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.

Lack of association between COX-2 8473T>C polymorphism and breast cancer risk: a meta-analysis

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Introduction

Breast cancer is the most common malignancy among females, and is the leading cause of cancer-related deaths in the general population [1]. To date, a number of studies have shown that some gene polymorphisms may modify breast cancer risk, such as XRCC3 Thr241Met [2], hMSH2 Gly322Asp [3], RAD51 135G>C [4], ERCC1 (ASE-1) [5] and BRCA2 [6]. It has been widely accepted that these common variants within genes involving breast carcinogenesis-related pathways are candidate loci for breast cancer susceptibility [7]. Cyclooxygenase-2 (COX-2), as an inducible enzyme, plays a role in catalyzing the conversion of arachidonic acid to prostaglandins, which are strong mediators of inflammation [8]. Over-expression of COX-2 reinforces carcinogenesis by inhibiting apoptosis, promoting cell proliferation, stimulating invasion, and suppressing immune responses [9–11]. Therefore, COX-2 may constitute a risk factor in the development and progression of breast cancer.

There are different single-nucleotide polymorphism (SNP) sites in the *COX-2* gene and some have been given more attention in the field of human tumor susceptibility, such as rs5275 (8473T>C), rs20417 (-765G>C), and rs689466 (-1195G>A). Rs5275 is a common T>C polymorphism at position 8473 in the 3'-untranslated region of the *COX-2* gene which is designated as PTGS2 [12]. To date, a number of studies have shown that 8473T>C is associated with several cancers in different ethnic populations [13–16], indicating that 8473T>C is an important determinant of mRNA stability and contributes to individual variation in the susceptibility to cancers [17]. Although numerous studies have demonstrated the association between 8473T>C polymorphism and breast cancer, the cumulative results are still inconclusive due to various ethnicities, histological types, age and so on. To conclude, this meta-analysis based on all eligible case-control studies we performed aimed to estimate the association between the polymorphism and breast cancer risk.

Material and methods

Literature search strategy

We searched PubMed, Embase and Cochrane Library (updated to March 1st, 2013) for relevant reports on the association between cyclooxygenase-2 polymorphism and breast cancer. The search terms used were as follows: 'cyclooxygenase-2 or cyclooxygenase 2 or COX-2 or COX 2 or prostaglandin synthase 2 or PTGS 2', '8473T>C or rs5275', 'breast', 'neoplasm or cancer or tumor or carcinoma' and 'polymorphism or polymorphisms or SNP or SNPs'. References of original studies and review articles were identified by hands-on searches for additional studies. No restrictions were applied on language.

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 χ^2 test-based Q-statistic and I^2-statistic [18] were used to analyze the heterogeneity (considered significant for $p \leq 0.10$). If the heterogeneity was not an issue, the fixed-effects model (Mantel-Haenszel method) was selected [19]. Otherwise, the random-effects model (DerSimonian-Laird method) was used [20].

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 $I^2 = 45.9\%$ for heterogeneity; for CC vs. TT: OR = 0.957, 95% CI: 0.803–1.140, $p = 0.62$, and $I^2 = 51\%$ for heterogeneity (Fig. 1); for TC vs. TT: OR = 0.964, 95% CI: 0.881–1.055, $p = 0.421$, and $I^2 = 33.7\%$ for heterogeneity; for CC + TC vs. TT: OR = 0.963, 95% CI: 0.880–1.053, $p = 0.406$, and $I^2 = 39.5\%$ for heterogeneity; for CC vs. TT + TC: OR = 0.978, 95% CI: 0.831–1.15, $p = 0.788$, and $I^2 = 49.2\%$ for heterogeneity). We also found

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

| First author	Year	Country	Ethnicity	Cases CC	Cases TC	Cases TT	Controls CC	Controls TC	Controls TT	Source of controls[a]	PHWE[b]	Frequency C allele in controls
Gao	2007	China	Asian	18	179	404	20	194	429	PB	0.733	0.182
Langsenlehner	2006	Austria	Caucasian	62	224	214	33	232	234	PB	0.014	0.299
Vogel	2006	Denmark	Caucasian	44	150	167	41	165	155	PB	0.770	0.342
Schonfeld	2010	USA	Caucasian	96	348	387	144	501	437	HB	0.983	0.365
Gallicchio	2006	USA	Caucasian	11	31	38	133	583	559	PB	0.293	0.333
Abraham	2009	UK	Caucasian	260	985	927	259	1010	996	PB	0.903	0.337
Cox 1	2007	USA	Caucasian	141	567	541	213	808	699	HB	0.383	0.359
Cox 2	2007	USA	Caucasian	30	131	140	81	259	270	HB	0.134	0.345
Cox 3	2007	USA	Caucasian	67	296	281	79	294	278	HB	0.925	0.347
Piranda	2010	Brazil	Mix	20	149	125	25	99	120	HB	0.496	0.305

aHB – hospital based, PB – population based, bHWE – 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	χ^2	p	I^2 (%)	
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	0.006	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
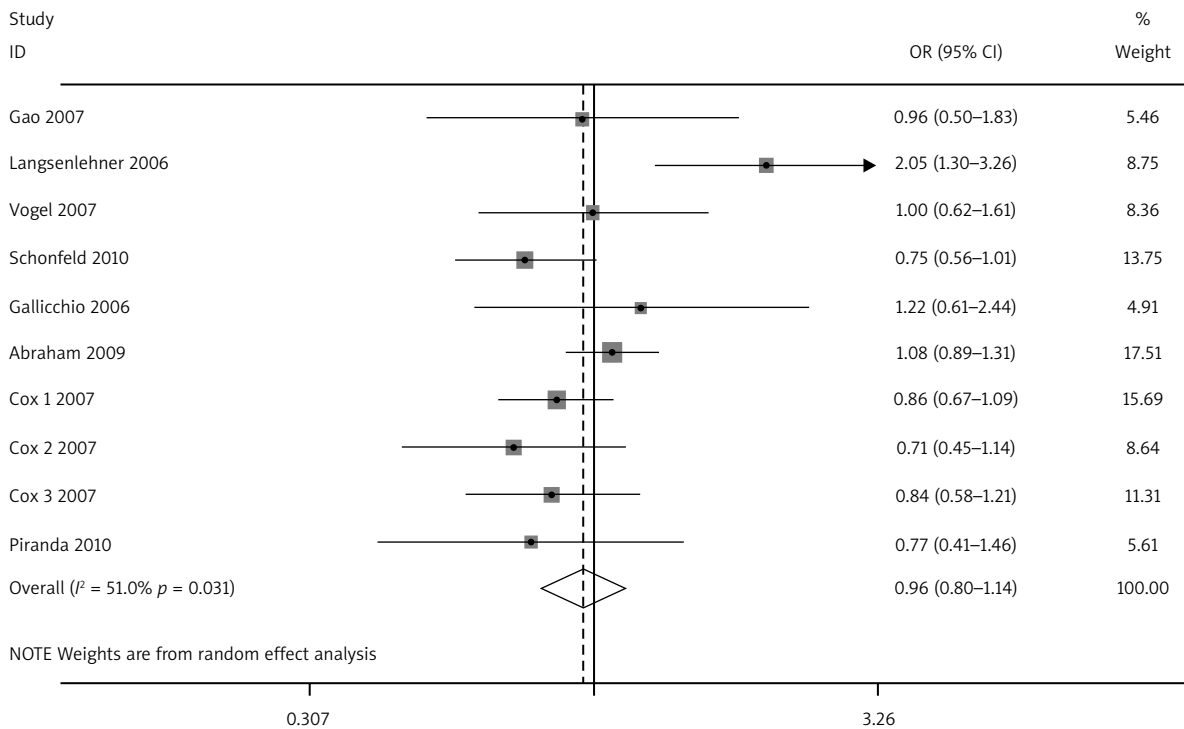
Fig. 1. Forest plot for the overall meta-analysis for Cox-2 8473T>C and breast cancer risk (CC vs. TT). The squares and horizontal lines correspond to the OR and 95% CI, and the diamond represents the pooled OR and 95% CI
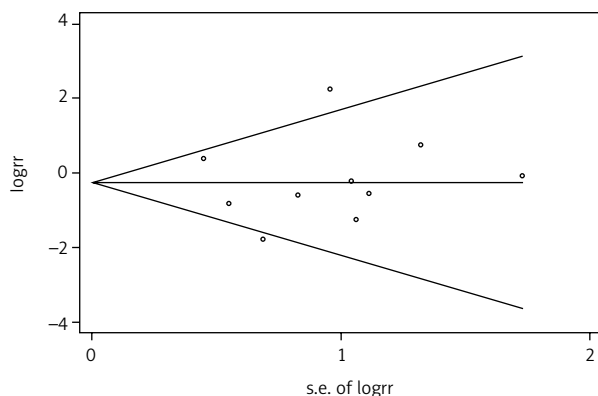
Fig. 2. Begg's funnel plot for publication bias test (C vs. T allele). Each point represents a separate study for the indicated association. Log [OR], natural logarithm of OR. Horizontal line, mean effect size

no significant relationship in all genetic models of the subgroup analyses by ethnicity (Caucasian) and source of controls (population-based [PB] and hospital-based [HB]), except the allelic model (C vs. T), the homozygote comparison (CC vs. TT) and the recessive model (CC vs. TT + TC) in the "hospital-based" studies.

Sensitivity analysis

By means of restricting the meta-analysis to studies conforming to HWE, we conducted sensitivity analysis to evaluate the robustness of the results. It turned out our meta-analysis was statistically stable since the corresponding ORs were not evidently varied (data not shown).

Publication bias

We also carried out Begg's funnel plot and Egger's regression test to assess the publication bias of the literature. The shapes of the funnel plots did not show significant 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 assess 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 article 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 population stratification, genotyping errors, and selection bias in the controls [34]. Secondly, the authors gave the genotype contrasts (the dominant and recessive model, the heterozygous and homozygous carriers). However, the allele (A genotype vs. T genotype) contrast was not included. Thirdly, subgroup analyses concerning the source of controls (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 polymorphism of *COX-2* and susceptibility to breast cancer. Unfortunately, we did not discover any significant association between *COX-2* 8473T>C polymorphism and breast cancer. Only among the analyses stratified by ethnicity and source of controls did we observe some associations in three studies from "hospital-based" settings. This phenomenon 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 evidence provides a negative result. The acceptable explanation 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 polymorphisms.

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 African 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 polymorphism 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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