.78	0.29	0.58
HER2 status				
Neg/pos	67/40	107/62	27/12	134/74
OR(95%CI)	1.00	0.97(0.59−1.60)	0.74(0.34−1.63)	0.93(0.57−1.50)
P value		0.91	0.46	0.75
Characteristics	rs2981582			
	C/C	C/T	T/T	C/T+T/T
Menopause				
post/premenopausal	68/80	48/95	8/14	56/109
OR(95%CI)	1.00	1.68(1.05−2.70)	1.49(0.59−3.76)	1.65(1.05−2.61)
P value		0.03	0.39	0.03
ER status				
Neg/pos	56/85	44/95	4/18	48/113
OR(95%CI)	1.00	1.42(0.87−2.33)	2.96(0.95−9.22)	1.55(0.96−2.50)
P value		0.16	0.05	0.07
PR status				
Neg/pos	61/80	55/84	5/17	60/101
OR(95%CI)	1.00	1.16(0.72−1.87)	2.59(0.91−7.42)	1.28(0.81−2.04)
P value		0.53	0.06	0.29
HER2 status				
Neg/pos	85/54	87/46	15/6	102/52
OR(95%CI)	1.00	0.83(0.51−1.36)	0.63(0.23−1.72)	0.80(0.50−1.29)
P value		0.47	0.36	0.37

in premenopausal subjects [13]. However, another report indicated that the association between three SNPs (including the two studied here) and the risk of breast cancer appeared to be strongest in premenopausal Chinese women [17]. In our study, rs2981582 TT and CT genotypes were more significantly associated with premenopausal patients than the CC genotype. Although we found no significant difference for the TT genotype between premenopausal and postmenopausal patients, this

study included only a few cases of premenopausal status and we cannot exclude the possibility that this masked any effect. Our results were similar to Liang et al. [17], which showed a stronger association in premenopausal women compared with postmenopausal women.

Additional analysis by ER status has shown that *FGFR2* variants (rs1200014, rs1219648, rs2420946, and rs2981582) were significantly associated with risk of ER-positive early-onset breast cancer but not with ER-negative breast cancer [18]. Other studies have demonstrated that rs1219648 and rs2981582 were strongly associated with ER-/PR-positive tumors and that rs2981582 appeared to have a much stronger association with ER-positive than ER-negative breast cancer [19]. A number of reports on SNPs and clinicopathological features have also confirmed that the two SNPs rs1219648 and rs2981582 were associated with breast cancer risk predominantly among ER-positive tumors [20–24]. In this study, rs2981582 was more strongly associated with ER-positive breast cancer than with ER-negative breast cancer, although the statistical significance of this association is marginal.

A haplotype block is a distinct section of a chromosome containing SNPs proven to be statistically linked or inherited together. The SNP rs2981582 lies within a 25-kb LD block in intron2 of *FGFR2* and has an r^2 of 1.0 with rs1219648 based on the HapMap Central European-like Utahns samples [25]. We also confirmed that rs2981582 and rs1219648 are in an LD block, which had an r^2 of 0.9 (data not shown). By haplotype analysis, we did not identified haplotype associated with breast cancer risk in Chinese.

Conclusion. In summary, the study showed that two SNPs, rs1219648 and rs2981582, in intron 2 of *FGFR2* had no association with breast cancer risk in Chinese women. A stratified analysis by clinicopathological characteristics demonstrated that the distribution of rs2981582 T alleles (including CT and TT genotypes) was significantly higher in premenopausal patients compared with postmenopausal patients. This analysis also showed that the TT genotype was also strongly associated with ER-positive tumors. The limitation in this study is its relatively small sample size. A study with a larger sample size is needed to confirm the associations between these SNPs in *FGFR2* and breast cancer risk.

ЗАВИСИМОСТЬ ПОЛИМОРФИЗМА ИНТРОНА 2 ИЗ *FGFR2* И РИСКА РАКА ГРУДИ У КИТАЙСКИХ ЖЕНЩИН

Z. Pan, Y. Bao, X. Zheng, W. Cao, W. Cheng, X. Xu

Недавние исследования с помощью GWAS показали, что генетическая изменчивость интрона 2 рецептора фактора роста фибробластов *FGFR2* является еще одним риском возникновения рака груди. Мы исследовали, ассоциированы ли SNPs rs1219648 и rs2981582 в интроне 2 из FGFR2 с риском рака груди у китайских женщин. Всего исследовали 340 пациенток и 400 здоровых женщин такого же возраста. Два SNPs были генотипированы с использованием matrix-assisted laser desorption/ionization масс-спектрометрии. Оба SNPs, rs1219648 и rs2981582, не показали связи с риском рака груди. Анализ подгрупп по статусу менопаузы показал, что распределение аллелей rs2981582 T, включая CT и TT генотипы, было существенно выше у пациенток с предменопаузой по сравнению с пациентками с постменопаузой. Генотип TT у rs2981582 был больше ассоциирован с ER-позитивными, чем с ER-негативными опухолями согласно анализу статуса ER. Анализ гаплотипов показал, что они не связаны с раком груди. Результаты не выявили связи между двумя SNPs, rs1219648 и rs2981582, и риском рака груди, хотя по данным стратифицированного анализа rs2981582 точно связан с ER-позитивными пациентками с раком груди и пременопаузой у китайских женщин.

REFERENCES

1. Jemal, A., Sieger, R., Ward, E., Hao, Y., Xu, J., and Thun, M.J., Cancer statistics 2009, *CA Cancer J. Clin.,* 2009, vol. 59, no. 4, pp. 225–249.
2. Pharoah, P.D., Dunning, A.M., Ponder, B.A., and Easton, D.F., Association studies for finding cancer-susceptibility genetic variants, *Nat. Rev. Cancer*, 2004, vol. 4, no. 11, pp. 850–860.
3. Katoh, Y., and Katoh, M., FGFR2-related pathogenesis and FGFR2-targeted therapeutics (Review), *Int. J. Mol. Med.*, 2009, vol. 23, no. 3, pp. 307–311.
4. Davies, H., Hunter, C., Smith, R. et al., Somatic mutations of the protein kinase gene family in human lung cancer, *Cancer Res.*, 2005, vol. 65, no. 17, pp. 7591-7595.
5. Jang, J.H., Shin, K.H., and Park, J.G., Mutations in fibroblast growth factor receptor 2 and fibroblast growth factor receptor 3 genes associated with human gastric and colorectal cancers, *Cancer Res.*, 2001, vol. 61, no. 9, pp. 3541–3543.
6. Udler, M.S., Meyer, K.B., Pooley, K.A. et al., FGFR2 variants and breast cancer risk: fine-scale mapping using African American studies and analysis

of chromatin conformation, *Hum. Mol. Genet.*, 2009, vol. 18, no. 9, pp. 1692–1703.
7. Hunter, D.J., Kraft, P., Jacobs, K.B. et al., A genome-wide association study identifies alleles in FGFR2 associated with risk of sporadic postmenopausal breast cancer, *Nat. Genet.*, 2007, vol. 39, no. 7, pp. 870–874.
8. Raskin, L., Pinchev, M., Arad, C. et al., FGFR2 is a breast cancer susceptibility gene in Jewish and Arab Israeli populations, *Cancer Epidemiol. Biomarkers Prev.*, 2005, vol. 17, no. 5, pp. 1060–1065.
9. Easton, D.F., Pooley, K.A., Dunning, A.M. et al., Genome-wide association study identifies novel breast cancer susceptibility loci, *Nature*, 2007, vol. 447, no. 7148, pp. 1087–1093.
10. Jia, C., Cai, Y., Ma, Y., and Fu, D., Quantitative assessment of the effect of FGFR2 gene polymorphism on the risk of breast cancer, *Breast Cancer Res. Treat.*, 2010, vol. 124, no. 2, pp. 521–528.
11. Zhang, J, Qiu, L.X., Wang, Z.H. et al., Current evidence on the relationship between three polymorphisms in the FGFR2 gene and breast cancer risk: a meta-analysis, *Ibid,* pp. 419–424.
12. Chen, X.H., Li, X.Q., Chen, Y., and Feng, Y.M., Risk of aggressive breast cancer in women of Han nationality carrying TGFB1 rs1982073 C allele and FGFR2 rs1219648 G allele in North China, *Breast Cancer Res. Treat.*, 2011, vol. 125, no. 2, pp. 575–582.
13. Liu, C.L., Hu, X.P., Guo, W.D., Yang, L., Dang, J., and Jiao, H.Y., Case-control study on the fibroblast growth factor receptor 2 gene polymorphisms associated with breast cancer in chinese han women, *J. Breast Cancer*, 2013, vol. 16, no. 4, pp. 366–371.
14. Long, J., Shu, X.O., Cai, Q. et al., Evaluation of breast cancer susceptibility loci in Chinese women, *Cancer Epidemiol. Biomarkers Prev.*, 2010, vol. 19, pp. 2357–2365.
15. Liang, J., Chen, P., Hu, Z. et al., Genetic variants in fibroblast growth factor receptor 2 (FGFR2) contribute to susceptibility of breast cancer in Chinese women, *Carcinogenesis*, 2008, vol. 29, no. 12, pp. 2341–2346.
16. Fu, F., Wang, C., Huang, M., Song, C., Lin, S., and Huang, H., Polymorphisms in second intron of the FGFR2 gene are associated with the risk of early-onset breast cancer in Chinese Han women, *Tohoku J. Exp. Med.*, 2012, vol. 226, no. 3, pp. 221–229.
17. Garcia-Closas, M., Hall, P., Nevanlinna, H. et al., Heterogeneity of breast cancer associations with five susceptibility loci by clinical and pathological characteristics, *PLoS Genet.*, 2008, vol. 4, no. 4, e1000054.
18. Slattery, M.L., John, E.M., Stern, M.C. et al., Associations with growth factor genes (FGF1, FGF2, PDGFB, FGFR2, NRG2, EGF, ERBB2) with breast cancer risk and survival: the Breast Cancer Health Disparities Study, *Breast Cancer Res. Treat.*, 2013, vol. 140, no. 3, pp. 587–601.
19. Cen, Y.L., Qi, M.L., Li, H.G. et al., Associations of polymorphisms in the genes of FGFR2, FGF1, and RBFOX2 with breast cancer risk by estrogen/progesterone receptor status, *Mol. Carcinog.*, 2013, vol. 52, Suppl 1, E52–59.
20. Chan, M., Ji, S.M., Liaw, C.S. et al., Association of common genetic variants with breast cancer risk and clinicopathological characteristics in a Chinese population, *Breast Cancer Res. Treat.*, 2012, vol. 136, no. 1, pp. 209–220.
21. Tapper, W., Hammond, V., Gerty, S. et al., The influence of genetic variation in 30 selected genes on the clinical characteristics of early onset breast cancer, *Breast Cancer Res.*, 2008, vol. 10, no. 6, R108.
22. Campa, D., Kaaks, R., Le Marchand, L. et al., Interactions between genetic variants and breast cancer risk factors in the breast and prostate cancer cohort consortium, *J. Natl Cancer Inst.*, 2011, vol. 103, no. 16, pp. 1252–1263.
23. Kawase, T., Matsuo, K., Suzuki, T., Hiraki, A., Watanabe, M., Iwata, H., Tanaka, H., and Tajima, K., FGFR2 intronic polymorphisms interact with reproductive risk factors of breast cancer: results of a case control study in Japan, *Int. J. Cancer*, 2009, vol. 125, no. 8, pp. 1946–1952.
24. Jurinke, C., van den Boom, D., Cantor, C.R., and Koster, H., Automated genotyping using the DNA MassArray technology, *Methods Mol. Biol.*, 2002, vol. 187, pp. 179–192.
25. Sole, X., Guino, E., Valls, J., Iniesta, R., and Moreno, V., SNPStats: a web tool for the analysis of association studies, *Bioinformatics*, 2006, vol. 22, no. 15, pp. 1928–1929.

Received 23.08.15