Gastric Cancer (2005) 8: 12–17
DOI 10.1007/s10120-004-0308-1

Original article

Association between *Helicobacter pylori* seropositivity and NAD(P)H:quinone oxidoreductase 1 (*NQO1*) C609T polymorphism observed in outpatients and health checkup examinees

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Abstract

Background. **Significant associations *of Helicobacter pylori* (*H. pylori*) seropositivity have been found with several host polymorphisms. This study investigated the associations of functional polymorphisms of the *NQO1*, *GSTM1*, and *GSTT1* genes of detoxification enzymes, with the seropositivity, as well as with pepsinogen levels, as markers of gastric atrophy.**

Methods. **The subjects were 241 noncancer outpatients who had participated in an *H. pylori* eradication program (HPE) at Aichi Cancer Center Hospital, and 465 health checkup examinees in Nagoya (HCE). The *NQO1* C609T, *GSTM1*, and *GSTT1* polymorphisms were determined by triplex polymerase chain reaction with confronting two-pair primers (PCR-CTPP).**

Results. **The sex- and age-group-adjusted odds ratio (OR) of *NQO1* C/C for *H. pylori* seropositivity relative to T/T was highly significant; OR, 1.92; 95% confidence interval (95% CI), 1.22–3.03. The ORs of the *GSTM1 present* type and *GSTT1 present* type for *H. pylori* seropositivity were not significant; OR, 0.87; 95% CI, 0.64–1.20 and OR, 1.14; 95% CI, 0.83–1.57, respectively. The association of the *NQO1* C/C genotype with *H. pylori* seropositivity was observed only for never-smokers; OR, 2.25; 95% CI, 1.33–3.79. The genotypes of the *NQO1*, *GSTM1*, and *GSTT1* genes were not associated with the development of atrophic gastritis among the *H. pylori*-seropositive subjects.**

Conclusion. **This is the first study to report a significant association of the *NQO1* C609T polymorphism with *H. pylori* seropositivity. The biological mechanism explaining the significant association with the seropositivity remains to be elucidated.**

Key words *NQO1* · Polymorphism · *Helicobacter pylori* · Atrophic gastritis

Offprint requests to: Y. Goto
Received: May 18, 2004 / Accepted: October 18, 2004

Introduction

Gastric cancer is the second most frequent cancer in the world, accounting for a large proportion of cancer cases in Asia, Latin America, and some countries in Europe [1]. The etiology of gastric cancer is not well established, although nutritional, microbial, and genetic factors have been suggested to be involved in a multistep and multifactorial process [2]. *Helicobacter pylori* has been reported to play a specific role in the development of atrophic gastritis that represents the most recognized pathway in multistep intestinal-type gastric carcinogenesis [3–5]. *H. pylori* infection is considered to occur primarily in childhood, through an oral-oral or fecal-oral route, mainly from the family [6–8], depending on sanitary conditions [7]. Because some individuals who are exposed are not infected with the bacterium, it is assumed that host factors have an important role in the infection and its maintenance. Recently, associations between HLA types [9] and polymorphisms of the *secretor* gene [10], *Lewis* gene [10], *interleukin 1B* (*IL-1B*) gene [11–14], *myeloperoxidase* (*MPO*) gene [15], *tumor necrosis factor A* (*TNF-A*) gene [16,17], and the *TCRBV6S1* gene [18] and *H. pylori* seropositivity have been reported.

Nicotinamide adenore dinucleotide phosphate, reduced (NAD(P)H):quinone oxidoreductase 1 (NQO1) is a cytosolic obligate two-electron reductase characterized by its capacity for utilizing NADH or NADPH as a reducing cofacor [19,20]. NQO1 prevents the generation of semiquinone free radical and reactive oxygen species, thus protecting cells from oxidative damage [21]. On the other hand, NQO1 activates nitrosoaromatic compounds and heterocyclic amines in tobacco smoke and food, which may work to induce carcinogenesis [22,23]. The activity level of the NQO1 enzyme is determined by a single C-to-T substitution at 609 (exon

6) of the *NQO1* cDNA that causes a Pro187Ser amino-acid change [24]. While the enzyme encoded by the C/C genotype has full enzyme activity, that encoded by the T/T genotype has complete lack of activity. The enzyme activity for the C/T genotype decreases to approximately one-third of that for the C/C genotype [24–27]. Associations between the T/T genotype and various cancers, including renal [28], urothelial [28], colorectal [29], cutaneous basal cell carcinomas [30], and pediatric leukaemia [31] have been reported in previous studies.

Glutathione S-transferase (GST) μ and GST θ are enzymes involved in the conjugation of reactive electrophiles to soluble glutathione and, therefore, they play an important role in the detoxification of endogeneous and exogeneous toxicants, including heterocyclic amines [32]. Individuals who are carriers of homozygous deletions in the *GSTM1* or *GSTT1* gene may have an impaired ability to metabolically eliminate carcinogenic compounds and may, therefore, be at an increased cancer risk [33].

This study aimed to evaluate the associations of polymorphisms of the *NQO1*, *GSTM1*, and *GSTT1* genes with *H. pylori* seropositivity and atrophic gastritis. We also investigated the effect of interactions between the *NQO1*, *GSTM1*, and *GSTT1* genes, and smoking on the risk of *H. pylori* seropositivity.

Subjects and methods

Study subjects

Two sets of subject data were used for the present analysis. The first subject group included 241 noncancer outpatients (118 male and 123 female), aged 39 to 69 years, who participated in an *H. pylori* eradication program (HPE) at Aichi Cancer Center Hospital between March and December, 1999. The group included 97 participants who were receiving medication for 107 diseases (23 gastric/duodenal ulcers; another 23, so-called gastritis; and 61 miscellaneous diseases) [34] and who participated in the program without knowing their infection status.

The second group of subjects included 468 health checkup examinees (128 male and 340 female), aged 32 to 85 years, who attented a health checkup (HCE) program supported by the Nagoya Municipal Government in Nagoya, in August and September, 2000. The examinees were inhabitants of the west ward of Nagoya City, who had had no chance to attend health checkups at their workplaces [13]. Out of 489 examinees invited to participate in the study, 468 agreed to provide their blood for genetic tests, along with the requested information. However, 3 subjects did not allow their blood samples to be used for DNA extraction; Therefore, the HCE group for this study included 465 subjects. Eleven participants had a cancer history, including 2 with gastric cancer. The subjects in both groups were each classified into two groups; never-smokers and ever-smokers (including current smokers and former smokers). The study protocols were approved by the Ethics Committee of the Aichi Cancer Center, and were subject to the draft version of the guidelines for research on the human genome, issued on March 29, 2001, by the collaboration of three Japanese ministries.

Tests for H. pylori *antibody and pepsinogens*

An anti-*HP* IgG antibody test, high-molecular weight Campylobacter-associated-protein (HM-CAP) enzyme-linked immunosorbent assay (ELISA; Enteric Products, Westbury, NY, USA) was conducted by SRL (Tokyo) to detect *H. pylori*-infected participants. The sensitivity of the HM-CAP is reported to be 98.7%, with a specificity of 100%, in the United States [35], though the sensitivity was not so high for Japanese [36]. An ELISA value of 2.3 or over was regarded as indicating *H. pylori* infection-positive status. Concentrations of pepsinogens I and II (PG1 and PG2) in sera from the HPE subjects and plasma from the HCE subjects were measured by radioimmunoassay, using a commercially available kit (Dinabot, Tokyo, Japan). Atrophic gastritis was defined as PG1 less than 70 ng/ml and a PG1/PG2 ratio of less than 3.

Genotyping

DNA was extracted from the buffy coat fraction with a Qiagen QIAamp DNA Blood Mini Kit (Qiagen, Valencia, CA, USA). Genotyping of the *NQO1* C609T, *GSTM1*, and *GSTT1* genes was conducted by polymerase chain reaction with confronting two-pair primers (PCR-CTPP). The primers and PCR conditions were described previously [37].

Statistical analysis

The strength of associations between *H. pylori* seropositivity and polymorphisms of the *NQO1*, *GSTM1*, or *GSTT1* genes were measured as odds ratios (ORs). ORs, adjusted for sex, age, and subject group, with 95% confidence intervals (CIs), were calculated using logistic regression analysis. Hardy-Weinberg equilibrium was tested for the *NQO1* C609T polymorphism. The equilibrium test was not applicable for the *GSTM1* and *GSTT1* genes, because the homozygous and heterozygous genotypes with the "present" allele are not distinguished. These calculations were performed

Table 1. Genotype distribution of *NQO1* C609T, *GSTM1*, and *GSTT1* genes according to subject group

	HPE[a]		HCE[b]		Total	
Polymorphism	*n* (%)	HP+%	*n* (%)	HP+%	*n* (%)	HP+%
NQO1 C609T						
T/T	48 (19.2)	54.2	83 (18.6)	48.2	131 (19.0)	50.4
C/T	107 (44.4)	58.9	210 (47.1)	57.1	317 (46.0)	57.7
C/C	86 (35.7)	72.1	153 (34.3)	55.6	239 (35.0)	61.5
GSTM1						
Null	125 (53.4)	64.8	207 (46.4)	56.0	332 (48.8)	59.3
Present	109 (46.6)	60.6	239 (53.6)	54.0	348 (51.2)	56.0
GSTT1						
Null	103 (44.0)	61.2	215 (48.2)	53.3	318 (46.8)	55.7
Present	131 (56.0)	64.2	231 (51.8)	56.7	362 (53.2)	59.4

HPE, Participants in a *Helicobacter pylori* eradication program; HCE, health checkup examinees; HP+%, *H. pylori*-seropositive percentage
[a] Eleven blood samples could not be genotyped for *GSTM1* and *GSTT1*
[b] Nineteen blood samples could not be genotyped

Table 2. Sex- and age-adjusted odds ratios (ORs) and 95% confidence intervals (95% CIs) for *Helicobacter pylori* seropositivity according to the *NQO1* C609T, *GSTM1*, and *GSTT1* genotypes in the HPE, and HCE groups, and the groups combined

	HPE		HCE		Combined	
Polymorphism	OR	95% CI	OR	95% CI	OR	95% CI
NQO1 C609T						
T/T	1	Reference	1	Reference	1	Reference
C/T	1.13	0.55–2.33	1.57	0.92–2.68	1.43	0.93–2.19
C/C	2.42	1.11–5.29	1.70	0.96–2.98	1.92	1.22–3.03
C/T+C/C	1.56	0.80–3.06	1.62	0.98–2.68	1.62	1.08–2.41
GSTM1						
Null	1	Reference	1	Reference	1	Reference
Present	0.84	0.48–1.46	0.90	0.61–1.33	0.87	0.64–1.20
GSTT1						
Null	1	Reference	1	Reference	1	Reference
Present	1.17	0.67–2.05	1.14	0.77–1.67	1.14	0.83–1.57

HPE, Participants in a *Helicobacter pylori* eradication program; HCE, health checkup examinees; combined, both groups combined, adjusted for sex, age, and group

with a computer program (STATA Version 7; STATA, College Station, TX, USA).

Results

The characteristics of the study subjects were described previously [13,34]. The genotype distributions of the *NQO1*, *GSTM1*, and *GSTT1* genes are shown in Table 1. The distribution of the *NQO1* gene was in Hardy-Weinberg equilibrium for HPE ($\chi^2 = 1.93$; $P = 0.17$), HCE ($\chi^2 = 0.64$; $P = 0.43$) and both groups combined ($\chi^2 = 2.14$; $P = 0.14$). The genotype distributions in the two datasets (HPE and HCE) were similar. As described in our previous articles [13,34], *H. pylori* seropositivity tended to increase with age and was highest for the more-than-60-year age group in any study group. The seropositivity rate was highest for those with the *NQO1* C/C in the HPE group, and for those with the *NQO1* C/T in the HCE group. When the two groups were combined, the seropositivity rate for those with the *NQO1* C/C was highest. There were no substantial differences in the seropositivity rates between the genotypes of the *GSTM1* and *GSTT1* genes.

Table 2 shows the sex- and age-adjusted ORs and 95% CIs for *H. pylori* seropositivity according to the genotypes of the *NQO1*, *GSTM1*, and *GSTT1* genes in the HPE and HCE groups, and both groups combined. The increased OR of the *NQO1* C/C for *H. pylori* seropositivity relative to T/T was statistically significant in the HPE group, and marginally significant in the HCE group. The ORs of the *GSTM1 present* type and *GSTT1 present* type for *H. pylori* seropositivity were not significant in either the HPE or the HCE group. When both

Table 3. Sex- and age-group-adjusted odds ratios (ORs) and 95% confidence intervals (95% CIs) for *Helicobacter pylori* seropositivity according to the *NQO1* C609T, *GSTM1*, and *GSTT1* genotypes in never-smokers and ever-smokers

	Never-smokers		Ever-smokers	
	OR	95% CI	OR	95% CI
NQO1 C609T				
T/T	1	Reference	1	Reference
C/T	1.50	0.92–2.45	1.14	0.45–2.86
C/C	2.25	1.33–3.79	1.06	0.41–2.75
C/T+C/C	1.77	1.12–2.81	1.10	0.47–2.59
GSTM1				
Null	1	Reference	1	Reference
Present	0.96	0.67–1.38	0.58	0.29–1.18
GSTT1				
Null	1	Reference	1	Reference
Present	1.23	0.86–1.77	0.80	0.40–1.59

Table 4. Sex- and age-group-adjusted odds ratios (ORs) and 95% confidence intervals (95% CIs) of the *NQO1* C609T, *GSTM1*, and *GSTT1* genotypes for atrophic gastritis (AG) among *Helicobacter pylori*-seropositive participants

	n	AG%	OR	95% CI
NQO1 C609T				
T/T	66	48.5	1	Reference
C/T	183	53.6	1.25	0.71–2.20
C/C	147	53.1	1.23	0.68–2.21
C/T+C/C	330	53.3	1.24	0.73–2.11
GSTM1				
Null	197	48.3	1	Reference
Present	195	56.4	1.35	0.90–2.02
GSTT1				
Null	177	52.4	1	Reference
Present	215	51.2	0.87	0.58–1.30

AG%, Percentage of atrophic gastritis

datasets were combined, the ORs of the *NQO1* C/C and C/C + T/T genotypes were 1.92 (95% CI, 1.22–3.03) and 1.62 (95% CI, 1.08–2.41), respectively. The combined ORs for the *GSTM1* and *GSTT1* genes were not statistically significant.

The sex- and age-group-adjusted ORs and 95% CIs for *H. pylori* seropositivity according to the genotypes of the *NQO1*, *GSTM1*, and *GSTT1* genes in never-smokers and ever-smokers are shown in Table 3. In the never-smokers, the ORs of the *NQO1* C/C and C/C + C/T genotypes were statistically significant for association with *H. pylori* positivity (OR, 2.25; 95% CI, 1.33–3.79 and OR, 1.77; 95% CI, 1.12–2.81, respectively), while the corresponding ORs were not significant among the ever-smokers. The OR for the interaction between the *NQO1* C/C genotype and smoking was 0.55 (95% CI, 0.25–1.19). The ORs of the *GSTM1 present* type and the *GSTT1 present* type for *H. pylori* seropositivity were not significant in either never-smokers or ever-smokers.

We additionally examined the ORs of the genotypes of the *NQO1, GSTM1,* and *GSTT1* genes for atrophic gastritis (Table 4). The ORs for the *NQO1* C/C, C/T, and C/C + C/T for atrophic gastritis were not significant among *H. pylori*-seropositivite participants. Similar to the findings above, the ORs for the genotypes of the *GSTM1* and *GSTT1* genes were also not significant.

Discussion

This study found that *NQO1 609*C/C with full enzyme activity was a high-risk genotype for *H. pylori* seropositivity, relative to *609*T/T, with lack of enzyme activity, especially for never-smokers. The *present* types of *GSTM1* and *GSTT1* genes, with similar detoxification enzyme activity, were not associated with *H. pylori* seropositivity. Gastric atrophy among the *H. pylori*-seropositive participants was not associated with any genotypes. To our knowledge, there have been no reports concerning the associations between these genetic polymorphisms and *H. pylori* seropositivity. Because inconsistent findings are not rare in the field of association studies on polymorphisms, two datasets were studied; one from outpatients who participated in an *H. pylori* eradication program (HPE) and the other from health checkup examinees in the same area (HCE). Although the association was marginally significant for the HCE group, fairly consistent findings were observed. We have been screening dozens of polymorphisms to find associations with *H. pylori* seropositivity in our exploratory analysis [38], where the nominal *P* value was not corrected in view of multiple comparisons, similarly to other exploratory studies. Because an exploratory study cannot confirm the nature of an association, confirmatory studies are required.

If an association exists, the biological mechanisms supporting the association should be considered. However, there have been no reports on the biological roles of NQO1 in bacterial infection. Because the *GSTM1* and *GSTT1* genes were not associated with *H. pylori* seropositivity, the function of NQO1 as a detoxification enzyme may not be relevant to the observed association.

To date, the factors found to favor persistent *H. pylori* infection have been those relating to gastric acid secretion. It is considered that gastric acid inhibition leads to *H. pylori* distribution to the corpus [39], making a favorable condition for *H. pylori* to survive in the stomach lumen. The associations of *H. pylori* seropositivity with the polymorphisms of genes encoding interleukin (IL)-1β and tumor necrosis factor (TNF)-α,

potent acid inhibitors [40], support this hypothesis. It is not clear whether the two-electron reduction by NQO1 has a certain function in gastric acid secretion.

The biological reason that the association was marked only for the never-smokers is unclear. It is well known that chemicals in smoke induce many substances, including inflammation-related markers [41,42]. The associations between *H. pylori* seropositivity and the genotypes of the *IL-1B* gene [11–14] and the *MPO* gene [15] were stronger among smokers than among nonsmokers in the HPE dataset. This study found that the association of *H. pylori* seropositivity with the *NQO1* C/C genotype was stronger in the never-smokers, suggesting that smoking can cancel the effect of NQO1 activity in the gastric mucosa. Because the interaction between smoking and *NQO1* C/C was not significant (OR, 0.55; 95% CI, 0.25–1.19), the difference in the OR between never-smokers and ever-smokers could have occurred by chance. Confirmation by both epidemiologic and biological approaches seems to be required.

In conclusion, our data suggested that *NQO1* gene polymorphism influences *H. pylori* seropositivity. Although epidemiologic findings, lacking information on the biological mechanism, could be the first step to elucidate the relationship between host factors and outcome, biological evidence will be required. Studies of a larger size are needed to confirm the association between this gene polymorphism and *H. pylori* seropositivity.

Acknowledgments The authors are grateful to Dr. Nobuyuki Katsuda for his field work assistance and Ms. Yoko Mitsuda and Ms. Mayumi Kato for their technical assistance.

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