Association of \( \text{NAD(P)H: quinone oxidoreductase 1 (NQO1) C609T polymorphism with esophageal squamous cell carcinoma in a German Caucasian and a northern Chinese population} \)

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\textbf{NAD(P)H: quinone oxidoreductase 1 (NQO1) is an antioxidant enzyme, important in the detoxification of environmental carcinogens. A single base substitution (C to T) polymorphism at nucleotide 609 (null-allele) of NQO1 gene impairs stability and function of the NQO1 protein. To investigate the association of this NQO1 polymorphism with susceptibility to esophageal squamous cell carcinoma (ESCC), the NQO1 C609T genotypes were determined by PCR–RFLP analysis in 450 patients with ESCC (257 German Caucasians and 193 northern Chinese) and 393 unrelated healthy controls (252 German Caucasians and 141 northern Chinese). Additionally, NQO1 protein expression was determined by immunohistochemistry in a subset of 74 ESCC (50 German, 24 Chinese). A significant difference in NQO1 C609T genotype distribution was observed between Caucasian healthy controls (C/C, 73.4%; C/T, 25.0%; T/T, 1.6%) and Chinese healthy controls (C/C, 34.0%; C/T, 49.7%; T/T, 16.3%) (\(\chi^2 = 68.40, P < 0.001\)). The NQO1 T/T genotype significantly increased the risk for developing ESCC in both Caucasian subjects (OR = 4.62, 95% CI = 1.54–13.86) and Chinese subjects (OR = 1.81, 95% CI = 1.04–3.15), compared with the combined C/C and C/T genotypes. In Chinese subjects, this increased susceptibility was pronounced in patients with family history of upper gastrointestinal cancers (OR = 2.18, 95% CI = 1.14–4.17). Immunohistochemical analysis showed NQO1 protein expression in 53 carcinomas, whereas 21 carcinomas were negative. Negativity for NQO1 expression correlated strongly with the NQO1 genotype, being present in 8.6% of cases with C/C, 22.2% of cases with C/T and 100% of cases with T/T genotype (\(\chi^2 = 16.60, P < 0.001\)). In summary, the association of the NQO1 C609T polymorphism with ESCC in genetically distinct populations makes a strong argument for its importance in carcinogenesis of ESCC in the German Caucasian and the northern Chinese population.

\textbf{Abbreviations:} CI, confidence interval; ESCC, esophageal squamous cell carcinoma; NQO1, NAD(P)H: quinone oxidoreductase 1; OR, odds ratio; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism analysis; UGIC, upper gastrointestinal cancer.

**Introduction**

Esophageal cancer is the sixth most common cancer worldwide (1). Squamous cell carcinoma is the predominant histological type of esophageal cancer in both western countries and developing countries (2). Esophageal squamous cell carcinoma (ESCC) is in general, considered to be caused by chemical carcinogens, with alcohol and tobacco as the most important risk factors for ESCC in western countries (3). Other risk factors such as nutrient deficiency, physical damage of the esophagus caused by ingesting hard foods or hot liquids, as well as environmental or dietary nitrosamines have been suggested to be associated with ESCC, especially in developing countries (4–6). Nevertheless, only a subset of individuals exposed to these exogenous risk factors will develop ESCC, suggesting a role of host susceptibility factors. The observation of familial clustering of ESCC patients in high incidence regions in northern China provides another clue to a genetic background in the development of the disease (7,8).

Many chemical carcinogens require metabolic activation to mutagens by host enzymes (9). On the other hand, carcinogens may be inactivated \textit{in vivo} by detoxifying enzymes such as glutathione \textit{S}-transferases. As many enzymes of xenobiotic metabolism are polymorphic, differences in carcinogen metabolism may influence individual susceptibility to chemically induced cancers (10–12). NAD(P)H: quinone oxidoreductase 1 (NQO1: EC 1.6.99.2), originally called DT-diaphorase, is a cytosolic enzyme which catalyzes the two-electron reduction of quinone compounds and prevents the generation of semi-quinone free radicals and reactive oxygen species, thus protecting cells from oxidative damage (13). On the other hand, NQO1 catalyzes the reductive activation of quinoid chemotherapeutic agents and of environmental carcinogens such as nitrosamines, heterocyclic amines and cigarette smoke condensate (13). The activity of the NQO1 enzyme strongly depends on polymorphisms at the NQO1 locus. The major polymorphism involves a single C to T substitution at nucleotide 609 of \textit{NQO1} cDNA that causes a Pro187Ser amino acid change thereby leading to three phenotypes, i.e. wild-type phenotype with complete enzyme activity, heterozygous phenotype with ~3-fold decreased activity and homozygous mutant (null) phenotype with a complete lack of enzyme activity (14,15). Decreased NQO1 enzyme activity is caused by enhanced polyubiquination and proteosomal degradation of the mutant protein (16). Marked differences in the ethnic distribution of the NQO1 C609T polymorphism have been described with the null-allele being approximately twice as common in Chinese than in Caucasians (17). Among patients who developed tumors known to be induced by chemical carcinogens such as urothelial cancer (18), renal cancer (18) and leukemias (19,20) the NQO1 609T allele has been found more frequently than among controls, whereas conflicting results have been published for lung cancer
(21,22). The association of NQO1 C609T polymorphism with susceptibility to ESCC has not been investigated so far.

In the current study, we investigated the NQO1 C609T genotypic distribution in ESCC patients from a German Caucasian and a northern Chinese population. This was done to determine whether a potential association between NQO1 genotype and susceptibility to ESCC could be reproduced in two independent cohorts of patients. Moreover, the analysis of two different ethnic groups provided an opportunity to determine whether this association might be relevant in individuals with different genetic and lifestyle backgrounds.

Materials and methods

Subjects

This case-control study recruited 450 patients with histologically confirmed ESCC (257 German Caucasians and 193 northern Chinese) and 393 unrelated healthy controls (252 German Caucasians and 141 northern Chinese). All ESCC patients that underwent esophagectomy without prior radio- and/or chemotherapy between 1978 and 1998 in the Department of Surgery of the Heinrich Heine University, Duesseldorf and between 2001 and 2002 in the Fourth Affiliated Hospital, Hebei Medical University, were included in this study. The German ESCC patients were from Duesseldorf and surrounding regions and the Chinese patients were residents of Shijiazhuang City or its surrounding regions. The healthy controls from the German Caucasian and northern Chinese population were unrelated blood donors from the same region as the ESCC patients. Information about sex, age, smoking habits and family history was obtained from the cancer patients’ hospital records and from the healthy controls by interview after blood donation. Smokers were defined as formerly or currently smoking > 5 cigarettes/day for at least 2 years. Individuals with at least one first-degree relative or at least two second-degree relatives with esophageal/cardiac/gastric cancer were defined as having family history of upper gastrointestinal cancer (UGIC). The smoking status was only available from part of the Caucasian subjects and no family history of UGIC was recorded in this population. The study was approved by the Ethics Committee of the Faculty of Medicine of the Heinrich Heine University and the Institutional Review Board of the Hebei Cancer Institute, Hebei Medical University. The demographic data of the recruited subjects are presented in Table I.

DNA extraction

Genomic DNA was extracted from 200 μl of venous blood of Caucasian healthy controls with the QIAamp blood kit (Qiagen, Hilden, Germany). From Caucasian ESCC patients, tumor-free formaldehyde-fixed tissue samples from distant resection margins of tumor specimens were selected for DNA preparation. Following deparaffinization, the tissues were suspended in TE-buffer (10 mM Tris–Cl; pH 7.5; 0.1 mM EDTA) and incubated overnight at 55°C with proteinase K (Merck, Darmstadt, Germany) at a concentration of 2.5 mg/ml. The samples were subsequently heated at 94°C for 8 min to inactivate proteinase K. In Chinese subjects, 5 ml of venous blood was drawn in Vacutainer tubes containing EDTA and stored at 4°C. The genomic DNA was extracted within 1 week after bleeding by using proteinase K digestion followed by a salting-out procedure according to the method published by Miller et al. (24).

NQO1 genotyping by PCR and restriction fragment analysis

NQO1 genotyping of all subjects was performed at the molecular laboratory of the Institute of Pathology, Heinrich Heine University, Duesseldorf. The base change (C to T) at nucleotide 609 of NQO1 cDNA creates a Hinfl restriction site, which can be exploited for genotyping by PCR and subsequent restriction fragment analysis (23). PCR was performed in a 25 μl volume containing 100 ng DNA template, 2.5 μl 10× PCR-buffer, 1 U Hotstar Taq-DNA-polymerase (Qiagen), 10 mM dNTPs and 10 pmol sense primer (5′-AAAGCCCGAC-CAACCTC-3′) and antisense primer (5′-ATTTGAATTGCGGCGTCTGCTG-3′). Initial denaturation for 1 min at 94°C was followed by 40 cycles at 94°C for 1 min, 56°C for 1 min and 72°C for 2 min. Subsequently, the PCR products were digested with 20 U of Hinfl (Boehringer, Mannheim, Germany) for 3 h at 37°C and separated on a 2% agarose gel. The NQO1 wild-type allele shows a 172 bp PCR product resistant to enzyme digestion, whereas the null allele shows a 131 bp and a 41 bp band. Each restriction fragment analysis included PCR product from the bladder cancer cell line RT112MMC, which is homozygous for the NQO1 null-allele (23) as an internal control of restriction digestion.

Table I. Demographic characteristics and NQO1 C609T polymorphism among ESCC patients and healthy controls

<table>
<thead>
<tr>
<th>Group</th>
<th>German caucasian</th>
<th>Northern Chinese</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>Control (n%)</td>
<td>ESCC (n%)</td>
</tr>
<tr>
<td>Male</td>
<td>193 (76.6)</td>
<td>204 (79.4)</td>
</tr>
<tr>
<td>Female</td>
<td>59 (23.4)</td>
<td>53 (20.6)</td>
</tr>
<tr>
<td>Age (mean ± SD)</td>
<td>39 ± 11.3</td>
<td>57 ± 8.7</td>
</tr>
<tr>
<td>Smoking status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ex- or current smoker</td>
<td>146 (59.3)</td>
<td>131 (90.3)</td>
</tr>
<tr>
<td>Non-smoker</td>
<td>100 (40.7)</td>
<td>14 (9.7)</td>
</tr>
<tr>
<td>Family history of UGIC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Negative</td>
<td>141 (100)</td>
<td>107 (55.4)</td>
</tr>
<tr>
<td>Genotype&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C/C</td>
<td>188 (73.4)</td>
<td>183 (71.2)</td>
</tr>
<tr>
<td>C/T</td>
<td>63 (25.0)</td>
<td>56 (21.8)</td>
</tr>
<tr>
<td>T/T</td>
<td>4 (1.6)</td>
<td>16 (7.0)</td>
</tr>
<tr>
<td>Allele type&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>433 (85.9)</td>
<td>422 (82.1)</td>
</tr>
<tr>
<td>T</td>
<td>71 (14.1)</td>
<td>92 (17.9)</td>
</tr>
</tbody>
</table>

ESCC, esophageal squamous cell carcinoma; UGIC, upper gastrointestinal cancer; ND, not determined.

<sup>a</sup>Information of smoking status was available from part of Caucasian subjects.
<sup>b</sup> Information of smoking status was available from part of Caucasian subjects.

NQO1 immunohistochemistry

NQO1 monoclonal antibody (IgG<sub>1</sub>)-secreting hybridomas (clones A180 and B771) were derived from a BALB-c mouse immunized with purified recombinant NQO1 protein as described previously (25). A total of 74 paraffin blocks (50 cases from Germany, 24 cases from China) containing representative areas of ESCC tissue were selected for subsequent immunohistochemical investigations. For the German ESCC patients, all paraffin-embedded cancer specimens from patients carrying the TT genotype were included (n = 8). The remaining 42 cases were randomly selected from individuals carrying the C/C or C/T genotypes. From Chinese ESCC patients, paraffin blocks containing tissue were available only from 24 cases, they were all analyzed immunohistochemically.

Four-micrometer thick sections from the paraffin blocks were mounted on glass-slides coated with 3-aminopropyltriethoxysilane. Following deparaffinization and rehydration, the endogenous peroxidase activity was eliminated by incubation in 3% H<sub>2</sub>O<sub>2</sub> and endogenous biotin was blocked with avidin and biotin (0.02% in TBS buffer; Sigma, St Louis, MO). Then the slides were incubated with a 1:1 mixture of cell culture supernatants from the two anti-NQO1 monoclonal antibody secreting hybridoma clones (A180 and B771) at a dilution of 1:10 for 30 min at room temperature. After a second incubation with a biotin-conjugated polyclonal antibody, slides were incubated with an avidin–biotin–peroxidase reagent (both from Scy Tek, Logan, USA). Reaction products were visualized by immersing slides in diaminobenzidine tetrachloride and finally counter-stained with hematoxylin. Immunohistochemical staining also included negative controls using cell culture supernatant from a non-specific IgG<sub>1</sub> secreting hybridoma (clone C100) (26). An ESCC sample with confirmed NQO1 protein expression served as positive control in every immunohistochemical experiment.

The immunostained slides were examined by a senior pathologist (M.S.) without knowledge of the results of the NQO1 genotyping. The number of stained tumor cells was determined semi-quantitatively. Each sample was semi-quantitatively assigned to one of the following categories by assessing the whole tissue section: 0 (0–positive tumor cells percent), I (5–25%), II (26–50%), III (51–75%), IV (76–100%).
Statistical analysis
Statistical analysis was performed using the SAS software package (SAS Institute Inc., Carey, NC). Hardy–Weinberg equilibrium analysis was performed to compare observed and expected genotype frequencies using \( \chi^2 \) test. The comparison of \( NQO1 \) genotype distribution in the study groups as well as the comparison between \( NQO1 \) genotype and \( NQO1 \) protein expression were performed by means of two-sided contingency tables using \( \chi^2 \) tests. A probability level of 5% was considered as statistically significant. The odds ratio (OR) and 95% confidence interval (CI) were calculated using an unconditional logistic regression model. As both control groups were younger than the corresponding case groups, the odds ratio was not adjusted for age in both groups. In addition, as the data on smoking status in German cases were not completely available, the odds ratio was only adjusted for sex in the German group and for sex and smoking status in the Chinese group.

Results
Subject characteristics
The gender distributions in ESCC patients from the two populations were similar to each other and comparable with the corresponding healthy controls. However, the mean age in both control populations was lower than in the corresponding case populations (Table I). Moreover, the frequency of smokers among ESCC patients and healthy controls was different in the two populations. Thus, smokers were significantly more common (90.3%) among Caucasian ESCC patients than among healthy controls (59.3%) (\( \chi^2 = 42.43, P < 0.001 \)). Smoking significantly increased the risk for developing ESCC in Caucasian subjects compared to non-smoking (OR = 6.4, 95% CI = 6.05–11.17). In contrast, the proportion of smokers among the Chinese ESCC patients (53.9%) was not significantly different from that among healthy controls (51.1%) (\( \chi^2 = 0.260, P = 0.610 \)). In addition, 86 of the Chinese ESCC patients (44.6%) but none of the Chinese controls had a family history of UGIC.

Distinctive distribution of the \( NQO1 \) polymorphism in the German Caucasian and the northern Chinese population
The \( NQO1 C609T \) genotyping was successfully performed in all study subjects. The observed \( NQO1 \) genotype frequencies did not significantly deviate from those expected from Hardy–Weinberg equilibrium in both Caucasian and Chinese controls (\( \chi^2 = 0.146 \) and 0.061; \( P = 0.930 \) and 0.970, respectively). A significant difference in genotype distribution was observed between Caucasian healthy controls (C/C, 73.4%, C/T, 25.0%, T/T, 1.6%) and Chinese healthy controls (C/C, 34.0%, C/T, 49.7%, T/T, 16.3%) (\( \chi^2 = 68.40, P < 0.001 \) (Table I). Accordingly, the \( NQO1 \) C609T null allele frequency was significantly higher in the Chinese controls (41.1%) than that in the Caucasian controls (14.1%) (\( \chi^2 = 72.96, P < 0.001 \)) (Table I). The \( NQO1 \) genotype distribution was not correlated with gender, age and smoking status in each study group (data not shown).

Association of the \( NQO1 C609T \) homozygous null genotype with increased susceptibility to ESCC in German Caucasian and in northern Chinese population
In both Caucasian and Chinese subjects, no significant difference of the overall \( NQO1 \) null-allele frequency was found between ESCC patients and healthy controls (\( P > 0.05 \)) (Table I). Moreover, the distribution of the \( NQO1 \) C/C and C/T genotypes was not significantly different from that in healthy controls in both Caucasian and Chinese subjects (\( P > 0.05 \)) (Table I). However, the T/T genotype was significantly more frequent among ESCC patients than among healthy controls in both Caucasian (7.0 versus 1.6%; \( \chi^2 = 9.03, P = 0.003 \)) and Chinese subjects (25.9 versus 16.3%; \( \chi^2 = 4.39, P = 0.036 \)) (Table II). Among Caucasian subjects, the odds ratio (adjusted for sex) for the ESCC development was 4.62-fold higher in individuals carrying the \( NQO1 T/T \) genotype than in those carrying C/C or C/T genotypes (95% CI = 1.54–13.86). Similarly, an odds ratio (adjusted for sex and smoking status) of 1.81 was observed in Chinese individuals with the \( NQO1 T/T \) genotype compared with individuals with the C/C or C/T genotype (95% CI = 1.04–3.15) (Table II).

As the German ESCC patients had been collected over a considerably longer period (21 years) than the Chinese ESCC patients (2 years), it was tested if the distribution of the \( NQO1 \) alleles among German cancer had changed over time. Among patients that underwent esophagectomy between 1978 and 1988 (\( n = 105 \)), 75 patients carried the C/C genotype, 21 the C/T genotype and six the T/T genotype. Of the 155 patients that underwent resection between 1989 and 1998, 108 had the C/C genotype, 35 the C/T genotype and 15 the T/T genotype. These genotype distributions were not significantly different according to the \( \chi^2 \)-test (\( \chi^2 = 0.54, P = 0.7619 \)).

Association between \( NQO1 C609T \) polymorphism and ESCC in relation to the family history of UGIC
To compare the genetic risk factors for ESCC between patients with and without familial history of UGIC, the \( NQO1 \) C609T genotype analysis was stratified accordingly in Chinese subjects. Among patients with family history of UGIC, the \( NQO1 T/T \) genotype was significantly more common (30.2%) than among healthy controls (16.3%) (\( \chi^2 = 6.12, P = 0.013 \)). The odds ratio of the \( NQO1 T/T \) genotype significantly increased the risk for developing ESCC among patients with family history of UGIC, compared with the combination C/C and C/T genotypes (OR = 2.18, 95% CI = 1.14–4.17) (Table II). In contrast, the \( NQO1 T/T \) genotype frequency between patients without family history of UGIC (22.4%) and the healthy controls (16.3%) was not significantly different [OR (95% CI): 1.56 (0.82–2.97)] (Table II).

Table II. Relative risk of the \( NQO1 C609T \) homogenous null for ESCC in German Caucasian and northern Chinese population

<table>
<thead>
<tr>
<th>Group</th>
<th>( NQO1 ) genotype</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>German Caucasian</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Healthy control</td>
<td>C/C + C/T n (%)</td>
<td>4 (1.6)</td>
<td></td>
</tr>
<tr>
<td>ESCC patients</td>
<td>C/T n (%)</td>
<td>4.62</td>
<td>(1.54–13.86)</td>
</tr>
<tr>
<td>Northern Chinese</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Healthy controls</td>
<td>T/T n (%)</td>
<td>1.81</td>
<td>(1.04–3.15)</td>
</tr>
<tr>
<td>Total ESCC patient</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive family</td>
<td></td>
<td>2.18</td>
<td>(1.14–4.17)</td>
</tr>
<tr>
<td>history of UGIC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative family</td>
<td></td>
<td>1.56</td>
<td>(0.82–2.97)</td>
</tr>
<tr>
<td>history of UGIC</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ESCC, esophageal squamous cell carcinoma; UGIC, upper gastrointestinal cancer.

\(^a\)The odds ratio of the \( NQO1 C609T \) homozygous null genotype (T/T) carriers against the combination of the heterozygote (C/T) and homozygous wild-type (C/C), adjusted for sex in the German group and for sex and smoking status in the Chinese group.
Table III. Correlation between NQO1 C609T genotype and NQO1 protein expression in ESCC tissues ($\chi^2 = 16.60, P < 0.001; \chi^2$-test)

<table>
<thead>
<tr>
<th>Protein expression</th>
<th>NQO1 genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C/C n (%)</td>
</tr>
<tr>
<td>Absent</td>
<td>3 (8.6)</td>
</tr>
<tr>
<td>Present</td>
<td>32 (91.4)</td>
</tr>
</tbody>
</table>

ESCC, esophageal squamous cell carcinoma.

NQO1 protein negativity was restricted to the cancer tissue and has to be regarded as loss of NQO1 protein expression in the tumor cells. No correlation between NQO1 genotype (C/C or C/T) and percentage of NQO1 protein-positive tumor cells was found. Moreover, no difference in NQO1 expression was found between German and Chinese ESCC samples after adjustment for NQO1 genotype (data not shown).

Discussion

In the present study, a significantly increased number of subjects carrying the NQO1 homozygous null allele genotype was found among German Caucasian and northern Chinese ESCC patients compared with two corresponding control cohorts. This result is in line with previous investigations, that showed an association between the NQO1 homozygous null genotype and other tumor types such as urothelial cancer (18), renal cancer (18), leukemias (19,20), lung cancer (21) and cutaneous basal cell carcinoma (27). The mechanism underlying the correlation of NQO1 C609T polymorphism with the increased risk for developing various tumors likely resides in the different enzyme activities encoded by the NQO1 alleles. Thus, lack of NQO1 activity encoded by the homozygous null genotype may result in a decreased detoxification of exogenous carcinogens and increased cellular damage by quinone redox cycling and reactions of semi-quinones with DNA, thereby increasing the disposition to chemically induced cancers such as ESCC.

The functional relevance of the NQO1 C609T polymorphism is underlined by our immunohistochemical findings, which show that no NQO1 protein is detectable by immunohistochemistry in normal esophageal tissue as well as in cancer tissue in individuals carrying the homozygous null genotype. The current study suggests the NQO1 C609T null allele to act in a recessive mode in the development of ESCC, demonstrated by the fact that the heterozygous genotype frequency among ESCC patients remains similar to that among the healthy controls. This indicates that in individuals with the NQO1 heterozygous genotype, the enzyme activity might be sufficient for protecting cells from damage by exogenous carcinogens important for the development of ESCC such as cigarette smoke condensates and alcohol in western countries (3) and/or nitrosamines or heterocyclic amines in the high incidence regions of China (6).

A strong association of increased risk for esophageal cancer with a positive family history of UGIC in the first-degree relatives has been reported earlier in the high incidence regions of China (7,8). The segregation analysis on high-risk nuclear families suggested that the ESCC occurrence best fitted to an autosomal recessive Mendelian inheritance mode (28). However, the underlying molecular genetic mechanisms for the familiar clustering of UGIC patients have not been elucidated so far. The present study suggests that the increased risk of the NQO1 C609T homozygous null genotype for the ESCC

NQO1 genotype is associated with NQO1 protein expression

Immunohistochemical investigations for NQO1 protein expression among non-neoplastic tissues of the esophagus showed NQO1 immunostaining of weak intensity in endothelial cells and in basal cells of the esophageal squamous epithelium. Of the 74 ESCC under investigation, 21 tumors (28.3%) were negative for NQO1 expression (category 0), four (5.4%) were in category I (5–25% positive tumor cells), seven (9.5%) in category II (26–50%), seven (9.5%) in category III (51–75%) and 35 (47.3%) in category IV (76–100%) (Figure 1). As a result of the relatively low number of cases, the NQO1 protein expression data had to be pooled for subsequent statistical analysis between genotype and phenotype (protein expression). Therefore, all NQO1 protein-positive cases were pooled and opposed to the NQO1 protein-negative cases. In this analysis, negativity for NQO1 expression correlated strongly with the NQO1 genotype, as it was present in 8.6% of cases with C/C genotype, 22.2% of cases with C/T genotype and 100% of cases with T/T genotype ($\chi^2 = 16.60, P < 0.001$; Table III). In cases with T/T genotype, the internal constitutive controls (e.g. endothelial cells and basal cells of the normal esophageal squamous epithelium) also showed no NQO1 immunoreactivity. In contrast, in all cases carrying the C/C or C/T genotype the internal constitutive controls were immunoreactive for the NQO1 protein. In this subset of cases,
development seems to be pronounced in patients with a positive family history of UGIC, indicating that this polymorphism may partly contribute to the familiar aggregation of UGIC patients in the high incidence regions of China.

The identification of genetic factors predisposing to the development of ESCC would be of great clinical importance since the prognosis of this tumor type is poor, particularly in advanced stages of the disease. Therefore, earlier detection of ESCC is very common in many areas of China; therefore, a practical approach for cost-effective tumor screening is urgently needed in this country. In addition, the greater proportion of people carrying the NQO1 C609T homozygous null genotype in Chinese population, as shown in this study, indicates that lack of NQO1 enzyme activity may be an important molecular factor for the high prevalence for ESCC in this population. On the other hand, due to the relatively rare occurrence of the T/T genotype in the population, it is clear that in clinical practice NQO1 genotyping may become of importance only in combination with other risk factors.

In summary, the present case-control study suggests that the NQO1 C609T homozygous null genotype increases the risk for developing ESCC in both the German Caucasian and northern Chinese populations. Determination of the NQO1 C609T genotype may become important as a stratification marker in prevention trials for ESCC, particularly in high-incidence regions of China.

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References


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