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Role of NAD(P)H:quinone oxidoreductase polymorphism at codon 187 in susceptibility to lung, laryngeal and oral/pharyngeal cancers

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NAD(P)H:quinone oxidoreductase (NQO1) has been proposed to play a protective role against the toxic effects of benzo[*a*]pyrene quinones. The $C^{609}T$ base change in the *NQO1* gene, resulting in a $Pro^{187}Ser$ amino acid change in the protein, has been associated with deficient enzyme activity. We examined whether this polymorphism modified the risks of smoking-related cancers in a case-control study involving patients with lung cancer ($n = 150$), laryngeal cancer ($n = 129$), oral/pharyngeal cancer ($n = 121$) and control individuals ($n = 172$), all Caucasian smokers. No statistically significant associations were observed between the *NQO1* genotypes and smoking-related cancers, although the *Ser*/*Ser* genotype was associated with a tendency towards increased risk for lung cancer (odds ratio [OR] = 2.2, 95% confidence interval [CI] 0.7–6.7) and for oral/pharyngeal cancer (OR = 2.3, 95% CI 0.6–8.2). No significant interaction between the *NQO1* genotype and either smoking exposure or *GSTM1* genotype was found. Our results are consistent with the hypothesis that lack of NQO1 activity may be involved in some smoking-related cancers. However, they were based on small numbers of individuals with the putative at-risk genotype, and the associations did not reach statistical significance. Moreover, these results contrast with those observed in some other ethnic populations, where a protective effect of the *NQO1 Ser* allele was found. Further studies are therefore clearly needed for a better understanding of the potential role of NQO1 activity in tobacco-related cancers.

Keywords: lung cancer, laryngeal cancer, oral cavity/pharyngeal cancer, *NQO1* genotype, epidemiology, tobacco smoking

Introduction

Several tobacco carcinogens are metabolized via complex enzymatic mechanisms. Benzo[*a*]pyrene, a major constituent of tobacco smoke, undergoes oxidative metabolism to produce highly reactive benzo[*a*]pyrene quinones (BPQs) (Workman 1994), which are further metabolized by competing enzymes. A one-electron reduction of BPQs by NADPH:cytochrome P450 reductase results in the formation of semiquinones and reactive oxygen species, leading to cellular damage (Joseph and Jaiswal 1994). On the other hand, the two-electron-reducing NAD(P)H:quinone oxidoreductase 1 (NQO1) catalyses the conversion of quinones

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to relatively stable hydroquinones, which are readily excreted from the cells after conjugation (Talalay *et al.* 1995). NQO1 has been shown to specifically prevent the formation of BPQ-DNA adducts generated by CYP1A1 and P450 reductase (Joseph and Jaiswal 1994). A C to T base substitution at position 609 of the *NQO1* cDNA results in a proline (Pro) to serine (Ser) amino acid change at position 187 of the protein (Traver *et al.* 1992, 1997, Roswold *et al.* 1995). NQO1 is expressed in normal lung tissue but is undetectable in lungs from individuals homozygous for the *NQO1 Ser* allele (Siegel *et al.* 1999). *NQO1* polymorphism may therefore be a particularly important modifier of susceptibility to smoking-related cancers.

There are only a few previous studies on the possible association between *NQO1* genotypes and lung cancer, and these have yielded inconclusive results (Roswold *et al.* 1995, Wiencke *et al.* 1997, Chen *et al.* 1999, Lin *et al.* 1999). Moreover, the relationship between *NQO1* polymorphism and cancers of the upper aerodigestive tract has not been previously examined.

We have conducted a hospital-based case-control study in France to investigate the possible role of several polymorphic genes of xenobiotic-metabolizing enzymes in the aetiology of smoking-related cancers among Caucasian smokers. Here, we extended the study to examine whether the *NQO1* genotypes modify the risks of lung, laryngeal and oral cavity/pharyngeal cancers, either alone or in combination with glutathione S-transferase M1 (GSTM1), which is known to be involved in detoxification of reactive metabolites of carcinogenic substances from tobacco smoke. Studies on lung cancer have suggested an association with the *GSTM1* null genotype (Vineis *et al.* 1999). We also investigated the potential modifying role of smoking exposure in the relationships between cancer risks and *NQO1* genotypes.

Materials and methods

Study population

The study subjects were recruited between 1988 and 1992 in 10 French hospitals, nine of which are located in Paris (Bouchardy *et al.* 1997, Jourenkova-Mironova *et al.* 1999). Cases were all Caucasian patients with histologically confirmed incident squamous cell carcinomas of the lung, larynx, oral cavity or pharynx. Patients with small cell carcinomas of the lung were also eligible. A control group, frequency matched on age, sex and hospital, consisted of Caucasian patients without previous or current malignant disease. All cases and controls had to be regular smokers, defined as individuals having smoked five cigarettes or more (or cigars or pipe) per day for at least 5 years. Subjects were recruited by seven trained interviewers who determined eligibility using a short questionnaire. Each interviewer had to include both cases and controls. Blood samples from individuals fulfilling these criteria were collected in ethylene diamine tetra-acetic acid (EDTA) tubes and stored at -20°C. The study population consisted of 150 patients with lung cancer (98 squamous cell carcinomas and 52 small cell carcinomas), 129 patients with cancers of the larynx (55 supraglottic, 47 glottic/subglottic, and 27 unspecified or unclassifiable laryngeal cancers), 121 patients with cancers of the oral cavity/pharynx (67 oral cancers, 50 oro- or hypopharyngeal cancers, and four unspecified or unclassifiable cancers of the oral cavity or pharynx), and 172 control individuals. The main medical diagnoses in the control population were rheumatological (33%) (of which 71% were lumbago and sciatica), infectious and parasitic (10%), respiratory (9%), cardiovascular (8%), digestive (6%) and traumatological (6%) diseases. For the other categories, the main admission reasons were related to general symptoms (7%).

Detailed information on demographic factors, medical history, lifetime tobacco and alcohol use, and occupational exposures was recorded during a personal standardized interview. The daily consumption of each type of tobacco was expressed in g/day (1 g for a cigarette, 2 g for a cigar, and 3 g for a pipe). The average daily consumption of tobacco was calculated by dividing the cumulative lifetime tobacco consumption by the overall duration of smoking. The consumption of alcoholic beverages was expressed in grams of pure ethanol (4.0, 9.4, 14.5 and 31.7 g for 0.1 l of beer, wine, cider, aperitif and hard liquor, respectively). For the drinkers, the average daily consumption of alcohol was calculated

Table 1. Main characteristics of the study population.

	Controls (n = 172)	Lung cancer (*n* = 150)	Laryngeal cancer (*n* = 129)	Oral/pharyngeal cancer (*n* = 121)
Number of males (%)	163 (95%)	104 (93%)	127 (98%)	113 (93%)
Age (years)				
Mean ± SD	54.9 ± 11.1	58.4 ± 9.9[a]	55.0 ± 9.4	54.4 ± 10.2
Range	25–88	36–81	22–85	25–89
Mean (±SD) tobacco consumption (g day^{-1})	25.1 ± 12.5	26.3 ± 13.4	30.4 ± 15.8[b]	28.2 ± 13.6[a]
Mean (±SD) duration of smoking (years)	32.2 ± 11.6	38.0 ± 9.4[c]	34.6 ± 8.8[a]	33.9 ± 10.2
Mean (±SD) alcohol consumption (g day^{-1})	77.1 ± 64.0	83.0 ± 80.2	98.1 ± 69.9[b]	111.8 ± 71.5[d]
Mean (±SD) duration of alcohol drinking (years)	29.0 ± 16.5	29.4 ± 16.5	30.1 ± 14.1	30.3 ± 12.6

[a] $p < 0.01$, *t*-test (comparison with control individuals).
[b] $p < 0.01$, Wilcoxon's rank-sum test (comparison with control individuals).
[c] $p < 0.0001$, *t*-test (comparison with control individuals).
[d] $p = 0.0001$, Wilcoxon's rank-sum test (comparison with control individuals).

by dividing the cumulative daily consumption of alcohol (the sum of the number of grams of ethanol per day multiplied by the number of years that the quantity was drunk) by the overall duration of drinking (Péquignot *et al*. 1988). The main characteristics of the study population are presented in table 1. Since only regular smokers were enrolled, the variability in daily tobacco consumption was, as might have been expected, not markedly different between cases and controls. The daily alcohol intake was significantly higher among patients with cancers of the upper aerodigestive tract than among controls.

Genotyping analyses

DNA was isolated using standard methods and stored at −20°C until use. The *GSTM1* genotypes had been analyzed earlier (Jourenkova *et al*. 1997). The *NQO1* genotypes were determined as described previously (Traver *et al*. 1997). Briefly, the sense (5′-TCCTCAGAGTGGCATTCTGC-3′) and antisense (5′-TCTCCTCATCCTGTACCTCT-3′) primers were used to amplify over the region of the *NQO1* gene comprising the polymorphic site. The *Hinf*I restriction site created by the $C^{609}T$ change was used to distinguish between the *NQO1 Ser* and *Pro* alleles. Analyses were performed by investigators who were blinded to the subjects' case or control status. To ensure laboratory quality control, two independent readers interpreted the results. Any sample with ambiguous results was re-tested, and a random selection of 20% of all samples were repeated.

Statistical analyses

Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated by unconditional logistic regression (Breslow and Day 1980). All risk estimates were adjusted for sex, age (< 50, 50–54, 55–59, 60–64 and ≥65 years) and smoking-related variables, i.e. smoking status (ex-smoker/current smoker), inhalation (never/ever), duration of smoking in years (≤25, 26–35 and > 35) and daily tobacco consumption in g day^{-1} (≤20, 21–30 and > 30). Multivariate analyses included additional terms for occupational exposures to asbestos (never/ever) or arsenic (never/ever) for lung cancer, and daily consumption of alcohol in g day^{-1} (< 40, 41–80, 81–120 and > 120) for cancers of the upper aerodigestive tract. All of the cut-off points were defined according to the distributions in the control population so that sufficient numbers of individuals were included in each subgroup. Associations between each site of cancer and *NQO1* genotype were evaluated by homogeneity tests, and the increase of risk with the number of *Ser* alleles was tested by linear trend tests (Breslow and Day 1980). Interactions between *NQO1* genotype and smoking-related variables were studied to test the equality of the effect of genotype across levels of smoking exposure. These interactive effects were assessed by likelihood ratio tests to compare the goodness of fit of the models with and without the interaction term, taking into account the above-mentioned adjusting factors. For that purpose, the average daily tobacco consumption and duration of smoking were expressed as categorical variables dichotomized at the median in the control population. Because of the small number of individuals homozygous for the *NQO1 Ser* allele, interaction analyses could not be conducted considering the *Ser*/*Ser* genotype separately. Therefore, heterozygous and homozygous *NQO1 Ser* allele-containing genotypes were

combined. Similar analyses were conducted to test interactions between the *NQO1* genotype and *GSTM1* genotype (homozygous null versus others).

Results and discussion

The *NQO1* genotype distribution in the control population was in Hardy–Weinberg equilibrium ($p = 0.24$) (table 2). The *NQO1 Ser* allele was present in 43.3% of lung cancer patients (allele frequency = 0.25), 45.0% of laryngeal cancer patients (allele frequency = 0.24), 40.5% of oral/pharyngeal cancer patients (allele frequency = 0.24) and 39.0% of control individuals (allele frequency = 0.21). The frequency of homozygotes for the *Ser* allele among lung cancer patients (6.7%) and among oral/pharyngeal cancer patients (7.4%) was twice that among control individuals (2.9%). However, the genotype distribution did not differ significantly between cases and controls ($p = 0.26$ and $p = 0.20$, respectively). The adjusted ORs of lung cancer were 1.1 (95% CI 0.7–1.8) for the *NQO1 Pro/Ser* genotype and 2.2 (95% CI 0.7–6.7) for the *Ser/Ser* genotype compared with the *Pro/Pro* genotype ($p_{trend} = 0.29$). The figures for oral/pharyngeal cancer were 0.8 (95% CI 0.5–1.5) and 2.3 (95% CI 0.6–8.2), respectively ($p_{trend} = 0.60$). The laryngeal cancer risks associated with *NQO1* genotypes were close to 1 (table 2).

We did not find any significant interaction between the *NQO1* genotype and either the *GSTM1* genotype, the duration of smoking (table 3) or the daily consumption of tobacco (data not shown).

NQO1 has been proposed to play a protective role against the toxic effects of BPQs and to prevent the formation of BPQ-DNA adducts generated during metabolic activation. In this study, the *Ser/Ser* genotype was associated with a tendency towards a two-fold increased risk for lung cancer and for oral/pharyngeal cancer, but not for laryngeal cancer. These results are consistent with the hypothesis that lack of NQO1 activity may be involved in some smoking-related cancers. However, they were based on small numbers of individuals with the putative at-risk genotype and the associations did not reach statistical significance.

A limitation of our study could be the use of hospital controls, especially if there are any associations between *NQO1* genotype and their diseases. However, the genotype distribution was not significantly different among the disease groups, although the power to detect such differences was low. Moreover, the frequencies of the *NQO1 Ser* allele and of the *Ser/Ser* genotype in our control population were in agreement with those previously reported in Caucasians (Kelsey *et al.* 1997, Gaedigk *et al.* 1998).

To our knowledge, only two previous studies have investigated the association of *NQO1* polymorphism with lung cancer risk in Caucasian populations. In one US study (Roswold *et al.* 1995), the *Ser* allele was twice as frequent in lung cancer patients, recruited in a cancer centre, than in controls recruited independently of cases (0.22 versus 0.13), but this difference disappeared when cancer patients were compared with employees from the same centre as the cases. Consistent with this, Chen *et al.* (1999) found no difference in the *NQO1 Ser* allele frequency between lung cancer patients and controls (0.22 and 0.20, respectively). However, these findings contrast with those observed in some other ethnic populations; a protective effect of the *NQO1 Ser* allele was found among Mexican-Americans and African-Americans (Wiencke *et al.* 1997), among Japanese (Chen *et al.* 1999) and among Taiwanese (Lin *et al.* 1999). Further studies are therefore clearly needed

Table 2. Distribution of individuals by *NQO1* genotype and ORs (95% CI) of cancer.

	Controls ($n = 172$)	Lung cancer ($n = 150$)		Laryngeal cancer ($n = 129$)		Oral/pharyngeal cancers ($n = 121$)	
NQO1 genotype	No. (%)	No. (%)	OR[a] (95% CI)	No. (%)	OR[b] (95%CI)	No. (%)	OR[b] (95%CI)
Pro/Pro	105 (61.1)	85 (56.7)	1 (Reference)	71 (55.0)	1 (Reference)	72 (59.5)	1 (Reference)
Pro/Ser	62 (36.1)	55 (36.7)	1.1 (0.7–1.8)	54 (41.9)	1.2 (0.7–2.1)	40 (33.1)	0.8 (0.5–1.5)
Ser/Ser	5 (2.9)	10 (6.7)	2.2 (0.7–6.7)	4 (3.1)	1.1 (0.2–5.9)	9 (7.4)	2.3 (0.6–8.2)
Pro/Ser or *Ser/Ser*	67 (39.0)	65 (43.3)	1.2 (0.7–2.0)	58 (45.0)	1.2 (0.7–2.1)	49 (40.5)	0.9 (0.6–1.6)

[a]Adjusted for sex, age, smoking and occupational exposures. Data on smoking are missing for four lung cancer cases and three controls.

[b]Adjusted for sex, age, smoking exposure and daily consumption of alcohol. Data on smoking and/or alcohol drinking were missing for six laryngeal cancer cases, six oral/pharyngeal cancer cases and eight controls.

Table 3. Number of cases/controls and ORs (95% CI) of cancer in relation to *NQO1* genotype by duration of smoking and *GSTM1* genotype.

	Lung cancer[a]		Laryngeal cancer[b]		Oral/pharyngeal cancer[c]	
	Pro/Pro	*Pro/Ser* or *Ser/Ser*	*Pro/Pro*	*Pro/Ser* or *Ser/Ser*	*Pro/Pro*	*Pro/Ser* or *Ser/Ser*
Duration of smoking ≤30 years						
No. of cases/controls	22/51	12/29	23/49	16/28	24/49	17/28
OR (95% CI)	1 (Reference)	1.0 (0.4–2.3)	1 (Reference)	1.5 (0.6–3.6)	1 (Reference)	1.3 (0.6–3.0)
Duration of smoking >30 years						
No. of cases/controls	62/54	52/38	46/50	38/37	46/50	38/37
OR (95% CI)	3.1 (1.4–6.8)	3.7 (1.7–8.2)	2.5 (1.1–5.7)	2.7 (1.2–6.2)	2.0 (0.9–4.3)	1.8 (0.8–4.0)
*GSTM1 p*ositive						
No. of cases/controls	41/47	28/35	29/43	20/33	34/43	25/33
OR (95% CI)	1 (Reference)	1.2 (0.6–2.5)	1 (Reference)	1.2 (0.5–2.7)	1 (Reference)	1.1 (0.5–2.2)
GSTM1 null						
No. of cases/controls	44/58	37/32	40/56	34/32	36/56	20/32
OR (95% CI)	1.1 (0.6–2.2)	1.4 (0.7–2.8)	1.5 (0.7–3.1)	1.8 (0.9–3.9)	0.9 (0.5–1.8)	0.8 (0.4–1.8)

[a]Adjusted for sex, age, smoking and occupational exposures. Data on smoking are missing for four lung cancer cases and three controls. Interaction test between *NQO1* genotype and duration of smoking (likelihood ratio test, 1d.f.), $p = 0.75$; interaction test between *NQO1*genotype and *GSTM1* genotype (likelihood ratio test, 1d.f.), $p = 0.95$.

[b]Adjusted for sex, age, smoking exposure and daily consumption of alcohol. Data on smoking and/or alcohol drinking were missing for six laryngeal cancer cases and eight controls. Interaction test between *NQO1*genotype and duration of smoking (likelihood ratio test, 1d.f.), $p = 0.54$; interaction test between *NQO1*genotype and *GSTM1* genotype (likelihood ratio test, 1d.f.), $p = 0.95$.

[c]Adjusted for sex, age, smoking exposure and daily consumption of alcohol. Data on smoking and/or alcohol drinking were missing for six oral/pharyngeal cancer cases and eight controls. Interaction test between *NQO1*genotype and duration of smoking (likelihood ratio test, 1d.f.), $p = 0.51$; interaction test between *NQO1*genotype and *GSTM1* genotype (likelihood ratio test, 1d.f.), $p = 0.82$.

for a better understanding of the potential role of NQO1 activity in tobacco-related cancers.

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