The NAD(P)H:quinone oxidoreductase 1 C609T polymorphism modifies the risk of Barrett esophagus and esophageal adenocarcinoma

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Purpose: The role of genetic susceptibility to esophageal adenocarcinoma and its precursor lesion Barrett esophagus has not been fully elucidated. This study investigated the effect of polymorphisms in the manganese superoxide dismutase (*MnSOD*) and NAD(P)H:quinone oxidoreductase 1 (*NQO1*) genes in modulating the risk of developing Barrett esophagus or esophageal adenocarcinoma. **Methods:** A total of 584 patients (146 esophagitis, 200 Barrett esophagus, 144 esophageal adenocarcinoma, and 94 controls) were genotyped for the *MnSOD* C14T and *NQO1* C609T polymorphisms using polymerase chain reaction and restriction fragment length polymorphism analysis. **Results:** The *NQO1* TT genotype was less common in Barrett esophagus (2.0%) and esophageal adenocarcinoma (1.4%) patients, compared with both esophagitis patients (7.6%) and controls (5.4%). After adjustment for sex, age, body mass index, reflux symptoms, and smoking status, patients with the homozygous TT genotype had a 4.5-fold decreased risk of developing Barrett esophagus (odds ratio = 0.22, 95% confidence interval = 0.07–0.76, $P = 0.01$) and a 6.2-fold decreased risk of esophageal adenocarcinoma (odds ratio = 0.16, 95% confidence intervals = 0.03–0.94, $P = 0.04$) compared with individuals with the TC and CC genotypes. No significant differences between groups were observed for the *MnSOD* polymorphism ($P = 0.289$). **Conclusions:** Overall, the results of this study suggest that the *NQO1* TT genotype may offer protection from reflux complications such as Barrett esophagus and esophageal adenocarcinoma. **Genet Med 2007;9(6):341–347.**

Key Words: adenocarcinoma, Barrett epithelium, esophagus, manganese superoxide dismutase, NAD(P)H:quinone oxidoreductase 1

Most esophageal adenocarcinomas (EACs) arise as the last stage of a multistep pathway beginning with a metaplastic change in a segment of esophageal mucosa, a condition known as Barrett esophagus (BE).¹ In this premalignant stage, the pluristratified esophageal epithelium acquires columnar, intestine-like characteristics, including the presence of goblet cells.² Over the past 30 years, a number of epidemiological studies have established a causative link between gastroesophageal reflux disease (GERD) and the development of BE and EAC, with the risk increased in individuals with the greatest severity, duration, and frequency of reflux.^{3,4} Despite the clear link between reflux and the risk of BE and EAC, only a minority of GERD patients develop reflux-related complications, and as

few as 0.5% of BE patients progress to cancer each year.⁵ As yet, the reasons for such heterogeneity in response to reflux remain to be clarified.

Accumulating evidence suggests that free radicals may be important mediators of the damaging effects of reflux. In animal models of GERD, for example, acid- and pepsin-induced esophagitis was significantly reduced by supplementation with the free radical scavenger superoxide dismutase (SOD).⁶ Furthermore, SOD supplementation also prevented progression of esophagitis to BE and EAC.⁷ Similarly, a diet rich in antioxidants such as vitamin C, β -carotene, and α -tocopherol, appears to reduce EAC risk, particularly in people with reflux.⁸ Oxidative damage in GERD patients may arise from the inflammatory process associated with reflux, with superoxide anion and hydrogen peroxide produced by activated leukocytes and macrophages.⁹ Exposure of the esophageal epithelium to these free radicals may explain the increased levels of DNA damage measured in Barrett epithelium compared with normal esophageal tissue.¹⁰ The consequent accumulation of DNA damage could contribute to the acquisition of genetic alterations and ultimately lead to malignant transformation in some patients. In addition to the inflammatory response induced by reflux, both endogenous and exogenous components

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of refluxate may also induce DNA damage. Short in vitro exposure of esophageal cells to bile acids, for example, is associated with a significant increase in the level of DNA strand breaks.¹¹ In addition, there is strong evidence that as a result of the acid environment created by reflux, the esophagus, and specifically the BE segment, may be the site of the conversion of nitrite to a number of mutagens, such as nitric oxide, nitrous acid and *N*-nitroso compounds.^{12–14}

In view of this mutagenic potential of reflux, the heterogeneity in GERD and BE outcome may be related to individual variation in antioxidant, detoxification, and DNA repair pathways, which could affect the risk of acquiring the genetic alterations observed in the progression of BE to malignancy. A number of case-control studies have investigated the association between specific genetic polymorphisms and esophageal squamous cell carcinoma in high-risk Asian populations.^{15–19} To date, however, only a few reports have been published regarding genetic susceptibility to EAC in Western countries; still fewer have addressed the role of genetic susceptibility to BE.

In this study, we examined the role of genetic polymorphisms in the manganese superoxide dismutase (*MnSOD*) and NAD(P)H:quinone oxidoreductase 1 (*NQO1*) genes in modulating susceptibility to BE and EAC in a UK population. *MnSOD* was selected for investigation because this enzyme provides an important antioxidant defense against the superoxide anions produced during inflammatory processes.^{8,20} The *MnSOD* C14T polymorphism results in an alanine (C allele) to valine (T allele) amino acid change within the signal peptide responsible for the transport of the mature protein to the correct subcellular location^{21,22} and has been associated with susceptibility to a variety of cancers.^{23–27} The phase II detoxification enzyme *NQO1* was chosen because it reacts with a number of natural and synthetic compounds including quinones, quinone-epoxides, quinone-imines, naphthoquinones, methylene blue, azo- and nitro-compounds²⁸ and could therefore metabolize a wide range of dietary carcinogens contained in reflux. The TT genotype corresponds to a null phenotype, as it results in the production of a mutated protein with reduced activity and increased half-life.²⁹ Previous studies have shown a link between this polymorphism and a number of cancers, including gastroesophageal malignancies.^{30,31}

The results of our study suggest that the *NQO1* C609T polymorphism may modulate the likelihood of patients with gastroesophageal reflux developing BE and EAC, whereas no association was found between the *MnSOD* C14T polymorphism and the risk of these diseases.

MATERIALS AND METHODS

Patient groups

After informed consent, a total of 584 patients were recruited from the dedicated dyspepsia and Barrett endoscopy clinics of two tertiary referral centers (Royal Hallamshire Hospital, Sheffield and Leeds General Infirmary, Leeds) in the Yorkshire region of the United Kingdom from 2000 to 2003. Information about age, sex, reflux symptoms, body mass index

(BMI), smoking history, alcohol consumption, and medication was collected at recruitment. DNA was extracted from blood ($n = 470$) or normal esophageal biopsy samples ($n = 114$), using a phenol/ethanol method or the TriReagent kit (Sigma-Aldrich Ltd., Gillingham, UK).

Based on endoscopic and histological evidence, patient groups included symptomatic controls ($n = 94$), esophagitis ($n = 146$), Barrett ($n = 200$), and cancer ($n = 144$) cases. All control patients were free of endoscopically visible esophageal disease, including esophagitis, columnar epithelium, BE, and EAC. However, as control individuals had been recruited from a dyspepsia endoscopy list, 44 of these (47%) reported reflux-related symptoms, such as heartburn and/or regurgitation. Other indications for endoscopy in the patients designated as controls were dyspepsia, epigastric abdominal pain, dysphagia, diarrhea, weight loss, nausea, and suspected celiac disease. Patients included in the esophagitis group had histologically confirmed reflux-related mucosal inflammation but no evidence of columnar mucosa (including BE), dysplasia, or EAC. The Barrett group comprised patients with any length of histologically confirmed specialized intestinal metaplasia containing goblet cells, of whom 28 (14%) also had histological evidence of low-grade dysplasia. Finally, the cancer group comprised patients with EAC (90%) but also 14 individuals with histologically confirmed high-grade dysplasia (HGD). The rationale for including HGD with EAC is that a high proportion of HGD patients are expected to be incident cases of EAC or likely to progress to EAC within a short period.^{32,33} All samples used in this study were collected after obtaining informed consent from patients. Ethical approval for the study was granted by the South Sheffield Research Ethics Committee and the United Leeds Teaching Hospitals Trust.

Polymerase chain reaction (PCR)

Genotyping for the *NQO1* C609T and *MnSOD* C14T polymorphisms was performed by PCR and restriction length fragment polymorphism analysis, as previously described,^{23,30} with minor modifications. Briefly, 1 μ L of DNA was amplified with 1 $\times$ PCR buffer (Qiagen Ltd., Crawley, UK), 12.5 pmol of forward and reverse primers (Invitrogen Ltd., Paisley, UK), 0.5 units of HotStarTaq DNA Polymerase (Qiagen Ltd.), and 200 μ M deoxyribonucleoside triphosphate (Invitrogen Ltd.), in a total volume of 20 μ L, using a GeneAmp PCR System 2700 thermocycler (Applied Biosystems, Warrington, UK). Primer sequences were 5'-aagccagaccaactct3' and 5'-attgaattcggcgctctgctg3' for *NQO1* and 5'-accagcaggcagctggcgccgg3' and 5'-gcgttgatgtgaggtccag3' for *MnSOD*. Reaction conditions were 15 minutes at 95°C to activate the HotStarTaq, followed by 38 cycles of 30 seconds at 95°C, 30 seconds at 60°C, and 30 seconds at 72°C, and 7 minutes of final elongation at 72°C. Nontemplate controls, in which DNA was omitted, were included in each amplification batch. The success of the PCR reaction and the lack of cross-contamination in the negative controls were checked by 1.5% agarose gel electrophoresis. Expected sizes of the PCR products were 174 bp for *NQO1* and 107 bp for *MnSOD*.

Restriction fragment length polymorphism analysis

Ten microliters of PCR product was digested overnight at 37°C with 5 units of *HinfI* (*NQO1*) or *NgoM IV* (*MnSOD*) (New England Biolabs Ltd., Hitchin, UK) in a total volume of 20 μ L. Genotypes were visualized by 3% (*NQO1*) or 4% (*MnSOD*) agarose gel electrophoresis of the PCR product in 0.5x Tris borate ethylene diamine tetracetic acid buffer containing 0.5 μ g/mL ethidium bromide (Sigma-Aldrich Ltd., Gillingham, UK). For the *NQO1* polymorphism, the C allele was visualized as a 174 bp band, whereas the T allele was digested into two fragments of 122 and 52 bp (Fig. 1). The T allele of the *MnSOD* polymorphism was identified by an uncut 107 bp product, whereas the C allele produced two fragments of 89 and 18 bp (Fig. 2). After confirmation of the correct genotype by sequencing (GRI Genomics, Rayne, UK), one sample for each of the three genotypes was included in all amplification and digestion batches as quality controls. Genotyping was repeated for 10% of the samples and demonstrated consistent results in all instances.

Statistical analysis

Differences in categorical variables (male/female gender, ever/never smoker) between study groups were assessed using the Pearson χ^2 test. The Kruskal-Wallis test was applied for the comparison of continuous variables (age, BMI, and alcohol intake) because these variables did not fit with a normal distribution, as indicated by the Kolmogorov-Smirnov test. Odds ratios (ORs) and 95% confidence intervals (CIs) associated with specific genotypes were calculated by multiple logistic regression, with and without adjustment for sex, age, BMI, reflux, and smoking status. Statistical analysis was performed using SPSS 12.0 (SPSS Inc., Chicago, IL) and STATA 7 (STATA Corporation, College Station, TX) software. In addition, genotype frequencies were tested for

Hardy-Weinberg equilibrium using χ^2 goodness-of-fit test (Graphpad Instat, 2.05). $P \leq 0.05$ was considered as significant.

RESULTS

Population characteristics

Information regarding patient gender, age, BMI, smoking status, and alcohol consumption is reported in Table 1. There were no statistical differences in gender composition between the esophagitis, Barrett, and cancer groups ($P = 0.281$), which as expected included a majority of male subjects. However, the number of males in the symptomatic controls was lower than in the other groups ($P < 0.001$). Significant differences in age were observed between patient groups ($P < 0.001$), with cancer patients tending to be older than Barrett patients, who in turn were older than symptomatic controls and esophagitis patients. No association was found between average BMI ($P = 0.144$), smoking status ($P = 0.068$), or alcohol intake ($P = 0.088$) and risk of BE and/or EAC.

Genotype frequencies and ORs

Overall, of the 584 samples included in the study, the number successfully genotyped was 578 for *NQO1* and 577 for *MnSOD* (99%). Genotype frequencies in all patient groups were in accordance with Hardy-Weinberg equilibrium ($P > 0.05$) except for the *NQO1* polymorphism in esophagitis patients ($P = 0.005$). No significant differences in the genotype frequencies for the *MnSOD* polymorphism were observed between groups ($P = 0.289$) (Table 2). In contrast, frequencies of the *NQO1* C609T ($P < 0.037$) polymorphism differed across patient groups. The TT *NQO1* genotype, was significantly less common in BE (2.0%, $P = 0.024$) and EAC (1.4%, $P = 0.030$) patients compared with individuals with esophagitis (7.6%). Based on the comparison of ge-

Fig. 1. *NQO1* C609T genotypes visualized by 3% agarose gel electrophoresis. The C allele appears as a 174 bp band, whereas the T allele is indicated by two fragments of 122 and 52 bp. A, CC control; B, CT control; C, TT control; N, nontemplate control; M, 100-bp ladder. Numbers across the top indicate digested PCR product for separate individuals.

Fig. 2. *MnSOD* genotypes detected by 4% agarose gel electrophoresis. The T allele is visualized as an undigested fragment of 107 bp, whereas the C allele is indicated by two fragments of 89 and 18 bp. A, CC control; B, TT control; C, CT control; M1 and M2, 100-bp ladder; N, nontemplate control. Numbers across the top indicate digested PCR product for separate individuals.

Table 1
Information on gender, age, BMI, smoking, and drinking habits for patients in the four study groups

	Symptomatic controls	Esophagitis	BE	EAC	<i>P</i>
Total no.	94	146	200	144	
Male patients, no. (%)	42 (44.7)	106 (72.6)	148 (74.0)	117 (81.2)	<0.001 ^a
Average age, y (range)	56 (29–88)	58 (22–88)	65 (25–97)	72 (41–94)	<0.001 ^b
Average BMI (range)	28.9 (16.8–55.3)	26.4 (14.0–41.2)	25.7 (17.0–37.6)	26.4 (15.4–51.5)	0.144
Ever smokers, no. (%)	50 (53.2)	87 (59.5)	127 (63.5)	89 (61.8)	0.068
Average units alcohol/wk (range)	9.0 (0–50)	13.8 (0–100)	11.7 (0–100)	9.7 (0–90)	0.088
Patients >21 units/wk, no. (%)	15 (15.9)	25 (17.1)	28 (14.0)	18 (12.5)	0.888

BE, Barrett esophagus; EAC, esophageal adenocarcinoma; BMI, body mass index.

^a*P* < 0.001 between control patients and the other groups; *P* = 0.281 between esophagitis, BE, and EAC.

^b*P* = 0.203 between control and esophagitis patients; *P* < 0.001 between all other groups.

Table 2
Genotype frequencies for the *MnSOD* C14T and *NQO1* C609T polymorphisms in esophagitis, BE, and EAC cases and in endoscopically normal control individuals

	Symptomatic controls	Esophagitis	BE	EAC	<i>P</i> ^a
<i>MnSOD</i> C14T					0.289
CC	36.6% (34/93)	23.6% (34/144)	26.5% (53/200)	25.0% (35/140)	
CT	41.9% (39/93)	46.5% (67/144)	47.0% (94/200)	52.1% (73/140)	
TT	21.5% (20/93)	29.9% (43/144)	26.5% (53/200)	22.9% (32/140)	
<i>NQO1</i> C609T					0.037
CC	59.1% (55/93)	68.1% (98/144)	67.0% (134/200)	68.1% (96/141)	
CT	35.5% (33/93)	24.3% (35/144)	31.0% (62/200)	30.5% (43/141)	
TT	5.4% (5/93)	7.6% (11/144) ^{b,c}	2.0% (4/200) ^b	1.4% (2/141) ^c	

BE, Barrett esophagus; EAC, esophageal adenocarcinoma.

^a*P* values indicate significance of differences in genotype frequencies across all groups.

^bEsophagitis vs. BE (*P* = 0.024).

^cEsophagitis vs. EAC (*P* = 0.030).

Table 3
Odds ratios and *P* values associated with the *NQO1* C609T genotypes (TT/CC+CT)

	OR (95% CI)		<i>P</i>	
	Unadjusted	Adjusted ^a	Unadjusted	Adjusted ^a
Esophagitis vs. symptomatic controls	1.45 (0.49–4.33)	0.80 (0.16–4.06)	0.500	0.790
BE vs. symptomatic controls	0.36 (0.09–1.36)	0.34 (0.05–2.25)	0.132	0.364
BE vs. esophagitis	0.24 (0.08–0.79)	0.22 (0.07–0.76)	0.018	0.016
EAC vs. symptomatic controls	0.25 (0.05–1.33)	0.12 (0.01–1.58)	0.105	0.107
EAC vs. esophagitis	0.17 (0.04–0.80)	0.16 (0.03–0.94)	0.025	0.043
EAC vs. BE	0.71 (0.13–3.92)	0.80 (0.13–4.92)	0.693	0.806

OR, odds ratio; CI, confidence interval; BE, Barrett esophagus; EAC, esophageal adenocarcinoma.

^aAdjusted for age, sex, reflux, body mass index, and smoking status.

notype frequencies between the esophagitis group and the BE and EAC groups, a significantly reduced risk of BE (OR = 0.24, 95% CI = 0.08–0.79, *P* = 0.018) and EAC (OR = 0.17, 95% CI = 0.04–0.80, *P* = 0.025) was observed in esophagitis patients with the TT *NQO1* genotype (Table 3). These results remained essentially unchanged after adjustment for age, sex, BMI, reflux symp-

toms, and smoking status, with the TT *NQO1* genotype associated to a 4.5-fold decreased risk of developing BE (OR = 0.22, 95% CI = 0.07–0.76, *P* = 0.01) and a 6.2-fold reduced risk of EAC (OR = 0.16, 95% CI = 0.03–0.94, *P* = 0.04) (Table 3). A similar trend was observed when genotype frequencies were compared between BE or EAC patients and symptomatic controls, with a

protective effect of the TT genotype against BE (OR = 0.34, 95% CI = 0.05–2.25) and EAC (OR = 0.12, 95% CI = 0.01–1.58), but these differences were not statistically significant ($P = 0.364$ and $P = 0.107$, respectively) (Table 3). The frequency of the TT *NQO1* genotype in the control and esophagitis groups were similar ($P = 0.167$) (Table 2), and the TT genotype was not associated with a significant risk of esophagitis (OR = 1.45, 95% CI = 0.49–4.33, $P > 0.500$) (Table 3).

DISCUSSION

Reflux symptoms are common in Western populations,^{34,35} but when such patients are referred for endoscopy, some have no visible pathology, some have developed esophagitis, and others have developed the precancerous lesion of BE,³⁶ which is associated with an increased risk of progression to EAC. In an attempt to improve EAC prognosis with an early diagnosis, the American College of Gastroenterology recommends the enrollment of all BE patients in endoscopic surveillance programs.³⁷ The development of genetic markers to identify the subset of BE patients more likely to progress to cancer, however, would allow surveillance to be targeted specifically at the individuals at higher risk. The aim of this study was therefore to assess whether genetic polymorphisms in genes related to free radical defense and xenobiotic detoxification could contribute to the heterogeneity in the development of esophageal pathology and could possibly be used as markers of higher risk of progression.

When we examined the effect of the C609T polymorphism in the gene encoding for the detoxification enzyme *NQO1*, we found no difference in genotype frequencies between control and BE or EAC patients. In contrast, individuals with esophagitis demonstrated a significantly higher frequency of the TT genotype compared with patients with intestinal metaplasia or cancer. This finding could suggest a protective effect of the T allele against the development of more severe reflux complications, namely, BE and EAC. The mechanism by which the rare T allele, resulting in a mutated protein with reduced activity,²⁹ may confer protection against BE and EAC could reside in the ability of *NQO1* to activate promutagens contained in reflux. Therefore, one explanation for the lack of significant differences in genotype frequencies across the control and the BE and EAC groups could relate to the fact that the protective effect of the TT genotype may only be apparent when both cases and controls are subject to the relevant environmental exposure, i.e., longstanding reflux. In our study, only half of the control individuals reported reflux symptoms. In contrast, esophagitis patients reported long-standing reflux symptoms, consistent with BE and EAC groups, and could therefore be argued to represent a more suitable control group.

The results of our study are consistent with those of previous investigations showing a similar protective effect of the null T allele against lung cancer^{38–41} possibly due to the reduced activating effects of *NQO1* toward carcinogens contained in tobacco smoke.⁴¹ Nevertheless, other studies suggest that the decrease in *NQO1* activity associated with the T allele may predispose to

gastric,³⁰ colon,⁴² bladder,⁴³ cervical,⁴⁴ and breast⁴⁵ cancer. The apparent contrasting role of this polymorphism in different malignancies may reflect the complex effects of *NQO1* on carcinogen metabolism and its ability to exert both antioxidant and pro-oxidant activities.^{29,46} *NQO1* detoxifies toxic quinones, such as some derivatives of benzene, by catalyzing their reduction into the more stable and readily excreted hydroquinones. The metabolism of other substances, however, may lead to intermediates that can generate free radicals or alkylating species. Some nitroso compounds contained in food and some toxic substances found in diesel exhaust, for example, are activated by *NQO1*.^{29,46}

Regarding the role of *NQO1* in esophageal carcinogenesis, in one investigation, the TT genotype was associated with an increased risk of squamous cell carcinoma.³¹ The contrast between the protective effect of high *NQO1* activity in esophageal squamous cell carcinoma and our findings of a predisposing role toward EAC could reflect the different etiology of these two histologically distinct types of esophageal cancer. *NQO1* may, for instance, activate specific carcinogens involved exclusively in EAC development, such as exogenous and endogenous compounds contained in reflux. Nitroso compounds could be a plausible target for such activation, in view of their emerging role in the development of BE and EAC.^{12–14}

In contrast with our results, a predisposing effect of the *NQO1* T allele toward EAC was previously suggested in a small study comprising 61 EAC cases,³⁰ although a subsequent larger investigation from the same group did not confirm these earlier findings.⁴⁷ The lack of association in the study by von Rahden and colleagues⁴⁷ may be due to the fact that in their investigation, EAC cases were compared with healthy volunteers without reflux. As discussed above, the protective effect of the TT genotype may be apparent only in the presence of procarcinogen contained in reflux. However, in view of these inconsistencies, the role of the *NQO1* polymorphism in esophageal carcinogenesis needs to be further clarified in subsequent investigations. In particular, it would be interesting to confirm whether this polymorphism influences the likelihood of progression to BE and EAC in the context of a large prospective study enrolling patients with chronic reflux symptoms.

In our study, we also investigated a polymorphism in the gene encoding for the free radical scavenger *MnSOD*. This enzyme plays a crucial role in defense against free radicals by catalyzing the dismutation of superoxide radicals to hydrogen peroxide, which is then converted to water by catalases and peroxidases.²⁰ A recent report has shown that *MnSOD* levels in the esophagus are altered during EAC development,⁴⁸ confirming a possible important protective role of this enzyme against esophageal carcinogenesis. The C to T polymorphism examined here results in the replacement of the alanine in position 14 of the *MnSOD* protein with valine, altering the efficiency of the transport of the mature protein to the correct subcellular localization.^{21,22} As a result, this polymorphism has been shown to modulate predisposition to a number of malignancies, including breast,^{23,24} ovarian,²⁵ bladder,²⁶ and lung²⁷ cancer. Although animal experiments have shown that SOD administration can reduce reflux-mediated esophageal damage,^{6,7} our results suggest that the

genotype at the *MnSOD* C14T locus does not have a major effect on the risk of developing reflux-related complications such as BE or EAC. However, we cannot exclude a small effect that could be detected in larger studies.

Both esophagitis and BE are common findings in patients with reflux symptoms and referred for endoscopy (37% and 23%, respectively).³⁶ As both patients and controls in this investigation were recruited from dyspepsia and Barrett endoscopy clinics, a major strength of our study is that all patients had undergone endoscopic examination to allow inclusion into the appropriate study group. As a result, however, our study is limited by the relatively small number of patients recruited, implying that the role of this polymorphism in esophageal carcinogenesis needs to be further clarified in the context of a larger investigation. Furthermore, although our results suggest that the *NQO1* TT genotype may protect from progression to EAC, its development as a genetic marker for the identification of patients at lower risk of progression appears unfeasible due to the low frequency of this genotype in disease-free individuals (7.6%–5.4%).

In conclusion, our results indicate that the *NQO1* C609T polymorphisms may have a role in predisposing GERD patients to reflux complications and that patients with the T allele may have a lower risk of progression to BE and EAC. Larger investigations are required to confirm these findings and to explore the interaction between this genetic polymorphism and specific exposures or with polymorphisms in related genes.

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