NQO1 C609T polymorphism and colorectal cancer susceptibility: a meta-analysis

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Abstract

Introduction: A few studies have reported an association between NADP(H): quinine oxidoreductase 1 (*NQO1*) C609T polymorphism and susceptibility to colorectal cancer (CRC). However, the results were inconsistent rather than conclusive. We performed a meta-analysis to examine this association in various populations.
Material and methods: Eligible articles were identified by a search of several databases up until June 30, 2013. Summary odds ratios (ORs) and 95% confidence intervals (CIs) were calculated to assess the strength of the association.
Results: Overall, 14 case-control studies with 4,461 cases and 5,474 controls were included in this meta-analysis. The results indicated that the *NQO1* C609T polymorphism was significantly associated with CRC susceptibility (summary ORs (95% CIs): 1.30 (1.07–1.59) for CT vs. CC, 1.64 (1.15–2.33) for TT vs. CC, 1.34 (1.10–1.64) for TT/CT vs. CC, and 1.43 (1.10–1.87) for TT vs. CT/CC). Subgroup analyses indicated that the T allele was significantly associated with CRC susceptibility in both Asians and Caucasians, and was also observed in high quality studies and hospital-based case-control studies. Specifically, we found a positive association between the *NQO1* C609T polymorphism and CRC susceptibility in smokers, but not in non-smokers.
Conclusions: The results of this meta-analysis suggest that the *NQO1* C609T polymorphism significantly contributes to increased susceptibility to CRC in both Asians and Caucasians.

Key words: colorectal cancer, NQO1, polymorphism, meta-analysis.

Introduction

The incidence of colorectal cancer (CRC) is differently shaped over time: the incidence is stabilizing or even gradually decreasing in some countries of Northern and Western Europe [1], while it is increasing in others [2]. It is reported that both environmental and genetic factors contribute to colorectal carcinogenesis [3, 4]. In the last decade, great efforts have been focused on unraveling the genetic underpinnings of CRC [5]; however, its driving genes and genetic determinants that contribute to the development of CRC so far remain elusive.

NADP(H): quinine oxidoreductase 1 (NQO1), also named diphtheria toxin diaphorase, is located on chromosome 16q22. NQO1 has been reported to play a crucial role in the detoxification of potentially mutagenic and carcinogenic quinones (derived from tobacco or the diet) and

catalyzing the two-electron reduction of quinoid compounds into hydroquinones, which can alleviate cancer development [6]. In addition, NQO1 is implicated in protection of cells against oxidative stress and carcinogenesis by maintaining antioxidant forms of ubiquinone [7].

Several polymorphisms have been discovered in the *NQO1* gene [8]. The most commonly studied polymorphism in this gene is the C609T polymorphism (dbSNP: rs1800566) at exon 6 of the gene, which leads to a proline to serine substitution in the protein sequence. This polymorphism occurs at a frequency of 50% in the human population, with 10% being homozygous for T alleles [9]. Phenotyping studies suggested that the homozygosity for the NQO1 protein has little or no activity (2–4% activity of the wild type). In contrast, the heterozygous (CT) genotype showed threefold decreased enzymatic activity compared with the wild-type allele.

The *NQO1* C609T polymorphism has been widely evaluated in relation to susceptibility of CRC across various ethnicities, yet with inconsistent results [10–14]. A case-control study carried out by Van der Logt *et al.* [13] showed that, compared with the CC genotype, the TT genotype was significantly associated with the risk of CRC, with the adjusted odds ratio (OR) (95% confidence interval (CI)) of 1.6 (1.03–2.4). Specifically, a recent study from China found that, in addition to the positive relationship with CRC susceptibility, an interaction between *NQO1* polymorphism and smoking was also observed [15]. However, other studies showed that there was no relationship between *NQO1* polymorphism and CRC susceptibility [10, 11, 14]. Recently, several meta-analyses [16–19] have evaluated the association between the *NQO1* C609T polymorphism and risk of colorectal neoplasm. The most recent analysis by Wang *et al.* [18] found an obvious association between NQO1 C609T polymorphism and colorectal cancer risk in both Caucasians and Asians. In another meta-analysis, Zhu *et al.* [19] found that the *NQO1* C609T polymorphism might have a significantly increased risk of upper digest tract cancer, but not risk of CRC. That study included 9 studies on CRC involving 4,461 cases and 4,825 controls.

In the current study, we aimed to conduct a comprehensive meta-analysis of the association between only invasive colorectal neoplasm and the risk of *NQO1* C609T polymorphism in both Caucasians and Asians. We also explored the interaction between NQO1 genotype and smoking status.

Material and methods

Data sources and searches

Data searches were conducted by two independent investigators (C.R. and Z.B.A.). A computerized literature search was conducted in several databases for all published reports on the association between *NQO1* polymorphisms and the risk of CRC from the indexing to 30 June, 2013. For English articles, MEDLINE and EMBASE databases were searched; and for Chinese articles, the CNKI database, the China WanFang database, and the China Weipu database were searched. We applied the following algorithm to both the Medical Subject Heading (MeSH) and the full text: 1) "quinone oxidoreductase" OR "DT-diaphorase" OR "quinone reductase" OR "NAD(P)H: quinone oxidoreductase 1" OR "NQO1" OR "DTD"; 2) "colorectal" OR "colon" OR "rectal"; 3) "cancer" OR "carcinoma" OR "adenocarcinoma" or "neoplasm"; AND 4) "polymorphism" OR "allele" OR "genotype" OR "variant" OR "variation". We also reviewed the reference lists of the relevant articles to identify additional studies. Unpublished studies were not considered.

Selection and exclusion criteria

Studies included in the meta-analysis must meet all the following inclusion criteria: 1) being an independent case-control, nested case-control, or cohort study; 2) evaluating the association between *NQO1* C609T polymorphism and the risk of colorectal cancer; 3) having sufficient data for calculating an OR with 95% CI; and 4) reported in English or in Chinese. Exclusion criteria were: 1) duplicate data; 2) abstract, case report, comment, review and editorial; 3) no sufficient genotyping data; 4) the outcome was benign tumors, precancerous lesions, and adenomas; and 5) family-based study.

Data extraction

The following information was collected from all eligible publications according to the criteria listed above: first author's last name, year of publication, countries or region of origin, ethnicity, sources of controls (population-based or hospital-based), numbers of cases and controls, and Hardy-Weinberg equilibrium (HWE) for the control group. Two of us (C.R. and Z.B.A.) assessed and extracted the data in a standardized data extraction form each publication. When discrepancies were found, a third investigator would make the definitive decision for study eligibility and data extraction. To retrieve the missing data, we also contacted the authors of primary studies. Only one study provided the relevant data, although communication with the authors had taken place [15].

Quality score assessment

Two reviewers (C.R. and Z.B.A.) assessed the quality of each selected study using the quality assessment criteria, which were modified from a pre-

viously published meta-analysis of molecular association studies [20, 21]. Any discrepancies were resolved by consultation with the third authors. We included the following factors related to both traditional epidemiological considerations and cancer genetic issues in terms of quality of the studies: representativeness of the cases, representativeness of the controls, ascertainment of outcome, matching of case and control participants, genotyping examination, and total sample size. The criteria are described in detail in Supplementary Table I, and the scores were defined as 0 to 2 points given to each component. A numerical score ranging from 0 to 12 was assigned as a quantitative measure of literature quality. Studies were categorized as "high quality" if the quality score was ≥ 7; otherwise, studies were categorized as "low quality".

Statistical analysis

All statistical analyses were performed using STATA, version 11.0 (STATA, College Station, TX, USA), and a p value < 0.05 was considered significant. We assessed the departure from the HWE for the control group in each study using Pearson's goodness-of-fit χ^2 test with 1 degree of freedom. Summary ORs and corresponding 95% CIs were used to estimate the association between the *NQO1* C609T polymorphism and CRC risk, which were calculated by several comparisons, that is, a homozygote model (TT vs. CC), a heterozygote model (CT vs. CC), a dominant model (CT/TT vs. CC), and a recessive model (TT vs. CT/CC).

Heterogeneity among studies was evaluated by the Cochran Q and I^2 statistics. For the Q statistic, a p value < 0.10 was considered statistically significant; for I^2, a value > 50% was considered a measure of severe heterogeneity. To summarize the risk estimation, we used the method of a random-effects model, which accounts for heterogeneity among studies [22]. The potential source of heterogeneity across studies was explored by stratified analyses, which were conducted by several study characteristics, including ethnicity, sources of controls, smoking status and the quality score of studies (quality score, < 7 and ≥ 7).

Sensitivity analyses were conducted by removing one study at a time to assess the stability of the results. Asymmetry funnel plots were inspected to assess potential publication bias. Both Begg's test and Egger's test [23] were used to assess publication bias. A p value of less than 0.10 was considered to indicate statistically significant publication bias.

Results

Characteristics of selected studies

Based on our search strategy, a total of 14 case-control studies with 4,461 CRC patients and 5,474 controls met the inclusion criteria. The detailed baseline characteristics of qualified studies are presented in Table I. Of these 14 studies, 9 studies were based on a hospital-based design and 5 studies on a population-based design. Twelve studies were published in English and 2 in Chinese. Of these 14 studies, 7 studies were conducted in Caucasian populations, 7 in Asian populations (4 in Chinese, 2 in Japanese and 1 in Indians). The polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) method was used to determine the genotype in all the included studies.

The genotype distributions of the *NQO1* gene C609T polymorphism were in agreement with the HWE among control groups of all but two studies [24, 25]. The HWE test in the study by Lafuente *et al.* was not mentioned [24]; we also could not perform the HWE test for the subjects (either cases or controls) in that study, because only the total number of the combined genotypes (TT vs. CT/CC) was available. Quality scores for the individual studies ranged from 4 to 11, with 64.3% (9 of 14) of the studies being classified as high quality (≥ 7).

Overall analyses

As shown in Table II, compared to the wild-type CC homozygous genotype, the TT homozygous and CT heterozygous genotype were significantly associated with an increased risk for CRC (CT vs. CC: summary ORs = 1.30, 95% CIs: 1.07–1.59, Figure 1 A; TT vs. CC: summary ORs = 1.64, 95% CIs: 1.15–2.33, Figure 1 B). A main effect was significant in the dominant model (CT/TT vs. CC: summary ORs = 1.34, 95% CIs: 1.10– 1.64, Figure 1 C) and the recessive model (TT vs. CT/CC: summary ORs = 1.43, 95% CIs: 1.10–1.87, Figure 1 D).

Subgroup analyses and sensitivity analyses

Ethnicity

Subgroup analysis by ethnicity showed that the association was significant in both Caucasians (CT vs. CC: ORs = 1.23, 95% CIs: 1.03–1.39; TT vs. CC: ORs = 1.20, 95% CIs: 1.01–1.47; CT/TT vs. CC: ORs = 1.14, 95% CIs: 1.00–1.29; TT vs. CT/CC: ORs = 1.38, 95% CIs: 1.01–1.79) and Asians (CT vs. CC: ORs = 1.51, 95% CIs: 1.08–2.12; TT vs. CC: ORs = 1.84, 95% CIs: 1.16–2.89; CT/TT vs. CC: ORs = 1.40, 95% CIs: 1.11–2.34; TT vs. CT/CC: ORs = 1.46, 95% CIs: 1.02–2.10; Table II).

Sources of controls

Additional stratification by sources of controls showed significant associations between the *NQO1* C609T polymorphism and CRC risk in these four genetic models for hospital-based subgroups, but not for population-based controls.

Table I. Main characteristics of all studies included in the meta-analysis

First author	Year	Recruitment period	Country	Ethnicity	Control source	Cases/controls (*n*)	Adjustments/matching	Quality score	Value of $p^{*,\dagger}$
Harth *et al.* [41]	2000	1996–1999	Germany	White	PB	323/205	Area	6	0.792
Lafuente *et al.* [24]	2000	1996–1997	Spain	White	HB	247/296	Age, sex, smoking	6	–
Mitrou *et al.* [42]	2002	1998–2000	UK	White	HB	206/345	None	8	0.932
Sachse *et al.* [14]	2002	1997–2001	UK	White	PB	490/593	Age, sex, study centre and general practitioner	9	0.555
Hamajima *et al.* [43]	2002	1999–2000	Japan	Asian	HB	146/640	Age, sex	5	0.076
Dai *et al.* [44]	2004	2001–2002	China	Asian	HB	101/103	None	8	0.749
Van der Logt *et al.* [13]	2006	2002–2004	Netherlands	White	PB	369/415	Age, sex	7	0.947
Begleiter *et al.* [9]	2006	–	Canada	White	PB	280/327	Age, sex	8	0.452
Hlavata *et al.* [10]	2010	2004–2006	Czech	White	HB	495/495	Age, sex, smoking, education, living area	8	0.849
Nisa *et al.* [11]	2010	2000–2003	Japan	Asian	PB	684/777	Age, sex, residence area, smoking, alcohol, BMI, type of job, physical activity	11	0.068
Sameer *et al.* [12]	2010	2008–2009	India	Asian	HB	86/160	Age, sex, smoking, dwelling	4	0.447
Xiane *et al.* [45]	2010	2005–2006	China	Asian	HB	286/286	Age, sex, race, dwelling	7	0.316
Su *et al.* [25]	2012	2008–2010	China	Asian	HB	76/160	Age, sex	5	0.017
Peng *et al.* [15]	2013	2006–2010	China	Asian	HB	672/672	Age, sex, income, marriage, job, education, smoking, alcohol intake, family history, and diet	10	0.241

*PB – population-based, HB – hospital-based, BMI – body mass index. *p-value of the χ^2 goodness of fit test for Hardy-Weinberg equilibrium (HWE) in controls; †the HWE test cannot be conducted because only the total number of genotypes (TT vs. CT/CC) was available, and the HWE test was not mentioned in this study [24]*

Table II. Subgroup analyses of *NQO1* gene C609T polymorphism with risk of colorectal cancer

Variables	*N*	No. of cases/ controls	Heterozygote			Homozygote			Dominant model			Recessive model		
			CT vs. CC			TT vs. CC			CT/TT vs. CC			TT vs. CT/CC		
			OR (95% CI)	P_{het}	I^2 (%)	OR (95% CI)	P_{het}	I^2 (%)	OR (95% CI)	P_{het}	I^2 (%)	OR (95% CI)	P_{het}	I^2 (%)
All	14	4461/5474	1.30 (1.07–1.59)	< 0.001	73.9	1.64 (1.15–2.33)	< 0.001	75.7	1.34 (1.10–1.64)	0.001	78.8	1.43 (1.10–1.87)	0.002	62.0
Design:														
HB	9	2315/3157	1.45 (1.11–1.90)	< 0.001	74.6	1.83 (1.19–2.82)	0.001	75.3	1.53 (1.13–2.08)	0.001	82.8	1.53 (1.11–2.12)	0.011	62.6
PB	5	2146/2317	1.09 (0.85–1.40)	0.048	62.0	1.20 (0.77–1.89)	0.163	41.4	1.12 (0.94–1.34)	0.139	42.3	1.19 (0.77–1.85)	0.165	41.1
Ethnicity:														
White	7	2410/2676	1.23 (1.03–1.39)	0.159	39.4	1.20 (1.01–1.47)	0.331	13.0	1.14 (1.00–1.29)	0.385	4.9	1.38 (1.01–1.79)	0.159	37.1
Asian	7	2051/2798	1.51 (1.08–2.12)	< 0.001	80.6	1.84 (1.16–2.89)	< 0.001	81.9	1.40 (1.11–2.34)	< 0.001	86.6	1.46 (1.02–2.10)	< 0.001	74.5
Quality of study:														
Low (< 7)	5	917/1451	1.41 (1.00–1.99)	0.025	64.0	1.59 (0.74–3.45)	0.001	82.1	1.47 (0.96–2.24)	0.001	79.4	1.34 (0.74–2.42)	0.004	73.6
High (≥ 7)	9	3544/4023	1.25 (0.96–1.61)	< 0.001	80.1	1.65 (1.09–2.51)	0.001	74.0	1.29 (1.01–1.63)	< 0.001	80.7	1.47 (1.08–2.00)	0.022	57.4
Smoking status:														
Nonsmokers	2	952/999	1.05 (0.78–1.42)	0.559	0	1.41 (0.92–2.15)	0.216	34.6	1.12 (0.85–1.49)	0.673	0	1.30 (0.90–1.87)	0.184	43.4
Smokers	2	952/999	2.22 (1.69–2.92)	0.01	83.1	5.05 (3.22–7.92)	0.045	75.0	2.57 (0.98–3.33)	0.001	83.4	2.83 (1.86–4.31)	0.583	0

PB – population-based, HB – hospital-based

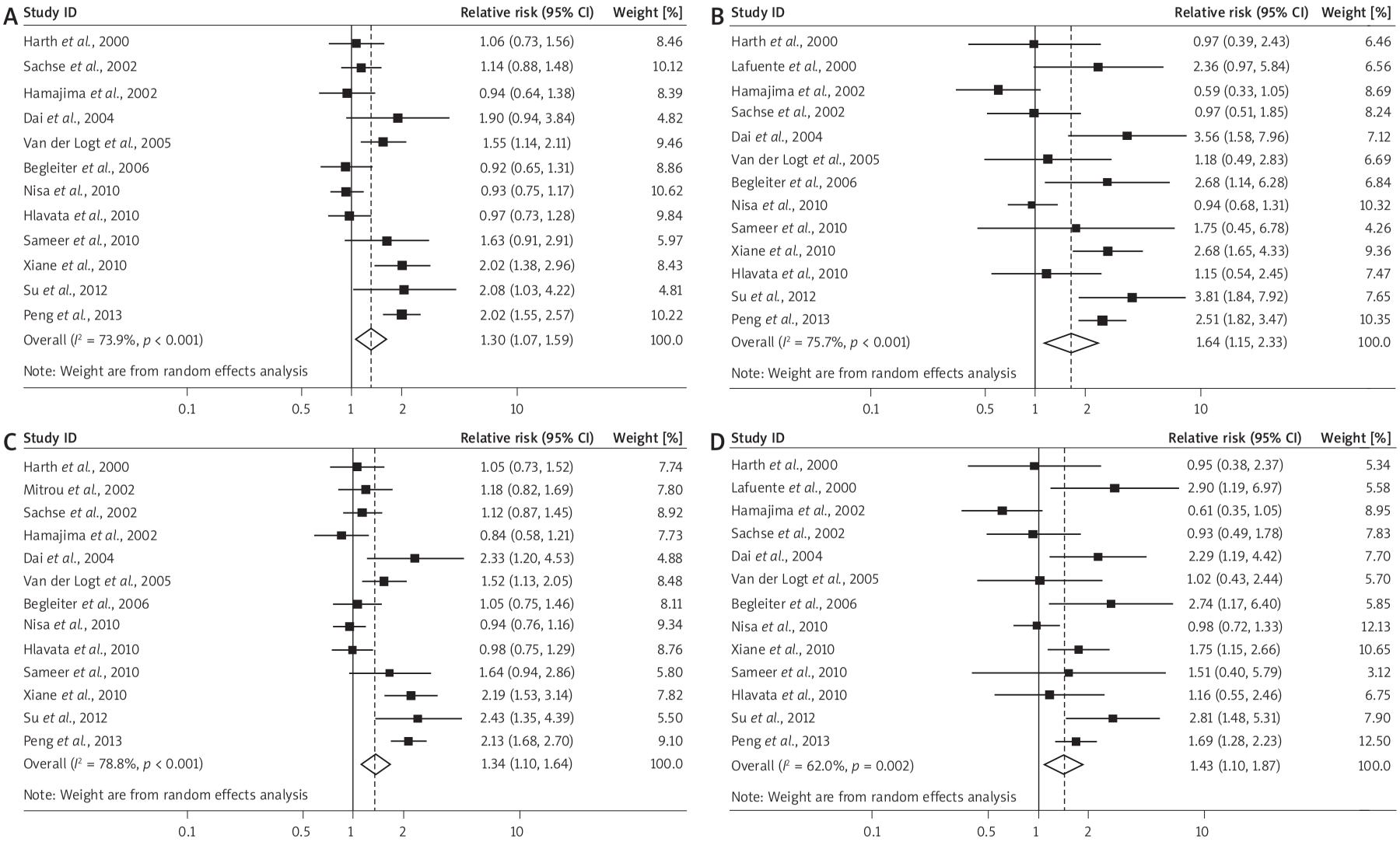

Figure 1. Study specific and summary odds ratios with 95% confidence intervals describing the association of *NQO1* C609T polymorphism with risk of colorectal cancer. The *NQO1* C609T polymorphism was associated with a modestly increased risk of colorectal cancer in heterozygous (CT vs. CC; **A**), homozygous (CT/TT vs. CC; **B**), dominant (CT/TT vs. CC; **C**) and recessive models (TT vs. CT/CC; **D**)

Study quality

After an analysis according to the quality score of studies, we found a significantly positive association of CRC for the four genetic models in the studies with a high quality score (≥ 7) (summary ORs (95% CIs), 1.41 (1.00–1.99) for TC vs. CC; 1.65 (1.09–2.51) for TT vs. CC; 1.29 (1.01–1.63) for CT + TT vs. CC; 1.47 (1.08–2.00) for TT vs. CT + CC), whereas no significant associations were found in these four genetic models based on a meta-analysis of low quality studies.

Smoking status

To determine the effect of smoking on the association between *NQO1* C609T polymorphism and the risk of CRC, we performed a stratified analysis by tobacco smoking. Based on two studies which reported these data [9, 15], we found a modified effect of smoking on this association: a positive association was found in smokers, but not in nonsmokers (Table II). In addition, compared with nonsmokers carrying the CC genotype, smokers carrying CT/TT genotypes had a significantly increased risk of colorectal cancer (summary OR = 2.74, 95% CI = 2.08–3.62).

Sensitivity analysis

We also conducted a sensitivity analysis by omitting one study at a time and calculating the pooled ORs for the remainder of studies, and found that there were no changes in the direction of effect when any one study was excluded. This analysis confirmed the stability of the positive association between the *NQO1* C609T polymorphism and the risk of CRC in these four genetic models.

Publication bias

The shape of the funnel plots did not reveal any evidence of the obvious asymmetry for all genetic models in the overall meta-analysis. Begg's test and Egger's test did not reveal any significant evidence of publication bias for any of the genetic models (CT vs. CC: P_{Begg} = 0.340 and P_{Egger} = 0.824 (Figure 2 A); TT vs. CC: P_{Begg} = 0.855 and P_{Egger} = 0.380 (Figure 2 B); CT/TT vs. CC: P_{Begg} = 0.428 and P_{Egger} = 0.771 (Figure 2 C); and TT vs. CT/CC: P_{Begg} = 0.855 and P_{Egger} = 0.434 (Figure 2 D)).

Discussion

The results of this meta-analysis suggested that the *NQO1* C609T polymorphism was associated with increased risk of CRC. Statistical significance was shown in the heterozygote, homozygote, dominant and recessive models. Subgroup analyses indicated that the C609T polymorphism was associated with increased risk of CRC in both Asians and Caucasians. In addition, an increased risk of CRC associated with the *NQO1* C609T poly-

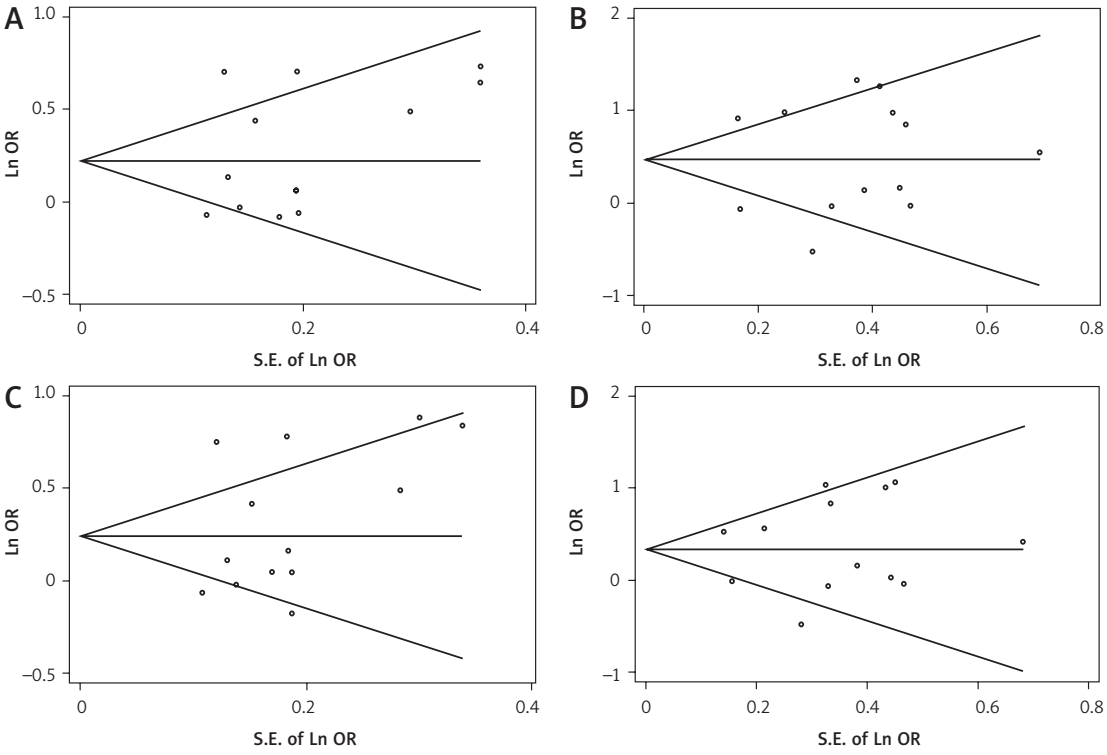

Figure 2. Funnel plot analysis to detect publication bias. No evident publication bias was detected for heterozygous (CT vs. CC; **A**), homozygous (CT/TT vs. CC; **B**), dominant (CT/TT vs. CC; **C**) or recessive models (TT vs. CT/CC; **D**)

morphism was only observed in smokers, but not in nonsmokers.

It has been reported that the *NQO1* C609T polymorphism is associated with the risk of many types of malignancies, such as cancers of the stomach [26], breast [27] and esophagus [28]. However, a meta-analysis by Guo *et al.* [29] involving 7,286 patients and 9,167 controls suggested that the *NQO1* C609T polymorphism was not associated with the risk of lung cancer for both Caucasians and Asians. Therefore, different primary sites of cancers may have different risk relationships between *NQO1* C609T polymorphism and malignancies. In terms of the association between *NQO1* C609T polymorphism and CRC risk, the most recent meta-analysis by Wang *et al.* [18] included 12 studies involving 4,026 cases and 4,855 controls, and found that the *NQO1* C609T polymorphism was positively associated with risk of CRC in both Caucasians and Asians. However, this study did not perform a study quality assessment and did not evaluate the modified role of smoking on this association. In addition, Zhu *et al.* [19] included 21 case-control studies and found that the *NQO1* C609T polymorphism might be associated with a significantly increased risk of upper digestive tract cancer, but not risk of CRC. The further subgroup analysis by ethnicity showed significant associations for colorectal cancer in Caucasians, but not in Asians [19]. The null association between the *NQO1* C609T polymorphism and CRC risk in Asians may be due to the limited number of studies included (n = 2 studies). In the current comprehensive meta-analysis, we identified a total of 14 case-control studies with 4,461 CRC patients and 5,474 controls and found a significant association between the *NQO1* C609T polymorphism and CRC risk in both Caucasians (n = 7 studies) and Asians (n = 7 studies). Moreover, we found that smoking had a modified role in this association: the increased risk of CRC associated with *NQO1* C609T polymorphism was only observed in smokers, but not in nonsmokers.

In terms of the biological functions of the NQO1 protein and gene, the results of our meta-analysis are biologically plausible and reliable. In vitro studies have indicated that *NQO1* C609T polymorphism is associated with decreased enzyme activity of NQO1 protein: homozygous variant cells have little or no NQO1 enzyme activity, and heterozygous variant cells have approximately half of the NQO1 enzyme activity of wild-type cells [30]. Wild-type *NQO1* has been shown to sensitize cells to undergo apoptosis and also to stabilize the p53 tumor suppressor protein, whereas heterozygous variant does not [31, 32]. Furthermore, *in vivo* studies have suggested that wild-type NQO1 could inhibit colon carcinogenesis at both the initiation and post-initiation stages by directly detoxifying colon carcinogens and tumor promoters [33]. Alternately, NQO1 knockout mice were reported to exhibit a significantly increased prevalence rate of skin carcinogenesis induced by 7,12-dimethylbenz(a)anthracene (DMBA) and benzo(a)pyrene (BP) [34].

Tobacco smoking is a source of carcinogenic compounds such as nitrosamines, benzene, BP, and vinyl chloride [35], which is an established risk factor in developing CRC [36]. In the current meta-analysis, we observed an interaction between smoking and *NQO1* C609T polymorphism in cancer risk: the risk with *NQO1* C609T polymorphism was elevated in smokers, but absent in nonsmokers. Similar interactions were also observed in previous studies on colorectal adenoma risk, though these did not reach statistical significance due to the small sample size [37, 38]. Hou *et al.* [37] recruited 725 Caucasian cases with advanced colorectal adenoma and 729 gender- and ethnicity-matched controls, and found that subjects carrying NQO1 Ser187 alleles were weakly associated with risk of colorectal adenoma; however, a higher risk of CRC was observed among recent (including current) (OR = 2.2, 95% CI: 1.5–3.2) and heavy cigarette smokers (> 20 cigarettes/day) (OR = 1.8, 95% CI: 1.2–2.7) compared with non-smokers who did not carry this variant. This genotype was unassociated with risk in non-smokers [37]. Similar results were also reported in the UKFSS study [38], which recruited 946 polyp-free controls and 894 colorectal adenoma cases. The mechanism by which tobacco smoking has a modified effect on the association between *NQO1* C609T variant and colorectal cancer risk may involve decreased NQO1 enzyme activity, leading to increased levels of BP metabolites in smokers and thus leading to an increased risk of CRC. Animal and human studies have suggested that dietary BP increased the risk of CRC [39, 40].

Our meta-analysis has some advantages. First, this is to date the largest analysis exploring the association of the *NQO1* gene C609T polymorphism with CRC risk, which included 14 studies; thus, the results of our study are more reliable. Second, the present meta-analysis comprehensively evaluated the quality score of the included studies according to the quality score criteria, and restricting analyses to high quality studies generated similar findings. Third, our meta-analysis explored the interactive role of tobacco smoking in this association, and we found a positive association between *NQO1* C609T polymorphism and the risk of CRC in smokers, but not in non-smokers.

Our meta-analysis has some limitations that may affect the interpretation of the results. First, the current meta-analysis included only 14 ret-

rospective studies. Because of the limited data, we could not determine a relationship between *NQO1* C609T polymorphism and the risk of CRC in Africans and in Latin Americans. Second, high between-study heterogeneity was detected, which would throw some doubt on the reliability of the summary OR estimates. Significant heterogeneity may exist in terms of ethnicity, sources of controls and study quality. By using sub-group analyses with random-effect models, we found the high heterogeneity may exist among Asian studies and hospital-based case-control studies; thus, our results should be interpreted with caution and further larger studies are needed. Third, an effect of the modified role of smoking in the association between the *NQO1* C609T polymorphism and the risk of CRC is likely to be present. Unfortunately, the information of smoking status was unavailable in the majority of the included studies, although we made our best efforts to contact the authors of primary studies. Fourth, as with all meta-analyses, publication bias might have occurred, although an appropriate search strategy was used to identify eligible studies. Because our analyses were based entirely on published studies from English- and Chinese-language journals, it is possible that some relevant unpublished studies, which may have met the inclusion criteria, were missed. However, neither the funnel plots nor standard statistical tests indicated remarkable publication bias in the meta-analysis.

In conclusion, this meta-analysis indicates that the *NQO1* C609T polymorphism is significantly associated with risk of CRC in both Caucasians and Asians, and in smokers but not in nonsmokers. However, because of the high heterogeneity and potential bias, the results should be interpreted with caution, and further well-designed studies with large sample sizes are warranted to confirm our findings.

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