hOGG1 Ser326Cys polymorphism, interaction with environmental exposures, and gastric cancer risk in Japanese populations

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Oxidative DNA damage caused by reactive oxygen species (ROS) generated by *Helicobacter pylori* (*H. pylori*) infection or smoking may be a cause of gastric cancer development. 8-Hydroxydeoxyguanine (8-OHdG) formation is one of the most common types of oxidative DNA damage, while human oxoguanine glycosylase 1 (hOGG1) is responsible for repairing 8-OHdG lesions. Among several *hOGG1* gene polymorphisms, the Ser→Cys polymorphism at position 326 is related to biological function. To investigate the association between Ser326Cys *hOGG1* polymorphism and gastric cancer in relation to the potential risk factors of gastric cancer and antioxidant dietary or nutrient intakes, we conducted a case-control study with 142 histologically-confirmed gastric cancer cases and 271 age, sex-matched healthy controls in Japanese populations. Overall, neither the *hOGG1* Ser/Cys nor the Cys/Cys genotype was associated with risk of gastric cancer, compared with the Ser/Ser genotype. A significant interaction was observed between *hOGG1* Ser/Cys or Cys/Cys genotype and atrophic gastritis (*P* for interaction=0.03). No significant interaction was found between *hOGG1* genotype and antioxidant dietary or nutrient intakes. The results of the present study suggest that patients with atrophic gastritis in conjunction with the *hOGG1* Cys allele might have a higher susceptibility to gastric cancer. (Cancer Sci 2004; 95: 977–983)

Although the incidence and mortality rates of gastric cancer in Japan have markedly decreased over the few past decades,[1] this cancer still shows the highest mortality rate among Japanese females and the second highest among Japanese males. Gastric carcinogenesis is a complex, multistep, multifactorial process.[2] Like many malignancies, this cancer is the result of interactions between genetic factors of the host and environmental factors. A large number of epidemiological studies have confirmed the effects of low consumption of fresh fruits and vegetables, high consumption of salty food, smoking, and *Helicobacter pylori* (*H. pylori*) infection on the development of gastric cancer.[3, 4] Although just how the biological mechanisms underlying these factors are involved in its development is not fully understood, one of the proposed mechanisms involves oxidative DNA damage caused by reactive oxygen species (ROS). Oxidative DNA damage occurs in a cell when the production of ROS exceeds the cell's antioxidant-defense capacity, producing a mutation that in turn can activate oncogenes or inactive tumor suppressor genes and eventually lead to cancer.[5, 6] Fresh fruits and vegetables contain micronutrients such as vitamin C and β-carotene that have antioxidative activity, and the consumption of those foods has been shown to be inversely associated with gastric cancer risk.[7] Exposure to tobacco smoke and *H. pylori* infection involve ROS generation and oxidative DNA damage, and both have been shown to be positively associated with gastric cancer risk.[8–10]

Among the many types of oxidative DNA damage, the 8-hydroxydeoxyguanine (8-OHdG) residue is one of the most abundant oxidative products of cellular DNA, and is a mutagenic agent causing GC to TA transversions, since 8-OHdG preferentially pairs with adenine instead of cytosine during DNA replication.[11–13] An increase in 8-OHdG content in DNA has been shown to elevate cancer risk.[14] The DNA repair enzyme, human oxoguanine glycosylase 1 (hOGG1), is responsible for repairing 8-OHdG lesions, and is ubiquitously expressed in a variety of organs.[15, 16] Several polymorphisms of the *hOGG1* gene have been identified, and the repair activities of the variant genotypes have been evaluated in many studies.[17] Among these *hOGG1* gene polymorphisms, a Ser→Cys polymorphism at position 326 is related to biological function. The activity for the repair of 8-OHdG was found to be approximately 7-fold greater in hOGG1-Ser^{326} protein than in hOGG1-Cys^{326} protein in a complementation assay of an *E. coli* mutant defective in the repair of 8-OHdG.[18] Recent studies have suggested that the Ser326Cys *hOGG1* polymorphism may be associated with an increased risk of lung,[19, 20] esophageal,[21] orolaryngeal,[16] and prostate cancers.[22] As for the association between gastric cancer and Ser326Cys *hOGG1* polymorphism, three studies have reported no significantly increased risk.[23–25] However, the association between gastric cancer and Ser326Cys *hOGG1* polymorphism was seen to be modulated by drinking habits, pickled vegetable consumption, and smoking status in two studies.[24, 25]

We hypothesized that Ser326Cys polymorphism in *hOGG1* is associated with gastric cancer development in conjunction with environmental risk factors thought to be associated with ROS generation and oxidative DNA damage. In this case-control study of 142 gastric cancer patients and 271 healthy controls matched by age, sex and residential area, we studied the influence of *hOGG1* genotype on gastric cancer incidence in relation to family history of gastric cancer, smoking status, salt intake, *H. pylori* infection, and atrophic gastritis. Additionally, we assessed the joint effects on gastric cancer of Ser326Cys *hOGG1* polymorphism and antioxidant dietary or nutrient intakes.

Materials and Methods

Subjects. A multi-center, hospital-based case-control study of

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Abbreviations: 8-OHdG, 8-hydroxydeoxyguanine; 95% CI, 95% confidence interval; FFQ, food-frequency questionnaire; hOGG1, human oxoguanine glycosylase 1; *H. pylori*, *Helicobacter pylori*; JA, Japan Agricultural Cooperative; OR, odds ratio; ROS, reactive oxygen species.

gastric cancer was conducted at four hospitals in Nagano Prefecture, Japan between October 1998 and March 2002.[26] Eligible cases were gastric cancer patients aged 20 to 74 with histologic confirmation, newly diagnosed during a survey at those hospitals. Two controls were matched to each case by sex, age (±3 years), and residential area during the same period in the same hospitals. The controls were selected from among those who visited any of the four hospitals for a health check-up, and who were confirmed to be free of cancer. For some exceptional cases, only one or more than two controls were selected. Consequently, we enrolled 153 gastric cancer cases and 302 healthy controls in the study. Histopathological grading and anatomical subsites of gastric cancers were determined according to the general rules for gastric cancer study.[27] Histopathological grading was divided into two groups, well-differentiated and undifferentiated adenocarcinomas. We used the term "m cancer" to refer to those which were limited to the mucosa; and "sm+ cancer" for those which had penetrated down to or beneath the submucosa. Gastric cancer cases were divided into the upper third around the cardia (designated here as "cardia cancer") and the other two-thirds (middle body and antrum; designated as "noncardia cancer"). We obtained written informed consent from all cases and controls. The study protocol was approved by the Institutional Review Board of the National Cancer Center, Tokyo.

Questionnaire survey. We asked the subjects to fill out a self-administered questionnaire that included general characteristics (age, sex, and socio-demographic characteristics), personal medical history, familial history of diseases including gastric cancer, smoking and drinking history. They were also asked to fill out a supplement use and semi-quantitative food-frequency questionnaire (FFQ). We also asked whether they belonged to the Japan Agricultural Cooperatives (JA) because most JA members in this area engage in agriculture; thus, they have better access to fresh vegetables than non-members and may possibly tend to consume vegetables more often. In addition, they are eligible for financial support for health check-ups from JA. Habitual consumption of food and beverages was assessed with a 141-item FFQ. Subjects reported their average frequencies of consumption and average portion sizes for those items during the previous year. If subjects showed any current symptoms, they provided their dietary habits during the year before the onset of those symptoms. The mean daily consumption of energy and nutrients was mainly calculated using the food composition table developed for this FFQ based on the Standard Tables of Food Composition in Japan, 4th revised edition.[28, 29] The estimated consumption was validated by a 14- or 28-day dietary record (DR).[30] The Spearman correlation coefficients of energy-adjusted intakes of vegetables, fruits, sodium, vitamin C, and β-carotene were 0.44, 0.55, 0.42, 0.46, and 0.45 in males, and 0.47, 0.29, 0.45, 0.44, and 0.47 in females, for intakes calculated by DR.[31]

Evaluations of atrophic gastritis and *H. pylori* infection. The study subjects were tested for serum pepsinogen I (PG I) and pepsinogen II (PG II), and for IgG antibody to *H. pylori* (Hp-Ab) and CagA (CagA-Ab). These antibodies were measured with an enzyme-linked immunosorbent assay kit (ELISA; Helico G, Porton-Cambridge, Oxford, UK) and CagA (RADIM S.A., Rome, Italy). Test results of 10 units per milliliter (U/ml) or more were considered as positive for both Hp-Ab and CagA-Ab. *H. pylori* infection was verified when one or both serum assays were positive. The serum PG I and PG II levels were measured by radioimmunometric assay kits (PG1/PG2 RIA-BEAD, Dainabot Co., Ltd., Tokyo). Atrophic gastritis was diagnosed according to the following criteria: PG I level below 70 ng/ml and PG I/PG II ratio below 3.0. The prevalence of serologically diagnosed atrophic gastritis using these criteria was well correlated (r=0.999, P<0.0001) with the age-adjusted mortality rates of gastric cancer among five Japanese populations.[32] These criteria have been adopted in other studies, and their reliability has been recognized.[33–35]

Genotyping of hOGG1 Ser326Cys polymorphism. Genomic DNA was obtained from the peripheral blood of gastric cancer patients and healthy controls. The DNA Extractor WB Kit (Wako, Osaka, Japan) was used to extract DNA from peripheral blood, according to the manufacturer's protocol. Genotyping of *hOGG1* Ser326Cys polymorphism was performed by the PCR amplification procedure using the primer set of 5′-CTGT-TCAGTGCCGACCTGCGCCGA-3′ and 5′-ATCTTGTTGTG-CAAACTGAC-3′. A single-base mismatch was introduced in the forward primer to create a *Mbo*I site in the wild allele. The amplified products were digested with *Mbo*I and analyzed by electrophoresis on a 10% polyacrylamide gel. The variant-allele had no *Mbo*I site and was characterized by a 247-bp fragment on the gel. The wild-type allele was characterized by 224- and 23-bp fragments. The heterozygous genotype had both alleles and was characterized by 247-, 224- and 23-bp fragments. All genotyping was performed by laboratory personnel unaware of case-control status. For validation purposes, 10% of cancer cases and controls were randomly selected and regenotyped, and reproducibility was 100%.

Statistical analysis. We excluded 1 non-adenocarcinoma case and 2 matched controls, 5 cases and 11 controls who did not provide blood samples, 1 case and 3 controls who failed to provide complete data on vegetable and fruits items, and 4 cases with 8 matched controls and 7 controls who had extreme caloric intakes (for men, 500> or >/=4000 kcal/day; for women, 400> or >/=3500 kcal/day), leaving a total of 142 adenocarcinoma cases and 271 matched controls. The χ^2 test was used for a categorical comparison of the data and for evaluating the probability of Hardy-Weinberg equilibrium. Characteristics of cases and controls were compared using the Mantel-Haenszel test with matched-pair strata. Odds ratios (OR) and 95 percent confidence intervals (95% CI) were obtained by conditional logistic regression analysis. In our regression models, we adjusted ORs for potential confounders including a family history of gastric cancer, smoking status (never, past, and current), salt intake (tertiles based on controls), *H. pylori* infection, atrophic gastritis, and JA membership. The joint effects between *hOGG1* genotypes and environmental factors were also estimated. Tests for the interaction of these joint effects were performed based on the differences between two log-likelihoods obtained from the models with and without the joint-effect interaction terms.[36] A P value less than 0.05 (two-tailed) was considered to be statistically significant. All the statistical analyses were performed using the SAS statistical software package (SAS Institute, Inc., Cary, NC).

Results

The baseline characteristics of the gastric cancer cases and controls are shown in Table 1. The case patients and control subjects appeared to be adequately matched as to age and sex. The mean age was 57.5 years for cases and 57.1 years for controls (P=0.86). The frequencies of a family history of gastric cancer and current-smoking status were significantly higher in cases than in controls (P=0.047, 0.001, respectively). Alcohol drinking habits, total energy intake, salt intake, and JA membership were not associated with gastric cancer. The incidence of *H. pylori* infection was significantly higher in cases (88.7%) than in controls (64.2%) (P<0.0001). The prevalence of atrophic gastritis was significantly higher in cases (38.7%) than in controls (15.1%) (P<0.0001).

The frequencies of *hOGG1* genotype associated with gastric cancer are shown in Table 2. The genotype frequencies in cases were Ser/Ser: 0.23, Ser/Cys: 0.53, and Cys/Cys: 0.25, against

Ser/Ser: 0.27, Ser/Cys: 0.52, and Cys/Cys: 0.21 in controls. The observed frequency of *hOGG1* genotype in controls was similar to those previously observed in Japanese control populations.[23–25, 37] Genotype distribution in controls was within the Hardy-Weinberg equilibrium (P=0.46). The crude and adjusted ORs for Ser/Cys and Cys/Cys genotypes compared with Ser/Ser showed no statistically significant difference. Furthermore, we compared the distribution of *hOGG1* genotype classified by histopathological tumor grading, tumor invasion, or tumor location in a case group, and found no significant difference in distribution among any of those classifications (data not shown).

In order to check the effect of *hOGG1* genotype in combination with potential risk factors for gastric cancer, we calculated the ORs for data that were classified by family history, smoking status (never, past, and current), salt intake (tertiles based on controls), *H. pylori* infection and atrophic gastritis, and gene genotypes (Table 3). Family history was positively associated with gastric cancer risk whether subjects had Ser/Ser or Ser/Cys+Cys/Cys genotype compared with subjects who did not have a family history involving Ser/Ser genotype (adjusted OR, 3.35; 95% CI 0.88, 12.77 vs. adjusted OR, 2.79; 95% CI 1.24, 6.32, respectively). Current smokers were also positively associated with an increased risk of gastric cancer whether they had Ser/Ser or Ser/Cys+Cys/Cys genotype compared with never-smokers with Ser/Ser genotype only (adjusted OR, 3.44; 95% CI 0.96, 12.26 vs. adjusted OR, 4.56; 95% CI 1.50, 13.25, respectively). As for salt intake, a positive association with gastric cancer was found among subjects with the Cys allele, whereas no such association was found among subjects with Ser/Ser genotype. The Cys allele among subjects with an *H. pylori* infection was associated with a significantly increased

Table 1. Baseline characteristics of gastric cancer cases and controls

	Cases (n=142) No. (%)	Controls (n=271) No. (%)	P for difference[1)]
Age, mean (SD), y	57.5 (±9.5)	57.1 (±9.5)	0.86
Sex			
Male	99 (69.7)	191 (70.5)	
Female	43 (30.3)	80 (29.5)	—
Family history of gastric cancer			
No	105 (73.9)	225 (83.0)	
Yes	37 (26.1)	46 (17.0)	0.047
Smoking habit			
Never	50 (35.2)	137 (50.6)	
Past	43 (30.3)	62 (22.9)	
Current	49 (34.5)	72 (26.6)	0.001
Alcohol drinking habit			
Never	39 (27.5)	72 (26.6)	
Ever	103 (72.5)	199 (73.4)	0.35
Total energy intake (kcal/day)			
<1859	57 (40.1)	89 (32.8)	
1859–2402	42 (29.6)	93 (34.3)	
>2402	43 (30.3)	89 (32.8)	0.58
Salt intake (g/1000 kcal, day)			
<5.6	51 (35.9)	90 (33.2)	
5.6–7.2	43 (30.3)	90 (33.2)	
>7.2	48 (33.8)	91 (33.6)	0.67
H. pylori antibody			
Negative	16 (11.3)	97 (35.8)	
Positive	126 (88.7)	174 (64.2)	<0.0001
Atrophic gastritis			
No	87 (61.3)	230 (84.9)	
Yes	55 (38.7)	41 (15.1)	<0.0001
JA membership			
No	61 (43.0)	92 (34.0)	
Yes	81 (57.0)	179 (66.1)	0.054

1) Analyzed by Mantel-Haenszel test with matched-pair strata.

Table 2. Frequency of *hOGG1* genotype and odds ratios among gastric cancer cases and controls

Genotype	Cases (n=142) n (%)	Controls (n=271) n (%)	Crude OR	Adjusted OR[1)]
hOGG1				
Ser/Ser	32 (22.5)	74 (27.3)	1	1
Ser/Cys	75 (52.8)	141 (52.0)	1.22 (0.74, 1.99)	1.19 (0.64, 2.20)
Cys/Cys	35 (24.7)	56 (20.7)	1.51 (0.83, 2.73)	1.37 (0.69, 2.74)
P for trend			0.18	0.37
Ser/Cys+Cys/Cys	110 (77.5)	197 (72.7)	1.29 (0.80, 2.07)	1.25 (0.71, 2.22)

1) ORs were adjusted for family history of gastric cancer, smoking status, salt intake, *H. pylori* infection, atrophic gastritis, and JA membership.

gastric cancer risk, showing an adjusted OR of 4.19 (95% CI 1.23, 14.29) compared with the Ser/Ser genotype among those who did not have an *H. pylori* infection. However, the interaction test did not reach statistical significance (P=0.15). Atrophic gastritis with the Cys allele was significantly associated with gastric cancer compared with the absence of atrophic gastritis and Ser/Ser genotype (adjusted OR, 5.83; 95% CI 2.52, 13.50). The interaction test was statistically significant (P=0.03).

Table 4 presents the joint effects on gastric cancer risk of *hOGG1* genotype and antioxidant dietary or nutrient intakes, including total vegetables, carotene-rich vegetables, cruciferous vegetables, total fruits, vitamin C, and β-carotene, all of which are considered possible protective factors for gastric cancer. Overall, we found inverse associations between these dietary or nutrient intakes and gastric cancer among subjects who had Ser/Ser genotype, whereas no such associations were found among subjects with the Cys allele. Significantly decreased gastric cancer risks were particularly evident among frequent cruciferous vegetable consumers with Ser/Ser genotype (adjusted OR of tertile 2, 0.24; 95% CI 0.06, 0.95, adjusted OR of tertile 3, 0.26; 95% CI 0.07, 0.95, respectively), whereas no inverse association was found between cruciferous vegetable intake and gastric cancer among those who had the Cys allele. No

Table 3. ORs for gastric cancer associated with *hOGG1* genotype according to potential gastric cancer risk factors

Variable	*n* (cases/controls)		Crude ORs (95% CI)			Adjusted ORs (95% CI)[1)]		
	hOGG1 genotype		*hOGG1* genotype		*P* for interaction	*hOGG1* genotype		*P* for interaction
	Ser/Ser	Ser/Cys+Cys/Cys	Ser/Ser	Ser/Cys+Cys/Cys		Ser/Ser	Ser/Cys+Cys/Cys	
Family history								
No	24/66	81/159	1.00 (reference)	1.37 (0.81, 2.33)		1.00 (reference)	1.36 (0.73, 2.55)	
Yes	8/8	29/38	2.63 (0.85, 8.19)	1.95 (1.01, 3.77)	0.34	3.35 (0.88, 12.77)	2.79 (1.24, 6.32)	0.51
Smoking status								
Never	11/38	39/99	1.00 (reference)	1.37 (0.63, 3.01)		1.00 (reference)	1.58 (0.63, 4.00)	
Past	10/15	33/47	3.51 (1.12, 10.98)	3.80 (1.52, 9.49)		4.02 (1.03, 15.64)	3.44 (1.24, 9.56)	
Current	11/21	38/51	2.85 (0.99, 8.27)	4.53 (1.75, 11.74)	0.85	3.44 (0.96, 12.26)	4.56 (1.50, 13.85)	0.74
Salt intake (g/1000 kcal, day)								
<5.6	14/24	37/66	1.00 (reference)	0.99 (0.46, 2.16)		1.00 (reference)	0.39 (0.15, 1.07)	
5.6–7.2	7/24	36/66	0.54 (0.19, 1.58)	0.94 (0.42, 2.10)		0.48 (0.14, 1.72)	0.75 (0.29, 2.00)	
>7.2	11/26	37/65	0.74 (0.28, 1.93)	0.98 (0.46, 2.10)	0.65	0.34 (0.10, 1.16)	1.12 (0.44, 2.87)	0.01
H. pylori antibody								
Negative	4/20	12/77	1.00 (reference)	0.83 (0.23, 3.02)		1.00 (reference)	0.49 (0.13, 1.93)	
Positive	28/54	98/120	3.15 (0.90, 10.94)	5.03 (1.53, 16.51)	0.36	2.83 (0.77, 10.37)	4.19 (1.23, 14.29)	0.15
Atrophic gastritis								
No	23/60	64/170	1.00 (reference)	0.89 (0.50, 1.58)		1.00 (reference)	0.85 (0.45, 1.63)	
Yes	9/14	46/27	1.85 (0.68, 5.06)	5.47 (2.57, 11.63)	0.04	1.57 (0.50, 4.92)	5.83 (2.52, 13.50)	0.03

1) ORs were mutually adjusted for family history of gastric cancer, smoking status, salt intake, *H. pylori* infection, atrophic gastritis, and JA membership.

Table 4. ORs for gastric cancer associated with *hOGG1* genotypes stratified by dietary or nutrient intakes

Variable (range; g/1000 kcal, day)	*n* (cases/controls)		Adjusted ORs (95% CI)[1)]		*P* for interaction
	hOGG1 genotype		*hOGG1* genotype		
	Ser/Ser	Ser/Cys+Cys/Cys	Ser/Ser	Ser/Cys+Cys/Cys	
Total vegetables (g/1000 kcal, day)					
Tertile 1 (<78.0)	13/20	39/69	1.00 (reference)	0.53 (0.19, 1.47)	
Tertile 2 (78.0–129.3)	8/25	39/67	0.45 (0.12, 1.72)	0.62 (0.21, 1.85)	
Tertile 3 (>129.3)	11/29	32/61	0.29 (0.07, 1.12)	0.71 (0.23, 2.19)	0.11
Vegetables containing carotenes≥600 μg/100 g (g/1000 kcal, day)					
Tertile 1 (<17.8)	13/24	40/64	1.00 (reference)	0.49 (0.18, 1.31)	
Tertile 2 (17.8–32.1)	9/25	32/69	0.26 (0.07, 1.02)	0.53 (0.19, 1.49)	
Tertile 3 (>32.1)	10/25	38/64	0.37 (0.10, 1.36)	0.78 (0.26, 2.34)	0.08
Cruciferous vegetables (g/1000 kcal, day)					
Tertile 1 (<27.7)	12/19	42/70	1.00 (reference)	0.42 (0.15, 1.17)	
Tertile 2 (27.7–46.2)	8/22	36/69	0.24 (0.06, 0.95)	0.45 (0.15, 1.36)	
Tertile 3 (>46.2)	12/33	32/58	0.26 (0.07, 0.95)	0.53 (0.17, 1.65)	0.053
Total fruit (g/1000 kcal, day)					
Tertile 1 (<42.6)	12/24	41/63	1.00 (reference)	0.79 (0.30, 2.08)	
Tertile 2 (42.6–92.5)	7/26	40/66	0.37 (0.09, 1.49)	0.89 (0.34, 2.34)	
Tertile 3 (>92.5)	13/24	29/68	0.69 (0.21, 2.25)	0.83 (0.29, 2.36)	0.35
Vitamin C (mg/1000 kcal, day)					
Tertile 1 (<47.4)	12/18	46/69	1.00 (reference)	0.48 (0.15, 1.51)	
Tertile 2 (47.4–77.7)	10/29	29/63	0.24 (0.06, 0.95)	0.34 (0.10, 1.14)	
Tertile 3 (>77.7)	10/27	35/65	0.28 (0.07, 1.20)	0.53 (0.16, 1.79)	0.20
β-Carotene (mg/1000 kcal, day)					
Tertile 1 (<0.64)	12/23	35/65	1.00 (reference)	0.44 (0.16, 1.24)	
Tertile 2 (0.64–1.14)	7/25	35/69	0.11 (0.03, 0.48)	0.49 (0.16, 1.44)	
Tertile 3 (>1.14)	13/26	40/63	0.67 (0.19, 2.44)	0.76 (0.25, 2.38)	0.057

1) ORs were adjusted for family history of gastric cancer, smoking status, salt intake, *H. pylori* infection, atrophic gastritis, and JA membership.

significant interaction was found between *hOGG1* genotype and these dietary or nutrient intakes.

Discussion

In the present study, a statistically significant interaction was found between Ser326Cys *hOGG1* genotype and atrophic gastritis (P=0.03). Furthermore, we divided atrophic gastritis into low (PG I level≤70 ng/ml and PG I/PG II ratio≤3.0) and high (PG I level≤30 ng/ml and PG I/PG II ratio≤2.0) severity of atrophic gastritis. Compared with the absence of atrophic gastritis and Ser/Ser genotype, very severe atrophic gastritis with the Cys allele was more strongly associated with gastric cancer (adjusted OR, 8.37; 95% CI 2.68, 26.12) than less severe atrophic gastritis with the Cys allele (adjusted OR, 4.73; 95% CI 1.87, 11.98). The interaction test between *hOGG1* genotype and the different severities of atrophic gastritis was also statistically significant (P=0.003). Atrophic gastritis led by superficial gastritis is a possible precursor of gastric cancer according to Correa's model.[38] Acute or chronic gastritis is characterized by infiltration of neutrophils, lymphocytes, macrophages/monocytes, and plasma cells into the gastric mucosa. These neutrophils and macrophages/monocytes produce ROS that could cause DNA damage. 8-OHdG is a major form of oxidative DNA damage induced by ROS,[13] and the presence of 8-OHdG residues in DNA produces GC-to-TA transversion. Therefore, the presence of 8-OHdG may lead to mutagenesis. Increased levels of 8-OHdG in gastric mucosal tissue have been associated with chronic atrophic gastritis, and significantly higher levels of 8-OHdG are found in tumor-adjacent and tumor tissues than in the normal tissue of gastric cancer patients.[39] hOGG1 is responsible for repairing 8-OHdG lesions, and several polymorphisms have been described in the *hOGG1* gene. One of these polymorphisms, a Ser-Cys substitution at codon 326, results in decreased DNA-repair activity, and decreased hOGG1 activity might be expected to increase gastric cancer risk, especially among those who are exposed to high levels of ROS generated by chronic gastritis. Our study revealed that the gastric cancer risk from atrophic gastritis was higher among those with the Cys allele than among those who have Ser/Ser genotype, indicating that those who have both decreased hOGG1 activity and atrophic gastritis have a strong susceptibility to gastric cancer.

As for the association between *hOGG1* genotype and antioxidant dietary or nutrient intakes in the present study, inverse associations were found between gastric cancer risk and intakes of total vegetables, carotene-rich and cruciferous vegetables, total fruits, total vitamin C and β-carotene among subjects who have *hOGG1* Ser/Ser genotype, whereas such associations were not found among subjects who have the *hOGG1* Cys allele. These results suggest that dietary antioxidant compounds might exert a strong protective effect against gastric cancer among subjects who have *hOGG1* Ser/Ser genotype. The mechanism underlying the absence of the protective effects of dietary antioxidant intake among subjects who have the *hOGG1* Cys allele remains unknown. The possibility cannot be excluded that the protective effects of these dietary or nutrient intakes on gastric cancer among those with *hOGG1* Ser/Ser genotype in the present study may actually be due to some other natural compound present at a high concentration in the same foods (phenols, flavonoids, isothiocyanates, etc.) rather than to antioxidant vitamins (vitamin C and β-carotene). The present study showed significantly decreased gastric cancer risks among frequent cruciferous vegetable consumers with Ser/Ser genotype (adjusted OR of tertile 2, 0.24; 95% CI 0.06, 0.95; adjusted OR of tertile 3, 0.26; 95% CI 0.07, 0.95, respectively). Cruciferous vegetables are the richest source of glucosinolates, which are converted by plant myrosinase and gastrointestinal microflora to isothiocyanates, which may protect against DNA damage by the induction of xenobiotic metabolizing enzymes.[40] Further studies are needed to evaluate the association between dietary or nutrient intakes and Ser326Cys *hOGG1* polymorphism.

To our knowledge, only three studies have reported on the association between Ser326Cys *hOGG1* polymorphism and gastric cancer, including our study, concluding that Ser326Cys *hOGG1* polymorphism is not related to the overall risk of gastric cancer.[23–25] Two previous studies examined the association between Ser326Cys *hOGG1* polymorphism and environmental factors that might be related to gastric cancer risk and/or oxidative stress.[24, 25] Hanaoka *et al*.[25] reported a significantly increased risk from smoking among non-Japanese Brazilians only among Ser/Ser genotype subjects, and no increased risk in those having that genotype together with the Cys allele. Takezaki *et al*.[24] reported that the ORs of habitual drinkers and frequent consumers of pickled vegetables and meat tended to be higher in Cys/Cys than in Ser/Ser or Ser/Cys carriers among Chinese subjects. No statistically significant interactions between Ser326Cys *hOGG1* polymorphism and environmental factors were found in the above two studies. In our study, gastric cancer risks from smoking did not differ between Ser326Cys *hOGG1* genotypes, and the ORs among drinkers with Ser326Cys *hOGG1* genotypes also did not differ (data not shown). We have no clear explanation for these discrepancies. Differences in genetic background or lifestyle patterns among non-Japanese-Brazilian, Chinese, and Japanese populations may explain to some extent the different risk estimates associated with Ser326Cys *hOGG1* genotype and environmental factors.

Our study has several strengths. First, we used a highly detailed questionnaire, including general characteristics, personal medical history, familial history of diseases, smoking and drinking history, and dietary factors. In the present study, significant differences between cases and controls were found among the factors of family history of stomach cancer, smoking status, *H. pylori* infection, and atrophic gastritis (P=0.047, 0.001, <0.0001, <0.0001, respectively), whereas only marginal differences were noted among JA members (P=0.054). Salt intake was not associated with gastric cancer risk in our study, though it could be a risk factor for gastric cancer, based on the results of many epidemiological studies.[41, 42] Thus, these factors were adjusted for when calculating ORs in relation to potential risk factors and dietary intakes. In addition to these factors, we further adjusted ORs for fruit and vegetable consumption (tertiles based on controls), since the antioxidative compounds derived from those foods may provide protection against gastric cancer. However, the gastric cancer risk did not change much in calculating the gastric cancer risk, whether fruit and vegetable consumption was adjusted for or not (data not shown). Second, serum was analyzed for PG I and PG II, and for IgG antibody to *H. pylori* and CagA in all subjects. *H. pylori* infection and atrophic gastritis are major risk factors for gastric cancer, and measurements of serum PG I and PG II, and of IgG antibody to *H. pylori* and CagA enabled us to diagnose whether or not subjects had an *H. pylori* infection or atrophic gastritis. Because *H. pylori* may disappear spontaneously with the progression of precancerous changes, the possibility of a lower estimation of *H. pylori* infection detected by anti-*H. pylori* IgG antibody has been suggested. CagA antibodies may persist longer than the antibodies detected by IgG ELISA, and thus the combination of IgG antibody with *H. pylori* and CagA is useful for evaluating the gastric cancer risk from *H. pylori* infection.[43] Since *H. pylori* infection was defined by both IgG antibody to *H. pylori* and CagA in our study, the possibility of a lower estimation of *H. pylori* infection might have been minimized.

One limitation of this study was the relatively small number

of subjects available for stratified analyses. Subdividing subjects into groups somewhat diminished the study's statistical power, and multiple comparisons also could have given rise to an α error. A second limitation is that, since exposure information was collected after the diagnosis of gastric cancer, differential dietary recall between cases and controls could bias results. These recall biases do not preclude the possibility of overestimating the protective effects of antioxidant dietary or nutrient intakes on gastric cancer. Finally, as in any retrospective study, selection bias also may have affected our results. As the controls were participants in a health check-up program, they were considered to be more health-conscious than the general population. The JA members were more familiar with health check-up programs than non-members. We performed careful multivariate adjustment for confounding factors, including JA membership, as mentioned above.

In this study, a significant gene-environmental interaction was found between Ser326Cys *hOGG1* genotype and atrophic gastritis. These findings suggested that those who have atrophic gastritis with *hOGG1* Ser/Cys or Cys/Cys genotype have a higher susceptibility to gastric cancer.

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