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NO ASSOCIATION BETWEEN *HELICOBACTER PYLORI* SEROPOSITIVITY AND *ORNITHINE DECARBOXYLASE (ODC)* A317G POLYMORPHISM, AND NO MODIFICATION BY *NAD(P)H:QINONE OXIDOREDUCTASE 1* (*NQO1*) C609T

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ABSTRACT

Our previous epidemiologic study reported that *NAD(P)H:quinone oxidoreductase 1* (*NQO1*) *609C/C* with full enzyme activity was a high risk genotype for *Helicobacter pylori* (*H. pylori*) seropositivity. Since NQO1 stabilizes ornithine decarboxylase (ODC), which attenuates the innate immune response through elevated polyamines, ODC functional polymorphisms may also influence *H. pylori* seropositivity. This study aimed to examine the association with *ODC* A317G polymorphism, as well as the modification by *NQO1* C609T. The two polymorphisms were determined by polymerase chain reaction with confronting two-pair primers (PCR-CTPP) among 465 health checkup examinees in Nagoya. The *ODC* A317G genotype frequency was 35.9% for *A/A*, 49.3% for *A/G*, and 14.8% for *G/G*. The sex-age-adjusted odds ratio (OR) of the *ODC* gene for *H. pylori* seropositivity was not significant (OR=1.09 for *G/A* and OR=1.02 for *G/G*, relative to *A/A*). Among subjects with any *NQO1* genotype, no association was observed between the *ODC* ploymorphism and *H. pylori* seropositivity. Results of the present study did not support the hypothesis that the different genetic traits in the ODC-polyamine pathway are associated with susceptibility to persistent *H. pylori* infection. The higher frequency of the *ODC* 317A allele in the Japanese population than that in the Caucasian population is firstly reported. The genetic traits through the ODC-polyamine pathway will be further investigated.

Key words; *NQO1*, *ODC,* Polymorphism, *Helicobacter pylori*

INTRODUCTION

Our previous epidemiologic study reported that *NAD(P)H:quinone oxidoreductase 1* (*NQO1*) *609C/C* with full enzyme activity was a high risk genotype for *Helicobacter pylori* (*H. pylori*) seropositivity.[1)]

A recent study suggested that NQO1 regulates the proteasomal degradation of ornithine decarboxylase (ODC) differently from the antizyme-dependent ODC degradation (Fig. 1).[2)] The

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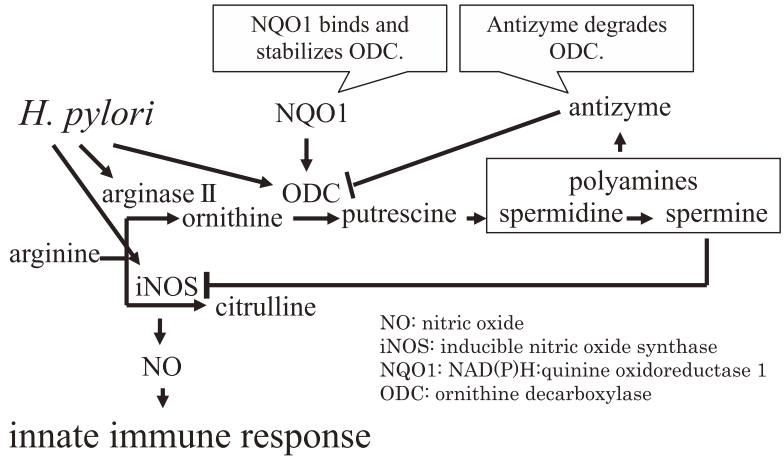

Fig. 1 Arginine-ornithine decarboxylase pathway leading to attenuation of innate immune response.

degradation of ODC is blocked by NQO1 and can be enhanced in the presence of dicoumarol, an inhibitor of NQO1.[2] Although the mechanism of ODC protection by NQO1 is not completely resolved, it requires their physical association.

Inducible nitric oxide synthase (iNOS)-derived NO is a central effector molecule in the innate immune response to pathogens, with essential antimicrobial functions in host defense. *H. pylori* induces both arginase II and ODC in macrophages.[3] Arginase converts L-arginine to L-ornithine, which is metabolized by ODC to produce putrescine that is converted to polyamines (spermidine and spermine). Spermine inhibits *H. pylori*-stimulated NO production in macrophages by a post-transcriptional effct on iNOS translation.[4] The previous study reported a mechanism of immune dysregulation induced by *H. pylori* in which stimulated spermine synthesis by the arginase-ODC pathway inhibits iNOS translation and NO production, leading to persistence of the bacterium.[4]

When macrophages were cocultured with *H. pylori*, the killing of bacteria was enhanced by transfection of ODC small interfering (si) RNA.[4] The *ODC* A317G polymorphism, located between the two E-boxes in intron 1, was reported to be functional.[5] Accordingly, we have investigated the association between *ODC* polymorphism and *H. pylori* seropositivity, and whether *NQO1* polymorphism modifies the association.

SUBJECTS AND METHODS

Study Subjects

The present subjects included 465 health checkup examinees (127 males and 338 females) aged 32 to 85 years, who attended a health checkup program in Nagoya supported by the Nagoya municipal government in August and September in 2000. The examinees were inhabitants of West ward of Nagoya City who had no chance to attend health checkups at their workplaces.[6] Out of 489 examinees invited to participate in the study, 468 agreed to provide their blood for genetic tests along with the requested information. Three blood samples were not available for DNA extraction. The remaining 465 including eleven participants with a cancer history (two with gastric cancer and nine with a miscellaneous cancer) were genotyped. The study protocols were

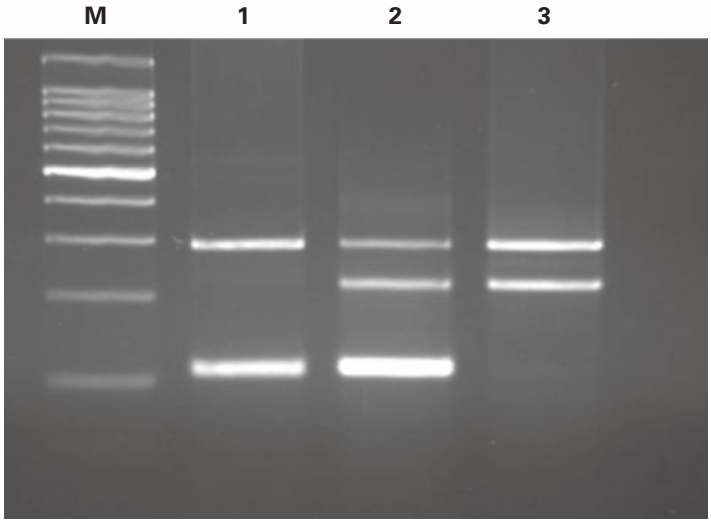

Fig. 2 Representative results of agarose gel electrophoresis for *ODC* A317G polymorphism. Lane M for a 100-bp DNA ladder; lane 1 for *A/A* (106 and 280 bp); lane 2 for *GA* (106, 212 and 280 bp); lane 3 for *G/G* (212 and 280 bp).

approved by the Ethics Committee of the Aichi Cancer Center, which was subject to the draft version of the guidelines for research on the human genome issued on March 29, 2001 by the collaboration of three ministries.

Tests for H. pylori antibody and pepsinogens

An anti-*HP* IgG antibody test, High-Molecular weight Campylobacter-Associated-Protein (HM-CAP) ELISA (Enteric Product, USA) was conducted for detecting *HP*-infected participants by SRL, Tokyo. The sensitivity of HM-CAP is reported to be 98.7% with a specificity of 100% in the US,[7] though the sensitivity was not that high for Japanese.[8]

Genotyping

DNA was extracted from the buffy coat fraction by Qiagen QIAamp DNA Blood Mini Kit (QIAGEN Inc.,Valencia,CA). The primers for *ODC* were F1: 5' GGC CGA GCG CTC CTG CG, R1: 5' GCT CGG CGA CCA CGT GTC TCC, F2: 5' GCC TCG CCG GCC TGC A, R2: 5' GCC CGG ATC ACC CTT ATC CAG C. The bases of the single nucleotide polymorphism are undelined. Genomic DNA was used per 25µl of reaction with 0.12mM dNTPs, 25 pmol of each primer, 0.5 units of "AmpliTaq Gold", and 2.5µl GeneAmp 10×PCR Buffer including 15 mM $MgCl_2$ (Perkin-Elmer Corp., Foster City, CA). Amplification conditions were 10 minutes of initial denaturation at 95°C, followed by 30 cycles of 1 minute at 95°C, 1 minute at 66°C and 1 minute at 72°C, then a 5-minute final extension at 72°C. The amplified DNA was visualized on a 2% agarose gel with ethidium bromide staining (Fig. 2). The primers for *NQO1* C609T were described previously.[9]

Statistical analysis

The strengths of associations of *H. pylori* seropositivity with the *NQO1* C609T and *ODC* A317G gene polymorphisms were measured as odds ratios (ORs). ORs adjusted for sex and

Table 1 Sex-age-adjusted odds ratios (ORs) and 95% confidence intervals (95% CIs) for *Helicobacter pylori* (*HP*) seropositivity according to the *ODC* A317G genotypes.

Polymorphism	N (%)	*HP*+ (%)	OR	95%CI
ODC A317G				
A/A	167 (35.9)	90 (53.9)	1	Reference
G/A	229 (49.3)	129 (56.3)	1.09	0.72–1.66
G/G	69 (14.8)	36 (52.2)	1.02	0.57–1.84
G/A+G/G	298 (64.1)	165 (55.4)	1.07	0.73–1.60

Table 2 Sex-age-adjusted odds ratios (ORs) and 95% confidence intervals of the *ODC* A317G genotypes for *Helicobacter pylori* seropositivity according to the *NQO1* C609T genotypes.

ODC A317G	*NQO1* C609T[a]					
	C/C		*C/T*		*T/T*	
	N	OR (95% CI)	N	OR (95% CI)	N	OR (95% CI)
A/A	59	1 (Reference)	76	1 (Reference)	26	1 (Reference)
G/A	72	1.28 (0.61–2.66)	103	1.18 (0.63–2.22)	43	1.07 (0.39–2.95)
G/G	22	0.94 (0.34–2.62)	31	1.00 (0.41–2.45)	14	1.24 (0.33–4.74)
G/A+G/G	94	1.19 (0.60–2.37)	134	1.14 (0.63–2.07)	57	1.11 (0.42–2.91)

[a]Nineteen blood samples could be not genotyped for *NQO1*

age with 95% confidence intervals (CIs) were calculated using logistic regression analysis. Hardy-Weinberg equilibrium was tested for *NQO1* C609T and *ODC* A317G polymorphism. These calculations were performed by the computer program STATA, Version 8 (STATA Corp., College Station, TX).

RESULTS

The characteristics of study subjects were described previously.[6,10] The distributions of the *ODC* A317G and *NQO1* C609T genes were in the Hardy-Weinberg equilibrium; χ^2=0.44 and P=0.51, χ^2=1.93 and P=0.17,[1] respectively. Table 1 shows the sex-age-adjusted ORs and 95% CIs of the *ODC* genotypes for *H. pylori* seropositivity; ORs of the *ODC* genotypes for *H. pylori* seropositivity were not significant. Table 2 shows the sex-age-adjusted ORs and 95% CIs of the *ODC* genotypes for *H. pylori* seropositivity according to the *NQO1* genotypes. The *ODC* gene was not associated with *H. pylori* seropositivity among subjects with any *NQO1* genotype.

DISCUSSION

ODC is a critical regulatory enzyme in the polyamine biosynthesis pathway. Myc is a member of a family of proteins that regulate transcription by binding to specific Myc-binding elements, termed E-boxes, of genes affecting both proliferation and apoptosis.[11] One transcriptional target

of c-Myc is the *ODC* gene whose transcription is activated by c-Myc.[12] In the many physiological/pathological contexts in which c-Myc expression is up-regulated, ODC expression is also up-regulated.

Guo *et al*. have reported that the single nucleotide polymorphism at base +317 in intron 1 of the *ODC* gene, between two closely spaced E-boxes, could have functional significance.[5] Those with *ODC A/A* genotype may be capable of greater ODC expression than *A/G* or *G/G*. Accordingly, we speculated that those with the *ODC A/A* might be more susceptible to persistent *H. pylori* infection. However, the present study showed that the *ODC* polymorphism did not affect *H. pylori* seropositivity. The function of the *ODC* polymorphism has not been previously investigated in Japan. We have found ethnic heterogeneity in *ODC* polymorphism, the frequency of *ODC A/A* was 6.8% in the Caucasian population[5] versus 35.9% in our subjects.

Each *NQO1* genotype has a different enzyme activity from others; full enzyme activity for *C/C*, a complete lack of it for *T/T,* approximately one third of *C/C* for *T/C*.[13-16] We stratified subjects according to the *NQO1* genotypes and investigated the effect on the relation between the *ODC* polymorphism and *H. pylori* infection. However, no modification was observed.

In conclusion, the present study did not strengthen the hypothesis that the different genetic traits in the ODC-polyamine pathway are associated with a susceptibility to persistent *H. pylori* infection. The higher frequency of *ODC* 317A allele in the Japanese than in the Caucasian population was first reported here. Genetic traits through the ODC-polyamine pathway will be further investigated.

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