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Modifying Effect of Chronic Atrophic Gastritis on Radiation Risk for Noncardia Gastric Cancer According to Histological Type

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The findings from previously published studies have suggested that radiation exposure is associated with increased mortality and incidence of gastric cancer. However, few cohort studies have incorporated risk factors such as *Helicobacter pylori* (*H. pylori*) infection or chronic atrophic gastritis (CAG). The current study is aimed at evaluating the modifying effect of CAG on radiation risk of noncardia gastric cancer by histological type, by reanalyzing data from a nested case–control study conducted within the longitudinal clinical cohort of atomic bomb survivors. The analysis was restricted to 297 intestinal- or diffuse-type noncardia cases and 873 controls rematched to the cases on gender, age, city, and time and type of serum storage, and countermatched on radiation dose. Multivariable-adjusted relative risks [95% confidence interval (CI)] of noncardia gastric cancer were 3.9 (2.1–7.2) for *H. pylori* IgG seropositivity with cytotoxin-associated gene A (CagA) IgG low titer, 2.6 (1.9–3.6) for CAG, 1.9 (1.3–2.8) for current smoking, and 1.4 (1.1–1.9) for 1 Gy irradiation. Among subjects without CAG, the relative risk (95% CI) of noncardia gastric cancer at 1 Gy was 2.3 (1.4–3.7), whereas relative risk (95% CI) at 1 Gy was 1.1 (0.8–1.5) among subjects with CAG (for the overall interaction, $P = 0.012$). By histological type, the risk at 1 Gy was high for diffuse type without CAG, with adjusted relative risk (95% CI) of 3.8 (2.0–7.6), but was not high for diffuse type with CAG or for intestinal-type irrespective of CAG status. The results indicate that radiation exposure is associated with increased risk of diffuse-type noncardia gastric cancer without CAG, and this association exists despite adjustment for *H. pylori* infection and smoking habit.

INTRODUCTION

Gastric cancer ranks as one of the most common malignancies and the third cause of cancer mortality worldwide (*1*). Estimates from GLOBOCAN 2018 indicate that nearly 1,000,000 new cases occurred in 2018 with an estimated 783,000 deaths (*1*). The International Agency for Research on Cancer (IARC) classified *Helicobacter pylori* (*H. pylori*) as a group 1 carcinogen in 1994, and almost 90% of noncardia gastric cancer (arising from distal regions of the stomach) are now estimated to be caused by chronic *H. pylori* infection (*2*). Gastric cancer can be divided into two histological types: 1. Intestinal or well-differentiated, which includes papillary and tubular adenocarcinomas; and 2. Diffuse or poorly differentiated, which includes poorly differentiated adenocarcinoma and signet cell carcinoma (*3*). Recently reported evidence indicates that environmental factors such as lifestyle (*4*) and genetic factors, as well as *H. pylori* infection, synergistically affect the genesis of the two types of gastric cancer (*3*). The relationship of noncardia gastric cancer with *H. pylori* infection is stronger than that of cardia gastric cancer (arising in the area adjoining the esophageal-gastric junction), and it is pronounced in the gastric corpus and pyloric antrum of intestinal- and diffuse-types of gastric cancer. In particular, in intestinal-type noncardia gastric cancer, the initial stage is chronic gastritis (superficial gastritis) after *H. pylori* infection, followed by chronic atrophic gastritis (CAG), intestinal metaplasia, dysplasia and eventually adenocarcinoma. On the other hand, the diffuse type occurs with a low-to-moderate degree of gastritis.

It is well established that exposure to radiation is associated with increased risks of malignant diseases, including solid cancers. Studies among patients receiving

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radiotherapy have shown that high doses of radiation are associated with increased risk of gastric cancer (*5–10*). Significant association has not been observed between gastric cancer and low doses of occupational and environmental radiation exposure (*11–17*), while positive radiation risk estimate for gastric cancer has been observed in a cohort study of nuclear workers (INWORKS) (*18*). The risk estimate from the Mayak worker cohort has also demonstrated marginally significant radiation effect on mortality of gastric cancer (*19*). An increased risk of gastric cancer with radiation dose among atomic bomb survivors has been reported based on Life Span Study (LSS) mortality studies (*20*), tumor registries (*21*) and pathology review (*22*), but important risk factors such as *H. pylori* infection or smoking were not considered in those analyses. The LSS cancer incidence study demonstrated that excess relative risk per Gy (ERR/Gy) for gastric cancer was 0.21 for men and 0.47 for women (*21*). The latest LSS cancer incidence study also showed that sex-averaged relative risk for gastric cancer at age 70 was 0.33 per Gy (*23*). A pathology review of gastric cancer cases indicated that the poorly-differentiated type is more frequently observed in survivors with high doses than in controls and survivors with low doses (*22*).

Our earlier study (*24*) further demonstrated that radiation exposure is associated with increased risk of diffuse-type noncardia gastric cancer among nonsmokers, but not with risk of diffuse-type among smokers or with risk of intestinal-type regardless of smoking status. However, the earlier study did not show a joint effect of radiation and CAG status by histological type upon gastric cancer risk in detail. Furthermore, the earlier study cases comprised cardia cases and noncardia cases of unknown histology or histological types other than intestinal- or diffuse-types, with three controls per case matched to the cases on gender, age, city, and time and type of serum storage, and countermatched on radiation dose.

We hypothesized that radiation exposure affects certain steps of the multi-stage carcinogenesis process of gastric cancer. We have investigated how radiation exposure interacts with risk factors such as *H. pylori* infection, CAG and smoking, for noncardia gastric cancer in the carcinogenic process, by means of the earlier nested case–control study conducted within the longitudinal clinical cohort of atomic bomb survivors. Furthermore, in the current study, to evaluate the modifying effect of CAG status on radiation risk of noncardia gastric cancer by histological type, the reanalysis was restricted to intestinal- or diffuse-type noncardia cases and the available controls were rematched to these cases.

MATERIALS AND METHODS

Cohorts

The Atomic Bomb Casualty Commission (ABCC) and its successor, the Radiation Effects Research Foundation (RERF), established the Adult Health Study (AHS) cohort in 1958 for a prospective, longitudinal study in which more than 20,000 gender-, age- and city-matched proximal and distal atomic bomb survivors and persons who were not in the cities at the time of the bombings have been examined biennially in outpatient clinics in Hiroshima and Nagasaki. From 1969, serum samples have been stored systematically on each occasion of participants' visits.

Cases and Controls

Incident cancer cases were identified through the Hiroshima Tumor and Tissue Registry and Nagasaki Cancer Registry, where the histological classification was based at first on the Japanese Society for Gastric Cancer classification until 1986 and then on the WHO coding system (ICD-O, ICD-O-2 and ICD-O-3). As described in our previously reported work (*24, 25*), there were 719 gastric cancer cases diagnosed between January 1970 and December 2001 among AHS participants who visited our clinic before their diagnosis and provided serum samples on at least two separate occasions. There were 423 primary gastric cancer cases with histological diagnosis via pathologic review by E. Tahara; second and third primary gastric cancer cases were excluded. Of the primary cases, 356 cases had serum samples stored at the time of AHS examination 1 to 5 years (mean, 2.3 years) before diagnosis. After we excluded 22 cardia cases, 34 noncardia cases of histological types other than intestinal- or diffuse-types, two noncardia cases of unknown histology, and one case of unknown location (59 cases total), three controls per case were reselected from among the controls previously selected from at-risk cohort members. Control selection was made in nested case–control fashion, with matching to cases on gender, age ($\pm$5 years) at the time of serum collection, city, and calendar time ($\pm$1 year) of serum collection, and method of serum storage, and counter-matching on radiation dose (*26*). Noncardia gastric cancer cases were classified by intestinal-type and diffuse-type on the basis of microscopic appearance and growth patterns defined by Lauren (*27*). Characteristics of the 297 noncardia gastric cancer cases and 873 controls with full serologic data are shown in Table 1.

Laboratory Tests

Details about laboratory tests for anti-*H. pylori* IgG and anti-cytotoxin-associated gene A (CagA) IgG are described elsewhere (*24, 25*). Grade of atrophic gastritis was diagnosed by the criteria of Miki *et al*. (*28*) using pepsinogen (PG) measurements. Details about laboratory tests for PG I and II are described elsewhere (*24, 25*). Grade II (moderate positive) CAG was defined as PG I of less than 50 μg/l and PG I/PG II ratio of less than 3.0. Grade III (severe positive) CAG was defined as PG I of less than 30 μg/l and PG I/PG II ratio of less than 2.0. In this study, subjects with moderate or severe CAG were categorized as having CAG, and subjects with no or mild CAG were categorized as being without CAG.

Smoking Habit and Other Epidemiologic Information

Information on smoking status, alcohol consumption, education history and dietary habits was obtained from AHS interviews and mail surveys, as described elsewhere (*24, 25*). Body mass index was calculated from height and weight measured at the last clinic visit before diagnosis.

Radiation Dose

Radiation dose to the stomach was estimated for each subject according to Dosimetry System 2002 (DS02) (*29*). A weighted sum of the gamma dose in Gy plus 10 times the neutron dose in Gy was used. For improved efficiency in estimating radiation effects, we used the counter-matching method for case–control selection to obtain controls with a broader distribution of radiation doses than would be obtained

TABLE 1
Characteristics of Noncardia Gastric Cancer Cases by Histological Type and Controls

Study variables	Cases		Controls
	Intestinal-type	Diffuse-type	
Matched variables			
Number	163	134	873
Male/female	99/64 (61%/39%)	79/55 (59%/41%)	520/353 (60%/40%)
Age at time of bombing (years)[a]	28 (10)	26 (12)	26 (11)
Age at serum storage (years)[a]	69 (9)	65 (11)	67 (10)
Age at cancer diagnosis (years)[a]	72 (9)	67 (11)	-
Hiroshima/ Nagasaki	108/55 (66%/34%)	75/59 (56%/44%)	537/336 (62%/38%)
Unmatched variables			
Anti-*H. pylori* lgG(+)	144 (88%)	123 (92%)	687 (79%)
Anti-CagA IgG (negative/low/high)	42/24/97 (26%/15%/59%)	23/21/90 (17%/16%/67%)	244/88/541 (28%/10%/62%)
CAG(+)[b] (%)	121 (74%)	85 (63%)	414 (47%)
Current smoker	88 (54%)	82 (61%)	434 (50%)
Former smoker	11 (7%)	7 (5%)	51 (6%)
Stomach dose[c] (mGy)	320 (0, 3029)	426 (0, 2891)	276 (0, 3182)

[a] Mean (SD: standard deviation).
[b] CAG(+): moderate or severe.
[c] Mean (minimum, maximum). Mean dose among controls is a weighted mean with weight being the inverse of the sampling probability to correct for dose-dependent sampling of controls.
Abbreviations. H. pylori = helicobacter pylori; CagA = cytotoxin-associated gene A; CAG = chronic atrophic gastritis

by simple random selection from the cohort, in which survivors with low does predominate (*24, 26*).

Ethical Considerations

This study (RERF Research Protocol 2-04) was reviewed and approved by the RERF Research Protocol Review Committee and by the RERF Human Investigation Committee (the committee that functioned as the IRB at that time).

Statistical Analyses

The nested case–control design (*26*) is based on risk sets formed by associating each case with a set of controls chosen at random from all persons in the AHS cohort of the same age who were at risk (i.e., had not developed a cancer) at the time when the case was diagnosed. The relative risk (RR) was estimated using the conditional logistic regression model: $RR = e^{\beta_1 x_1 + \beta_2 x_2 + \cdots}(1 + \beta_0 D)$, where D is the radiation dose in Gy, the $x_i s$ are the other risk factor variables, and the $\beta_i s$ are coefficients to be estimated. In other words, relative risk for radiation was estimated via a linear excess relative risk, whereas relative risks for other factors were estimated via log relative risks. To estimate the radiation dose response separately for sub-groups (such as those with or without CAG), or those in four categories formed by cross-classification (by 1. CAG versus no CAG; and 2. intestinal- versus diffuse-type), we created an indicator variable for each sub-group and estimated a parameter for a term in the model consisting of that indicator times the radiation dose (multiplicative interaction term).

Conditional logistic regression models were fitted using the GMBO package of Epicure software (Risk Sciences International, Metcalfe, Canada). Confidence intervals (CI) are based on profile likelihood, and P values are for likelihood ratio tests.

RESULTS

Characteristics of Cases and Controls

By design, cases and controls were comparable with respect to gender, age, city, and time and method of serum storage. Because of counter-matching on radiation dose, doses among controls are not representative of doses among non-cases in the cohort, but the known sampling fractions can be used to correct for the biased sampling (Table 1). Mean ages at cancer diagnosis of intestinal-type and diffuse-type cases were 72 and 67 years, respectively, and approximately 60% were males among each histological type. Prevalence of *H. pylori* infection status and proportions of current smokers and persons with CAG among noncardia gastric cancer cases was higher than among controls. A higher proportion of diffuse-type cases were current smokers and a higher proportion of intestinal-type cases had CAG. Mean stomach dose was higher among diffuse-type cases than among intestinal-type cases, and mean doses among both case groups were higher than mean dose among controls.

Risk of Noncardia Gastric Cancer for H. pylori Infection Status, CAG, Smoking Habit and Radiation

In our earlier analysis (*24*), preliminary results suggested that potential and well-known risk factors (*30*) such as body mass index, education, alcohol consumption and dietary habits were not associated with risk of noncardia gastric cancer. Thus, we omitted these variables from the final model in the current analyses. After we excluded participants who had incomplete information on the risk factors used in our analysis, 287 strata (case–control sets) remained. Multivariable-adjusted relative risks (95% CI) of noncardia gastric cancer were 3.9 (2.1–7.2) and 1.9 (1.2–3.2) for *H. pylori* IgG seropositivity with CagA IgG low titer and high titer, 2.6 (1.9–3.6) for CAG, 1.9 (1.3–2.8) for current smoking, and 1.4 (1.1–1.9) for radiation at 1 Gy (Table 2). Multivariable-adjusted relative risks of noncardia gastric cancer were also

TABLE 2
Multivariable-Adjusted Relative Risks of Noncardia Gastric Cancer for Individual Factors

	Relative risk (95% CI)[a]	*P* value
H. pylori$^-$ CagA$^-$	(Reference)	
H. pylori$^+$ CagA$^-$	2.1 (1.2–3.9)	0.011
H. pylori$^+$ CagA^{low}	3.9 (2.1–7.2)	<0.001
H. pylori$^+$ CagA^{high}	1.9 (1.2–3.2)	0.004
CAG(−)	(Reference)	
CAG(+)	2.6 (1.9–3.6)	<0.001
Never smoker	(Reference)	
Current smoker	1.9 (1.3–2.8)	0.001
Former smoker	1.4 (0.4–2.8)	0.28
Stomach dose (at 1 Gy)	1.4 (1.1–1.9)	0.007

[a] With simultaneous adjustment for all factors: *H. pylori* IgG and CagA IgG status, CAG, smoking habit, and radiation dose to the stomach. Relative risk (at 1 Gy) was estimated using a linear excess relative risk model.

Abbreviations. *H. pylori* = *helicobacter pylori*; CagA = cytotoxin-associated gene A; CAG = chronic atrophic gastritis.

greater than unity for *H. pylori* IgG seropositivity with CagA IgG low titer, CAG, current smoking and stomach dose at 1 Gy (Table 2), indicating that these factors are associated with higher risk of noncardia gastric cancer even when they are mutually adjusted [which should control for confounding or indirect (mediating) effects].

Interaction between CAG and Radiation on Noncardia Gastric Cancer Risk by Histological Type

Multivariable-adjusted relative risk for 1 Gy dose for all noncardia gastric cancer without CAG (Table 3) was 2.3 (95% CI, 1.4–3.7; $P < 0.001$) based on a linear model for excess relative risk, with adjustment for *H. pylori* IgG and CagA IgG status and for smoking habit, whereas relative risk of all noncardia gastric cancer with CAG at 1 Gy was not statistically significant with similar adjustment (relative risk, 1.1; 95% CI, 0.8–1.5). Additional analyses were performed by adding indicators for the 159 intestinal-type case sets and the 128 diffuse-type case sets to examine the interaction between CAG and radiation on gastric cancer risk separately by histological type. Again, with adjustment for the other factors, exposure to radiation was associated with increased risk of diffuse-type noncardia gastric cancer without CAG: relative risk at 1 Gy was 3.8 (95% CI, 2.0–7.6, $P < 0.001$). However, statistically significant associations were not found between radiation and diffuse-type with CAG, or between radiation and intestinal-type irrespective of CAG status. The overall interaction of radiation and CAG for all noncardia gastric cancer (Table 3) was significant ($P = 0.012$). By histological type, the interaction of radiation and CAG in the diffuse-type stratum was statistically significant ($P = 0.002$), while that in the intestinal-type stratum was not ($P > 0.5$).

Interaction between CAG and Noncardia Gastric Cancer Risk Factors

Multivariable-adjusted relative risks in cases with CAG were greater than those in cases without CAG (Table 4), indicating that each of the factors is associated with higher risk of noncardia gastric cancer even with mutual adjustment. Furthermore, *H. pylori* infection and current smoking were significant risk factors for gastric cancer irrespective of CAG status, whereas relative risk of former smoking without CAG was not significant. Multivariable-adjusted relative risks for noncardia gastric cancer showed no significant difference between *H. pylori* IgG seropositivity with CAG and *H. pylori* IgG seronegativity with CAG, except for *H. pylori* IgG seropositivity with CagA low titer, with adjusted relative risk (95% CI) of 16.6 (7.4–39.7). These results indicate that CAG, *H. pylori* infection, and current smoking are important risk factors of gastric cancer occurrence, and the combination of *H. pylori* IgG seropositivity with CagA IgG low titer and CAG may exert a stronger effect on gastric cancer risk.

TABLE 3
Relative Risks for Radiation of Noncardia Gastric Cancer by Histological Type

	Adjusted[a] RR (95% CI)	*P* value	*P* value for interaction
All noncardia gastric cancer			
Stomach dose (at 1 Gy) with CAG(−)[b]	2.3 (1.4–3.7)	<0.001	0.012
Stomach dose (at 1 Gy) with CAG(+)[b]	1.1 (0.8–1.5)	>0.5	
Intestinal-type (159 strata)			
Stomach dose (at 1 Gy) with CAG(−)[c]	1.1(0.6–2.4)	>0.5	>0.5
Stomach dose (at 1 Gy) with CAG(+)[c]	1.2 (0.8–1.8)	0.41	
Diffuse-type (128 strata)			
Stomach dose (at 1 Gy) with CAG(−)[c]	3.8 (2.0–7.6)	<0.001	0.002
Stomach dose (at 1 Gy) with CAG(+)[c]	0.8 (0–1.5)	>0.5	

[a] Adjusted for *H. pylori* IgG and CagA IgG status, subtype, subtype-CAG interaction, and smoking habit. Relative risk (at 1 Gy) was estimated by using a linear excess relative risk model.

[b] An interaction between stomach dose and CAG status was included.

[c] An interaction between stomach dose and CAG status cross-classified by subtype was included.

Abbreviations. RR = relative risk; CAG = chronic atrophic gastritis.

TABLE 4
Unadjusted and Multivariable-Adjusted Relative Risks for Noncardia Gastric Cancer Risk Factors by CAG Status

Variable	CAG status	Unadjusted[a] relative risk (95% CI)	Adjusted[b] relative risk (95%CI)
H. pylori$^-$ CagA$^-$	CAG(−)	1 (reference)	1 (reference)
	CAG(+)	5.2 (2.2–12.7)	6.1 (2.5–15.5)
H. pylori$^+$ CagA$^-$	CAG(−)	3.2 (1.2–8.2)	3.0 (1.1–8.1)
	CAG(+)	7.7 (3.6–17.1)	8.3 (3.9–19.0)
H. pylori$^+$ CagA^{low}	CAG(−)	4.0 (1.4–11.0)	4.2 (1.5–12.0)
	CAG(+)	18.1 (8.3–42.4)	16.6 (7.4–39.7)
H. pylori$^+$ CagA^{high}	CAG(−)	3.2 (1.7–6.7)	3.3 (1.7–7.1)
	CAG(+)	6.2 (3.3–12.6)	6.7 (3.5–14.1)
Never smoker	CAG(−)	1 (reference)	1 (reference)
	CAG(+)	3.1 (2.1–5.2)	2.7 (1.8–4.6)
Current smoker	CAG(−)	1.9 (1.3–3.4)	1.9 (1.3–3.4)
	CAG(+)	5.5 (3.7–9.8)	5.0 (3.3–9.0)
Former smoker	CAG(−)	2.0 (0.7–5.2)	1.8 (0.6–4.8)
	CAG(+)	3.9 (1.6[c]–9.7[c])	3.3 (1.3[c]–8.2)

[a] Not adjusted for other factors, except that an interaction of CAG(+) with the reference category was included to allow for estimating the other interactions with the reference category for each factor, CAG interaction being the combination of that factor's reference category and CAG(−).

[b] For each factor (*H. pylori* IgG and CagA IgG status, smoking habit and stomach dose), the interaction with CAG is estimated as in the previous column with additional adjustment for the other two factors (but not including the CAG interaction for the adjustment factors).

[c] The likelihood bound could not be computed, so the Wald bound (based on the standard normal approximation for the estimate divided by its standard error) is reported.

Abbreviations. *H. pylori* = *helicobacter pylori*; CagA = cytotoxin-associated gene A; CAG = chronic atrophic gastritis.

DISCUSSION

Our in-depth reanalysis of data from a nested case–control study demonstrated that radiation exposure is associated with increased risk of noncardia gastric cancer among atomic bomb survivors despite concomitant adjustment for *H. pylori* infection and smoking habit. Specifically, risk of diffuse-type noncardia gastric cancer increased with radiation dose among participants without CAG but not among participants with CAG, and there was no association between intestinal-type noncardia gastric cancer and radiation dose irrespective of CAG status.

Epidemiological studies based on the LSS cohort of atomic bomb survivors have revealed that mortality (*20*) and incidence (*21, 23*) of gastric cancer increase in association with radiation dose. Sakata *et al*. demonstrated that sex-averaged ERR is 0.33 per Gy [relative risk (at 1 Gy) 1.33] at age 70. Their result was not adjusted for *H. pylori* IgG or CAG, but was adjusted for smoking. In the current study, the relative risk (at 1 Gy) was 1.4 (Table 2), with adjustment for smoking, *H. pylori* IgG and CAG. The average age at examination in the current study was 66 years for men and 68 for women. Thus, the overall relative risk in the current study (without interaction with either *H. pylori* IgG or CAG) is comparable to the estimate of Sakata *et al*.

Matsuura *et al*. (*22*) observed a tendency for the frequency of poorly-differentiated-type adenocarcinoma (poorly differentiated adenocarcinoma and signet ring cell carcinoma) to be in the high-dose group, based on 997 cases diagnosed between 1950 and 1977. The results based on 231 cases between 1964 and 1986 reported by Ito *et al*. (*31*) were similar, but the relationship between the frequency of poorly-differentiated-type adenocarcinoma and radiation dose was significant. Additionally, our previously published analysis conducted in the AHS cohort (*24*) showed that radiation risk is significant for diffuse-type noncardia gastric cancer among non-smokers (relative risk 4.0; $P = 0.046$), but not for diffuse-type among smokers or for intestinal-type irrespective of smoking status. However, multivariable analyses of interaction between histological type and other possible risk factors, except for CAG, did not indicate that radiation risk differs between diffuse- and intestinal-types.

H. pylori induces a chronic inflammatory reaction that causes intestinal metaplasia, which plays an important role in the multi-step process of human gastric carcinogenesis (*3, 32*). Carcinogenesis of the stomach is a multi-stage process and progression of epithelial cells from normal to tumor cells after *H. pylori* infection involves several stages: chronic gastritis (superficial gastritis), CAG, intestinal metaplasia, dysplasia, and adenocarcinoma. CAG might cause long-term oxidative stress in cancer progenitor cells or stimulate their proliferation, which is important for the progenitor cells to progress through the steps of multi-stage carcinogenesis (*33*). In the general population, diffuse-type gastric cancer develops within normal mucosa or chronic gastritis, whereas intestinal-type gastric cancer develops accompanied by CAG (*34*). In both histological types, *H. pylori* infection might play a role in development of gastric cancer, because the prevalence of *H. pylori*-negative gastric cancer, based on strict methods such as anti-body titer, microscopic observation, urea breath test, or rapid urease test, has been calculated to be only 0.66% in Japan (*35*). Radiation exposure might shorten the elapsed time of some steps in the multi-stage process of carcinogenesis by increasing genetic instability in cancer progenitor cells. However, it is unclear in which steps of the carcinogenic process radiation exposure might affect the onset of gastric cancer. It is also unclear whether radiation exposure is associated with the development of gastric cancer through progress to CAG.

Thus, we investigated whether radiation exposure is directly associated with increased risk of noncardia gastric cancer despite adjustment for *H. pylori* infection, CAG and smoking habit. We evaluated whether CAG status modifies risk of noncardia gastric cancer for radiation by histological type. Radiation exposure was associated with increased risk of diffuse-type noncardia gastric cancer without CAG. This result is consistent with previously published findings that risk of poorly differentiated (diffuse-type) gastric cancer is

higher in persons with higher radiation doses (*22, 31*). This suggests that radiation exposure might affect one or more carcinogenesis steps leading to diffuse-type noncardia gastric cancer, those that usually occur in the chronic gastritis stage in nonirradiated subjects. Our earlier studies also suggested that LTA genotypes (*25*) or *IL-10* genotypes (*36*) might be involved in the development of radiation-related diffuse-type gastric cancer. Such immune/inflammatory-related genetic factors may be associated with carcinogenesis steps in the chronic gastritis mucosa, and may alter individual differences in radiation-related cancer risk.

As with the results reported previously (*24, 25*), among *H. pylori* IgG seropositive subjects, those with CagA IgG low titer showed higher and more significant risk for future noncardia gastric cancer than those with CagA IgG seronegative or high titer. In this study, the combination of *H. pylori* IgG seropositivity with CagA IgG low titer and CAG showed a stronger effect on gastric cancer risk. We also found no significant difference between *H. pylori* IgG seropositivity with CAG and *H. pylori* IgG seronegativity with CAG, except for CagA IgG low titer with CAG. In this study, subjects with moderate or severe CAG were categorized as having CAG based on the criteria of Miki *et al*. (*28*) and thus, their risks of gastric cancer might have been underestimated. These results nevertheless are similar to those of a meta-analysis of studies conducted in Eastern Asians (*37*) or another Japanese cohort study (*38*).

The main strengths of our study include its prospective cohort-based design, which partially avoids selection bias, and the use of data obtained from stored sera and epidemiological information obtained prior to gastric cancer diagnosis. It is difficult and expensive to perform full cohort serum analyses, whereas the nested case–control design utilized here can provide substantial reductions in effort with little loss of statistical efficiency (*39*). Another strength is that gastric cancer cases categorized by histological type were identified through well-established cancer registries, and misclassification was reduced because the data were supplemented by additional cases detected by way of pathological review of materials from cases of related diseases. The novelty of the current analysis is that we newly evaluated the modifying effect of CAG on radiation risk for noncardia gastric cancer by histological type.

The main limitation of our study is the method used to diagnose gastric atrophy. Atrophic gastritis is usually diagnosed with endoscopy and biopsies, whereas the serum PG method has recently been used instead of photofluorography for screening to evaluate gastric mucosal status in Japan (*40, 41*). A cutoff value for PG I/II ratio of less than 3.0 would have identified intestinal metaplasia with a sensitivity of 71.7% and a specificity of 66.7% in participants who were positive for *H. pylori* (*42*). In the current study, we used serum pepsinogens as a marker of the topography of CAG, because it was difficult to perform an invasive examination, such as endoscopy, in the AHS health examinations. Although the PG method is useful to noninvasively assess atrophic change in gastric mucosa, the precision of diagnosis of CAG based on the PG method is inferior to that based on invasive endoscopy and biopsies. Another potential limitation, which exists in all observational studies, is unmeasured confounding. Although age and sex are not directly associated with radiation dose, they (in addition to city of residence at the time of exposure) are the potential correlates of gastric cancer most likely to be associated with unmeasured factors that are associated with radiation exposure, and they have been adjusted through the control-selection process. As for the interaction between radiation dose and CAG status, as explained by VanderWeele (*43*), heterogeneity of risk for radiation exposure can be established if confounders of the association between radiation and gastric cancer are adjusted, and if confounders of the association between CAG and gastric cancer are also adjusted, the interaction can be assumed to be causal. Age, sex and smoking are the most likely confounders of this latter relationship, and all of them have been adjusted.

In conclusion, radiation exposure was directly associated with increased risk of noncardia gastric cancer even after adjusting for *H. pylori* infection, CAG and smoking habit. In particular, radiation exposure was a significant risk factor for diffuse-type noncardia gastric cancer without CAG. The mechanism underlying the association between radiation exposure and diffuse-type gastric carcinogenesis from normal mucosa or chronic gastritis could not be examined. However, an in-depth understanding of the mechanisms by which radiation exposure contributes to the development of diffuse-type noncardia gastric cancer without CAG may lead to prevention, early diagnosis, and better therapeutic strategies, especially for those exposed to radiation.

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