RESEARCH COMMUNICATION

The NQO1 C609T Polymorphism and Risk of Lung Cancer: a Meta-analysis

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Abstract

Objective: NAD(P)H: quinone oxidoreductase 1 (NQO1) is a cytosolic flavoprotein that catalyzes the two-electron reduction of quinoid compounds into hydroquinones. A single base substitution (C→T) polymorphism at 609 in the NQO1 gene reduces quinone reductase activity. Published data on the association between NQO1 C609T polymorphism and lung cancer risk are conflicting. Methods: To derive a more precise estimation of the relationship, a meta-analysis was performed. Results: A total of 23 studies including 5,575 cases and 9,132 controls were assessed. The pooled result showed that the NQO1 polymorphism was not associated with a clear increased risk of lung cancer (OR = 1.009, 95% CI: 0.943-1.078; $P_{heterogeneity}$=0.049). In the subgroup analysis by ethnicity, no clear increased risk was found among Asians for TT/CT versus CC (OR = 1.005; 95% CI = 0.890-1.135; $P_{heterogeneity}$=0.024). However, the TT and CT genotypes combined were associated with significantly increased risk of lung cancer in Chinese (OR = 1.237, 95% CI: 1.029-1.486; $P_{heterogeneity}$=0.061) among whom the variant allele is common. The variant genotype of NQO1 was also associated with modestly increased risk of lung cancer among white populations (OR = 1.017, 95% CI: 0.936-1.105; $P_{heterogeneity}$=0.101). However, no significant association was found in Africans with all genetic models. Conclusions: Our meta-analysis suggests that the variant NQO1 C609T genotype may affect individual susceptibility to lung cancer. This meta- analysis suggests that the NQO1 609T allele is a low penetrant risk factor for developing lung cancer in Chinese.

Keywords: NQO1 - polymorphism - lung cancer - meta-analysis - Chinese - ethnicity

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Introduction

Lung cancer is currently one of the most common cancers and a major cause of cancer-related death in the world. The mechanism of lung cancer is still not fully understood. Genetic factors that affect lung cancer susceptibility have been searched to establish novel and efficient ways of preventing the disease.

NAD(P)H: quinone oxidoreductase 1 (NQO1), formerly referred to as diphtheria toxin diaphorase (DT-diaphorase), is a cytosolic flavoprotein that catalyzes the two-electron reduction of quinoid compounds into the readily excreted hydroquinones (Smith,1999). The NQO1 gene is located on chromosome 16q22. A genetic polymorphism of this gene is related to C609T (rs1800566) of exon 6 of the NQO1 cDNA that encodes for a proline to serine substitution at position 187 in enzyme's amino acid sequence (Vineis et al., 2007). NQO1 is also known to bioactivate some environmental procarcinogens present in tobacco smoke and foods, such as nitroaromatic compounds and heterocyclic amines (Nebert et al.,2002). A number of studies have examined the relationship between NQO1 genetic polymorphism and lung cancer risk, but the results are inconsistent. To clarify the effect of NQO1 C609T on the risk of lung cancer, our study undertakes a meta-analysis of the published case–control observational studies.

Materials and Methods

Literature Serach

The electronic databases of PubMed, Embase, Web of Science, and China National Knowledge Infrastructure (CNKI) were searched for studies to include in the present meta-analysis, by using the terms "quinone oxidoreductase", "NQO1", "DT-diaphorase", "DTD", "quinone reductase", "NAD(P)H dehydrogenase (quinone)" in combination with "lung cancer", "lung carcinoma". An upper date limit of June 30, 2011 was applied; we used no lower date limit. The search was done without restriction on language but was focused on studies conducted on human subjects. We also reviewed the Cochrane Library for relevant articles. The reference lists of reviews and retrieved articles were hand-searched simultaneously. Only published studies with full text articles were included. When more than one of the same patient population was included in several publications, only the most recent or complete study was used.

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Inclusion Criteria

The included studies met the following criteria: (1) evaluated NQO1 polymorphism and lung cancer risk; (2) case–control studies; (3) supplied the number of individual genotypes in lung cancer cases and controls; and, (4) indicated that the distribution of genotypes among controls were in Hardy–Weinberg equilibrium.

Data Extraction

Information was carefully extracted from all eligible publications independently by two of the authors according to the inclusion criteria listed above. Disagreement was resolved by discussion between the two authors. If these two authors could not reach a consensus, another author was consulted to resolve the dispute and a final decision was made by the majority of the votes. The following data were collected from each study: first author's surname, year of publication, ethnicity of study population, characteristics of matching criteria in controls, genotyping methods, total number of cases and controls and numbers of cases and controls with the CC, CT and TT genotypes, respectively. Different ethnicity descents were categorized as white population, Asian and African. When studies included subjects of more than one ethnicity, genotype data were extracted separately according to ethnicities for subgroup analyses. We did not define any minimum number of patients as a criterion for a study's inclusion in our meta-analysis.

Statistical Methods

The meta-analysis examined the overall association for the allele contrasts, the contrast of homozygotes, and the recessive and dominant models. Odds ratios (OR) with 95% confidence interval (CI) were used to assess the strength of association between the NQO1 C609T polymorphism and lung cancer risk. The OR of lung cancer associated with NQO1 genotype, the T allele carriers (T/C +T/T) versus C/C genotype was calculated. Heterogeneity assumption was tested by the chi-square-based Q-test. A P value greater than 0.10 for the Q-test indicates a lack of heterogeneity among studies, so that the pooled OR estimate of each study was calculated by the fixed-effects model (the Mantel–Haenszel method). Otherwise, the random-effects model (the DerSimonian and Laird method) was used. To evaluate the ethnicity-specific effect, subgroup analyses were performed by ethnic group. One-way sensitivity analyses were performed to assess the stability of the results, namely, a single study in the meta-analysis was deleted each time to reflect the influence of the individual data-set to the pooled OR. An estimate of potential publication bias was carried out by the funnel plot, in which the standard error of log (OR) of each study was plotted against its log (OR). An asymmetric plot suggests a possible publication bias. Funnel plot asymmetry was assessed by the method of Egger's linear regression test, a linear regression approach to measure the funnel plot asymmetry on the natural logarithm scale of the OR. The significance of the intercept was determined by the t-test suggested by Egger ($P < 0.05$ was considered representative of statistically significant publication bias). All of the statistical tests were performed with STATA version 10.0 (Stata Corporation, College Station, TX).

Results

Study Characteristics

A total of 25 publications met the inclusion criteria (Wiencke et al., 1997; Chen et al., 1999; Rosvold et al., 1995; Lin et al., 1999; 2000; 2003; Benhamous et al., 2001; Lewis et al., 2001; Xu et al., 2001; Yin et al., 2001; Hamajima et al., 2002; Alexandrie et al., 2004; Lan et al., 2004; Liang et al., 2004; Bock et al., 2005; Lawson et al., 2005; Saldivar et al., 2005; Sørensen et al., 2005; Skuladottir et al., 2005; Cote et al., 2009; Su et al., 2009; Timofeeva et al., 2010). We excluded one study due to the lack of genotype information (Rosvold et al.,1995). Study subjects in Lin et al (Lin et al.,1999) seemed to overlap with the subjects in Lin et al (Lin et al.,2003); therefore, the former was excluded. Hence, the remaining 23 studies included a total of 5,575 cases and 9,132 controls were used in the pooled analyses. Table 1 lists the selected study characteristics. There were eight studies of white populations, ten studies of Asian populations, and five studies included multiple ethnic/racial groups. Almost all the cases were histologically confirmed, and the control groups were mainly healthy populations and matched for age and gender.

Meta-analysis Results

Table 2 lists the main results of this meta-analysis. Overall, for the T allele carriers (C/T +T/T), the pooled OR for all the 23 studies combined 5,575 cases and 9,132 controls was 1.009 (95% CI = 0.943–1.078; P = 0.049 for heterogeneity), when compared with the homozygous wild-type genotype (C/C). In the stratified analysis by ethnicity, a modestly increased risk was suggested for the variant homozygotes in whites (OR T/T+C/T versus C/C, 1.017; 95% CI=0.936–1.105; P = 0.101 for heterogeneity). In the subgroup analysis by ethnicity, no clear increased risk was found among Asians for C/T+T/T versus CC (OR = 1.005; 95% CI = 0.890–1.135; P = 0.024 for heterogeneity). In the subgroup analysis by source of controls, statistically significantly increased cancer risks were found among Chinese groups with population-based controls for C/T+T/T versus CC (OR = 1.237, 95% CI: 1.029–1.486, P = 0.061 for heterogeneity). Among Africans, no significant association was found in the T allele carriers versus C/C (OR = 0.926, 95% CI =0.687–1.248; P = 0.809 for heterogeneity). The meta-analysis showed no effect of carrying at least one variant allele in African-American populations.

Sensitivity Analyses

A single study involved in the meta-analysis was deleted each time to reflect the influence of the individual data-set to the pooled ORs, and the corresponding pooled ORs were not materially altered (data not shown).

Publication Bias

Begg's funnel plot and Egger's test were performed to access the publication bias of literatures. The shapes

Table 1. Main Characteristics of all Studies Included in the Meta-analysis

First author Year	Country	Ethnicity	Source of controls	Cases/controls	Genotyping method
Lewis 2001	United Kingdom	White	Hospital	94/165	PCR-RFLP
Xu 2001	United States	White	Friends/Family	814/1123	PCR-RFLP
Benhamou 2001	France	White	Hospital	150/172	PCR-RFLP
Alexandrie 2004	Sweden	White	Laboratory workers	524/530	PCR-RFLP
Sorensen 2005	Denmark	White	Population based	256/269	TaqMan
Skuladottir 2005	Denmark	White	Mixed	232/346	TaqMan
Lawson 2005	Finland	White	Population based	353/360	PCR-RFLP
Timofeeva 2010	Germany	White	Population based	638/1300	PCR-RFLP
Wiencke 1997	United States	White	Community	61/161	PCR-RFLP
		African		116/136	
Chen 1999	United States	White	Population based	218/273	PCR-RFLP
		Asian		109/167	
Bock 2005	United States	White	Population based	130/144	PCR-RFLP
		African		31/29	
Saldivar 2005	United States	White	Private physican group	719/719	PCR-RFLP
		African		107/107	
Cote 2009	United States	White	Population based	387/405	TaqMan
		African		113/121	
Lin 2000	China	Asian	Hospital	95/136	PCR-RFLP
Yin 2001	China	Asian	Hospital	84/84	PCR-RFLP
Liang 2004	China	Asian	Hospital	152/152	PCR-RFLP
Lan 2004	China	Asian	Population based	119/109	PCR-RFLP
Su 2009	China	Asian	Community	396/465	PCR-RFLP
Lin 2003	Taiwan	Asian	Hospital	198/332	PCR-RFLP
Yang 2007	Korean	Asian	Hospital	314/347	PCR-RFLP
Eom 2009	Korean	Asian	Hospital	387/387	PCR-RFLP
Sunaga 2002	Japan	Asian	Hospital	198/152	PCR-RFLP
Hamajima 2002	Japan	Asian	Hospital	192/640	PCR-RFLP

Table 2. Main Results of Pooled ORs in the Meta-analysis

Ethnicity	Number of cases/controls	TT+CT versus CC OR(95% CI)	I2 (%) value	P Q test
Total	5,575/9,131	1.009 (0.943–1.078)		
33.3	0.049			
White	2,964/5,768	1.017 (0.936–1.105)	35.2	0.101
African	367/393	0.926 (0.687–1.248)	0.0	0.809
Asian	2,244/2,971	1.005 (0.890–1.135)	53.0	0.024
Chinese only	949/1,142	1.237 (1.029–1.486)	52.6	0.061

of the funnel plots did not reveal any evidence of obvious asymmetry (Figures not shown). Then, the Egger's test was used to provide statistical evidence of funnel plot symmetry. The results still did not suggest any evidence of publication bias.

Discussion

It is well recognized that individual susceptibility to the same kind of cancer can vary, even with the same environmental exposure. Host factors, including polymorphism in the genes involved in carcinogenesis may have accounted for this difference. Therefore, genetic susceptibility to cancer has been the focus of research in the scientific community. Recently, genetic variants of the NQO1 gene have been subject to increasing attention in the etiology of lung cancer (Asher et al., 2001). Several studies have reported that the role of NQO1 609 C/T polymorphism in lung cancer risk, but the conclusions have been contradictory. Therefore, to better understanding of the association between the polymorphisms and lung cancer risk, a pooled analysis with a large sample, subgroup analysis performed, and heterogeneity explored is necessary.

This meta-analysis summarized all the available data on the association between NQO1 C609T and lung cancer, including a total of 5,575 cases and 9,132 controls. Our results indicated that NQO1 C609T is a modest risk factor for lung cancer in Asians especially in Chinese populations and white population. However, in African population, the association with NQO1 polymorphism and lung cancer risk was not found for all genetic models. The risk appeared to be more evident in the white and Chinese but not in Africans who even had a possible protective effect from the same genotypes, suggesting a possible role of ethnic differences in genetic backgrounds and the environment they lived in. Population stratification is an area of concern, and it can lead to spurious evidence for the association between the genetic marker and the disease, suggesting a possible role of ethnic differences in genetic backgrounds and environments.

This finding is biologically plausible. NQO1 is an oxidoreductase that has dual functions of both activating and detoxifying carcinogens. Synthetic antioxidants, such as butylated hydroxyanisole, and extracts of cruciferous vegetables, including broccoli, have been shown to be potent inducers of NQO1 (Smith, 1999, Begleiter and Fourie, 2004). This inducibility suggests that NQO1 plays an important role in cancer chemoprevention. NQO1 catalyzes the reduction of quinones to hydroquinones without the intermediate formation of the free semiquinone radical. Because NQO1 induction appears to protect against chemical carcinogenesis and mutagenesis, it would seem

logical that individuals lacking NQO1 activity because of inheritance of homozygous mutant alleles would be at higher risk of developing certain cancers (Smith, 1999). Moreover, gene–gene and gene–environment interactions should also be considered in the analysis. Genetic polymorphisms within genes encoding metabolic enzymes probably act as lung cancer susceptibility loci because of their potential role in the activation and/or detoxification of lung carcinogens (Tsvetkov et al.,2010).

Our data were consistent with the results of a previous meta-analysis by Chao et al. (2006) that showed an association between NQO1 polymorphism and lung cancer risk. This analysis included 19 studies published before January 2006, and seven Asian population studies. They concluded that there is no clear association between the NQO1 C609T polymorphism and lung cancer risk in Asian group. We updated that previous meta analysis by including more recent related studies and used a more comprehensive search strategy. And we reduced the problem of missing data by contacting authors. We also explored heterogeneity and potential publication bias in accordance with published guidelines.

Some limitations of this meta-analysis should be acknowledged. First, lack of the original data of the reviewed studies limited our further evaluation of potential interactions, because the interactions between gene–gene, gene–environment, and even different polymorphic loci of the same gene may modulate cancer risk. Second, our result was based on unadjusted estimates, while a more precise analysis should be conducted if more detailed individual data were available, which would allow for an adjusted estimate by other factors such as age, ethnicity, family history, environmental factors, and lifestyle. Lacking of the information for the data analysis may cause serious confounding bias. Third, the controls were not uniformly defined. Although most of the controls were selected mainly from healthy populations, some may not be truly representative of the underlying source populations,

Despite these limitations, this meta-analysis suggests that the NQO1 C609T allele is a low-penetrant risk factor for developing lung cancer, especially in Chinese and white populations. Future studies should use standardized unbiased genotyping methods and homogeneous cancer patients and well-matched controls and include multi-ethnic groups. Moreover, gene–gene and gene–environment interactions should also be examined in the future analysis.

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