Published in final edited form as:

Breast Cancer Res Treat. 2008 July ; 110(1): 169–176. doi:10.1007/s10549-007-9691-8.

***ERBB2* genetic polymorphism and breast cancer risk in Chinese women: a population-based case-control study**

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Abstract

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A polymorphism at codon 655 (ATC/isoleucine to GTC/valine [Ile⁶⁵⁵Val], rs1801200) in the transmembrane domain-coding region of human *ERBB2* gene has been previously evaluated for its association with breast cancer risk with mixed results. We evaluated this polymorphism in association with breast cancer in a group of women who participated in a large-scale, population-based, case-control study of breast cancer in Shanghai, China, followed by an in vitro analysis of the function of this polymorphism. Genomic DNA from 3,012 patients with breast cancer and 3,004 healthy controls was examined for the Ile⁶⁵⁵Val polymorphism using a TaqMan genotyping method. Adjusted odds ratios (OR) were derived from multiple logistic regression. In vitro analyses were carried out to examine whether the Ile⁶⁵⁵Val polymorphism affect *ERBB2* expression and the activity of its downstream targets. Approximately 2% of study subjects carry the Val/Val genotype. Compared with women with the Ile/Ile (76%) genotype, women who had the Ile/Val (22%) or Val/Val genotype did not have an elevated risk of breast cancer. Stratified analyses by age and menopausal status revealed no apparent association with this polymorphism in any subgroups of women. In a series of biochemical analyses, we found that the Ile⁶⁵⁵Val substitution did not alter ErbB2 and its downstream signaling molecule activity. These study results suggest that Ile⁶⁵⁵Val polymorphism of the *ERBB2* gene do not alter its activity and may not be associated with increased breast cancer risk among Chinese women.

Keywords

ERBB2; Genetic polymorphism; Breast cancer risk

Introduction

ErbB2 (HER2/Neu), a member of the ErbB family of transmembrane receptor tyrosine kinases which include the epidermal growth factor receptor (EGFR, ErbB1), ErbB3 (HER3), and ErbB4 (HER4), plays an important role in mammary gland development, transformation, and cancer progression [1, 2]. Binding of their cognate ligands to the ectodomain of receptors results in the formation of receptor homodimers or heterodimers and activates receptors by phosphorylation of specific tyrosine residues within the cytoplasmic domain. These phosphorylated residues serve as docking sites for a range of signal molecules and lead to the activation of cascades of intracellular signaling pathways including the mitogen-activated protein kinase (MAPK) and phosphatidylinositol-3 kinase (PI3K) pathways [3]. ErbB2 has no activating ligand, thus it can only signal as a heterodimer activated by the other ErbB receptor ligands. Despite this apparent limitation, ErbB2 is the favorite receptor for heterodimerization and offers more potent mitogenic and transforming signals within the ErbB receptor network [4]. ErbB2 is overexpressed in approximately 20–30% breast cancer, primarily due to gene amplification and this feature correlates with poor patient prognosis [5–7]. Mutation in the juxta-transmembrane region (Val⁶⁶⁴Glu) of Neu (the rat homologue of human ErbB2) results in the development of focal mammary tumors [8–10]. Although this mutation has not been identified in humans [11, 12], a common genetic variant in the nearby transmembrane region at codon 655 was identified in the human *ERBB2* gene. This polymorphism encodes either isoleucine (Ile; ATC) or valine (Val; GTC) [13]. Several epidemiologic studies have been conducted during the past several years to investigate *ERBB2* Ile⁶⁵⁵-Val polymorphism in relation to breast cancer risk [14–

24]. However, the findings from these studies have been inconsistent, and no convincing conclusions have been drawn. Herein, we report the results from a combination of an in vitro functional study and a large population-based case-control study to evaluate the role of *ERBB2* Ile⁶⁵⁵Val genetic polymorphisms in breast cancer risk, as well as the modifying effects of this polymorphism on the association between estrogen exposure and breast cancer risk.

Materials and methods

Study subjects

Biological samples and relevant clinical and survey data were collected from more than 3,000 breast cancer patients and controls who participated in the Shanghai Breast Cancer Study (SBCS). The SBCS is a two-phase population-based case-control study; details of study design have previously been described elsewhere [25]. Briefly, in the phase I study, 1,602 eligible cases (women between 25 and 64 years of age with newly diagnosed breast cancer) were identified between 1996 and 1998 through a rapid case-ascertainment system supplemented by the Shanghai Cancer Registry. Of these, 1,459 (91.1%) participated in the study. Controls were frequency-matched to cases by age (5-year interval) and randomly selected from the Shanghai Resident Registry. Of 1,724 eligible controls, 1,556 (90.3%) were interviewed. The phase II cases and controls were recruited between April 2002 and February 2005 using a protocol similar to that describe above. The age range of the second stage of the study was between 20 and 70 years. A total of 1,999 cases and 1,918 controls were recruited, with participation rates of 83.7% for cases and 70.4% for controls. The study protocol was approved by committees of relevant institutes for the use of human subjects in research.

All participating cases and controls completed a face-to-face interview. A structured questionnaire was used to elicit detailed information on demographic factors, menstrual and reproductive history, medication use, dietary habits, prior disease history, physical activity, tobacco and alcohol use, weight, and family history of cancer. All participants were measured for their current weight, height, and circumference of the waist and hips. All measurements were taken twice by trained interviewers using a standard protocol. A third measurement was taken if the difference from the two measurements exceeded the tolerance level. Medical charts were reviewed to obtain information on cancer diagnosis, tumor-node-metastasis (TNM) stage, estrogen receptor (ER) and progesterone receptor (PR) status, and cancer treatments. Pathologic slides for all cases were reviewed independently by two senior pathologists to confirm cancer diagnosis. Many quality control procedures were implemented in the study to assure the high quality of data collection.

In addition to the in-person interviews, a blood sample or exfoliated buccal cell sample was obtained from the majority of study subjects. Blood samples were collected in Vacutainer tubes containing EDTA or heparin, and buccal cell samples were collected by a modified mouth washing method. All samples were processed on the same day, typically within 6 h, at the Shanghai Cancer Institute and stored at -70°C until relevant bioassays could be conducted. In the initial stage of the study during 1996 and 1998, 1,193 (82%) cases and 1,310 (84%) controls donated a blood sample. In the second stage of the study during 2002

and 2005, virtually all cases (96.5%) and controls (94.3%) provided a blood sample or a buccal cell sample. These subjects were included in the current study.

Genomic DNA was extracted from buffy coats or buccal cells using a Puregene (Gentra Systems, Minneapolis, MN) or Qiagen (Valencia, CA) DNA purification kit following the manufacturer's protocol. The allelic discrimination of the *ERBB2* gene Ile⁶⁵⁵Val polymorphism was assessed with the ABI PRISM 7900 Sequence Detection Systems (Applied Biosystems, Foster City, CA) using the TaqMan assay (assay ID: C_7452451_1_). The final volume for each reaction was 5 µl, consisting of 2.5 µl TaqMan Universal PCR Master Mix, 0.25 µl primers/TaqMan probes mix, and 5 ng genomic DNA. The PCR profile consisted of an initial denaturation step at 95 °C for 10 min and 40 cycles of 92°C for 15 s and 60°C for 1 min. The fluorescence level was measured with the ABI PRISM 7900HT sequence detector (Applied Biosystems). Allele frequencies were determined by ABI SDS software. Genotyping data were obtained from 3,012 cases and 3,004 controls.

The laboratory staff was blind to the identity of the subjects. Quality control samples were included in geno-typing assays. Each 384-well plate contained two water, eight CEPH 1347-02 DNA, eight blinded quality control DNA, and eight unblinded quality control DNA samples. The blinded and unblinded quality control samples were taken from the second tube of study samples included in the study. The genotypes of quality control samples and study samples were in 98% agreement. In addition, 45 DNA samples of the Chinese participants used in HapMap and 24 DNA samples used in Perlegen were genotyped as an additional quality control. The genotypes of the same samples generated from our study were compared to data downloaded from HapMap (<http://www.hapmap.org>) and/or Perlegen (<http://genome.perlegen.com>). The concordance rates between the data generated in our lab and the data from the above database was 100%.

Cell lines, plasmids, and viruses

MCF10A cells and its derivative cells were grown in DMEM/F12 medium containing 5% horse serum, EGF (20 ng/ml), Hydrocortizone (0.5 µg/ml), Cholera toxin (100 ng/ml), insulin (10 µg/ml), and Pen/Strep as described elsewhere [26]. Phoenix-Ampho cells were grown in Dulbecco's modified Eagle's medium (DMEM; Cellgro) containing 10% FBS (Hyclone) in a humidified 5% CO₂ incubator at 37°C.

Several ErbB2 variants, Ile⁶⁵⁴Ile⁶⁵⁵ (II), Val⁶⁵⁴Val⁶⁵⁵ (VV), Ile⁶⁵⁴Val⁶⁵⁵ (IV), and Val⁶⁵⁹Glu (VE), which correspond to Val⁶⁶⁴Glu in oncogenic neu, were generated by using QuikChange Multi Site Directed Mutagenesis Kit (Stratagene, CA). The template is parental *ERBB2* construct, which contains Val⁶⁵⁴Val⁶⁵⁵ amino acid residues in the transmembrane domain and is cloned in the pBabe retroviral vector. Oligonucleotides GCCAGCCCTCTGACGTCCATCGTCTCTGCGGTGGTTG and CAACCACCGCAGAGACGATGGACGTCAGAGGGCTGGC were used to generate ErbB2 IV mutant. Oligonucleotides GCCAGCCCTCTGACGTCCATCATCTCTGCGGTGGTTGGC and GCCAACCACCGCAGAGATGATGGACGTCAGAGGGCTGGC were used to generate ErbB2 II mutant. Oligonucleotides CATCATCTCTGCGGTGGAGGGCATTCTGCTGGTCGTG and

CACGACCAGCAGAATGCCCTCCACCGCAGAGACGAC were used to generate ErbB2 VE mutant. The clones were verified by sequence analysis. Phoenix-Ampho cells were transfected by corresponding plasmid DNA to produce retroviruses expressing ErbB2 variants and the empty pBabe vector. Cells infected by retroviruses were selected in puromycin (1 µg/ml) 24 h after infection.

Immunoblot analysis

About 24 h after plating, the stably transduced MCF10A cells were serum starved for 24 h in serum-free DMEM/F12 medium. Some cells were treated with 10 ng/ml EGF for 45 min before being lysed. Cells were washed twice with ice-cold PBS and lysed on ice in NP-40 lysis buffer (20 mM Tris [pH 7.4], 150 mM NaCl, 1% Nonidet P-40, 0.1 mM EDTA, plus protease and phosphatase inhibitors). After sonication for 10 s and centrifugation (14,000 rpm), the protein concentration in supernatants was measured using the BCA protein assay reagent (Pierce, IL). About 25 µg of total cell protein were separated by 10% SDS-PAGE and transferred onto nitrocellulose membranes (BioRad, Hercules, CA). Primary antibodies included P-HER2Y1248, ErbB2, Erk1/2, AKT, and phosphorylated AKT (Ser⁴⁷³) (Cell Signaling Technology, Beverly, MA) along with P-MAPK (Promega, WI) and β-actin (Sigma, St. Louis, MO). Immunoreactive bands were detected by enhanced chemiluminescence after incubation with horse-radish peroxidase-conjugated secondary antibodies (Promega).

Soft agarose colony-forming assay

Base layers consisting of a growth medium containing 0.8% low-melting point agarose (Gibco, Carlsbad, CA) and 10 mM HEPES (pH 7.5) were poured onto 6-well plates and allowed to solidify. Cells (3×10^4 per well) were plated in triplicate in top layers consisting of a growth medium containing 0.4% agarose and 5 mM HEPES. Colonies were photographed after 13–18 days.

Statistical analysis

Statistical methods for analysis of case-control data were utilized with SAS (version 9.1; SAS Institute, Cary, NC). Chi-square test or t-test were used to compare demographic factors and major risk factors between cases and controls. Unconditional logistic regression analysis was used to estimate odds ratios (ORs) and corresponding 95% confidence intervals (CIs) for measuring the strength of the association between *ERBB2* polymorphisms and the breast cancer risk. Trend tests for dose-response relationships between breast cancer risk and the number of the Val allele were performed by treating an ordinal-score variable (with a value of 0, 1, or 2 for number of Val alleles) as a continuous variable in logistic regression. All statistical tests were two-sided.

Results

Ile⁶⁵⁵Val polymorphism of *ERBB2* was not associated with breast cancer risk

The distributions of demographic characteristics and major risk factors for breast cancer are shown in Table 1 for case patients and control subjects. Breast cancer cases and controls were comparable in age due to the frequency-match study design by age. There were

significant differences between cases and controls in education and most of the known risk factors for breast cancer [27]. The associations of these risk factors with breast cancer were similar in the two phases of study, although women in the phase II study were slightly older and slightly better educated than those recruited in the phase I study.

Genotype frequencies among cases and controls are listed in Table 2. The distribution of the *ERBB2* genotype in cases and controls was consistent with Hardy-Weinberg equilibrium. Among subjects recruited in phase I, women with the Val/Val genotype had a slightly elevated risk of breast cancer (OR = 1.5; 95% CI = 0.8–2.7), and the association was seen only in pre-menopausal women (OR = 2.3; 95% CI = 1.0–5.0). This positive association was not replicated in the phase II study. Overall, this polymorphism was not related to the risk of breast cancer in combined or stratified analysis.

Ile⁶⁵⁵Val polymorphism does not affect ErbB2 expression and its activity

The ErbB2 Ile⁶⁵⁵Val polymorphism, located in the transmembrane domain, can affect protein integrity and abundance as well as ErbB2 activity. To investigate the effects of Ile⁶⁵⁵Val substitution on ErbB2 function, in vitro biochemical analyses were carried out with human mammary epithelial cells. To accomplish this, we generated retroviral vectors encoding several ErbB2 variants with Ile or Val at residue 654 and 655 (because both amino acid residues appeared in the ErbB2 sequences in NCBI database and other publications). Human immortalized MCF10A mammary epithelial cells were then stably transduced with the retrovirus expressing wild type ErbB2 Ile⁶⁵⁴ Ile⁶⁵⁵ (II), ErbB2 Ile⁶⁵⁴Val⁶⁵⁵ (IV), ErbB2 Val⁶⁵⁴ Val⁶⁵⁵ (VV). The cells were also transduced with a previously characterized oncogenic ErbB2 Val⁶⁵⁹Glu (VE), a point mutation in the transmembrane domain resulting in the substitution of valine (V) with glutamic acid (E) at residue 659 [28]. After selection, transgene expression and its downstream effectors, AKT and Mapk (Erk1/2), were detected by immunoblot under serum starvation and EGF stimulation condition, respectively. Under both conditions, the results showed that transduced MCF10A cells expressed an equal level of ErbB2 (II), ErbB2 (VV), ErbB2 (IV), and a lower level of ErbB2 (VE) (Fig. 1A). MCF10A cells with ErbB2 Val⁶⁵⁹ Glu exhibited a higher level of phosphorylated ErbB2 and elevated phosphorylated AKT (only under serum starvation condition) and Erk1/2, two important ErbB2 downstream targets which regulate survival and proliferation signaling. The cells containing other ErbB2 variant exhibited equal levels of a tyrosine phosphorylated ErbB2, phosphorylated AKT, and Erk1/2 (Fig. 1A and B). These results indicate that Ile⁶⁵⁵Val substitution in ErbB2 did not alter ErbB2 and its downstream signal molecules activity.

We also examined whether ErbB2 Ile⁶⁵⁵Val substitution results in enhanced transformation ability. The anchorage-independent colony-forming assays were carried out with MCF10A mammary epithelial cells transduced with *ERBB2* variants. The transforming activity of the protein encoded by ErbB2 IV was compared with wild type ErbB2 II, ErbB2 VV, and previously characterized transforming ErbB2 VE. The results showed cells expressing ErbB2 II, ErbB2 IV and ErbB2 VV failed to transform MCF10A mammary epithelial cells, whereas expression of oncogenic ErbB2 VE resulted in induction of foci (Fig. 2). These

results indicate that Ile⁶⁵⁵Val substitution in ErbB2 does not alter ErbB2 transforming activity.

Discussion

Over the past few years, several epidemiologic studies including our study in Shanghai, China, have investigated the association of Ile⁶⁵⁵Val polymorphism in *ERBB2* gene with breast cancer risk [14–24]. The results, however, were inconsistent. In the present study, we conducted a two-phase case-control study and in vitro experiments to examine the function of Ile⁶⁵⁵Val substitution in ErbB2. Although a positive association was suggested with the Val allele in the initial set of subjects, particularly for those with an early-onset of breast cancer, this association was not replicated in the second set of subjects. Overall, no association was found between Ile⁶⁵⁵Val variant in the *ERBB2* gene and breast cancer risk. In vitro experiments show no apparent evidence that Ile⁶⁵⁵Val substitution in ErbB2 may affect its function. The results from this study suggest that this *ERBB2* polymorphism may not contribute to the susceptibility of breast cancer risk.

Considering the role of ErbB2 in breast cancer and that Val⁶⁵⁹Glu (VE) substitution in Neu resulted in neu activation and rapid tumor progression in an animal model [28], we further evaluated whether the Ile⁶⁵⁵Val substitution in ErbB2 could result in its activity change and influence breast cancer risk. To this aim, we first carried out in vitro biochemical analyses to examine whether Ile⁶⁵⁵Val substitution in ErbB2 had any effects on *ERBB2* expression and its activity through examining ErbB2 autophosphorylation (at Tyr1248) and colony-forming assays. Our results showed that ErbB2 carrying Ile⁶⁵⁵Val substitution (IV) is expressed at the same level as the wild type ErbB2 (II) and ErbB2 (VV) in MCF10A mammary epithelial cells. They were also phosphorylated at the same degree. The two major downstream signaling molecules AKT and MAPK, which regulate cell survival and proliferation, were also not affected. In contrast, the oncogenic ErbB2 VE showed higher autophosphorylation and higher phosphorylated AKT (under starvation condition only) and MAPK, which resulted in transformation of MCF10A mammary epithelial cells. Those results are not surprising since (1) both isoleucine and valine have similar aliphatic side chains and substituting one with another probably will not dramatically change the characteristics of underlying protein; and (2) only substituted glutamic acid (E) or glutamine (Q) residues at amino acid positions 664 activate the neu gene, not positions 663 or 665. Even substitution of valine with histidine (H) or lysine (K) at 664 was insufficient to activate the neu, while substitution of valine with aspartic acid (D) only resulted in a small number of foci [29]. We then evaluated whether the Ile⁶⁵⁵Val substitution altered its oncogenic ability. Results from our in vitro colony forming assays clearly indicated that Ile⁶⁵⁵Val substitution in ErbB2 does not have any effect on clonogenicity compared to the oncogenic ErbB2 VE controls.

The current study has several strengths compared to our previous study and other studies. Most importantly, a two-phase design was used to reduce type I errors, and the sample sizes in both stages were large so that type II errors were reduced. The population-based case-control study design and high participation rate minimized selection bias. Extensive information on anthropometrics and lifestyle factors were collected in this study for adjusting for confounding factors. Additionally, Chinese women living in Shanghai are

relatively homogeneous in ethnic background; over 98% are classified into a single ethnic group (Han Chinese). Therefore, the potential confounding effect by ethnicity is not a major concern for our study. Furthermore, our in vitro biological functional study further confirms the null association results observed in the case-control study.

Taken together, the results from this study do not support a potential role of the *ERBB2* Ile⁶⁵⁵Val polymorphism in the etiology of breast cancer that was suggested in several previous studies [17–21, 23], including our own study [14] conducted in a smaller set of subjects, in which the possibility for genotyping and type I errors could be a concern. Our study underscores the importance of replication using independent samples to reduce type I errors and in vitro functional studies in the evaluation of low penetrance genetic factors.

Acknowledgments

We thank Dr. Jirong Long, Regina Courtney, Hongmei Wu, and Qing Wang for technical assistance in genotyping; Bethanie Hull and Brandy Venuti for assistance in the preparation of this article; as well as all study participants and research staff of the Shanghai Breast Cancer Study for their support. This research was supported by NIH grants RO1 CA64277 and RO1 CA90899 from the National Cancer Institute.

Abbreviations

CI	Confidence interval
OR	Odds ratios
BMI	Body mass index
EGFR	Epidermal growth factor receptor
ER	Estrogen receptors
MAPK	Mitogen-activated protein kinase
PI3K	Phosphatidylinositol-3 kinase
PR	Progesterone receptors
SBCS	Shanghai Breast Cancer Study
TNM	Tumor-node-metastasis

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Fig. 1. *ERBB2* Ile⁶⁵⁵Val polymorphism does not alter *ERBB2* expression, activity and its downstream signaling. (A) Immunoblot. Cell lysates from MCF10A mammary epithelial cells transduced by different ErbB2 variants, Ile⁶⁵⁴Ile⁶⁵⁵ (II), Val⁶⁵⁴Val⁶⁵⁵ (VV), Ile⁶⁵⁴Val⁶⁵⁵ (IV), and Val⁶⁵⁹Glu (VE), were subjected to immunoblotting. Levels of total ErbB2 and phosphorylated ErbB2 (P-ErbB2), Akt and phosphorylated Akt (P-Akt), Erk1/2 and phosphorylated Erk1/2 (P-ERK) were analyzed by immunoblotting. Note cells transduced by ErbB2 II, IV, and VV expressed equal levels of ErbB2 protein, whereas the

ErbB2 VE level was lower. **(B)** Relative expression or activity to wild type ErbB2 II. The proteins were quantified by BioQuant NOVA Prime imaging analysis software (BioQuant, Nashville, TN). Normalized phosphorylation of ErbB2, AKT and Erk1/2 were cumulative results of three independent experiments. Tublin serves as loading control. * $P < 0.05$ compared with other ErbB2 variants

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Fig. 2.

The clonogenic assay of different ErbB2 variants. Equivalent numbers of MCF10A mammary epithelial cells expressing the indicated ErbB2 variants were plated in 6 well plates. After 13 days of growth, cells were stained with crystal violet and photographed. In comparison to parental MCF10A and pBabe vector transduced mammary epithelial cells, cells expressing ErbB2 Ile⁶⁵⁴ Ile⁶⁵⁵ (II), ErbB2 Ile⁶⁵⁴ Val⁶⁵⁵ (IV) and ErbB2 Val⁶⁵⁴ Val⁶⁵⁵

(VV) failed to transform MCF10A mammary epithelial cells, whereas oncogenic ErbB2 Val⁶⁵⁹Glu (VE) transduction resulted in induction of foci

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Comparison of cases and controls by selected demographic characteristics and major breast cancer risk factors, Shanghai Breast Cancer Study, 1996–2005

Table 1

Subject characteristic	Phase I study		Phase II study		Combined study				
	Cases (n = 1,106)	Controls (n = 1,205)	P-value ^a	Cases (n = 1,906)	Controls (n = 1,799)	P-value ^a	Cases (n = 3,012)	Controls (n = 3,004)	P-value ^a
Age (years), mean ± SD	47.6 ± 8.0	47.2 ± 8.8	0.27	50.9 ± 8.3	51.8 ± 8.3	0.003	49.7 ± 8.4	49.9 ± 8.8	0.31
Education (%)									
Elementary school or below	12.5	14.7		6.0	11.8		8.4	13.0	
Middle or high school	75.3	75.3		77.8	77.0		76.9	76.3	
College or above	12.2	10.0	0.10	16.2	11.2	<0.01	14.7	10.7	<0.01
Breast cancer in first-degree relative (%)	3.3	2.4	0.22	5.5	3.1	0.01	4.6	2.8	<0.01
Ever had breast fibroadenoma, %	9.6	5.2	<0.01	10.1	5.5	<0.01	9.9	5.4	<0.01
Age at menarche (years), mean ± SD	14.5 ± 1.6	14.7 ± 1.7	<0.01	14.4 ± 1.7	14.7 ± 1.8	<0.01	14.4 ± 1.7	14.7 ± 1.8	<0.01
Ever had a live birth (%)	94.8	95.9	0.21	95.0	96.3	0.04	94.9	96.1	0.02
Number of live births, mean ± SD	1.5 ± 0.8	1.5 ± 0.9	0.14	1.2 ± 0.7	1.4 ± 0.8	<0.01	1.3 ± 0.7	1.4 ± 0.8	0.14
Age at first live birth (years), mean ± SD	26.8 ± 4.1	26.2 ± 3.8	<0.01	26.3 ± 3.6	25.7 ± 3.9	<0.01	26.5 ± 3.8	25.9 ± 3.8	<0.01
Post-menopausal (%)	33.0	36.1	0.11	43.9	49.8	<0.01	39.9	44.4	0.11
Age at menopause (years), mean ± SD	48.1 ± 4.6	47.5 ± 4.9	0.07	48.5 ± 4.4	48.3 ± 4.6	0.24	48.4 ± 4.5	48.0 ± 4.7	0.04
Physically active past 10 years (%)	19.4	25.8	<0.01	29.4	34.2	<0.01	25.7	30.8	<0.01
BMI (kg/m ²), mean ± SD	23.6 ± 3.4	23.2 ± 3.4	0.02	23.7 ± 3.3	23.4 ± 3.2	<0.01	23.7 ± 3.3	23.4 ± 3.3	<0.01
Waist-to-hip ratio, mean ± SD	0.81 ± 0.06	0.80 ± 0.06	<0.01	0.83 ± 0.05	0.82 ± 0.06	<0.01	0.82 ± 0.06	0.81 ± 0.06	<0.01

^aFrom χ^2 test (categorical variables) or *t*-test (continuous variables)

Table 2

The distributions of ErbB2 rs1801200 genotypes and breast cancer risks

Genetic variants	Phase I ^a			Phase II ^a			Combined ^b		
	Cases (%)	Controls (%)	OR (95% CI)	Cases (%)	Controls (%)	OR (95% CI)	Cases (%)	Controls (%)	OR (95% CI)
All subjects									
AA (Ile/Ile)	839 (76)	905 (75)	1.0	1459 (77)	1347 (75)	1.0	2298 (76)	2252 (75)	1.0
AG (Ile/Val)	239 (22)	280 (23)	0.9 (0.8–1.1)	409 (21)	407 (23)	0.9 (0.8–1.1)	648 (22)	687 (23)	0.9 (0.8–1.0)
GG (Val/Val)	28 (2)	20 (2)	1.5 (0.8–2.7)	38 (2)	45 (2)	0.8 (0.5–1.2)	66 (2)	65 (2)	1.0 (0.7–1.4)
Premenopausal women									
AA (Ile/Ile)	549	573	1.0	826	684	1.0	1375	1257	1.0
AG (Ile/Val)	169	185	0.9 (0.8–1.2)	220	196	0.9 (0.7–1.1)	389	381	0.9 (0.8–1.1)
GG (Val/Val)	20	9	2.3 (1.0–5.0)	23	22	0.9 (0.5–1.6)	43	31	1.3 (0.8–2.0)
Postmenopausal women									
AA (Ile/Ile)	288	329	1.0	633	663	1.0	921	992	1.0
AG (Ile/Val)	67	94	0.8 (0.6–1.2)	189	210	0.9 (0.7–1.2)	256	304	0.9 (0.7–1.1)
GG (Val/Val)	8	11	0.8 (0.3–2.1)	15	23	0.7 (0.4–1.3)	23	34	0.7 (0.4–1.2)
Ages < 45 years									
AA (Ile/Ile)	369	429	1.0	349	309	1.0	718	738	1.0
AG (Ile/Val)	110	137	0.9 (0.7–1.2)	115	98	1.0 (0.8–1.4)	225	235	1.0 (0.8–1.2)
GG (Val/Val)	13	6	2.5 (0.9–6.7)	10	13	0.7 (0.3–1.6)	23	19	1.2 (0.7–2.3)
Age > 45 years									
AA (Ile/Ile)	470	476	1.0	1110	1038	1.0	1580	1514	1.0
AG (Ile/Val)	129	140	0.9 (0.7–1.2)	294	309	0.9 (0.7–1.1)	423	452	0.9 (0.8–1.0)
GG (Val/Val)	15	14	1.1 (0.5–2.3)	28	32	0.8 (0.5–1.4)	43	46	0.9 (0.6–1.4)

^a adjusted for age^b adjusted for age and study phase