RESEARCH ARTICLE

The NQO1 rs1800566 Polymorphism and Risk of Bladder Cancer: Evidence from 6,169 Subjects

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Abstract

Objective: The NAD(P)H:quinone oxidoreductase 1 (NQO1) rs1800566 polymorphism, leading to proline-toserine amino-acid and enzyme activity changes, has been implicated in bladder cancer risk, but individually published studies showed inconsistent results. We therefore here conducted a meta-analysis to summarize the possible association. Methods: A systematic literature search up to August 27, 2012 was carried out in PubMed, EMBASE and Wanfang databases, and the references of retrieved articles were screened. Crude odds ratios (ORs) with 95% confidence intervals (CIs) were analyzed for homozygote contrast (TT vs. CC), additive model (T vs. C), dominant model (TT+CT vs. CC), and recessive model (TT vs. CC+CT) to assess the association using fixed- or random-effect models. Results: We identified 12 case-control studies including 3,041 cases and 3,128 controls for the present meta-analysis. Significant association between NQO1 rs1800566 genetic polymorphism and risk of bladder cancer was observed in the additive model (OR = 1.15, 95% CI = 1.01-1.30, p = 0.030). Moreover, in the subgroup analysis stratified by ethnicity, significant associations were observed in Asians (OR = 1.26, 95% CI = 1.08–1.47, p = 0.003 for T vs. C; OR = 1.68, 95% CI = 1.21-2.32, p = 0.002 for TT vs. CC; OR = 1.50, 95% CI = 1.13-1.98, p = 0.005 for TT vs. CT+CC) but not in Caucasians. Conclusions: The results suggest that NQO1 rs1800566 genetic polymorphism may contribute to bladder cancer development, especially in Asians.

Keywords: NQO1 - polymorphism - bladder cancer - meta-analysis

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Introduction

Urothelial bladder cancer (UBC) ranks ninth in worldwide cancer incidence (Ploeg et al., 2009). It is the seventh most common malignancy in men and seventeenth in women (Ploeg et al., 2009). An estimated 386,300 new cases and 150,200 deaths from bladder cancer occurred in 2008 worldwide (Jemal et al., 2011). Although the etiology of this disease remains largely elusive, cigarette smoking, occupational exposure to arylamines and schistosomal infection are generally considered possible risk factors for this cancer (Murta-Nascimento et al., 2007; Jemal et al., 2011; Burger et al., 2012). In addition, increasing evidence suggests a significant influence of genetic predisposition on bladder incidence (Burger et al., 2012); the role of genetic factors in the etiology of bladder cancer is estimated to be about 31% (Lichtenstein et al., 2000).

NAD(P)H:quinone oxidoreductase 1 (NQO1), also known as diphtheria toxin diaphorase (DT-diaphorase), is an obligate two-electron reductase, which reduces reactive quinones to less reactive and less toxic hydroquinones (Siegel et al., 2004). The quinones are a class of compounds ubiquitous in nature as combustion by-products. In addition, diet can also be an indirect source of exposure to these compounds as some procarcinogens present in food can be metabolized in cells to quinone intermediates (O'Brien, 1991; Workman, 1994). This two-electron reduction prevents the formation of semiquinone free radicals and reactive oxygen species (ROS), thus protecting cells against oxidative stress, cytotoxicity, and mutagenicity (Tsvetkov et al., 2010). Furthermore, both in vivo and in vitro studies have confirmed that NQO1 regulates the stability of the tumor suppressor p53 (Asher et al., 2002; Anwar et al., 2003). NQO1-deficient mice show reduced p53 induction and apoptosis, increased sensitivity to chemically induced tumors (Long et al., 2000; Iskander et al., 2005). Therefore, NQO1 is considered as an anticancer enzyme.

The NQO1 gene is located on chromosome 16q22.1 and contains 6 exons and 5 introns. A number of single nucleotide polymorphisms (SNPs) have been discovered in this gene (Nebert et al., 2002), of which rs1800566 polymorphism, a C-to-T transition at nucleotide position 609 in exon 6, has been studied by various researchers. Genotype-phenotype studies demonstrated that this kind of polymorphism is associated with a decreased activity of NQO1 enzymatic activity and shows a phenotypic gene-dose effect (Siegel et al., 1999; Basu et al., 2004; Ross et al., 2004). Because of this SNP's functional consequence, many case-control studies were

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conducted to evaluate the association of NQO1 rs1800566 polymorphism with bladder cancer risk. However, the observed associations of these studies remain inconsistent and contradictive, partially because a single study might be too underpowered to detect a possible small effect of the polymorphism on bladder cancer, especially when the sample size is relatively small.

A meta-analysis conducted by Chao published in 2006 found a significant association between this polymorphism with bladder cancer risk (Chao et al., 2006). But there were only eight studies with limited sample size included in that study and the results should be treated with caution. Therefore, we performed a meta-analysis of published data to obtain a more precise estimation of the association.

Materials and Methods

Publication search

We carried out a search in PubMed, EMBASE and Wanfang databases, covering all the papers published from their inception to August 27, 2012, using the following search algorithm: (quinone oxidoreductase or NQO1 or DT-diaphorase or DTD or quinone reductase or NAD(P)H dehydrogenase (quinone)) and (bladder Cancer or bladder tumor or bladder neoplasm or urothelial cancer or urinary tract cancer) and (polymorphism or mutation or variation or variant or genotype or gene). We evaluated potentially relevant publications by examining their titles and abstracts and all the studies matching the eligible criteria were retrieved. We also checked the references from retrieved articles and reviews to identify any additional relevant study.

Inclusion criteria

Studies included in this meta-analysis had to meet all the following criteria: (a) evaluation of the NQO1 rs1800566 polymorphism and the risk of bladder cancer, (b) had a case-control design, (c) there were sufficient data for calculating an odds ratio (OR) with 95% confidence interval (CI). If multiple publications from the same study population were available, the most recent and detailed study was eligible for inclusion in the meta-analysis.

Data extraction

Information was carefully extracted independently by two authors according to the inclusion criteria noted above. For each study, the following characteristics were collected: study name (together with the first author's name and year of publication), the country in which the study was carried out, ethnicity, numbers of cases and controls, genotyping methods, genotypes, and allele frequency information.

Quality Assessment

The quality of each study was assessed by the same two investigators using the quality assessment criteria, which was modified on the basis of previously published meta-analysis of molecular association studies (Thakkinstian et al., 2011; Yu et al., 2012). The criteria consist of seven parameters of quality: representativeness of the cases, representativeness of the controls, ascertainment of bladder cancers, control selection, genotyping examination, Hardy–Weinberg equilibrium, and total sample size (The criteria are described in detail in Table 1). Scores were ranged from 0 (worst) to 15 (best). Studies scoring < 9 were classified as low quality, and those ≥ 9 as high quality. Disagreements were resolved by a joint reevaluation of the original article with a third investigator.

Table 1. Score of Quality Assessment

Criteria	Score
Representativeness of case	
Selected from population cancer registry	2
Selected from hospital	1
No method of selection described	0
Representativeness of control	
Population-based	3
Blood donors	2
Hospital-based (cancer-free patients)	1
Not described	0
Ascertainment of bladder cancer	
Histopathologic confirmation	2
by patient medical record	1
Not described	0
Control selection	
Controls matched with cases by age and sex	2
Controls matched with cases only by age or by sex	1
Not matched or not descried	0
Genotyping examination	
Genotyping done blindly and quality control	2
Only genotyping done blindly or quality control	1
Unblinded and without quality control	0
HWE	
HWE in the control group	1
HWD in the control group or not mentioned	0
Total sample size	
Larger than 1000	3
Larger than 500, but less than 1000	2
Larger than 200, but less than 500	1
Less than 200	0

Statistical methods

Hardy–Weinberg equilibrium in control groups of each study was examined using goodness-of-fit test. The odds ratios (ORs) and their 95% confidence intervals (CI) were used to calculate and assess the strength of association between NQO1 rs1800566 polymorphism and the risk of bladder cancer. If a statistical heterogeneity among studies exists, the combined ORs and 95% CI were estimated by the DerSimonian and Laird (DerSimonian et al., 1986) random effects models. Otherwise, the ORs were obtained by Mantel–Haenszel method (Mantel et al., 1959) in a fixed effect model. The pooled ORs were analyzed for the homozygote contrast (TT vs. CC), additive model (T vs. C), dominant model (TT+CT vs. CC), and recessive model (TT vs. CC+CT). Stratified analysis was also conducted by ethnicity (Asian and Caucasian).

Homogeneity of ORs across studies was tested by a Chi-square-based Q statistic and the I^2 score. Heterogeneity was considered significant if the P-value is < 0.10. The value of I^2 is used to assess the degree of heterogeneity (I^2 < 25% no heterogeneity; I^2 = 25–50% moderate heterogeneity; I^2 > 50% large or extreme heterogeneity). Meta-regression analysis was used to

Table 2. Main Characteristics of the Studies Included in the Meta-analysis

Author	Year	Ethnicity	Country	Sample size (Frequency of T allele %)		OR	HWE in	Genotyping	quality
				Case	Control	(95% CI for T allele, %)	control	method	score
Schulz	1997	Caucasian	Germany	99 (18.18)	260 (13.27)	1.45 (0.45-2.93)	Yes	PCR-RFLP	7
Park	2003	Caucasian	USA	232 (21.12)	239 (17.99)	1.22 (0.88-1.69)	Yes	PCR-RFLP	9
Choi	2003	Asian	Korea	177 (35.03)	170 (35.88)	0.96 (0.71-1.31)	Yes	PCR-RFLP	7
Sanyal	2004	Caucasian	Sweden	299 (16.89)	124 (19.35)	0.85 (0.58-1.24)	Yes	PCR-RFLP	8
Moore	2004	Caucasian	Argentina	106 (25.00)	108 (25.00)	1.00 (0.65-1.55)	Yes	PCR-RFLP	11
Hung	2004	Caucasian	Italy	201 (25.12)	214 (21.50)	1.23 (0.89-1.69)	Yes	PCR-RFLP	10
Terry	2005	Caucasian	USA	235 (18.72)	214 (16.36)	1.18 (0.83-1.66)	Yes	MALDI-TOF	8
Broberg	2005	Caucasian	Sweden	61 (18.85)	156 (16.67)	1.16 (0.68-2.00)	Yes	MALDI-TOF	11
Wang	2008	Asian	Taiwan.	300 (52.00)	300 (46.00)	1.27 (1.01-1.60)	Yes	PCR-RFLP	10
Figueroa	2008	Caucasian	Spain	1128 (21.90)	1123 (23.33)	0.92 (0.80-1.06)	Yes	GoldenGate	12
Fu	2009	Asian	China	99 (50.51)	100 (39.00)	1.60 (1.07-2.38)	Yes	PCR-CTPP	8
Pandith	2011	Asian	India	104 (32.21)	120 (23.33)	1.56 (1.03-2.37)	Yes	PCR-RFLP	9

OR, odds ratio; CI, confidence interval; HWE, Hardy–Weinberg equilibrium

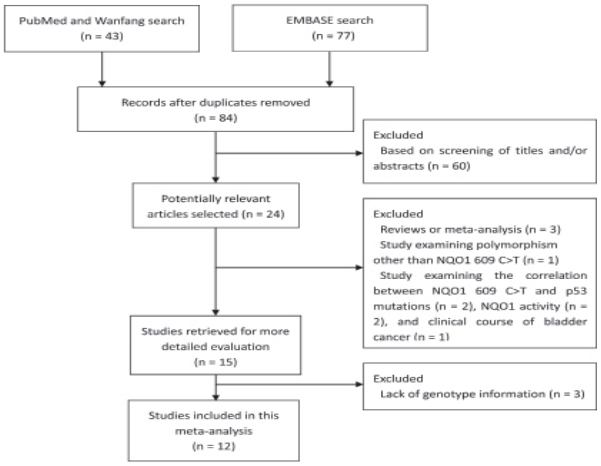

Figure 1. Flowchart of Study Assessment and Selection

explore the influence of ethnicity, genotyping method, and quality score of studies (quality score < 9 and ≥ 9) in the heterogeneity.

Moreover, for each statistically significant association, we estimated the false positive report probability (FPRP) using the method reported by Wacholder et al (Wacholder et al., 2004) to assess the robustness of the findings. Wacholder et al suggested that estimating statistical power based on the ability to detect an OR of 1.5 (or 0.67 = 1/1.5 for an OR less than 1.0), with an alpha level equal to the observed P-value. And an FPRP < 0.2 was considered as a noteworthy association (Wacholder et al., 2004).

Evaluation of publication bias

Publication bias was assessed using Begg's test (rank correlation method) (Begg et al., 1994) and Egger's test (linear regression method) (Egger et al., 1997). P < 0.05 was considered to be representative of a significant statistical publication bias. All of the statistical analyses were performed with STATA 11.0 (StataCorp, College Station, TX), using two-sided P-values.

Results

Characteristics of all included studies

As of August 27, 2012, we had identified 24 potentially eligible studies that have investigated the association between the NQO1 rs1800566 polymorphism and the risk of bladder cancer. After further retrieved, 12 articles were excluded because of the following reasons: three were for review or meta-analysis articles (Gonzalez, 1997; Chao et al., 2006; Ersoy Tunali et al., 2011); one did not focus on the NQO1 rs1800566 polymorphism but on NQO2 (exon 3, T14055C) polymorphism (Wen et al., 2009); two were for the correlation between the NQO1 rs1800566 polymorphism and NQO1 activity (Basu et al., 2004; Jamieson et al., 2007); two were for the correlation between the NQO1 rs1800566 polymorphism and p53 mutations (Martone et al., 2000; Ryk et al., 2006); one was for the correlation between the NQO1 rs1800566 polymorphism and clinical course of bladder cancer (Sanyal et al., 2007); Three studies did not report gene frequencies (Vineis et al., 2007; Dhaini et al., 2012; Ersoy Tunali et al., 2012). Therefore, 12 studies (Schulz et al., 1997; Choi et al., 2003; Park et al., 2003; Hung et al., 2004; Moore et al., 2004; Sanyal et al., 2004; Broberg et al., 2005; Terry et al., 2005; Figueroa et al., 2008; Wang et al., 2008; Paonessa et al., 2009; Pandith et al., 2011) were included in our meta-analysis. The detailed characteristics of the included studies were shown in Table 2. Figure 1 shows the flow diagram of the study selection process.

Among 12 eligible case-control studies including 3,041 cases and 3,128 controls, eight studies were conducted in Caucasian populations and four in Asian populations. Cancers were confirmed histologically in all studies. Diverse genotyping methods were used, including PCR–RFLP, PCR-CTPP, MALDI-TOF and GoldenGate assay. All the studies included in this meta-analysis were in Hardy–Weinberg equilibrium. Quality scores for the individual studies ranged from 7 to 12, with 58.3% (7 of 12) of the studies being classified as high quality (≥ 9).

Quantitative synthesis

There was a wide variation in the NQO1 rs1800566 polymorphism variant allele (T allele) frequency across different ethnicities, ranging from 0.13 in a Caucasian population to 0.46 in an Asian population. The mean frequency of T allele was 0.36 for Asians, which was significantly higher than the mean frequency in Caucasians which was 0.19 (P < 0.01, Figure 2).

As showed in Table 3, NQO1 rs1800566 polymorphism

Table 3. Meta-analysis of the Association Between the NQO1 rs1800566 Genetic Polymorphism and the Risk of Bladder Cancer

Polymorphism	Study	Sample size		No. of	Test of association				Test of heterogeneity	
		Case	Control	studies	OR (95% CI)	p-value	z	Model	I^2 (%)	p-value
T vs. C	Overall	3041	3128	12	1.15 (1.01-1.30)	0.030	2.17	R	42.1	0.061
	Asian	680	690	4	1.26 (1.08-1.47)	0.003	2.97	F	42.7	0.155
	Caucasian	2361	2438	8	1.02 (0.93-1.13)	0.656	0.45	F	20.0	0.271
TT vs. CC	Overall	3041	3128	12	1.33 (0.98-1.81)	0.066	1.84	R	41.2	0.067
	Asian	680	690	4	1.68 (1.21-2.32)	0.002	3.14	F	0.0	0.719
	Caucasian	2361	2438	8	0.99 (0.75-1.30)	0.932	0.08	F	38.6	0.122
TT vs. CT+CC	Overall	3041	3128	12	1.29 (0.96-1.73)	0.092	1.69	R	42.9	0.057
	Asian	680	690	4	1.50 (1.13-1.98)	0.005	2.83	F	6.1	0.362
	Caucasian	2361	2438	8	0.98 (0.75-1.28)	0.869	0.16	F	39.3	0.117
TT+CT vs. CC	Overall	3041	3128	12	1.14 (0.98-1.34)	0.098	1.65	R	44.4	0.048
	Asian	680	690	4	1.29 (0.84-1.97)	0.250	1.15	R	70.2	0.018
	Caucasian	2361	2438	8	1.04 (0.92-1.17)	0.545	0.60	F	6.3	0.382

F, fixed-effect model; R, random-effect model

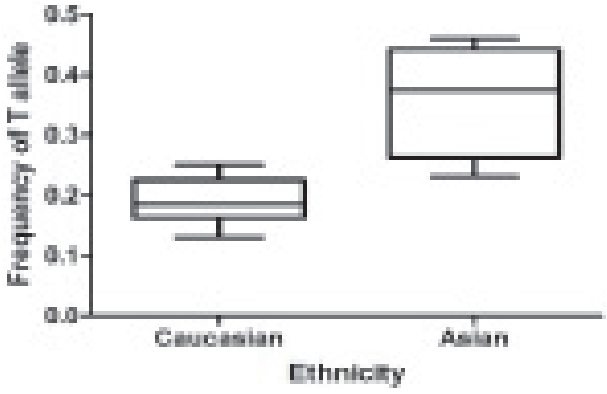

Figure 2. Frequencies of the Variant Alleles (T allele) among Controls Stratified by Ethnicity

Table 4. False Positive Reporting Probability Values forAssociations Between the NQO1 rs1800566 Polymorphism and Bladder Cancer Risk

Genotype	OR (95% CI)	Prior probability				
		0.25	0.1	0.01	0.001	0.0001
All subjects						
T vs. C	1.15 (1.01-1.30)	0.071	0.186	0.716	0.962	0.996
Asians						
T vs. C	1.26 (1.08-1.47)	0.010	0.029	0.249	0.770	0.971
TT vs. CC	1.68 (1.21-2.32)	0.020	0.056	0.397	0.869	0.985
TT vs. CT+CC	1.50 (1.13-1.98)	0.025	0.070	0.454	0.894	0.988

was associated with a modestly increased risk for bladder cancer in all subjects (OR = 1.15, 95% CI = 1.01–1.30, p = 0.030 for T vs. C; OR = 1.33, 95% CI = 0.98–1.81, p = 0.066 for TT vs. CC; OR = 1.29, 95% CI = 0.96–1.73, p = 0.092 for TT vs. CT+CC; OR = 1.14, 95% CI = 0.98–1.34, p = 0.098 for TT+CT vs. CC).

In the subgroup analysis, an increased risk of NQO1 rs1800566 polymorphism was more pronounced in Asians (OR = 1.26, 95% CI = 1.08–1.47, p = 0.003 for T vs. C; OR = 1.68, 95% CI = 1.21-2.32, p = 0.002 for TT vs. CC; OR = 1.50, 95% CI = 1.13-1.98 for, p = 0.005 TT vs. CT+CC; OR = 1.29, 95% CI = 0.84-1.97, p = 0.250 for TT+CT vs. CC), but not in Caucasians (OR = 1.02, 95% CI = 0.93-1.13, p = 0.656 for T vs. C; OR = 0.99, 95% CI = 0.75-1.30, p = 0.932 for TT vs. CC; OR = 0.98, 95% CI = 0.75-1.28, p = 0.869 for TT vs. CT+CC; OR = 1.04,

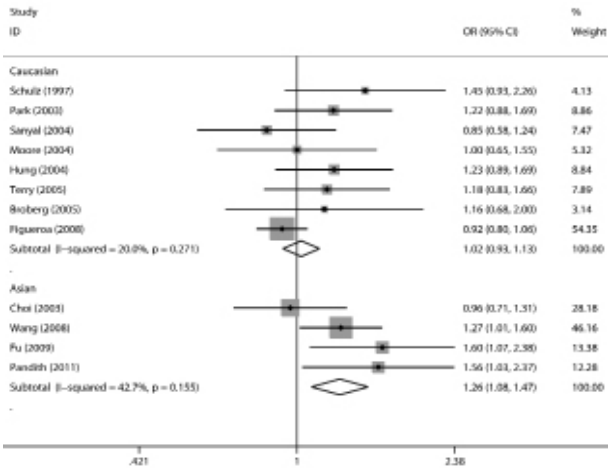

Figure 3. Forest Plot for the Association Between NQO1 rs1800566 Polymorphism and Bladder Cancer Risk for Fixed Effects Stratified by Ethnicity (T vs. C). The size of the black square corresponding to each study is proportional to the sample size and the center of each square represents the OR. Horizontal line shows the corresponding 95% CI of the OR. Pooled OR is represented by hollow diamond

95% CI = 0.92-1.17, p = 0.545 for TT+CT vs. CC).

We also calculated false positive report probability (FPRP) for each statistically significant result (Table 4). With the assumption of a moderate prior probability of 0.1 and the OR for the specific genotype was 1.5, the FPRP values for the significant findings in the additive model (T vs. C) in all subjects, and in the additive model (T vs. C), homozygote contrast (TT vs. CC) and recessive model (TT vs. CT+CC) in Asians were 0.186, 0.029, 0.056, 0.070, respectively.

Evaluation of heterogeneity

In this meta-analysis, we used the Q test and the I^2 index to evaluate the heterogeneity across studies. As shown in Table 3, a low to high heterogeneity across studies presented in most of comparisons. In addition, we assessed heterogeneity across studies by ethnicity, genotyping method, and quality of studies using meta-regression. However, none of these above was identified as a possible source of heterogeneity in the overall meta-analysis.

Publication bias

There was no evidence of significant publication bias either with the Begg's test (Figure 3, P = 0.631 for additive model; P = 0.193 for homozygote contrast; P = 0.193 for recessive genetic model; P = 0.837 for dominant genetic model) or with Egger's test (P = 0.050 for additive model; P = 0.182 for homozygote contrast; P = 0.235 for recessive genetic model; P = 0.162 for dominant genetic model).

Discussion

The rs1800566 polymorphism is the most well characterized NQO1 polymorphism, but the reported associations with bladder cancer risk among studies are inconsistent. Our present meta-analysis incorporating 12 case–control studies (3,041 cases and 3,128 controls) suggests that the NQO1 rs1800566 polymorphism is significantly associated with increased bladder cancer risk in Asians, but not in Caucasians.

In this meta-analysis, publication bias was not observed. And the FPRP analyses showed that with the assumption of a prior probability of 0.1, the FPRP values for all the significant findings were below 0.2. It indicated that the results of our meta-analysis were statistically robust.

The results of the present study are in contrast with a previous meta-analysis conducted by Chao et al (Chao et al., 2006), who found an association between the NQO1 rs1800566 polymorphism and an increased risk for bladder cancer in Caucasians, but not in Asians. However, their study only included eight studies with limited sample size (1,410 cases and 1,485 controls) and only one conducted in Asia, thus it may lack sufficient statistical power to detect the real association and may have generated a fluctuated risk estimate.

Our findings have some biological plausibility. As a multifactor disease, both genetic and environmental factors are involved in the development of bladder cancer. Previous studies reported that NQO1 enzyme can not only detoxify carcinogenic compounds (Siegel et al., 2004), but can also bioactivate several kinds of procarcinogen (Danson et al., 2004). As a result, decreased activity of NQO1 enzyme may have dual effect on carcinogenesis. The relevant environmental exposures in the Caucasians population differ from Asian. There may be some alternative ways in the Caucasians population to detoxify carcinogenic compounds which can compensate more effectively for the loss of NQO1 enzyme activity. Specific environmental factors, such as cigarette smoking, occupational exposure to arylamines and schistosomal infection, which are now more common in Asians than Caucasians, are also confirmed to be risk factors for developing bladder cancer (Jemal et al., 2011). In fact, a number of other studies also showed that NQO1 rs1800566 polymorphism plays a different role in different cancer site and ethnicity. For example, Ding et al found that NQO1 rs1800566 polymorphism was associated with increased risk of colorectal cancer in Caucasians but not in Asians (Ding et al., 2012), while Zhang et al reported that NQO1 rs1800566 polymorphism is only associated with increased gastric cancer risk in Asians but not in Caucasians (Zhang et al., 2012). On the other hand, there were only four studies involving Asians that were included in our meta-analysis; the relative small sample size may have affected the results.

Several limitations of this meta-analysis should be discussed. First, although the Begg's test and Egger's test did not show any publication bias, some inevitable publication bias may exist, because only studies published in English and Chinese were included in our meta-analysis. Second, the number of selected studies was still relatively small, especially in Asians, and the significant between-study heterogeneity was detected in some comparisons, which may distort the meta-analysis. Third, because of the lack of the individual original data, our results were just based on unadjusted estimates and a more precise analysis has not been performed.

In summary, despite the limitations, results of our meta-analysis suggest that the minor allele T of the NQO1 rs1800566 polymorphism may contribute to bladder cancer development, especially in Asians. Whether it could be applied to genotyping for clinical assessment requires large-scale population studies among different ethnicities and regions.

Acknowledgements

The authors declare that there is no potential conflict of interest with this work.

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