Aim of the study: Results of recent published studies on the association between the COX-2 8473T>C polymorphism and the risk of breast cancer have often been conflicting. To make a more precise estimation of the potential relationship, a meta-analysis was performed.

Material and methods: A total of seven case-control studies with 7,033 cases and 9,350 controls were included in the current meta-analysis through searching the databases of PubMed, Embase, and Cochrane Library (up to March 1st, 2013). The odds ratio (OR) and 95% confidence interval (95% CI) were calculated to assess the strength of the association. The meta-analysis was conducted in a fixed/random effect model.

Results: We found no significant associations for all genetic models after all studies were pooled into the meta-analysis (for C vs. T: OR = 0.974, 95% CI: 0.906–1.047, \(p = 0.471\); for CC vs. TT: OR = 0.957, 95% CI: 0.803–1.140, \(p = 0.62\); for TC vs. TT: OR = 0.964, 95% CI: 0.881–1.055, \(p = 0.421\); for CC + TC vs. TT: OR = 0.963, 95% CI: 0.880–1.053, \(p = 0.406\); for CC vs. TT + TC: OR = 0.978, 95% CI: 0.831–1.15, \(p = 0.788\)). We also observed no obvious associations in the subgroup analyses by ethnicity (Caucasian) and source of controls (population based, PB) for all genetic models.

Conclusions: Current evidence suggests that the COX-2 8473T>C polymorphism is not associated with breast cancer risk.

Key words: breast cancer, polymorphism, meta-analysis, COX-2.
Inclusion and exclusion criteria

Studies were included if they met the following criteria: 1) evaluation of 8473T>C (rs5275) polymorphism of COX-2 and breast cancer risk; 2) retrospective case-control studies or prospective cohort studies; 3) sufficient data to examine an odds ratio (OR) with 95% confidence interval (CI); 4) conforming to Hardy-Weinberg equilibrium (HWE) in the control group. Studies were excluded when: 1) not case-control studies; 2) case reports, letters, reviews, editorial articles, and animal studies; 3) duplicate or insufficient data; 4) family-based design; 5) controls were not in HWE.

Data extraction

Data from published studies were extracted independently and carefully by two reviewers (Jiang J. and Quan X.F.). For each study, we collected the following information: first author, year of publication, country, ethnicity, numbers of cases and controls of different genotypes, source of controls, evidence of HWE and quality control.

Statistical analysis

The strength of the association between the 8473T>C polymorphism and breast cancer risk was calculated by ORs with 95% confidence intervals (95% CIs). We evaluated the risk of the dominant model (CC + TC vs. TT), the recessive model (CC vs. TT + TC), the homozygote comparison (CC vs. TT), the heterozygote comparison (TC vs. TT), and the allelic model (C vs. T). We also performed subgroup analyses including ethnicity and source of controls. The χ² test-based Q-statistic and I²-statistic [18] were used to analyze the heterogeneity (considered significant for \( p \leq 0.10 \)). Potential publication bias was investigated by funnel plot [21], and funnel plot asymmetry was assessed by the method of Egger’s linear regression test (bias considered significant for \( p < 0.05 \)) [22]. All statistical tests were performed with STATA version (Stata Corporation College Station, TX, USA). All the \( p \) values were two-sided.

Results

Study characteristics

According to the inclusion and exclusion criteria, a total of nine publications were included in this meta-analysis [23–31]. However, there is one study [29] just presenting the information for genotypes of TC + CC and TT, without data for other genotypes; we were unable to identify whether it fulfills Hardy-Weinberg equilibrium in the control group. Thus, this publication was excluded. We noticed that Cox et al. validated their primary results in two other independent populations [30] and each validation group was considered separately in pooling analyses. Therefore, ten studies including 7,033 cases and 9,350 controls from eight publications were finally selected in this meta-analysis [23–28, 30, 31]. Characteristics in this meta-analysis are summarized in Table 1.

Meta-analysis results

Table 2 presents the results of meta-analysis and the heterogeneity test. Clearly, no association can be found between the COX-2 8473T>C polymorphism and the risk of breast cancer in the total population (for C vs. T: OR = 0.974, 95% CI: 0.906–1.047, \( p = 0.471 \), and I² = 45.9% for heterogeneity; for CC vs. TT: OR = 0.957, 95% CI: 0.803–1.140, \( p = 0.62 \), and I² = 51% for heterogeneity (Fig. 1); for TC vs. TT: OR = 0.964, 95% CI: 0.881–1.055, \( p = 0.421 \), and I² = 33.7% for heterogeneity; for CC + TC vs. TT: OR = 0.963, 95% CI: 0.880–1.053, \( p = 0.406 \), and I² = 39.5% for heterogeneity; for CC vs. TT + TC: OR = 0.978, 95% CI: 0.831–1.15, \( p = 0.788 \), and I² = 49.2% for heterogeneity). We also found

| First author | Year | Country | Ethnicity | Cases | Controls | Source of controls | PHWE* | Frequency C allele in controls |
|--------------|------|---------|-----------|-------|----------|-------------------|-------|-----------------------------|
| Gao          | 2007 | China   | Asian     | 18    | 20       | PB                | 0.733 | 0.182                       |
| Langsenlehner| 2006 | Austria | Caucasian | 62    | 33       | PB                | 0.014 | 0.299                       |
| Vogel        | 2006 | Denmark | Caucasian | 44    | 41       | PB                | 0.770 | 0.342                       |
| Schonfeld    | 2010 | USA     | Caucasian | 96    | 144      | HB                | 0.983 | 0.365                       |
| Gallicchio   | 2006 | USA     | Caucasian | 11    | 33       | PB                | 0.293 | 0.333                       |
| Abraham      | 2009 | UK      | Caucasian | 260   | 259      | PB                | 0.903 | 0.337                       |
| Cox 1        | 2007 | USA     | Caucasian | 141   | 213      | HB                | 0.383 | 0.359                       |
| Cox 2        | 2007 | USA     | Caucasian | 30    | 81       | HB                | 0.134 | 0.345                       |
| Cox 3        | 2007 | USA     | Caucasian | 67    | 79       | HB                | 0.925 | 0.347                       |
| Piranda      | 2010 | Brazil  | Mix       | 20    | 25       | HB                | 0.496 | 0.305                       |

*HB – hospital based, PB – population based, HWE – Hardy-Weinberg’s equilibrium, N.A. – not available
### Table 2. Summary of Pooled ORs in the meta-analysis

| Study groups (n) | Comparison     | Test of association | Test of heterogeneity | Model |
|------------------|----------------|---------------------|-----------------------|-------|
|                  |                | OR (95%)            | Z                     | p     | χ²       | p     | I² (%) |       |
|                  |                |                     |                       |       | χ²       | p     |       |       |
|                  |                |                     |                       |       | F (%)    |       |       |       |
|                  |                |                     |                       |       |          |       |       |       |
| Total (10)       | C vs. T        | 0.974 (0.906–1.047) | 0.72                  | 0.473 | 16.64    | 0.055 | 45.90  | R     |
|                  | CC vs. TT      | 0.957 (0.803–1.140) | 0.5                   | 0.62  | 18.38    | 0.031 | 51.00  | R     |
|                  | TC vs. TT      | 0.964 (0.881–1.055) | 0.8                   | 0.421 | 13.58    | 0.138 | 33.70  | R     |
|                  | CC + TC vs. TT | 0.963 (0.880–1.053) | 0.83                  | 0.406 | 14.88    | 0.094 | 39.50  | R     |
|                  | CC vs. TT + TC | 0.978 (0.831–1.151) | 0.27                  | 0.788 | 17.71    | 0.039 | 49.20  | R     |
| Ethnicity        |                |                     |                       |       |          |       |       |       |
| Caucasian (8)    | C vs. T        | 0.967 (0.889–1.052) | 0.78                  | 0.435 | 16.04    | 0.025 | 56.40  | R     |
|                  | CC vs. TT      | 0.973 (0.797–1.187) | 0.27                  | 0.787 | 17.92    | 0.012 | 60.90  | R     |
|                  | TC vs. TT      | 0.949 (0.883–1.021) | 1.41                  | 0.159 | 8.45     | 0.294 | 17.20  | F     |
|                  | CC + TC vs. TT | 0.942 (0.856–1.037) | 1.22                  | 0.223 | 11.66    | 0.112 | 40.00  | R     |
|                  | CC vs. TT + TC | 0.988 (0.889–1.099) | 0.22                  | 0.826 | 15.84    | 0.027 | 55.80  | R     |
| Source           |                |                     |                       |       |          |       |       |       |
| PB (5)           | C vs. T        | 1.048 (0.978–1.122) | 1.34                  | 0.182 | 4.91     | 0.296 | 18.60  | F     |
|                  | CC vs. TT      | 1.204 (0.922–1.573) | 1.36                  | 0.173 | 7.18     | 0.127 | 44.30  | R     |
|                  | TC vs. TT      | 1.006 (0.914–1.107) | 0.12                  | 0.906 | 2.83     | 0.586 | 0      | F     |
|                  | CC + TC vs. TT | 1.031 (0.942–1.129) | 0.66                  | 0.509 | 3.26     | 0.515 | 0      | F     |
|                  | CC vs. TT + TC | 1.226 (0.943–1.594) | 1.52                  | 0.128 | 7.52     | 0.111 | 46.80  | R     |
| HB (5)           | C vs. T        | 0.908 (0.849–0.972) | 2.77                  | 0.066 | 3.35     | 0.501 | 0      | F     |
|                  | CC vs. TT      | 0.803 (0.690–0.934) | 2.83                  | 0.004 | 0.77     | 0.943 | 0      | F     |
|                  | TC vs. TT      | 0.959 (0.819–1.124) | 0.52                  | 0.606 | 9.37     | 0.052 | 57.30  | R     |
|                  | CC + TC vs. TT | 0.920 (0.805–1.051) | 1.23                  | 0.218 | 7.47     | 0.113 | 46.40  | R     |
|                  | CC vs. TT + TC | 0.860 (0.746–0.993) | 2.06                  | 0.039 | 0.97     | 0.914 | 0      | F     |

### Study ID

| Study ID            | OR (95% CI) | Weight |
|---------------------|-------------|--------|
| Gao 2007            | 0.96 (0.50–1.83) | 5.46  |
| Langsenlehner 2006  | 2.05 (1.30–3.26) | 8.75  |
| Vogel 2007          | 1.00 (0.62–1.61) | 8.36  |
| Schonfeld 2010      | 0.75 (0.56–1.01) | 13.75 |
| Gallicchio 2006     | 1.22 (0.61–2.44) | 4.91  |
| Abraham 2009        | 1.08 (0.89–1.31) | 17.51 |
| Cox 1 2007          | 0.86 (0.67–1.09) | 15.69 |
| Cox 2 2007          | 0.71 (0.45–1.14) | 8.64  |
| Cox 3 2007          | 0.84 (0.58–1.21) | 11.31 |
| Piranda 2010        | 0.77 (0.41–1.46) | 5.61  |
| Overall (I² = 51.0% p = 0.031) | 0.96 (0.80–1.14) | 100.00 |

**NOTE** Weights are from random effect analysis

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**Fig. 1.** Forest plot for the overall meta-analysis for Cox-2 8473T>C and breast cancer risk (CC vs. TT). The squares and horizontal lines correspond to the OR and 95% CI, and the diamond represents the pooled OR and 95% CI.
no significant relationship in all genetic models of the sub-
group analyses by ethnicity (Caucasian) and source of 
controls (population-based [PB] and hospital-based [HB]), 
except the allelic model (C vs. T), the homozygote com-
parison (CC vs. TT) and the recessive model (CC vs. TT + TC) in 
the “hospital-based” studies.

Publication bias

We also carried out Begg’s funnel plot and Egger’s re-
gression test to assess the publication bias of the litera-
ture. The shapes of the funnel plots did not show signif-
ificant asymmetry (Fig. 2), and Egger’s test did not reveal 
any statistical evidence of publication bias (for C vs. T: 
$p = 0.983$; for CC vs. TT: $p = 0.894$; for TC vs. TT: $p = 0.982$; 
for CC + TC vs. TT: $p = 0.981$; for CC vs. TT + TC: $p = 0.897$).

Discussion

Numerous in vitro and in vivo experiments with respect 
to COX-2 polymorphism have been conducted. In many 
cancers, the association of over-expression of COX-2 
and tumor progression is established. Moreover, COX-2 
expression may be correlated with cancer prognosis [32]. 
Therefore, COX-2 polymorphism has received widespread 
attention, and many meta-analyses have been reported to 
assess the relationship between the polymorphism and 
human cancers. However, the association in the field of 
breast cancer remains unclear and its discovered is eagerly 
awaited.

Only one meta-analysis has been conducted to as-
ss the strength of the association between the COX-2 
8473T>C polymorphism and susceptibility to breast cancer 
[33]. However, several issues should be considered after 
carefully reading the report.

Firstly, though one of the inclusion criteria in that ar-
ticle was fulfilling Hardy-Weinberg equilibrium (HWE) in 
the control group ($p > 0.01$ was eligible), one case-control 
study without sufficient available data to calculate the 
$p$ value of HWE was eventually included [29]. Evidence 
suggested that HWE might reflect the presence of popu-
lation stratification, genotyping errors, and selection bias 
in the controls [34]. Secondly, the authors gave the geno-
type contrasts (the dominant and recessive model, the 
heterozygous and homozygous carriers). However, the al-
lele (A genotype vs. T genotype) contrast was not included. 
Thirdly, subgroup analyses concerning the source of con-
trols (HB and PB) were not performed. In order to reach 
a more precise conclusion, we present this meta-analysis 
to seek the association of breast cancer risk and the COX-2 
8473T>C polymorphism.

The present meta-analysis, including 7,033 cases and 
9,350 controls from 10 case-control studies, was intended to 
explore the association between the 8473T>C polymor-
phism of COX-2 and susceptibility to breast cancer. Unfor-
unately, we did not discover any significant association 
between COX-2 8473T>C polymorphism and breast can-
cer. Only among the analyses stratified by ethnicity and 
source of controls did we observe some associations in 
three studies from “hospital-based” settings. This phe-
nomenon may be due to small-study bias.

Although it is theoretically plausible that 8473T>C 
polymorphism could increase the susceptibility to breast 
cancer by influencing COX-2 expression, the current evi-
dence provides a negative result. The acceptable expla-
nation is that one single gene or polymorphism may have 
a limited impact on the effect of the risk of breast cancer, 
and susceptibility is decided by multiple genes or poly-
morphisms.

We should also be aware of some limitations in this 
meta-analysis. First, the overall outcomes were based on 
individual unadjusted ORs. The unadjusted ORs may lead 
to confounding bias due to lack of individual information 
of each study, such as joint effects of SNP-SNP or gene-
environment factors. Second, there was no study of an Afri-
can population and only one study of an Asian population. 
Thus, publication bias might exist. Third, the majority of 
controls were selected from a healthy population in which 
some may have potential benign breast disease. Fourth, 
recall and selection bias may exist since the meta-analysis 
is a type of retrospective study.

In conclusion, we found that the 8473T>C polymor-
phism of the COX-2 gene might not be a risk factor for 
breast cancer among Caucasians. Larger, well-designed, 
and more comprehensive multicenter studies based on 
African and Asian population should be performed, and 
other SNPs of the COX-2 gene in breast carcinogenesis are 
worthy of further research.

The authors declare no conflict of interest.

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