

RESEARCH COMMUNICATION

No Association of an SDHC Gene Polymorphism with Gastric Cancer

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Abstract

It is widely reported that reactive oxygen species (ROS) cause apoptosis and carcinogenesis. Marked infiltration of activated leukocyte and enhanced production of ROS appear to occur in the gastric mucosa infected with *Helicobacter pylori* (*H. pylori*). The previous studies reported that the mutation of the succinate dehydrogenase subunit C (SDHC) gene caused the increase in superoxide anion (O_2^-) and oxidative stress. To extend these findings, we epidemiologically investigated the association of a SDHC polymorphism at 3'-untranslated region of exon 6 (JST173800) with *H. pylori* infection, gastric atrophy and gastric cancer risk in Japan. The subjects consisted of 454 health checkup examinees without a history of cancer and 202 gastric cancer patients. The SDHC polymorphism was not associated with *H. pylori* infection seropositivity, gastric atrophy, and cancer risk in this study. Although the polymorphism at the 3'-untranslated region could be hypothesized to be functional, this study did not demonstrate any significant association of the SDHC gene polymorphism with gastric atrophy and cancer.

Key Words: SDHC polymorphism – *H. pylori* infection seropositivity - gastric atrophy - gastric cancer

Asian Pacific J Cancer Prev, 7, 525-528

Introduction

Gastric cancer is the fourth most frequent cancer in the world, accounting for a large proportion of cancer cases in East Asia (China, Japan), Eastern Europe, and parts of Central and South America. In terms of cancer mortality, it is the second most common cause of deaths from cancer (Parkin et al., 2005). Although the etiology of gastric cancer is not completely understood, nutritional, microbial and genetic factors acting in a multistep, multifactorial process have been suggested (Correa, 1992).

Metabolic oxidative stress results from an imbalance between steady-state levels of prooxidants and the cellular antioxidants (Sies, 1991). Gradual accumulation of oxidative damage to critical biomolecules caused by metabolic oxidative stress is believed to contribute to many aging-related diseases including cancer (Finkel et al., 2000; Spitz et al., 2000). Mitochondrial electron transport chains (METC) are believed to be one of the major metabolic sources of superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2) (Turrens et al., 1985; Ahmad et al., 2005). The METC is mediated by five multimeric complexes (complexes I-IV) that are embedded in the inner membrane of the mitochondrion. Mitochondrial succinate-ubiquinone reductase (complex II) catalyses electron transport from

succinate to ubiquinone and is composed of succinate dehydrogenase (SDH), which is composed of the flavin protein, the iron-sulfur protein and two other subunits containing cytochrome b560. It was reported that electrons leaking from complexes I and mainly complex III convert oxygen to O_2^- (Turrens, 1997; Finkel et al., 2000; Raha et al., 2000). Ishii et al reported that complex II deterioration may also produce O_2^- (Ishii et al., 2006).

The SDH genes encode subunits of the heterotetrameric succinate dehydrogenase complex, a component of both complex II and the Krebs cycle. SDHA and SDHB encode the two catalytic subunits, the flavoprotein and the iron-sulfur protein, respectively. SDHC and SDHD encode transmembrane proteins that anchor complex II in the inner mitochondrial membrane, and contain a ubiquinone binding site.

Phaeochromocytomas are catecholamine-secreting tumors that usually arise within the adrenal medulla with hypertension and most of these tumors are benign. Extra-adrenal phaeochromocytomas are often referred to as paragangliomas. Inherited cancer syndromes with phaeochromocytomas as a component feature include von Hippel-Lindau syndrome (VHL), multiple endocrine neoplasia type 2 (MEN 2), and, less commonly, neurofibromatosis type 2. It is now known that germline

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mutations in SDHB, SDHC, and SDHD are implicated in the genesis of hereditary paragangliomas (Niemann et al., 2000; Maher et al., 2002; Gimm et al., 2004). A mutation in the genes coding for SDH subunits was reported to increase steady-state levels of reactive oxygen species (ROS), resulting in genomic instability and tumorigenesis (Ishii et al., 2005; Slane et al., 2006).

It is widely known that ROS cause apoptosis and carcinogenesis. Oberley et al reported that O₂[•], one of ROS, played a role in the etiology of cancer, differentiation, and aging (Oberley et al., 1980; Oberley et al., 1988). Marked infiltration of activated leukocyte and enhanced production of ROS appear to occur in the gastric mucosa infected with *H. pylori* (Mai et al., 1991). Eradication of the bacteria reduced the activity of leukocytes and oxidative stress (Drake et al., 1998; Pignatelli et al., 2001). These indicated that ROS were involved in the pathogenesis of *H. pylori* infection. Mashimo et al also suggested that ROS production was enhanced in peripheral blood by *H. pylori* infection (Mashimo et al., 2006). Ishii et al succeeded in establishing transgenic mouse cell lines (SDHC E69) with a single amino acid substitution in SDHC gene (Ishii et al., 2005). Their study of these cell lines provided direct evidence that O₂[•] production from mitochondria results in oxidative stress leading to apoptosis and tumorigenesis.

Although many SNPs at the exon/intron in the SDHC gene have been reported in NCI dbSNP, the frequencies among Japanese were unknown. Accordingly, we examined a C-to-G SNP at 3'-untranslated region of exon 6 reported in the Japan Single Nucleotide Polymorphisms (JSNP) database (<http://snp.ims.u-tokyo.ac.jp>) based on 48 chromosome genotyping, being identified as JST 173800. In this case-control study, the associations of the SDHC polymorphism with *H. pylori* seropositivity, gastric atrophy development, and gastric cancer risk.

Materials and Methods

Study Subjects

The characteristics of the controls and cases were described in our previous papers. Briefly, the controls were 454 inhabitants (126 males and 328 females) aged 35 to 85 years with no history of cancer who attended a health checkup program supported by the Nagoya Municipal Government in August and September, 2000 (Katsuda et al., 2003). The case group consisted of 202 patients (134 males and 68 females) aged 33 to 94 years with a pathologically confirmed diagnosis of gastric adenocarcinoma who underwent tumor resection at hospitals in Nagoya (Goto et al., 2006). Informed consent was obtained from all subjects. Approval for the study was given by the Ethics Committees of Nagoya University.

Tests for *H. pylori* antibody and pepsinogens

Anti-*H. pylori* IgG antibody tests, high-molecular-weight campylobacter-associated-protein (HM-CAP) ELISA (Enteric Products Inc., Westbury, NY) and HM-CAP with

antigens extracted from clinically isolated Japanese *H. pylori* strains (J-HM-CAP) ELISA (Kyowa Medex, Tokyo, Japan), were used for the identification of *H. pylori*-infected participants. An ELISA value of 2.3 or over was regarded as positive for both tests. The infection was confirmed in all gastric cancer cases by culture and bacteriological tests (Gram-negative, oxidase, catalase, and urease test-positive spiral, curved rods) using biopsy specimens before gastric resection. Pepsinogens I and II (PG I and PG II) in plasma were measured by radioimmunoassay using a commercially available kit (DINABOT, Tokyo, Japan). Gastric atrophy was defined as PG I < 70 ng/ml and PG I/PG II ratio < 3. These parameters for atrophy are in wide use in Japan and have been validated against histological confirmatory studies.

SDHC Genotyping

DNA was extracted from the buffy coat fraction with the Qiagen QIAamp DNA Blood Mini Kit (QIAGEN Inc., Valencia, CA, USA). A single nucleotide polymorphism at exon 6 of SDHC, named JST 173800 in JSNP database, was genotyped by PCR-CTPP (polymerase chain reaction with confronting two-pair primers). The primers were F1: 5' CAG ATG TGG GAC CTA GGA AAA GG, R1: 5' GTA CTC TAC TGC TCC AAG GAG ATC TG, F2: 5' GGG AAA AGT TCT CCT TAT TTG TTT AGA TCC TTT TGT ATT TTG, and R2: 5' CCT CTC TTC TTT CAA TAA CAC TGT GTA GGT G. The underlined are the bases of the single nucleotide polymorphism. Genomic DNA was applied in a volume of 25 ul with 0.12 mM dNTPs, 25 pmol of each primer, 0.5 units of AmpliTaq Gold (Perkin-Elmer Corp., Foster City, CA, USA), and 2.5 ul 10 x PCR buffer including 15 mM MgCl₂. The PCR was performed with initial denaturation at 95°C for 10 min, followed by 30 cycles of denaturation at 95°C for 1 min, annealing at 68°C for 1 min and extension at 72°C for 5 min. Final extension was at 72°C for 5 min. Figure 1 shows the results of PCR-CTPP for the SDHC polymorphism. PCR product was visualized on a 2% agarose gel with ethidium bromide staining.

Statistical Analysis

We used a χ^2 test. Odds ratios (ORs) adjusted for sex and age with 95% confidence intervals (CIs) were calculated using unconditional logistic regression analysis. Hardy-Weinberg equilibrium was tested for the SDHC polymorphism. Calculations were performed using the computer program STATA v. 8 (STATA Corp, College Station, TX, USA).

Results

The genotype distribution of the control group fitted the Hardy-Weinberg equilibrium ($\chi^2=3.38$, $P=0.07$). Table 1 shows that the genotype frequency and ORs with 95% CIs of SDHC genotype for *H. pylori* seropositivity in the controls. There was no significant association between them. No association was found between this polymorphism and

Table 1. Genotype Frequency of the SDHC Polymorphism (JST 173800) and Sex-age-adjusted Odds Ratios (ORs) with 95% Confidence Intervals (95% CIs) for *H. pylori* Seropositivity (HP+) in Healthy Checkup Examinees

Genotype	n*	HP+ (%)	OR (95%CI)
C/C	269	147 (54.7)	1.00 (Reference)
G/C	168	94 (56.0)	0.96 (0.64-1.44)
G/G	15	8 (53.3)	0.92 (0.31-2.76)

*Two healthy controls could not be genotyped.

Table 2. Sex-age-adjusted ORs and 95% CIs of the SDHC Polymorphism (JST 173800) Genotypes for Gastric Atrophy among *H. pylori* Seropositive Controls

Polymorphism	n*	GA (%)	OR (95%CI)
C/C	147	78 (53.1)	1.00 (Reference)
G/C	94	55 (58.5)	1.26 (0.75-2.14)
G/G	8	3 (37.5)	0.51 (0.12-2.22)

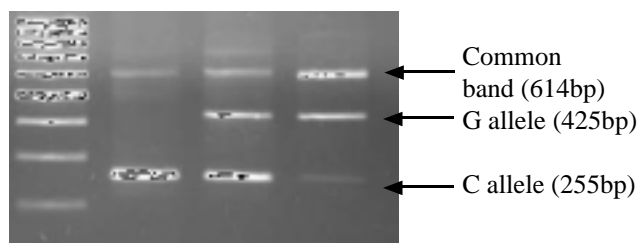
*One subject could not be genotyped.

gastric atrophy (Table 2). Table 3 shows that the sex-age-adjusted ORs and 95% CIs of the SDHC genotypes for gastric cancer. Compared with the whole healthy controls, the ORs were not significant. The prevalence of *H. pylori* seropositivity in the cases and in the healthy controls was 100% and 55.1%, respectively. On the other hand, atrophy was present in 34.8% of 454 controls, 21 of which were seronegative. We have got controls narrowed down to *H. pylori* seropositive controls or gastric atrophy positive controls and compared them with cases, the corresponding ORs being nonsignificant.

Discussion

Two of 454 healthy controls could not be genotyped. The increased production of ROS in the gastric mucosa of *H. pylori*-infected individuals has been reported (Mai et al., 1991). ROS induced by neutrophil infiltration in response to *H. pylori* infection can injure epithelial cells and damage their DNA, resulting in contributing to the initiation and promotion of tumors. In addition, the electron transport system is the major endogenous source of ROS, such as hydrogen peroxide (H₂O₂), O₂⁻, and hydroxyl radical (.OH) (Nohl et al., 1978).

A single amino acid substitution in SDHC gene (of a transgenic mouse cell line) provided direct evidence that production from mitochondria results in oxidative stress

**Figure 1. 2 % Agarose Gel Showing the Different Genotypes for the SDHC Polymorphism at Exon 6 (JST 173800)**

leading to apoptosis and tumorigenesis (Ishii et al, 2005). Accordingly, we hypothesized that SDHC gene polymorphisms are associated with gastric atrophy and cancer via the increase of ROS, if the polymorphisms are functional. Since there are no reports on the functions of polymorphisms of SDHC gene, a polymorphism JST173800 at 3'-untranslated region of exon 6 common among Japanese was selected as a possible candidate in this study. The analysis, however, revealed no associations with, *H. pylori* seropositivity, gastric atrophy development, and gastric cancer risk. To our knowledge, the genotype frequency was not reported for this gene polymorphism in any ethnic groups. This is the first report on the genotype frequency, with the result that we could not compare the genotype frequency with those in other studies.

It may take decades for superficial gastritis to progress to atrophic gastritis. *H. pylori* infection is highly associated with this progression (Faisal et al., 1990). Loss of serological markers of *H. pylori* infection following onset of severe atrophy and intestinal metaplasia is a well described phenomenon, including in Japan (Asaka et al., 2001). Considering these facts, the comparison with the controls with *H. pylori* seropositivity or with gastric atrophy might be adequate to evaluate the effect of the genotype on *H. pylori*-related gastric cancer risk among those infected with *H. pylori*. But, the significant OR was not observed in this comparison.

These findings could indicate that the JST173800 polymorphism is nonfunctional, or limitedly functional. Another interpretation is that SDHC is not directly involved in gastric carcinogenesis. The biological studies are needed to resolve this question. The present study revealed that this polymorphism or linked polymorphisms were not associated with the susceptibility to *H. pylori* infection and *H. pylori*-related diseases, gastric atrophy and cancer. This is the first study to examine the associations between the SDHC gene

Table 3. Sex-age-adjusted ORs and 95% CIs of the SDHC Polymorphism (JST 173800) Genotype for Gastric Cancer

SDHC Genotype	Cases (n=202)	Controls* (n=452)		Controls** (n=269)			
		OR	95% CI	OR	95% CI		
C/C	125 (61.9%)	269 (59.5)	1.00	Reference	159 (59.1%)	1.00	Reference
G/C	70 (34.7%)	168 (37.2)	0.77	0.52-1.13	102 (37.9%)	0.81	0.54-1.23
G/G	7 (3.47%)	15 (3.3)	0.99	0.35-2.75	8 (3.0%)	1.19	0.39-3.65

*Two healthy controls could not be genotyped **Controls with Helicobacter pylori-seropositivity or gastric atrophy, two of them not being genotyped.

polymorphism and the *H. pylori*-related conditions. Although no associations were found epidemiologically, further studies are required to delineate the role of SDHC genotypes in human genetic traits of gastric cancer.

Acknowledgements

The authors are grateful to Dr. Nobuyuki Katsuda for his field work assistance and Ms. Yoko Mitsuda for her technical assistance. This work was supported in part by a Grant-in-Aid for Cancer Research from the Japanese Ministry of Health, Labour and Welfare.

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