ABCB1-C3435T polymorphism and breast cancer risk: a case-control study and a meta-analysis
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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
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 was 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 CR-RFLP. ABCB1 fragment was amplified with the use of 0.35 µM MgCl2, Taq polymerase and template DNA

Statistics

Hardy-Weinberg equilibrium (HWE) for both the cases and controls were 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) 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 I² 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 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.

![Figure 1. Study identification diagram.](image-url)
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.
The overall results of the meta-analysis are summarized in Table 3. Overall, for the 13 pooled studies, a significant association between AB
cB1-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=1.199, 95%CI=1.011-1.488, p=0.039) models (Figure 2). Results of meta-analysis in Asian and Caucasian 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-C5435T 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\textsubscript{heterogeneity} < 0.001; I\textsuperscript{2}=76%), TT vs CC (P\textsubscript{heterogeneity} < 0.001; I\textsuperscript{2}=68%), CT+TT vs CC (P\textsubscript{heterogeneity}=0.012; I\textsuperscript{2}=53%), and TT vs CC+CT (P\textsubscript{heterogeneity}=0.004; I\textsuperscript{2}=59%) models (Table 4). In Asian studies, a high heterogeneity was found in T vs C (P\textsubscript{heterogeneity}<0.001; I\textsuperscript{2}=79%) model (Table 4). Also, in Caucasian studies a true heterogeneity was found in T vs C (P\textsubscript{heterogeneity}<0.001; I\textsuperscript{2}=79%) model (Table 4). In addition,
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in Latinos studies, a true heterogeneity was ob-
served in T vs C ($P_{\text{heterogeneity}}=0.077; I^2=68\%$), TT vs
CC ($P_{\text{heterogeneity}}=0.055; I^2=75\%$), and TT vs CT+CC
($P_{\text{heterogeneity}}=0.074; I^2=69\%$).

The Egger’s test suggested a publication bias for
the T vs C ($P_{\text{Egger}}=0.03$), CT vs CC ($P_{\text{Egger}}=0.04$),
and CT+TT vs CC ($P_{\text{Egger}}=0.02$) models in overall
meta-analysis and for the T vs C ($P_{\text{Egger}}=0.01$), TT vs
CC ($P_{\text{Egger}}=0.01$), CT+TT vs CC ($P_{\text{Egger}}=0.05$), and TT
vs CC+CT ($P_{\text{Egger}}=0.001$) models in Caucasian met-
a-analysis, whereas the shapes of funnel plot for
all the 5 models in the Asian subgroup seemed
approximately symmetrical (Figure 5). The distrib-
ution 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 malignan-
cy 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 (ap-
proximately 1% annually) [25-27] and the causes
of this disease are poorly understood. Risk factors
such as age, geographical variation, family histo-
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 nucle-
otide polymorphisms (SNP), which affect the ex-
pression and structure of proteins, could be help-
ful 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 biolog-
ically 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=1.256-2.535, p=0.002), 3435CT genotype
(OR=1.661, 95%CI=1.017-2.713, p=0.042), and
3435TT genotype (OR=3.399, 95%CI=1.236-2.535,
p=0.002) with breast cancer risk. Several
al 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 power-
ful statistical tool, provides reconciliation of re-
results by analyzing pooled inconsistent data from
several studies [32]. The present meta-analysis
revealed that an association exists between AB-
CB1-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 sub-
group. These might be ascribed to different en-
vvironments, lifestyles, and genetic backgrounds
among different ethnicities.

There are 4 meta-analyses over the AB-
CB1-C3435T gene polymorphism and breast
cancer risk [31,33-35] which evaluate the asso-
ciation of ABCB1-C5435T 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 poly-
morphism and increased risk of breast cancer.
Also Sheng et al. (2012) [34] carried out a me-
aplasis between ABCB1-C3435T polymor-
phism and cancer risk. They also observed, with
strong evidence, an association between AB-
CB1-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-anal-
ysis with different ethnicity subgroups (Asian,
Caucasian and Latinos).

Previous bioinformatics studies showed
that SNPs may affect mRNA structure [36,37],
protein function [38-40] and gene expression
[41]. ABCB1-C3435T is a synonymous transition
and has no effect on the function and structure
of the protein, but it may affect mRNA. SNPs in
gene lead to formation of two different forms
of mRNA and 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 orig-
inal data, such as age, drinking, smoking and fam-
ily history might affect the precision of the asso-
ciation of ABCB1-C3435T and breast cancer. Also,
this meta-analysis lacks any data from African
populations.

In conclusion, the present meta-analysis sug-
gests 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, stud-
ies with gene-environment and gene-gene inter-
actions are essential.

Conflict of interests

The authors declare no conflict of interests.
References

33. Lu PH, Wei MX, Yang J et al. Association between two polymorphisms of ABCB1 and breast cancer risk in...


