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The Association of Radiation Exposure with Stable Chromosome Aberrations in Atomic Bomb Survivors Based on DS02R1 Dosimetry and FISH Methods

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The frequency of stable chromosome aberrations (sCA) in lymphocytes is a recognized radiation biological dosimeter. Its analysis can provide insights into factors that affect individual susceptibility as well as into the adequacy of radiation dose estimates used in studies of atomic bomb survivors. We analyzed the relationship between atomic bomb radiation exposure using the most recent DS02R1 dose estimates and the frequency of sCA as determined by FISH in 1,868 atomic bomb survivors. We investigated factors that may affect the background sCA rate and the shape and magnitude of the dose response. As in previous analyses of sCA in atomic bomb survivors that were based on Giemsa staining methods and used older DS86 dose estimates, the relationship between radiation dose and sCA rate was significant ($P < 0.0001$) with a linear-quadratic relationship at lower doses that did not persist at higher doses. As before, age at the time of the bombing and type of radiation shielding were significant dose-effect modifiers ($P < 0.0001$), but in contrast the difference in dose response by city was not so pronounced ($P = 0.026$) with a city effect not evident at doses below 1.25Gy. Background sCA rate increased with age at the time of examination ($P < 0.0001$), but neither sex, city, nor smoking was significantly associated with background rate. Based on FISH methods and recent dosimetry, the relationship between radiation dose and sCA frequency is largely consistent with previous findings, although the lesser importance of city as an effect modifier may reflect better dosimetry as well as more reproducible scoring of sCA. The persisting difference in sCA dose response by shielding category points to remaining problems with the accuracy or precision of radiation dose estimates in some A-bomb survivors.

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INTRODUCTION

The frequency of chromosome aberrations in blood lymphocytes is a well-established and useful biological dosimeter (*1–3*). At the Radiation Effects Research Foundation (RERF), a successor of the former Atomic Bomb Casualty Commission (ABCC), a cytogenetics program was initiated in the late 1960s (*4*), and under this program the association between the frequency of chromosome aberrations and radiation exposure has been studied. These investigations are useful for understanding the biological effect of radiation on the human body, but in addition they provide insight into the adequacy of radiation dose estimates that derive from complex computational modeling of input data based on self-reported location and shielding as opposed to a bioassay that can be measured on the affected individual.

Although unstable chromosome aberrations, represented by dicentrics and rings, are relatively simply to detect using simple solid Giemsa methods, they are known to disappear with a half-life of a few years (*5, 6*). When the cytogenetics study plan was launched in the late 1960s, more than 20 years had already passed since the exposure to radiation, and thus unstable aberrations had mostly disappeared. However, stable chromosome aberrations (sCA), such as translocations, complex changes and inversions, persist and often could be detected using the Giemsa staining method provided that the observers had good skill in understanding the human karyotype and could detect subtle changes in length and/or arm ratios of each chromosome (*7*).

A previous large study of sCA in blood lymphocytes detected using the Giemsa staining method examined over 3,000 A-bomb survivors over the course of approximately 25 years (*8*). In the analysis of these data, which used the previous RERF DS86 dose estimates, a radiation dose-response for sCA was detected several decades after exposure. A linear-quadratic dose response model of sCA frequency described the data well up to doses of about 1 sievert, above which the linear-quadratic increase did not persist. A piecewise linear-quadratic (LQ) spline model with an inflection point at 1 sievert (i.e., different LQ functions below and above 1 sievert) significantly improved the fit

across the full dose range compared to a simple linear-quadratic model. Furthermore, when assessing effect modification using a subsample of survivors with estimated doses below 1.5 Gy, it was found that the dose response in Nagasaki was substantially lower than that in Hiroshima, that there was heterogeneity among different age at time of bombing (age ATB) groups, and that each of the other shielding categories examined showed a lower dose response than those inside 9P structures, the subset of the cohort with the most detailed shielding information and dosimetry calculation based on a sophisticated nine-parameter physical model of Japanese structures typical of the era. This was especially true of Nagasaki factory workers, who showed the lowest dose response and have long been suspected of greater potential dose uncertainty due to complications in shielding and dose reconstruction.

However, the study of chromosome aberrations detected using the Giemsa staining method had some limitations. Because of the difficulty of visually identifying translocations with this method, it could only reliably be used to determine whether each cell exhibited one or more translocations, so that the outcome was simply the proportion of cells with aberrations as opposed to the total number of aberrations, although the latter may provide more information for assessing the dose response. It was also found that background rates of detected aberrations differed by city and time period, suggesting that technicians became more adept at identifying aberrations over time, and not identically between the Hiroshima and Nagasaki laboratories (*9*). While this source of bias was identified and adjusted for in subsequent analyses, residual sources of error may have remained.

During the late 1980s to early 1990s, a new technique was developed which used in situ hybridization of chromosome-specific DNA probes labeled with fluorescent dyes on metaphase chromosome spreads so that different chromosomes may be colorized with specific colors — a technique named fluorescent in situ hybridization (FISH) (*10*). Using this method, translocations can be seen clearly as bi-colored chromosomes under a fluorescence microscope. A study of chromosome aberrations using the FISH method was launched at RERF in the early 1990s, and a pilot study that examined chromosome aberration frequencies in 230 survivors comparing Giemsa staining to FISH methods showed a reasonably close correlation between the two measurements, although for translocations Giemsa staining detection frequency was 73% of the genome-equivalent rate using FISH (*11*). To prevent the lab-specific biases associated with the previous Giemsa study, new procedures were introduced in which blood samples gathered in Hiroshima and Nagasaki were all processed (including cell culture, slide preparation, and FISH analysis) only in the Hiroshima laboratory. Furthermore, chromosome aberrations were much more easily identified via the two-color FISH method and could thus be scored as total counts instead of proportions, which likely increases the power and precision to detect the radiation association.

In this paper we analyze the relationship between the frequency of stable chromosome aberrations detected by FISH and radiation exposure as estimated by the latest RERF DS02R1 dosimetry in blood lymphocytes collected from atomic bomb survivors. The major foci of the analysis are the strength and shape of the dose response and factors that appear to modify the effect of radiation exposure on induction of stable chromosome aberrations.

MATERIALS AND METHODS

Study Subjects

Blood samples were collected between 1989 and 2014 from survivors in Hiroshima and Nagasaki. Subjects originally targeted for the study comprised 800 individuals (500 Hiroshima, 300 Nagasaki) randomly selected from the RERF Adult Health Study (AHS) cohort (*12*) from within DS86 (*13*) dose strata according to RERF research proposal 8-93. This original sample was supplemented to include 84 individuals (1999–) who were tooth donors for electron resonance spin (ESR) analysis, 237 parents (1999–) who were part of the F1 molecular genetics study, 378 survivors (2003–) exposed to radiation between 0 and 5 years of age, and additional subjects exposed to >0.5 Gy along with a corresponding systematic sample of controls. Individuals from whom samples were successfully obtained are the subject of this report.

Lymphocyte Culture and Slide Preparation

Two milliters of blood were collected into sodium/heparin tubes and stored at room temperature until cultured. Samples collected in Nagasaki were shipped to the Hiroshima laboratory under temperature-controlled conditions. Blood was cultured for 48 h at 37°C in 10 ml of RPMI-1640 medium (Sigma-Aldrich, Tokyo) supplemented with glutamine (Nissui, Tokyo), 20% heat-inactivated fetal bovine serum (Gibco-BRL, Tokyo), and phytohemagglutinin (PHA, 0.15 mg/ml, Murex, Tokyo). Colchicine (0.15 ug/ml, Wako, Osaka) was added 2 h before harvest (*14*). Cells were harvested and treated with a hypotonic solution (a mixture of 1 part of 1% sodium citrate and 3 parts of 0.075 M KCl) for 15 min at 37°C and were fixed three times with a methanol/acetic acid mixture (3:1, v/v) (*7*). Air-dried slides were kept in the freezer (–20°C) before use. All slides were coded, and microscopic examinations were performed without knowledge of individual radiation doses.

FISH Method (Staining)

Details of the FISH study methodology were described previously (*11*). In brief, chromosomes 1, 2 and 4 were painted yellow using FITC labeled chromosome specific DNA probes, while all chromosomes were counterstained as red by propidium iodide (PI). Cells bearing six painted centromeric segments were selected for the analysis, and bicolored chromosomes were scored as structural aberrations, including translocations (t), dicentrics (dic), insertions (ins) and complex exchanges (cx). For translocations, both one-way and two-way translocations were counted as single events. Insertions were counted as single exchange events. Complex exchanges as denoted here are those aberrations with three or more breaks on two or more chromosomes, at least one of which is painted. Among the complex aberrations, those consisting of one to two color junctions were counted as single events, three to four junctions as two events, five or six junctions as three events, and so on.

All aberrations or suspected aberrations were photographed, and their X and Y coordinates on the microscope were recorded. To reduce

the influence of inter-observer variations, 4 to 6 observers (YK, KH, and other scientists and technicians under the supervision of YK) examined 100 to 125 metaphases each so that a total of 500 to 600 cells could be examined per sample. The total count (t + ins + cx) of sCA was multiplied by gender-specific values (2.771 for males and 2.806 for females) to scale them to the full genome according to the equation developed by Lucas et al. (*15*).

Clonal Chromosome Aberrations

Clonal chromosome aberrations were defined as three or more cells with identical aberrations in a single culture. The number of three was set to exclude possible in vitro artefactual clones consisting of two daughter cells derived from an aberrant cell which had undergone second mitosis during the 48-h culture period (*14*).

For the confirmation of clonal aberrations, stepwise effort was made to identify the non-painted counterpart chromosome involved and the approximate breakpoints, first by re-staining the same slides with the Q-banding method (*16*), followed by FISH staining with new probes for the suspected counterpart chromosome which were labeled with fluorescent colors other than yellow (FITC) and red (PI) (Cambio, Cambridge, UK). Among the suspected clonal cells, over 90% of the cases could be confirmed as clones. These clonal aberrations were counted as single events.

Occasionally, clonal aberrations involving two painted chromosomes [e.g., t(1p+;2q-)] or within a painted chromosome [e.g., inv(1p+q-)] were recognized. They were found by their characteristic changes in the arm lengths or arm ratios. They were counted as one event in the denominator but zero in the numerator because they were not bicolor chromosomes. A portion of these data were reported previously (*14*).

Radiation Dose

Absorbed radiation doses to bone marrow were estimated by the Dosimetry System 2002 Revision 1 (DS02R1) (*17*). A fixed weighting factor of 10 was used for neutrons to calculate weighted dose in Gy. Radiation doses were adjusted to reduce bias due to random dosimetry error (*18*) assuming 35% error variation. In a supplemental analysis the previous DS86 doses (*13*) were substituted for DS02R1 doses to assess the impact of this important change on the results.

Other Covariates

Information on participant city, sex, age at the time of the bombing (ATB), and age at examination (ATE) was obtained from RERF records. Smoking status at the time of examination was categorized based on information periodically collected since 1963 through interviews and mail surveys.

Shielding [see ref. (*19*), chapter 7) categories were obtained from the dosimetry system records and broadly categorized as: Inside, 9P structure; inside, other; outside, with shielding; outside, in open; Nagasaki factory; and unknown. In brief, for individuals closer to the hypocenter exposed in Japanese style structures, very detailed so-called 9 parameter (9P) models of Japanese wooden houses were used to assess the subject specific shielding of radiation by the surrounding structure. For individuals farther away and exposed in structures, average shielding values were used. For individuals outside, shielding by adjacent buildings was accounted for to varying extents, whereas others were exposed in the open without surrounding structure shielding. Finally, several individuals were in factories in Nagasaki, which required special consideration because of the material used in factory structures and equipment. The small number of survivors without shielding information who were essentially unexposed with estimated doses of 0.001 Gy or less were grouped with ''Inside, 9P structure'' for analysis.

Statistical Analysis

The primary endpoint for analysis was the frequency of sCA over all cells that were scored, scaled to reflect sex-specific whole genome frequencies as described above. sCA frequency was modelled as quasi-Poisson (*20*). Specifically, if x_i is the number of sCA counted in n_i cells for subject i, then the expectation and variance of x_i were:

$$E(x_i) = \mu_i$$

$$V(x_i) = \theta\mu_i$$

where the constant θ represents extra-Poisson variation. The mean μ_i was modelled as

$$\mu_i = \mu_0\left(Z_i^0\right) \cdot \left\{1 + ERR(D_i) \cdot G\left(Z_i^1\right)\right\}$$

where D_i is dose in Gy, Z_i^0 and Z_i^1 are vectors of covariates, and where

$$\mu_0\left(Z_i^0\right) = e^{A'Z_i^0}$$

and

$$G\left(Z_i^1\right) = e^{B'Z_i^1}$$

where A and B are parameter vectors, and

$$ERR(D_i) = \left(\gamma_1 D_i + \gamma_2 D_i^2\right) \cdot e^{\gamma_3[D_i - D_0]^+}$$

where D_0 is a fixed dose cutpoint, $[u]^+$takes values 0 for $u \leq 0$ and u for $u > 0$, and γ_1, γ_2 and γ_3 are parameters. The choice of this parametric form for $ERR(D_i)$ will be justified below.

Here $\mu_0\left(Z_i^0\right)$ is the background (zero dose) sCA rate, $ERR(D_i)$ is the excess relative rate, and $G\left(Z_i^1\right)$ is a dose-effect modifier.

Background covariates were city (Hiroshima, Nagasaki), sex (male, female), linear and quadratic age ATE (centered at age 70, scaled to 5-year units), and smoking status at the time of examination (never, former, current, or unknown). These covariates were retained in the background irrespective of their statistical significance because of prior research indicating that they may be important determinants of aberration frequency (*8, 21*)

The adequacy of a simple linear-quadratic dose-response model without effect modification was initially examined after controlling for background factors. This simple model did not fit well over the entire dose range but fit well under approximately 1.25 Gy, above which the linear-quadratic increase did not persist. Empirical examination of the data indicated that the exponential decay model shown above with $D_0 = 1.25$ fit the data well. This model was used as the basis for examination of dose effect modifiers.

Dose response effect modification was examined by adding to the exponential term of $G(\cdot)$ a main effect and interaction (with $[D_i - D_0]^+$) for the variate of interest and performing a simultaneous test of these main effect and interaction terms. Inclusion of the interaction term sometimes resulted in an estimated dose response above D_0 which was not biologically coherent, which might have been rectified with additional modelling assumptions, but these terms were retained as is so as not to bias estimates of effect modification at lower doses, which is of primary interest.

The quasi-Poisson model was fitted using the ''gnm'' package of R [R: A language and environment for statistical computing. 2021, R Core Team, R Foundation for Statistical Computing: Vienna, Austria.] The parameter θ was estimated by method-of-moments as the ratio of the Pearson chi-square statistics to its degrees of freedom. Covariates were retained in or excluded from the ERR and effect modification terms of the model based on the approximate F-test (*22*) with criterion P value of 0.05. P values in the text are reported with numerator degrees of freedom (df) of the corresponding F-test. There was no adjustment for multiple testing (*23*). Confidence intervals for individual parameters were computed via profile F-test. Goodness of

fit of continuous variable representations for age ATE and dose was assessed by testing the significance of adding to the model categorical versions of these variables. Confidence intervals for ERR at 1 Gy were determined by taking the 2.5th and 97.5th percentiles of the empirical distribution of 2.5×10^5 ERR values generated randomly from the multivariate normal distribution of dose- and effect-modifier-related parameters conditional on background parameters, with the parameter estimates and estimated variance-covariance matrix take as the mean and variance of the unconditional distribution. Confidence intervals for background sCA rates as a function of age ATE were determined similarly but using the unconditional normal distribution of background parameters.

Additional statistical computations were performed using Stata (StataCorp, Stata Statistical Software: Release 17. 2021, StataCorp LLC: College Station, TX).

Ethical Considerations

This study was approved by the RERF Institutional Review Board via approval of Research Protocols 8-93 ''Cytogenetic study in the Adult Health Study by fluorescence *in situ* hybridization (FISH)''

RESULTS

Sample Characteristics

Among 1,985 subjects on whom samples for FISH assay were collected, 102 were excluded:

- 77 received radiation therapy prior to sample collection
- 23 had missing DS02R1 dosimetry
- 1 was not in the city at the time of the bombing
- 1 was an F1 offspring of A-bomb survivor parents and was not directly exposed

Of the 2,206 samples in the remaining 1,883 subjects, 338 were excluded:

- 35 had insufficient response to PHA mitogen yielding insufficient metaphase cells
- 21 in which the assay was not completed – sCAs were not counted
- 282 were repeat assays for clonal confirmation. Only the first successful assay was used for analysis.

This resulted in the exclusion of 15 additional subjects. The analysis set therefore comprises 1,868 subjects and assays.

Table 1 describes the study participants by relevant factors such as sex, smoking status, age ATB, age ATE, DS02R1 weighted bone marrow dose, and shielding category, by city.

The majority of subjects were female, equally in the two cities. The median age at exposure was 15 years, with exposure from early childhood to young adult ages. Blood was drawn at least 45 years after the bombing, so that the median age at exam was 69 years, with most subjects classifiable as senior citizens at the time of exam. More than half of individuals were never smokers, and 80% not current smokers at the time of exam.

Sampling was such as to favor subjects exposed at higher doses, so that 70% of individuals in the sample were exposed to 100 mGy or greater, compared to the RERF Life Span Study (LSS), where only 25% of individuals fall into this category. Almost 70% of subjects were inside of structures at the time of the bombing, slightly more in Hiroshima than in Nagasaki. Figure 1 shows the distribution of DS02R1 weighted marrow doses by shielding category. Note that subjects exposed outside in the open, and in particular those in factories, have estimated doses in a higher, more restricted range compared to the other shielding categories. Also, the majority of those exposed inside other structures had very low exposure doses, reflecting that these subjects were at greater distances from the hypocenter.

Total cells scored ranged from 291 to 700, with median 500. Scaled total aberrations detected ranged from 0 to 601 with median 28. Only 18 subjects (<1%) had zero aberrations detected.

Assessment of Background Factors

Sex, city, age at time of examination (ATE), and smoking at the time of examination were considered possible determinants of background sCA rate. In an analysis of 388 subjects exposed to <0.005 Gy, only age ATE was significant ($P < 0.0001$, 2 df) and remained significant in the final complete-data model ($P = 0.003$, 2 df) (Table 2). The test of departure from the linear-quadratic representation of age ATE was not significant in either the background cases-only model or the full model ($P = 0.53$, $P = 0.16$, 5 df, respectively).

Overall Radiation Dose Response

Figure 2 shows the ERR estimated from several models that do not include effect modification but all which control for city, sex, age ATE, and smoking status in the background as described above. Compared to separate ERR estimates within dose category (points plus error bars), a simple LQ function of dose over the entire dose range (solid curve) does not describe the data well, although an LQ model using only subjects with doses between 0 Gy and 1.25 Gy (dashed curve) fits the data well in this range, after which the LQ increase in ERR does not persist. However, a model with an added exponential decay component after 1.25 Gy in the ERR term, as described above, provides a good fit over the entire dose range (dotted curve). A comparison of the simple LQ model and the LQ model with exponential decay to these same two models with added categorical dose was used to assess fit. In the former, adding categorical dose significantly improved model fit ($P < 0.0001$, 8 df), whereas in the later, the addition of categorical dose was no longer significant ($P = 0.41$, 8 df). Hence the LQ model with exponential decay after 1.25 Gy was adopted as the reference model for exploring effect modification.

TABLE 1
Participant Characteristics

Variable		Exposure city		
		Hiroshima	Nagasaki	Total
	Total	1179 (100%)	689 (100%)	1868 (100%)
Sex	Male	445 (38%)	255 (37%)	700 (37%)
	Female	734 (62%)	434 (63%)	1168 (63%)
Age ATB[3] (years)	[0, 5)[1]	271 (23%)	155 (22%)	426 (23%)
	[5, 10)	148 (13%)	101 (15%)	249 (13%)
	[10, 15)	189 (16%)	112 (16%)	301 (16%)
	[15, 20)	231 (20%)	168 (24%)	399 (21%)
	[20, 25)	151 (13%)	67 (10%)	218 (12%)
	[25, 30)	87 (7%)	51 (7%)	138 (7%)
	[30, +)	102 (9%)	35 (5%)	137 (7%)
Age ATE[3] (years)	[-, 60)[1]	139 (12%)	68 (10%)	207 (11%)
	[60, 65)	226 (19%)	103 (15%)	329 (18%)
	[65, 70)	298 (25%)	145 (21%)	443 (24%)
	[70, 75)	221 (19%)	176 (26%)	397 (21%)
	[75, 80)	125 (11%)	95 (14%)	220 (12%)
	[80, +)	170 (14%)	102 (15%)	272 (15%)
Weighted marrow dose (Gy)	[0, 0.005)[1]	244 (21%)	144 (21%)	388 (21%)
	[0.005, 0.1)	112 (9%)	47 (7%)	159 (9%)
	[0.1, 0.25)	129 (11%)	41 (6%)	170 (9%)
	[0.25, 0.5)	106 (9%)	62 (9%)	168 (9%)
	[0.5, 0.75)	146 (12%)	100 (15%)	246 (13%)
	[0.75, 1.0)	127 (11%)	113 (16%)	240 (13%)
	[1.0, 1.5)	122 (10%)	107 (16%)	229 (12%)
	[1.5, 2.0)	76 (6%)	34 (5%)	110 (6%)
	[2.0, +)	117 (10%)	41 (6%)	158 (8%)
Shielding category	Inside, 9P structure	580 (49%)	307 (45%)	887 (47%)
	Inside, other	258 (22%)	120 (17%)	378 (20%)
	Outside, with shielding	275 (23%)	99 (14%)	374 (20%)
	Outside, in open	58 (5%)	24 (3%)	82 (4%)
	Nagasaki factory	0 (0%)	126 (18%)	126 (7%)
	Unknown[2]	8 (1%)	13 (2%)	21 (1%)
Smoking status	Never	639 (54%)	424 (62%)	1063 (57%)
	Past	281 (24%)	155 (22%)	436 (23%)
	Current	242 (21%)	99 (14%)	341 (18%)
	Unknown	17 (1%)	11 (2%)	28 (1%)

[1] [x,y) means greater than or equal to x but less than y; [x,+) means greater than or equal to x.
[2] ''Unknown'' category is grouped with ''Inside, 9P structure'' category for regression analysis.
[3] ATB = ''At time of bombing''. ATE = ''At time of examination.''

Assessment of Effect Modification

City, sex, age ATB, and shielding history were each examined as possible dose response effect modifiers. There was no evidence of effect modification by sex ($P = 0.50$, 2 df). However, age ATB ($P < 0.0001$, 12 df; $P < 0.0001$, 6 df for interaction with $[D_i - D_0]^+$), shielding history ($P < 0.0001$, 8 df; $P < 0.0001$, 4 df for interaction), and city ($P = 0.026$, 2 df; $P = 0.026$, 1 df for interaction) were all significant (Table 2).

For age ATB, in all categories, the slope of the dose response after 1.25 Gy is less than would be expected from a pure linear-quadratic dose response (Fig. 3A). Effect modification above 1.25 Gy does not maintain the same proportionality among age ATB groups as under 1.25 Gy. An *ad hoc* observation is that there is a greater change in the effect modification after 1.25 Gy in groups with the fastest initial rise in sCA rate (Spearman rank correlation –0.75, $P = 0.052$). In the lower dose range the effect of dose on sCA rate was least in young and older ages ATB (Fig. 3B).

For city, the effect modification was evident exclusively above 1.25 Gy, with little apparent effect at lower doses: Hiroshima ERR at 1 Gy = 4.6 (95% CI 4.1, 5.1); Nagasaki ERR at 1 Gy = 4.4 (95% CI 3.9, 5.0) (Fig. 4). A supplemental analysis that substituted DS86 doses for DS02R1 doses in the final model with the current FISH data also found significant effect modification by city ($P = 0.038$, 2 df), although with somewhat more of this effect evident at lower doses: Hiroshima ERR at 1 Gy = 6.6 (95% CI 5.9, 7.3); Nagasaki ERR at 1 Gy = 5.2 (95% CI 4.6, 5.9). The pattern of effect modification by age ATB and shielding was otherwise similar to the primary analysis using DS02R1 (data not shown).

For shielding, the effect modification tends to maintain the same proportionality among groups above 1.25 Gy as below, except for those exposed while inside other

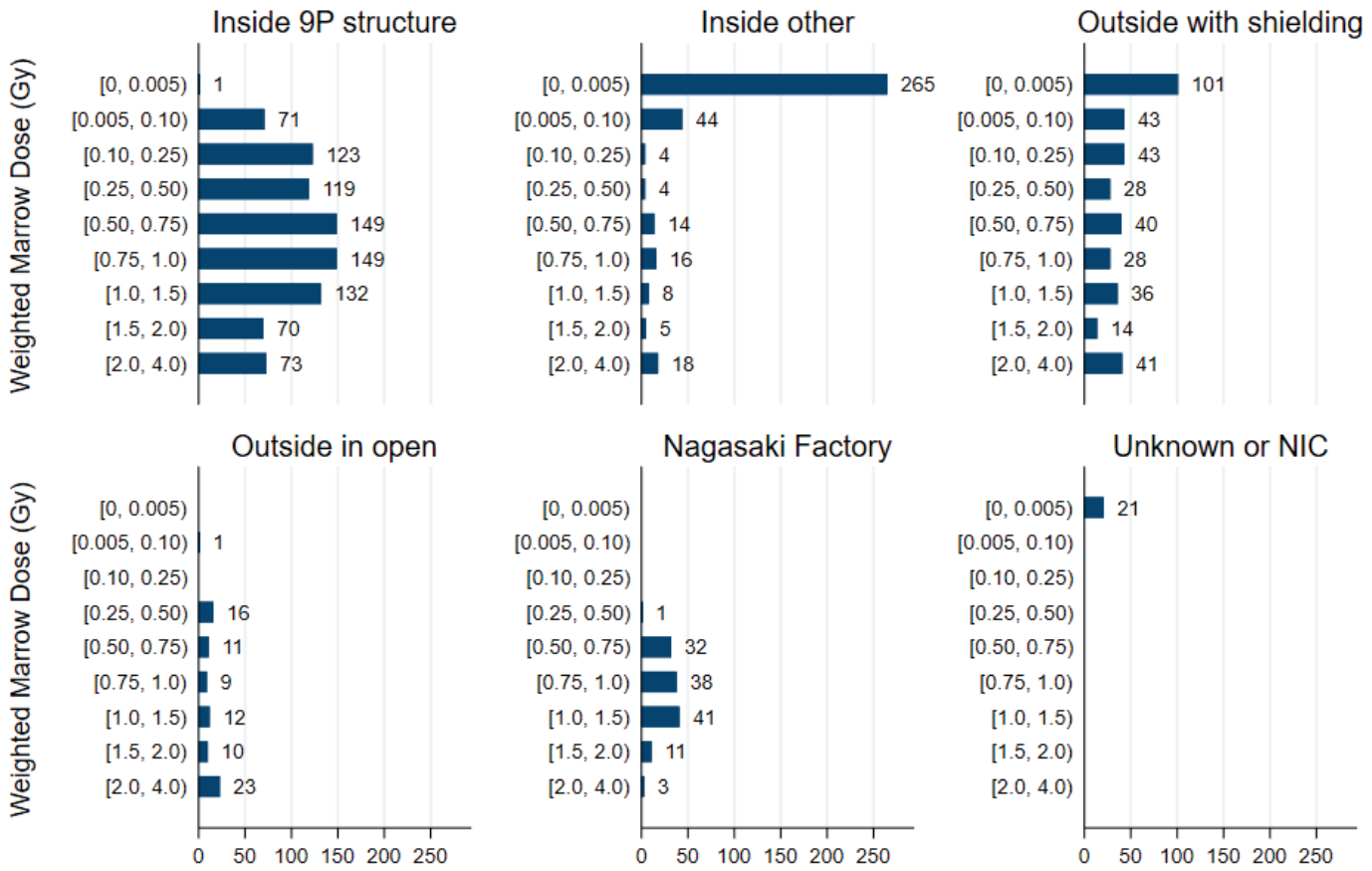

FIG. 1. Distribution of weighted bone marrow dose within shielding category.

structures where the estimated dose response above 1.25 Gy is based on a very small number of subjects (Fig. 5A). Individuals exposed outside in the open or exposed in factories in Nagasaki have the shallowest dose response among shielding groups (Fig. 5B). A model was examined that added city-specific effect modification terms both as main effect and interaction with dose greater than 1.25 Gy. While these added parameters were nominally significant ($P = 0.038$, 6 df), this was mostly due to city-specific effect modification above 1.25 Gy, as eliminating the main effect term did not degrade the model ($P = 0.81$, 3 df). Figures from this model analogous to Figs. 3 and 4 were virtually identical, and the pattern of within-city ERR at 1 Gy estimates by shielding category was not qualitatively different between Hiroshima and Nagasaki or from Fig. 5B (Supplemental Fig. S1; https://doi.org/10.1667/RADE-22-00154.1.S).

DISCUSSION

The present study is another in a series of analyses of the frequency of stable chromosome aberrations in lymphocytes of atomic bomb survivors and its relationship to radiation dose (*7–9*). There are three major differences in the current study compared to previous studies. The first is that the current study uses FISH technology rather than Giemsa-staining methods to identify chromosome aberrations, which provides much more sensitive and reproducible identification of chromosome aberrations and the ability to identify multiple aberrations per cells as well as clonal aberrations which should not be counted as individual occurrences. The second is that all cell cultures and sCA scoring occurred in a single laboratory. The third is that the current study uses the improved radiation dose estimates from the RERF DS02R1 dosimetry rather than the previous DS86 dose estimates (*13, 17*). Despite these differences, the results of the current study are largely confirmatory of previous findings, but with some notable differences.

As in the most recent previous analysis of stable chromosome aberrations (*8*), there was a significant increase in background sCA frequency by age at examination. Also, the overall shape of the dose response was consistent with that found in this most recent prior analysis, with an initial linear-quadratic increase in frequency by dose, which was attenuated at higher doses, a phenomenon that might be attributed to uncertainty in dose estimates. Under the assumption that the relationship between the frequency of chromosome aberrations and radiation dose should be linear quadratic throughout the dose range if individual marrow doses were known exactly, in the presence of random dosimetry error, since the LD50 for humans without

TABLE 2
Parameter Estimates for Final Quasi-Poisson Regression Model

Variable	Term	Estimate	Std. error	LB 95%[1]	UB 95%[1]	P value (df)[2]
Background term						
	Intercept	–3.59	0.0708	–3.73	–3.45	
Age ATE (years)	Linear term	0.0763	0.0154	0.0461	0.106	<0.0001 (2)[a]
	Quadratic term	0.00159	0.00456	–0.00744	0.0104	0.003 (2)[b]
Sex	Male	Reference				0.87 (1)[a]
	Female	–0.0495	0.0394	–0.127	0.0276	0.40 (1)[b]
City	Hiroshima	Reference				0.38 (1)[a]
	Nagasaki	–0.0154	0.0925	–0.198	0.164	
Smoking	Non-smoker	Reference				0.13 (3)[a]
	Past smoker	0.0696	0.0424	–0.0137	0.152	
	Current smoker	0.0122	0.046	–0.0782	0.102	
	Unknown	0.0249	0.108	–0.193	0.23	
ERR TERM						
Dose (Gy)	Linear term	1.28	0.449	0.458	2.26	<0.0001 (3)[c]
	Quadratic term	3.33	0.378	2.64	4.11	
	[Dose - 1.25 Gy]$^+$	–0.575	0.0766	–0.726	–0.426	<0.0001 (1)[c]
Effect modifier term						
City	Hiroshima	Reference				
	Nagasaki	–0.042	0.132	–0.301	0.216	
Age ATB (years)	[0, 5)[3]	–0.234	0.0947	–0.422	–0.0506	
	[5, 10)	–0.119	0.0909	–0.299	0.0582	
	[10, 15)	–0.0505	0.081	–0.212	0.108	
	[15, 20)	Reference				
	[20, 25)	0.0312	0.0858	–0.138	0.197	
	[25, 30)	–0.203	0.108	–0.418	0.00389	
	[30, +)	–0.379	0.138	–0.659	–0.113	
Shielding	Inside, 9P structure	Reference				
	Inside, other	0.112	0.104	–0.101	0.311	
	Outside, with shielding	–0.118	0.0718	–0.262	0.0196	
	Outside, in open	–0.409	0.125	–0.673	–0.168	
	Nagasaki Factory	–0.534	0.0992	–0.733	–0.343	
High dose × city	[Dose - 1.25 Gy]$^+$, Hiroshima	Reference				0.026 (2)[d]
	[Dose - 1.25 Gy]$^+$, Nagasaki	–0.142	0.064	–0.268	–0.017	0.026 (1)[e]
High dose × age ATB	[Dose - 1.25 Gy]$^+$, [0, 5)[3]	0.369	0.0807	0.212	0.528	<0.0001 (12)[d]
	[Dose - 1.25 Gy]$^+$, [5, 10)	0.286	0.0964	0.0957	0.474	<0.0001 (6)[e]
	[Dose - 1.25 Gy]$^+$, [10, 15)	0.403	0.085	0.237	0.571	
	[Dose - 1.25 Gy]$^+$, [15, 20)	Reference				
	[Dose - 1.25 Gy]$^+$, [20, 25)	–0.218	0.116	–0.449	0.00595	
	[Dose - 1.25 Gy]$^+$, [25, 30)	0.169	0.128	–0.0857	0.418	
	[Dose - 1.25 Gy]$^+$, [30, +)	0.442	0.152	0.135	0.735	
High Dose × shielding	[Dose - 1.25 Gy]$^+$, Inside, 9P structure	Reference				<0.0001 (8)[d]
	[Dose - 1.25 Gy]$^+$, Inside, other	–0.676	0.128	–0.935	–0.427	<0.0001 (4)[e]
	[Dose - 1.25 Gy]$^+$, Outside, with shielding	–0.0133	0.0656	–0.142	0.115	
	[Dose - 1.25 Gy]$^+$, Outside, in open	–0.224	0.112	–0.448	0.000366	
	[Dose - 1.25 Gy]$^+$, Nagasaki factory	–0.32	0.278	–0.965	0.176	

[1] Lower (LB) and upper (UB) bounds of 95% profile F-test confidence intervals.
[2] P value and numerator degrees of freedom (df) for approximate F-test. P values in the final model for tests of main effects alone are not shown for variable for which interactions with other variables exist in the model.
[3] [x,y) mean greater than or equal to x but less than y; [x,+) means greater than or equal to x.
[a] P value is relative to the model of background factors only in N=388 subjects with dose < 0.005 Gy.
[b] P value is relative to the current final model shown.
[c] Tests of all dose terms (3 df) and of the exponential decay term only (1 df) in a model without effect modification.
[d] Refers to the simultaneous test of both main effect and interaction with dose in the EM term.
[e] Refers to the test of only the interaction with dose in the EM term.

medical intervention after exposure to high-dose-rate radiation is 3 to 4 Gy (*24*), subjects who survive exposure at higher doses will tend to have estimated doses higher that their actual dose received, resulting in flattening of the observed dose response at these higher estimated doses.

There was significant effect modification by age ATB, although the pattern of the effect modification was different, with a previous study noting general heterogeneity without a discernible pattern (*9*), whereas in the current study there is the appearance of a more regular pattern with steeper dose

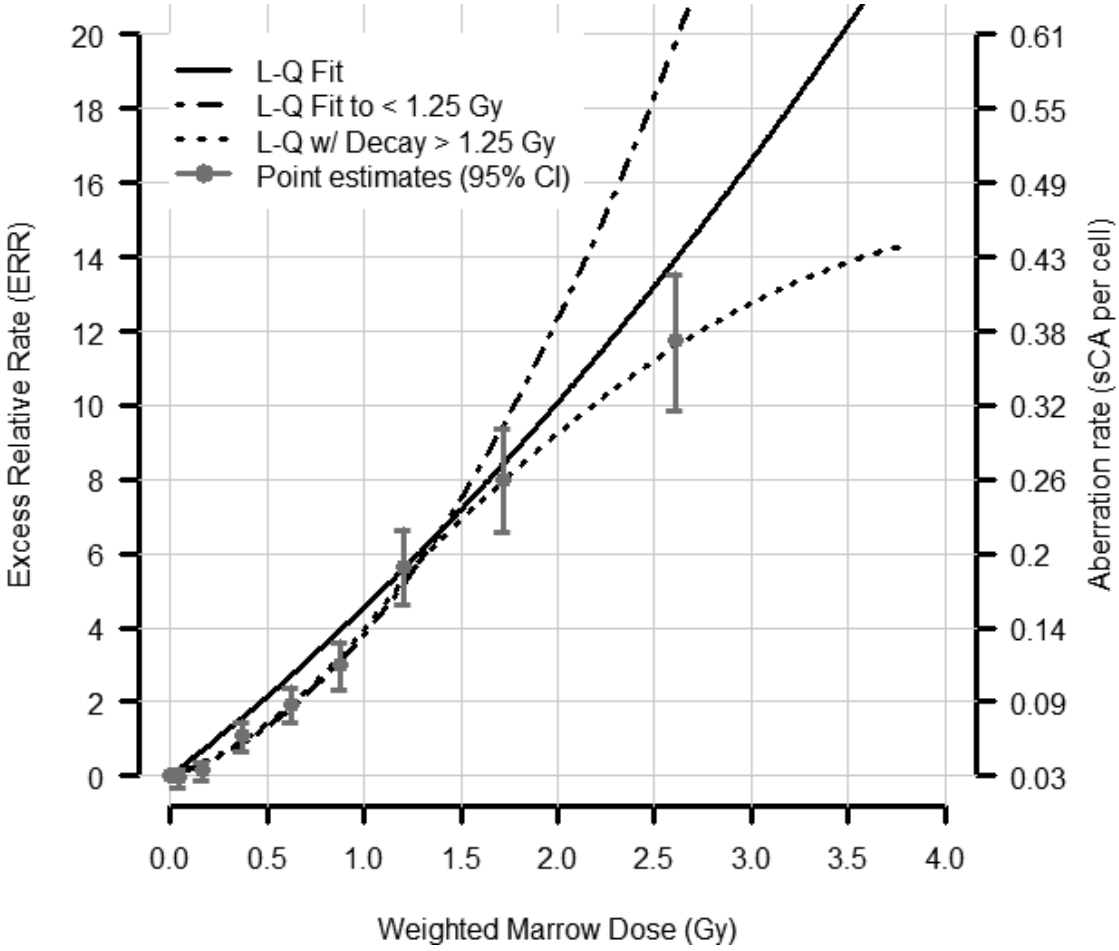

FIG. 2. Assessment of goodness of fit of the dose response model. Left scale – excess relative rate (ERR). Right scale - corresponding aberration rate computed from background rate in Hiroshima males aged 70 at exam.

response with increasing age ATB from childhood but then a shallower dose response at the older ages ATB.

One major difference in the current analysis versus the previous analysis is that at lower doses there was no discernible effect modification by city, whereas Kodama et al. (*8*) found significant 1.4 to 1.5 times larger initial slope in Hiroshima compared to Nagasaki across shielding categories [(*8*) see table 4]. A supplemental analysis that substituted DS86 dose for DS02R1 dose in our final model also showed a significant effect modification by city, but with more of this evident as a lower initial slope in Nagasaki than in Hiroshima. The difference was not as pronounced as that found by Kodama et al., however. This suggests that some of the previous observed difference in dose response between cities could be due to deficiencies in the DS86 dosimetry that were improved in the DS02R1 dosimetry — for example the reduction in neutron kerma component in Nagasaki (*13*) — and some due to differences in the Giemsa-based sCA detection efficiency between the Hiroshima and Nagasaki laboratories.

The most important finding in this updated study of the association between stable chromosome aberrations and atomic bomb radiation exposure is that the dose response differs by shielding category. The significant effect modification by shielding category was such that Nagasaki factory workers and those exposed outside had shallower dose response than those exposed inside of structures. This finding by shielding category, consistent with the previous DS86-based analysis (*8*), suggests possible remaining inaccuracies in the shielding input data or the shielding dosimetry models that results in overestimating the effect of structure shielding to attenuate exposure doses, or alternatively or in addition, random errors in the input data that are larger in those exposed outside or in factories and that are not accounted for in the current dose-error adjustment (*18*), thus differentially biasing the estimated dose response for individuals in different shielding categories.

It should be noted that the broad shielding categories utilized in this analysis obscures some fine details of shielding that will be important to consider in subsequent investigations of the implications of the finding of shielding effect modification. For example, the ‘‘outside with shielding’’ group comprises two main subgroups, with about 60% having detailed subject-level data about the type of and proximity to structures and terrain that provided shielding and thus whose dose estimates may be closer in

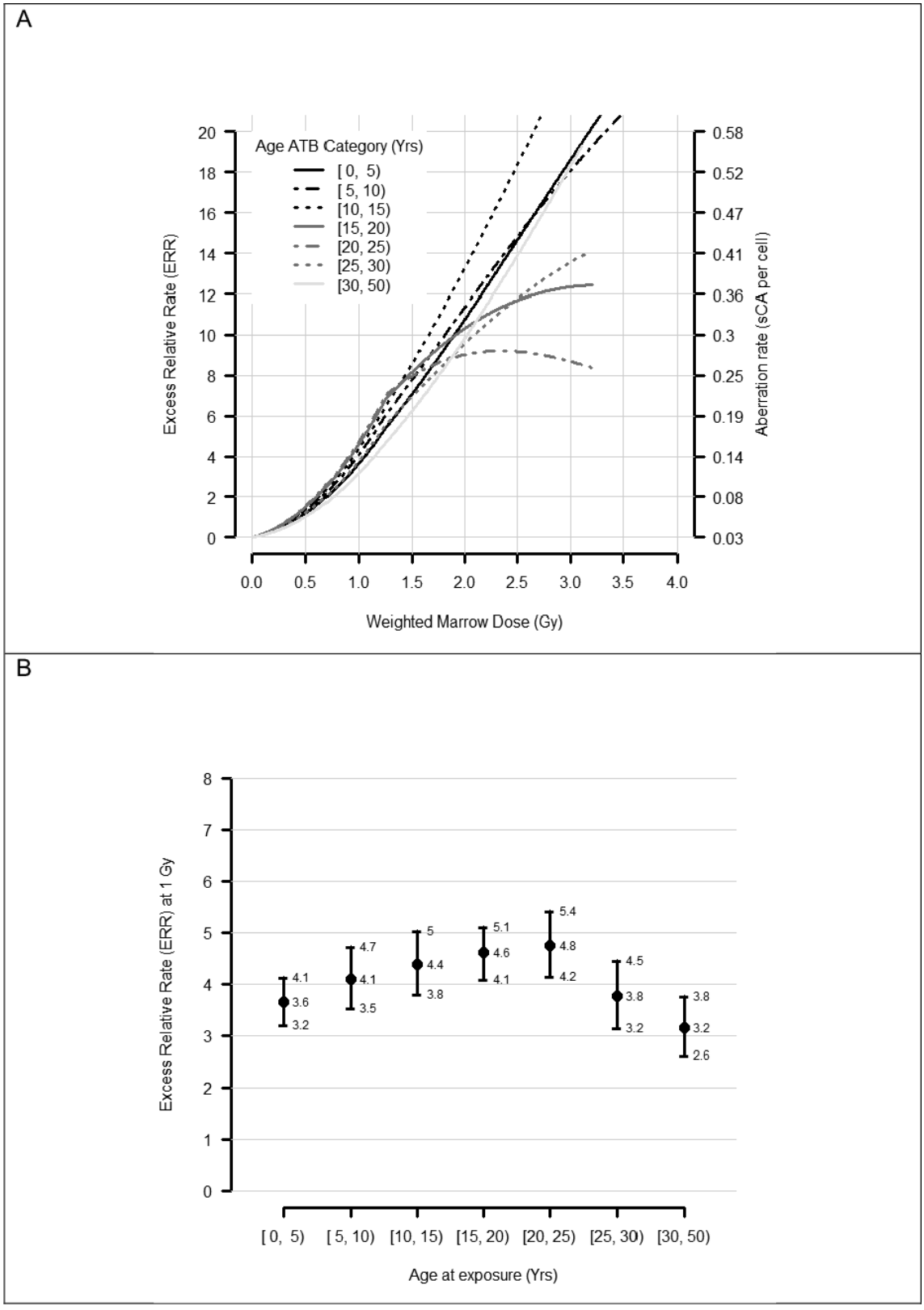

FIG. 3. Excess relative rate (ERR) of sCA aberrations by age ATB category. Panel A: Left scale – ERR. Right scale - corresponding aberration rate computed from background rate in Hiroshima males aged 70 at exam. Panel B: ERR at 1 Gy (95% CI).

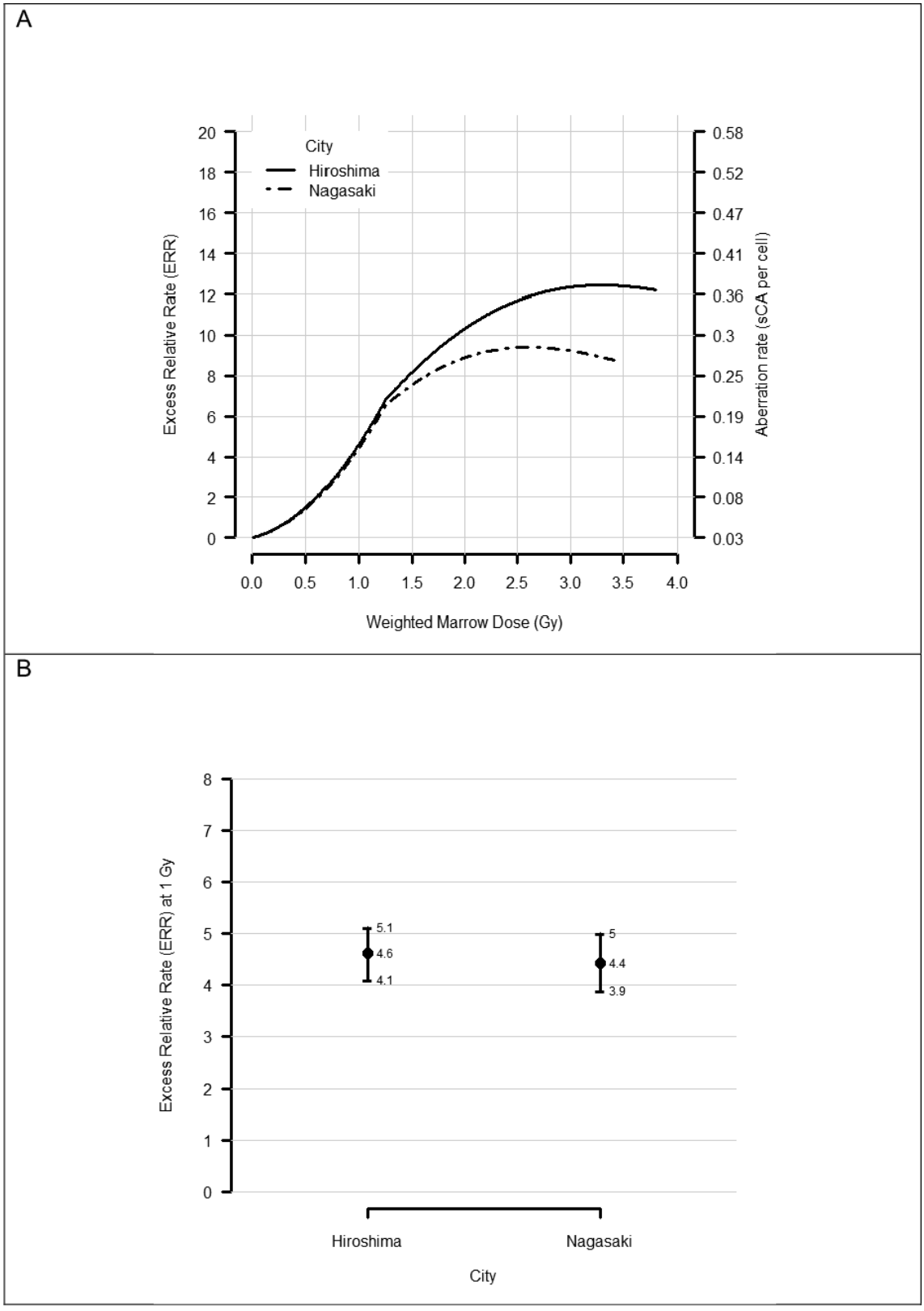

FIG. 4. Excess relative rate (ERR) of sCA by city. Panel A: Left scale – ERR. Right scale - corresponding aberration rate computed from background rate in Hiroshima males aged 70 at exam. Panel B: ERR at 1 Gy (95% CI).

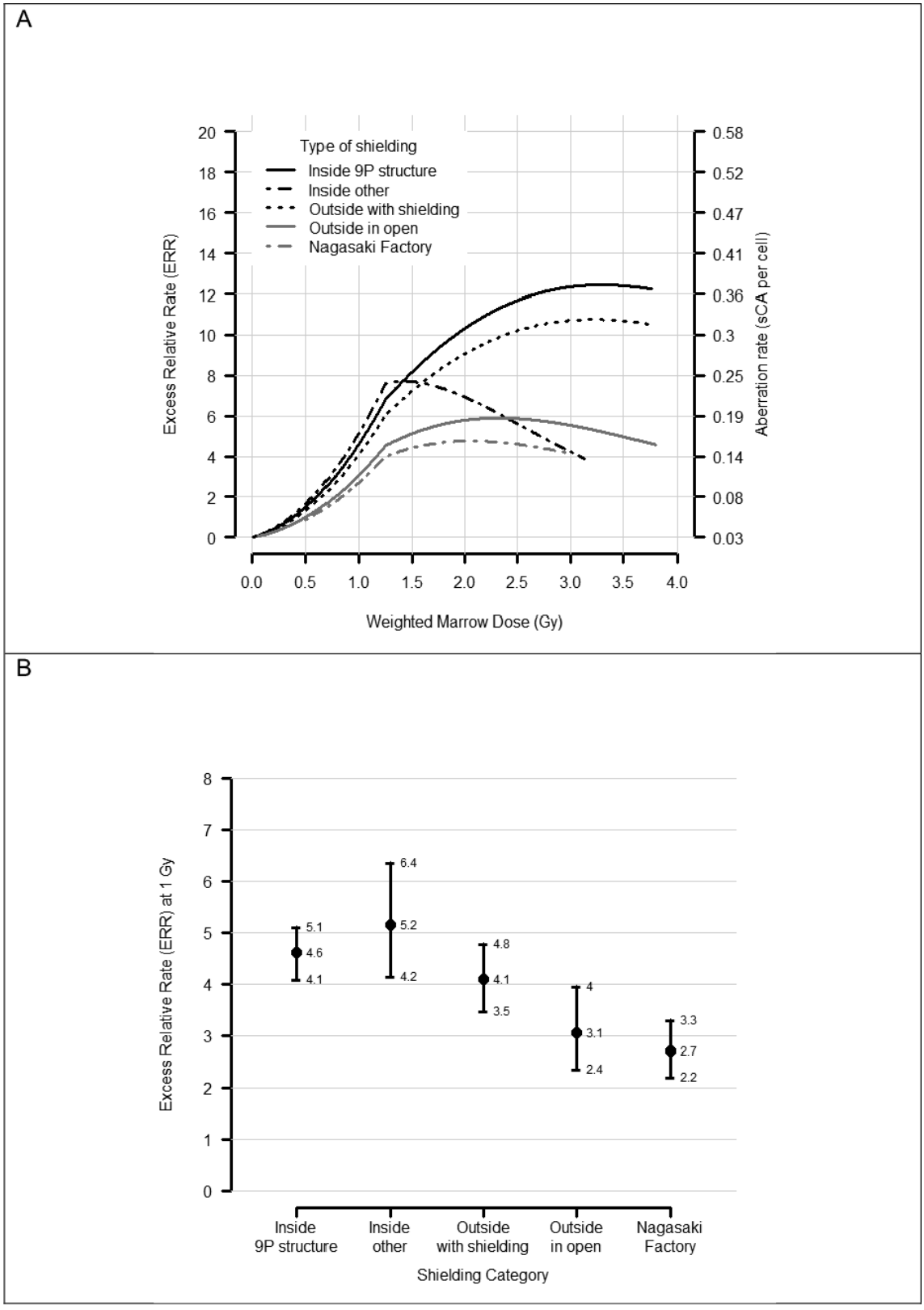

FIG. 5. Excess relative rate (ERR) of sCA by shielding category. Panel A: Left scale – ERR. Right scale - corresponding aberration rate computed from background rate in Hiroshima males aged 70 at exam. Panel B: ERR at 1 Gy (95% CI).

accuracy to those exposed in 9P houses, whereas the remainder lack this subject level data and are assigned average shielding factors, and would be expected to have more uncertainty in their dose estimates.

SUPPLEMENTARY MATERIAL

Supplementary Fig. S1. Excess relative rate at 1 Gy with shielding category for (panel A) Hiroshima and (panel B) Nagasaki.

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