

A single-nucleotide polymorphism of the beta 2-adrenergic receptor gene can predict pathological complete response to taxane- and platinum-based neoadjuvant chemotherapy in breast cancer

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Background: Germline genetic polymorphisms in certain genes are associated with the response to anthracycline- and taxane-based neoadjuvant chemotherapy in breast cancer (BC). This translational study aims to evaluate the potential role of rs1042713 in the beta 2-adrenergic receptor (*ADRB2*) gene in predicting pathological complete responses (pCRs) to taxane- and platinum-based neoadjuvant chemotherapy in locally advanced breast cancer (LABC).

Materials and methods: The distribution frequencies of rs1042713 were genotyped in LABC patients who received taxane- and platinum-based neoadjuvant chemotherapy. Associations between tumor-relevant biomarkers, genotypes and pCRs were evaluated using Student's *t*-test for continuous variables and Chi-square or Fisher's exact test for categorical variables. For univariate analysis, the relationship between the rs1042713 polymorphism and pCR was analyzed by Chi-square or Fisher's exact test. The modified ORs with their 95% CIs were calculated by a multivariate logistic regression analysis to explore the association between genotype and pCR.

Results: There was a significant correlation of the rs1042713 genotype with estrogen receptor (ER) status ($P=0.008$). Significant differences were detected in the rs1042713 genotypes of pCR and non-pCR patients ($P=0.046$). The pCR rate was 18.2% in patients with *ADRB2* rs1042713 AA genotypes and 38.7% in AG+GG genotypes. Women carrying the AG+GG (OR=2.91, 95% CI: 1.02–8.29, $P=0.046$) genotype had a higher pCR rate than those with the AA genotype.

Conclusion: rs1042713, which is located in the *ADRB2* gene, could predict pCR to taxane- and platinum-based neoadjuvant chemotherapy in LABC. This finding suggests that rs1042713 could play a potential role as a predictive marker in clinical settings.

Keywords: beta 2-adrenergic receptor gene, locally advanced breast cancer, neoadjuvant chemotherapy, pathological complete response, polymorphism

Introduction

Neoadjuvant chemotherapy is a widely accepted treatment modality in women with newly diagnosed early breast cancer (BC), especially in locally advanced breast cancer (LABC).^{1,2} Neoadjuvant chemotherapy helps reduce tumor volume, thus enabling more patients to receive breast-conserving surgery. In addition, it offers the opportunity to assess tumor response in the resection specimen.³ Pathological complete response (pCR) is defined as the absence of invasive cancer. Previous studies have demonstrated

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that pCR after neoadjuvant chemotherapy is associated with better long-term survival.⁴⁻⁶ Among women who receive neoadjuvant chemotherapy, the clinical response rate is up to 70%, but only ~15%–30% of patients achieve pCR.^{1,2,7} Novel predictive biomarkers that can predict tumor response prior to the initiation of chemotherapy are valuable in making individualized treatment decisions and maximizing efficacy in cancer patients. Germline genetic polymorphisms include minor changes in DNA sequences that can modify the structure, expression or activity of the proteins encoded by genes.⁸ Previous research has demonstrated that germline genetic polymorphisms located in *WT1*, *IGF1R*, *MDM2* and *CD24* genes are associated with a response to anthracycline- and taxane-based neoadjuvant chemotherapy in BC.^{3,9-11}

ADRB2 is localized to the 5q32 chromosomal region. The encoded protein, the beta 2-adrenergic receptor, is a member of the G protein-coupled receptor superfamily.¹² The association between polymorphisms in the *ADRB2* genes and the risk of several diseases has been previously studied. Different polymorphic forms, point mutations, and/or downregulation of this gene are associated with nocturnal asthma, obesity, and type 2 diabetes.¹²⁻¹⁵ Huang et al demonstrated that the presence of the 27Glu (rs1042714) mutation in *ADRB2* or the 64Arg mutation in *ADRB3* (rs4994) was associated with a decreased risk for BC, especially when both mutations were present.¹⁶ rs1042713 is an A/G single-nucleotide variation at nucleotide position 46 in the *ADRB2* gene (AGA to GGA). The mutation substitutes the 16th amino acid of the translated protein chain from arginine to glycine. It is still unclear whether this polymorphism in the *ADRB2* gene has a predictive role in response to neoadjuvant chemotherapy in LABC.

ADRB2 is one of the target genes of the microRNA (miRNA) let-7 family.¹⁷ It has been reported that resistance to paclitaxel and cisplatin is partly mediated by miRNA let-7.¹⁸ We hypothesized that rs1042713 in the *ADRB2* gene could predict response to taxane- and platinum-based neoadjuvant chemotherapy in BC. In this translational study, we identified the single-nucleotide polymorphism (SNP) in the rs1042713 mutation from participants of two prospective studies in which patients received weekly paclitaxel and cisplatin as neoadjuvant chemotherapy.

Materials and methods

Study populations

From October 2013 until November 2016, 130 patients with LABC were recruited for this study. The inclusion criteria were females between the ages of 18 and 70 years with histologically confirmed but untreated large operable BC

(T size ≥ 2 cm and N0-2). Patients who suffered from other malignancies were excluded. Other inclusion and exclusion criteria have been described elsewhere.¹⁹ Patients enrolled in this study were participants from two open-label, prospective trials (SHPD001 and SHPD002). All participants from the two trials who were willing to take part in this study and provide informed consent were enrolled in this study. SHPD001 (NCT02199418) was a Phase II study evaluating weekly treatment with paclitaxel in combination with cisplatin as neoadjuvant therapy for LABC patients. SHPD002 (NCT0222199) is a prospective, randomized, open-label study comparing preoperative weekly treatment with paclitaxel and cisplatin with or without endocrine therapy in patients with operable hormone receptor-positive and triple-negative LABC. In these two trials, patients were scheduled to receive intravenous weekly infusions of 80 mg/m² paclitaxel on day 1 for 16 weeks and 25 mg/m² cisplatin on days 1, 8 and 15, every 28 days. Patients with HER2-positive cancer were allowed to use concomitant trastuzumab on a weekly basis. Written informed consent was obtained from each subject. For each subject, a 5 mL peripheral blood sample was collected and stored at -80°C . DNA was extracted from the peripheral blood samples of all subjects using Qiagen DNA blood kits (Qiagen NV, Venlo, the Netherlands) according to the manufacturer's protocol. This study was approved by the ethics committee of Renji Hospital, Shanghai Jiao Tong University. This study was conducted in accordance with the Declaration of Helsinki. Among the 130 patients in this study, 97 (74.6%) were estrogen receptor (ER)-positive and 33 (25.4%) were ER-negative.

Histopathologic response

pCR was evaluated in surgical specimens following chemotherapy and defined as the absence of invasive cancer in the breast, with or without residual noninvasive intraductal carcinoma. All cases with residual invasive cancer of any size were classified as non-pCR.

Selection of SNP in *ADRB2* gene

SNPs of the *ADRB2* gene were surveyed in the NCBI-dbSNP (www.ncbi.nlm.nih.gov/SNP/) and Hapmap (www.hapmap.org) databases. These databases have genotyped a large number of SNPs in different populations and have provided a set of tag SNPs (tSNPs) representing evolutionally linked genetic variants.²⁰ Using the SNPbrowser™ v4.0 software developed by ABI Company, we selected one tSNP (rs1042713) from the exon of the *ADRB2* gene.

SNP genotyping

SNP genotyping was performed at Shanghai Bene-gene Biotechnology Co., Ltd. (Shanghai, China), using the MassARRAY system (Sequenom, San Diego CA, USA) by matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry. Detailed information on the primer is shown in Table 1.

Statistical analyses

Genotype distributions were tested for adherence to the Hardy–Weinberg equilibrium (HWE). Associations between tumor-relevant biomarkers, genotype and pCR were evaluated using Student's *t*-test for continuous variables and Chi-square or Fisher's exact test for categorical variables. The relationship between the rs1042713 polymorphism and pCR was analyzed by Chi-square or Fisher's exact test. Modified ORs with 95% CIs were calculated by univariate and multivariate logistic regression analyses to explore the association between genotype and pCR. All statistical analyses were conducted with Stata 14.0 (StataCorp LP, College Station, TX, USA).

Results

Associations of genotype with clinicopathologic parameters

The genotypic distribution for rs1042713 was tested for deviation from HWE and showed no deviation (data not shown). There was a correlation between the rs1042713 genotypes and the clinicopathologic parameters of primary BC.

There was a significant correlation between the codominant model of the rs1042713 genotype and ER status ($P=0.008$). In ER-positive BC, more women carried the AA genotype than the AG and GG genotypes, whereas in ER-negative BC, more women carried the AG genotype than the AA and GG genotypes. There were no significant correlations between the rs1042713 genotype and other clinicopathologic features, including age, menstrual status, PR and HER2 status. The detailed associations between the rs1042713 genotype and ER are shown in Table 2.

Associations of genotype with response to neoadjuvant chemotherapy

Thirty-six out of 130 (27.7%) patients had pCR following neoadjuvant chemotherapy. The pCR rate was 18.2% in patients with the ADRB2 rs1042713 AA genotype, 38.6% and 38.9% in patients with the AG and GG genotypes, respectively, and 38.7% in patients with the AG+GG genotype. In the univariate analysis, significant differences were detected in the dominant model of rs1042713 between pCR and non-pCR patients ($P=0.046$). In the multivariate analysis, significant differences were detected in the dominant model of rs1042713 between these two groups ($P=0.046$). This finding suggests that the genotypes of this SNP are associated with response to neoadjuvant chemotherapy. Genotype distributions in the pCR and non-pCR patients are shown in Table 3. Women carrying the AG+GG (OR =2.91, 95% CI: 1.02–8.29, $P=0.046$) genotype of ADRB2 rs1042713 had a higher pCR rate than those carrying the AA genotype

Table 1 Detailed information of primers in the study

SNP	Primer1	Primer2	Extension of primer	Single-base primer
Rs1042713	ACGTTGGATGACCCAC ACCTCGTCCCTT	ACGTTGGATGAGCGCCTT CTTGCTGGCA	R	ACGTTGGATGAGCGCCTT CTTGCTGGCA

Abbreviation: SNP, single-nucleotide polymorphism.

Table 2 Associations between genotypes of rs1042713 and ER in patients with locally advanced breast cancer

SNPs	Model	Genotype	ER positive	ER negative	χ^2	P-value
rs1042713	Codominant	AA	45 (46.4)	10 (30.3)	3.503	0.174
		AG	38 (39.2)	19 (57.6)		
		GG	14 (14.4)	4 (12.1)		
	Dominant	AA	45 (46.4)	10 (30.3)	2.611	0.106
		AG+GG	52 (53.6)	23 (69.7)		
	Recessive	AA+AG	83 (85.6)	29 (87.9)	0.110	0.740
		GG	14 (14.4)	4 (12.1)		
	Overdominant	AA+GG	59 (60.8)	14 (42.4)	3.386	0.066
		AG	38 (39.2)	19 (57.6)		

Abbreviations: ER, estrogen receptor; SNP, single-nucleotide polymorphism.

(Table 3). Further analysis showed that there were significant differences in rs1042713 between the pCR and non-pCR patients in luminal type ($P=0.035$) and HER2-positive breast cancers ($P=0.045$) (Figure 1).

Discussion

In this translational study, we report for the first time that *ADRB2* rs1042713 could predict pCR to taxane- and platinum-based neoadjuvant chemotherapy in LABC, especially in luminal type and HER2-positive breast cancers. In the neoadjuvant setting, it is important to identify nonresponders to avoid ineffective treatments and provide alternative

therapies such as surgery or switching to a noncross resistant chemotherapy regimen.

In this study, we showed that the *ADRB2* rs1042713 genotypes were associated with ER status. Recent studies have found that similarities and interactions exist between the signaling pathways of ERs ($ER\alpha$, $ER\beta$, GPR30) and β adrenoceptors (β_1 , β_2 and β_3 adrenoceptors).²¹ GPR30, a recently discovered receptor, shares structural features with the β adrenoceptors. GPR30 has sites for protein kinase A phosphorylation, PDZ binding motifs, and interacts with A-kinase anchoring protein 5 (AKAP5), all of which enable its interaction with the β adrenoceptors

Table 3 Associations between genotypes of rs1042713 and pCR after neoadjuvant chemotherapy in patients with locally advanced breast cancer

SNPs	Model	Genotype	pCR	Non-pCR	OR (95% CI) ^a	P-value
rs1042713	Codominant	AA	10 (18.2)	45 (81.8)	1.00	
		AG	22 (38.6)	35 (61.4)	2.92 (0.96–8.87)	0.059
		GG	7 (38.9)	11 (61.1)	2.87 (0.65–12.71)	0.165
	Dominant	AA	10 (18.2)	45 (81.8)	1.00	
		AG+GG	29 (38.7)	46 (61.3)	2.91 (1.02–8.29)	0.046 ^b
	Recessive	AA+AG	32 (28.6)	80 (71.4)	1.00	
		GG	7 (38.9)	11 (61.1)	1.63 (0.43–6.19)	0.471
	Overdominant	AA+GG	17 (23.3)	56 (76.7)	1.00	
AG		22 (38.6)	35 (61.4)	1.63 (0.43–6.19)	0.129	

Notes: ^aOR and 95% CI was analyzed by logistic regression and adjusted by age, menses status, ER, PR, and HER2 status. Common genotype was taken as reference. ^b $P<0.05$. **Abbreviations:** pCR, pathological complete response; SNP, single-nucleotide polymorphism.

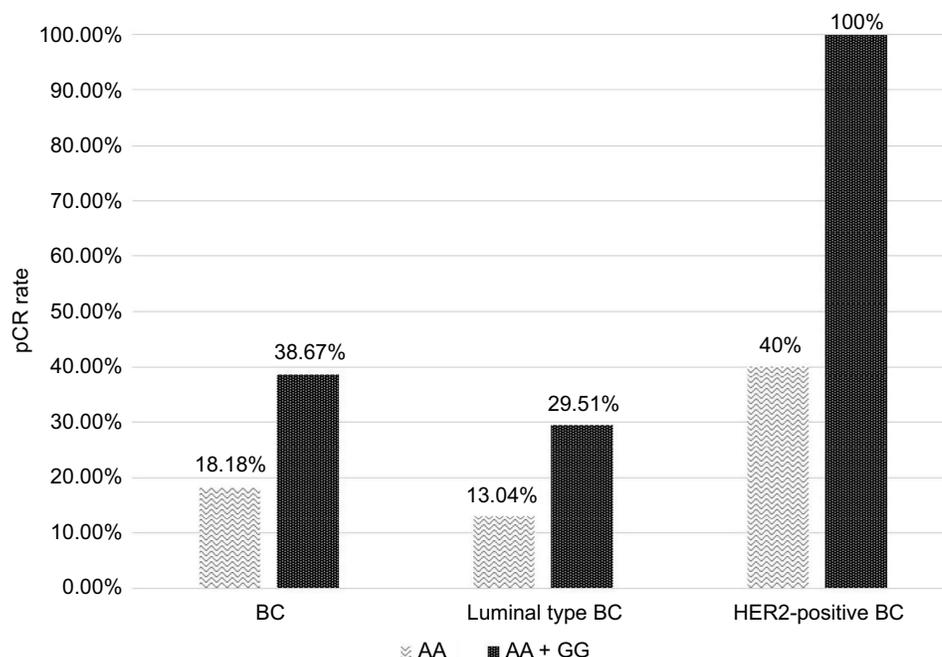


Figure 1 The relationship between genotypes of rs1042713 and pCR in patients with BC, luminal type BC and HER2-positive BC. **Abbreviations:** BC, breast cancer; pCR, pathological complete response.

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pathways.²¹ Our previous study investigated the association between β_2 adrenoceptor (encoded by the *ADRB2* gene) and clinical outcomes in BC; we found that in hormone receptor-positive BC patients, strong β_2 adrenoceptor expression was correlated with better disease-free survival than weak β_2 adrenoceptor expression.²² These findings were consistent, indicating that there are interactions between ERs and β adrenoceptors that help coordinate one another.

Some studies have demonstrated that germline polymorphisms have an effect on patient response to therapy for malignant diseases.^{23–25} However, the association between polymorphisms in the *ADRB2* gene and response to neoadjuvant chemotherapy in BC has not yet been reported. Our data showed that *ADRB2* rs1042713 can predict pCR to taxane- and platinum-based neoadjuvant chemotherapy in BC. Wu et al used modified miRNA probes to detect let-7a via in situ hybridization in formalin-fixed and paraffin-embedded sections from BC patients who received epirubicin-based neoadjuvant chemotherapy; they found that lower let-7a expression was associated with epirubicin resistance in BC tumors.²⁶ They also showed that let-7a upregulation in an epirubicin-resistant SK-3rd sphere cell line sensitized this cell line to epirubicin and upregulated let-7a expression, increasing the apoptotic cell population in the SK-3rd sphere cells compared to the negative control groups.²⁶ Additionally, Wang et al found that the *ADRB2* gene was a target of let-7f.¹⁷ Under static conditions, let-7f actively repressed translation and established basal levels of β_2 adrenoceptor expression. During agonist activation of β_2 adrenoceptor, let-7f levels decreased, resulting in depressed *ADRB2* gene silencing and a subsequent attenuation of agonist-promoted downregulation of β_2 adrenoceptor.¹⁷ As a downstream molecule of let-7, *ADRB2* may be involved in the regulation of miRNAs in let-7-related gene networks. Finally, changes in the function of *ADRB2* itself may also influence the regulation of the let-7 response to chemotherapy. The correlation between response to chemotherapy and miRNA in let-7-related gene networks will be further investigated in our future work.

We acknowledge that the present study has some limitations. The sample size of the patient cohort was small. Our findings indicate that the SNP *ADRB2* rs1042713 was associated with response to neoadjuvant chemotherapy and may have a potential role as a predictive marker in clinical settings. However, we acknowledge that we did not investigate other SNPs located in the *ADRB2* gene or miRNAs in the let-7-related gene networks.

Conclusion

The results of the present study showed that *ADRB2* rs1042713 can predict pCR to taxane- and platinum-based neoadjuvant chemotherapy in LABC. A significant association was detected between the rs1042713 genotypes and ER status. Further work is needed to clarify the molecular mechanisms underlying this relationship.

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Disclosure

The authors report no conflicts of interest in this work.

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