

# NQO1 C609T polymorphism correlated to colon cancer risk in farmers from western region of Inner Mongolia

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**Objective:** To investigate the relationship between NAD(P)H:quinone oxidoreductase 1 (*NQO1*) C609T polymorphism and colon cancer risk in farmers from western region of Inner Mongolia.

**Methods:** Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was performed to analyze *NQO1* C609T polymorphism from 160 healthy controls and 76 colon cancer patients.

**Results:** Among the colon cancer patients, the incidence of *NQO1* T allele (53.29%) was significantly higher than it in control group (33.75%,  $P < 0.001$ ). The individuals with *NQO1* T allele had higher risk [2.239 (95% CI: 1.510-3.321) times] to develop colon cancer than individuals with *NQO1* C allele. The incidence of *NQO1* (T/T) (34.21%) in colon cancer patients was higher than that in control group (15.62%,  $P < 0.001$ ). Odds ratios (OR) analysis suggested that *NQO1* (T/T) and *NQO1* (T/C) genotype carriers had 3.813 (95% CI: 1.836-7.920) times and 2.080 (1.026-4.219) times risk compared with wild-type *NQO1* (C/C) gene carriers in developing colon cancer. Individuals with *NQO1* (T/T) genotype had 2.541 (95% CI: 0.990-6.552) times, 3.713 (95% CI: 1.542-8.935) times, and 3.471 (95% CI: 1.356-8.886) times risk than individuals with *NQO1* (T/C) or *NQO1* (C/C) genotype in well-differentiated, moderately-differentiated, and poorly-differentiated colon cancer patients, respectively.

**Conclusions:** *NQO1* gene C609T could be one of risk factors of colon cancer in farmers from western region of Inner Mongolia.

**Key Words:** Colon cancer; NAD(P)H:quinone oxidoreductase 1; gene polymorphism; Inner Mongolia



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## Introduction

Quinone is a noxious chemical compound, existing generally through nature, in exhaust of automobile, benzene, tobacco smoke, which is involved in development of tumor, mutation and necrosis in mammalian cells. NAD(P)H: quinone oxidoreductase 1 (*NQO1*) catalyzes two-electron reduction of quinones and nitrogen oxides (1) and turns quinones to hydroquinones directly. Most hydroquinones are more stable and can be metabolized within cells. Therefore, *NQO1* can prevent organs from carcinogen-induced tumorigenesis. Previous studies (2,3) showed that *NQO1* has protected against carcinogenesis and mutation induced by quinone and its derivants. Studies (4-6) also found that the expression of *NQO1* is elevated in many tumor cells and human cancer tissues, suggesting that *NQO1* might

be the target of drugs for cancer therapy. Polymorphism of *NQO1* gene occurs at the site of 609 with cytosine changed into thymine. Although it would not affect synthesis of RNA, the protein product of *NQO1* T609 is ubiquitinated and subsequently degraded, which may lead to null *NQO1* (7). Our previous study (8) showed that the incidence of *NQO1* C609T in gastric cancer patients is higher than that in controls. Individuals with *NQO1* (T/C) and *NQO1* (T/T) genotypes have higher onset risk with odds ratios (OR) of 2.080 and 3.813 respectively.

Colon cancer is one of the most common cancers, and it is the third cause of cancer related death. Various factors, multiple genes and pathways are involved in colon carcinogenesis and progress. *K-ras*, adenomatous polyposis coli (*APC*), tumor protein P53 (*TP53*) and mismatch

repair (*MMR*) genes (9-12), chromosomal instability, microsatellite instability (13,14) and wnt-hedgehog pathway (15,16) are reported to be involved in initiation and progress of colon cancer. Diet factors and carcinogens play roles in influencing onset risk of colon cancer (17,18). The molecular mechanism of colon cancer has been better understood, however, the prognosis of colon cancer has not been improved too much. Therefore we carried out a case-control study in farmers from western region of Inner Mongolia to examine the association between *NQO1* C609T polymorphism and risk of developing colon cancer. Farmers from local region have their unique eating habits. They consume lots of pickled meat and vegetables which contain high levels of salt and nitrite. They are used to have the animal oils. The diet mostly consists of grain and less fresh fruits and vegetables. Most of them are alcohol users. As a result of specific diet of farmers, we study the *NQO1* polymorphism and susceptibility to colon cancer, which may also improve the treatment of local colon cancer patients.

## Materials and methods

### Subjects

All 76 colon cancer patients were hospitalized cases from the Affiliated Hospital of Inner Mongolia Medical College from 2007-2008, which were all diagnosed by histopathological examination, and did not undergo anti-cancer treatment. Of the 76 patients, 46 were male and 30 were female, with a mean age of  $60.13 \pm 10.96$  years (range, 39-79 years). One hundred and sixty healthy individuals who matched the age and sex of the colon cancer patients were selected by routine physical examination. The healthy controls did not have either tumors, tumor-related diseases detected by physical examination, or history of familial colon cancer. All individuals involved in the study gave informed consent.

### DNA extraction and polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP)

DNA was extracted from 1 ml venous blood of the investigated objects, and then PCR was performed with the primers of 5'-AAGCCCAGACCAACTTCA-3' and 5'-GCGTTTCTTCCATCCTT-3' and under following conditions: initial denaturation at 94 °C for 5 min, followed by 33 circles of denaturation at 94 °C for 1min, annealing at 54 °C for 45 s and extension at 72 °C for 45 s, with a final extension of 10 min at 72 °C. The PCR products were digested with restriction enzyme *Hinf*I. The full size of

PCR product was 195 bp. There was no enzyme site of *Hinf*I in PCR product of wild-type *NQO1* (C/C). If *NQO1* C609→T base substitution occurred, the PCR product was digested into two fragments (119 bp and 76 bp). There were 2 fragments in *NQO1* (T/T) and 3 fragments in *NQO1* (T/C).

### Statistical analysis

The frequency of *NQO1* (C/C), *NQO1* (T/C) and *NQO1* (T/T) was compared in control group *vs.* colon cancer patients and according to clinical characteristics by Chi-square test. OR [95% confidence interval (95% CI)] was calculated. All the statistical analyses were performed by SPSS 11.1 statistic software (SPSS Inc., Chicago, IL, USA).

## Results

We investigated the incidence of *NQO1* T609 allele in colon cancer patients and control group. We found that the incidence of *NQO1* T609 allele in colon cancer patients was 53% which was significantly higher than it in control group (33.75%). The individuals with *NQO1* T609 allele had higher risk [2.239 (95% CI: 1.510-3.321) times] to develop colon cancer compared to individuals with *NQO1* C609 allele.

Distribution of *NQO1* C609T polymorphism was different between colon cancer patients and control group. In the control group, the frequency of *NQO1* (C/C), *NQO1* (T/C) and *NQO1* (T/T) was 48.13%, 36.25% and 15.62%, respectively. While in colon cancer patients, the frequency of *NQO1* (C/C), *NQO1* (T/C) and *NQO1* (T/T) was 27.63%, 38.16%, and 34.21% (Table 1), respectively. There was a significantly increased incidence of *NQO1* (T/T) in colon cancer patients compared with control group, ( $\chi^2=13.566$ ,  $P<0.001$ ), while the incidence of *NQO1* (T/C) was not increased in colon cancer patients compared with control group. OR analysis showed that individuals with *NQO1* (T/T) or *NQO1* (T/C) genotype had higher risk [3.813 (95% CI: 1.836-7.920) time and 2.080 (95% CI: 1.026-4.219) time respectively] to develop colon cancer than individuals with wild type *NQO1* (C/C) (Table 1).

We investigated the distribution of *NQO1* C609T polymorphism in the differentiation of colon cancer. The frequency of *NQO1* (T/T) was found that it is increased in colon cancer patients with the increased malignance (32.00% in well-differentiated colon cancer patients, 40.00% in moderately-differentiated colon cancer patients