specific risks exists, while current analyses suggest some differences much of the observed variability is consistent with random variation because formal statistical tests generally lack the power to detect real differences.

In summary, the updated solid cancer incidence data indicate that the shape of the dose response is well described by a linear model. Solid cancer excess rates increased throughout life for all ages, while excess relative risks decreased with increasing age. Excess risks for all solid cancers were higher for women than men, and lifetime risk estimates were considerably larger than for leukemia. The relatively small number of cancers for most individual sites made it difficult to identify statistically significant differences in age-time patterns. While overall patterns were similar to those seen in previous analyses, we continue to find new results with each new follow-up.

A large proportion of the radiation-associated excess solid cancers are likely to occur over the next 15 to 20 years. We therefore expect that the accumulating data will continue to offer important new insights into radiation effects on cancer risks. Continued follow-up is necessary to understand risk patterns for persons less than age 20 years at the time of the bombings. Additional site-specific incidence studies incorporating pathological reviews will provide needed information on the radiation-sensitivity of specific histologies. With close collaboration among statisticians, epidemiologists, biologists and pathologists; we should be able to improve our understanding of these data and their implications for radiation protection.

RESEARCH COMMUNICATION

Minimal Sizes of Cases with a Susceptible Genotype and Minimal Odds Ratios among Susceptible Individuals in Case-control Studies

Nobuyuki Hamajima[1], Hironori Mutoh[2], Hidetaka Eguchi[3], Hiroyuki Honda[2]

Abstract

Objective: Disease risk elevation due to an environmental factor only for individuals with a susceptible genotype is a typical example of gene-environment interaction. In order to identify risk factors interacting with susceptible genotypes in case-control studies, presumptions on minimal size of cases with the susceptible genotype (S_{min}) and odds ratio (OR) among the susceptible individuals ($OR_{susceptible}$) are useful.

Model: Proportion of exposed cases (P_1) and OR for whole cases (OR_{whole}) statistically detectable in a case-control study can be calculated in a conventional method. P_1 was assumed to be a weighted sum of the exposed among cases with the genotype (P_x) and cases without the genotype (equal to proportion of the exposed among controls, P_0), i.e., $S\ P_x + (1 - S)\ P_0$, where S is the size (proportion) of cases with the genotype. For each calculated P_1, S became the minimum (S_{min}) in case of $P_x = 1$. $OR_{susceptible}$ was calculated by $\{P_x (1 - P_0)\} / \{(1 - P_x) P_0\}$.

Results: S_{min} and $OR_{susceptible}$ were listed for the combinations of the above components. For example, a detectable P_1 was 0.638 for P_0=0.5 in a case-control study with 200 cases (N_1) and 200 controls (N_0), when α error of a two-sided test was 0.05 with an 80% of power. In case of P_1=0.638, OR_{whole} was 1.77, producing S_{min}=0.277 for infinite $OR_{susceptible}$. It indicates that an environmental factor cannot be detected in case that a high-risk genotype frequency is less than 0.277.

Interpretation: If the size of cases with a susceptible genotype is expected to be less than S_{min}, case-control studies are unlikely to detect a significant OR of the environmental factor.

Key Words: gene-environment interaction – genetic polymorphism – sample size – case-control studies

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Introduction

Recent development of genotyping methods allows us to examine the hypothesis that environmental factors cause a disease for individuals with a susceptible genotype. Although not perfect, it was exemplified by the finding that smoking causes lung cancer more frequently in those with low enzyme activity genotypes of carcinogen detoxification enzyme genes (Kiyohara et al., 2002; Mohr et al., 2003). Epidemiologically, such phenomena are termed as a gene-environment interaction, which is defined with a relative risk ratio of environmental exposure for those with a genotype relative to those without it, or a relative risk ratio of genotype for the exposed relative to the unexposed (Khoury and Flanders, 1996; Hamajima et al., 1999; Brennan, 2002). Since the elucidation of the interactions is useful for individualized disease prevention, researches on the interactions have been becoming popular in the field of epidemiology (Mucci et al., 2001; Kang, 2003). The targeted genotypes are selected from commonly observable ones, which are called "polymorphism" genotypes.

When the genotype interacting with an environmental factor is known, a sample size to detect the odds ratio (OR) of the factor in a case-control study can be calculated based

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on the genotype frequency with a conventional method (Hwang et al., 1994; Garcia-Closas and Lubin, 1999). On the contrary, the sample size cannot be calculated in case that the genotype frequency is unknown. In order to detect environmental factors in case-control studies including both subjects with and without the susceptible genotype, we had better have presumptions on the size (proportion) of individuals with the genotype and the OR among them. This paper aims to demonstrate minimal size of cases with the susceptible genotype to detect a significant environmental factor in case-control studies, as well as minimal required OR for individuals with the susceptible genotype.

Statistical Models

We recognized that there was a subgroup of cases with a genotype susceptible to an environmental factor. In order to calculate minimal detectable odds ratios of the environmental factor among those with the genotype ($OR_{susceptible}$), the following steps were made, as shown in Chart.

2.1. A proportion of exposed cases (P_1) producing a significant result in a case-control study with N_0 controls and N_1 cases was calculated based on a significance level (α), statistical power ($1-\beta$), and proportion of exposed controls (P_0), using the below conventional formula for a sample size calculation (Donner, 1984).

$$N_0 = \frac{[\,Z_\alpha\sqrt{(1+M)\,\bar{P}\,(1-\bar{P})} + Z_\beta\sqrt{M\,P_0\,(1-P_0) + P_1\,(1-P_1)}\,]^2}{M\,(P_0 - P_1)^2}$$

where P is defined with $(P_0 + M\,P_1) / (1 + M)$, M with the ratio of N_1 / N_0, and Z_α and Z_β with the values derived from a normal distribution with mean=0 and variance=1 for a given significance level (α) and statistical power ($1-\beta$), respectively.

2.2. Odds ratio for whole subjects (OR_{whole}) was obtained by $P_1\,(1-P_0) / P_0\,(1-P_1)$.

2.3. P_1 was also defined with a weighted average calculated by $S\,P_x + (1 - S)\,P_0$, as shown in Fig 1. In this formula, P_x and P_0 were the proportions for the exposed in cases with and without the susceptible genotype, respectively. S was the size in proportion for cases with the genotype. It was assumed that the environmental exposure does not elevate the risk of disease for cases without the genotype. Accordingly, the proportion of the exposed among them was set to be the same as that among the controls, i.e., P_0.

2.4. S_{min} was defined as the S in case of $P_x=1$. It was the minimum of S, because P_x was the maximum at 1.

2.5. $OR_{susceptible}$ was calculated with $\{P_x\,(1-P_0)\}/\{P_0\,(1-P_x)\}$.

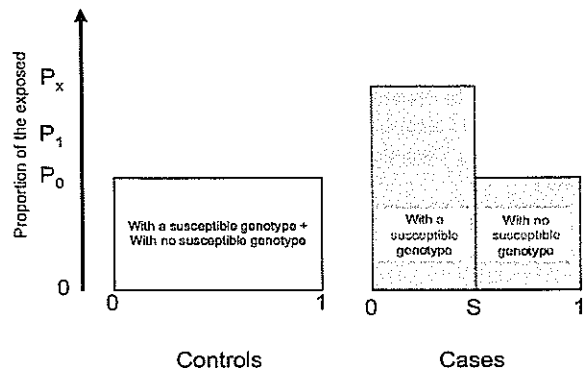


Figure 1. Proportions of the Exposed among Controls (P_0) and Cases (P_1). P_1 is the Average Proportion for Cases with a Susceptible Genotype (P_x) and Cases with no Susceptible genotype (P_0). The Area Surrounded by a Dotted Line is the Same as the Shadowed Areas. S is the Size in Proportion of Cases with a Susceptible Genotype.

Results

Since a large number of combinations exist, those with α=0.05 in a two-sided test (Z_α=1.96), $1-\beta$=0.80 (Z_β=0.842), and $N_0=N_1$ (M=1) were calculated as examples. Table 1 shows the calculated P_1, OR_{whole}, and S_{min}, when N_0 is fixed to be 200, 500, 1,000, or 2,000, and P_0 to be 0.05, 0.1, 0.3, 0.5 or 0.8. For example, a detectable P_1 was 0.638 for P_0=0.5 in a case-control study with 200 cases (N_1) and 200 controls (N_0), when α error of a two-sided test was 0.05 with an 80% of power. In case of P_1=0.638, OR_{whole} was 1.77, producing S_{min}=0.277 for infinite $OR_{susceptible}$. It indicates that an environmental factor cannot be detected in case that a high-risk genotype frequency is less than 0.277. Figure 2 depicts the relationship between S_{min} and N_0 for given P_0. The minimal size of cases with the genotype (S_{min}) increased with the proportion of the exposed in controls (P_0) and decreased with the number of controls (N_0).

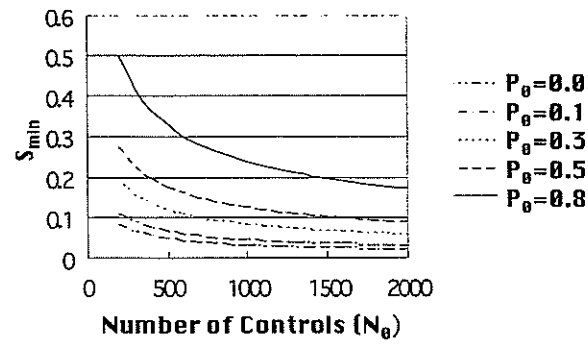


Figure 2. Minimal Size of Susceptible Cases Enabling to Detect a Significant Odds Ratio (S_{min}) According to Sample Sizes (N_0, in Case of $N_0=N_1$) and Proportion of the Exposed among Controls (P_0)

Table 1. Detectable Proportion of the Exposed among Cases (P_1), Odds Ratio for Whole Subjects (OR_{whole}), Minimal Size of Cases with a Susceptible Genotype (S_{min}) according to Number of Controls (N_0) and Proportion of Exposed Controls (P_0), under a Significance Level (α) = 0.05 for a Two-sided Test with Statistical Power (1-β) =0.8

N_0	P_0=0.05	P_0=0.1	P_0=0.3	P_0=0.5	P_0=0.8
			P_1		
200	0.130	0.200	0.435	0.638	0.900
500	0.096	0.160	0.384	0.588	0.866
1,000	0.081	0.141	0.359	0.563	0.848
2,000	0.071	0.128	0.341	0.544	0.834
			OR_{whole}		
200	2.84	2.25	1.79	1.77	2.25
500	2.02	1.71	1.45	1.43	1.62
1,000	1.67	1.47	1.31	1.29	1.39
2,000	1.46	1.32	1.21	1.19	1.26
			S_{min}		
200	0.084	0.111	0.192	0.277	0.499
500	0.048	0.066	0.120	0.176	0.330
1,000	0.033	0.045	0.084	0.125	0.239
2,000	0.022	0.031	0.059	0.088	0.171

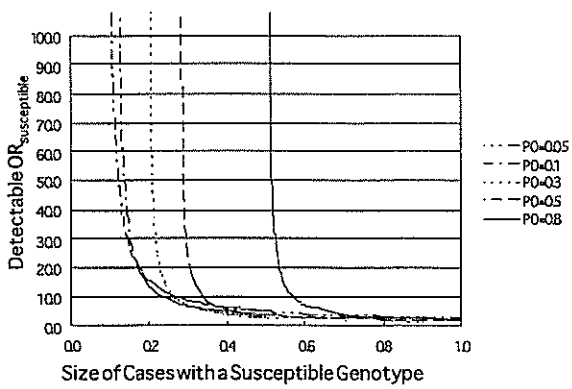
Figure 3 shows $OR_{susceptible}$ in a case-control study with 200 cases and 200 controls according to size of cases with the genotype (S) and proportion of the exposed controls (P_0). Since all the cases with the genotype were to be the exposed at S_{min}, the $OR_{susceptible}$ was infinite at S_{min}. In case of $S > S_{min}$, the $OR_{susceptible}$ decreased with S, and was equal to OR_{whole} at S=1. Figure 4 shows $OR_{susceptible}$ in case of P_0= 0.5 according to N_0 (=N_1). As N_0 was larger, $OR_{susceptible}$ was smaller in a given S. Table 2 lists the detectable $OR_{susceptible}$ according to S for different P_0 and N_0.

The above results can be used for the following examples. 1) When a case-control study has only 200 cases (N_1) and 200 controls (N_0), smoking can not be evaluable as a risk factor of male colon cancer in the following condition. Those with the susceptible genotype (S) are assumed to be 20% among the cases, and smokers are 50% among the controls (P_0). Table 1 provides S_{min} = 0.277 for N_0=N_1=200 and P_0=0.5, which is larger than the assumed S (0.2). 2) When a 30% of male colon cancer cases (S) have a genotype susceptible to smoking, $OR_{susceptible}$ more than 3.85 would be detected in a case-control study with 500 male cases (N_1) and 500 male controls (N_0), in an area where smokers are 50% among the male population (P_0) as indicated in Table 2.

Discussion

We know intuitively that risk factors affecting a small proportion of individuals may not be detected in a study, because of the effect dilution. Accordingly, even with a high penetrance, rare genotypes are not examined in association studies. As Shpilberg et al stated, "A twofold risk for 1000 exposed versus nonexposed people could be an average twofold risk for all 1000 exposed or a 20-fold risk for 100 exposed individuals" (Shpilberg et al., 1997). In case-control studies, however, there were no reference tables on the proportion of susceptible individuals. To date, several papers have been reporting required sample sizes for unmatched case-control studies to detect a gene-environment or gene-gene interaction (Hwang et al., 1994; Garcia-Closas and Lubin, 1999; Gauderman, 2002a; Gauderman, 2002b, Selinger-Leneman et al., 2003). But, their view is different from the present report. Tables and Figures presented in this paper provide useful information to avoid studies impossible to detect the significant results. The newly introduced concept, S_{min}, is an important measure when case-control studies are planned taking account of a susceptible subgroup in the study subjects.

In the present paper, the size of susceptible cases was



Figire 3. Detectable Minimal $OR_{subgroup}$ in a Case-control Study with 200 Cases and 200 Controls According to Size of Cases with a Susceptible Genotype (S) and Proportion of the Exposed among Controls (P_0)

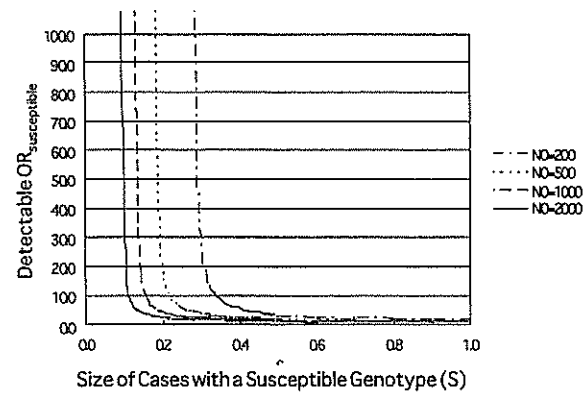


Figure 4. Detectable Minimal $OR_{subgroup}$ in a Case-control Study with Half of the Controls Exposed (P_0=0.5), According to Size of Cases with a Susceptible Genotype (S) and Number of Controls (N_0)

Table 2. Detectable OR for Individuals with a Genotype Susceptible to Environmental Factor ($OR_{susceptible}$) according to Size of Cases with the Susceptible Genotype (S), Proportion of Exposed Controls (P_0), and Number of Controls (N_0), under a Significance Level (α) = 0.05 for a Two-sided Test with Statistical Power (1-β) =0.8

N_0	S=0.1	S=0.2	S=0.3	S=0.5	S=0.7	S=1
P_0=0.05						
200	107	15.5	8.80	5.05	3.73	2.84
500	19.8	7.40	4.86	3.15	2.49	2.02
1,000	10.7	4.90	3.44	2.40	1.98	1.67
2,000	6.72	3.50	2.60	1.93	1.66	1.46
P_0=0.1						
200	N.E.	13.4	6.86	3.85	2.88	2.25
500	20.5	5.94	3.83	2.52	2.04	1.71
1,000	9.28	3.93	2.78	2.00	1.69	1.47
2,000	5.55	2.86	2.16	1.67	1.47	1.32
P_0=0.3						
200	N.E.	85.4	6.96	3.09	2.26	1.79
500	N.E.	6.00	3.22	2.05	1.69	1.45
1,000	18.6	3.42	2.30	1.67	1.46	1.31
2,000	5.81	2.40	1.82	1.45	1.31	1.21
P_0=0.5						
200	N.E.	N.E.	24.7	3.48	2.31	1.77
500	N.E.	15.9	3.85	2.09	1.67	1.43
1,000	N.E.	4.33	2.43	1.67	1.43	1.29
2,000	16.4	2.59	1.84	1.43	1.29	1.19
P_0=0.8						
200	N.E.	N.E.	N.E.	584	4.10	2.25
500	N.E.	N.E.	N.E.	3.43	2.12	1.62
1,000	N.E.	N.E.	5.85	2.14	1.65	1.39
2,000	N.E.	8.43	2.66	1.65	1.41	1.26

N.E.: ORsusceptible does not exist.

used, not of susceptible controls which represent the population without disease under study. Generally, the size of susceptible cases is larger than the size of susceptible controls ($S_{control}$). Although Tables and Figures could similarly be made using $S_{control}$, the size of susceptible cases (S) was adopted here. The S seems easier to be understood and estimated by clinicians, who are faced with patients.

In conclusion, this paper provided the useful figures when case-control studies on environmental factors interacting with genotypes are designed. These figures are applicable for OR of a genotype interacting with environmental factors, and also for gene-gene interactions to be derived from case-control studies based on high-throughput SNP analysis (Marnellos, 2003; McLeod and Yu, 2003).

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Chart for the Calculation Steps

1. Calculation of P_1 to obtain a significant result from given P_0, N_0, N_1, significance level, and statistical power.
2. Calculation of OR_{whole} from P_0 and P_1.
3. Calculation of P_x from P_0, P_1, and given S.
4. Calculation of S_{min} in case of $P_x = 1$.
5. Calculation of $OR_{susceptible}$ from P_0, P_x, and S.

N_0: Number of controls
N_1: Number of cases
P_0: Proportion of the exposed among controls
P_x: Proportion of the exposed among cases with a susceptible genotype
P_1: Proportion of the exposed among cases, which is defined with $S P_x + (1 - S) P_0$
S : Size (proportion) of cases with the susceptible genotype
S_{min}: The minimal S, i.e., S in case of P_1=1
OR_{whole}: Odds ratio for whole cases
$OR_{susceptible}$: Odds ratio for individuals with the susceptible genotype.

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Long-term effects of radiation dose on inflammatory markers in atomic bomb survivors

Tomonori Hayashi, PhD, Yukari Morishita, MSc, Yoshiko Kubo, Yoichiro Kusunoki, PhD, Ikue Hayashi, PhD, Fumiyoshi Kasagi, PhD, Masayuki Hakoda, MD, Seishi Kyoizumi, PhD, Kei Nakachi, PhD

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THE AMERICAN JOURNAL of MEDICINE®

BRIEF OBSERVATION

Long-term effects of radiation dose on inflammatory markers in atomic bomb survivors

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Atomic bomb survivors have a persistently increased risk of cancer, hepatitis, and cardiovascular and autoimmune diseases.[1-4] There is no clear explanation for these late effects of radiation exposure. One hypothesis is that radiation causes chronic low-grade inflammation, with elevated circulating levels of cytokines. Proinflammatory cytokines, such as interleukin 6, tumor necrosis factor (TNF) α, and interferon γ, and anti-inflammatory cytokines, such as interleukin 10, are synthesized predominantly by macrophages and lymphocytes, and regulate the inflammatory response.[5,6] Interleukin 6 in turn induces the synthesis of acute-phase plasma proteins, such as C-reactive protein.[7] Increased levels of inflammatory cytokines, even within the normal range, have been associated with an increased risk of cardiovascular disease.[8] Chronic low-grade inflammation may also influence the production of immunoglobulins by B cells.[9]

We therefore analyzed the effects of presumed radiation dose on inflammatory parameters in atomic bomb survivors.

Methods

Subjects

We studied subjects from Hiroshima who had participated in an epidemiological follow-up study of atomic bomb survivors, which collected health information from 2436 survivors during biennial medical examinations.[10] Peripheral blood samples were collected between March 1995 and April 1997. We obtained institutional approval from the human investigation committee and informed consent from participants. We excluded subjects with a history of cancer or diseases that have been associated with inflammation (e.g., current upper respiratory tract infection, chronic bronchitis, collagen disease, arthritis, or myocardial infarction). We classified the other participants into four radiation dose groups: nonexposed, low dose (0.005 to 0.7 Gy), medium dose (0.7 to 1.5 Gy), and high dose (>1.5 Gy). Estimated bone marrow doses were based on the 1986 Dosimetry System.[11] Doses were for whole-body exposure, mainly from gamma rays but with a small neutron component. We selected 180 subjects from the nonexposed group and 90 from each of the other

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Table 1 Characteristics of the study subjects*

Characteristic	Nonexposed (n = 179)	Radiation Exposure (Gy) 0.005–0.7 (n = 87)	0.7–1.5 (n = 88)	>1.5 (n = 88)
	Number (%) or mean ± SD			
Radiation dose (Gy)	0	0.3 ± 0.2	1.1 ± 0.2	2.1 ± 0.5
Age (years)	68 ± 11	69 ± 11	67 ± 10	68 ± 10
Female sex	96 (54)	50 (58)	52 (59)	47 (53)
Body mass index (kg/m^2)	23 ± 3	23 ± 3	22 ± 4	23 ± 4
Current smokers	44 (25)	17 (20)	23 (26)	21 (24)

*Among atomic bomb survivors from Hiroshima, Japan.

groups, such that the age and sex distributions were similar in the four groups. Data were missing for 8 subjects; these subjects were excluded from all analyses.

Measurements

We measured plasma TNF-α, interferon γ, and interleukin 10 levels in duplicate using a highly sensitive enzyme-linked immunosorbent assay kit (Quantikine HS; R&D Systems, Minneapolis, Minnesota). Mean values of duplicate measurements were reported for all assays. We quantitated immunoglobulin levels using standard kits (Bethyl Lab. Inc., Montgomery, Texas). The interassay and intra-assay coefficients of variations of these enzyme-linked immunosorbent assay kits were lower than 10%. The erythrocyte sedimentation rate was measured using standard methods.

Statistical analysis

We estimated the effects of changes in several predictor variables (linear radiation dose, age, and sex), adjusted for current smoking and body mass index (in kg/m^2) using a multivariate linear regression model based on the log of the outcome variables (biological markers). We present results as percentage changes in the outcome variables with 95% confidence intervals. All analyses were performed using SAS software (Cary, North Carolina).

Results

There were no significant differences in age, sex, body mass index, or current smoking among the four groups (Table 1). Interferon γ levels and the erythrocyte sedimentation rate increased significantly with radiation dose (Figure). Tumor necrosis factor α and interleukin 10 levels also increased slightly but not significantly with radiation dose. The levels of immunoglobulin (Ig) A and IgM increased significantly with radiation dose, but those of IgG and IgE did not.

In multivariate models, the levels of TNF-α, interferon γ, and interleukin 10, and the erythrocyte sedimentation rate, increased significantly with radiation dose, as did IgA, IgM, and total immunoglobulin levels (Table 2). The levels of TNF-α, interleukin 10, IgG, IgA, and total immunoglobulins, and the erythrocyte sedimentation rate, increased significantly with age.

Discussion

Tumor necrosis factor α, interleukin 6, interferon γ, and interleukin 10 coordinate the inflammatory response. In the present study, plasma levels of inflammatory cytokines and biomarkers (TNF-α and the erythrocyte sedimentation rate) increased with radiation dose and with age. Plasma levels of other cytokines (interferon γ and interleukin 10) and immunoglobulins (IgA and IgM) increased with radiation dose. Combined with previous results on other inflammatory signs, such as increased white blood cell counts, and sialic acid and C-reactive protein levels,[12,13] our results provide evidence of persistent inflammatory responses in atomic bomb survivors more than 50 years after radiation exposure.

In light of these studies, we hypothesized that radiation exposure accelerated aging. To test the hypothesis, we calculated radiation exposure as a function of age using inflammatory status as an index. We estimated that exposure to 1 Gy was equivalent to an increase in age of about 9.0 years. Noting that the mean exposure among atomic bomb survivors was about 0.2 Gy, we inferred that mean accelerated aging among atomic bomb survivors was about 2 years (range, 1 to 2.5 years). Others have reported that the decrease of CD4-expressing T cells was about 4% per 10 years and 2% per Gy, implying that the decrease per Gy is equivalent to about 5 years of aging.[14] Furthermore, interleukin 6 levels correlate

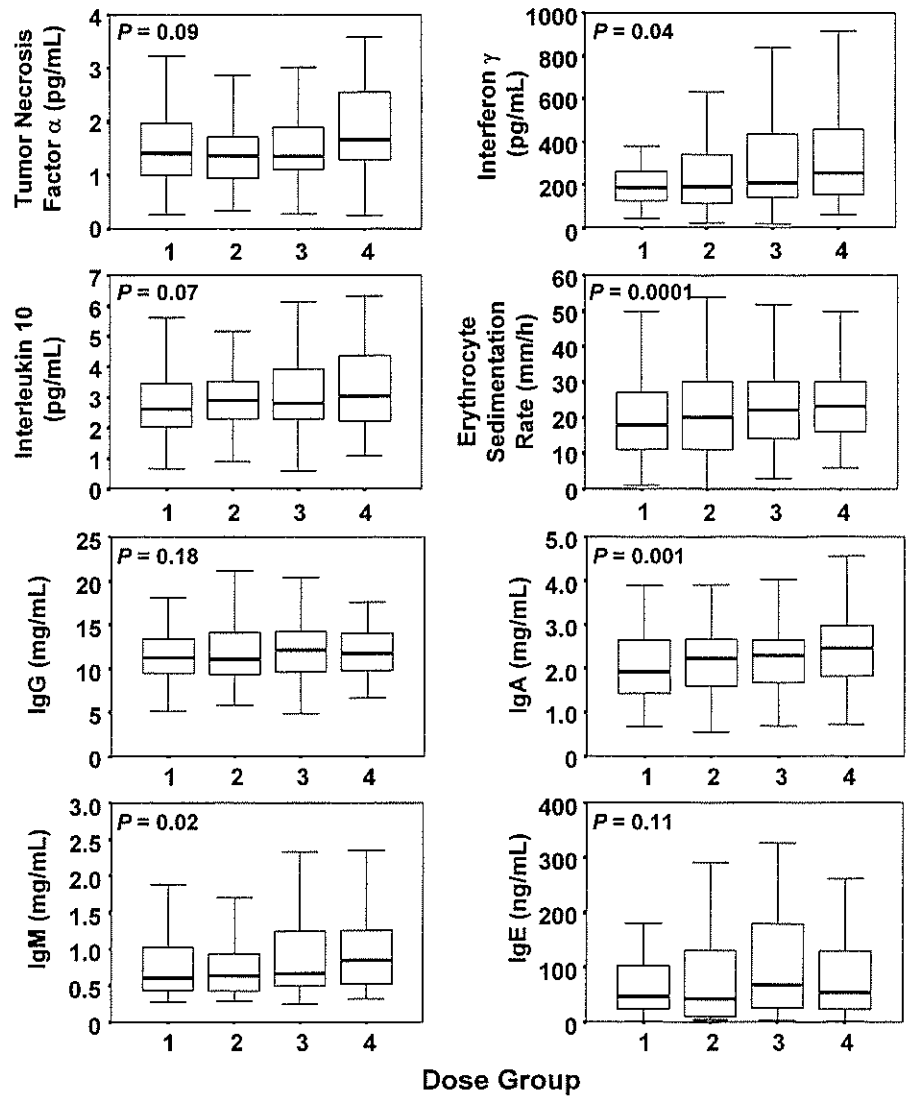

Figure 1 Box plot of inflammatory biomarker levels and erythrocyte sedimentation rate among atomic bomb survivors. The horizontal line inside the box represents the median. Lower and upper boundaries represent the 25th and 75th percentiles. Whiskers represent the smallest and largest values that are less than 1.5 box-length from the 25th and 75th percentiles. 1 indicates nonexposed; 2 indicates radiation exposure of 0.005 to 0.7 Gy; 3 indicates exposure of 0.7 to 1.5 Gy; and 4 indicates exposure of >1.5 Gy. Ig = immunoglobulin.

negatively with the percentage of CD4 T cells.[13] Thus, acceleration of immunological aging may also be involved in radiation effects on the inflammatory status in humans.

Increased mortality and morbidity from cardiovascular disease has been observed in atomic bomb survivors,[10] and elevated plasma levels of inflammatory markers, including interleukin 6, have been associated with an increased risk of cardiovascular disease.[8] Indeed, C-reactive protein and complement are mediators of ischemic myocardial injury.[15] Further, the percentage of CD4 T cells in the blood is markedly lower among atomic bomb survivors who have a history of myocardial infarction.[16] We hypothesize that modification of cytokine production may be involved in the onset or progression of some of the conditions, such as hepatitis and cardiovascular disease, which are more common in atomic bomb survivors.

Several studies have shown that radiation causes short-term inflammatory effects, such as increased plasma levels of proinflammatory cytokines, among patients who received radiation therapy.[17-19] In addition, radiation for cancer or Hodgkin's disease leads to long-term depletion of naïve CD4 T cells,[20,21] and pathologic cardiac changes.[22] Our results suggest that radiation exposure may also produce long-term adverse effects by generating a persistent inflammatory status, manifested by cytokines and other inflammatory markers along with long-lasting impairment of CD4 T cells. Given the potential implication of our findings, follow-up of radiotherapy-treated patients is warranted to assess the asso-

Table 2 Multivariate models of the effects of age, sex, and radiation dose on inflammatory biomarkers and immunoglobulins

Variable	Tumor Necrosis Factor α	Interferon γ	Interleukin 10	Erythrocyte Sedimentation Rate	Total Ig	IgG	IgA	IgM	IgE
	Percentage Increment (95% Confidence Interval)								
Age per 10 years	15 (9 to 20)	4 (−4 to 12)	8 (4 to 13)	15 (9 to 20)	3 (1 to 6)	3 (1 to 6)	5 (2 to 9)	−6 (−11 to 14)	2 (−11 to 14)
Female sex*	15 (2 to 30)	−8 (−23 to 10)	6 (0 to 12)	17 (9 to 24)	5 (0 to 10)	7 (1 to 13)	−9 (−17 to −1)	14 (1 to 28)	−51 (−63 to −34)
Radiation dose per Gy	7 (1 to 15)	12 (2 to 23)	6 (0 to 12)	17 (9 to 24)	3 (1 to 6)	2 (−1 to 5)	8 (3 to 13)	9 (2 to 15)	14 (−3 to 32)

Ig = immunoglobulin.
*Compared with men.

ciation between inflammatory status and the occurrence of inflammation-associated diseases.

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Individual Variation of Somatic Gene Mutability in Relation to Cancer Susceptibility: Prospective Study on Erythrocyte Glycophorin A Gene Mutations of Atomic Bomb Survivors

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Abstract

It has previously been reported that hemizygous mutant fraction (Mf) at the glycophorin A (*GPA*) locus in erythrocytes increased with radiation dose in heterozygotes among Hiroshima and Nagasaki atomic bomb survivors. In the present study, we analyzed the relationship between GPA Mf and cancer risk using newly developed cancers among previously cancer-free subjects whose GPA Mf had been measured between 1988 and 1996. Among 1,723 survivors (1,117 in Hiroshima and 606 in Nagasaki), we identified 186 subjects who developed a first cancer by the end of 2000. We compared the radiation dose responses of GPA Mf between cancer and cancer-free groups using a linear-quadratic model fit by multiple regression analysis in combination with age, sex, and city. The slope of the GPA Mf dose-response curve was significantly higher in the cancer group than in the cancer-free group among Hiroshima subjects. Moreover, no significant difference of GPA Mf between cancer and cancer-free groups was found in unexposed controls in the two cities. The same conclusions were obtained using a linear dose-response model and by further analysis using Cox regression of cancer incidence. These findings suggest that there might be interindividual variation in mutability of somatic genes and that Hiroshima survivors who have higher mutability in response to radiation exposure would be expected to have a higher probability of suffering radiation-related cancer. (Cancer Res 2005; 65(12): 5462-9)

Introduction

Interindividual variability in human responses to mutagen exposures, including ionizing radiation, is believed to be a critical element in determining individual risk of cancer as well as the incidence of cancer in a population. At least a part of such interindividual variability of cancer susceptibility may be attributed to capacity of responses to oxidative DNA damage generated by mutagens (1). Multistaged defense mechanisms may exist in the responses to oxidative DNA damage, involving the initial defense against reactive oxygen species by superoxide dismutase and catalase, inhibition of incorporating oxidized bases into DNA by hydrolase, and repair of DNA damage (i.e., base excision repair, transcription-coupled repair, global genome repair, mismatch repair, translesion synthesis, homologous recombination, and nonhomologous end joining). Among them, much attention has been paid to several DNA repair genes. There is increasing evidence that mild reductions in DNA repair capacity, assumed to be the consequence of common genetic variation, affect cancer predisposition (2, 3). Currently, molecular epidemiologic studies are being conducted in many laboratories to define the roles that polymorphisms in DNA repair genes play in individual cancer susceptibility (3–5). In contrast to such genetic markers, phenotypic markers of DNA repair capacity and cancer susceptibility comprise both genetically and environmentally determined fractions and express integrated effects of complicated processes where a number of gene products are involved. Thus, phenotypic markers have often played vital roles in cancer research, specifically in prospective cohort studies, assessing the exposure levels (biodosimetry) as well as cancer risk (2, 6).

Many phenotyping assays have been developed using blood cells and skin fibroblasts for quantifying *in vivo* somatic mutations and *in vitro* DNA repair capacity (6). The erythrocyte glycophorin A (GPA) mutation assay, which can enumerate hemizygous mutants at the *GPA* locus in long-lived hematopoietic stem cells of heterozygous donors, provides one useful phenotypical end point for the assessment of cancer risk. This was supported by the findings that highly elevated GPA mutant fractions (Mf) were detected in patients with cancer-prone diseases, such as ataxia telangiectasia (7), Bloom's syndrome (8, 9), Fanconi's anemia (8, 10), and Werner syndrome (11, 12). These patients have defects in genes that are involved in several pathways of DNA repair mechanisms. It was also reported that GPA Mfs can be used as an assessment marker for the development of secondary induced leukemia in patients treated for childhood acute lymphoblastic leukemia (13). These findings suggest that the GPA Mf may, in some way, reflect individual repair capacity and cancer risk.

To clarify the association between radiation-induced mutation and cancer risk, prospective studies are critical to exclude the role of cancer itself in the association, such as through chemotherapy and radiation therapy. Because the atomic bomb (A-bomb) survivor population is an epidemiologically well-controlled cohort in terms of dose estimation (14) and cancer follow-up (15), such an analysis is feasible in this population. We previously measured hemizygous GPA Mf in ~1,200 heterozygous A-bomb survivors in Hiroshima and Nagasaki between 1988 and 1993 and analyzed the dose response of GPA Mf and the relationship between GPA Mf and cancer risk (16). It was found that the doubling dose of GPA Mf was similar to that of solid-cancer incidence in A-bomb survivors. Furthermore, the dose response was significantly higher in persons who had been diagnosed with cancer than in cancer-free

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individuals among Hiroshima survivors. This suggests an earlier onset of cancer due to enhanced mutagenesis or a higher radiation sensitivity in the cancer group. However, although we attempted to exclude all survivors who had undergone chemotherapy and radiotherapy, we may have missed some of them due to incompleteness of the medical records. Thus, because we could not completely exclude the possible effect of the therapies on GPA Mf, a prospective study was desired.

We have extended the GPA Mf measurements to ~1,900 survivors in total as of 1996 and followed them until 2000 to identify newly developed cancers among the previously cancer-free subjects. Based on these prospective data, we reanalyzed the relationship between GPA Mf and cancer development. In the present report, we show the reproducibility of the previous findings and discuss interindividual variation of susceptibility to radiation-induced mutagenesis, which may be associated with subsequent cancer risk.

Materials and Methods

Study subjects. Blood samples were obtained randomly from 1,902 survivors whose MN blood types were heterozygous by the hemagglutination test, who were participating in the Radiation Effects Research Foundation (RERF) Adult Health Study from June 1988 to August 1996. We excluded 179 survivors who were diagnosed with cancer before the GPA measurements and observed subsequent cancer development. Subject ages ranged from 43 to 100; mean ages were 63 for males and 67 for females.

Survivors in Hiroshima and Nagasaki, who have been diagnosed with malignant tumors (n = 186; 118 in Hiroshima; 68 in Nagasaki) through December 2000, were identified from the RERF tumor registry (17). Diagnoses and medical treatment histories for these survivors were also confirmed from the Adult Health Study medical charts. Identified malignant tumors included stomach (n = 32), colon (n = 31), lung (n = 19), liver (n = 18), breast (n = 10), rectum (n = 10), pancreas (n = 8), prostate (n = 7), gall bladder (n = 6), esophagus (n = 5), thyroid (n = 5), and other (n = 35) cancers.

The distribution of subjects by DS86 bone marrow doses (14), sex, and city are shown in Table 1. This distribution is similar to that of the total Adult Health Study population. The estimated dose includes both neutron and γ-ray components. The analyses described in the present report were based on weighted bone marrow doses computed as the γ dose plus 10 times the neutron dose and adjusted for the effect of imprecision on regression analyses (18). The weighting factor will be called the relative biological effectiveness of neutrons, and weighted doses are expressed in sieverts (Sv). This study population consists of Hiroshima and Nagasaki survivors who were exposed to significant radiation doses of ≥0.004 Sv because of their location within 2 km of the hypocenter plus a second group whose exposures were at distances in excess of 3 km from the hypocenter and as a result led to them receiving radiation doses of <0.004 Sv (i.e., doses that are indistinguishable from background). The latter group of distally exposed survivors includes the most appropriate controls for all of our studies of the effects of A-bomb radiation exposures, including the present one.

Measurement of glycophorin A mutation frequency. Using the GPA mutation assay, four types of mutant cells, Mϕ, Nϕ, MM, and NN cells, can be detected among the erythrocyte populations of MN heterozygous donors. Hemizygous Mϕ and Nϕ cells are caused by deactivation of *N* or *M* alleles of the *GPA* gene, respectively. Homozygous MM and NN cells may be induced by somatic recombination between the two chromosomes on which the *M* and *N* alleles reside. Among these four types of mutants, the reproducibility of NN cells was low, probably due to carbohydrate modification of the GPA molecules (19, 20). Also, MM mutant frequency is significantly affected by overlapping of Mϕ mutants in the MM mutant window on the flow cytogram, particularly for the high-dose exposed who have high Mϕ Mfs. Thus, in this report, statistical analysis was undertaken for the mean of Mϕ and Nϕ hemizygous Mf (GPA Mf).

The detailed method for the flow cytometric measurement of mutant erythrocytes has been described previously (20). Briefly, using a single-beam cell sorter, FACStar (Becton Dickinson Immunocytometry System, San Jose, CA), four types of variants lacking the expression of one *GPA* allele were distinguished from normal MN heterozygous cells. By two-color staining with the GPA (M + N)–specific monoclonal antibody (mAb) 10F7 and the GPA (M)–specific mAb 6A7, two mutant cell types, hemizygous Nϕ and homozygous NN cells, from MN heterozygous donors can be detected simultaneously. By combining the GPA (M)–specific mAb 9A3 and the GPA (N)–specific mAb NN3, hemizygous Mϕ and homozygous MM cells can be measured simultaneously. 10F7 and 9A3 mAbs were directly labeled with fluorescein and mAbs 6A7 and NN3 were conjugated with biotin followed by labeling with streptavidin-conjugated phycoerythrin (Biomeda, Foster City, CA). Mutant cells displaying a hemizygous or homozygous phenotype were sorted onto a glass slide. Cells showing typical erythrocyte morphology with fluorescein fluorescence matched for the mutant phenotype were counted under a fluorescence microscope. Typically, ~10^6 total erythrocytes were assayed per sample.

Table 1. Distribution of subjects excluding persons who had malignant cancer before GPA Mf measurement

City	Sex	No. subjects	Survivor bone marrow dose (Sv, neutron RBE = 10)				
			<0.004*	0.004-0.499	0.500-0.999	1.000-1.499	1.500+
Hiroshima	Male	360 (54)†	129 (22)	132 (13)	47 (12)	32 (3)	20 (4)
	Female	757 (64)	311 (19)	292 (25)	93 (9)	29 (7)	32 (4)
	Total	1,117 (118)	440 (41)	424 (38)	140 (21)	61 (10)	52 (8)
Nagasaki	Male	221 (36)	84 (14)	53 (7)	45 (7)	25 (5)	14 (3)
	Female	385 (32)	168 (8)	88 (10)	77 (8)	38 (2)	14 (4)
	Total	606 (68)	252 (22)	141 (17)	122 (15)	63 (7)	28 (7)
Total	Male	581 (90)	213 (36)	185 (20)	92 (19)	57 (8)	34 (7)
	Female	1,142 (96)	479 (27)	380 (35)	170 (17)	67 (9)	46 (8)
	Total	1,723 (186)	692 (63)	565 (55)	262 (36)	124 (17)	80 (15)

Abbreviation: RBE, relative biological effectiveness.
*According to the DS86 dosimetry system, survivors whose dose estimation would result in a free-in-air kerma <5 mGy were automatically assigned doses of zero. However, most of the persons in this category were too far from the hypocenter to have received significant radiation exposure.
†Numbers of persons with cancer diagnosed subsequent to GPA Mf measurement are in parentheses.

Statistical analysis. Dose responses for GPA Mf and incidence proportion of cancer were fit using ordinary (least squares) regression. Weighted, adjusted bone marrow dose was used as described above. Age at examination was centered at its mean (65 years). City, sex, and cancer status were treated as indicator variables. Application of least-squares regression to the GPA Mf and radiation dose-response data for purposes of statistical testing would necessitate logarithmic transformation of both the GPA Mf and radiation dose variables (16) to achieve approximate normality and constant variance of the response variable (log GPA Mf) and approximately uniform distribution of the predictor variable (log radiation dose), but we desired to mimic standard radiobiological practice and fit linear or linear-quadratic dose responses. Thus, we did not transform the variables for the purpose of estimating the radiation dose response and instead verified the fit of the least-squares regression using nonparametric curve-fitting methods. As a further check on adequacy of the dose-response fit, individual observations with large influence on the regression analysis were identified through single-deletion regression diagnostics and regression models were refit after excluding such points. As a result, the small number of subjects with GPA Mf values >400 was not used in estimating the GPA Mf dose response. For fitting the cancer incidence proportion to radiation dose, least squares regression was applied to the binary indicator of cancer status. Approximately homogeneous variance and fit to the data were confirmed by comparing the fitted regression to a plot of binomial proportions grouped on radiation dose with approximately equal numbers of subjects (Fig. 1).

Follow-up for incident cancer subsequent to GPA measurement was analyzed using Cox regression with age as the time scale and adjustment for year of birth and age at examination. The effect of GPA Mf on cancer incidence was assessed using either the logarithm of continuous GPA Mf to reduce the influence of the small number of points with large values of GPA Mf, or the untransformed GPA Mf excluding the subjects with values larger than 400. For graphic presentation of the results of the Cox regression, summary plots of cumulative incidence (proportion of subjects who were free of cancer) were produced by dividing subjects into two strata—low and high values of GPA Mf—based on the median GPA Mf among cancer cases.

All analyses were conducted using S-plus version 2000 (Mathsoft, Inc., Seattle, WA).

Results

Cancer prevalence in the study cohort. One hundred eighty-six subjects developed a first cancer between the GPA Mf measurement and the end of 2000. There was a statistically significant increase in cancer incidence proportion with dose after adjustment for city, sex, and age (Fig. 1). This suggests that the subjects of the present study developed cancers in a dose-dependent manner even >40 years after exposure.

Effects of cancer status and city difference on dose responses of glycophorin A mutant fraction. Figure 2 shows nonparametric curves for the GPA Mf (the mean of hemizygous Mϕ and Nϕ Mf) values according to bone marrow dose among all study subjects (n = 1,902), including cancer cases diagnosed before GPA Mf measurements. The plots suggest that the GPA radiation dose response is steeper among cancer patients in Hiroshima, particularly among those whose cancer was diagnosed subsequent to GPA measurement (Fig. 2, *left*). Persons who had cancer diagnosed before the time of GPA measurement may not be representative, because some individuals with cancer might have been censored—debilitated or deceased—and unable to attend the Adult Health Study examination. In contrast, no apparent differences were observed between cancer and noncancer groups among Nagasaki survivors (Fig. 2, *right*).

To evaluate further the possible difference in cancer-related GPA dose response between the two cities, we did standard regression on GPA Mf using bone marrow dose, cancer status, sex, and age at examination as covariates, excluding subjects with values of GPA Mf >400 and those who had cancer diagnoses before GPA measurement (Table 2). Background GPA Mf (estimate for 0 Gy exposure) was lower in Nagasaki than in Hiroshima and lower in females than in males. An increase in GPA Mf with age of the subject at examination was only marginally significant, probably because age-dependent increase in GPA Mf reaches a plateau after about 50 years of age (21). There was an initial increase in the GPA response with bone marrow dose, followed by an attenuation in the slope (negative quadratic term). Although the quadratic term was statistically significant (P = 0.033), there was little quantitative difference in the results with or without the quadratic term except for a slightly lower dose-response slope without the quadratic term. There was no significant difference in background GPA Mf between male subjects who subsequently developed cancer and those who did not, but among females the background GPA Mf was lower among those with cancer.

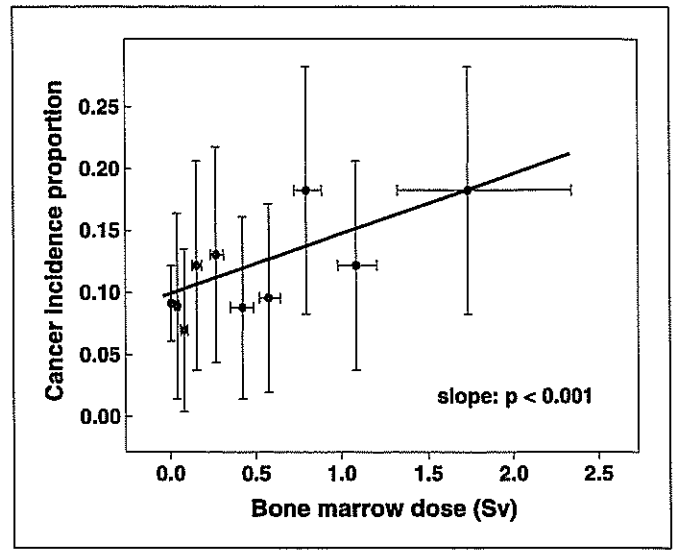

Figure 1. Dose response of cancer incidence proportion subsequent to GPA Mf measurement adjusted for city, sex, and age at examination. The total number of subjects is 1,723. *Points*, crude proportions in 10 dose groups with approximately equal numbers of subjects (n = 115) except for unexposed controls (n = 691); *bars*, SE of the estimated proportions and the quartiles of dose.

The fitted linear-quadratic regression models for each of the two cities, adjusted for sex and age, are shown in Fig. 3. The dose response of GPA Mf was significantly higher in Hiroshima subjects who subsequently developed cancer than in those who did not. Whether there was a difference in dose response by cancer status depended significantly on city (a three-way interaction between city, dose, and cancer status; P = .0081), with no difference in Nagasaki. Seven points were identified that had a high influence on the value of the city × cancer × dose three-way interaction term. Upon deleting these points, the value of the interaction term decreased somewhat but remained statistically significant. Among Nagasaki subjects, the initial slope of the GPA dose response in the linear-quadratic model adjusted for the average values of the other factors was 22.6 in cancer-free subjects and 22.3 in subjects with subsequent cancer (P = 0.29). In Hiroshima, the similarly adjusted initial slopes were 35.8 in cancer-free subjects and 51.3 in subjects with subsequent cancer (P = 0.0039). There was no evidence that the quadratic term of the dose response differed according to cancer status.

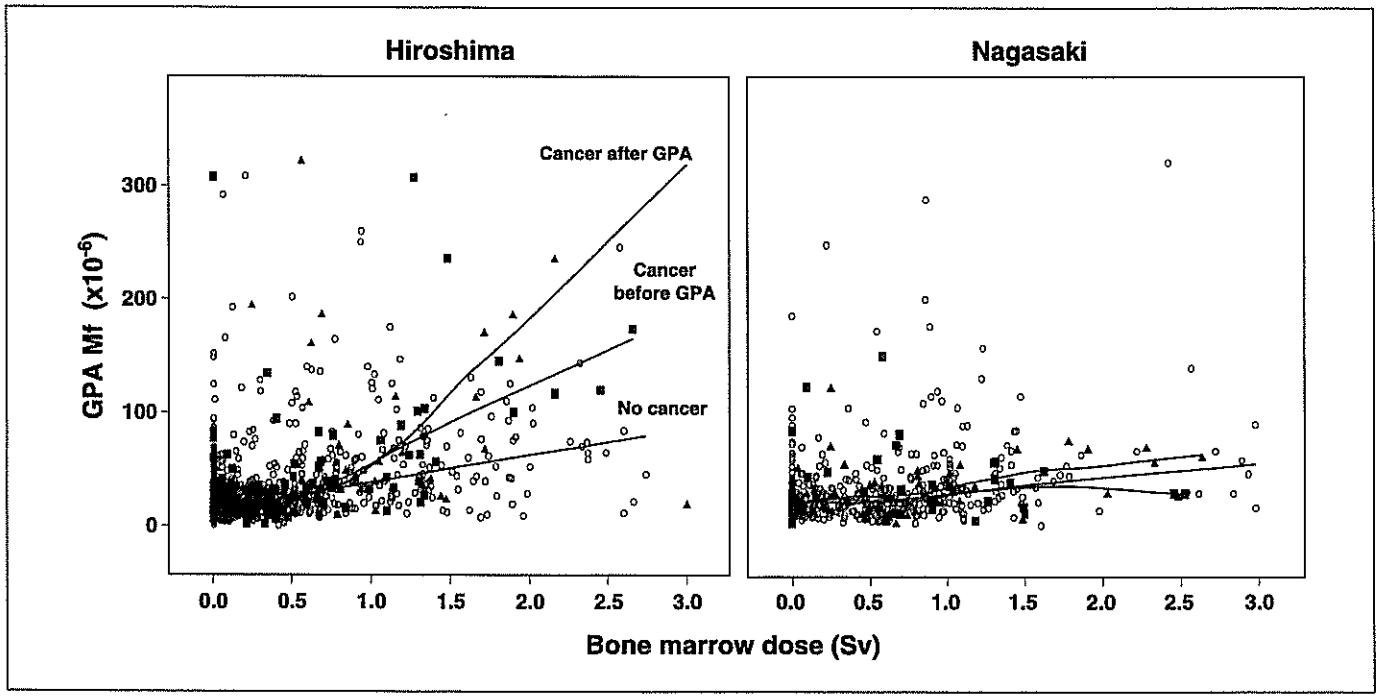

Figure 2. Dose response of GPA Mf based on nonparametric curve fitting for cancer (either before or after GPA Mf measurement) and noncancer groups in Hiroshima and Nagasaki. Symbols indicate subjects who were diagnosed with cancer before GPA Mf measurement (■), those who were diagnosed with cancer after GPA Mf measurement (▲), and those who were not diagnosed with cancer (○). Lines denote fitted dose-response curves for cancer and noncancer groups.

Difference of glycophorin A mutant fraction between cancer and noncancer groups among high dose–exposed subjects. Because the GPA Mf values are highly skewed, even when restricted to $\leq 400 \times 10^{-6}$, the results of statistical tests of parameters from the regression analysis may not be reliable.

Table 2. Regression analysis of GPA Mf using linear and linear-quadratic dose-response models

Term	Estimated value ± SE [$\times 10^{-6}$] (P value)	
	Linear model	Linear-quadratic model
City (Nagasaki)	−7.1 ± 3.0 (0.018)	−7.5 ± 3.0 (0.013)
Sex (female)	−6.5 ± 2.1 (0.0016)	−6.4 ± 2.1 (0.0020)
Age at exam (per year)	0.13 ± 0.076 (0.078)	0.13 ± 0.076 (0.099)
Dose (initial slope, per SV)	26 ± 2.0 (<0.0001)	32 ± 3.5 (<0.0001)
Dose (quadratic term)	—	−3.6 ± 1.7 (0.033)
Cancer (present)	5.7 ± 4.6 (0.21)	5.5 ± 4.6 (0.23)
Sex-cancer interaction	−12 ± 4.8 (0.013)	−12 ± 4.8 (0.012)
City-dose interaction	−5.5 ± 3.1 (0.071)	−5.0 ± 3.1 (0.10)
Cancer-dose interaction	15 ± 5.3 (0.0049)	15 ± 5.3 (0.0038)
City-dose-cancer interaction	−21 ± 7.9 (0.0068)	−21 ± 7.9 (0.0081)

NOTE: Persons with GPA Mf >400 × 10^{-6} were excluded.

Therefore, we compared log-transformed GPA Mf values between high dose and unexposed persons, thereby avoiding assuming any particular dose-response model, using either a t test (Fig. 4) or regression analysis with adjustment for sex and age (Table 3). Among unexposed subjects, there was no difference in log GPA Mf between persons with and without cancer in either city. The log GPA Mf was significantly higher in subjects with subsequent cancer than without cancer among the heavily exposed (>1.5 Sv) subjects in Hiroshima (t test $P = 0.012$, regression $P = 0.0057$) but was not significantly different between subjects with and without cancer in Nagasaki (t test $P = 0.52$, regression $P = 0.21$).

The analysis of cancer onset rates during the follow-up period by Cox regression confirmed the finding that cancer risk was related to GPA Mf level among high-dose–exposed subjects in Hiroshima but not in Nagasaki (Fig. 5). Whereas there was no association between log GPA Mf level and cancer onset rate among unexposed persons or Nagasaki high-dose–exposed persons, there was significantly higher risk of cancer with higher GPA Mf value in Hiroshima high-dose–exposed persons ($P = 0.043$). The estimated relative risk of cancer for a 10-unit difference in GPA Mf (i.e., a 50% increase over the median) was 1.13 (95% confidence interval, 1.00-1.27; Table 4). Relative risks of cancer for GPA for all individual dose groups by city are shown in Table 4. There was no change in the significance of the results when log GPA was used.

The t tests and Cox regression analyses were repeated after excluding persons whose follow-up was <1 year. There was no change in the pattern of results. The association among high dose and cancer on log GPA Mf was significant in Hiroshima ($P = 0.024$) but not in Nagasaki ($P = 0.41$). The effect of GPA Mf on cancer incidence was significant in high-dose persons in Hiroshima ($P = 0.041$) but not in Nagasaki ($P = 0.66$).

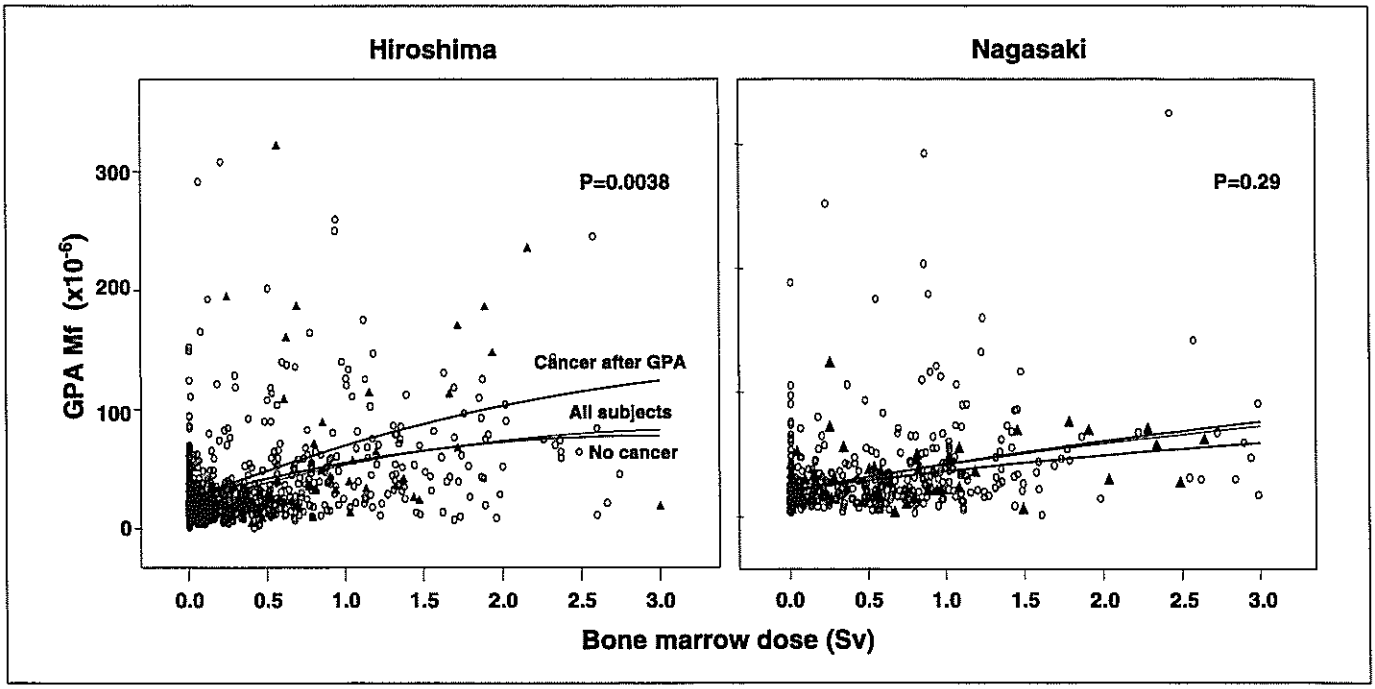

Figure 3. Dose response of GPA Mf among cancer (post-GPA Mf measurement) and noncancer groups based on the linear-quadratic regression model in Hiroshima and Nagasaki. The number of subjects in each group is listed in Table 1. Symbols indicate subjects who were diagnosed with cancer after GPA Mf measurement (▲) and subjects without cancer (○). Lines denote fitted dose-response curves for cancer and noncancer groups. *P* values represent the statistical significance of difference of dose response (quadratic term) between cancer post-GPA Mf measurement and noncancer groups.

Discussion

It is increasingly accepted that the accumulation of multiple abnormalities in cancer-associated genes of a target cell is required for the development of cancer (22–24). Although the mechanism of radiation carcinogenesis is still unknown, some evidence has been presented that A-bomb radiation seems to reduce the number of gene changes needed for cancer induction, thereby inducing earlier onset of cancer in the exposed, compared with the unexposed (25). If GPA Mf reflects nonspecific mutability of all somatic cells in an individual, it can be presumed that GPA Mf may also reflect the prevalence of mutations at cancer-associated genes. Among survivors exposed to the same dose, those who have a higher GPA Mf would be expected to have a higher probability of suffering cancer at any given point in time. In fact, as shown in the present study, the dose response of GPA Mf in the cancer group among Hiroshima survivors was found to be significantly higher than that in the noncancer group. The dose response among persons with cancer diagnosis before GPA measurement was intermediate to that of the groups with no cancer or cancer after GPA measurement, which is further consistent with a higher cancer risk for high GPA Mf radiation–exposed subjects because persons at high risk of cancer would more likely be censored (unobserved due to death or debility) before GPA measurement. The individual difference of GPA Mf in the same dose group might be explained by individual variation in the capacity to repair radiation-induced DNA damage. We discuss below the validity and feasibility of these hypotheses regarding individual differences in DNA repair capacity among A-bomb survivors.

A potentially important source of interindividual variability in relation to cancer risk is DNA repair capacity, including the DNA repair–defective cancer-prone diseases, such as ataxia telangiectasia, Bloom's syndrome, Fanconi's anemia, and Werner syndrome. Apart from these rare and extreme familial cases, there is increasing evidence that a moderate reduction in DNA repair capacity contributes to the sporadic incidence of cancer in the general population (2, 3). Conventional phenotype assays have detected considerable interindividual variation in DNA repair capacity (2, 26, 27). These reduced repair capacity phenotypes have been associated with an increased risk of cancer (2, 28, 29). Evidence of the importance of moderate reduction in DNA repair capacity is also accumulating from mouse models, which have provided results regarding cancer risk increased by heterozygous knock-out in DNA repair genes (30, 31) and those regarding strain differences in cancer susceptibility (32, 33). Furthermore, a number of molecular epidemiologic studies have been initiated using the data from systematic screening of populations for common variants in DNA repair genes (3, 34). Associations of common variants in several repair genes with increased cancer risk have been reported in case-control studies (5). In general human populations, it has been suggested that individual differences in peripheral blood T cell chromosome aberration frequencies may be associated with individual differences in cancer susceptibility (35, 36). These accumulating data are consistent with the hypothesis that interindividual variation in DNA repair capacity has an impact on cancer risk.

Statistical analysis in this study showed that there was a city difference (i.e., significant interaction among radiation, cancer, and city in their association with GPA MF; Table 2). This does not necessarily imply that there is a significant correlation between GPA Mf dose response and cancer in Hiroshima subjects but not in Nagasaki subjects. Small numbers of cases in Nagasaki make it difficult to clearly state the apparent negative finding. However, the observed city difference may be, at least in part, due to possible differences in ethnic background between Hiroshima and Nagasaki, which were suggested by the previous biochemical genetic study of

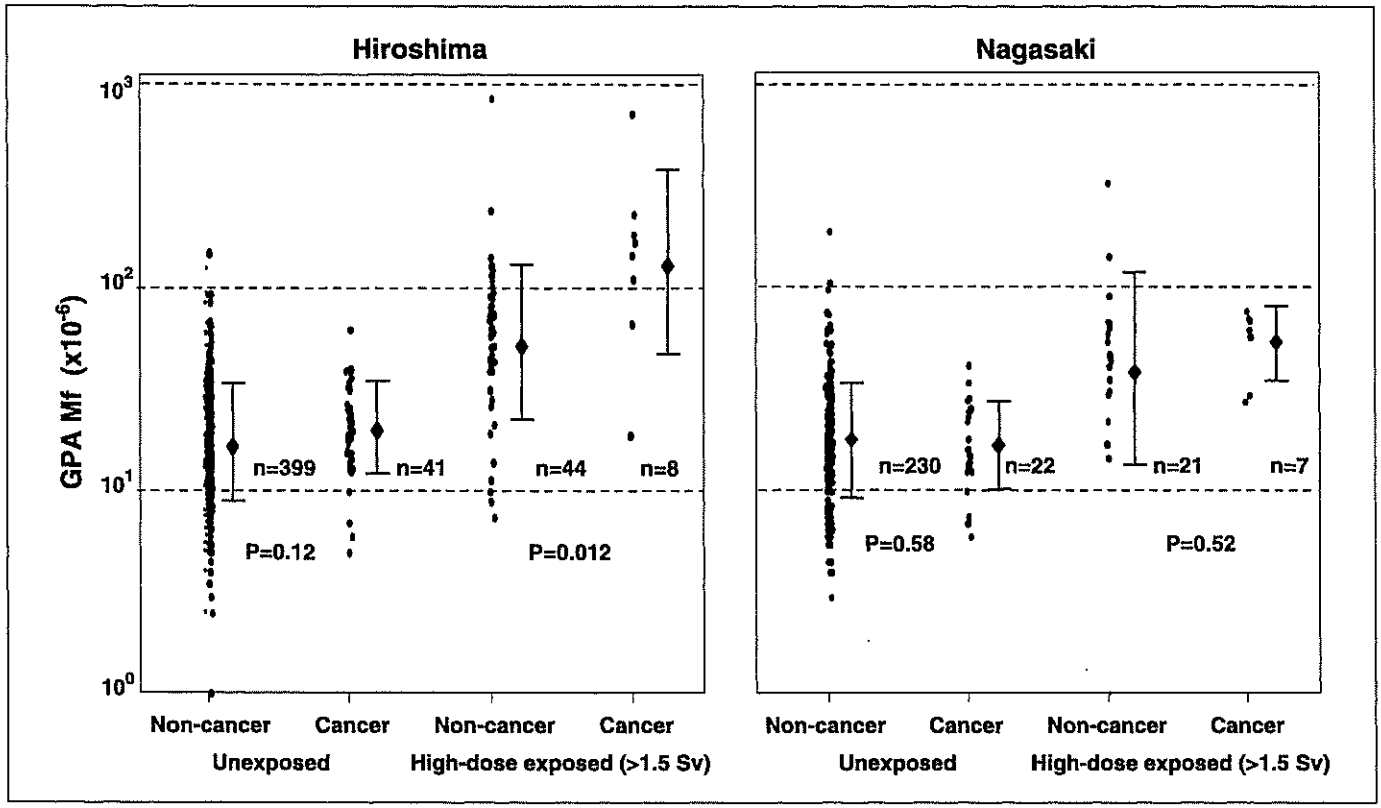

Figure 4. Comparison of GPA Mf between cancer and noncancer groups within unexposed and high dose exposed (>1.5 Sv), Hiroshima and Nagasaki. ◆, mean of GPA Mf; *bars*, SE. *P* values represent the statistical significance of difference of GPA Mfs between cancer and noncancer groups within each dose group.

A-bomb survivors and their children (37). We might presume that the proportion of individuals who have higher mutability of somatic genes is somewhat larger in Hiroshima than in Nagasaki. Interestingly, the background GPA Mf of Hiroshima is significantly higher than that of Nagasaki, as shown in the present study. Such a city difference was also reported for the background solid-tumor mortality (38) and chronic myelogenous leukemia (CML) of survivors (39). City differences of dose response (lower in Nagasaki) were also suggested for the solid tumor mortality (15), the chromosome aberration frequencies in lymphocytes (40), and the incidence of CML (39). These city differences have been attributed to dose estimation errors and/or to a qualitative difference in the radiation produced by the bombs (i.e., the difference in the amount of neutron and γ-ray components), assuming that there is no city difference in radiation sensitivity of A-bomb survivors (15, 40). Because recent genomic analyses have shown extensive interindividual—including ethnic—variations in gene polymorphisms, as mentioned above, this assumption should be reassessed.

Table 3. Relationship between log GPA Mf and cancer status in high dose–exposed survivors

City	Term	Estimated difference in log GPA Mf	SE	*P*
Hiroshima	Exposed	0.503	0.0477	<0.0001
	Cancer	0.0368	0.0495	0.46
	Exposure-cancer interaction	0.342	0.123	0.0057
Nagasaki	Exposed	0.365	0.0670	<0.0001
	Cancer	−0.0622	0.0634	0.33
	Exposure-cancer interaction	0.177	0.141	0.21

NOTE: Results of fitting log GPA Mf, comparing high dose (>1.5 Sv) with nonexposed with adjustment for sex and age at examination.

The following possible caveats of the present study should be kept in mind. We conducted a prospective study, which is critical to exclude possible effects of chemotherapy and/or radiotherapy on *in vivo* somatic mutations. Nevertheless, because the period between GPA Mf measurement and cancer diagnosis is rather short (average: about 3.7 years; range: 22 days-9.4 years; $n = 187$), it is possible that tumors of preclinical size had already developed before GPA Mf measurement. Tumor burden, even with a very small lesion, might increase somatic mutations through high metabolic rate and excessive endogenously generated oxidative stress. However, this may not be the case because GPA Mf values were nearly constant in the cancer subjects ($n = 29$) whose Mf were measured more than twice during the 8-year examination period before cancer diagnosis (data not shown). Further, the radiation dose responses of GPA Mf between cancer and cancer-free groups did not change after excluding all persons whose follow-up ended within 1 year of GPA measurement (data not shown). Another factor complicating the interpretation is uncertainty in dose estimation, because persons with radiation-related outcomes, such as cancer, are more likely to have underestimated dose than persons without. However, our comparison between high-dose–exposed

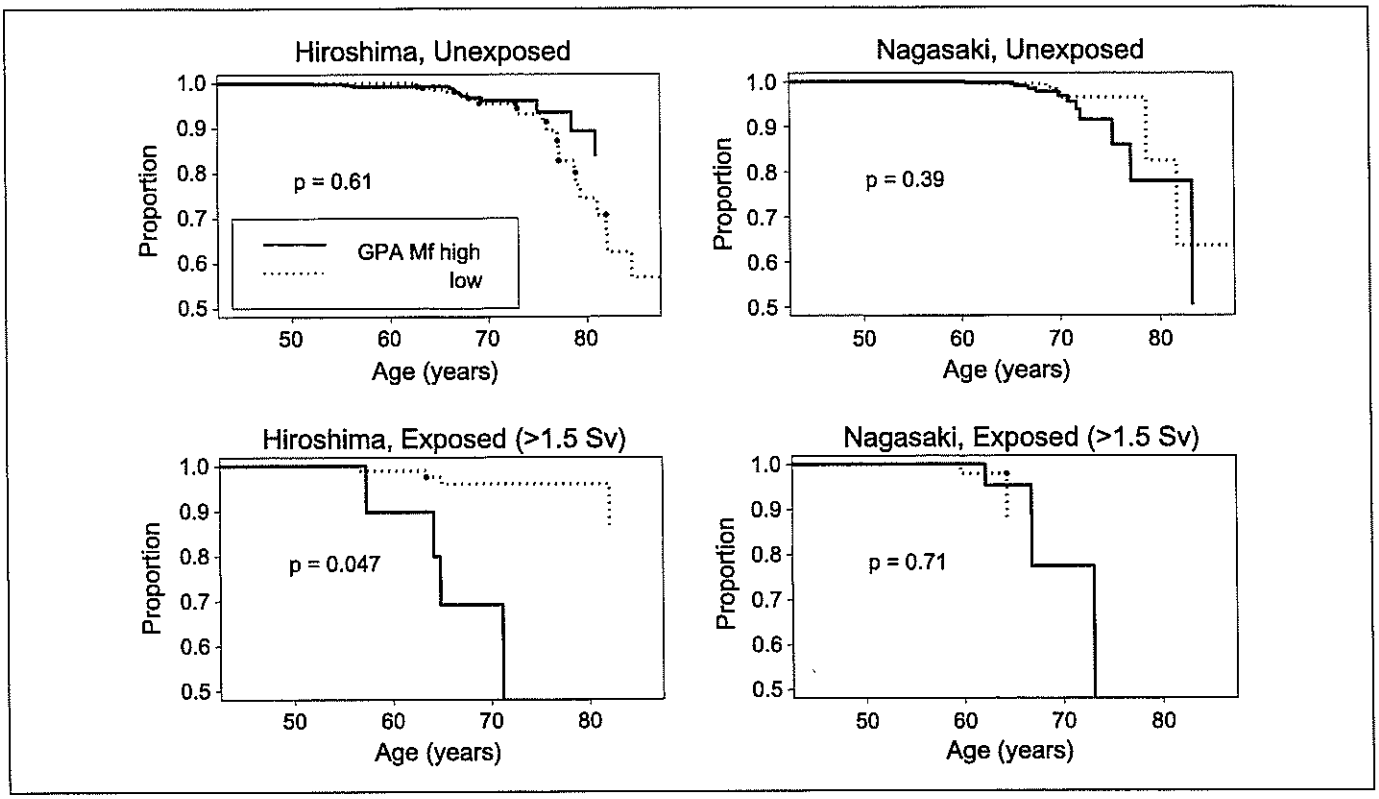

Figure 5. Cox regression analyses of cancer incidence following GPA Mf measurement within unexposed and high dose exposed (>1.5 Sv) in Hiroshima and Nagasaki. *Dashed line,* low GPA Mf; *solid line,* high GPA Mf; low and high are defined as below or above the median GPA Mf level among cancer cases. Numbers of cases of cancer are as shown in Fig. 4. *P* values represent the statistical significance of difference of cancer incidence between high and low GPA Mf groups within each dose group.

and nonexposed persons, which is unaffected by dose uncertainties, did not change the conclusions.

Although our hypothesis is valid, it is far from proved based on the present study alone. We believe that the evolving ability to study polymorphisms in DNA repair genes can contribute to understanding about the relationship between DNA repair capacity and cancer risk in A-bomb survivors. Because the difference in GPA Mf between cancer and cancer-free groups is significant only for high dose–exposed survivors, a candidate polymorphic gene affecting interindividual variability could be one involved in repair of DNA double-strand breaks induced by high-dose irradiation. Double-strand breaks are potentially cytotoxic to cells and mutagenic. At least two molecular mechanisms are involved in the pathway of double-strand break repair: homologous recombination repair and nonhomologous end joining (3, 5, 41, 42). Homologous recombination repair occurs predominantly in the S or G_2 phase of cell division and exchanges DNA strands between the damaged chromatid and the intact sister chromatid. Nonhomologous end joining repair involves

Table 4. Relative risk of cancer for GPA (relative risk for 10-unit difference in GPA Mf)

Exposure category	Hiroshima	Nagasaki	Both cities
Zero dose (<0.004 Sv)	0.96 (0.80, 1.16)	0.82 (0.57, 1.18)	0.91 (0.77, 1.07)
	$P = 0.69$	$P = 0.28$	$P = 0.26$
All exposed (≥0.004 Sv; no dose adjustment)	1.03 (0.99, 1.08)	1.00 (0.93, 1.09)	1.02 (0.99, 1.06)
	$P = 0.15$	$P = 0.92$	$P = 0.20$
All exposed (≥0.004 Sv; with dose adjustment)	1.03 (0.98, 1.08)	0.98 (0.89, 1.08)	1.02 (0.98, 1.06)
	$P = 0.23$	$P = 0.68$	$P = 0.45$
Low dose (≤1.5 Sv)	1.01 (0.95, 1.07)	0.99 (0.89, 1.10)	1.00 (0.96, 1.05)
	$P = 0.80$	$P = 0.81$	$P = 0.87$
High dose (>1.5 Sv)	1.13 (1.00, 1.27)	0.98 (0.82, 1.17)	1.06 (0.98, 1.14)
	$P = 0.043$	$P = 0.82$	$P = 0.14$

NOTE: Relative risk of cancer for GPA is based on Cox regression analysis of cancer risk by age, with adjustment for sex and year of birth, and relative risk for 10-unit difference in GPA Mf is restricted to GPA values of 400×10^{-6} and below.

direct ligation of the two double-strand break ends. These two pathways are thought to involve numerous molecules. Although extensive polymorphic variation in double-strand breaks repair genes has been reported, only a few common polymorphisms of these genes have been examined in epidemiologic studies for their association with cancer risk (43–48). We are planning to study the A-bomb survivors further to elucidate the relationship between DNA repair gene polymorphisms and risk of radiation-induced cancer. The phenotypical data, such as those obtained in the present study, will provide valuable information toward drawing conclusions from genotype-cancer association analyses in future studies.

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