Effects of the NQO1 609C>T Polymorphism on Leukemia Susceptibility: Evidence from a Meta-analysis

Fei-Fei Han¹*, Chang-Long Guo²,³*, Li-Li Gong¹, Zhu Jin¹, Li-Hong Liu¹*

Abstract

A functional polymorphism in the NQO1 gene, featuring a 609C>T substitution, leading to proline to serine amino-acid and enzyme activity changes, has been implicated in cancer risk. However, individually published investigations showed inconclusive results, especially for leukemia. In this study, we therefore performed a meta-analysis of 21 publications with a total of 3,634 cases and 4,827 controls, mainly for leukemia. We summarized the data on the association between the NQO1 609C>T polymorphism and risk of leukemia and performed subgroup analyses by ethnicity and leukemia type. We found that the variant TT homozygous genotype was associated with a modestly increased risk of leukemia (TT versus CT/CC: OR = 1.23, 95% CI = 1.00 - 1.51, heterogeneity = 0.76; I² = 0%). Following further stratified analyses, increased risk was only observed in subgroups of Caucasians. This meta-analysis suggests that the NQO1 609T allele is a high-penetrance risk factor for leukemia in Caucasians. The effect on leukemia may be modified by ethnicity and leukemia type, and the small sample sizes of the subgroup analyses suggest that further larger studies are needed.

Keywords: NQO1 609C>T polymorphism - leukemia - meta-analysis

Introduction

NQO1 (NAD(P)H dehydrogenase, quinone 1) is a member of the NAD(P)H dehydrogenase family and encodes a cytoplasmic 2-electron reductase. This FAD-binding protein forms homodimers and reduces quinones to hydroquinones (Snyder et al., 1996; North et al., 2011). In organism, NQO1 functions as a gatekeeper of the 20S proteasomes (Asher et al., 2005), it binds to a subset of short-lived proteins (such as p53, p73 and ornithine decarboxilase) and protects them from 20S proteasomal degradation. The NQO1 609C>T polymorphism is characterized by a single proline-to-serine amino acid substitution, that decreases the half time of NQO1 from 18 h (wild-type) to only 1.2 h via ubiquitination and proteasome pathways. Moreover, other research demonstrated that cell lines and tissues genotyped as homozygous for the NQO1 609C>T polymorphism are deficient in NQO1 activity (Siegel et al., 2001).

NQO1 protein prevents one electron reduction of quinones that results in the production of radical species. Mutations in this gene have been associated with tardive dyskinesia (TD) (Pae et al., 2004; Pae, 2008), an increased risk of hematotoxicity after exposure to benzene (Iskander et al., 2005; Ross, 2005), increased risk of childhood asthma (David et al., 2003; Li et al., 2009), susceptibility to various forms of cancer (Sameer et al., 2010; Pandith et al., 2011; Goode et al., 2013; Malik et al., 2013; Yang et al., 2013) and Alzheimer’s disease (AD) (SantaCruz et al., 2004; Bian et al., 2008). Previous researches have revealed the association between NQO1 609C>T polymorphism and leukemia susceptibility. However, the results were conflicting, including an increased risk (Wiemels et al., 1999; Smith et al., 2001; Lanciotti et al., 2005; Yamaguti et al., 2009; Yamaguti et al., 2010; Yamaguti et al., 2010), a reduced risk (Malik et al., 2006; Silveira Vda et al., 2010; Yeoh et al., 2010), and no association (Seedhouse et al., 2002; Kracht et al., 2004; Wu et al., 2004; Zhang, 2005; Clavel et al., 2005; Eguchi-Ishimae et al., 2005; Guillem et al., 2007; Vosso et al., 2007; Gra et al., 2007; Bolufer et al., 2007; Begleiter et al., 2009; Chan et al., 2011; Lozic et al., 2011).

The aim of this article is to review and evaluate the association between NQO1 609C>T polymorphism and leukemia risk, mainly focusing on different ethnicity types.

Materials and Methods

Identification and eligibility of relevant studies

To identify all published articles that examined the association between NQO1 609C>T polymorphism and leukemia risk, we conducted a search in the PubMed database (before 2012-12-15). We identified 90 articles...
with the search terms (“NAD(P)H Dehydrogenase (Quinone)” [MeSH] or “NQO1”) and “leukemia” and limiting the search to studies in human populations. Articles with the following characteristics were excluded from the review: 1) non-English articles; 2) review articles; 3) non-epidemiological studies (e.g., studies on animals or cell culture); 4) treatment outcome studies (Figure 1); 5) studies with control that did not meet Hardy-Weinberg equilibrium (HWE). As of December 15, 2012, we had identified 22 published studies describing the association between NQO1 polymorphisms and leukemia included case-control analyses.

Data extraction and assessment of study quality

Two authors (Fei-fei Han and Chang-long Guo) extracted data and reached a consensus on all of the eligibility items, including author, journal and year of publication, location of study, selection and characteristics of cancer cases and controls, control source, age grades of patients, ethnicity, and leukemia types.

Meta-analysis

The results (odds ratios, OR) of cancer associated with NQO1 609C>T polymorphism were estimated for each study independently. Also we estimated the risk for the NQO1 609C>T polymorphism and breast cancer, colorectal cancer separately.

Statistical analysis

The meta-analysis was performed in a fixed/random effect model. The OR and its 95% CI were estimated for each study independently. Also we estimated the risk for the NQO1 609C>T polymorphism and breast cancer, colorectal cancer separately.
Table 2. Meta-Analysis of the Risk of Leukemia for NQO1 609 C>T Polymorphism

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Populations</th>
<th>OR</th>
<th>P (heterogeneity)</th>
<th>P</th>
<th>Model</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ser/Ser + Pro/Ser</td>
<td>All populations</td>
<td>1.21 (1.02, 1.44)</td>
<td>&lt;0.00001</td>
<td>0.03</td>
<td>random</td>
</tr>
<tr>
<td>versus Pro/Pro</td>
<td>Asian</td>
<td>1.19 (0.87, 1.62)</td>
<td>0.006</td>
<td>0.41</td>
<td>random</td>
</tr>
<tr>
<td></td>
<td>Caucasian</td>
<td>1.22 (1.00, 1.49)</td>
<td>0.003</td>
<td>0.05</td>
<td>random</td>
</tr>
<tr>
<td>AML populations</td>
<td>Asian</td>
<td>0.96 (0.64, 1.44)</td>
<td>&lt;0.00001</td>
<td>0.85</td>
<td>random</td>
</tr>
<tr>
<td></td>
<td>Caucasian</td>
<td>0.94 (0.76, 1.16)</td>
<td>0.23</td>
<td>0.57</td>
<td>fixed</td>
</tr>
<tr>
<td>ALL populations</td>
<td>Asian</td>
<td>1.08 (0.81, 1.45)</td>
<td>0.69</td>
<td>0.58</td>
<td>random</td>
</tr>
<tr>
<td></td>
<td>Caucasian</td>
<td>0.93 (0.59, 1.46)</td>
<td>0.04</td>
<td>0.75</td>
<td>random</td>
</tr>
<tr>
<td></td>
<td>Asian</td>
<td>1.36 (0.9, 2.06)</td>
<td>0.03</td>
<td>0.15</td>
<td>random</td>
</tr>
<tr>
<td></td>
<td>Caucasian</td>
<td>1.13 (0.83, 1.55)</td>
<td>0.0006</td>
<td>0.44</td>
<td>random</td>
</tr>
<tr>
<td></td>
<td>Asian</td>
<td>1.01 (0.57, 1.79)</td>
<td>0.02</td>
<td>0.98</td>
<td>random</td>
</tr>
<tr>
<td></td>
<td>Caucasian</td>
<td>1.41 (0.95, 2.09)</td>
<td>0.07</td>
<td>0.09</td>
<td>random</td>
</tr>
<tr>
<td>Ser/Ser versus Pro</td>
<td>All populations</td>
<td>1.25 (1.02, 1.53)</td>
<td>0.94</td>
<td>0.03</td>
<td>fixed</td>
</tr>
<tr>
<td>Pro/Pro</td>
<td>Asian</td>
<td>1.19 (0.87, 1.62)</td>
<td>0.76</td>
<td>0.27</td>
<td>fixed</td>
</tr>
<tr>
<td></td>
<td>Caucasian</td>
<td>1.44 (1.02, 2.01)</td>
<td>0.77</td>
<td>0.04</td>
<td>fixed</td>
</tr>
<tr>
<td>AML populations</td>
<td>Asian</td>
<td>0.93 (0.64, 1.33)</td>
<td>0.4</td>
<td>0.68</td>
<td>fixed</td>
</tr>
<tr>
<td></td>
<td>Caucasian</td>
<td>1.09 (0.69, 1.72)</td>
<td>0.55</td>
<td>0.72</td>
<td>fixed</td>
</tr>
<tr>
<td>ALL populations</td>
<td>Asian</td>
<td>1.29 (0.99, 1.67)</td>
<td>0.47</td>
<td>0.06</td>
<td>fixed</td>
</tr>
<tr>
<td></td>
<td>Caucasian</td>
<td>1.17 (0.84, 1.65)</td>
<td>0.73</td>
<td>0.36</td>
<td>fixed</td>
</tr>
<tr>
<td></td>
<td>Asian</td>
<td>2.35 (1.27, 4.37)</td>
<td>0.64</td>
<td>0.007</td>
<td>fixed</td>
</tr>
<tr>
<td></td>
<td>Caucasian</td>
<td>1.28 (0.96, 1.71)</td>
<td>0.82</td>
<td>0.1</td>
<td>fixed</td>
</tr>
<tr>
<td>Ser/Ser versus Pro</td>
<td>All populations</td>
<td>1.23 (0.84, 1.80)</td>
<td>0.56</td>
<td>0.31</td>
<td>fixed</td>
</tr>
<tr>
<td>Pro/Pro</td>
<td>Asian</td>
<td>2.03 (0.84, 4.93)</td>
<td>0.68</td>
<td>0.12</td>
<td>fixed</td>
</tr>
</tbody>
</table>

Figure 2. Forest Plot of Leukemia Risk Associated with NQO1 609 C>T Polymorphism Analysis

with P < 0.10 (Yuan et al., 2010). The heterogeneity was quantified by F metric (F = 100% x (Q-df)/Q), which is independent of the number of studies in the meta-analysis (F < 25% no heterogeneity; F = 25–50% moderate heterogeneity; F ≥ 50% extreme heterogeneity) and P value (P > 0.1 no heterogeneity). Publication bias was investigated by funnel plot and Egger’s linear regression test (Egger et al., 1997). The significant of asymmetry was determined by t test and P < 0.05 was considered as a significant publication bias. Hardy-Weinberg equilibrium (HWE) was tested by the Chi-square test. Meta-analysis was performed using Review Manager 5.0 software. Sensitivity analysis was performed by sequential remove (statistics of study remove) of individual studies (Review Manager 5.0 software).

Results

Eligible studies for meta-analysis

This study is focusing on NQO1 609C>T polymorphism and leukemia risk. After a careful evaluation of the published literature, only 22 studies met our inclusion criteria for this meta-analysis (Table 1). The retrieved papers were then read in entirety to assess their appropriateness for the inclusion in this study. The basic information, including leukemia type, ethnicity, the number of cases and controls of each study, are listed in Table I. In all studies, the controls were free of leukemia. In the total 22 studies, 8 articles provided the data of AML (acute myeloid leukemia) patients and 10 articles provided the data of ALL (acute lymphoblastic leukemia) patients. All of the researches were then conducted in different ethnicity, mainly Asian and Caucasian: 13 studies provided Caucasian and 5 studies provided Asian data.

Leukemia susceptibility analysis

22 studies (3700 cases and 5027 controls) examining the association between NQO1 609C>T polymorphism and leukemia were included. Significant heterogeneity was observed in dominant genetic model (Ser/Ser + Pro/Ser versus Pro/Pro) and the original data were combined by means of the random effect model. In this model there showed no association of NQO1 609C>T polymorphism with leukemia (OR = 1.21, 95% CI = 1.02-1.44, Heterogeneity <0.00001; F = 69%)), and there is an association of NQO1 609C>T polymorphism with leukemia in recessive genetic model (Ser/Ser versus Pro/Pro or Pro/Pro: OR = 1.25, 95% CI = 1.02-1.53, Heterogeneity =0.94; F = 0%). The forest plot (Figure 3A) showed that the distribution of the ORs from individual studies (Review Manager 5.0 software).
results were shown in Table 2.

For ethnicity in AML and ALL populations. The was symmetric in funnel plot. Similarly, we performed an analysis for ethnicity in AML and ALL populations. The OR = 1.33, 95% CI = 1.02-1.75, P = 0.04; T/T versus C/T+C/C OR = 1.28, 95% CI = 0.96-1.7, P = 0.1). The forest plot (Figure 3C) showed that the distribution of the ORs from individual studies in relation to their respective standard deviation was symmetric in funnel plot.

**Ethnicity analysis**

In different ethnicity populations we found the results are different. Both results of dominant genetic model and recessive genetic model showed the associations of NQO1 609 C>T polymorphism with leukemia (Ser/Ser + Pro/Ser versus Pro/Pro OR = 1.22, 95% CI = 1.22-1.45, P = 0.05; Ser/Ser versus Pro/Ser + Pro/Pro OR = 1.25, 95% CI = 1.02-1.53, P = 0.04) in Caucasian population. The Egger’s test provided no evidence of publication bias in reviewed studies (t = 0.36, P = 0.772 for recessive genetic model and t = 0.22, P = 0.832 for dominant genetic model). In Asian population there are no significant results (Ser/Ser + Pro/Ser versus Pro/Pro OR = 1.19, 95% CI = 0.78-1.82, P = 0.41; Ser/Ser versus Pro/Ser + Pro/Pro OR = 1.19, 95% CI = 0.87-1.62, P = 0.27).

**Leukemia type analysis**

Most of studies involved in this research provided the data of AML and ALL. 7 studies (1111 cases and 1829 controls) examining the association between NQO1 609C>T polymorphism and leukemia were included. We found no association of NQO1 609C>T polymorphism with leukemia (Ser/Ser + Pro/Ser versus Pro/Pro OR = 0.96, 95% CI = 0.64-1.44, P = 0.85; Ser/Ser versus Pro/Ser + Pro/Pro OR = 0.93, 95% CI = 0.64-1.33, P = 0.68). 10 studies (1248 cases and 1489 controls) examining the association between NQO1 609C>T polymorphism and ALL population were included. And we found an association of NQO1 609C>T polymorphism with ALL in recessive genetic model but not in dominant genetic model (Ser/Ser + Pro/Ser versus Pro/Pro OR = 1.18, 95% CI = 0.77-1.81, P = 0.45; Ser/Ser versus Pro/Ser + Pro/Pro OR = 1.33, 95% CI = 1.02-1.75, P = 0.04). The forest plot showed that the distribution of the ORs from individual studies in relation to their respective standard deviation was symmetric in funnel plot. Similarly, we performed an analysis for ethnicity in AML and ALL populations. The results were shown in Table 2.

**Age phase analysis**

Because of some studies applied the data of children or pediatric, we performed an analysis of NQO1 609C>T polymorphism and children leukemia. Nine studies (1248 cases and 1489 controls) examining the association between NQO1 609C>T polymorphism and children leukemia were included. We found there is no association between NQO1 609C>T polymorphism and children leukemia (Ser/Ser + Pro/Ser versus Pro/Pro OR = 1.13, 95% CI = 0.83-1.55, P = 0.44; T/T versus C/T+C/C OR = 1.28, 95% CI = 0.96-1.7, P = 0.1). The forest plot (Figure 3C) showed that the distribution of the ORs from individual studies in relation to their respective standard deviation was symmetric in funnel plot.

**Discussion**

The NQO1, which is generally involved in Xenobiotic-Metabolizing, has been studied extensively on its relationship with different types of cancer, such as breast cancer, colorectal cancer, leukemia and so on. Previous conclusions of numerous studies on association between NQO1 609C>T polymorphism and leukemia remained conflicting and contradictory, this was largely attributed to the small samples or the relatively low statistical power of published studies. Meta-analysis is a powerful method for resolving inconsistent findings with a relatively large number of subjects. So, this meta-analysis was applied to provide a quantitative approach for combining the different results. To the author’s knowledge, this is the most comprehensive meta-analysis investigating the genetic susceptibility of NQO1 gene C609T polymorphism to leukemia.

In the present meta-analysis with 3700 cases and 5027controls, the variant TT homozygous genotype and the combined CT/TT genotype of the NQO1 609C>T polymorphism was found to be associated with a increased risk of leukemia, especially in Caucasian populations. These findings suggested that the NQO1 609C>T polymorphism may modify the risk of leukemia mainly in Caucasian populations but not in Asian populations. Publication bias was not observed in this study. In the subgroup analysis of age phase we found that NQO1 609C>T polymorphism was not associated with children leukemia.

Several limitations of this meta-analysis should be pointed out. First, although the Begg’s test and Egger’s test did not show any publication bias, selection bias could have occurred, because only studies published in English and Chinese were included in our meta-analysis. Second, this analysis was based on unadjusted published estimates, and hence, it was unable to adjust them by possible confounders such as sex, smoking status and living environment risk factors. Furthermore, due to a limited number of published studies available to be included, it was unable to perform further subgroup analyses for AML in Asian populations.

In summary, this meta-analysis provided robust evidence of the association between NQO1 609 C>T polymorphism and leukemia risk on Caucasian population, supporting the results of published paper that NQO1
609 C>T polymorphism is a strong susceptibility marker of leukemia, especially in Caucasian population. Moreover, sophisticated gene-gene interaction should be considered in future analysis, which would lead a better, comprehensive understanding of the association between NQO1 609 C>T polymorphism and leukemia risk.

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