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Effects of *IL-10* Haplotype and Atomic Bomb Radiation Exposure on Gastric Cancer Risk

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Gastric cancer (GC) is one of the cancers that reveal increased risk of mortality and incidence in atomic bomb survivors. The incidence of gastric cancer in the Life Span Study cohort of the Radiation Effects Research Foundation (RERF) increased with radiation dose (gender-averaged excess relative risk per Gy = 0.28) and remains high more than 65 years after exposure. To assess a possible role of gene-environment interaction, we examined the dose response for gastric cancer incidence based on immunosuppression-related *IL-10* genotype, in a cohort study with 200 cancer cases (93 intestinal, 96 diffuse and 11 other types) among 4,690 atomic bomb survivors participating in an immunological substudy. Using a single haplotype block composed of four haplotype-tagging SNPs (comprising the major haplotype allele *IL-10-ATTA* and the minor haplotype allele *IL-10-GGCG*, which are categorized by *IL-10* polymorphisms at −819A>G and −592T>G, +1177T>C and +1589A>G), multiplicative and additive models for joint effects of radiation and this *IL-10* haplotyping were examined. The *IL-10* minor haplotype allele(s) was a risk factor for intestinal type gastric cancer but not for diffuse type gastric cancer. Radiation was not associated with intestinal type gastric cancer. In diffuse type gastric cancer, the haplotype-specific excess relative risk (ERR) for radiation was statistically significant only in the major homozygote category of *IL-10* (ERR = 0.46/Gy, *P* = 0.037), whereas estimated ERR for radiation with the minor *IL-10* homozygotes was close to 0 and nonsignificant. Thus, the minor *IL-10* haplotype might act to reduce the radiation related risk of diffuse-type gastric cancer. The results suggest that this *IL-10* haplotyping might be involved in development of radiation-associated gastric cancer of the diffuse type, and that *IL-10* haplotypes may explain individual differences in the radiation-related risk of gastric cancer.

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INTRODUCTION

Even now, more than 60 years after the atomic bombings, rates of certain cancers in atomic bomb survivors are significantly elevated in a dose-dependent manner (*1–4*). Widely accepted mechanisms of radiation carcinogenesis include direct damage to cellular oncogenes and tumor suppressor genes, and other late effects by which mutations of these genes occur in later years (e.g., bystander effects; genomic instability), eventually leading to malignant transformation of directly-exposed, surrounding and offspring cells. No definite conclusion has been reached concerning whether the immune system is involved in these mechanisms. In the study of late effects of atomic bomb radiation, it is important to elucidate gene-radiation interaction—the impact of past radiation exposure may work differently on cancer risks of individuals who have different genetic backgrounds, particularly related to immune and inflammatory responses.

Some reports suggest that functional polymorphisms in genes regulating the immune and inflammatory response may contribute to susceptibility to, and clinical outcome with, gastric cancer (GC) (*5–7*). Interleukin-10 (IL-10) is an important immunoregulatory cytokine mainly produced by activated T cells, monocytes, B cells and thymocytes. It has important anti-inflammatory and immunosuppressive activities, including the ability to downregulate T helper 1 (Th1) cytokine and macrophage costimulatory molecule expression (*8*). IL-10 has been shown to inhibit various immune functions, such as antigen presentation, cytokine production, macrophage activation and antigen-specific T-cell proliferation (*9, 10*). By interfering with antigen-presenting cells, IL-10 reduces antigen-specific T-cell proliferation. It has been postulated that IL-10 plays a key role in the

oncogenic and metastatic ability of neoplasms (*11, 12*). However, a large body of evidence in different animal tumor models shows that IL-10 can favor immune-mediated cancer rejection (*13–17*). The *IL-10* gene comprises five exons and is located on chromosome 1q31-32 (*18*). The promoter region contains at least 40 polymorphic sites according to dbSNP (http://www.ncbi.nlm.nih.gov/sites/snp). Three polymorphic promoter variants of IL-10, located at positions −1082 (A>G), −819 (T>C) and −592 (A>C), are associated with IL-10 production (*19, 20*) and gastric cancer risk (*21, 22*). It has also been reported that *IL-10 −819 TT* genotype is protective against gastric cancer relative to the *CC* and *TC* genotypes in Asians (*23*) and that the IL-10 −1082 G allele is associated with an increased risk of cardiac gastric cancer in Asians (*24*).

Several studies have demonstrated a possible involvement of IL-10 in the pathogenesis of gastric cancer (*22, 25–28*). A nested case–control study previously carried out within the Adult Health Study (AHS) cohort of atomic bomb survivors at the Radiation Effects Research Foundation, using stored sera and blood cells (*29, 30*), reported that radiation increases the risk of noncardia gastric cancer of diffuse type but not intestinal type (*31*). The aim of the present study was to examine the relationship between risk of intestinal- or diffuse-type gastric cancer and radiation dose based on inflammation-related *IL-10* gene polymorphisms among atomic bomb survivors. The effects of *IL-10* genotype and radiation exposure on plasma IL-10 levels were also examined.

MATERIALS AND METHODS

Study Population

The Radiation Effects Research Foundation (RERF) conducted a cohort study within the AHS of approximately 20,000 atomic bomb survivors. The AHS is a clinical research program based on biennial health examinations established in July, 1957 as the clinical subcohort of the Life Span Study (LSS) cohort of atomic bomb survivors in Hiroshima and Nagasaki, Japan. AHS subjects were selected from the LSS cohort stratified according to distance from the hypocenter at the time of bombing and presence or absence of acute symptoms. Subjects for a broad immunology study including the genome study were selected from the AHS subjects because blood samples are not available from other members of the LSS. The clinical data collected during these examinations facilitate long-term follow-up studies of disease incidence and changes in physiological and biochemical endpoints, which benefit participants and contribute to health management of the atomic bomb survivors. Between 1981–2002, we obtained blood samples from 7,131 AHS participants who visited the clinic for examinations as part of the broader immunology study. After excluding subjects who had a history of first primary cancer at the time of blood collection, whose radiation dose could not be estimated, who were exposed *in utero* (organ doses not estimable), who were aged 80 or older at the time of blood collection or who refused to provide informed consent (84 subjects), 4,690 subjects remained for analysis [3,175 subjects provided informed consent (1,515 subjects who were deceased after blood collection were approved for this study by the RERF Ethical Committee)]: we call this the ''cancer and immuno-genome (IMG) cohort''. As a part of the LSS study, incident cancer cases were detected by the Hiroshima Tumor and Tissue Registries and the Nagasaki Cancer Registries, which also provided data on histological classification. Histological classification of gastric cancer was based on the Japanese Research Society for gastric cancer classification until 1986, and subsequently on the WHO coding system (ICD-O, ICD-O-2 and ICD-O-3), which was converted into Lauren's classification as reported elsewhere (*32, 33*). Baseline for follow-up was defined as the date of the first blood sample for the IMG study (collection began in 1981). The end of follow-up was December 31, 2001, the latest date of complete cancer ascertainment as of the time the data analysis was initiated. Follow-up of individual subjects ended on the date of first primary cancer onset, date of death, or the end of cohort follow-up, whichever occurred first. During the follow-up period (maximum 21 years), 200 gastric cancer cases (93 intestinal, 96 diffuse and 11 other types) were identified.

Baseline characteristics of cases and cohort subjects are shown in Table 1. We analyzed linkage disequilibrium (LD) with 300 controls, performed association analysis between *IL-10* haplotypes and plasma IL-10 levels in 644 noncancer cohort members, and conducted risk estimation for radiation and *IL-10* haplotyping using 200 cases amidst the 4,690 IMG cohort subjects.

Ethical Consideration

This study was approved by the Ethical Committee (Human Investigation Committee) and by the Ethics Committee for Genome Research at the RERF.

Measurement of Plasma IL-10 Levels

We measured plasma IL-10 levels in the IMG cohort members using a highly sensitive enzyme-linked immunosorbent assay kit (Quantikine HS, R&D systems, Minneapolis, MN). The minimum detectable dose of IL-10 was 0.5 pg/mL. Six hundred forty-four subjects who did not have a cancer history were randomly selected from the IMG cohort members to exclude an effect of cancer history on the relationship between *IL-10* haplotype and plasma IL-10 levels.

Identification and Genotyping of SNPs

The Celera Genomic database including Asian populations (*34, 35*) was used to screen haplotype-tagging (ht) SNPs in the *IL-10* gene region, along with the detection of novel SNPs over the region using the NCBI database. We selected the 19 htSNPs with allele frequency >5% among Japanese. After examining allele frequency in the study population, we found that ten of the 19 htSNPs showed variant allele frequencies >5% in our study population. We therefore selected these ten htSNPs: *IL-10* −92952C>T (IL10-1, rs1400986), −64871T>C (IL10-2, rs2073186), −32510A>G (IL10-3, rs4347211), −23055T>C (IL10-4, rs11583394), −14378T>C (IL10-5, rs880790), −1082T>C (IL10-6, rs1800896), −819G>A (IL10-7, rs1800871), −592T>G (IL10-8, rs1800872), +1177T>C (IL10-9, rs1518111), and +1589A>G (IL10-10, rs1554286). To reduce time and labor, 300 noncancer cohort members from the nonexposed group in the IMG cohort were analyzed to identify *IL-10* haplotypes for these 10 htSNPs. Primers and probes for these htSNPs were designed using Primer Express software, version 2.1 (Applied Biosystems, Foster City, CA). The TaqMan-Allelic Discrimination method was used for the detection of SNPs. All of the assays were conducted in 384-well PCR plates. The principle of TaqMan Real-Time PCR assay system using fluorogenic probes and the 5′ nuclease is described by Livak (*36*). Amplification reactions (5 μl) were carried out in duplicate with 10 ng of template DNA, 1× TaqMan Universal Master Mix buffer (Applied Biosystems), 300 n*M* of each primer and 200 n*M* of each fluorogenic probe. Thermal cycling was initiated with 2 min incubation at 50°C, followed by a first denaturation step of 10 min at 95°C, and then by 40 cycles of 15 s at 95°C and of 1 min at 60°C. After PCR was completed, the plates were brought to room temperature and read in an ABI PRISM 7900 Sequence Detection

TABLE 1
Characteristics of the Study Subjects within the RERF Immuno-Genome Cohort and Others

	Cases		Cohort		Subjects other than IMG cohort	
Total[a]	200 (100)		4,690 (100)		15,476 (100)	
Age at the time of bombings[b]	21 (1–43)		18 (0–43)		33 (0–79)	
Age at entry[c]	59 (38–80)		56 (37–80)			
Gender[a]						
Men	111 (55.5)		1,642 (35.0)		6,328 (40.9)	
Women	89 (44.5)		3,048 (65.0)		9,148 (59.1)	
City[a]	Men	Women	Men	Women	Men	Women
Hiroshima	77 (69.4)	65 (73.0)	1,013 (61.7)	2,064 (67.7)	4,362 (68.9)	6,731 (73.6)
Nagasaki	34 (30.6)	24 (27.0)	629 (38.3)	984 (32.3)	1,966 (31.1)	2,417 (26.4)
Radiation dose[a]	Men	Women	Men	Women	Men	Women
<5 mGy	53 (47.7)	28 (31.5)	710 (43.2)	1,246 (40.9)	3,736 (59.0)	5,328 (58.2)
5–728[d]	27 (24.3)	32 (36.0)	423 (25.8)	943 (30.9)	1,250 (19.8)	2,121 (23.2)
≥728	31 (27.9)	29 (32.6)	509 (31.0)	859 (28.2)	1,342 (21.2)	1,699 (18.6)
Smoking status[a]	Men	Women	Men	Women		
Nonsmoking	41 (36.9)	64 (71.9)	728 (44.3)	2,672 (87.7)		
Quit smoking	9 (8.1)	3 (3.4)	233 (14.2)	110 (3.6)		
Smoking	46 (41.4)	8 (9.0)	636 (38.7)	190 (6.2)		
Unknown	15 (13.5)	14 (15.7)	45 (2.7)	76 (2.5)		
Histological type[e]						
Intestinal type	93 (46.5)					
Diffuse type	96 (48.0)					
Other types	11 (5.5)					
IL-10 haplotype						
Major homozygote	71 (35.5)		2,048 (43.8)			
Heterozygote	93 (46.5)		1,988 (42.5)			
Minor homozygote	33 (16.5)		492 (10.5)			
Others[a,f]	3 (1.5)		145 (3.1)			

[a]Number (%).
[b]Median (range).
[c]Median (5–95% percentiles).
[d]728 mGy: median dose in exposed cohort members.
[e]Gastric cancer cases are classified by intestinal, diffuse and other types (the intestinal type included tubular and papillary carcinomas, the diffuse type included adenocarcinomas, mucinous adenocarcinomas, and signet-ring adenocarcinomas, and the other types were five cases with cancer in adenoma, one with squamous or adenosquamous carcinoma and five not otherwise specified cases). These histological types are counted individually.
[f]Seventeen of 4,690 subcohort subjects could not be determined one or two genotypes in the *IL-10* haplotype.

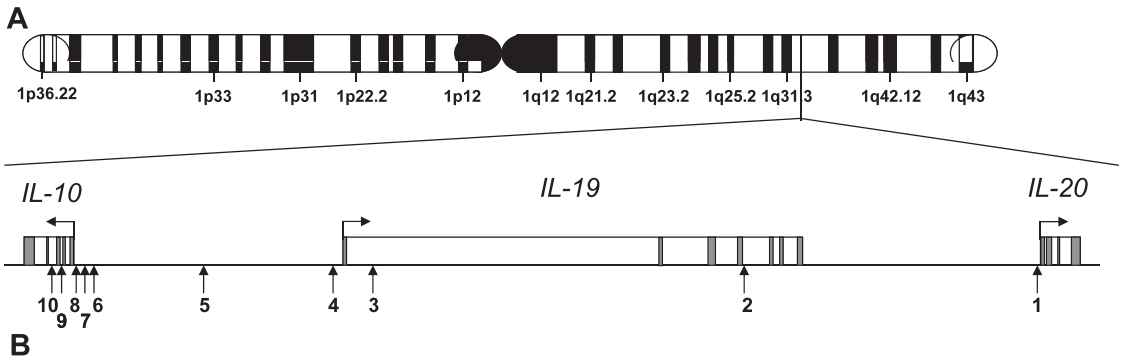
System (Applied Biosystems). Results were analyzed using the Allelic Discrimination software (SNPAlyze, Dynacom, Yokohama, Japan).

Haplotype and Radiation Risk Analysis

Rate ratios or excess rate ratios for gastric cancer incidence were estimated using general risk models extending the standard proportional hazards time-to-event analysis for cohort follow-up data, with attained age as the underlying time axis and left truncation on age at the time of blood collection (*37, 38*). Analyses were performed using the Cox regression module (Peanuts) of Epicure (version 1.81, Hirosoft Inc., Seattle, WA). All statistical models were stratified on gender and included adjustment for year of birth, city (Hiroshima or Nagasaki) and smoking intensity (cigarettes per day). City was included in the background in all analyses to adjust for any potential confounders, since *IL-10* haplotype-specific risk for radiation may possibly differ by city due to potential involvement of other gene polymorphisms that are mechanistically associated with *IL-10* functions and differ by city. Information on smoking was obtained by interview at the time of blood collection. *IL-10* haplotypes were determined as described below. Atomic bomb radiation dose in weighted Gray (wGy) was estimated using the DS02 dosimetry system (*39*), based on weighted skin dose computed as the gamma dose plus 10 times the neutron dose. A radiation dose <0.005 Gy was called nonexposed when performing analyses based on dose group.

Regression analysis of gastric cancer incidence was based on histological subtype – intestinal or diffuse. Cases in this study were primary gastric cancers, which included 17 synchronous multiple cancer cases that experienced primary cancer(s) of other organs within one year before or after gastric cancer diagnosis. Censoring was imposed at the time of other primary cancer diagnosis, death or the end of cancer follow-up (December 31, 2001). An excess relative risk (ERR: excess rate ratio) model was used for radiation risk estimation with adjustment for gender using stratification and adjustment for smoking intensity and year of birth using a log-linear model.

For *IL-10* haplotypes, indicator variables for the three categories (major homozygote, heterozygote and minor homozygote) were used to estimate either the rate ratio (RR, in the case of multiplicative models using the standard log-linear Cox model for *IL-10* haplotypes) or ERR (in the case of additive models for the joint effect of haplotyping and radiation), with the major homozygotes treated as the reference category. In this study, we considered only heterozygotes involving the most frequent (major) and second-most frequent (minor) alleles among the numerous heterozygotes that appeared in the process



B

	IL10-1	IL10-2	IL10-3	IL10-4	IL10-5	IL10-6	IL10-7	IL10-8	IL10-9
IL10-1									
IL10-2	0.23								
IL10-3	0.01	0.00							
IL10-4	0.04	0.00	0.67						
IL10-5	0.03	0.00	0.58	0.85					
IL10-6	0.00	0.01	0.01	0.01	0.02				
IL10-7	0.04	0.01	0.05	0.09	0.10	0.09			
IL10-8	0.04	0.01	0.05	0.09	0.10	0.09	1.00		
IL10-9	0.04	0.01	0.05	0.09	0.10	0.09	1.00	1.00	
IL10-10	0.03	0.01	0.05	0.09	0.11	0.09	0.99	0.99	0.99

FIG. 1. Identification of haplotype block. Panel A: 10 SNPs examined in the 95 kb region spanning the *IL-10*, *IL-19* and *IL-20* loci (arrows with numbers from 1 to 10). Panel B: LD analysis of the *IL-10* htSNPs. The r^2 values between the ten htSNPs are shown, indicating strong LD among IL10-7, −8, −9 and −10.

of LD analysis. Four genomic models for *IL-10* haplotype effects were considered: arbitrary (no structure among the three *IL-10* haplotypes; the ''categorical'' genomic model); ''linear'' (a codominant model where the effect in heterozygotes is assumed to be one-half of the effect in minor homozygotes); ''dominant'' (the presence of one or two minor haplotype alleles confers the same risk); and ''recessive'' (risk occurs only in minor homozygotes). Statistical interaction (effect estimate modification) between radiation and *IL-10* haplotypes was tested on both multiplicative and additive scales (*40*). The multiplicative joint risk model is $r(g,d) = e^{\delta g}[1 + \beta d]$ and the additive joint risk model is $r(g,d) = 1 + \phi g + \gamma d$, with g being the haplotype (a two- or three-level factor) and d the radiation dose (a continuous variable). Both models specify a rate ratio $r(g,d)$ that multiplies the background incidence (incidence among nonexposed persons).

RESULTS

Identification of Haplotype Blocks

The ten htSNPs (IL-10-1 to −10) are located on one gene region spanning about 95 kb in length (Fig. 1A). When we examined all combinations between the ten htSNPs for LD, four of ten htSNPs formed one haplotype block with r^2 values greater than 0.9 (Fig. 1B) and generated two major haplotype alleles, wild allele *IL-10 –ATTA* and variant one *IL-10 -GGCG* (IL-10-7, −8, −9 and −10). These two haplotype alleles constituted 98.1% of all haplotype alleles available in this block, 65.9% for wild *IL-10 -ATTA* and 32.2% for variant *IL-10 -GGCG*. Posterior probabilities for these two haplotype alleles exceeded 0.99, so these haplotypes were treated as known.

IL-10 Haplotypes and Plasma IL-10 Levels

As these htSNPs are located in untranslated regions including the promoter regions, we examined the relationship between *IL-10* haplotypes and plasma IL-10 levels in 664 cancer-free IMG cohort members. Mean plasma IL-10 protein levels were 2.79 pg/ml (SD 1.85, CV 0.68) among persons with the major homozygote *IL-10* haplotype, 3.02 pg/ml (SD 2.36, CV 0.78) among heterozygotes and 3.69 pg/ml (SD 2.97, CV 0.81) among minor homozygotes. Using log transformed IL-10 protein levels, regression analysis revealed no difference in IL-10 protein levels between major homozygotes and heterozygotes ($P = 0.39$), but minor homozygotes had significantly higher levels ($P = 0.030$). Using a linear model for *IL-10* haplotype, there was a significant trend in number of minor haplotyope alleles ($P = 0.034$). Radiation dose was related to log plasma IL-10 protein levels with a relative change in actual protein levels (not log transformed) of $e^{0.1278} = 1.14$ (14% increase) at 1 Gy ($P < 0.001$). These results suggest that plasma IL-10 levels varied not only by genetic factors but also by radiation exposure, and might therefore be closely associated with gastric cancer risk.

TABLE 2
Relative Risk of Intestinal Type Gastric Cancer for Radiation and *IL-10* Haplotypes using a Multiplicative Model

Model	Haplotype RR (95% CI, *P* value) (log-linear model, adjusted for radiation)				
P value is for heterogeneity test with categorical haplotype or for comparison with categorical haplotype	Major homozygote (*AA*)	Heterozygote (*Aa*)	Minor homozygote (*aa*)	Radiation ERR (95% CI, *P* value) (adjusted for haplotypes)	Interaction *P* value
Numbers of cases	31	41	20		
Categorical haplotype ($P = 0.071$) (*aa*) or (*Aa*) vs. *AA*[ref] AIC[b] = 910.022	1 (referent)	1.38 (0.83 – 2.33, 0.21)	**2.22 (1.10 – 4.25, 0.027)**	−0.05 (−0.19 – 0.23, >0.5)	NA[a]
Linear ($P > 0.5$) *aa*(1) > *Aa*(½) > *AA*[ref] AIC = 908.106	1 (referent)	exp {log(RR[*aa*]) × ½} = 1.47	**2.16 (1.11 – 4.17, 0.024)**	−0.05 (−0.19 – 0.23, >0.5)	>0.5
Dominant ($P = 0.17$) (*aa*,*Aa*) vs. *AA*[ref] AIC = 909.950	1 (referent)	1.55 (0.96 – 2.53, 0.071)		−0.05 (−0.19 – 0.23, >0.5)	0.32
Recessive ($P = 0.20$) *aa* vs. (*Aa*,*AA*)[ref] AIC = 909.646	1 (referent)		1.87 (0.98 – 3.30, 0.057)	−0.06 (−0.19 – 0.21, >0.5)	0.30

[a]NA, could not be estimated.
[b]Akaike's information criteria.

The incidence rate ratio of all gastric cancer combined for women compared with men was 0.41 [95% confidence interval (CI) 0.28, 0.58; $P < 0.001$]. Incidence increased with smoking intensity (RR = 1.24 among smokers of 10 cigarettes/day compared with nonsmokers; 95% CI 1.07, 1.43; $P < 0.001$) and year of birth (rate increased with more recent years of birth; $P < 0.001$). With those adjustments, crude RRs for *IL-10* haplotypes were: 1.26 (*ATTA*/*GGCG* heterozygote; 95% CI 0.91, 1.75; $P = 0.17$) and 1.59 (minor homozygote; 95% CI 0.98, 2.50; $P = 0.062$), with heterogeneity test $P = 0.13$. The linear genomic model was statistically significant: risk for the minor homozygote was 1.60 (95% CI 1.03, 2.48; $P = 0.038$), with the risk for the *ATTA*/*GGCG* heterozygote being one-half of the risk for minor homozygote on the log scale (i.e., RR = exp{[ln(1.60)]/2} = 1.26). The other two types of one-parameter genomic models (dominant and recessive) did not produce significant effects ($P = 0.066$ with the dominant model, $P = 0.13$ with the recessive model).

There was no evidence of interaction between gender and *IL-10* haplotypes ($P = 0.23$). Crude (adjusted only for gender, smoking and birth year, but not for *IL-10* haplotypes) ERR of all gastric cancers for radiation was 0.11 (95% CI −0.05, 0.34; $P = 0.22$) and after adjustment for *IL-10* haplotypes using the categorical genomic model the ERR for radiation was 0.12 (95% CI −0.05, 0.36; $P = 0.19$). The main effect of *IL-10* haplotypes after adjustment for radiation was essentially the same as the crude risk with each genomic model. The best fitting genomic model, and only statistically significant parameter, was with the linear genomic model. However, the apparent effects of radiation and *IL-10* haplotypes with all gastric cancers combined reflect large differences in their effects according to subtypes (see below), hence results for interaction between these two factors with all gastric cancers combined are not reported.

Results for Intestinal Type Gastric Cancer

Crude proportions of cohort members with intestinal type gastric cancer increased with number of variant (*GGCG*) haplotype allele: 1.5% among those with the major (*ATTA*) *IL-10* homozygote, 2.0% with the *ATTA*/*GGCG* heterozygote, and 3.9% with the minor homozygote. Incidence was about twice as high in Hiroshima as in Nagasaki (RR for Nagasaki 0.41; 95% CI 0.22, 0.71; $P = 0.001$) and was significantly related to smoking intensity (RR for smoking 10 cigarettes/day 1.38; 95% CI 1.12, 1.66; $P = 0.004$).

The cohort distribution of haplotypes (major homozygote, *ATTA*/*GGCG* heterozygote, minor homozygote) was similar in the two cities: (0.45, 0.44, 0.11) in Hiroshima and (0.46, 0.43, 0.11) in Nagasaki. Given no apparent association between city and *IL-10* haplotypes, the city difference would not be expected to confound the *IL-10* risk, and indeed there was no noticeable difference in *IL-10* risk parameters depending on whether or not city was included in the background model. There was no evidence of an effect of radiation: after adjustment of background incidence for gender, birth year, city and smoking (not adjusting for *IL-10* haplotypes), radiation ERR/Gy was −0.06 (95% CI −0.19, 0.21; $P > 0.5$).

Tables 2 and 3 show the jointly estimated risks for radiation and *IL-10* haplotypes with intestinal type gastric cancer. Main effects for radiation and haplotypes were similar with either the multiplicative or the additive model, so ERRs for radiation and haplotypes in the additive model

TABLE 3
Excess Relative Risk of intestinal Type Gastric Cancer for Radiation by *IL-10* Haplotypes using an Additive Model

Model[b]	Radiation ERR with interaction (95% CI, *P* value) (radiation ERR specific to haplotypes)			
P value is for heterogeneity test with categorical haplotype or comparison with categorical haplotype (crude risk)	Major homozygote (*AA*)	Heterozygote (*Aa*)	Minor homozygote (*aa*)	Interaction *P* value
Numbers of cases	31	41	20	
Categorical haplotype ($P = 0.071$) (*aa*) or (*Aa*) vs. *AA*[ref] AIC[b] = 910.022	−0.13 (NA – 0.20, 0.30)	0.25 (−0.21, 1.00, >0.5)	−0.58 (NA[a] – NA, 0.25)	0.23
Codominant ($P > 0.5$) *aa*(1) > *Aa*(½)>*AA*[ref] AIC = 908.337	−0.11 (NA – 0.28, 0.42)	—	0.14 (NA – NA, NA)	>0.5
Dominant ($P = 0.17$) (*aa*,*Aa*) vs. *AA*[ref] AIC = 909.950	−0.13 (−0.21 – 0.20, 0.30)	0.09 (−0.33 – 0.72, >0.5)		0.39
Recessive ($P = 0.20$) *aa* vs. (*Aa*,*AA*)[ref] AIC = 909.646	−0.02 (NA – NA, NA)		−0.53 (NA– NA, NA)	0.29

[a]NA, could not be estimated.
[b]Akaike's information criteria.

are not shown. With intestinal type gastric cancer, there was evidence of a significant effect of *IL-10* haplotypes after adjustment for radiation (Table 2). As with all gastric cancers combined, the fit of the categorical genomic model was similar to that of the linear genomic model, but the RRs were larger than with all gastric cancers combined. The linear genomic model had the lowest value of Akaike information criterion (AIC), and the categorical model produced risk estimates for the minor homozygote and the *ATTA/GGCG* heterozygote that were consistent with those from the linear model (compare $\exp\{\ln[2.16]/2\} = 1.47$ from the linear model with 1.55 for major heterozygotes).

Although the intestinal subtype evidenced no overall effect of radiation, it is possible that radiation effects might exist if there were biologically significant interaction between radiation and *IL-10* haplotypes. It was not possible to estimate or test interaction between radiation and particular *IL-10* haplotypes with the categorical genomic model, but none of the estimable radiation ERRs with interaction in the multiplicative model were very far from 0 (not shown) and none of the interaction tests for the one-parameter genomic models produced significant results (Table 2). With the additive model, again there were no statistically significant interactions (Table 3) and there was no evidence of trend in radiation ERRs with frequency of minor haplotype allele (radiation ERR was higher with the *ATTA/GGCG* heterozygote relative to the major homozygote but lower with the minor homozygote; Table 3).

Results for Diffuse Type Gastric Cancer

Crude proportions of cohort members with diffuse type gastric cancer evidenced no association with *IL-10* haplotypes: 1.9% with the major (*ATTA*) homozygote, 2.3% with the *ATTA/GGCG* heterozygote and 2.0% with the minor (*GGCG*) homozygote. Incidence of diffuse type did not depend on city ($P = 0.49$) and there was no impact on rate ratios for *IL-10* haplotypes depending on whether or not city was included in the background model. Smoking was not significantly related to diffuse type gastric cancer: RR for smoking 10 cigarettes/day compared with nonsmokers was 1.15 (95% CI 0.91, 1.40; $P = 0.23$). However, smoking was left in the analyses to avoid potential confounding. The crude ERR for radiation (after adjustment for gender, birth year, city and smoking but not for *IL-10* haplotypes) was statistically significant (ERR/Gy = 0.33; 95% CI 0.03, 0.83; $P = 0.027$).

Tables 4 and 5 show the jointly estimated risks for radiation and haplotype for diffuse type gastric cancer. With diffuse type, there was no evidence of an effect of *IL-10* haplotypes after adjustment for radiation (Table 4). There was no significant evidence of interaction between radiation and *IL-10* on either the multiplicative (Table 4) or additive (Table 5) scale. However, the haplotype-specific ERR for radiation was statistically significant only in the major homozygote category of *IL-10* (Table 5), being higher than the overall ERR for radiation, whereas estimated ERR for radiation with the minor *IL-10* homozygote was close to 0 and nonsignificant. Thus, the minor *IL-10* haplotype might act to reduce radiation-related risk of diffuse type gastric cancer.

DISCUSSION

We investigated the association between gene polymorphisms in the anti-inflammatory cytokine *IL-10* gene region

TABLE 4
Relative Risk of Diffuse Type Gastric Cancer for Radiation and *IL-10* Haplotypes using a Multiplicative Model

Model	Haplotype RR (95% CI, *P* value) (log-linear model, adjusted for radiation)				
P value is for heterogeneity test with arbitrary haplotype or for comparison with arbitrary haplotype	Major homozygote (*AA*)	Heterozygote (*Aa*)	Minor homozygote (*aa*)	Radiation ERR (95% CI, *P* value) (adjusted for haplotypes)	Interaction *P* value
Numbers of cases	38	46	10		
Categorical haplotype ($P > 0.5$) (*aa*) or (*Aa*) vs. *AA*[ref] AIC[a] = 1,039.889	1 (referent)	1.15 (0.73 – 1.82) >0.5	0.87 (0.35 – 1.84, >0.5)	**0.33 (0.03 – 0.83, 0.027)**	>0.5
Codominant ($P = 0.46$) *aa*(1) > *Aa*(½) > *AA*[ref] AIC = 1,038.444	1 (referent)	exp{log(RR[*aa*]) × ½} = 1.01	1.02 (0.52 – 1.94, >0.5)	**0.32 (0.03 – 0.82, 0.029)**	>0.5
Dominant ($P > 0.5$) (*aa*,*Aa*) vs. *AA*[ref] AIC = 1,038.338	1 (referent)	1.09 (0.71 – 1.71, >0.5)		**0.33 (0.03 – 0.82, 0.028)**	>0.5
Recessive ($P > 0.5$) *aa* vs. (*Aa*,*AA*)[ref] AIC = 1,038.153	1 (referent)	0.81 (0.34 – 1.64, >0.5)		**0.32 (0.03 – 0.82, 0.029)**	>0.5

[a]Akaike's information criteria.

and gastric cancer risk using a cohort study setting. *IL-10* haplotypes were significantly related to the risk of intestinal type gastric cancer, but not to the diffuse type. On the other hand, radiation was significantly associated with an increased risk of diffuse type gastric cancer. Regarding statistical interaction between radiation and *IL-10*, there was a suggestion of a trend towards lower radiation risk of the diffuse type with an increased number of variant *IL-10* haplotype allele, and only the *IL-10* major homozygote evidenced a statistically significant risk elevation by radiation when the radiation ERR was separately estimated by *IL-10* haplotype category. In addition, plasma IL-10 levels among noncancer cohort members were associated with *IL-10* haplotypes and increased with radiation dose, demonstrating the functional significance of this *IL-10* haplotyping and implying a joint effect of genetic factors and radiation in IL-10 expression.

Although the linear model was the best-fitting genomic model, we cannot conclude based on statistical criteria that it is the correct model. Furthermore, four genomic models were tested, leading to the possibility of spurious findings due to multiple testing. A Bonferroni correction would result in reducing the significance level from 0.05–0.0125, but due to a high degree of correlation among the genomic models the Bonferroni correction is inappropriate and a multiple-testing correction taking the correlation into account would not result in much change of significance level. We are therefore confident that the effect of *IL-10* for intestinal type gastric cancer based on the linear genomic model ($P = 0.038$) is unlikely to be a spurious result.

Observational studies lack power for detecting statistical interactions (*40*), and biological interactions—usually defined as departure from additive effects (*41*)—can therefore go undetected. The advantages of our study are long-term follow-up, detailed dosimetry reconstruction, and a well-defined radiation-exposed population. A limitation is the small number of subjects, particularly cases of gastric cancer, due to the size of the original cohort and exclusion criteria. Thus, lack of statistical significance does not necessarily imply lack of meaningful biological interaction. With intestinal type gastric cancer, not only were the interaction tests not statistically significant, the haplotype-specific radiation ERR estimates did not suggest any consistent pattern, being higher among the major heterozygotes and lower among the minor homozygotes, compared with the major homozygote. Nevertheless, were such a pattern biologically significant, it could explain the absence of overall radiation effect for intestinal type gastric cancer because the haplotype-specific ERRs would tend to cancel in the overall (no interaction) radiation ERR estimate. With diffuse type gastric cancer, there was some evidence of lack of additivity for joint effects of *IL-10* and radiation, with radiation risk being high and statistically significant among the major homozygote but low and nonsignificant among the minor homozygote. The lack of statistically significant interaction test in the additive model could be due to lack of power. Indeed, there was no statistically significant interaction on either the additive or multiplicative scale, but one or the other must require a statistical interaction term depending on which is the more appropriate model representing the joint biological effects (*42*).

To increase statistical power we included synchronous gastric cancer cases diagnosed within one year of other, first-primary cancer diagnoses. Although some of these cases could have been diagnosed through greater surveillance resulting from prior cancer, we think that is unlikely in

TABLE 5
Excess Relative Risk of Diffuse Type Gastric Cancer for Radiation by *IL-10* Haplotypes using an Additive Model

Model [b]	Radiation ERR with interaction (95% CI, *P* value) (radiation ERR specific to haplotypes)			
P value is for heterogeneity test with arbitrary haplotype or comparison with arbitrary haplotype (crude risk)	Major homozygote (*AA*)	Heterozygote (*Aa*)	Minor homozygote (*aa*)	Interaction *P* value
Numbers of cases	38	46	10	
Categorical haplotype ($P > 0.5$) (*aa*) or (*Aa*) vs. *AA*[ref] AIC[b] = 1,039.889	**0.46** **(0.02 – 1.43, 0.037)**	0.29 (−0.25 – 1.14, 0.33)	0.12 (NA[a] – NA, >0.5)	>0.5
Codominant ($P = 0.46$) *aa*(1) > *Aa*(½)>*AA*[ref] AIC = 1,038.444	**0.45** **(0.02 – 1.33, 0.034)**	—	0.08 (NA – 1.08, >0.5)	>0.5
Dominant ($P > 0.5$) (*aa*,*Aa*) vs. *AA*[ref] AIC = 1,038.338	**0.46** **(0.02 – 1.43, 0.037)**	0.25 (−0.23 – 0.97, 0.33)		>0.5
Recessive ($P > 0.5$) *aa* vs. (*Aa*,*AA*)[ref] AIC = 1,038.153	**0.34** **(0.03 – 0.88, 0.027)**		0.10 (NA – NA, >0.5)	>0.5

[a]NA, could not be estimated.
[b]Akaike's information criteria.

the case of gastric cancer. It is also unlikely that such cases are due to treatment for prior cancer because of the induction and latency periods for cancer growth and detection. However, we repeated the analyses of Tables 2–5 using as cases only first primary gastric cancers, with multiple cancer diagnoses (other cancer diagnosed at the same time as gastric cancer) or nonfirst-primary gastric cancers (gastric cancer diagnosed within one year of prior first-primary cancer) treated as censored at the time of first cancer diagnosis (the same as was done with all other cancer diagnoses in the analysis). There was no significant impact on results, except for slightly lower precision as would be expected with fewer cases. For example, with diffuse type gastric cancer, numbers of first-primary cases were 36, 44 and 10 (versus 38, 46 and 10 in Tables 4 and 5). The radiation ERR among major homozygotes was the same (0.45) but with slightly wider confidence interval (0.02–1.36 vs. 0.02–1.33) and slightly higher *P* value (0.038 vs. 0.034).

It is difficult to directly compare our results with those of previous studies in the atomic bomb survivors (*31*) because the nested case-control study that examined subtypes of gastric cancer involves a separately selected sample of cases with different follow-up period and the Life Span Study incidence analyses do not include subtype. Subtype will be considered in future LSS analyses but results are not yet available.

The Gram-negative bacterium Helicobacter pylori (H. pylori) is a well established etiologic factor, which works to elicit a chronic inflammatory response in the gastric mucosa and closely relates to the development of intestinal type gastric cancer (*43*). Differing inflammatory responses among hosts may help to explain different outcomes for persons infected with H. pylori, and therefore, consideration of *H. pylori* infectious status as well as gene polymorphisms involved in the host response to this infection might help further estimation of the radiation risks for *H. pylori*-infected and noninfected populations. This will be considered in future studies.

Regulatory mechanisms of immune suppression and inflammatory cytokine production inhibition of IL-10 are not fully understood. However, immune suppression of IL-10 may play an important role in the development of gastric cancer. From these results, we assessed the possibility of measurement of surrogate biomarkers, such as plasma IL-10 levels, for real-time estimation of gastric cancer risk as a model for prevention of gastric cancer. This idea came to us because the findings suggested that plasma IL-10 level is affected by both genetic and environmental factors, such as radiation exposure, and is closely associated with gastric cancer risk. We hope that further details of the relationship between gastric cancer risk and biomarkers clarified by future studies will enable us to better estimate gastric cancer risk, contributing to innovative preventive measures in atomic bomb survivors as well as other populations exposed to high levels of radiation in general, on the basis of both genetic and radiation factors.

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