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ORIGINAL ARTICLE

Genetic polymorphism 609C > T in *NAD(P)H:quinone oxidoreductase 1* enhances the risk of proximal colon cancer

Jolien JM Freriksen[1], Jody Salomon[1], Hennie MJ Roelofs[1], Rene HM te Morsche[1], Jos WJ van der Stappen[2], Polat Dura[1], Ben JM Witteman[3], Martin Lacko[4] and Wilbert HM Peters[1]

Gastrointestinal (GI) cancer is responsible for the majority of deaths among all types of cancer. Lifestyle factors may not only be the main risk factor for GI cancer but reactive oxygen species (ROS) may also be involved. The single-nucleotide polymorphisms (SNPs) 609C > T (rs1800566) and 465C > T (rs1131341) in the *NAD(P)H:quinone oxidoreductase 1* (*NQO1*) gene lead to a decline in NQO1 enzyme activity. NQO1 catalyzes the two-electron reduction of quinones to hydroquinones, thereby preventing the formation of ROS. Such polymorphisms in *NQO1* may increase the risk of GI cancer. The aim of this study was to evaluate the influence of the SNPs rs1800566 and rs1131341 in the *NQO1* gene on the risk of GI cancer in the Netherlands. Real-time polymerase chain reaction techniques were conducted to determine the *NQO1* genotypes of 1457 patients with GI cancer and 1457 age- and gender-matched controls in a case–control study. Binary logistic regression analyses showed no statistically significant difference in genotype distributions between patients and controls: odds ratios (ORs) with 95% confidence interval (CI) for rs1800566 were 1.09 (0.93–1.28) and 1.17 (0.77–1.77) for the CT and TT genotypes, respectively. ORs for rs1131341 CT and TT genotypes were 1.21 (0.90–1.63) and 0.54 (0.05–5.94), respectively. For rs1800566, a significant association between the CT genotype and proximal colon cancer was detected (OR = 1.60; 95% CI = 1.09–2.35). The *NQO1*2* T allele of SNP rs1800566 was found associated with an increased risk for proximal colorectal cancer, whereas SNP rs1131341 was rare in our Dutch population and was not associated with GI cancer.
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INTRODUCTION

Gastrointestinal (GI) cancer is a prominent cause of death of all types of cancer. The incidence of patients with GI cancer, including head–neck cancer (HNC), esophageal cancer (EC) and colorectal cancer (CRC), increased between 2001 and 2011 in the Netherlands. In 2001, a total of 12 349 new patients were diagnosed with HNC, EC and CRC, whereas this number was increased to 18 669 new patients in 2011, being 2970 patients with HNC, 2445 with EC and 13 254 with CRC. The mortality rate of these GI cancers was also high, 7588 deaths in 2011, accountable for 5.4% of the total mortality in the Netherlands.[1] Worldwide, approximately 560 000 patients do get HNC, resulting in about 300 000 deaths every year. EC affects more than 450 000 patients every year and results in more than 380 000 deaths, whereas CRC is the most common cancer of the GI tract, with 1 058 000 new cases every year, contributing to 9.4% of the total cancer cases in the world. There is a great variety in the incidence of CRC worldwide, with the highest incidence in the most affluent (Western) countries.[2]

GI carcinogenesis is a complex multistep event, in which many dietary and lifestyle factors are involved; such as, cigarette smoking, heavy alcohol drinking, high body mass index, less physical exercise, consumption of less fruits and vegetables, etc.[3,4] Except for shared risk factors, different GI cancers may also have different risk factors and thus different etiologies. For example, Barrett's esophagus is a key risk factor for esophageal carcinoma[5] and colorectal adenomas is a risk factor for CRC (http://www.health.am/cr/colorectal-cancer/#2).

Genetic factors are increasingly recognized as modulators to GI cancer risk, including single-nucleotide polymorphisms (SNP).[6] SNPs in the *NAD(P)H:quinone oxidoreductase 1* (*NQO1*) gene have been studied extensively; a decline in the NQO1 enzyme activity because of the 609C > T or 465C > T polymorphism has been associated with an increased risk of various types of cancer, including GI cancer.[7] NQO1 is also known as DT-diaphorase, a cytosolic flavoenzyme that is able to catalyze the two-electron reduction of quinones to hydroquinones. Quinones are aromatic compounds present in the environment and in our body. The quinones are mainly derived from endogenous quinones; such as, vitamin E quinine and ubiquinone, and exogenous

[1]Department of Gastroenterology, Radboud University Nijmegen Medical Center, Nijmegen, The Netherlands; [2]Department of Clinical Chemistry, Canisius-Wilhelmina Ziekenhuis, Nijmegen, The Netherlands; [3]Department of Gastroenterology, Hospital Gelderse Vallei, Ede, The Netherlands and [4]Department of Otorhinolaryngology, Head and Neck Surgery, Maastricht University Medical Center, Maastricht, The Netherlands
Correspondence: Dr WHM Peters, Department of Gastroenterology, Radboud University Nijmegen Medical Center, PO Box 9101, 6500HB Nijmegen, The Netherlands.
E-mail: w.peters@mdl.umcn.nl
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quinones; such as, exhaust gas, cigarette smoke or diet.[8,9] They are destructive for human cells. The two-electron reduction is beneficial because it bypasses the one-electron reduction of quinones. In a one-electron reduction of quinones, by enzymes such as NADPH-cytochrome *P*-450 reductase, semiquinones are formed, which provoke the production of reactive oxygen species (ROS). Therefore, NQO1 is important for two-electron reduction of quinones, thereby contributing to the prevention of ROS formation.

The enzyme NQO1 is present in all body tissues and is highly expressed in case of oxidative or electrophilic stress. In addition to the role of NQO1 in detoxifying potentially mutagenic and carcinogenic quinones, NQO1 is also important for the bioactivation of chemotherapeutic quinones and for the stabilization of p53, especially under circumstances of oxidative stress.[10] Polymorphic *NQO1* with a C-to-T transition is unable to stabilize p53. Polymorphisms in *NQO1* affecting the activity of the enzyme are thought to increase the risk of developing several types of cancer.[10,11]

The most extensively studied polymorphism *NQO1*2* of *NQO1* is a C-to-T transition at nucleotide position 609 in exon 6 (609C>T; c559C>T; rs1800566), which results in a proline-to-serine amino-acid substitution at codon 187. The *NQO1*2* allele, which codes for an enzyme with low or deficient activity compared with the wild-type allele,[12] is thought to be related to the development of different types of GI cancer. A recent study reported that carriers of a single T allele (CT) have an increased risk of GI cancer (odds ratio (OR): 1.13) and for homozygous carriers (TT) this risk is even higher.[13]

The second most important polymorphism in the *NQO1* gene, *NQO1*3*, is the cytosine to thymine change at position 465 (465C>T; c415C>T; rs1131341). This SNP is located in exon 4 and is responsible for the substitution of arginine to tryptophan at position 139.[14] The frequency of the polymorphic *NQO1*3* allele in the European population is very low compared with the wild-type allele, 0.03 and 0.97, respectively (National Center of Biotechnology Information Sd. Cluster Report: rs1131341). The enzyme activity corresponding of the *NQO1*3* gene product depends on the substrate used and a decline in NQO1 enzyme activity up to 60% was revealed.[15,16] We now conducted a large case–control genetic association study, including 1457 patients with GI cancer in comparison with an equal number of matched controls. The study was set up to answer the question: What is the influence of the 609C>T and 465C>T polymorphisms in the *NQO1* gene on the risk of GI cancer in the Netherlands?

MATERIALS AND METHODS

Study population

Patients with either HNC, EC or CRC were included. All patients and controls enrolled in this study provided written informed consent for their participation. The investigations were approved by the Medical Ethical Review Committees of the Maastricht University Medical Center and Radboud University Nijmegen Medical Center.

Blood of 438 patients with HNC was obtained from the University Hospital Maastricht (AZM) (Maastricht, The Netherlands). The control samples matched with these patients were also from the Maastricht area. Blood or tissue of 475 patients with EC was obtained from the Radboud University Nijmegen Medical Center (RUNMC), the Canisius-Wilhelmina Hospital Nijmegen (CWZ), the Rijnstate Hospital Arnhem and the Gelderse Vallei Hospital Ede. Blood of 544 patients with CRC was obtained from the RUNMC and the Gelderse Vallei Hospital Ede. Controls for both EC and CRC were recruited from the Nijmegen area, by advertisements in local papers. The controls used for this experiment were matched with the patients based on geographical area, gender and age to create two most similar populations. No data were available concerning the lifestyle of the patients and controls.

DNA isolation

DNA was isolated from blood, from leukocyte suspensions or healthy tissue by using the 'High Pure PCR Template Preparation Kit' (Roche Diagnostics GmbH, Mannheim, Germany) according to the instructions of the manufacturer. The concentration of DNA was measured with the Tecan Infinite M200 PRO NanoQuant plate reader (Männedorf, Switzerland). The DNA samples were stored at 4 °C until use.

Real-time polymerase chain reaction

*NQO1*2* and *NQO1*3* polymorphisms were established by means of real-time-polymerase chain reaction techniques. A specific set of primers, which flank the region of the SNPs, was used to amplify the DNA. In the TaqMan assay, two probes, labeled with a fluorophore at the 5′ end and a quencher at the 3′ end of the probe, were added to the PCR mixture. The designed PCR primers and TaqMan probes were checked for polymorphisms in their binding sites using SNPCheck version 3 (https://ngrl.manchester.ac.uk/SNPCheckV3/snpcheck.htm) and were synthesized by Sigma Aldrich Chemie BV (Zwijndrecht, The Netherlands). The sequences of the primers and probes are given in Table 1. The 6-fluorescein amidite and hexachloro-fluorescein amidite were covalently bound to the probes for the most common alleles and the less common alleles, respectively. The real-time polymerase chain reactions were performed with the CFX96 Real-Time system (Bio-Rad Laboratories, Hercules, CA, USA) and the results were analyzed with the data analysis software Bio-Rad CFX Manager 2.0 (Bio-Rad Laboratories).

Statistical analyses

The results from the real-time polymerase chain reaction, followed by allelic discrimination, were analyzed with IBM SPSS Statistics version 20 (International Business Machines Corp., Armonk, NY, USA). First, the genotype frequencies of the controls were tested for Hardy–Weinberg equilibrium. With independent sample *T*-tests, the mean age of the patient and control groups were compared. Unconditional logistic regression analyses were performed to determine the association between genotype and GI cancer. ORs were given with 95% confidence interval (95% CI). Then, the analyses were conducted on the cancer subtypes (HNC, EC and CRC) and also on the histology or localization of the tumor for EC and CRC, respectively. The effect of the *NQO1* genotypes on the development of GI cancer was also examined for male and female separately.

Table 1 Sequences of the primers and probes used in RT–PCR

SNP	*Sequence*	
*NQO1*2* (rs1800566)	Forward primer	5′-AGAGTGGCATTCTGCATTTC-3′
	Reverse primer	5′-TTTCTCCTCATCCTGTACCTC-3′
	'Most common' probe	5′-(FAM)TTCCAAGTCTTAGAA**C**CT CAACTGACATAT(BHQ1)-3′
	'Variant' probe	5′-(HEX)TTCCAAGTCTTAGAA**T**CTC AACTGACATAT(BHQ1)-3′
*NQO1*3* (rs1131341)	Forward primer	5′-GATGTCTTCTGTCCCACAGT-3′
	Reverse primer	5′-AGAAGCTGGCTGTCAGAG-3′
	'Most common' probe	5′-(FAM)TTC**C**GGGTAGGTGGA TGGTTC(BHQ1)-3′
	'Variant' probe	5′-(HEX)TTC**T**GGGTAGGTGGA TGGTTC(BHQ1)-3′

Abbreviations: BHQ1, black hole quencer 1; FAM, fluorescein amidite; HEX, hexachloro-fluorescein amidite; NQO1, NAD(P)H:quinone oxidoreductase 1; RT–PCR, real-time-polymerase chain reaction.
The difference between the probes is indicated with the bold C and T. The 'most common' probe recognizes the C allele and the 'variant' probe recognizes the T allele.
Underlined nucleotides indicate the locations of the SNPs.

RESULTS

The characteristics of the patients with GI cancer and controls are given in Table 2. Controls were matched with the patients in the cancer subgroups based on geographical area, gender and age; however, the latter did not succeed completely in the HNC subgroup. The independent sample *T*-test showed significant differences in age between the total patient and control group ($P = 0.001$) and between the HNC patient group and their controls ($P = 0.000$). Therefore, the binary logistic regression analyses performed on the overall GI cancer group and on the HNC subgroup were adjusted for age.

Patients with EC could be subdivided by the histology of the tumor. The majority of the patients had esophageal adenocarcinoma (EAC), whereas approximately one-third beared esophageal squamous cell carcinoma (ESCC). One patient suffered from both EAC and ESCC, and this patient was included for statistical analysis in both EAC and ESCC subgroups. For some patients, the histology of the tumor was unknown and these cases were not included in the statistical analyses of the EAC or ESCC subgroups.

Patients with CRC could be subdivided according to the localization of the tumor: cancer of the cecum, ascending colon, transverse colon, descending colon, sigmoid colon or rectum. Some patients with CRC, who had cancer at multiple locations, were included in more than one subgroup for statistical analyses. When the localization of the tumor was unknown, patients were not included in the statistical analysis based on localization.

Genotype distribution of both SNPs was tested for deviation from Hardy–Weinberg equilibrium in the whole GI cancer population as well as in the cancer subgroups, and no deviation was noticed (all $P > 0.05$).

Table 2 Characteristics of patients and controls

Cancer type	*Patients*	*Controls*
GI cancer		
Total	1457	1457
Male (%)	1054 (72.3)	1054 (72.3)
Female (%)	403 (27.7)	403 (27.7)
Age (years ± s.d.)*	63.8 (11.5)	62.4 (11.3)
HNC		
Total	438	438
Male (%)	345 (78.8)	345 (78.8)
Female (%)	93 (21.2)	93 (21.2)
Age (years ± s.d.)*	60.9 (11.3)	56.5 (6.7)
EC		
Total	475	475
Male (%)	384 (80.8)	384 (80.8)
Female (%)	91 (19.2)	91 (19.2)
Age (years ± s.d.)	65.1 (11.0)	65.1 (11.3)
CRC		
Total	544	544
Male (%)	325 (59.7)	325 (59.7)
Female (%)	219 (40.3)	219 (40.3)
Age (years ± s.d.)	65.0 (11.7)	64.7 (12.5)

Abbreviations: CRC, colorectal cancer; EC, esophageal cancer; GI, gastrointestinal; HNC, head and neck cancer.
Mean age plus/s.d. is given.
All *P*-values are >0.05, unless otherwise indicated.
*P-value <0.01.

Binary logistic regression analyses were conducted to determine the association between *NQO1* genotype and GI cancer, with the most common genotype CC as reference. Table 3 shows the *NQO1*2* genotype distribution and ORs with corresponding 95% CI. No statistical significant association between the different *NQO1*2* genotypes and the development of GI cancer was noticed. This also accounts for the different cancer subtypes. When males and females were analyzed separately, the presence of the *NQO1*2T* allele in men was significantly associated with an increased risk for CRC (OR: 1.35; 95% CI: 1.02–1.80). Further associations were not seen (data not shown). In women the same tendency was seen; however, no statistical significance was reached, possibly because of lower numbers of female patients.

Table 4 shows the genotype distribution and ORs with corresponding 95% CI for *NQO1*3*. Comparable with the results on *NQO1*2* genotypes, binary logistic regression analyses showed no statistical significant association between the *NQO1*3 CT* genotype and the development of GI cancer. No ORs could be calculated for the homozygous TT genotypes in the cancer subgroups because there were either no patients or controls bearing this genotype. When males and females were analyzed separately, no effect of *NQO1*3* alleles or genotypes on the development of GI cancer could be revealed (data not shown).

For the large majority of patients with EC, the histology of the tumor was known. Binary logistic regression analyses on the EAC and ESCC subtypes of EC revealed no statistically significant risk modulation of both the *NQO1*2* or *NQO1*3* genotypes on the development of cancer.

Cancers of the cecum, ascending colon and transverse colon were considered to be proximal colon cancers. Cancers of the descending colon, sigmoid colon and rectum were considered to be distal colon cancers. For patients with CRC, a statistically significant association was found for the *NQO1*2* heterozygous CT genotype and the development of proximal colon cancer (OR: 1.60; 95% CI: 1.09–2.35; Table 5). This association was even stronger, however not statistically significant, for TT genotypes (OR: 2.4; 95% CI: 0.93–6.23). Analyses on distal colon cancer showed no statistically significant association with *NQO1*2*. For *NQO1*3*, no statistically significant association was found with CRC tumor localization. Here no ORs could be calculated for the homozygous TT genotype because there were either no patients or no controls bearing this genotype.

DISCUSSION

Prior studies reported a reduced NQO1 enzymatic activity associated with both polymorphisms studied here[12,15,16] and carriers of the variant allele showed an increased risk of developing GI cancer.[7,11,13] In contrast, the results of our study did not show any statistically significant associations between *NQO1*2* or *NQO1*3* and GI cancer. According to the site of the tumor, numerous studies suggested that the presence of the T allele in the *NQO1*2* polymorphism is a risk factor in the development of EC[17–22] or CRC,[23–27] whereas studies on HNC reported no significant associations with the *NQO1*2* and *NQO1*3* polymorphisms.[28,29] Our data only support the increased risk for proximal CRC of the *NQO1*2* T allele.

A possible explanation for the differences in results is the study population. The meta-analyses included more patients and controls than our case–control study, which leads to more reliable results. Besides, a considerable number of studies included were focused on the Chinese and Indian populations, in contrast to our Dutch Caucasian study population. The *NQO1*2* T allele

Table 3 Genotype distribution and ORs of *NQO1*2* with corresponding 95% CI

			*NQO1*2→rs1800566*		
Cancer type	*Genotype*		*Patients (%)*	*Controls (%)*	*OR (95% CI)*
GI cancer	Most common	CC	914 (63.4)	949 (62.3)	Reference
	Heterozygote	CT	478 (33.2)	460 (31.6)	1.09 (0.93–1.28)[a]
	Variant	TT	49 (3.4)	45 (3.1)	1.17 (0.77–1.77)[a]
	Total		1441[b]	1454[b]	
Head–neck	Most common	CC	274 (63.3)	290 (66.2)	Reference
	Heterozygote	CT	140 (32.3)	132 (30.1)	1.09 (0.81–1.47)[a]
	Variant	TT	19 (4.4)	16 (3.7)	1.23 (0.61–2.47)[a]
	Total		433[b]	438	
Esophagus	Most common	CC	298 (63.8)	288 (60.8)	Reference
	Heterozygote	CT	158 (33.8)	170 (35.9)	0.90 (0.68–1.18)
	Variant	TT	11 (2.4)	16 (3.4)	0.67 (0.30–1.47)
	Total		467[b]	474[b]	
Colorectum	Most common	CC	342 (63.2)	371 (68.5)	Reference
	Heterozygote	CT	180 (33.3)	158 (29.2)	1.25 (0.97–1.63)
	Variant	TT	19 (3.5)	13 (2.4)	1.64 (0.80–3.38)
	Total		541[b]	542[b]	

Abbreviations: CI, confidence interval; GI, gastrointestinal; NQO1, NAD(P)H:quinone oxidoreductase 1; OR, odds ratio.
[a]Adjusted for age.
[b]Note that for some patients and controls analysis did not succeed.

Table 4 Genotype distribution and ORs of *NQO1*3* with corresponding 95% CI

			*NQO1*3→rs1131341*		
Cancer type	*Genotype*		*Patients (%)*	*Controls (%)*	*OR (95% CI)*
GI Cancer	Most common	CC	1336 (92.8)	1358 (93.7)	Reference
	Heterozygote	CT	103 (7.2)	89 (6.1)	1.21 (0.90–1.63)[a]
	Variant	TT	1	2 (0.1)	0.54 (0.05–5.94)[a]
	Total		1440[b]	1449[b]	
Head–neck	Most common	CC	395 (91.4)	399 (91.5)	Reference
	Heterozygote	CT	37 (8.6)	36 (8.3)	1.07 (0.65–1.76)[a]
	Variant	TT	0	1 (0.2)	—
	Total		432[b]	436[b]	
Esophagus	Most common	CC	437 (74.8)	446 (94.9)	Reference
	Heterozygote	CT	27 (5.8)	23 (4.9)	1.17 (0.66–2.07)
	Variant	TT	0	1 (0.2)	—
	Total		464[b]	470[b]	
Colorectum	Most common	CC	504 (92.6)	513 (94.5)	Reference
	Heterozygote	CT	39 (7.2)	30 (5.5)	1.38 (0.84–2.26)
	Variant	TT	1 (0.2)	0	—
	Total		544	543[b]	

Abbreviations: CI, confidence interval; GI, gastrointestinal; NQO1, NAD(P)H:quinone oxidoreductase 1; OR, odds ratio.
[a]Adjusted for age.
[b]Note that for some patients and controls analysis did not succeed.

frequency varies between ethnic populations and there may be differences in lifestyle, which could influence the effect of *NQO1* polymorphisms.

Our findings seem to be consistent with studies that revealed no significant association between *NQO1*2* and HNC.[28,29] It is also in line with the results from two meta-analyses where also no significant ORs were found for EC and CRC.[11,13] In a recent meta-analysis, however, with 1217 EC patients and 1560 controls, a statistically significant association between the *NQO1*2* TT genotype and EC was reported.[22] This study included both patients with ESCC and EAC,

Table 5 Results of binary logistic regression analyses for *NQO1*2* and *NQO1*3* genotypes performed on proximal and distal colon cancer

			CRC		
Localization	*Genotype*		*Patients*	*Controls*	*OR (95% CI)*
*NQO1*2 (rs1800566)*					
Proximal[a]	Most common	CC	87/152 (57.2)	371/542 (68.5)	Reference
	Heterozygote	CT	58 (38.2)	158 (29.2)	**1.60 (1.09–2.35)**
	Variant	TT	7 (4.6)	13 (2.4)	2.41 (0.93–6.23)
Distal[b]	Most common	CC	237/364 (65.1)	371/542 (68.5)	Reference
	Heterozygote	CT	115 (31.6)	158 (29.2)	1.15 (0.86–1.54)
	Variant	TT	12 (3.3)	13 (2.4)	1.48 (0.66–3.29)
*NQO1*3 (rs1131341)*					
Proximal[a]	Most common	CC	141/154 (91.6)	513/543 (94.5)	Reference
	Heterozygote	CT	13 (8.4)	30 (5.5)	1.75 (0.88–3.46)
	Variant	TT	0	0	—
Distal[b]	Most common	CC	339/365 (92.9)	513/543 (94.5)	Reference
	Heterozygote	CT	25 (6.8)	30 (5.5)	1.29 (0.74–2.23)
	Variant	TT	1 (0.3)	0	—

Abbreviations: CI, confidence interval; CRC, colorectal cancer; GI, gastrointestinal; NQO1, NAD(P)H:quinone oxidoreductase 1; OR, odds ratio.
[a]Cancers of the cecum, ascending colon and transverse colon were considered to be proximal colon cancers.
[b]Cancers of the descending colon, sigmoid colon and rectum were considered to be distal colon cancers.
Bold and underlined indicates significant difference.

and a similar effect was found on these subtypes (ESCC: OR 2.03, 95% CI 1.29–3.19; EAC: OR 1.61, 95% CI 1.01–2.56).

Meta-analyses that focused on CRC in Caucasians revealed a significant effect of *NQO1*2* on CRC,[24,25] whereas three other meta analysis studies also including Asian patients did not reveal such an association.[7,11,13] The divergent *NQO1*2* allele frequencies between different ethnic groups[30] and lifestyle differences might have a role in this discrepancy. Carriers of the *NQO1*2* T allele might have an increased risk of developing proximal CRC; heterozygous CT genotype: OR, 1.60 (95% CI 1.09–2.35), whereas the *NQO1*2* TT genotypes shows a logical tendency, although not significant: OR, 2.41 (95% CI 0.93–6.23). These results need to be interpreted with caution because of the low number of patients with proximal CRC ($n = 152$). Research with larger sub-populations must be performed to further elucidate the effect of *NQO1*2* T allele on the development proximal colon cancer.

Developmental and biologic differences may cause differing susceptibilities to neoplastic transformation of proximal and distal colon.[31] Differences in proximal and distal colorectal cancer suggest that each may arise through different pathogenetic mechanisms. For instance, proximal CRC appear to be more associated with hereditary nonpolyposis coli, caused by mutations in DNA repair genes, whereas distal CRC may develop through the same mechanisms that underlie familial adenomatous polyposis, caused by mutations in the *APC* gene.[32] Presence of the *NQO1*2* T allele may lead to reduced detoxification and higher oxidative stress, which may be associated with more DNA damage. As the proximal colon may be more susceptible for disturbed DNA repair mechanisms, as demonstrated by the increased incidence of proximal CRC in hereditary nonpolyposis coli patients, this could mean that the proximal colon is also more susceptible for DNA damage as a result of the *NQO1*2 T* mutation.

No statistically significant differences could be found in the *NQO1*3* genotype distribution between patients with GI cancer and controls. This also accounts for the different cancer subtypes, when males and females are analyzed separately and for the specific types of cancer subdivided by histology or localization. No previous studies were performed that determined the effect of *NQO1*3* on the development of GI cancer. The *NQO1*3* T allele may lead to a low NQO1 expression,[15,16] and low levels of NQO1, which leads to less detoxifying potential and a disability to stabilize p53, may increase cancer risk. However, the T allele frequency was found very low and the hypothesis that the *NQO1*3* polymorphism might contribute to the development of GI cancer is unlikely. Our analyses were also conducted for males and females separately and no statistical evidence for a risk modulation effect of a particular *NQO1*2* or *NQO1*3* containing genotype was found. Presence of the *NQO1*2* T allele in men, however, significantly increased the risk for CRC: OR: 1.35, 95% CI: 1.02–1.80. No other studies specified gender effects of *NQO1* polymorphisms.

The strengths of this study include the relatively large sample size of this case–control study and the fact that two important functional *NQO1* SNPs were studied simultaneously. In addition, despite the fact that many studies reported on the modulating effects of *NQO1*2* on CRC, localization of the tumor seems to be an item that was hardly dealt with. However, several limitations might have influenced the results of our study. Information about lifestyle of patients and controls was missing. It is unknown whether; for example, smoking patterns were comparable between GI cancer patients and controls. A recent study that focused on CRC suggests that there is no interaction between *NQO1*2* and tobacco or alcohol use.[23] On the other hand, a study that was focused on the relation between *NQO1*2*, smoking and HNC, reported that *NQO1*2* (CT and TT genotypes) were associated with a tobacco dose-dependent increase in risk of HNC.[33] Thus, it appears that the effect of smoking may differ among the various types of GI cancer. Therefore, lifestyle information should have strengthened the outcome of this study.

Further research is necessary to elucidate the effect of *NQO1*2* and *NQO1*3* on the development of GI cancer and for HNC, EC and CRC specifically. Reliable and representative results could be of great clinical value and could be used for a screening program for persons at high risk for GI cancer. Especially in Western countries, the exogenous risk factors are highly prevalent. When the presence of the variant T alleles is detected, one could advice to avoid exposure to risk

factors. Several studies reported that the *NQO1*2* is associated with many other types of malignancies; for example; breast cancer, lung cancer and cervical cancer.[34–36]

In conclusion, the *NQO1*2* T allele was found associated with an increased risk for proximal colorectal cancer, whereas the *NQO1*3* T allele was rare in our Dutch population and does not seem to contribute to the development of GI cancer.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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