A germline alteration of *ERBB2* increases the risk of breast cancer in Chinese Han women with a familial history of malignant tumors

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Abstract. Previous studies have demonstrated that a family history of breast cancer is considered a risk factor, and hereditary factors may be involved in breast cancer pathogenesis. Next-generation sequencing techniques were used to analyze 111 cancer-associated genes in patients with breast cancer with a familial history of malignant tumors in the pre-experiment and a novel variant, *receptor tyrosine-protein kinase erbB-2* (*ERBB2)* c.338G>A: p.R113Q was identified in two cases of breast cancer. *ERBB2* is considered an important oncogene, and overexpression or mutation of the *ERBB2* gene may lead to the occurrence or metastasis of tumors. To assess a potential association between rs185670819 and breast cancer, 117 patients with breast cancer and a familial history of any cancer, who were diagnosed by experienced pathologists at the Xijing Hospital (Shaanxi, China) between July 2015 and December 2016, were recruited. The presence of the missense variant was confirmed using bi-directional Sanger sequencing of samples from the patients with breast cancer and 250 healthy controls. The effects of the missense mutation on the structure and function of *ERBB2* were analyzed *in silico*. The missense variant, R113Q, in patients with breast cancer with a familial history of malignant tumors in China, was present in 8 patients [6.8% (95% CI: 3.21-13.45)] and 3 of 250 healthy controls [1.2% (95% CI: 0.31-3.76; OR=6.04, 95% CI: 1.573-23.214, P=0.009)]. Of the 8 patients with the R113Q variant, 6 patients had a family history of cancer of the digestive system. The present study suggests that *ERBB2* c.338G>A: p.R113Q may be a potential risk factor in the development and progression of breast cancer.

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Introduction

Breast cancer is the most common malignancy and the leading cause of cancer-associated mortality in women worldwide (1). Genetic alterations serve a critical role in the etiology of cancer, disrupting gene function and increasing the risk of cancer (2). Germline mutations in the *BRCA1* and *BRCA2* genes are the most common causes of breast cancer susceptibility, with *BRCA1* conferring a risk of breast cancer of ~67% and 66% for *BRCA2*, by the age of 80 (3). Although other moderate-to-low-penetrance genes, such as *CHEK2*, *PALB2* and *ATM*, serve an important role in breast cancer predisposition (4), *BRCA1/2* mutations account for 10% of all cases in the Chinese population (5).

Previous studies have indicated that a family history of other types of cancer, such as colon cancer, prostate cancer, and ovarian cancer, is associated with an increased risk of breast cancer occurrence (6-8). However, studies on the presence of germline mutations in patients with breast cancer with a family history of tumors are limited, to the best of our knowledge. To determine a comprehensive spectrum of genetic factors for patients with breast cancer with a family history of malignant tumors in the Chinese population, targeted exome sequencing was performed (the judgment standard of next-generation-sequencing is shown in Appendix S1) and 111 cancer-associated genes from 13 patients with breast cancer with a family history of malignant tumors were identified. In addition, a novel allele, *ERBB2* c.338G>A: p.R113Q that was enriched in two cases of breast cancer was identified.

Receptor tyrosine-protein kinase ebb (HER/ERBB)2 is part of the *ERBB*-like oncogene family and is considered a critical oncogene, mutations of which may initiate the onset of breast cancer. HER2 is capable of forming a homodimer on its own or heterodimers with other receptors, including HER1 and HER3, which results in the autophosphorylation of tyrosine residues of the kinase domains. These reactions subsequently trigger intracellular signaling cascades, including mitogen-activated protein kinase, phosphoinositide 3-kinase, protein kinase C

and signal transducer and activator of transcription, which primarily promote cell proliferation and prevent apoptosis (9).

Next-generation sequencing studies have demonstrated that somatic mutations in *ERBB2* are present in a considerable number of tumors, including in breast cancer (10,11). Overexpression gene editing experiments have designated a number of these mutations as activating and oncogenic elements, that is, acting as carcinogens (11,12). Therefore it is hypothesized that *ERBB2* c.338G>A: p.R113Q may be a genetic risk factor of breast cancer. The aim of the present study was to sequence the loci and determine whether there was an association between rs185670819 and breast cancer risk.

Materials and methods

Patients. A total of 117 patients with breast cancer with a familial history of cancer, all of whom were diagnosed by experienced pathologists at The Xijing Hospital (Shaanxi, China) were recruited for the present study between July 2015 and December 2016. The inclusion criteria were as follows: i) Women aged between 18 and 74 years old with primary breast cancer, regardless of histological type or stage; and ii) patients had at least one first-degree, or two second-degree relatives with cancer (no limitation on tumor type). Exclusion criteria were as follows: i) Patients with breast cancer with no familial history of cancer; and ii) patients with breast cancer without informed consent forms. Within the same time period, a total of 250 healthy volunteers were included in the study as a control group. The mean ages of the case and control groups were 52.5±9.7 and 52.1±9.9 years, respectively. All participants enrolled in our research were from unrelated families and provided written informed consent. The Ethics Committee of Xijing Hospital approved the study protocol. Peripheral blood samples were drawn and stored in EDTA-coated tubes at -80˚C.

DNA extraction and genotyping. DNA was extracted from peripheral blood using commercially available kits, in accordance with the manufacturer's protocol (E.Z.N.A.® Blood DNA Mini kit, Omega Bio-Tek, Norcross, GA, USA). A 300 bp fragment within the extracellular domain of *ERBB2* was amplified using PCR. Primer sequences were: Forward 5'-ACGTGCTCA TCGCTCACAAC-3' and reverse 5'-CCCAGAAGGGACACC ATTTC-3'. The PCR product was confirmed using electrophoresis in a 1.5% agarose gel and subsequently purified using the E.Z.N.A.® Gel Extraction kit (Omega Bio-Tek). The purified PCR product sequencing was performed with a BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems; Thermo Fisher Scientific, Inc., Waltham, MA, USA) on the 3730xl DNA Analyzer system (Applied Biosystems; Thermo Fisher Scientific, Inc.), and the data obtained were analyzed with the 3730xl Genetic Analyzer Data Collection Software v3.0 (Applied Biosystems; Thermo Fisher Scientific, Inc.).

In silico analysis of ERBB2 encoded by the ERBB2 c.338G>A variant. The scores of SIFT (13), PolyPhen v2 (14), MutationTaster (15), and GEPR++ (16) was collected from dbNSFP (version 2.6) (17) to predict the possible impact of an amino acid substitution on the structure and the function of HER2. PyMOL 2.0 software (https://pymol.org/2/) (18) was used for the analysis of the 3D structure of HER2 encoded by rs185670819. Conservation analysis among a range of vertebrate species was performed using data obtained from University of California Santa Cruz Genome Browser [Human Dec. 2013 (GRCh38/hg38) assembly; http://genome.ucsc.edu] (19).

Table I. Clinical and pathological characteristics of patients with breast cancer.

Characteristic	Value
Mean age ± SD, years	52.5 ± 9.7
Stage, n (%)	
I	34 (29.1)
IIA	44 (37.6)
IIB	14 (12.0)
IIIA	21 (17.9)
IIIC	3 (2.6)
IV	1 (0.9)
Tumor size, n (%)	
≤2 cm	47 (40.2)
>2 cm	70 (59.8)
Lymph nodes, n (%)	
Negative	67 (57.2)
Positive	50 (42.8)
Distant metastasis, n (%)	
Negative	116 (99.1)
Positive	1 (0.9)
Pathology, n (%)	
Invasive ductal carcinoma	103 (88)
Ductal carcinoma *in situ*	8 (6.8)
Invasive lobular carcinoma	2 (1.7)
Invasive mucinous carcinoma	2 (1.7)
Invasive medullary carcinoma	2 (1.7)
HER2 expression, n (%)	
Negative (score 0 and 1)	54 (46.2)
Uncertain (score 2)	25 (21.4)
Positive (score 3)	27 (23.1)
Unknown	11 (9.4)

HER2, receptor tyrosine-protein kinase erbB-2.

Statistical analysis. A χ^2 test was used to assess the differences in genotype frequencies between cases and controls. A one-sided P-value and odds ratios (OR) with 95% confidence interval (95% CI) were calculated to demonstrate an association between the allelic *ERBB2* and breast cancer risk. P<0.05 was considered to indicate a statistically significant difference. The Hardy-Weinberg equilibrium (HWE) test was performed to confirm the samples are representative of the population according to a method previously described (20).

Results

Study population. To examine whether *ERBB2* c.338G>A: p.R113Q was a potential risk factor for patients with breast

Table II. Variant of *ERBB2* present in Chinese patients with breast cancer.

Base change	Protein change	Variant class	Genotype	Frequency in patients (%)	Frequency in controls (%)	P-value	OR (95% CI)	Hardy-weinberg equilibrium
NM_001005862.2: c.338G>A	p.Arg 113Gln	Missense	heterozygous	8/117 (6.8)	3/250 (1.2)	0.009	6.04 (1.573-23.214)	0.70

ERBB2, *receptor tyrosine-protein kinase erbB-2*; OR, odds ratio; CI, confidence interval.

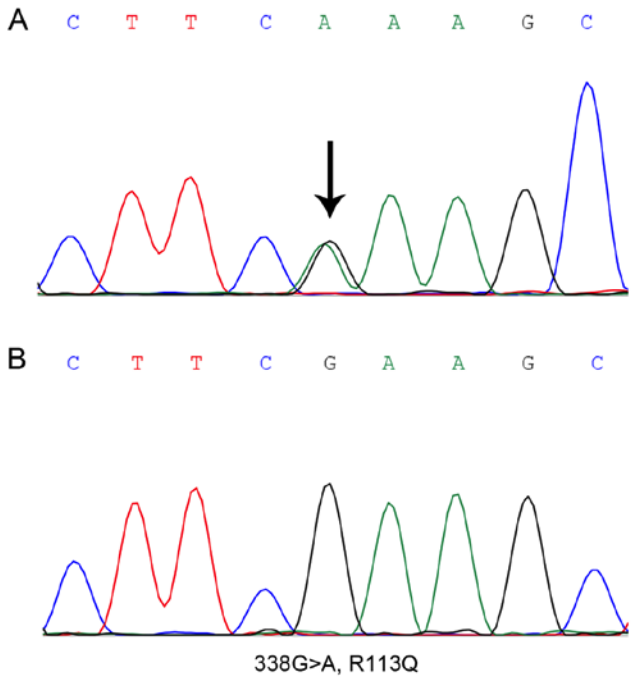

Figure 1. R113Q allele identified in the *ERBB2* gene. (A) The wild-type sequences of *ERBB2*. (B) R113Q allele detected in the present sudy: G→A substitution (338G>A, black arrow). *ERBB2*, *receptor tyrosine-protein kinase erbB-2*.

cancer with a familial history of malignant tumors, *ERBB2* c.338G>A was sequenced using the DNA isolated from peripheral blood samples of 117 Chinese women with breast cancer. Both the case and control groups consisted of individuals from the Han ethnic community of China, and there were no relationships between the recruited patients. The clinical and pathological characteristics of the patients with breast cancer are presented in Table I. The majority of the subjects had early-stage breast cancer (stage I, stage IIA and stage IIB); accounting for 78.6% of the overall participants, and 99.1% of the patients had no distant metastasis. The histological type of invasive ductal carcinoma accounted for 88%, which was the most common among the recruited patients. Almost one-half of the patients with breast cancer were negative for HER2 expression (n=54, 46.2%).

ERBB2 c.338G>A: p.R113Q variant. The R113Q variant that was detected in the present study (Table II; Fig. 1) resulted in an amino acid alteration from arginine to glutamine in the receptor L domain of ERBB2. All the R113Q carriers had heterozygous genotypes (Table II). The frequency of R113Q occurrence was significantly higher in patients with breast cancer with a family history of tumors (8/117, 6.8%) compared with the healthy group (3/250, 1.2%; χ^2=8.712, P=0.003; Table II). The risk of breast cancer increased significantly with the occurrence of R113Q carriers (OR=6.04; 95% CI 1.573-23.214, P=0.009; Table II). There was no deviation in the HWE of R113Q (P=0.70), which indicated the population was representative.

Additionally, the results of the 1,000 Genomes Project indicated that the frequency of occurrence of this allele accounts for 1.9% of the Han people from Beijing and the south of China (Table III), a figure similar to our finding (1.2%). Furthermore, the R113Q allele was present at a lower frequency in Japanese populations and was not detected at all in Europeans (Table III) (21).

Clinical characteristics of the carriers. Out of the eight carriers with family histories of cancer, six of the patients had at least one relative with tumors of the digestive system. The rate of R113Q-associated breast cancer with a family history of tumors of the digestive system was 75% (Table IV). In the present study, the R113Q was more prevalent in patients with breast cancer who did not present with amplification of ERBB2 (Table IV).

In silico analysis of the R113Q mutation. The R113Q variant in the *ERBB2* gene was predicted to be involved in the extracellular ligand-binding domain, a domain that is highly conserved in biological evolution. The mutation-induced structural change of the extracellular domain was thus hypothesized to cause a deficit in function. The arginine at residue 113 of ERBB2 was highly conserved among different vertebrates (Fig. 2) where a red box indicates residue 113 of HER2 in each of the vertebrates. Although the predicted effects of the R113Q mutation from four different programs were inconsistent, two of the *in silico* tools predicted its pathogenicity, although specific details were not provided (Table V). The results of the 3D structure suggested that the alteration of the amino acid at position 113 (Fig. 3) led to a structural change in the side chain of ERBB2. Accordingly, the R113Q variant was predicted to have a potentially damaging effect on the function of the protein, the mechanism of which will be investigated in future studies.

Discussion

In the present study a novel susceptible locus allele, *ERBB2* c.338G>A: p.R113Q, which may increase breast cancer risk

Table III. Frequency of the *ERBB2* c.338G>A: p.R113Q according to the 1,000 Genomes project.

Population	Population description	Super population	Allele frequency		Allele Count		Genotype frequency		Genotype count	
			G	A	G	A	GlG	AlG	GlG	AlG
1000GENOMES:phase_1_ALL			0.9984	0.0016	5,000	8	1.0000	0.0032	2504	8
1000GENOMES:phase_1_CHB	Han Chinese from Beijing, China	EAS	0.9903	0.0097	204	2	1.0000	0.0194	103	2
1000GENOMES:phase_1_CHS	Southern Han Chinese	EAS	0.9905	0.0095	208	2	1.0000	0.0190	105	2
1000GENOMES:phase_1_CLM	Colombians from Medellin, Colombia	AMR	1.0000	0.0000	188	0	1.0000	0.0000	94	0
1000GENOMES:phase_1_FIN	Finnish from Finland	EUR	1.0000	0.0000	198	0	1.0000	0.0000	99	0
1000GENOMES:phase_1_GBR	British from England and Scotland	EUR	1.0000	0.0000	182	0	1.0000	0.0000	91	0
1000GENOMES:phase_1_IBS	Iberian population from Spain	EUR	1.0000	0.0000	214	0	1.0000	0.0000	107	0
1000GENOMES:phase_1_JPT	Japanese from Tokyo	EAS	0.9952	0.0048	207	1	1.0000	0.0096	104	1
1000GENOMES:phase_1_PUR	Puerto Ricans from Puerto Rico	AMR	1.0000	0.0000	208	0	1.0000	0.0000	96	0
1000GENOMES:phase_1_TSI	Population from Toscani in Italy	EUR	1.0000	0.0000	214	0	1.0000	0.0000	104	0
1000GENOMES:phase_1_YRI	Yoruba in Ibadan, Nigeria	AFR	1.0000	0.0000	216	0	1.0000	0.0000	102	0

EAS, East Asian; AMR, America; EUR, Europe; AFR, Africa.

in Chinese women with a family history of the tumor, was identified. The R113Q was detected in 6.8% of the patients with familial history of malignant tumors, increasing the risk of breast cancer 6.04-fold. The association between the R113Q variant and the risk of breast cancer in Chinese women with a familial history of malignant tumors was reported for the first time, to the best of our knowledge. *In silico* analysis of the R113Q variant suggested that the R113Q variant may exhibit a potentially pathogenic effect. The single nucleotide polymorphism was additionally more prevalent in patients with breast cancer with a familial history of different types of cancer of the digestive system.

The data presented in the present study support previous studies which have demonstrated an *ERBB2* polymorphism as a potential risk factor for the development of breast cancer, including the best investigated mutation, I655V (rs1136201), which conferred a 1.1-2.3-fold risk of developing breast cancer (22-24). Yamamoto *et al* (25) additionally demonstrated the potential oncogenicity of a mutation in HER2 (V659E), which caused hereditary lung adenocarcinomas in a Japanese family. The present data, therefore, provide a new marker to determine an increased risk of breast cancer in individuals.

The 113th residue of HER2 is located on the receptor L domain of the extracellular domains (ECDs). Dimerization of the extracellular domains (ECDs) results in phosphorylation of the intracellular domains and initiates signal transduction of the ERBBs. A previous study demonstrated that HER2 can only be activated without a ligand through structural alterations when HER2 is overexpressed (26), with the ECD playing an important role in the dimerization process (27,28). Another study additionally demonstrated that a mutation in the ECDs results in the loss of dimerization and subsequent phosphorylation (29).

R113Q is located on the L domain of the ECDs and is a highly conserved amino acid across the listed vertebrates. As the relationship between the mutation and disease severity had not been established, software was used to predict the effects of the amino acid substitution on protein function, and also generate the three-dimensional shape of the protein coded by *ERBB2*. Mutation Taster, GEPR++, and assessment of the three-dimensional structure all predicted the mutation at this locus to be potentially pathogenic.

A limitation of the present study is that the identification of the R113Q allele was from a small cohort. It is necessary to expand the cohort in the future to include larger samples of the breast cancer patient population, as well as to identify any potential link(s) that may have remained undetected with the relatively small sample size used. *In vivo* models, such as transgenic mice, to further investigate the molecular mechanisms of the *ERBB2* variation at extracellular domains may be required.

The present study identified a novel R113Q allele that may contribute to an increased risk of breast cancer in the Chinese population with a family history of tumors, especially tumors of the digestive system. The presence of the R113Q allele was highlighted in 6.8% of the patient cohort, conferring a 6.04-fold increased risk of breast cancer compared to non-carriers. The structure of the mutant *ERBB2* was predicted *in silico* to have a potential pathogenic effect in breast cancer. Therefore R113Q may be a novel susceptibility factor for Chinese women with a family history of tumors.

Table IV. Characteristics of the 8 patients with breast cancer who carried the *ERBB2* c.338G>A: p.R113Q variant.

ID	Age at diagnosis	ER	PR	HER2	Family cancer history
20150828001	37	-	-	-	Paternal uncle, EC; paternal uncle, EC; paternal cousin, EC; father, GC
20151014001	38	-	-	+	Mother, SC; father, SC
20151223001	42	+	+	+	Father, EC
201602170134	76	+	+	-	Maternal uncle, SC; maternal uncle, LC
201603080153	56	-	-	-	Father, SC; grandson, LK
201606080274	60	+	+	-	Mother, BC
201610090471	50	-	-	+	Father, LV; sister, LK
F0017BRCA201609020028	51	+	+	-	Father, LC

ERBB2, *receptor tyrosine-protein kinase erbB-2*; BC, breast cancer; EC, esophageal cancer; SC, stomach cancer; LC, lung cancer; LK, leukemia; LV, liver cancer; GC, gastric cancer.

Table V. *In silico* prediction outcomes of R113Q.

Variation	Variation ID	SIFT (score)	PolyPhen_2 (score)	Mutation Taster (score)	GERP++ (score)
p.Arg 113Gln	rs185670819	Tolerated (0.061)	Benign (0.162)	Disease causing (1)	Conserved (5.21)

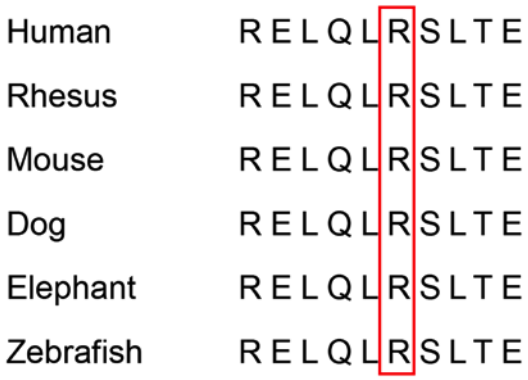

Figure 2. Conservation of the amino acid sequence of ERBB2 in the extracellular domain among six vertebrae species.

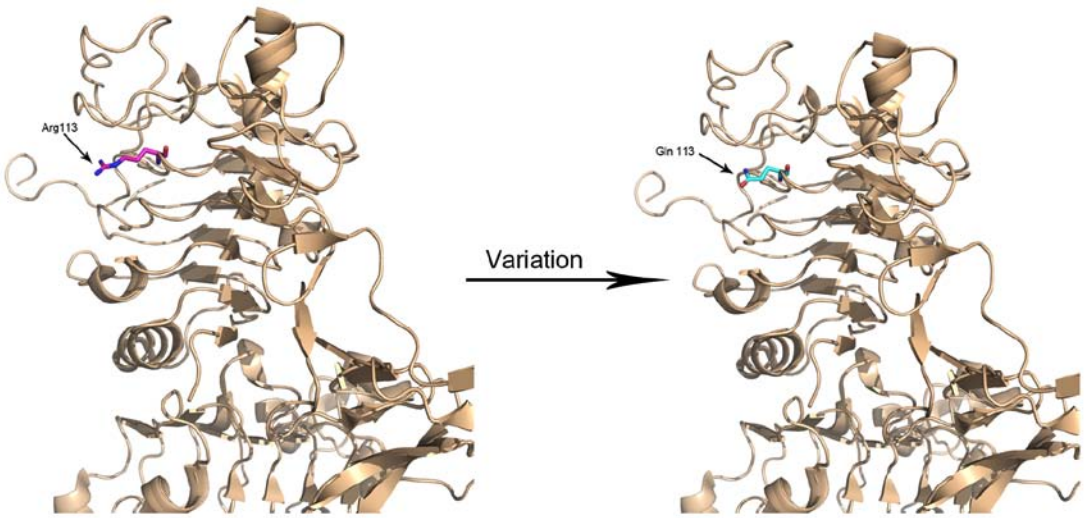

Figure 3. Predicted 3D structure of an amino-acid alteration from Arg to Gln at residue 113 of ERBB2. Arg, arginine; Gln, glutamine.

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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

HS and LL participated in the design of the study and wrote the original protocol for the research. YJ and LW conducted the experiments, analyzed the data, and drafted the manuscript. ST analyzed the data and revised the manuscript. RS, SY, and XG collected the data and revised the manuscript. All authors directly provided their contribution, read and approved the final manuscript.

Ethics approval and consent to participate

The Ethics Committee of Xijing Hospital (Shaanxi, China) approved the present study. All participants enrolled in the present study provided written informed consent.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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