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ORIGINAL ARTICLE

ABCB1-C3435T polymorphism and breast cancer risk: a case-control study and a meta-analysis

Alireza Sharif[1], Davood Kheirkhah[2], Mohammad Reza Sharif[3], Mohammad Karimian[4], Zahra Karimian[5]

[1]Department of Infectious Disease, Kashan University of Medical Sciences, Kashan; [2]Department of Pediatrics, Kashan University of Medical Sciences, Kashan; [3]Trauma Research Center, Kashan University of Medical Sciences, Kashan; [4]Anatomical Sciences Research Center, Kashan University of Medical Sciences, Kashan; [5] Student Research Committee, School of Nursing and Midwifery, Shahroud University of Medical Sciences, Shahroud, Iran

Summary

Purpose: *To investigate the association of ABCB1-C3435T transition with breast cancer risk which was followed by a meta-analysis.*

Methods: *In a case-control study we collected blood samples from 290 women (including 150 breast cancer patients and 140 healthy controls). ABCB1-C3435T genotyping was performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. A meta-analysis was performed for a total of 13 eligible studies involving 5,835 cases and 8,178 controls.*

Results: *The results of case-control study revealed a significant association between T allele (OR=1.770, 95%CI=1.236-2.535, p=0.002), CT genotype (OR=1.661, 95%CI=1.017-2.713, p=0.042), and TT genotype (OR=3.399, 95%CI=1.409-8.197, p=0.006) with breast cancer risk. Data from meta-analysis revealed a significant association between ABCB1-C3435T polymorphism and breast cancer risk in allelic (OR=1.243, 95%CI=1.079-1.432, p=0.003), co-dominant (OR=1.349, 95%CI=1.042-1.746, p=0.023), dominant (OR=1.204, 95%CI=1.019-1.422, p=0.029), and recessive (OR=1.226, 95%CI=1.011-1.488, p=0.039) models.*

Conclusions: *The results suggest that the ABCB1-C3435T gene polymorphism might be a genetic risk factor and a potential biomarker for breast cancer.*

Key words: *ABCB1 gene, breast cancer, genetic polymorphism, meta-analysis*

Introduction

Breast cancer is most widespread malignant disease in females worldwide [1]. In the last decades, extensive progress has taken place in the diagnosis and therapy of breast cancer. Some efforts were realized to identify environmental and genetic risk factors that contribute to the development of breast cancer [2,3]. Complex interactions between genes (including *BRCA1*, *BRCA2*, *PTEN*, and *p53*) and epigenetic modifications (such as DNA methylation, chromatin remodeling), contribute to breast cancer genesis. Genetic polymorphisms are a helpful tool to identify susceptibility genes as prognostic biomarkers [4]. Furthermore, some studies revealed that genetic variants of the adenosine triphosphate-binding cassette B1 (*ABCB1*) gene, are associated with cancer susceptibility [5,6].

ABCB1, also known as P-glycoprotein (P-gp), is encoded by *MDR1* (multi-drug resistance) gene, and it is one of the most broadly studied ATP-binding membrane transporters. This molecule is an ATP-dependent drug efflux pump for a range

Correspondence to: Davood Kheirkhah, MD. Department of Pediatrics, Kashan University of Medical Sciences, Kashan, Iran. Tel/fax: +98 31 55621158, E-mail: kheirkhah.d@kaums.ac.ir
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of carcinogens and antineoplastic agents, and it plays an important role in the defense against exogenous and endogenous toxins [7,8]. The *ABCB1* gene is located on chromosome 7 (7q21.1). Single-nucleotide polymorphisms (SNPs) may reduce the function of ABCB1. There are more than 50 variants in the *ABCB1* gene. C3435T transition (with ID: rs1045642) which is located in exon 26 is one of the most common polymorphisms in *ABCB1* gene [9].

The aim of this study was to investigate the association of *ABCB1*-C3435T polymorphism with risk of breast cancer development, followed by a meta-analysis.

Methods

Subjects

Blood samples of 150 Iranian women with sporadic breast cancer were collected from the Shahid Beheshti Hospital (the first Affiliated Hospital of Kashan University of Medical Sciences, Kashan, Iran). The cases were diagnosed by clinical tests as well as mammographic and pathological studies. We excluded patients with previous cancer and previous chemotherapy or radiotherapy. Blood samples from 140 age-matched healthy women (controls) from the same geographical area without history of familial malignancies were collected. All the participants provided written informed consent and this study complied with the principles outlined in the Declaration of Helsinki and approved by the Hospital's Ethics Committee.

SNP genotyping

Genomic DNA for genotype analyses was isolated from blood samples, using a DNGplus Kit (Cinnagen, Iran). For PCR, the entire genome sequence of *ABCB1* gene was deduced from NCBI database. The oligonucleotide primers for *ABCB1* gene containing C3435T transition were designed by oligo7 software (http://www.oligo.net/). The sequences of forward and reverse primers were F: 5'- GTGAACTCTTGTTTTCAGCTG-3' and R: 5'-CTTACATTAGGCAGTGACTCG-3', respectively. *ABCB1*-C3435T genotyping was done by polymerase chain reaction-restriction fragment length (PCR-RFLP). *ABCB1* fragment was amplified with the use of 0.35 µM of each forward and reverse primers, 0.5 µl dNTPs mix, and 1.5 µM $MgCl_2$, Taq polymerase and template DNA in a total volume (all PCR reagents) were purchased from Fermentas Co., Germany. PCR was carried out in Eppendorf thermal cycler (Eppendorf, Germany) with the following program: initial denaturation at 94°C for 5 min, 35 repetitive cycles of 30 sec at 94°C, 30 sec at 53°C, 45 sec at 72°C, and final extension at 72°C for 5 min.

PCR products were digested by *Sau3A*I at 37°C for 16 hrs, and then the enzyme mixture was electrophoresed onto 8% polyacrylamide gels and visualized by silver nitrate (AgNO3) staining. The digested samples presented 3 altered patterns: CC genotype, representing 167 and 33-bp fragments; TT genotype with 230-bp fragments; and CT genotype with 230,167, and 33-bp fragments.

Statistics

Hardy-Weinberg equilibrium (HWE) for both the cases and controls was calculated. The frequencies of alleles and genotypes were calculated by direct counting. Comparisons of the alleles and genotypes frequencies were assessed using the chi-square test. The associations of each genotypes and alleles with breast cancer risk were estimated by odds ratios (ORs) and 95% confidence intervals (CIs). A two-tailed p value <0.05 was considered as a statistically significant difference.

Meta-analysis

We tried to detect all studies that investigated the association of *ABCB1*-C3435T polymorphism with breast cancer. To find related studies, we carried out a comprehensive systematic search through PubMed, ScienceDirect, Google Scholar, and other databases on Sep 2015. The following words were searched: "ABCB1", "MDR1", "polymorphism", "rs1045642", and "breast cancer". Moreover, some studies were recognized by a manual exploration of the reference lists in the main articles. Of the studies with overlapping or similar data from the same researchers, the complete or most recent articles were included. Included studies had to satisfy the following criteria: (i) studies on humans; (ii) investigation of the *ABCB1*-C3435T polymorphism and breast cancer risk; (iii) case-control study design; (iv) HWE equilibrium should be established in control groups; (v) valid data were accessible to estimate the odds ratio (OR) and its 95%CI.

Two of the authors extracted the data independently according to the aforementioned inclusion criteria. Inconsistencies around inclusion of articles and explanation of data were decided by discussion and negotiation with a specialist. The following data were extracted from each study: the first author's name, year of publication, subject ethnicity, number of cases and controls, genotype frequency, and genotyping method. Different ethnicity was classified as Asian and Caucasian.

Chi-square test was used to assess the HWE in the control groups. The groups that deviated from the HWE had a p value <0.05. The strength of the associations between *ABCB1*-C3435T and breast cancer risk was evaluated by OR and 95%CI. Meta-analysis was carried out by the 5 following models: (1) T vs C allele (allelic model), (2) TT vs CC genotype (co-dominant model), (3) CT vs CC genotype (co-dominant model), (4)

Table 1. Genotype and allele frequencies of *ABCB1*-C3435T in cases and controls

Genotype	*No. and percentage*		*OR (95% CI)*	*p value*
	Control (n=140) n (%)	*Case (n=150) n (%)*	*n (%)*	
CC	79 (56.43)	61 (40.67)	-	-
CT	53 (37.86)	68 (45.33)	1.661 (1.017-2.713)	0.042
TT	8 (05.71)	21 (14.00)	3.399 (1.409-8.197)	0.006
CT+TT	61 (43.57)	89 (59.33)	1.889 (1.185-3.013)	0.008
Allele				
C	211 (75.36)	190 (63.33)	-	-
T	69 (24.64)	110 (36.67)	1.770 (1.236-2.535)	0.002

OR: odds ratio, CI: confidence interval

TT+CT genotype vs CC genotype (dominant model), and (5) TT vs CT+CC (recessive model). The heterogeneity test was done by the Q test and estimated I^2 score [10], and p<0.1 was considered as a significant difference. When the p value of the Q test was >0.1, the fixed model was used [11], otherwise the random model was used [12]. Sensitivity analysis was done to evaluate the stability of meta-analysis by excluding an individual study each time. The Begg's funnel plot and Egger's test were used to assess the possible publication bias and p<0.05 was considered as presence of such bias [13,14]. OpenMeta[Analyst] (http://www.cebm.brown.edu/open_meta/download) and Comprehensive Meta Analysis ver.2 software (http://www.meta-analysis.com/index.php) were used to perform the statistical analysis.

Results

Distribution of ABCB1-C3435T alleles and genotypes

The *ABCB1* genotypes distribution for C3435T transition was in HWE in the case and control groups. The alleles and genotypes frequencies for the C3435T in case and control groups are provided in Table 1. The frequencies of 3435CC, 3435CT, and 3435TT genotypes in the breast cancer group were 40.67, 45.33 and 14.00%, while these ratios in the control group were 56.43, 37.86 and 05.71%, respectively. The 3435C and 3435T allele frequencies in the case group were 63.33 and 36.67%, while these ratios in the control group were 75.36 and 24.64%, respectively. Genotype analysis revealed that there were significant associations between CT (OR=1.661, 95%CI=1.017-2.713, p=0.042) and TT (OR=3.399, 95%CI=1.409-8.197, p=0.006) genotypes and breast cancer risk. Furthermore, carriers of T allele (CT+TT) showed

Table 2. Characteristics of included studies

Country (ethnicity)	*Genotype frequencies*						*PHWE*	*Genotyping method*	*Author [Reference]*
	Controls			*Cases*					
	CC	*CT*	*TT*	*CC*	*CT*	*TT*			
Turkey	18	23	9	7	33	17	0.73	PCR–RFLP	Turgut et al. 2007 [5]
Norway	40	17	52	33	9	51	<0.01	PCR-RFLP	Nordgard et al. 2007 [15]
Spain	85	162	54	35	70	30	0.13	PCR–RFLP	Henríquez-Hernández et al. 2009 [16]
Iran	12	45	20	16	57	33	0.11	PCR–RFLP	Tatari et al. 2009 [17]
India	15	32	21	8	39	39	0.67	PCR–RFLP	George et al. 2009 [18]
Iran	10	27	13	10	30	14	0.55	PCR–RFLP	Taheri et al. 2010 [19]
Slovak	35	54	24	46	108	67	0.71	PCR–RFLP	Cizmarikova et al. 2010 [20]
Germany	1228	2736	1522	730	1543	902	0.98	MALDI-TOF MS	Abbas et al. 2010 [21]
Poland	52	103	50	48	96	65	0.94	PCR–RFLP	Rubiś et al. 2012 [22]
China	440	624	180	388	565	220	0.08	PCR–RFLP	Wu et al. 2012 [23]
Mexico	37	103	43	32	83	13	0.09	PCR–RFLP	Macías-Gómez et al. 2014 [24]
Mexico	56	72	24	82	133	33	0.92	PCR–RFLP	Gutierrez-Rubio et al. 2015 [9]
Iran	79	53	8	61	68	21	0.82	PCR-RFLP	This study

HWE:Hardy–Weinberg equilibrium, PCR:polymerase chain reaction, RFLP:restriction fragment length polymorphism. A Hardy–Weinberg equilibrium in the control group with p value <0.05 did not satisfy the Hardy–Weinberg equilibrium.

most significant association with breast cancer risk (OR=1.889, 95%CI=1.185-3.013, p=0.008). Also, allele analysis showed a significant association between 3435T allele and breast cancer risk (OR=1.770, 95%CI=1.236-2.535, p=0.002).

Meta-analysis

According to inclusion criteria a total of 12 eligible articles were included in the meta-analysis [5,9,15-24]. Also the data from our study were added to the meta-analysis. A flow chart presenting the study selection procedure is shown in Figure 1. As a result, 13 studies were included in the meta-analysis with 5,835 cases and 8,178 healthy controls. Table 2 shows the main features of all studies of the meta-analysis. There were 6 studies with Caucasians, 5 with Asians and 2 with Latinos. One study applied MALDI-TOF MS to SNP genotyping whereas the others applied PCR-RFLP.

The overall results of the meta-analysis are summarized in Table 3. Overall, for the 13 pooled studies, a significant association between *ABCB1*-C3435T and breast cancer was observed in T vs C (OR=1.243, 95%CI=1.079-1.432, p=0.003), TT vs CC (OR=1.349, 95%CI=1.042-1.746, p=0.023), CT+TT vs CC (OR=1.204, 95%CI=1.019-1.422, p=0.029), and TT vs CC+CT (OR=1.226, 95%CI=1.011-1.488, p=0.039) models (Figure 2). Results of meta-analysis in Asian and Caucasian

Table 3. Association results in the meta-analysis

Group	*T vs C*		*TT vs CC*		*CT vs CC*		*CT+TT vs CC*		*TT vs CC+CT*	
	OR (95% CI)	*P*	*OR (95% CI)*	*P*	*OR (95% CI)*	*P*	*OR (95% CI)*	*P*	*OR (95% CI)*	*P*
Total	1.24 (1.08- 1.43)	0.003	1.35 (1.04- 1.75)	0.023	1.02 (0.94- 1.11)	0.615	1.20 (1.02- 1.42)	0.029	1.23 (1.01- 1.49)	0.039
Asian	1.54 (1.11-2.13)	0.010	1.51 (1.22- 1.87)	< 0.001	1.10 (0.94- 1.30)	0.222	1.20 (1.03- 1.39)	0.018	1.42 (1.18- 1.71)	< 0.001
Caucasian	1.21 (1.01- 1.45)	0.035	1.42 (1.01- 2.00)	0.047	1.11 (0.84- 1.47)	0.462	1.24 (0.93- 1.64)	0.139	1.08 (0.99- 1.19)	0.082

OR: odds ratio, CI: confidence interval

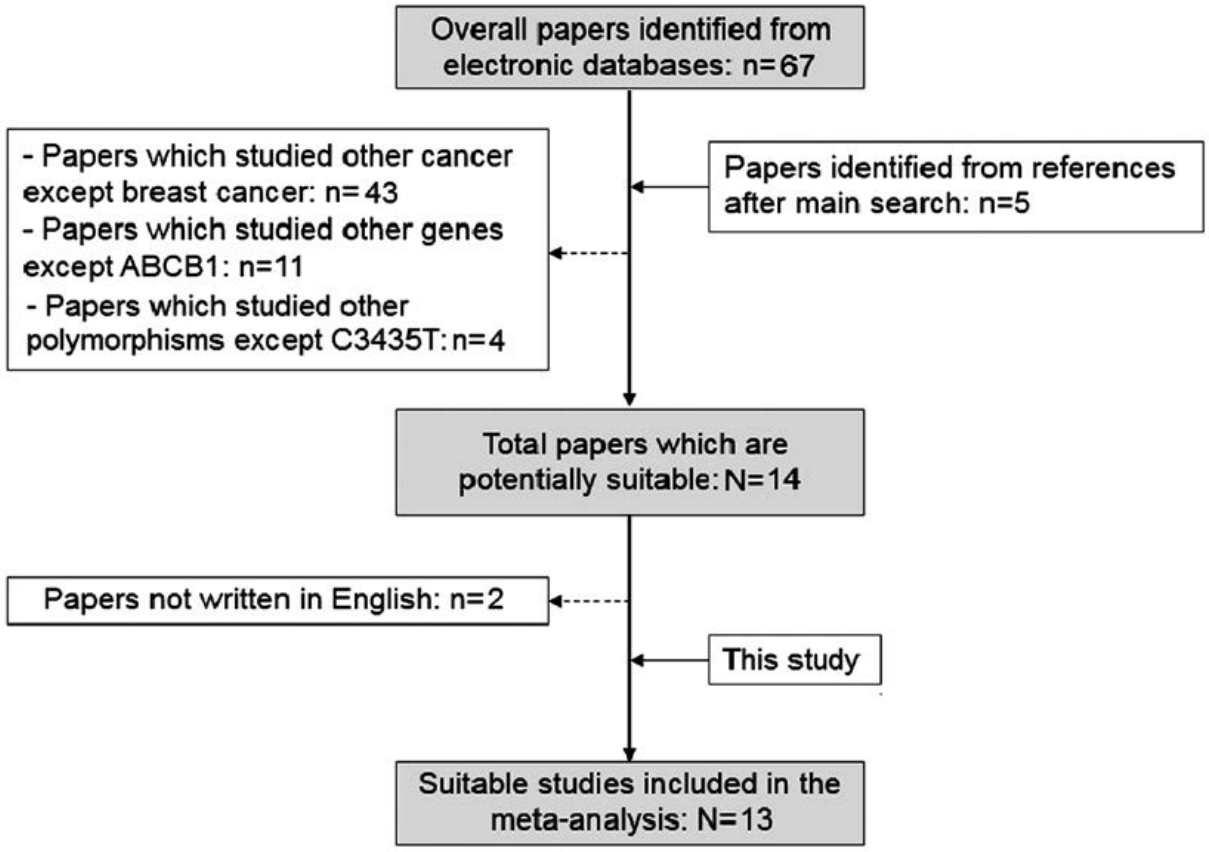

Figure 1. Study identification diagram.

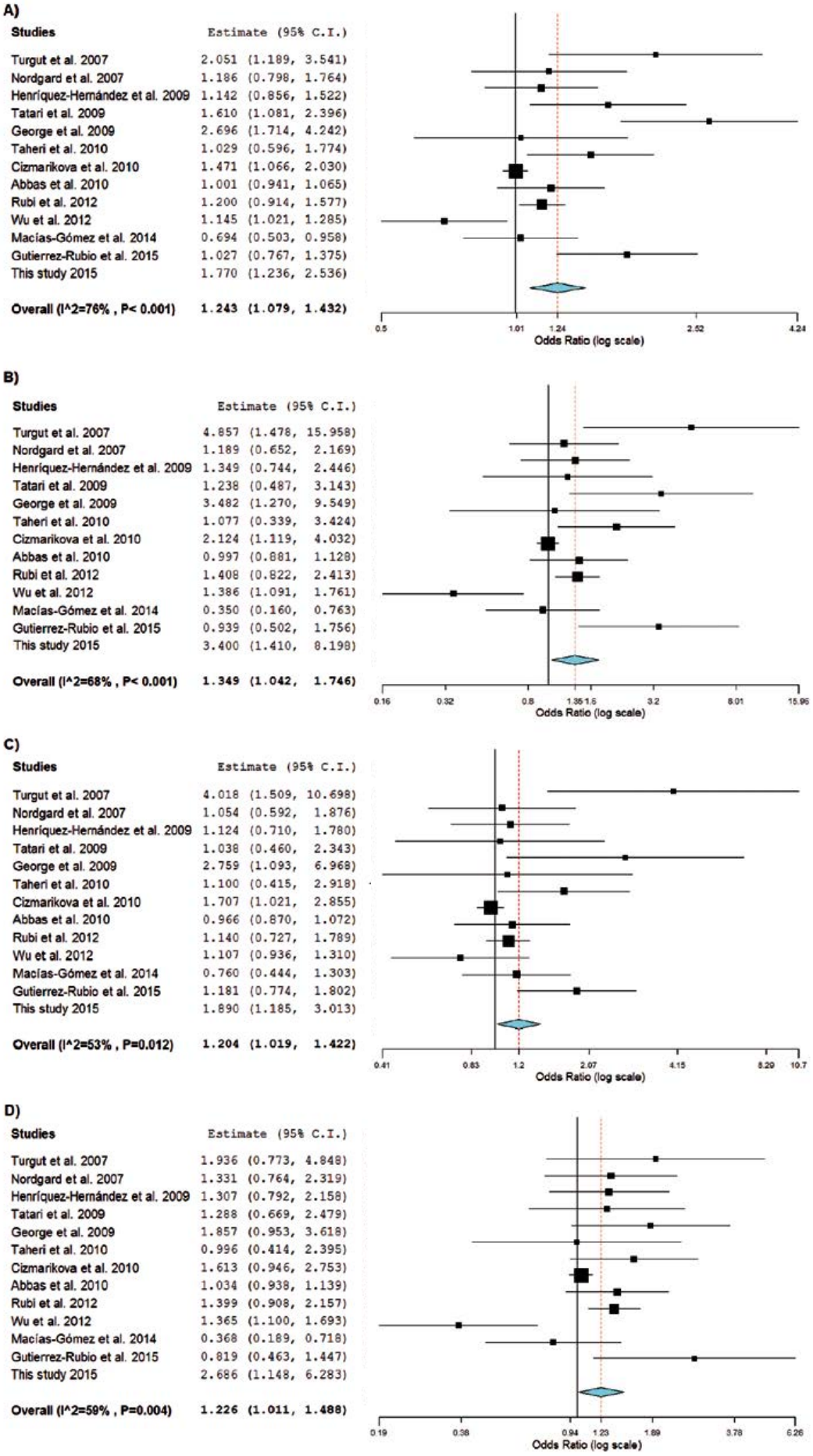

Figure 2. Forest plot for association of *ABCB1*-C3435T with breast cancer risk in overall meta-analysis. **A)** T *vs* C model; **B)** TT *vs* CC model; **C)** CT+TT *vs* CC model; **D)** TT *vs* CC+CT model.

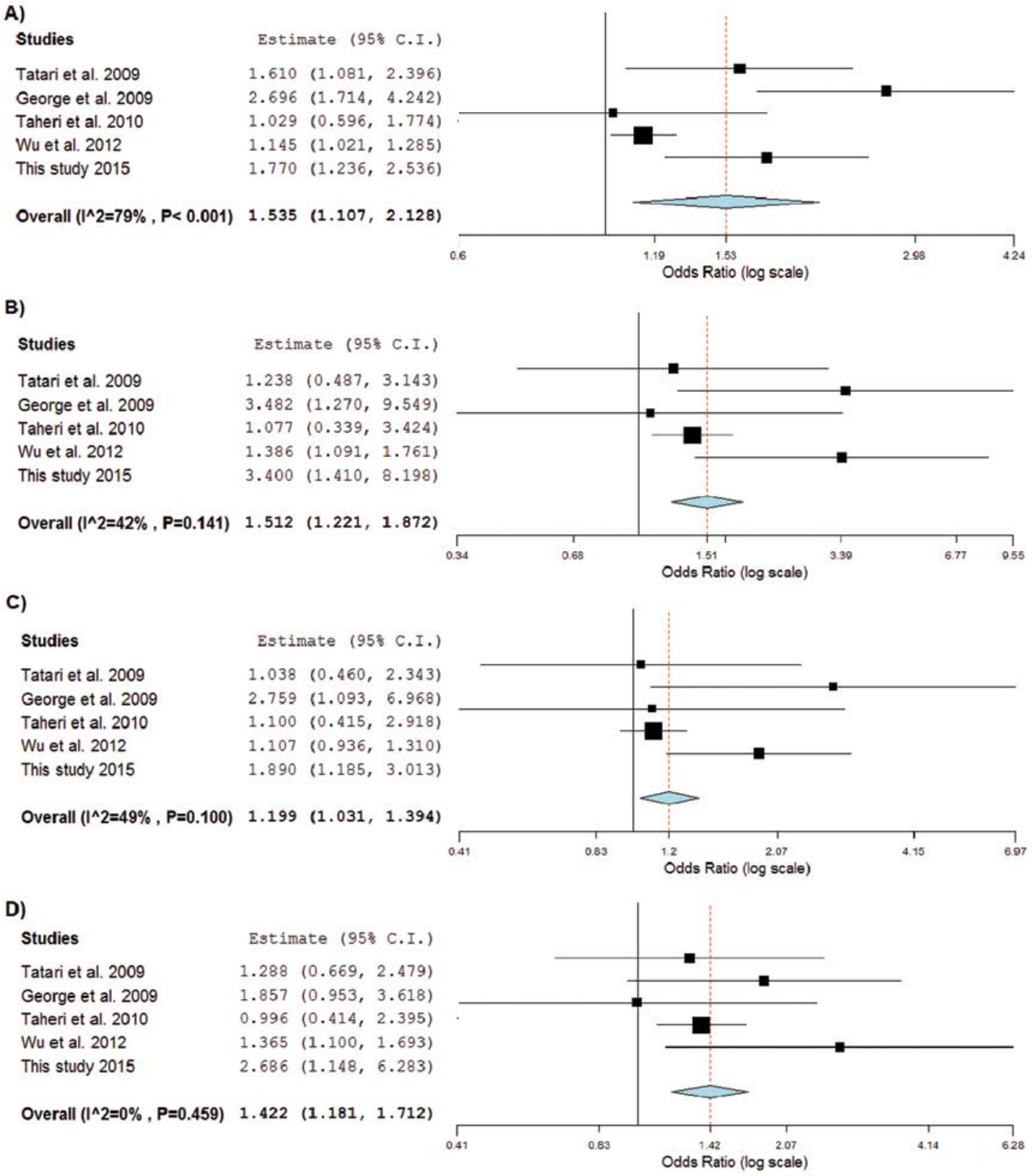

Figure 3. Forest plot for association of *ABCB1*-C3435T with breast cancer risk in Asian. **A)** T *vs* C model; **B)** TT *vs* CC model; **C)** CT+TT *vs* CC model; **D)** TT *vs* CC+CT model.

subgroups are summarized in Table 3. The results revealed that the *ABCB1*-C3435T was associated with breast cancer risk in Asian population in T vs C (OR=1.535, 95%CI=1.107-2.128, p=0.010), TT vs CC (OR=1.512, 95%CI=1.221-1.872, p<0.001), CT+TT vs CC (OR=1.199, 95%CI=1.031-1.394, p=0.018), and TT vs CC+CT (OR=1.422, 95%CI=1.181-1.712, p<0.001) models (Figure 3). Also, we observed a significant association between *ABCB1*-C3435T and breast cancer risk in Caucasian population in T vs C (OR=1.214, 95%CI=1.014-1.454, p=0.035) and TT vs CC (OR=1.419, 95%CI=1.005-2.002, p=0.047) models (Figure 4). No significant association between *ABCB1*-C3435T and breast cancer risk was observed in the Latinos subgroup (data not shown).

In overall meta-analysis, there was a high heterogeneity for T vs C ($P_{heterogeneity}$< 0.001; I^2=76%), TT vs CC ($P_{heterogeneity}$<0.001; I^2=68%), CT+TT vs CC ($P_{heterogeneity}$=0.012; I^2=53%), and TT vs CC+CT ($P_{heterogeneity}$=0.004; I^2=59%) models (Table 4). In Asian

Table 4. Results of heterogeneity and publication bias in the meta-analysis

Group	T vs C			TT vs CC			CT vs CC			CT+TT vs CC			TT vs CC+CT		
	Ph	I^2	*Pe*	*Ph*	I^2	*Pe*	*Ph*	I^2	*Pe*	*Ph*	I^2	*Pe*	*Ph*	I^2	*Pe*
Total	< 0.001	76%	0.03	< 0.001	68%	0.11	0.110	34%	0.04	0.012	53%	0.02	0.004	59%	0.25
Asian	< 0.001	79%	0.90	0.141	42%	0.38	0.235	28%	0.29	0.100	49%	0.31	0.459	0%	0.55
Caucasian	0.017	64%	0.01	0.019	63%	0.01	0.071	51%	0.25	0.025	61%	0.05	0.222	28%	0.001

Ph: P heterogeneity (p< 0.1) was considered as a significant difference. Pe: P Egger (p< 0.05) was considered as a significant difference

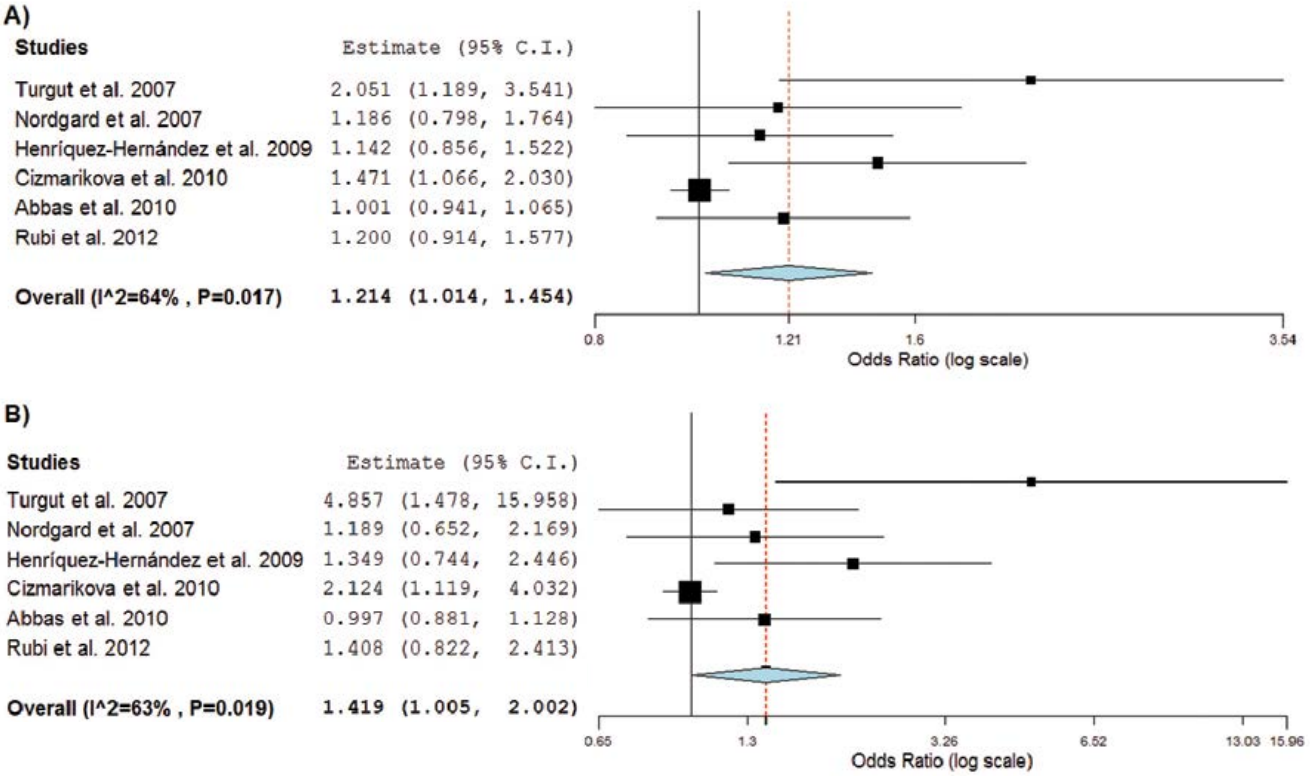

Figure 4. Forest plot for association of *ABCB1*-C3435T with breast cancer risk in Caucasian. **A)** T *vs* C model; **B)** TT *vs* CC model.

studies, a high heterogeneity was found in T vs C ($P_{heterogeneity}$<0.001; I^2=79%) model (Table 4). Also, in Caucasian studies a true heterogeneity was found in T vs C ($P_{heterogeneity}$=0.017; I^2=64%), TT vs CC ($P_{heterogeneity}$=0.019; I^2=63%), CT vs CC ($P_{heterogeneity}$=0.071; I^2=51%), and CT+TT vs CC ($P_{heterogeneity}$=0.025; I^2=61%) models (Table 4). In addition, in Latino studies a true heterogeneity was observed in T vs C ($P_{heterogeneity}$=0.077; I^2=68%), TT vs CC ($P_{heterogeneity}$=0.053; I^2=73%), and TT vs CT+CC ($P_{heterogeneity}$=0.074; I^2=69%).

The Egger's test suggested a publication bias for the T vs C (P_{Egger}=0.03), CT vs CC (P_{Egger}=0.04), and CT+TT vs CC (P_{Egger}=0.02) models in overall meta-analysis and for the T vs C (P_{Egg}=0.01), TT vs CC (P_{Egger}=0.01), CT+TT vs CC (P_{Egger}=0.05), and TT vs CC+CT (P_{Egger}=0.001) models in Caucasian meta-analysis, whereas the shapes of funnel plot for all the 5 models in the Asian subgroup seemed approximately symmetrical (Figure 5). The distribution of the studies on the funnel plot did not show any evidence of asymmetry, suggesting lack of publication bias. The lack of publication bias was confirmed by the Egger's test (Table 4).

Sensitivity test was performed by excluding a study at one time and the results revealed that the estimates before and after the omission of each study were similar. These results suggested that this meta-analysis was stable (data not shown).

Discussion

Breast cancer is the most common malignancy in women in both developed and developing countries. This disease is a growing problem that affects about one in 8 women in their lifetime. The occurrence of breast cancer is on the increase (approximately 1% annually) [25-27] and the causes of this disease are poorly understood. Risk factors such as age, geographical variation, family histo-

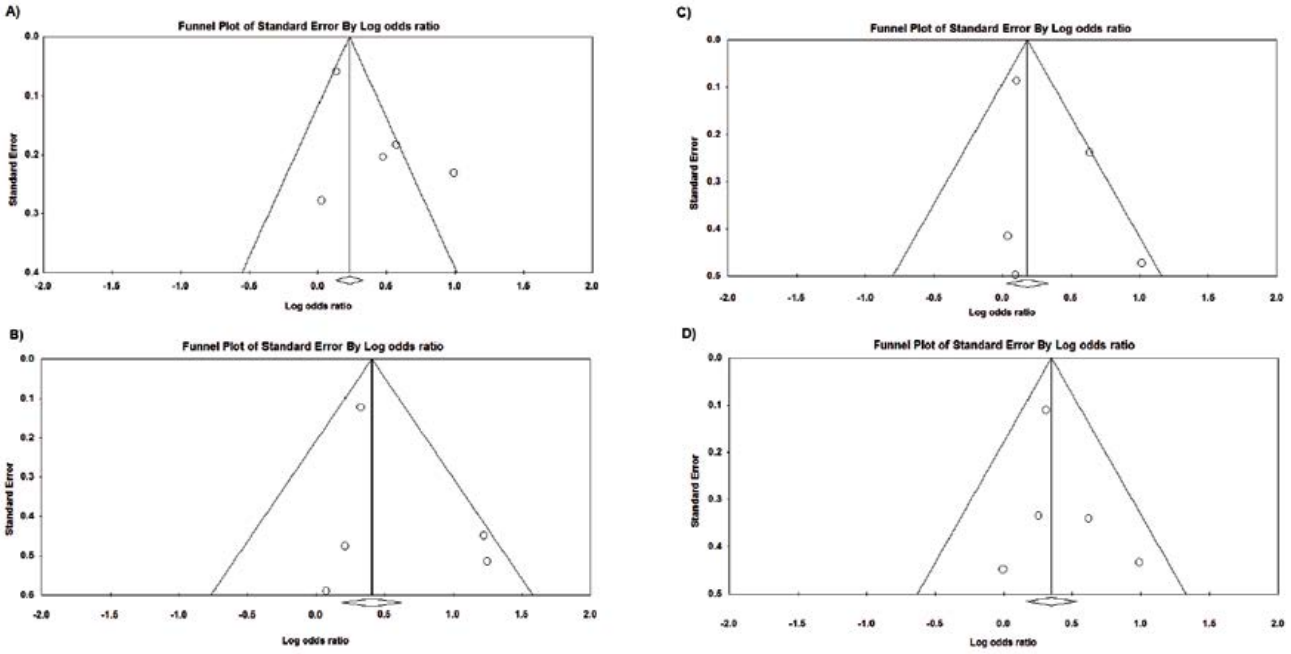

Figure 5. Funnel plot for publication bias test in Asian meta-analysis. **A)** T *vs* C model; **B)** TT *vs* CC model; **C)** CT+TT *vs* CC model; **D)** TT *vs* CC+CT model.

ry, lifestyle, and genetic factors play a key role in the genesis of this disease. Gene polymorphisms are increasingly considered as key risk factors for breast cancer occurrence in specific populations [28]. The identification of some key single nucleotide polymorphisms (SNP), which affect the expression and structure of proteins, could be helpful to predict susceptible individual for cancer risk [29].

ABCB1, as an ATP-dependent pump, can drive cytotoxic drugs and intracellular toxins out of the cell [30]. Since ABCB1 results in resistance to toxic xenobiotic and cytotoxic drugs, it is biologically possible that the C3434T transition might change the protective effects of the proteins [31].

Our case-control study revealed that there is a significant association of 3435T allele (OR=1.770, 95%CI=1.236-2.535, p=0.002), 3435CT genotype (OR=1.661, 95% CI=1.017-2.713, p=0.042), and 34 35TT genotype (OR=3.399, 95%CI=1.409-8.197, p= 0.006) with breast cancer risk. Several studies have investigated the association of *ABCB1*-C3435T SNP and breast cancer risk with inconsistent results. For example Turgut et al. (2007) [5] and Tatari et al. (2009) [17] reported an association between *ABCB1*-3435T allele and breast cancer, whereas Henríquez-Hernández et al. (2009) [16] and Taheri et al. (2010) [19] didn't find any association. Meta-analysis, as a powerful statistical tool, provides reconciliation of results by analyzing pooled inconsistent data from several studies [32]. The present meta-analysis revealed that an association exists between *ABCB1*-C3435T polymorphism and breast cancer risk both in Asian and Caucasian studies. Also, our study revealed heterogeneity in the overall meta-analysis. After stratified meta-analysis, we didn't find a true heterogeneity in the Asian subgroup. These might be ascribed to different environments, lifestyles, and genetic backgrounds among different ethnicities.

There are 4 meta-analyses over the *ABCB1*-C3435T gene polymorphism and breast cancer risk [31,33-35] which evaluate the association of *ABCB1*-C3435T and *ABCB1*-rs2214102 polymorphisms with breast cancer risk. Wang et al. (2012) [35] assessed the association of *ABCB1*-C3435T and overall risk of cancer and reported an association between C3435T polymorphism and increased risk of breast cancer. Also Sheng et al. (2012) [34] carried out a meta-analysis between *ABCB1*-C3435T polymorphism and cancer risk. They also observed, with strong evidence, an association between *ABCB1*-C3435T and breast cancer risk. There was some incorrect data in previous studies which were corrected in the Wang et al. (2013) [31] study. Herein we report an updated meta-analysis with different ethnicity subgroups (Asian, Caucasian and Latinos).

Previous bioinformatic studies showed that SNPs may affect mRNA structure [36,37], protein function [38-40], and gene expression [41]. *ABCB1*-C3435T is a synonymous transition, therefore it has no effect on the function and structure of the protein, but it may affect the mRNA. SNPs

in gene leads to the formation of two different forms of mRNA. mRNA with different nucleotide at SNP location may vary in some features such as mRNA maturation, transport, degradation, and translation [42]. So it is fitting to employ *in silico* tools for evaluation of the deleterious effects of ABCB1-C3435T transition on the mRNA.

There are some limitations in our study that must be considered. For example, absence of original data, such as age, drinking, smoking and family history might affect the precision of the association of *ABCB1*-C3435T and breast cancer. Also, this meta-analysis lacks any data from African populations.

In conclusion, the present meta-analysis suggests that the *ABCB1*-C3435T polymorphism may be associated with breast cancer risk, however large sample size studies are required to confirm our results. To evaluate the precise results, studies with gene-environment and gene-gene interactions are essential.

Conflict of interests

The authors declare no confict of interests.

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