

Null Results in Brief

Association of the NAD(P)H:Quinone Oxidoreductase (*NQO1*) 609C→T Polymorphism with Lung Cancer Risk among Male Smokers

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Introduction

NAD(P)H:quinone oxidoreductase (*NQO1*, DT-diaphorase) is an enzyme involved in both the detoxification and activation of a variety of substrates (1). This obligate two-electron reductase has chemoprotective properties including reduction of quinone to hydroquinone forms (1, 2), allowing avoidance of semiquinone free radical production and subsequent oxidative damage, as well as metabolism of vitamin E, catalyzing the production of a vitamin E antioxidant metabolite, vitamin E hydroquinone (3). Conversely, *NQO1* is also involved in bioactivation of procarcinogens such as nitroaromatic compounds and heterocyclic amines (1, 4). A base pair change in the *NQO1* gene (*NQO1**2) results in a proline to serine substitution in the encoded protein, causing the production of a variant protein with only 2% enzymatic activity of the wild-type that is quickly degraded, leaving a lack of detectable protein in those homozygous for the *NQO1**2 variant (5-7). Reports on the association between this *NQO1* polymorphism and lung cancer risk are mixed (8-20), although most show increased risk associated with the wild-type (9-11, 15, 16, 20) or show a null association (12, 14, 17, 19). We examined the association between the *NQO1* polymorphism and lung cancer risk in the Alpha-Tocopherol Beta-Carotene Cancer Prevention Study and assessed any interaction with vitamin E supplementation.

Materials and Methods

We conducted a nested case-control study within the Alpha-Tocopherol Beta-Carotene Cancer Prevention Study cohort of male smokers in Finland. The trial was a randomized, placebo-controlled primary prevention trial investigating the efficacy of supplemental α -tocopherol (50 mg/d) and β -carotene (20 mg/d) in reducing the incidence of lung and other cancers. The original cohort, recruited between 1985 and 1988, consisted of 29,133 men, ages 50 to 69 years, who reported smoking at least five cigarettes per day. Study design and main outcomes have been reported previously, as have main trial results (21, 22). Cases and controls for the current study were chosen based on

availability of a whole blood sample collected during the study. Genotype data were obtained on 353 individuals with incident lung cancer (ICD9-162) diagnosed through July 2001. Cases were identified by the Finnish Cancer Registry and the Register of Causes of Death, and histologic information was available for 345 cases (58 adenocarcinoma, 59 small cell, 157 squamous cell, 56 other, and 15 unknown). Genotype data were also obtained on 360 control subjects. Medical, habitual diet, smoking information, and a fasting blood sample (stored at -70°C) were collected at baseline. DNA was isolated from a whole blood sample collected during follow-up, as previously described (23). PCR was used to amplify DNA using 5'-TCC TCA GAG TGG CAT TCT GC-3' and 5'-TCT CCT CAT CCT GTA CCT CT-3' primers. Restriction enzyme digestion was used to assess variant status, as the *NQO1**2 (T) minor allele introduces a *HinfI* restriction cut site. Odds ratios (OR) and 95% confidence intervals (95% CI) were estimated using unconditional logistic regression with adjustment for age at randomization, cigarettes smoked per day, years smoked, and intervention assignment (α -tocopherol versus no α -tocopherol, β -carotene versus no β -carotene). Those heterozygous or homozygous for the minor allele were combined in the analysis, as *NQO1* levels are diminished for both genotypes depending on presence of the allele (6) and due to the fact that only 3.7% of the sample was homozygous for the minor allele. Effect modification was assessed using cross-product interaction terms in the above model and by stratified analyses. The study had 90% power ($\alpha = 0.05$) to detect an OR of at least 1.6 for wild-type (CC) versus having one or two copies of the minor allele (CT + TT), as seen in previous studies (9, 10, 16). Statistical analysis was done using STATA 8.0 (Stata Inc., College Station, TX).

Results

The *NQO1* genotypes were in Hardy-Weinberg equilibrium and the allele frequency of the variant (T) was 0.18. Cases and controls did not differ in any characteristics analyzed, except for those related to smoking status. Cases reported a longer lifetime duration of smoking and more cigarettes smoked per day.

The *NQO1* CC genotype was not associated with risk of lung cancer after adjustment for age, number of cigarettes smoked per day, duration of smoking, and intervention assignment (Table 1). There was no association between genotype and advanced disease (tumor-node-metastasis cancer stage III-IV: OR, 1.03; 95% CI, 0.71-1.50; $n = 206$ cases) or nonadvanced disease (tumor-node-metastasis stage I-II: OR, 1.14; 95% CI, 0.73-1.78; $n = 136$ cases). In addition, no

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Table 1. OR and 95% CIs for lung cancer and histology-specific lung cancer by *NQO1* genotype

	<i>NQO1</i> genotype	
	CT + TT	CC
All		
Cases	109	244
Controls	117	243
Age-adjusted OR (95% CI)	1.00 (reference)	1.09 (0.80-1.50)
Multivariate OR (95% CI)*	1.00 (reference)	1.07 (0.78-1.49)
Adenocarcinoma		
Cases	18	40
Controls	117	243
OR (95% CI)*	1.00	1.10 (0.60-2.02)
Small-cell carcinoma		
Cases	20	39
Controls	117	243
OR (95% CI)*	1.00	0.97 (0.53-1.75)
Squamous cell carcinoma		
Cases	50	107
Controls	117	243
OR (95% CI)*	1.00	1.03 (0.68-1.55)

*Estimated by unconditional logistic regression and adjusted for age at randomization, number of cigarettes smoked per day, years smoked, and intervention assignment.

relationship was seen between genotype and histology-specific lung cancer (Table 1). Neither trial intervention assignment, baseline serum vitamin E level, smoking, age at randomization, age at diagnosis, alcohol intake, nor dietary α -tocopherol, vitamin C, or flavonoid intake had effect on the relationship between *NQO1* and lung cancer risk.

Discussion

The functional *NQO1* polymorphism was not related to lung cancer risk in this population of male smokers, despite having a substantial number of cases compared with previous studies and adequate statistical power to test the hypothesis. Although some studies reported a positive association between the *NQO1**2 minor allele and lung cancer risk (8, 13, 18), most studies have shown a significant (9, 10, 15, 16, 20) or statistically nonsignificant (11) positive association for the *NQO1* common allele. Our results are consistent with four studies showing no association between the polymorphism and lung cancer risk (12, 14, 17, 19).

We failed to substantiate the role of smoking in the relationship between the *NQO1* polymorphism and lung cancer risk, and found no effect modification by antioxidant status as assessed by baseline serum vitamin E concentrations, dietary antioxidants, or randomized supplementation with α -tocopherol or β -carotene, an interaction we had hypothesized a priori. The lack of effect modification by smoking status could be due to limited variability in smoking exposure in this population, as all participants in the Alpha-Tocopherol Beta-Carotene Cancer Prevention Study were smokers. The association for *NQO1* was also not modified by age at diagnosis, as seen by Bock et al. (20), possibly due to enrollment having been restricted to men of ages 50 to 69 years at baseline, with median age at diagnosis of 66 years. Finally, no association was seen between genotype and specific histologies such as seen for small-cell carcinoma (13), squamous cell carcinoma (14, 18), or adenocarcinoma (11, 16, 17) in previous studies.

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