Original Article

The impact of *EGFR* gene polymorphisms on the response and toxicity derived from neoadjuvant chemotherapy for breast cancer

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Background: Neoadjuvant chemotherapy is usually used for treating locally advanced breast cancer. However, not all patients achieve pathologic complete response (pCR). In this study, we selected two epidermal growth factor receptor (EGFR) single nucleotide polymorphism (SNP) sites, rs1468727 and rs845552, to investigate the association between the genotypes and the response and toxicity derived from neoadjuvant chemotherapy for breast cancer.

Methods: All participants took part in clinical trial SHPD001 and SHPD002. For univariate analyses, the association between SNP and pCR or toxicity was analyzed by Chi-square or Fisher's exact test. For multivariate analyses, logistic regression was used instead.

Results: In all, one hundred and eighteen patients were enrolled. We found that the frequency of AA genotype in rs845552 was higher than that of other genotypes in HER2-positive breast cancer (AA *vs.* AG, P=0.039; AA *vs.* GG, P=0.005; AA *vs.* AG+GG, P=0.009). Multivariate logistic regression analyses showed that pCR was more difficult to be achieved in patients with a CT genotype in rs1468727 compared to those with a CC+TT genotype (OR =0.288, 95% CI: 0.109–0.762, P=0.012) or a CC genotype (OR =0.254, 95% CI: 0.076–0.849, P=0.026). Moreover, we demonstrated that both rs1468727 and rs845552 were associated with toxicity that results in complications such as increased total bilirubin, skin rash, peripheral neuropathy, and alopecia (P<0.05).

Conclusions: Our study reported for the first time, that in treating breast cancer with neoadjuvant chemotherapy, EGFR SNP rs1468727 is associated with treatment response, and that both rs1468727 and rs845552 are related to treatment-derived toxicity. In addition, we also found that rs845552 may be related to the status of HER2 in breast cancer.

Keywords: Breast cancer; single nucleotide polymorphisms (SNP); epidermal growth factor receptor (EGFR); neoadjuvant chemotherapy

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Introduction

Neoadjuvant chemotherapy has been used as a standard method for treating locally advanced breast cancer. It can not only improve the success rates of surgical removal and breast conservation, but also provide valuable information about the sensitivity of patients in response to chemotherapy regimens. Pathologic complete response (pCR) rate has been utilized as an effective indicator to evaluate the efficacy of neoadjuvant chemotherapy. To date, the application of neoadjuvant chemotherapy has achieved a considerable rate in several clinical trials, including a preliminary trial of SHPD001 recently accomplished by our department where the participants received a weekly chemotherapy regimen of paclitaxel combined with cisplatin. The results achieved from the trial were promising, with an overall pCR rate reaching 34.4% and a pCR rate reaching 64.7% in triple-negative breast cancer (1). However, pCR could not be achieved in all patients receiving neoadjuvant chemotherapy. Patients who achieved a pCR after neoadjuvant chemotherapy have been shown to exhibit a higher survival rate compared with those without pCR (2). Therefore, in order to facilitate the identification of patients who may respond better to neoadjuvant chemotherapy, the discovery of effective predictive biomarkers is essential.

Epidermal growth factor receptor (EGFR), or human epidermal growth factor receptor 1 (HER1) is a membrane receptor with tyrosine kinase activity that belongs to the epidermal growth factor receptor (HER) family alongside HER2, HER3 and HER4. Playing an essential role in cell proliferation, EGFR has been found to be widely distributed in many malignant tissues such as breast cancer, stomach cancer and lung cancer (3). EGFR overexpression has been shown to promote cell division, leading to an uncontrolled cell growth (4,5). Studies have shown that EGFR overexpression is frequently found in triple-negative breast cancer (6), and is related to poor prognosis (7,8). A study has shown that both the expression level and gene copy number of EGFR were independent adverse prognostic factors for esophageal adenocarcinoma patients treated with neoadjuvant chemotherapy (9). Choura *et al.* have identified three specific single nucleotide polymorphisms (SNPs) in *EGFR* gene (rs17337451, rs1140476, rs17290699) that are related to the protein stability of the EGFR dimer structure and hence a potential attribution to the risk of developing breast cancer (10). Moreover, recent studies have shown that certain SNPs are associated with the response and toxicity from chemotherapy in breast cancer (11-14). Chen *et al.* have reported that f*ibroblast growth factor receptor (FGFR) 4* rs1966265 and *FGFR2* rs2981578 are related to the response and prognosis of breast cancer patients receiving chemotherapy based on docetaxel-epirubicin-cyclophosphamide combinations (11). Therefore, it is theoretically feasible to identify SNPs in the *EGFR* gene which are associated with the efficacy of neoadjuvant chemotherapy for breast cancer.

Although the EGFR expression has been proven to have a level of close connection with breast cancer, there has been limited research that focused on the association between *EGFR* gene SNPs and the efficacy and toxicity derived from neoadjuvant chemotherapy for breast cancer. Sobral-Leite *et al.* have reported a study focused on the evaluation of the *EGFR* SNP (rs2227983) located in exons in neoadjuvantly treated breast cancer patients from Brazil and Netherlands (15). However, the regimens in their study were complicated including different kinds such as 5-fluorouracil/doxorubicin/cyclophosphamide (FAC), docetaxel, doxorubicin/cyclophosphamide (AC) and paclitaxel/carboplatin., and they did not report the relationship between SNPs and toxicity derived from neoadjuvant chemotherapy. In this study, two SNP sites, rs1468727 and rs845552, located in *EGFR* intron 13 and intron 19 respectively were selected, which were reported to have clinical significance in the risk of glioma, and in predicting the therapeutic effect and prognosis of glioma (16-20). However, these two SNP sites have not been studied in breast cancer. We hypothesized that these two sites might be associated with the sensitivity to chemotherapy in breast cancer. To verify this hypothesis, we conducted this exploratory analysis.

We present the following article in accordance with the TRIPOD Reporting Checklist. Available at http://dx.doi.org/10.21037/gs-20-330.

Methods

Study population

All patients included in this study participated in either clinical trial SHPD001 (NCT02199418) or SHPD002 (NCT02221999), and were admitted to Renji Hospital, School of Medicine, Shanghai Jiaotong University, between 2013 and 2016. These programs were approved by the ethics committee of Renji Hospital, School of Medicine, Shanghai Jiaotong University and was in strict adherence to the relevant regulations. The trial was conducted in accordance

Table 1 Sequences of primers

SNP	Primer 1	Primer 2
Rs1468727	ACGTTGGATGTTTACTCTCTGGGCATGGAC	ACGTTGGATGGCCTATCAGCTAAAGGATTC
Rs845552	ACGTTGGATGGCAAGCATGCTTGGTATTCC	ACGTTGGATGTCCAACTGTGCGCTCTGCCT

with the Declaration of Helsinki (as revised in 2013). And an informed consent was signed by each participant.

All participants were female patients aged between 18 and 70 years old, who were pathologically diagnosed with primary breast cancer. In order to be included in this study, all the patients were required to undergo pre-treatment hematological examination, and achieve the following results: white blood cell count (WBC) ≥4.0×10^9/L, neutrophil count (ANC) ≥1.5×10^9/L, platelet count (PLT) ≥100×10^9/L, hemoglobin (Hb) ≥90 g/L, AST (SGOT)/ALT (SGPT) ≤1.5 times the upper limit of normal (ULN), creatinine ≤1.5 times ULN, and total bilirubin ≤ 1.5 times ULN. Patients who were pregnant, had metastatic breast cancer, or had a history of medical conditions such as uncontrolled cardiovascular disease or severe infection that suggested intolerance to neoadjuvant chemotherapy were excluded from the study.

A core needle biopsy from the mass of breast from each patient was taken. Hormone receptor (HR) positive was defined as ER ≥1% or PR ≥1%. HER2 positive was defined as immunohistochemical HER2+++ or FISH amplification according to ASCO/CAP HER2 testing guideline (21).

Neoadjuvant chemotherapy

Each patient in clinical trial SHPD001 and SHPD002 was scheduled to intravenously receive weekly dosage of paclitaxel at 80 mg/m^2 for 4 weeks (d1, d8, d15, d22) and weekly dosage of cisplatin 25 mg/m^2 for three weeks (d1, d8, d15), in a cycle of every 4 weeks. All patients underwent chemotherapy for four cycles. In addition, trastuzumab was recommended for HER2 positive patients at a weekly basis. HR positive patients in SHPD002, were randomized to receive endocrine therapy or not. Endocrine therapy included aromatase inhibitor for postmenopausal women and gonadotropin releasing hormone agonist for premenopausal counterparts. All patients received mastectomy with axillary lymph node dissection within 2 weeks following neoadjuvant chemotherapy. pCR was defined as the absence of carcinoma in the breast and axillary lymph nodes.

SNP detection

Five milliliters of peripheral blood samples were taken from all participants before neoadjuvant chemotherapy for DNA extraction and SNP genotyping, which were performed by Shanghai Benegene Biotechnology Co., Ltd (Shanghai, China) using MassARRAY system (Sequenom, San Diego, CA, USA). Detailed primer information is shown in *Table 1*.

Statistical analysis

Genotype distributions of all patients were assessed for their adherence to the Hardy–Weinberg equilibrium (HWE). Comparisons of genotype and allele frequencies between groups were performed using a two-sided chi-square test. The risk odds ratio (OR) and the 95% confidence interval (CI) were determined by multivariate logistic regression analyses. The test level was P<0.05 and all P values were bilateral. Statistical analysis was performed using statistical software Stata 14.0 (StataCorp LP, College Station, TX, USA).

Results

Basic information of SNPs

Both SNPs were found to be located in the short arm of chromosome 7 within the intron of the *EGFR* gene (*Table 2*). Whilst rs1468727 was identified in intron 13 with a major allele C and a minor allele T, rs845552 was found in intron 19 with a major allele A and a minor allele G. Both SNPs were consistent with the HWE law (P>0.05).

Association between SNPs and characteristics of patients

Among all the eligible patients, pretreatment peripheral blood samples were available for 118 patients (*Table S1*). Our results revealed that rs845552 genotype was related to the HER2 status in co-dominant model and dominant model (*Table 3*), and that the AA genotype was more prevalent in HER2-positive breast cancer (AA *vs.* AG, P=0.039; AA *vs.* GG, P=0.005, in co-dominant model; AA *vs.* AG+GG, P=0.009, in dominant model). No significant correlation was

Table 2 Basic information and frequency of SNPs

SNP ID	Location	Position	Gene	Region	Major allele	Minor allele	Minor allele frequency (%)
Rs1468727	7p11.2	55230105	EGFR	Intron 13	C	T	51.3
Rs845552	7p11.2	55245507	EGFR	Intron 19	A	G	59.7

Table 3 Relationship between genotypes of SNPs and characteristic

SNP ID	Model	Geno-type	HER2, n (%)			HR, n (%)			Ki67, n (%)		
			Positive	Negative	χ^2-P	Positive	Negative	χ^2-P	≥14%	<14%	χ^2-P
Rs1468727	Co-dominant	CC	14 (51.9)	13 (48.1)		21 (77.8)	6 (22.2)		24 (88.9)	3 (11.1)	
		CT	23 (37.7)	38 (62.3)	0.215	54 (88.5)	7 (11.5)	0.190	54 (90.0)	6 (10.0)	1.000
		TT	9 (30.0)	21 (70.0)	0.093	29 (96.7)	1 (3.33)	0.108	28 (93.3)	2 (6.7)	0.660
	Dominant	CC	14 (51.9)	13 (48.1)		21 (77.8)	6 (22.2)		24 (88.9)	3 (11.1)	
		CT+TT	32 (35.2)	59 (64.8)	0.118	83 (91.2)	8 (8.8)	0.058	82 (91.1)	8 (8.9)	0.714
	Recessive	CC+CT	37 (42.1)	51 (57.9)		75 (85.2)	13 (14.8)		78 (89.7)	9 (10.3)	
		TT	9 (30.0)	21 (70.0)	0.243	29 (96.7)	1 (3.33)	0.113	28 (93.3)	2 (6.7)	0.727
	Over-dominant	CC+TT	23 (40.4)	34 (59.6)		50 (87.7)	7 (12.3)		52 (91.2)	5 (8.8)	
		CT	23 (37.7)	38 (62.3)	0.768	54 (88.5)	7 (11.5)	0.892	54 (90.0)	6 (10.0)	0.820
Rs845552	Co-dominant	AA	13 (65.0)	7 (35.0)		16 (80.0)	4 (20.0)		18 (90.0)	2 (10.0)	
		AG	21 (38.2)	34 (61.8)	0.039*	47 (85.5)	8 (14.5)	0.723	48 (88.9)	6 (11.1)	1.000
		GG	12 (27.9)	31 (72.1)	0.005*	41 (95.4)	2 (4.6)	0.180	40 (93.0)	3 (7.0)	0.649
	Dominant	AA	13 (65.0)	7 (35.0)		16 (80.0)	4 (20.0)		18 (90.0)	2 (10.0)	
		AG+GG	33 (33.7)	65 (66.3)	0.009*	88 (89.8)	10 (10.2)	0.253	88 (90.7)	9 (9.3)	1.000
	Recessive	AA+AG	34 (45.3)	41 (54.7)		63 (84.0)	12 (16.0)		66 (89.2)	8 (10.8)	
		GG	12 (27.9)	31 (72.1)	0.062	41 (95.4)	2 (4.6)	0.081	40 (93.0)	3 (7.0)	0.744
	Over-dominant	AA+GG	25 (39.7)	38 (60.3)		57 (90.5)	6 (9.5)		58 (92.1)	5 (7.9)	
		AG	21 (38.2)	34 (61.8)	0.858	47 (85.5)	8 (14.5)	0.400	48 (88.9)	6 (11.1)	0.558

HER2, human epidermal growth factor receptor 2; HR, hormone receptor. *, P<0.05.

found between rs1468727 genotype and HER2 status, HR status, Ki67 index and T stage (P>0.05, *Table 3*).

Association between genotype of SNPs and response to neoadjuvant chemotherapy

Univariate chi-square analyses showed that different genotypes of rs1468727 in dominant (CC *vs.* CT+TT, P=0.012), over-dominant (CC+TT *vs.* CT, P=0.023) and co-dominant models (CC *vs.* CT, P=0.006) exhibited significant correlation with the efficacy of neoadjuvant chemotherapy (*Table 4*). Multivariate logistic regression analyses showed that genotypes of rs1468727 were associated with pCR rate in the over-dominant model and the co-dominant model following parameter adjustment in the status of HER2, HR, menopause, and mass size. We showed that patients with a CT genotype were more difficult to achieve pCR compared to patients with a CC+TT genotype (OR =0.288, 95% CI: 0.109–0.762, P=0.012, in over-dominant model, *Table 4*) or a CC genotype (OR =0.254, 95% CI: 0.076–0.849, P=0.026, in co-dominant model, *Table 4*). None of the genotypes of rs845552 was found to exhibit a significant association with the pCR rate of neoadjuvant chemotherapy (P>0.05,

Table 4 Associations between genotypes and pCR rate

SNP ID	Model	Genotype	pCR, n (%)	Non-pCR, n (%)	χ^2-P	OR (95% CI)	Logit-P
Rs1468727	Co-dominant	CC	13 (48.1)	14 (51.9)		1	
		CT	12 (19.7)	49 (80.3)	0.006*	0.254 (0.076–0.849)	0.026*
		TT	9 (30.0)	21 (70.0)	0.160	0.958 (0.247–3.715)	0.951
	Dominant	CC	13 (48.1)	14 (51.9)		1	
		CT+TT	21 (23.1)	70 (76.9)	0.012*	0.400 (0.143–1.116)	0.080
	Recessive	CC+CT	25 (28.4)	63 (71.6)		1	
		TT	9 (30.0)	21 (70.0)	0.868	2.047 (0.687–6.097)	0.199
	Over-dominant	CC+TT	22 (38.6)	35 (61.4)		1	
		CT	12 (19.7)	49 (80.3)	0.023*	0.288 (0.109–0.762)	0.012*
Rs845552	Co-dominant	AA	9 (45.0)	11 (55.0)		1	
		AG	13 (23.6)	42 (76.4)	0.072	0.519 (0.144–1.870)	0.316
		GG	12 (27.9)	31 (72.1)	0.180	1.180 (0.288–4.845)	0.818
	Dominant	AA	9 (45.0)	11 (55.0)		1	
		AG+GG	25 (25.5)	73 (74.5)	0.079	0.648 (0.206–2.045)	0.460
	Recessive	AA+AG	22 (29.3)	53 (70.7)		1	
		GG	12 (27.9)	31 (72.1)	0.869	1.744 (0.639–4.764)	0.278
	Over-dominant	AA+GG	21 (33.3)	42 (66.7)		1	
		AG	13 (23.6)	42 (76.4)	0.246	0.475 (0.185–1.219)	0.122

*, P<0.05.

Table 4).

In the HER2-negative subgroup, the rs1468727 genotype was found to be associated with pCR in the addictive model (CC *vs.* CT *vs.* TT, P=0.031 in chi-square analyses, and P=0.028 in multivariate logistic regression analyses). Our results showed that pCR was more difficult to be achieved in patients with allele T (OR =0.183, 95% CI: 0.403–0.829, *Table 5*). Meanwhile, in the HR positive subgroup, multivariate logistic regression analyses showed that the rs1468727 genotype was associated with pCR in the addictive model (CC *vs.* CT *vs.* TT, OR =0.322, 95% CI: 0.114–0.907, P=0.032, *Table 5*). No significant correlation was found between the genotypes of rs845552 and pCR rate in each subgroup (*Table 6*).

Relationship between genotype of SNPs and toxicities of neoadjuvant chemotherapy

We further investigated the association between SNPs and toxicity derived from chemotherapy. Complete information of adverse events was obtained from 109 patients. We found that most patients receiving paclitaxel plus cisplatin developed common adverse events, such as anemia, leukopenia, neutropenia, increased total bilirubin, alopecia, and peripheral neuropathy. Other common grade 3 to grade 4 adverse events included anemia, leukopenia, and neutropenia.

Our analysis revealed that genotypes of the two SNPs were associated with adverse events, including increased total bilirubin, skin rash, peripheral neuropathy, and alopecia (date shown in *Tables 7-10*). The rs1468727 genotype was associated with increased total bilirubin and skin rash. Multivariate logistic regression analyses revealed that, compared with other genotypes, the TT genotype showed a higher risk of grade 2 to grade 4 increased total bilirubin (CC *vs.* TT, OR =17.183, 95% CI: 1.524–193.721, P=0.021, in co-dominant model; CC+CT *vs.* TT, OR =9.006, 95% CI: 2.402–33.773, P=0.001, in recessive model), while univariate chi-square analyses demonstrated a lower risk of grade 2 to grade 4 skin rash (CC *vs.* TT, P=0.034, in co-

Table 5 Associations between genotypes of rs1468727 in addictive model and pCR rate in subgroups

rs1468727 (C/T)	χ^2-P	Logit-P	OR (95% CI)
HER2+	0.238	0.184	0.404 (0.106–1.538)
HER2–	0.031*	0.028*	0.183 (0.403–0.829)
HR+	0.068	0.032*	0.322 (0.114–0.907)
HR–	0.266	0.303	0.174 (0.006–4.833)
HER2+, HR–	0.429	/	/
HER2+, HR+	0.433	0.292	0.479 (0.122–1.884)
HER2–, HR+	0.071	0.052	0.183 (0.033–1.013)
HER2–, HR–	0.486	0.303	0.174 (0.006–4.833)

HER2, human epidermal growth factor receptor 2; HR, hormone receptor. *, P<0.05.

Table 6 Associations between genotypes of rs845552 in addictive model and pCR rate in subgroups

rs845552 (A/G)	χ^2-P	Logit-P	OR (95% CI)
HER2+	0.504	0.377	0.665 (0.270–1.642)
HER2–	0.477	0.139	2.507 (0.742–8.463)
HR+	0.332	0.852	1.070 (0.525–2.182)
HR–	0.441	/	/
HER2+, HR–	1.000	/	/
HER2+, HR+	0.838	0.501	0.727 (0.288–1.837)
HER2–, HR+	0.263	0.287	2.025 (0.553–7.418)
HER2–, HR–	1.000	/	/

HER2, human epidermal growth factor receptor 2; HR, hormone receptor.

dominant model; CC+CT *vs.* TT, P=0.034, in recessive model). The rs845552 genotype was found to be associated with increased total bilirubin, peripheral neuropathy, and alopecia. The GG genotype was demonstrated through multivariate logistic regression analyses to display a higher risk of grade 2 to grade 4 increased total bilirubin (AA *vs.* GG, OR =10.876, 95% CI: 1.059–111.680, P=0.045, in co-dominant model; AA+AG *vs.* GG, OR =7.833, 95% CI: 1.976–31.047, P=0.003, in recessive model), while multivariate logistic regression analyses revealed a lower risk of grade 2 to grade 4 alopecia (AA+AG *vs.* GG, OR =0.418, 95% CI: 0.187–0.936, P=0.034, in recessive model). Meanwhile, the AG genotype was shown by multivariate logistic regression analyses to exhibit a higher risk of grade 2 to grade 4 peripheral neuropathy (AA *vs.* AG, OR= 3.570, 95% CI: 1.052–12.119, P=0.041, in co-dominant model; AA+GG *vs.* AG, OR =3.061, 95% CI: 1.348–6.953, P=0.008, in over-dominant model) and alopecia (AA+GG *vs.* AG, OR =2.996, 95% CI: 1.340–6.697, P=0.008, in over-dominant model). No obvious association was found between the two SNP genotypes and the risk of anemia, leukopenia and neutropenia (data not shown).

Discussion

To the best of our knowledge, this is the first report that analyzed *EGFR* SNPs rs1468727 and rs845552 in breast cancer. In this study, we reported for the first time that the genotype of rs1468727 could predict the treatment response from neoadjuvant chemotherapy for breast cancer. We found that the genotypes of rs1468727 and rs845552 were associated with the degree of neoadjuvant chemotherapy-derived adverse events, such as increased total bilirubin, skin rash, peripheral neuropathy and alopecia. In addition, our

Table 7 Associations between genotypes and grades of increased total bilirubin

SNP ID	Model	Genotype	Grades 0–1, n (%)	Grades 2–4, n (%)	χ^2-P	OR (95% CI)	Logit-P
Rs1468727	Co-dominant	CC	23 (95.8)	1 (4.2)		1	
		CT	51 (92.7)	4 (7.3)	1.000	2.902 (0.280–30.034)	0.372
		TT	21 (70.0)	9 (30.0)	0.031*	17.183 (1.524–193.721)	0.021*
	Dominant	CC	23 (95.8)	1 (4.2)		1	
		CT+TT	72 (84.7)	13 (15.3)	0.297	4.954 (0.578–42.450)	0.144
	Recessive	CC+CT	74 (93.7)	5 (6.3)		1	
		TT	21 (70.0)	9 (30.0)	0.002*	9.006 (2.402–33.773)	0.001*
	Over-dominant	CC+TT	44 (81.5)	10 (18.5)		1	
		CT	51 (92.7)	4 (7.3)	0.093	0.289 (0.079–1.054)	0.060
Rs845552	Co-dominant	AA	18 (94.7)	1 (5.3)		1	
		AG	45 (93.8)	3 (6.2)	1.000	1.809 (0.161–20.274)	0.631
		GG	32 (76.2)	10 (23.8)	0.148	10.876 (1.059–111.680)	0.045*
	Dominant	AA	18 (94.7)	1 (5.3)		1	
		AG+GG	77 (85.6)	13 (14.4)	0.456	4.004 (0.465–34.463)	0.206
	Recessive	AA+AG	63 (94.0)	4 (5.97)		1	
		GG	32 (76.2)	10 (23.8)	0.016*	7.833 (1.976–31.047)	0.003*
	Over-dominant	AA+GG	50 (82.0)	11 (18.0)		1	
		AG	45 (93.8)	3 (6.2)	0.087	0.260 (0.065–1.036)	0.056

*, P<0.05.

Table 8 Associations between genotypes and grades of skin rash

SNP ID	Model	Genotype	Grades 0–1, n (%)	Grades 2–4, n (%)	χ^2-P	OR (95% CI)	Logit-P
Rs1468727	Co-dominant	CC	20 (83.3)	4 (16.7)		1	
		CT	47 (85.5)	8 (14.5)	1.000	1.020 (0.257–4.051)	0.978
		TT	30 (100.0)	0 (0)	0.034*	/	/
	Dominant	CC	20 (83.3)	4 (16.7)		1	
		CT+TT	77 (90.6)	8 (9.4)	0.295	0.606 (0.156–2.353)	0.469
	Recessive	CC+CT	67 (84.8)	12 (15.2)		1	
		TT	30 (100.0)	0 (0)	0.034*	/	/
	Over-dominant	CC+TT	50 (92.6)	4 (7.4)		1	
		CT	47 (85.5)	8 (14.5)	0.360	2.415 (0.656–8.883)	0.185
Rs845552	Co-dominant	AA	16 (84.2)	3 (15.8)		1	
		AG	42 (87.5)	6 (12.5)	0.706	0.813 (0.171–3.861)	0.794
		GG	39 (92.9)	3 (7.14)	0.364	0.686 (0.110–4.277)	0.687
	Dominant	AA	16 (84.2)	3 (15.8)		1	
		AG+GG	81 (90.0)	9 (10.0)	0.436	0.724 (0.168–3.120)	0.665
	Recessive	AA+AG	58 (86.6)	9 (13.4)		1	
		GG	39 (92.9)	3 (7.14)	0.364	0.557 (0.139–2.236)	0.409
	Over-dominant	AA+GG	55 (90.2)	6 (9.84)		1	
		AG	42 (87.5)	6 (12.5)	0.659	1.325 (0.393–4.469)	0.650

*, P<0.05.

Table 9 Associations between genotypes and grades of peripheral neuropathy

SNP ID	Model	Genotype	Grades 0–1, n (%)	Grades 2–4, n (%)	χ^2-P	OR (95% CI)	Logit-P
Rs1468727	Co-dominant	CC	16 (66.7)	8 (33.3)		1	
		CT	30 (54.5)	25 (45.5)	0.315	2.417 (0.811–7.203)	0.113
		TT	21 (70.0)	9 (30.0)	0.793	0.852 (0.255–2.850)	0.795
	Dominant	CC	16 (66.7)	8 (33.3)		1	
		CT+TT	51 (60.0)	34 (40.0)	0.553	1.680 (0.618–4.571)	0.309
	Recessive	CC+CT	46 (58.2)	33 (41.8)		1	
		TT	21 (70.0)	9 (30.0)	0.259	0.594 (0.238–1.482)	0.264
	Over-dominant	CC+TT	37 (68.5)	17 (31.5)		1	
		CT	30 (54.5)	25 (45.5)	0.134	2.175 (0.955–4.956)	0.064
Rs845552	Co-dominant	AA	14 (73.7)	5 (26.3)		1	
		AG	23 (47.9)	25 (52.1)	0.064	3.570 (1.052–12.119)	0.041*
		GG	30 (71.4)	12 (28.6)	1.000	1.518 (0.406–5.667)	0.535
	Dominant	AA	14 (73.7)	5 (26.3)		1	
		AG+GG	53 (58.9)	37 (41.1)	0.303	2.458 (0.777–7.776)	0.126
	Recessive	AA+AG	37 (55.2)	30 (44.8)		1	
		GG	30 (71.4)	12 (28.6)	0.091	0.508 (0.219–1.180)	0.115
	Over-dominant	AA+GG	44 (72.1)	17 (27.9)		1	
		AG	23 (47.9)	25 (52.1)	0.010*	3.061 (1.348–6.953)	0.008*

*, P<0.05.

Table 10 Associations between genotypes and grades of alopecia

SNP ID	Model	Genotype	Grades 0–1, n (%)	Grades 2–4, n (%)	χ^2-P	OR (95% CI)	Logit-P
Rs1468727	Co-dominant	CC	12 (50.0)	12 (50.0)		1	
		CT	23 (41.8)	32 (58.2)	0.501	1.744 (0.613–4.962)	0.297
		TT	17 (56.7)	13 (43.3)	0.625	0.782 (0.254–2.407)	0.668
	Dominant	CC	12 (50.0)	12 (50.0)		1	
		CT+TT	40 (47.1)	45 (52.9)	0.799	1.339 (0.517–3.468)	0.548
	Recessive	CC+CT	35 (44.3)	44 (55.7)		1	
		TT	17 (56.7)	13 (43.3)	0.248	0.632 (0.268–1.491)	0.295
	Over-dominant	CC+TT	29 (53.7)	25 (46.3)		1	
		CT	23 (41.8)	32 (58.2)	0.214	1.788 (0.812–3.937)	0.149
Rs845552	Co-dominant	AA	10 (52.6)	9 (47.4)		1	
		AG	16 (33.3)	32 (66.7)	0.144	2.482 (0.782–7.882)	0.123
		GG	26 (61.9)	16 (38.1)	0.495	0.851 (0.266–2.726)	0.786
	Dominant	AA	10 (52.6)	9 (47.4)		1	
		AG+GG	42 (46.7)	48 (53.3)	0.636	1.567 (0.555–4.427)	0.396
	Recessive	AA+AG	26 (38.8)	41 (61.2)		1	
		GG	26 (61.9)	16 (38.1)	0.019*	0.418 (0.187–0.936)	0.034*
	Over-dominant	AA+GG	36 (59.0)	25 (41.0)		1	
		AG	16 (33.3)	32 (66.7)	0.008*	2.996 (1.340–6.697)	0.008*

*, P<0.05.

results demonstrated for the first time that rs845552 may be related to HER2 status in breast cancer.

We showed that patients with an AA genotype were more likely to be HER2 positive compared to those with a AG or a GG genotype. Rs845552 is known to be located in the intron of *EGFR* gene. Previous studies have shown that the expression of EGFR can be altered following changes in the intron sequences. The repeat length of *CA simple sequence repeat 1* located at the intron 1 of *EGFR* gene has been reported to be associated with EGFR expression in breast cancer cells (22,23). Consistently, a previous study has reported that both *EGFR* and *HER2* genes tend to be overexpressed simultaneously in cancer patients (24). Thus, the rs845552 genotype may reflect the HER2 status to some extent.

In our study, the rs1468727 genotype was observed to be associated with the treatment response derived from neoadjuvant chemotherapy. In agreement with our results, a previous study has demonstrated a close association between the expression of EGFR protein with the efficacy of neoadjuvant chemotherapy for breast cancer (25). Liu *et al.* have found that the patients with a high level of EGFR expression can achieve a higher pCR rate while receiving neoadjuvant chemotherapy with docetaxel and epirubicin (26). Tanioka *et al.* have reported that a high level of EGFR mRNA was associated with the efficacy of neoadjuvant chemotherapy using anthracycline, taxane and trastuzumab (27). Thus, rs1468727, which is a SNP located in the intron region of *EGFR*, may have an impact on the expression of EGFR at mRNA or protein level, and ultimately impact the sensitivity of neoadjuvant chemotherapy for breast cancer. However, further research is required to verify this hypothesis.

Furthermore, our study suggested that the genotypes of rs1468727 and rs845552 were associated with the degree of adverse events resulting from neoadjuvant chemotherapy, such as increased total bilirubin, skin rash, peripheral neuropathy and alopecia. This could be due to myelin, a protein closely related to the HER family proteins that is localized in the peripheral nervous system (28,29). In agreement with this, a study has reported that EGFR activation is associated with peripheral neuropathy in mice (30). In this study, rs845552 genotype, a SNP of *EGFR*, was also found to be associated with the occurrence and severity of peripheral neuropathy following chemotherapy, suggesting that EGFR and the peripheral nervous system in patients are closely associated. In addition, we found that genotypes of rs1468727 and rs845552 were associated with the incidence and severity of common adverse events due to chemotherapy, such as increased total bilirubin, skin rash and alopecia, however the specific underlying mechanism remains unclear and demands further studies.

A couple of limitations were identified in our study. Firstly, the sample size in this study was relatively small. However, as an exploratory analysis based on prospective clinical trials, we can get new clues of intrinsic association between these SNPs and the efficacy and toxicity of neoadjuvant chemotherapy for further study. Of course, it is required to expand the sample size to further verify this conclusion in the future. Secondly, the specific mechanism behind the association between the SNPs with the efficacy and toxicity derived from neoadjuvant chemotherapy was unexplored. In future research, we plan to further explore whether and how different SNP genotypes will affect the level of mRNA transcription or protein expression of EGFR, in order to verify its correlation with the efficacy and toxicity of neoadjuvant chemotherapy.

Conclusions

Our study found that, in treating breast cancer with neoadjuvant chemotherapy, *EGFR* SNP rs1468727 associated with treatment response; while both SNP rs1468727 and rs845552 were attributed to toxicity, such as increased total bilirubin, skin rash, peripheral neuropathy and alopecia. In addition, we also showed that SNP rs845552 may be related to the status of HER2 in breast cancer. Our findings provide important information that may facilitate further studies to dissect the specific mechanism of EGFR in breast cancer.

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Footnote

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Ethics Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards (as revised in 2013). The study was approved by the Institutional Review Board (IRB) of Renji Hospital, School of Medicine, Shanghai Jiaotong University (IRB approval number: [2014]14K and [2017]088). Informed consent was obtained from all individual participants included in the study.

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Supplementary

Table S1 Characteristics of patients in this study

Variable	N (%)
Age	
≤50 years	47 (39.8)
>50 years	71 (60.2)
Menopausal status	
Premenopausal	48 (40.7)
Postmenopausal	70 (59.3)
Hormone receptor	
Positive	104 (88.1)
Negative	14 (11.9)
HER2	
Positive	46 (39.0)
Negative	71 (60.2)
Unknown	1 (0.8)
Clinical tumor stage	
T1	12 (10.2)
T2	60 (50.8)
T3 and T4	46 (39.0)
Trastuzumab	
No	80 (67.8)
Yes	38 (32.2)
pCR	
No	84 (71.2)
Yes	34 (28.8)

HER2, human epidermal growth factor receptor 2; pCR, pathologic complete response.