RESEARCH ARTICLE

The NAD(P)H: Quinine Oxidoreductase 1 (*NQO1*) Gene 609 C>T Polymorphism is Associated with Gastric Cancer Risk: Evidence from a Case-control Study and a Meta-analysis

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Abstract

The association between the NAD(P)H:quinone oxidoreductase 1 (*NQO1*) gene C609T polymorphism (rs1800566) and gastric cancer has been widely evaluated, but a definitive answer is so far lacking. We first conducted a case-control study to assess this association in a large Han Chinese population, and then performed a meta-analysis to further address this issue. Although our case-control association study indicated no significant difference in the genotype and allele distributions of C609T polymorphism between gastric cancer patients and controls, in the meta analysis involving 4,000 subjects, comparison of alleles 609T and 609C indicated a significantly increased risk (46%) for gastric cancer (95% confidence interval (95%CI) for odds ratio (OR)=1.20-1.79) in individuals with the T allele. The tendency was similar to the homozygote (OR=1.81, 95%CI: 1.16-2.84), dominant models (OR=1.41, 95%CI: 1.12-1.79), as well as recessive model (OR=1.58, 95%CI: 1.06-2.35). Stratified analysis by study design demonstrated stronger associations in population-based than in hospital-based studies. And ethnicity-based analysis demonstrated a significant association in Asians. We conclude that the *NQO1* gene C609T polymorphism increases the risk for gastric cancer, especially in Asian populations.

Keywords: Gastric cancer - *NQO1* gene - polymorphism - risk - meta-analysis

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Introduction

Gastric cancer is the second most common cause of cancer-related mortality in the world (Parkin et al., 2005), especially in East Asian countries (Long et al., 2010). Evidence is mounting suggesting that the cause of gastric cancer is multifactorial, and part is due to genetic defects. In the last decade, exhaustive efforts have been devoted to unraveling the genetic underpinnings of gastric cancer; however, its driving genes and genetic determinants that attribute to the development of gastric cancer so far remain elusive.

The gene encoding NAD(P)H:quinone oxidoreductase 1 (*NQO1*) is a promising candidate in the pathogenesis of gastric cancer. *NQO1* is a cytosolic enzyme, and catalyzes the reduction of two electrons of quinoid compounds to generate the less-toxic hydroquinones, which can alleviate cancer carcinogenesis (Rauth et al., 1997). Animal models using the *NQO1*-knockout mice suggested that the *NQO1* gene deficiency increased susceptibility to cancer (Long et al., 2000; Iskander et al., 2005). Recently, an exonic polymorphism, C609T (also known as Pro187Ser) in *NQO1* gene has been identified and widely evaluated in various malignancies such as esophageal cancer (Wang et al., 2012), Leukemia (Han et al., 2013) and Bladder Cancer (Guo and Feng, 2012) et al. In addition, the *NQO1* C609T polymorphism, was reported to associated with Helicobactor pylori seropositivity in Japanese (Goto et al., 2005), and with intestinal metaplasia, an important precursor lesion in the development of gastric cancer in a Singapore-Chinese population (Zhu et al., 2009). Although some studies have attempted to link the *NQO1* gene C609T polymorphism with gastric cancer, data are not often reproducible,possibly due to the insufficient sample sizes, genetic backgrounds, and selection of study populations.

In this study, we first decided to assess the assocation of C609T polymorphism of *NQO1* gene with gastric cancer risk in a large Han Chinese population. Then, given the accumulating data and to shed some light on current uncertain claims, we sought to conduct a comprehensive meta-analysis of this association from both English and Chinese literature.

Materials and Methods

Study population

This was a hospital-based case-control study with a total of 1050 subjects consecutively recruiting from Shanghai Ruijin hospital, China from May 2009 to

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December 2012. The study population included 448 unrelated cases with histopathologically confirmed gastric cancer and 602 cancer-free controls, and all subjects were local residents of Han descent. This study was approved by the Ethics Committee of Shanghai Jiaotong University School of Medicine, and was conducted according to the Declaration of Helsinki Principles. All subjects signed the written informed consent.

Genotyping

Blood samples (1 mL) were collected, and genomic DNA was extracted from white blood cells using the TIANamp Blood DNA Kit [Tiangen Biotect (Beijing) Co., LTD]. Genotyping was conducted using the PCR-LDR (ligase detection reactions) method by ABI 9600 system (Applied Biosystems, USA). Cycling parameters were as the following: 94°C for 2 min; 35 cycles of 94°C for 15 s; 60°C for 15 s; 72°C for 30 s; and a final extension step at 72°C for 5 min. Two specific probes to discriminate the specific bases and one common probe were synthesized (available upon request). The common probe was labeled at the 3' end with 6-carboxy-fluorescein and phosphorylated at the 5' end. The reacting conditions of LDR were: 94°C for 2 min, 20 cycles of 94°C for 30 s and 60°C for 3 min. After reaction, 1 mL LDR reaction products were mixed with 1 mL ROX passive reference and 1 mL loading buffer, and then denatured at 95°C for 3 min, and chilled rapidly in ice water. The fluorescent products of LDR were differentiated using ABI sequencer 377 (Applied Biosystems, USA).

Statistical analysis

Comparisons between gastric cancer patients and controls were conducted by unpaired t-test for continuous variables and by χ^2 test for categorical variables. To avoid gross genotyping error, C609T polymorphism was checked for consistency with Hardy-Weinberg equilibrium by χ^2 test.Genotypes were compared by conditional logistic regression analysis under assumptions of additive, dominant and recessive models of inheritance, respectively. Statistical significance was accepted as $p<0.05$.

Meta analysis

Studies were identified by searches of the PubMed, EMBASE, ISI Web of Knowledge, as well as and China WANFANG (www.wanfangdata.com.cn) databases for relevant articled published as of October 2013. Searching key subjects in Boolean combinations were (quinone oxidoreductase OR DT-diaphorase OR quinone reductase OR NAD(P)H: quinine oxidoreductase 1 OR *NQO1* OR DTD) AND (gastric cancer OR gastric carcinoma) AND (polymorphism OR allele OR genotype OR variant OR variation). Search results were restricted to human populations and articles written in English or Chinese language. The full text of the retrieved articles and reviews was scrutinized to decide whether information on the topic of interest was included. The reference lists of original studies and review articles were also checked to determine whether citations of articles that were not initially identified. If more than one geographic or ethnic heterogeneous groups were reported in one article, each group was treated separately.

Studies were qualified if they met the following criteria: (i) on a retrospective or nested case-control design; (ii) adopt validated genotyping method; (iii) provide genotype counts of *NQO1* gene C609T polymorphism between patients with gastric cancer and controls.

In the meta-analysis, We assessed the association of *NQO1* gene 609T allele with gastric cancer relative to the 609C allele (allelic model), as well as the homozygous contrast, the dominant model and the recessive model, respectively. Unadjusted OR and 95%CI were used to compare contrasts of alleles or genotypes between patients and controls. The random-effects model using the DerSimonian & Laird method was implemented to bring the individual effect-size estimates together, and the estimate of heterogeneity was taken from the Mantel-Haenszel model (Cohn and Becker, 2003).

Satisfaction of C609T genotypes with Hardy-Weinberg proportions was calculated using the χ^2 test or Fisher's exact test in control groups. Possible heterogeneity between the results of individual studies or in groups defined by race or by study design was assessed using the inconsistency index I^2 statistic (ranging from 0 to 100%) with higher values suggesting the existence of heterogeneity (Higgins and Thompson, 2002; Higgins et al., 2003). In the case of between-study heterogeneity, we examined the study characteristics that can stratified the studies into subgroups with homogeneous effects.

Funnel plot and Egger regression asymmetry test were used to examine publication bias. Probability less than 0.05 was judged significant except for the I^2 statistic and publication Egger's statistic, where a significance level of less than 0.1 was chosen. Data management and statistical analyses were performed using STATA version 11.0 for Windows.

Results

Single-locus analysis

The success rates of genotyping for C609T polymorphism were 98.44% and 99.17% in patients and controls, respectively. Genotype distributions of examined polymorphism respected Hardy-Weinberg equilibrium in controls ($p>0.05$). There was no significant difference in the genotype and allele distributions of C609T polymorphism between gastric cancer patients and

Table 1. The Alleles and Genotype Distributions of *NQO1* Gene C609T Polymorphism between Cases (n=441) and Controls (n=597)

Status	C609T genotypes (number)			C609T alleles (%)	
	CC	CT	TT	C	T
Cases	128	222	91	54.20	45.80
Controls	171	282	144	52.26	47.74
Additive model (a)		Dominant model(a)		Recessive model(a)	
0.93; 0.78-1.10; 0.389		0.98; 0.75-1.29; 0.893		0.82; 0.61-1.10; 0.183	

*p values were calculated using χ^2-test from a series of 3×2 contingency tables for genotype data and 2×2 contingency tables for allele data; (a) Data are expressed as odds ratio; 95% confidence interval; p values for genetic modes of inheritance

controls, and this non-significance was also mirrored under assumptions of the additive (OR=0.93; 95%CI: 0.78-1.10; *p*=0.389), dominant (OR=0.98; 95%CI: 0.75-1.29; *p*=0.893) and recessive (OR=0.82; 95%CI: 0.61-1.10; *p*=0.183) models (Table 1).

Eligible articles for meta-analysis and study characteristics

The initial search yielded 28 potentially relevant articles. After applying the inclusion/exclusion criteria, 7 articles were eligible for inclusion. The retrieved articles were published between 2002 and 2011, with 4 articles written in English and three in Chinese.

In total, 7 separate studies plus the present study encompassing a total of 1662 patients with gastric cancer and 2375 controls were finally meta-analyzed, with 7 studies performed in Asians, 1 in Caucasians. Besides the present study, five studies were conducted in hospital-based design (Hamajima et al., 2002; Sarbia et al., 2003; Zhang et al., 2003; Malik et al., 2011) and 3 studies in population-based design (Ren et al., 2006; Chen et al., 2007; 2011).The frequencies of 609T allele varied widely, which were exceedingly higher in Asians than in Caucasians for both patients (34.72 to 55.0% versus 20.31%) and controls (25.13 to 47.74% versus 14.09%). Baseline characteristics of qualified studies are shown in Table 3. The genotype distributions of the *NQO1* gene 609 C>T polymorphism were in agreement with the Hardy-Weinberg equilibrium among control groups of all studies.

Meta-analysis results

The pooled OR from all included studies indicated a significant association between *NQO1* C609T polymorphism and increased gastric cancer risk in allelic comparison (OR=1.38, 95%CI: 1.10-1.73; *p*=0.006) (Figure 1)with low possibility of publication bias as reflected by the suggestive asymmetry of funnel plot (Figure 2) and the Egger's test (*p*=0.22), although there was strong evidence of between-study heterogeneity (I^2=79.8%, *p*<0.001). The magnitude of OR in allele comparison was similar to the homozygote (OR=1.81, 95%CI: 1.16-2.84; *p*=0.009), dominant models (OR=1.41, 95%CI: 1.12-1.79; *p*=0.004), as well as recessive model (OR=1.58, 95%CI: 1.06-2.35, *p*=0.024).

To account for potential sources of heterogeneity, we conducted a set of subgroup analyses according to ethnicity and source of controls (Table 3). Notably, upon stratification by source of controls, significant association was found in allele and genetic models, in the population-based subgroup, while no obvious association existed in the hospital-based subgroup, whereas heterogeneity tangled the latter.

Further subgroup analysis by ethnicity suggested heterogeneous associations of C609T polymorphism with gastric cancer, by showing that there was significant association among Asian populations in allele comparison as well as all genetic models, whereas no significance was observed in Caucasians in either homozygous model or recessive model.

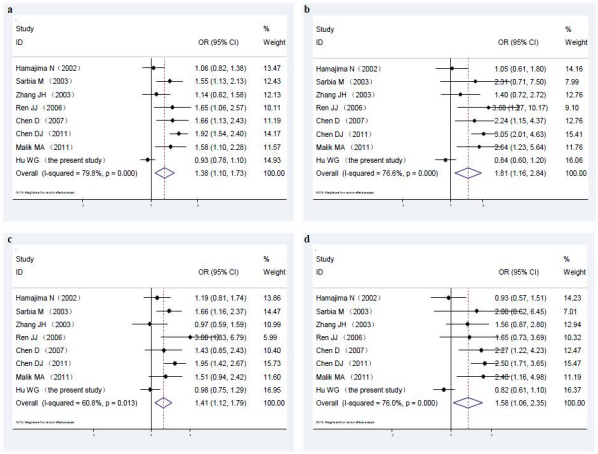

Figure 1. Overall Risk Estimates of NQO1 609 C>T Polymorphism for Gastric Cancer (a 609 T allele vs. 609 C allele; b 609 TT vs. 609 CC; c dominant model; d recessive model)

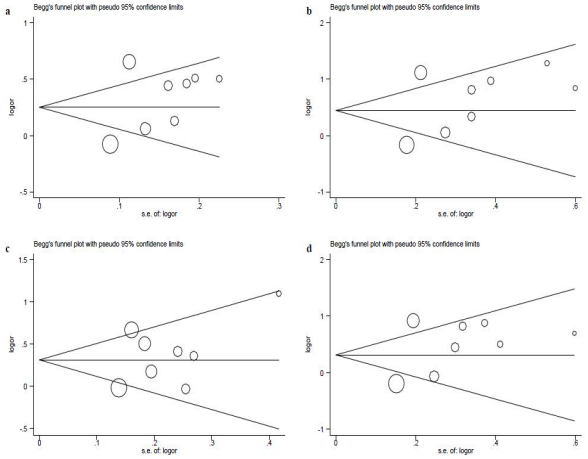

Figure 2. Begg's Funnel Plot of Publication Bias Test for the NQO1 609 C>T Polymorphism (a 609 T allele vs. 609 C allele; b 609 TT vs. 609 CC; c dominant model; d recessive model)

Table 2. The Baseline Characteristics of All Eligible Studies

References	Ethnicity	Country	Sources of Con.	No. of Ca.	No. of Con.	CC Ca./Con.	CT Ca./Con	TT Ca./Con	MAF(%) Ca/Con	HWE
Hamajima N et al. (2002)	Asian	Japan	HCC	143	640	48/240	71/286	24/114	41.61/40.16	>0.05
Sarbia M et al. (2003)	Caucasian	Germany	HCC	320	252	200/185	110/63	10/4	20.31/14.09	>0.05
Zhang JH et al. (2003)	Asian	China	HCC	124	165	40/52	55/86	29/27	45.56/42.42	>0.05
Ren JJ et al. (2006)	Asian	China	PCC	80	80	10/24	52/44	18/12	55.00/42.50	>0.05
Chen D et al. (2007)	Asian	China	PCC	112	112	47/57	28/35	37/12	45.54/33.48	>0.05
Chen DJ et al. (2011)	Asian	China	PCC	334	334	107/160	125/124	102/50	49.25/33.53	>0.05
Malik MA et al. (2011)	Asian	India	HCC	108	195	51/112	39/68	18/15	34.72/25.13	>0.05
Hu WG et al.(the present study)	Asian	China	HCC	441	597	128/171	222/282	91/144	45.81/47.74	>0.05

*Abbreviations: HCC=Hospital-based case-control study; PCC=Population-based case-control study; Ca=Case; Con=Control; HWE=Hardy-Weinberg equilibrium in the control group; MAF=minor allele frequency

Table 3. Overall and Subgroup Analysis of *NQO1* Gene C609T Polymorphism and Gastric Cancer

Subgroup		Study no.	T vs C				TT vs CC				Dominant				Recessive			
			OR (95%CI)	p	I^2 (%)	$p_{heterogeneity}$	OR (95%CI)	p	I^2 (%)	$p_{heterogeneity}$	OR (95%CI)	p	I^2 (%)	$p_{heterogeneity}$	OR (95%CI)	p	I^2 (%)	$p_{heterogeneity}$
Total		8	1.38 (1.10,1.73)	0.006	79.8	<0.001	1.81 (1.16,2.84)	0.009	76.6	<0.001	1.41 (1.12,1.79)	0.004	60.8	0.013	1.58 (1.06,2.35)	0.024	76	<0.001
Area	Asia	7	1.36(1.05,1.75)	0.019	81.9	<0.001	1.78 (1.10,2.87)	0.018	79.6	<0.001	1.38 (1.06,1.80)	0.018	63.8	0.011	1.55 (1.02,2.36)	0.041	79.1	<0.001
	Europe	1	1.55 (1.13,2.13)	0.006	-	-	2.31 (0.71,7.50)	0.163	-	-	1.66 (1.16,2.37)	0.006	-	-	2.00 (0.62,6.45)	0.246	-	-
Design	HCC	5	1.19 (0.96,1.48)	0.118	67.7	0.015	1.32 (0.86,2.01)	0.208	57.1	0.054	1.22(0.97,1.52)	0.084	41.3	0.146	1.25 (0.83,1.90)	0.286	62.9	0.029
	PCC	3	1.82 (1.53,2.17)	<0.001	0	0.725	2.87 (2.05,4.02)	<0.001	0	0.674	1.89(1.41,2.54)	<0.001	14	0.313	2.30 (1.71,3.11)	<0.001	0	0.656

*Abbreviations: HCC=Hospital-based case-control study; PCC=Population-based case-control study; OR=odds ratio; CI=confidence interval

Discussion

However, this case-control study in Han Chinese failed to find the association between *NQO1* gene C609T polymorphism with gastric cancer occurrence, after a comprehensive evaluation of *NQO1* gene C609T polymorphism over 4000 subjects, we provided evidence that *NQO1* 609T allele was associated with a significantly increased risk to gastric cancer occurrence, especially in Asians, although this finding might suffer from the disturbance of significant heterogeneity. To the authors' knowledge, this is the most comprehensive meta-analysis investigating the genetic susceptibility of *NQO1* gene C609T polymorphism to gastric cancer. Furthermore, the relatively large samples examined and low probability of publication bias as reflected by visual inspection of the funnel plots along with Egger's tests indicated the robustness of our results.

It is inevitable to encounter genetic heterogeneity in any disease identification strategy (Hemminki et al., 2006). As exemplified in the present study, frequency of -260T allele in patients differed remarkably between Asians and Caucasians (34.72 to 55.0% versus 20.31% in patients and 25.13 to 47.74% versus 14.09% in controls), leaving open the question that divergent genetic backgrounds or linkage disequilibrium patterns may account for this difference. Meanwhile, the possibility of *NQO1* gene C609T being in close linkage with different nearby causal variants in different populations cannot be excluded. Additionally, it is widely believed that genetic markers in predisposition to gastric cancer vary across geographical and racial groups. As evidenced in our ethnicity-stratified analyses, *NQO1* gene C609T polymorphism showed significant heterogeneous associations with gastric cancer between Asians and Caucasians, with positive association in Asians for all genetic models, whereas negative association in Caucasians in either homozygous model or recessive model, suggesting that *NQO1* C609T might exert a pleiotropic impact in the pathogenesis of gastric cancer or interact with other genetic or environmental factors. However, considering the relatively small sample sizes in Caucasians, more and more large, well-designed studies are required to understand the genetic variability of gastric cancer.

In addition, study design might also be a potential source of between-study heterogeneity for NOQ1 C609T polymorphism. It is universally believed that control for population stratification remains an important consideration in hospital-based studies (Salanti et al., 2005), because in this meta-analysis, most studies have recruited subjects from only one hospital, and thereby there might be a narrow socioeconomic profile for both patients and controls. Moreover, in hospital-based studies, poor comparability between cases and controls might exert a confounding effect on the true association in light of a regional specialty for the disease under study and the differential hospitalization rates between cases and controls (Ruano-Ravina et al., 2008). In contrast, subjects drawn from community or a fixed group might be representative of the true population, leading us to believe that results from population-based studies might hold the water. Our results showed that more stronger association observed in population-based studies relative to hospital-based subgroup, reinforcing the quality of our conclusion.

Of course, some limitations should be considered when interpreting our findings. First, as with all meta-analyses, publication bias might have occurred because our analyses were based entirely on published studies from English- and Chinese-language journals. Second, the single-locus-based meta-analysis precluded the possibility of gene-gene and gene-environment interactions, as well as haplotype-based effects.Third, data on circulating levels of *NQO1* protein or its catalyzed products were unavailable, precluding a more robust assessment of sources of heterogeneity, and making us incapable of comparing their levels across genotypes. Given these limitations, we cannot jump to a conclusion until further verification of our findings *in vitro*, *in vivo* and in large prospective studies.

In summary, we conducted a case-control study and expanded previous individual studies on gastric cancer by indicating that the *NQO1* C609T polymorphism may increased the risk of gastric cancer, especially in Asians. Also our observations leave open the question regarding the heterogeneous effects of C609T allele across different ethnic populations. Nonetheless, for practical reasons, we hope that this study will not remain just another endpoint of research instead of a beginning to establish the background data for further investigation on pathphysiological mechanisms of *NQO1* gene on gastric cancer.

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