ABCB1 gene polymorphisms and response to chemotherapy in breast cancer patients: a meta-analysis

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1 ABSTRACT

The ABCB1 gene encodes the P-glycoprotein, an efflux pump for some antineoplastic agents which acts as a resistance mechanism to chemotherapy. Three SNPs (C3435T, C1236T and G2677T/A), are the most widely studied in ABCB1. The inconsistent conclusions about the association of these polymorphisms and the response to chemotherapy in breast cancer (BC) patients prompted us to conduct a meta-analysis.

A total of nine (770 patients), five (566 patients) and three studies (367 patients) relating the ABCB1 C3435T, C1236T and G2677T/A polymorphisms respectively, were included.

The main analysis revealed a lack of association between ABCB1 polymorphisms and response to chemotherapy in every genetic model: C3435T (dominant OR: 0.888; 95%CI: 0.558-1.413), C1236T (dominant OR: 1.968; 95%CI: 0.609-6.362) and G2677T/A (GG vs GT+GA+TT+TA+AA OR: 0.854; 95%CI: 0.418-1.744). Stratification by ethnicity, cancer type and response criteria did not change the pattern of results.

The available evidence indicates that three polymorphisms within ABCB1; C3435T, C1236T and G2677T/A, cannot be considered a reliable predictor of response to chemotherapy in BC patients.[1]

2 KEYWORDS

META-ANALYSIS

ABCB1

[1] ABCB1 (ATP-binding cassette, B1), ACT (Adjuvant Chemotherapy), BC (Breast Cancer), FAC (Fluouracil-Adryamicin-Cyclophosphamide), FEC (Fluouracil-Epirubicin-Cyclophosphamide), LABC (Locally Advance Breast Cancer), MBC (Metastatic Breast Cancer), NACT (Neoadjuvant Chemotherapy), SNP (Single Nucleotide Polymorphism)

C3435T

C1236T

BREAST

CANCER

3 INTRODUCTION

Breast cancer (BC) is the most common cause of cancer-related death in women in the world [1]. In the recent decades, substantial progress has been made in the treatment of BC. BC patients are treated neoadjuvantly (prior surgery) or adjuvantly with chemotherapy, HER2-targeted drugs (if HER2-positive), hormonal therapy, or a combination of these. The chemotherapeutic drugs which are most commonly used against BC include anthracyclines (doxorubicin and epirubicin) and taxanes (paclitaxel and docetaxel). These can be used in combination with fluorouracil (5-FU), cyclophosphamide and/or carboplatin [2]. Chemotherapy with anthracyclines or taxanes has shown impressive response rates. Polychemotherapy with anthracycline and cyclophosphamide showed better results compared to the cyclophosphamide-methotrexate-fluorouracil scheme [3]. Patients treated with taxane-based schemes at first or second-line have shown high response rates [2]. However, there is a low to moderate risk of recurrence [4].

Clinical factors including stage, size of the tumour, number of lymph nodes involved, hormonal receptors or HER2 status, alter the response to chemotherapy [5]. Other mechanisms, such as genetic differences in drug transporters, may contribute to the inter-individual variations in treatment outcomes [6]. The ATP-binding cassette, B1 (ABCB1) gene encodes the P-glycoprotein, an efflux pump for some antineoplastic agents (anthracyclines, taxanes or tamoxifen) which acts as a resistance mechanism to chemotherapy [7,8]. Three single nucleotide polymorphisms (SNPs); C1236T (rs1128503), C3435T (rs1045642) and G2677T/A (rs2032582), are the most widely

studied SNPs in ABCB1 [9]. While the ABCB1-2677G>T/A gene polymorphism results in an amino-acid change from Ala to Ser/Thr at codon 893, the 1236C>T (Gly412Gly) and 3435C>T (Ile1144Ile) SNPs are synonymous [10].

3.1 ABCB1- C3435T

The association between the ABCB1-C3435T gene polymorphism and treatment outcomes response in BC patients treated with neoadjuvant chemotherapy remains controversial. A meta-analysis published in 2012, including 7 studies (464 patients), found no influence between the ABCB1-C3435T gene polymorphism and treatment response in BC patients [11]. Recent studies have shown an association of the ABCB1-C3435T gene polymorphism with treatment response, although they differ in the positive effect of the T-allele on the response [12,13]. In 148 BC patients treated with neoadjuvant chemotherapy, the ABCB1-C3435T T-allele carriers had a higher response (T-allele vs CC: 43.4% vs 23.8%, OR: 2.695; p = 0.02) [12], whereas in 153 BC patients also treated with neoadjuvant chemotherapy, the C-allele was associated with higher response (C-allele vs TT: 71.2 vs 33.3%, p = 0.001) [13].

A more recent meta-analysis including 8 studies (608 cases) found no association between the treatment response and the dominant model (CT + TT vs CC) of the ABCB1-C3435T gene polymorphism (OR: 1.13; 95% CI: 0.58-0.37; p = 0.71) [14]. More recently, no association has been found either in a study of Kurdish BC patients (100 cases/200 controls) [15] or in 83 BC patients treated with chemotherapy plus trastuzumab [16].

3.2 ABCB1- C1236T

T-allele carriers of the ABCB1-C1236T gene polymorphism showed worse response (T-allele vs CC: 58.3% vs 85%; OR non-responders/responders: 4.63; p = 0.021) in 100 BC patients treated with neoadjuvant chemotherapy [14]. These results were further confirmed in other studies (Agarwal: 2015fk, Tulsyan: 2014cy}. However, a meta-

analysis conducted in 2013 by these authors, which included 3 studies (373 patients), found no association between the dominant model of the ABCB1-C1236T gene polymorphism, but a trend towards response to treatment (OR 1.77, 95% CI: 1.01-3.1; p = 0.05) [14].

Recent studies not included in this meta-analysis have shown conflicting results. One study found an association between TT and CT genotypes and poor response to chemotherapy (OR: 0.35; 95 CI%: 0.13-1.90; p= 0.03) [17] in 100 Arabic BC patients treated with anthracycline-based chemotherapy. Another study performed in 83 Caucasian HER2-positive BC patients treated with chemotherapy plus trastuzumab showed no association, which was likely due to the smaller sample size [16].

3.3 ABCB1-2677 G>T/A

The ABCB1-G2677T/A gene polymorphism was not associated with response to chemotherapy in 103 Korean MBC patients treated with adjuvant anthracycline plus paclitaxel [18]. This result was confirmed in a another Asian cohort of 153 BC patients treated with neoadjuvant anthracycline-based chemotherapy [13] and in an Indian study of 111 of BC patients treated with neoadjuvant/adjuvant chemotherapy [19].

BC patients (61.5% HER2-positive) with GG + GT + GA genotypes of the ABCB1-G2677T/A gene polymorphism treated with the fluorouracil-doxorubicin-cyclophosphamide scheme, showed higher resistance to treatment than TT + TA patients. The multivariate analysis showed an OR of 3.19 (95% CI: 0.98 - 10.39; p = 0.053) [20].

The inconsistent conclusions in the association of the ABCB1 C1236T, C3435T and G2677T/A gene polymorphisms and the response to chemotherapy in BC patients led us to conduct a meta-analysis encompassing evidence from all published studies, in order to provide a more precise estimation of the association.

4 Material and methods

4.1 Study design

A meta-analysis was carried out.

4.2 Literature search strategy

A literature search was carried out using PubMed, Embase, Ovid and Scielo, and included all papers published until December 20th 2016, containing the keywords “ABCB1”, “polymorphism”, "breast”, “cancer" and “response”. In order to achieve a comprehensive literature, we also identified additional studies by screening reference lists of key studies or reviews. The literature retrieval was performed in duplication by two independent reviewers (AMP and MCG).

The inclusion criteria used for literature selection were:

1 Original papers.

2 Performed in humans.

3 Studies that explore the association between the three selected SNPS and chemotherapy response.

4 Papers with sufficient data to calculate odds ratio and 95% confidence interval.

5 Chemotherapy response evaluation by Response Evaluation Criteria In Solid Tumors (RECIST)/ World Health Organisation (WHO) criteria.

6 Papers written in English or Spanish.

The exclusion criteria were:

1 Studies reporting overlapping data.

2 Studies with incomplete data.

3 Studies with duplicated data

4.3 Data extraction

Data was carefully extracted from all eligible studies independently by two investigators

according to the aforementioned inclusion criteria. The following information of the studies were extracted: first author, published year, country, ethnicity, numbers of patients included in the study, genotype frequencies according to chemotherapy response, clinical stage, treatment protocols, genotyping methods and response evaluation criteria. Two reviewers completed the data extraction and reached consensus on all of the extracted data (AMP and MCG). Any discrepancies in the data extraction following this were resolved by a third reviewer (ASP) undertaking data extraction and analysis.

4.4 Statistical analysis

The strength of the association between the ABCB1 C3435T, C1236T and G2677T/A gene polymorphisms and the response to BC treatment therapies was measured with the ORs and their 95% confidence intervals. First, treatment response was estimated with all the available studies for each polymorphism according to the dominant (CT + TT vs CC), recessive (TT vs CC + CT) and co-dominant (CT vs CC and TT vs CC) models in the case of the ABCB1 C3435T and C1236T gene polymorphisms. Several models were used for the ABCB1-G2677T/A gene polymorphism (GG + GT + GA vs TT + TA + AA; GG vs GT + GA + TT + TA + AA; GG vs GT + GA; GG vs TT + AA + AT; GT + GA vs TT + AA + AT).

For the meta-analysis, the heterogeneity of the studies was determined using the Cochran Q chi-square test ($p < 0.05$ was considered as significant heterogeneity), alongside the degree of inconsistency presented as the I^2 index where I^2 of 0-25% = no heterogeneity; I^2 of 25-50% = moderate heterogeneity; I^2 of 50-75% = high heterogeneity and I^2 of 75-100% = extreme heterogeneity. The combined OR of all studies was calculated using the DerSimonian-Laird random effects model. The effect of the study was considered statistically significant if the confidence interval of the combined OR did not contain 1. The data of each analysis were graphically represented using funnel plots generated by the STATA program (Stata Software

Package version 10 (StataCorp LP, College Station, Texas, USA)). The bias of publication was analysed by the Begg`s and Egger`s tests.

We analysed the overall effect of all studies for each of the ABCB1 gene polymorphisms, - C3435T, C1236T and G2677T/A, according to ethnicity, tumor type and response criteria. The software used for the meta-analysis was Metadisc [21].

5 RESULTS

5.1 Study characteristics

A total of 44 studies were found with the keywords "ABCB1", "polymorphism", "breast", "cancer", "response".

We excluded 19 studies because they were written in Japanese, they included only pharmacokinetic data or because the analysis was performed in non-BC patients.

Seventeen studies were selected after eliminating all meta-analyses, one article whose drug in study was liposomal doxorubicin, one which considered survival as the dependent variable instead of response, and another which assessed only modifications in the expression of ABCB1. Of these 17, three further articles were discarded. Two had not used the RECIST/WHO criteria as a response measure and the third did not have the response data available for later use. Of the remaining articles, three had been developed by the same research group. The first,(Tulsyan et al. [27]) was excluded because all data had been included in the study by Agarwal et al. [19]. This study [19] was kept for the general analysis as it was the most recently published article and included patients with various types of BC. The third article, by Chaturvedi et al. [14], had also been published by the same research group. This study was used for the subgroup analysis which focused on the type of cancer, as its patients had locally advanced breast cancer (LABC) or metastatic breast cancer (MBC). The information from the final 12 studies included in analysis was extracted according to the gene polymorphism. This resulted in nine articles being included for the ABCB1-

C3435T gene polymorphism, five for ABCB1-C1236T and three for ABCB1-G2677T/A. All characteristics for each of the studies are summarised in tables 1 and 2.

Table 1. Characteristics of the studies included in the meta-analysis.

Reference	SNP	Cancer type	N	Age	NACT/ ADJ	Scheme	Etnia	Genotyping Method	Response Criteria
Kafka et al. 2003 [22]	C3435T	LABC	68	Mean 53 (27-78)	NACT	ANTHRACYCLINE-BASED	GERMAN (CAUCASIAN)	PCR-SEQ	RECIST
Ashariati et al. 2008 [23]	C3435T	LABC	19	Median 46.5	NACT	NA	INDONESIAN (ASIAN)	PCR-SEQ	RECIST
Chang et al. 2009 [18]	C3435T G2677T/A	MBC	103	Median 49	ACT	ANTHRACYCLINE + PACLITAXEL	KOREAN (ASIAN)	PCR-SEQ	RECIST
George et al. 2009 [24]	C3435T	LABC	76	Mean 48.7	NACT	FAC	INDIAN	PCR-RFLP	RECIST
Cizmarikova et al. 2010 [25]	C3435T	LABC	38	Mean 55	NACT	ANTHRACYCLINE-BASED	SLOVAK (CAUCASIAN)	PCR-RFLP	RECIST
Zhang et al. 2011 [26]	C3435T C1236T	LABC	119	Median 49	NACT	ANTHRACYCLINE-BASED	CHINESE (ASIAN)	PCR-RFLP	RECIST
Ji et al. 2012 [13]	C3435T C1236T G2677T/A	PRIMARY	153	Median 48	NACT	ANTHRACYCLINE-BASED	CHINOS (ASIAN)	PCR-RFLP	WHO
Alsaif et al. 2013	C1236T	DE TODOS	100	Mean 51.5	ACT	ANTHRACYCLINE-BASED	ARABIC	TAQMAN	WHO

[17]									
Chaturvedi et al. 2013 [14]	C3435T C1236T	LABC y MBC	100	NA	ACT / NACT	FAC/FEC	INDIAN	PCR-RFLP	RECIST
Agarwal et al. 2015 [19]	C3435T C1236T G2677T/A	ALL TYPES	111	Mean 48.92	ACT / NACT	ANTHRACYCLINE-BASED	INDIAN	PCR-RFLP	RECIST
Madrid et al. 2016 [16]	C3435T C1236T	ALL TYPES	83	Mean (52 ± 13)	ACT/ NACT	ANTHRACYCLINE-BASED	SPANISH (CAUCASIAN)	TAQMAN	RECIST

ACT (Adjuvant chemotherapy), BC (Breast Cancer), LABC (Locally Advance Breast Cancer), MBC (Metastatic Breast Cancer), FAC (Fluorouracil-adriamycin-cyclophosphamide), FEC (Fluorouracil-epirubicin- cyclophosphamide), NA: Not available, NACT (Neoadjuvant Chemotherapy), Seq (Sequencing)

Table 2. Genotype distribution of the studies included in the meta-analysis.

Reference	Genotypes C3435T			Genotypes C1236T			Genotypes G2677T/A		
	CC	CT	TT	CC	CT	TT	GG	GT or GA	TT or AA or AT
Kafka et al. 2003 [22]	14	39	15						
Ashariati et al. 2008 [23]	0	5	14						
Chang et al. 2009 [18]	53	10	40				23	44	36
George et al. 2009 [24]	8	35	23						
Cizmarikova et al. 2010 [25]	5	25	8						
Zhang et al. 2011 [26]	45	53	21	16	64	41			
Ji et al. 2012 [13]	34	98	21	19	56	78	26	86	41
Alsaif et al. 2013 [17]				73	11	16			
Chaturvedi et al. 2013 [14]	10	49	41	20	44	36			
Agarwal et al. 2015 [19]	15	50	46	23	48	40	3	54	54
Madrid et al. 2016 [16]	21	51	11	33	42	8			

5.2 Meta-analysis

Nine articles were included for the ABCB1-C3435T gene polymorphism (770 patients) to calculate the combined OR (Table 1). There was no association of this polymorphism with the response to chemotherapy in patients with BC in any of the models (dominant, recessive, codominant) (Table 3).

Table 3. Response to chemotherapy depending on different ABCB1-C3435T genetic models and groups of patients (ethnicity/cancer type).

Model	Total or subgroup of patients	N	OR (95% CI)	I^2 (%)	P
Dominant (CT+TT vs CC)	Global	8	0.888 (0.558 - 1.413)	21.2	0.261
	Caucasian	3	1.263 (0.193-8.253)	58.4	0.090
	Asian	3	0.797 (0.497-1.280)	1.7	0.362
	Indian	2	1.168 (0.334 - 4.084)	41.2	0.192
	All types + primary	3	1.217 (0.643 - 2.302)	6.7	0.342
	LABC + MBC	6	0.961 (0.436- 2.119)	46	0.099
	RECIST	7	0.807 (0.471 - 1.383)	22.4	0.258
Recessive (TT vs CT + CC)	Global	8	0.993 (0.519 - 1.900)	61.3	0.008
	Caucasian	3	0.875 (0.316-2.420)	0	0.510
	Asian	3	0.965 (0.267-3.483)	78.9	0.003
	Indian	2	0.948 (0.394 - 2.285)	54.1	0.140
	All types + primary	3	2.007 (0.695 - 5.798)	60.7	0.078
	LABC + MBC	6	0.830 (0.455 - 1.513)	45.1	0.091
	RECIST	8	0.812 (0.484 - 1.361)	30.9	0,182
CT vs CC	Global	8	0.834 (0.538 - 1.292)	0	0.467
	Caucasian	3	1.077 (0.544-2.131)	0	0.760
	Asian	3	0.709 (0.412-1.220)	75.3	0.017

	Indian	2	1.262 (0.468 - 3.404)	0	0.361
	All types + primary	3	1.008 (0.535 - 1.898)	0	0.466
	LABC + MBC	6	1.004 (0.426 - 2.368)	38.8	0.147
	RECIST	7	0.808 (0.467 - 1.397)	8.4	0,364
TT vs CC	Global	8	1.146 (0.511 - 2.571)	57	0.023
	Caucasian	3	1.244 (0.096-16.124)	65	0.057
	Asian	3	1.238 (0.366-4.190)	0	0.609
	Indian	2	1.083 (0.215 - 5.444)	60.3	0.113
	All types + primary	3	2.327 (0.756 - 7.161)	41.8	0.179
	LABC + MBC	6	0.830 (0.455 - 1.513)	45.1	0.091
	RECIST	7	0.848 (0.415 - 1.734)	34.0	0.168
N: studies, All types (BC of all stages)					

Five studies, with a total of 566 patients were selected for analysis of the ABCB1-C1236T gene polymorphism. No association of this polymorphism with the response to chemotherapy was found in patients with BC, in any model (Table 4).

Table 4. Response to chemotherapy depending on different ABCB1-C1236T genetic models and groups of patients (ethnicity/cancer type).

Model	Total or subgroup of patients	N	OR (95% CI)	I^2 (%)	P
Dominant (CT+TT vs CC)	Global	5	1.968 (0.609 - 6.362)	79.9	0.001
	Asian	2	0.956 (0.453-2.020)	0	0.640
	All types	4	2.507 (0.596 - 10.543)	82.5	0.001
	LABC + MBC	2	1.846 (0.323 - 10.545)	76.3	0.040
	RECIST	3	1.22 (0.53 – 2.82)	39.6	0.1912

	WHO	2	1.029 (0.429 - 2.469)	0	0.5473
Recessive (TT vs CT + CC)	Global	5	1.951 (0.690 - 5.519)	82.2	0.000
	Asian	2	1.045 (0.390 - 2.804)	72.5	0.057
	All types	4	2.753 (0.814 - 9.307)	80.7	0.001
	LABC + MBC	2	0.845 (0.446 - 1.601)	20.3	0.263
	RECIST	3	0.909 (0.531 -1.556)	0	0,399
	WHO	2	1.376 (0.814 - 2.325)	0	0,480
CT vs CC	Global	5	1.384 (0.576 - 3.323)	55.4	0.062
	Asian	2	0.867 (0.390 -1.928)	0	0.827
	All types	4	1.578 (0.498 - 5.001)	64.9	0.036
	LABC + MBC	2	2.117 (0.399 - 11.233)	72	0.059
	RECIST	3	1.275 (0.362 - 4.487)	68.1	0.044
	WHO	2	1.720 (0.278 -10.632)	63.7	0,097
TT vs CC	Global	5	2.472 (0.610 -10.019)	80.5	0.000
	Asian	2	0.963 (0.408-2.271)	13.1	0.283
	All types	4	3.660 (0.754 - 17.757)	79.1	0.002
	LABC + MBC	2	1.498 (0.227 - 9.897)	76.3	0.040
	RECIST	3	1.246 (0.320 - 4.861)	60	0.082
	WHO	2	7.106 (0.278 - 181.59)	91.7	0.001
N: studies, All types (BC of all stages)					

After the stratification by ethnicity, type of cancer and the criteria to measure the response according to the dominant, recessive or codominant models, no influence of these factors was found to be associated with the response to chemotherapy in BC

patients for either the ABCB1 C3435T (Table 3, Figures 1 and 2) or C1236T (Table 4, Figure 3) gene polymorphisms.

Figure 1. Forest plot of the dominant model of ABCB1-C3435T gene polymorphism by ethnicity.

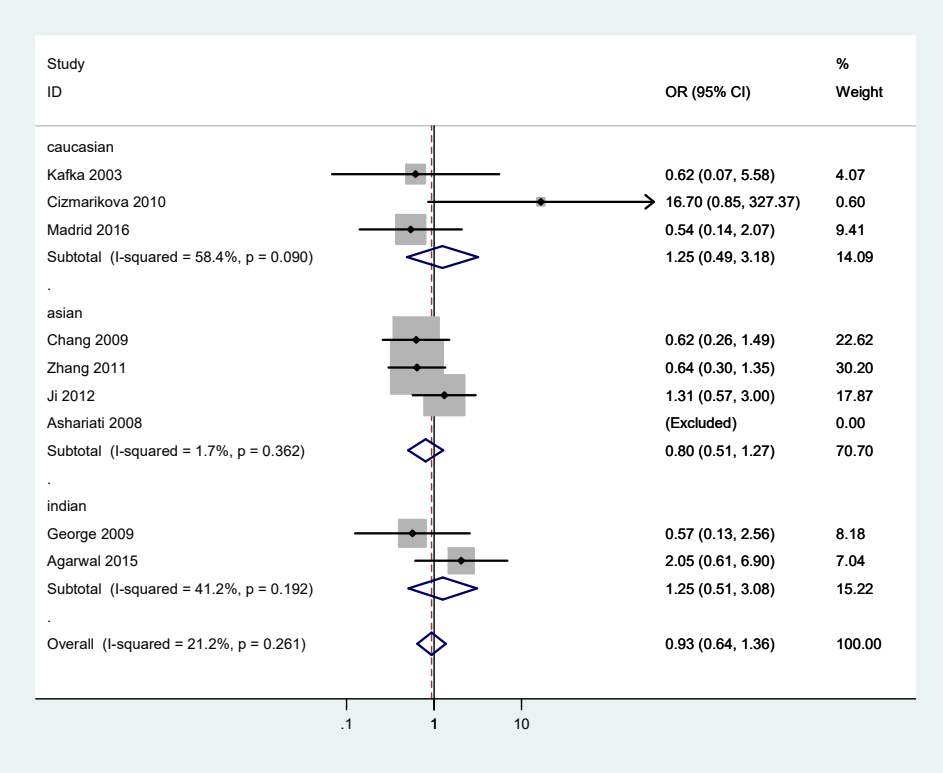


Figure 2. Forest plot of the dominant model of ABCB1-C3435T gene polymorphism by cancer type.

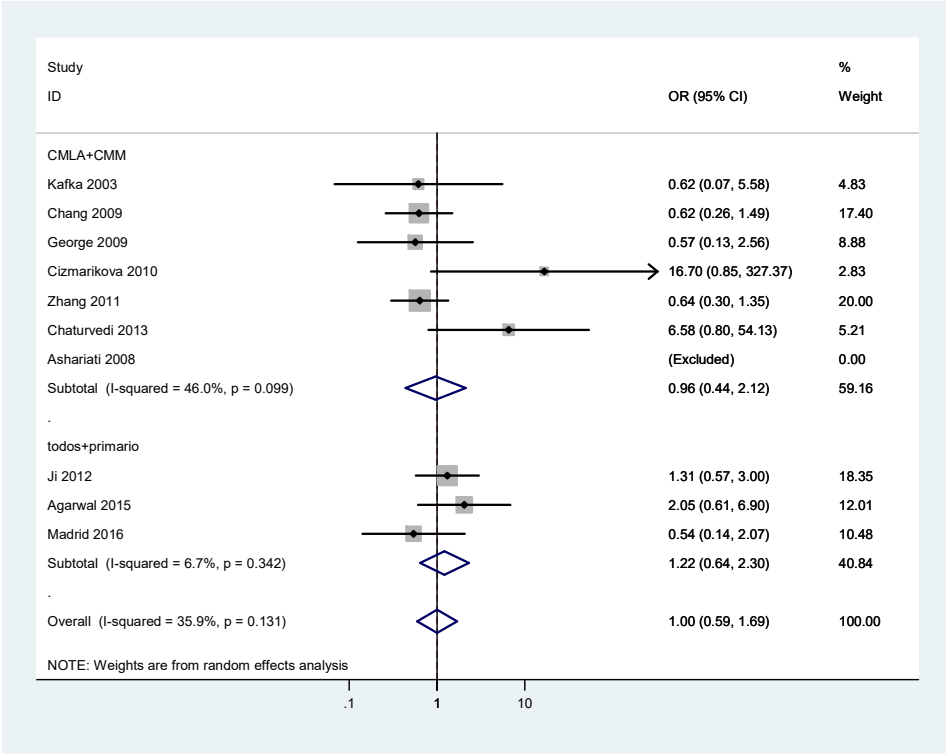
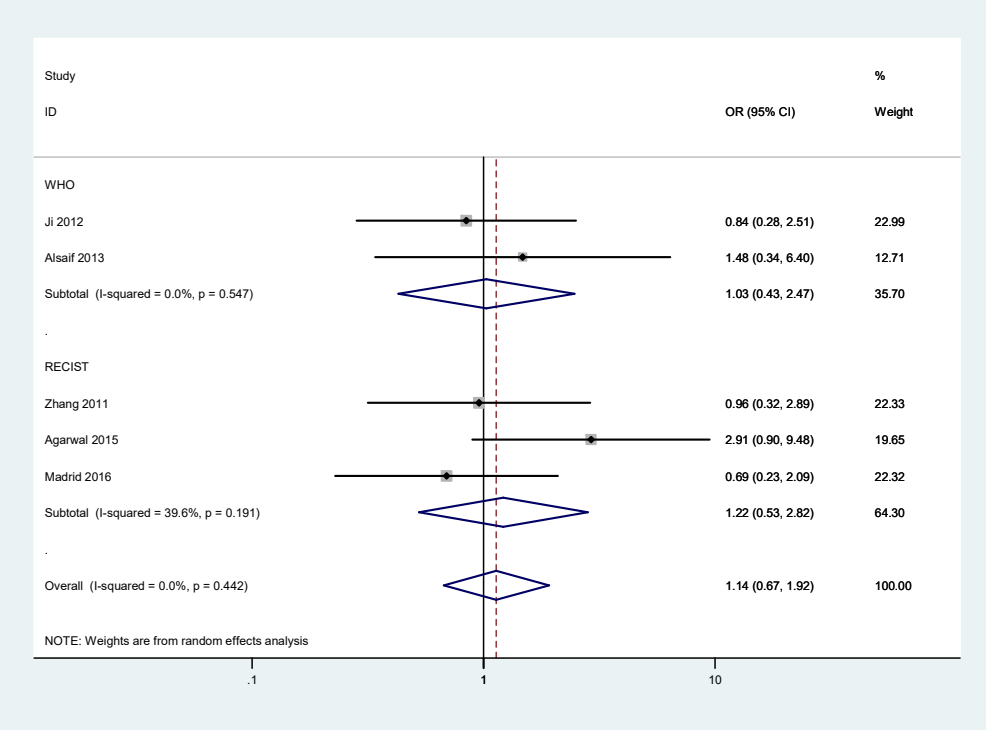


Figure 3. Forest plot of the dominant model of ABCB1-C1236T gene polymorphism by the criteria measurement of the response.



The meta-analysis for ABCB1-G2677T/A which included three studies and 367 patients, did not reveal an association between the polymorphism and the response to chemotherapy (table 4). No association between the ABCB1-G2677T/A gene polymorphism and response to chemotherapy was found after stratification by ethnicity (two studies in Asian population) or cancer type (two studies of all types of cancer) in any model (table 5).

Table 5. Response to chemotherapy depending on different ABCB1-G2677T/A genetic models and groups of patients (ethnicity/cancer type).

Model	Total or subgroup of patients	N	OR (95% CI)	I^2 (%)	P
GG + GT + GA vs TT + TA + AA	Global	3	0.804 (0.506-1.278)	0	0.912
	Asian	2	0.779 (0.434 – 1.400)	0	0.694

	All types	2	0.851 (0.500 - 1.450)	0	0.986
GG vs GT + GA + TT + TA + AA	Global	3	0.854 (0.418-1.744)	3.1	0.356
	Asian	2	0.934 (0.442 - 1.973)	8.3	0.296
	All types	2	0.602 (0.246 – 1.475)	0	0.444
GG vs GT + GA	Global	3	0.929 (0.374-2.308)	25.6	0.261
	Asian	2	1.071 (0.406 - 2.825)	39.2	0.200
	All types	2	0.614 (0.243 - 1.550)	0	0.453
GG vs TT + AA + AT	Global	3	0.708 (0.321-1.560)	0	0.575
	Asian	2	0.780 (0.344 - 1.769)	0	0.573
	All types	2	0.557 (0.203 – 1.533)	0	0.456
GT + GA vs TT + AA + AT	Global	3	0.824 (0.511 - 1.330)	0	0.673
	Asian	2	0.765 (0.414 - 1.412)	0	0.422
	All types	2	0.928 (0.538 – 1.599)	0	0.997
N: studies, All types (BC of all stages)					

5.3 Test for heterogeneity

The heterogeneity of the studies used in each comparison of the ABCB1 C3435T and C1236T gene polymorphisms and response to chemotherapy, were diverse. These are detailed in Tables 3, 4 and 5 and include the degree of inconsistent data (I^2 index) and the Cochran Q chi-square test (heterogeneity: $p<0.05$). Heterogeneity was higher for the ABCB1-C1236T polymorphism models.

5.4 Publication bias

The dominant model of ABCB1-C3435T did not present publication bias, calculated by the Begg test (p = 0.293; p continuity corrected = 0.368) and the Egger test results (p = 0.891).

Similarly, there was no publication bias in the dominant model of ABCB1-C1236T, according to the Begg (p = 0.73; p continuity corrected = 0.462) and the Egger test (p = 0.470). The graphical representations of these tests are shown below (Figures 4 and 5).

Figure 4. Begg's funnel plot for publication bias test (ABCB1-C3435T dominant model).

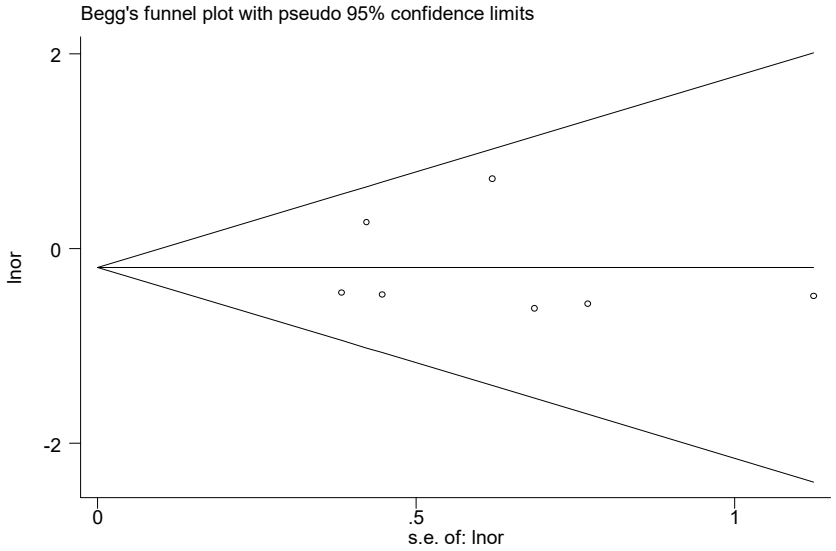
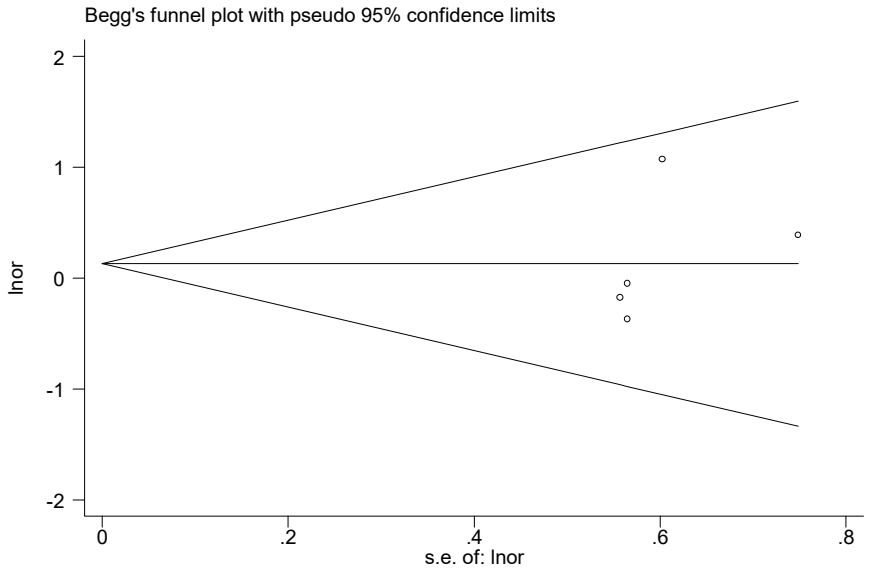
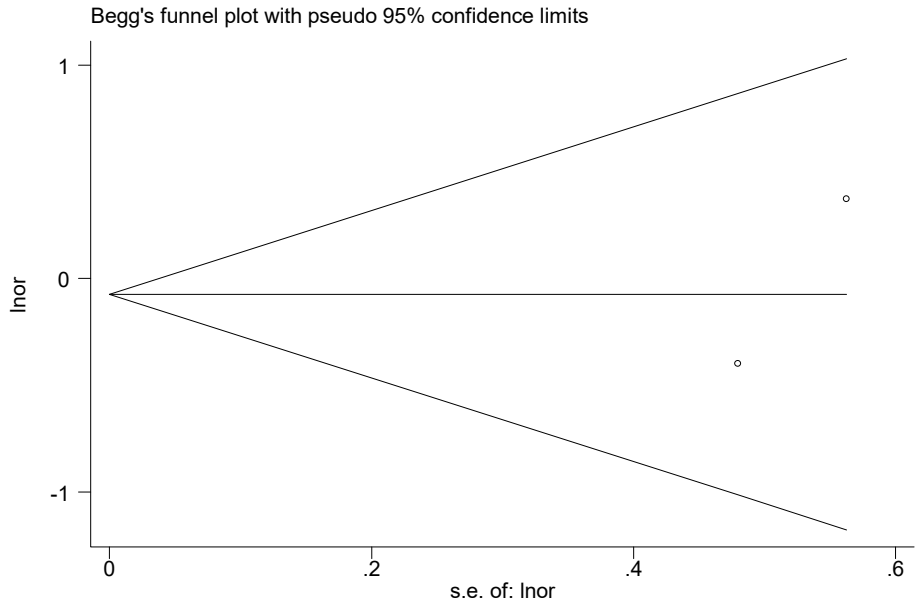


Figure 5. Begg's funnel plot for publication bias test (ABCB1-C1236T dominant model).



The model (GG vs GT + GA + TT + TA + AA) of ABCB1-G2677T/A did not present publication bias as showed by the Begg test (p = 0.317; p continuity corrected = 1.0) (Figure 6).

Figure 6. Begg's funnel plot for publication bias test (ABCB1-G2677T/A GG vs GT + GA + TT + TA + AA model).



6 DISCUSSION

Current therapeutic management of patients with HER2 positive BC, including various combinations of chemotherapeutic agents, HER2 targeting drugs and hormone therapy, has greatly improved the outcomes in these patients. Although these schemes often achieve favorable results, response to treatments remains a challenge for clinicians. ABCB1 gene polymorphisms have been proposed as potential predictors of response to chemotherapy. In the present study, we performed a meta-analysis to evaluate the association between the ABCB1 gene polymorphism and response to chemotherapy.

The association of the ABCB1-C3435T polymorphism and response in BC patients treated with neoadjuvant chemotherapy was initially suggested in a study of 68 patients with locally advanced BC who showed better clinical response in patients carrying the TT genotype of ABCB1-C3435T (OR: 4.38, 95% CI: 1.2-16.1, p = 0.029) [22]. In two meta-analyses, one published in 2012 which included 464 patients [11] and the second published in 2013 which included 608 individuals {Chaturvedi: 2013ch}, the ABCB1-C3435T gene did not influence the response to treatment. Other studies have attempted to refute this lack of association with response to treatment, but have failed to find consistency in the effect of the T allele on clinical outcome. In a study of Chinese BC patients, those with the ABCB1-C3435T T allele showed an improved response to neoadjuvant chemotherapy (43.4% vs 23.8%, OR: 2.695, p = 0.02) [12]. Conversely, patients with TT genotype of ABCB1-C3435T showed a poorer response to neoadjuvant chemotherapy (33.3 vs. 71.2%, p = 0.001) [13].

Our meta-analysis included 770 patients from nine studies confirmed the absence of the association between the response to chemotherapy and ABCB1-C3435T. These results are in agreement with the meta-analysis by Chen et al. [11] and Chaturvedi et al. [14]. The meta-analysis by Chen et al. included seven studies (464 patients), three of which included Caucasian populations, three which included Asian populations and

one included a mixed ethnic group (Brazilians). All patients had BC in advanced stages (6 LABC and 1 MBC), and the sample size of the studies varied between 19-119 cases. The meta-analysis published by Chaturvedi et al. encompassed 608 patients from 8 studies. Each of the included studies, were also assessed in the meta-analysis by Chen et al., with the exception of the study by Ji et al. [13], which was conducted in an Asian population, and data from their own study, focused on an Indian population [14].

In our meta-analysis, the stratified analysis by types of cancer (primary + all types vs LABC + MBC), also showed no association with the response to chemotherapy in any genetic model of the ABCB1-C3435T polymorphism, with a high heterogeneity, especially in the subgroup of patients with LABC + MBC (I^2 = 46.0%; p = 0.099).

Other polymorphisms in ABCB1 have been explored as biomarkers of response. The T-allele of the ABCB1-C1236T gene polymorphism was associated with a poorer response (58.3% vs 85%, OR $_{\text{non-responders/responders}}$: 4.63; p = 0.021) in 100 Indian BC patients treated with neoadjuvant chemotherapy, cyclophosphamide plus epirubicin or doxorubicin) [15,19]. A similar trend was demonstrated by carriers of the T allele in a small cohort of 58 patients treated with neoadjuvant chemotherapy (57.4% vs. 81.8%, OR $_{\text{non-responders/responders}}$: 3.33; p = 0.150), but without a statistically significant association, which is most likely due to the small sample size [27]. In our meta-analysis, the ABCB1-C1236T genetic polymorphism did not influence the response. The different genetic models (dominant, recessive and co-dominant) of ABCB1-C1236T were not associated with the response to chemotherapy in the global meta-analysis. Stratification by ethnicity, cancer type and response measurement criteria did not provide information about the possible influence of these variables on the response to chemotherapy in BC patients. This data is in line with the meta-analysis including 373 patients, conducted by an Indian research group, comprising three studies, two in Asian population, and their results in Indian population. They did not find an

association between the ABCB1-C1236T gene polymorphism and response to chemotherapy in patients with BC, although a trend was shown (OR: 1.77; 95% CI: 1.01-3.10; p = 0.05) [14]. Our meta-analysis included all studies to date with a total of five (566 patients), increasing the accuracy of the study, and thus confirming the non-association of this polymorphism with the response to chemotherapy in BC patients.

The ABCB1-G2677T/A gene polymorphism was not associated with response to chemotherapy in 103 Korean MBC patients treated with adjuvant anthracycline plus paclitaxel [18]. This result was confirmed in a another Asian cohort of 153 BC patients treated with neoadjuvant anthracycline-based chemotherapy [13] and in an Indian study of 111 of BC patients treated with neoadjuvant/adjuvant chemotherapy [19]. Our meta-analysis included three studies (two in Asian population and one in an Indian population), and did not find an association between the ABCB1-G2677T/A, different genetic models and the response to chemotherapy.

These meta-analyses showed no association between ABCB1 polymorphisms and response to chemotherapy, despite some studies having shown contradictory results. Possible causes of these differences may be the complexity of the phenomenon and gene-gene interactions [28]. Some intracellular alterations, as other unknown ABCB1 polymorphisms and errors in the posttranslational modification in the protein structure of P-glicoprotein, might balance the effect of these polymorphisms.

In our meta-analysis, one limitation is the high heterogeneity that has been found in the different comparisons, especially in relation to the ABCB1-C1236T gene polymorphism. This heterogeneity could be due to the fact that the analysis encompasses few studies, five in the global analysis, and fewer in the subgroup analyses.

The evidence provided by this work indicates that the ABCB1 C3435T, C1236T and G2677T/A gene polymorphisms are not associated with the response to chemotherapy in BC patients. No influence of ethnicity, cancer type and response criteria has been

found. Subsequent studies focused on other polymorphisms could clarify the importance of this gene in the response to chemotherapy.

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