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Chromosome Aberrations Determined by FISH in Radiation Workers from the Sellafield Nuclear Facility

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Workers from the Sellafield nuclear facility (Cumbria, UK) with occupational exposures to external sources of ionizing radiation were examined for translocation frequencies in peripheral blood lymphocytes using fluorescence *in situ* hybridization (FISH). This is an extension of an earlier study of retired workers, and includes analyses of additional samples from the earlier collection, bringing the total to 321. Another 164 samples from both current and retired employees, including 26 repeat samples, were obtained from a new collection, thus giving a combined dataset of 459 workers. This all-male population of workers was divided into 6 dose groups comprising 97 with recorded external occupational doses <50 mGy, 118 with 50–249 mGy, 129 with 250–499 mGy, 89 with 500–749 mGy, 17 with 750–999 mGy and 9 with >1,000 mGy. Univariate analysis showed a significant association between external dose and translocation frequency ($P < 0.001$) with the estimate of slope ± standard error being $1.174 \pm 0.164 \times 10^{-2}$ translocations per Gy. Multivariate analysis revealed that age increased the rate of translocations by $0.0229 \pm 0.0052 \times 10^{-2}$ per year ($P < 0.001$). However, the impact of age adjustment on the radiation dose response for translocation frequencies was minor with the new estimate of slope ± standard error being $1.163 \pm 0.162 \times 10^{-2}$ translocations per Gy. With the dose response for the induction of translocations by chronic *in vivo* low-LET radiation now well characterized, cytogenetic analysis can play an integral role in retrospective dose reconstruction of chronic exposure in epidemiological studies of exposed populations. © 2015 by Radiation Research Society

INTRODUCTION

Chromosome aberrations are an established marker of exposure to ionizing radiation and their presence in peripheral blood lymphocytes is a widely used measure of radiation exposure (*1*). Chromosome changes also play a major role in carcinogenesis (*2*) and there is increasing evidence that their presence in peripheral blood lymphocytes provides a marker of cancer risk (*3*). For the assessment of historical and chronic exposures to ionizing radiation the translocation is the aberration of choice (*1, 4–6*). Translocations are stable aberrations that persist through cell division and their presence in peripheral blood lymphocytes is maintained because descendants of irradiated bone marrow stem cells carrying translocations survive and appear in the circulating blood. However, translocations are monocentric, making it difficult to distinguish them from normal chromosomes, and it was only when fluorescence *in situ* hybridization (FISH) techniques were introduced in the mid-1990s that easy and accurate detection became possible. This led to suggestions that translocation frequency in peripheral blood lymphocytes could provide an integrated measure of past radiation exposure (*7*). Early application of the technique gave conflicting results. Some studies showed that doses estimated from translocation frequencies compared well with documented doses (*7–10*) and remained constant (*11, 12*). Other studies suggested a temporal decline in translocation frequencies, thus calling into question the validity of the technique and whether dose estimates were reliable in samples taken from people many years after exposure or from chronically exposed individuals (*13–18*). However, it became apparent that in cases where an initial drop in translocation frequencies was observed, this could be attributed to loss of cells that, in addition to carrying translocations, also carried dicentrics, centric rings and acentrics, thereby making them unstable (*4, 19, 20*). Restricting analysis to translocations in stable cells resulted in no appreciable reduction in yield. Chronic exposure to low doses of low-linear energy transfer (LET) radiation (<1

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Gy) will result in very few complex aberrations and chromosomally aberrant cells will predominantly contain only one simple exchange, i.e., a dicentric or a translocation (*21*). Thus for protracted low-LET exposure, translocation frequencies should increase with cumulative dose and provide a good measure of total dose. Indeed it has been suggested that the main application for FISH translocation analysis is in assessing long-term low-dose-rate cumulative exposure and thus, it is a useful tool in dose reconstruction for epidemiological studies (*5*).

The Sellafield nuclear reprocessing facility situated in Cumbria, UK has been in operation for over 60 years and many workers have spent their entire working lives employed at the site. Some of these individuals have accumulated relatively large occupational doses of radiation, albeit within regulatory limits operating at the time. Initial studies of current workers with recorded cumulative external occupational doses >500 mSv used FISH (*22*) and G banding (*23*) to detect stable chromosome aberrations and reported a positive dose response. A subsequent study of retired workers (*24*) focused primarily on translocations and was conducted according to guidelines derived by a consortium of laboratories funded by a European Commission Concerted Action on fluorescence *in situ* hybridization biological dosimetry (*4*). While a previous study (*24*) reported a linear dose response in line with expectations from *in vitro* work, this was driven by doses >500 mSv and there was still uncertainty regarding the response below 350 mSv. This uncertainty has implications for the application of FISH translocation analysis for dose estimation of low-dose-rate exposure and also for the interpretation of risk and the current practice of deriving risks for low-dose protracted exposure by extrapolation from high-dose, high-dose-rate studies. The current study was therefore initiated to reduce the statistical uncertainties at these lower doses by undertaking sampling on additional workers and undertaking a combined analysis with results from the earlier collection.

MATERIALS AND METHODS

Current Study Population

The male radiation workers included in this new collection were part of a wider investigation, which also examined *in vitro* chromosome radiosensitivity in relationship to previously reported *in vivo* translocation frequencies (*25*) and minisatellite mutations in families of radiation workers (*26*). The rationale for this was to reduce intrusion caused by multiple visits and to use a single blood sample for all studies. Prior to commencement, a presentation was made to workforce representatives at the Sellafield facility outlining the reasons for the study and how it would be undertaken. Having gained their support, ethical approval was obtained from North Cumbria Local Research Ethics Committee in May 2003 and the study started in January 2004. Initially the study focused on retired workers, however, in 2005 permission was sought from the Local Research Ethics Committee to include current workers. Approval was received in May 2005. Enrollment in the study and collection of blood samples continued until September 2008. Letters were sent to 825 radiation workers and included an information sheet and a reply/consent form. Separate permission was sought for participation in the different studies and also for the banking of DNA and lymphocytes for future studies. Potential participants were identified based on total cumulative occupational doses from external sources of radiation as measured by film badge readings (*27*). In previous studies of external radiation exposure we reported effective doses in mSv whereas here we report absorbed doses in mGy. Since the quality factor for gamma irradiation is one, the two are equivalent. Of the 825 radiation workers approached, 324 provided blood samples for FISH chromosome studies. These included workers with known intakes of plutonium, who will also be the subject of a future study, and repeat samples from workers with external radiation exposure who had been studied previously (*24*). Those consenting were visited by the study genetic nurse who took blood samples. These were transported to Westlakes Research Institute for lymphocyte culture and chromosome analysis. For the purpose of this study, FISH chromosome analysis was performed on samples from 138 men with external radiation exposure who had not been previously examined, and 26 men from the previous study (*24*) who provided a new blood sample. On completion of the study, individual dosimetry records were re-examined to confirm external doses and determine the extent of any internal alpha-particle dose. Only those with internal alpha-particle doses <10 mGy were included, this being recognized from previous *in vitro* (*28*) and *in vivo* studies (*29*) as likely to result in <0.5 translocations per 1,000 cells. The current study population of 164 comprised 12 control individuals with cumulative external doses <50 mGy, 63 with doses 50–249 mGy, 65 with doses 250–499 mGy and 24 with doses >500 mGy.

Cell Culture and sFISH Protocol

For each sample, multiple blood cultures were set up by the addition of 0.5 ml whole blood to 4.5 ml RPMI-1640 culture media supplemented with 10% heat inactivated fetal calf serum (Invitrogen Limited, Paisley, UK), and 1% phytohemagglutinin (PHA, M form; Invitrogen Limited). Bromodeoxyuridine (BrdU) (Invitrogen Limited) was added to a final concentration of 40 μ*M* 24 h after the initiation of the cultures to check cell cycling. The cultures were incubated at 37°C for 47 h. Colcemid (0.2 μg ml^{-1}) (Invitrogen Limited) was added for the final 4 h of culture to block the cells at metaphase. Cells were harvested using standard procedures with suspension in hypotonic solution (0.075 *M* KCl) (VWR International, Leicestershire, UK) for 15 min at 37°C followed by 3 fixes in cold methanol:acetic acid (3:1). Slides were prepared after storage of the fixed preparations for at least 24 h at −20°C.

Fluorescence *in situ* hybridization was performed on metaphase spreads using a cocktail of biotinylated probes for chromosomes 1, 3 and 5 and a pancentromeric probe directly labeled with Cy3 (*30, 31*). In this respect, the probe for chromosome 5 replaced that for chromosome 4 used in earlier studies. Briefly, slides were aged for 2–3 days at room temperature before being denatured in 70% formamide/2X SSC at 65°C. After denaturation of the chromosome (65°C) and pancentromeric probes (85°C), both were applied together to the denatured slides and allowed to hybridize overnight. Excess paint was subsequently washed off and the biotinylated chromosome probes detected with alternate layers of 4 μg ml^{-1} avidin-FITC and 5 μg ml^{-1} biotinylated goat anti-avidin. The chromosomes were counterstained with 4′, 6-diamidino-2-phenylindole (DAPI) in Vectashield anti-fade solution.

Chromosome Aberration Analysis

Analysis was carried out using a Nikon microscope with epifluorescence attachment and a computerized image analysis system (ISIS MetaSystems Group Inc., Newton, MA) was used to capture and confirm all aberrations. Only intact cells with ~46 chromosomes and all painted material present were included in the analysis. All aberrations were recorded and described according to the PAINT nomenclature (*32*). Exchanges involving one painted and one

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TABLE 1
Data on Age, Smoking, External Dose and Chromosome Aberration Frequencies by Dose Group

	Dose group (mGy)		
	<50	50–249	250–499
Current study			
Number of individuals	12	63	65
Mean age (years) (range)	75 (63–82)	66 (53–87)	65 (50–87)
Number of ever smokers (%)	11 (91.7)	35 (55.6)	43 (66.2)
Mean external dose (mGy) (range)	20 (0–46)	140 (57–246)	377 (251–490)
Number of cells scored	19,421	66,816	66,681
Number of genome equivalents	6,661	22,851	22,805
Number of translocations	111	265	317
Simple translocations per genome equivalent ± SE × 10^{-3}	16.66 ± 1.58	11.60 ± 0.71	13.90 ± 0.78
Total number of dicentrics (dicentric equivalents)	7 (1)	25 (9)	26 (15)
Total dicentrics per genome equivalent ± SE × 10^{-3}	1.05 ± 0.40	1.09 ± 0.22	1.14 ± 0.22
Number of unstable complexes (rogue cells)	7 (1)	15 (3)	26 (2)
Unstable complex aberrations per total cells ± SE × 10^{-3}	0.36 ± 0.14	0.22 ± 0.06	0.39 ± 0.08
Number of stable complexes	9	10	16
Stable complex aberrations per total cells ± SE × 10^{-3}	0.46 ± 0.15	0.15 ± 0.05	0.24 ± 0.06
Previous study			
Number of individuals	93	59	70
Mean age (years) (range)	69 (51–82)	69 (52–90)	69 (55–86)
Number of ever smokers (%)	64 (68.8)	39 (66.1)	48 (68.6)
Mean external dose (mGy) (range)	22 (0–50)	209 (57–250)	325 (251–499)
Number of cells scored	113,279	62,885	75,327
Number of genome equivalents	39,081	21,695	25,988
Number of translocations	397	197	301
Simple translocations per genome equivalent ± SE × 10^{-3}	10.16 ± 0.51	9.08 ± 0.65	11.58 ± 0.67
Total number of dicentrics (dicentric equivalents)	47 (24)	20 (11)	33 (15)
Total dicentrics per genome equivalent ± SE × 10^{-3}	1.20 ± 0.18	0.92 ± 0.21	1.27 ± 0.22
Number of unstable complexes (rogue cells)	52(15)	24 (3)	31 (4)
Unstable complex aberrations per total cells ± SE × 10^{-3}	0.46 ± 0.06	0.38 ± 0.08	0.41 ± 0.07
Number of stable complexes	38	12	21
Stable complex aberrations per total cells ± SE × 10^{-3}	0.34 ± 0.05	0.19 ± 0.06	0.28 ± 0.06
Combined data set[a]			
Number of individuals	97	118	129
Mean age (years) (range)	69 (51–82)	67 (52–90)	67 (50–87)
Number of ever smokers (%)	67 (69.1)	72 (61.0)	89 (69.0)
Mean external dose (mGy) (range)	22 (0–50)	174 (57–250)	354 (251–499)
Number of cells scored	132,700	129,701	142,008
Number of genome equivalents	45,742	44,546	48,793
Number of translocations	508	462	618
Simple translocations per genome equivalent ± SE × 10^{-3}	11.11 ± 0.49	10.37 ± 0.48	12.67 ± 0.51
Total number of dicentrics (dicentric equivalents)	54 (25)	45 (20)	59 (30)
Total dicentrics per genome equivalent ± SE × 10^{-3}	1.18 ± 0.16	1.01 ± 0.15	1.21 ± 0.16
Number of unstable complexes (rogue cells)	59 (16)	39 (6)	57 (6)
Unstable complex aberrations per total cells ± SE × 10^{-3}	0.44 ± 0.06	0.30 ± 0.05	0.40 ± 0.05
Number of stable complexes	47	22	37
Stable complex aberrations per total cells ± SE × 10^{-3}	0.35 ± 0.05	0.17 ± 0.04	0.26 ± 0.04

[a] A number of workers were sampled in both the current and previous studies. For the combined data set, they have been counted only once with their mean age and dose given and their chromosome results added to provide one result.

nonpainted chromosome were classified as simple while rearrangements with three or more breaks in two or more chromosomes were recorded as complex. Apparently simple translocations were recorded as one-way and two-way but for the purposes of this analysis were added together. Dicentrics were scored as simple if they occurred singly in a cell or dicentric equivalents if they occurred as part of more complex aberrations. Dicentrics were only recorded if they took place between a painted and nonpainted chromosome. Counterstained chromosomes in all cells with a painted rearrangement were examined for the presence of other aberrations to help identify complex aberrations and to establish stability status, the presence of a dicentric, centric ring or acentric conferring instability. Where rogue cells (*1*) were observed these were recorded separately and no attempt was made to resolve the aberrations. Such cells were placed in the unstable complex cell category. The presence of two or more cells with identical aberrations was considered as evidence of a clone and the aberrant cell was scored only once. In the main, approximately 1,000 cells were analyzed for each individual but in 24 cases this was extended to between approximately 1,500 and 3,000 cells, since these participants were intended for inclusion in other studies as well, and in 3 cases only between 700–1,000 cells were suitable for analysis.

In addition to results from 164 current study participants, the chromosome analysis data from the 294 individuals who were the subject of earlier reported studies were reassessed to ensure consistency of aberration categorization and their dosimetry and medical history reviewed. From 95 control individuals previously

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TABLE 1
Extended.

Dose group (mGy)		
500–749	750–999	>1,000
20	4	0
68 (53–80)	71 (65–76)	
13 (65.0)	4 (100.0)	
604 (500–741)	830 (755–897)	
24,009	5,141	
8,222	1,758	
157	17	
19.10 ± 1.52	9.67 ± 2.35	
13 (8)	0	
1.58 ± 0.44	0	
15 (1)	0	
0.62 ± 0.16	0	
11	1	
0.46 ± 0.14	0.19 ± 0.19	
75	15	9
70 (57–89)	70 (61–82)	73 (70–78)
50.0 (66.7)	13 (86.7)	3 (33.3)
611 (501–741)	845 (755–954)	1160 (1,039–1,563)
76,816	16,402	9,050
26,502	5,659	3,122
502	127	75
18.94 ± 0.85	22.44 ± 1.99	24.02 ± 2.77
55 (20)	9 (4)	4 (4)
2.08 ± 0.28	1.59 ± 0.53	1.28 ± 0.64
57 (21)	5 (2)	1 (1)
0.74 ± 0.10	0.30 ± 0.14	0.11 ± 0.11
38	6	4
0.49 ± 0.08	0.37 ± 0.15	0.44 ± 0.22
89	17	9
69 (53–89)	70 (61–82)	73 (70–78)
58 (65.2)	15 (88.2)	3 (33.3)
608 (500–741)	844 (755–954)	1,160 (1,039–1,563)
100,825	21,543	9,050
34,723	7,417	3,122
659	144	75
18.98 ± 0.74	19.42 ± 1.62	24.02 ± 2.77
68 (28)	9 (4)	4 (4)
1.96 ± 0.24	1.21 ± 0.40	1.28 ± 0.64
72 (22)	5 (2)	1 (1)
0.71 ± 0.08	0.23 ± 0.10	0.11 ± 0.11
49	7	4
0.49 ± 0.07	0.32 ± 0.12	0.44 ± 0.22

reported (*24*), six men were now excluded after a review of their medical history, and four were added to the group, one with <1,000 cells scored, one who was formerly classified as a plutonium worker and two who were previously classified as having doses >50 mGy prior to obtaining up-to-date dose data, resulting in a final control group from the earlier sampling of 93 workers with cumulative doses <50 mGy. For 21 of these men the analysis was extended from approximately 1,000 (*24*) to approximately 2,000 cells per individual (*33*). From the 199 exposed individuals reported previously (*24*), seven men were excluded after a review of their medical history and the aforementioned two moved to the control group, leaving a total of 190 men, five of whom had their analysis extended to approximately 2,000 cells. Additionally, when reevaluating the data from the previous study (*24*), results were found for an additional four individuals who had between 700–1,000 cells analyzed and three more men who had approximately 1,000 cells analyzed after publication of the study results. These men were added to the exposed group. Also added to the exposed group were results from 31 men with between 1,000–2,000 cells analyzed. While these 31 men were originally classified as plutonium workers (*34*), a reassessment of their doses showed that they had internal red bone marrow α-particle doses of <10 mGy. This brought the final exposed group from the earlier sampling to 228 individuals, and thus the total number now included in the results from the earlier sampling was 321.

Statistical Analysis

The AMFIT module of EPICURE software (*35*) was used to construct linear Poisson regression models for associations between cumulative occupational red bone marrow dose and translocation frequency. The models were of the general form, $\lambda(z, d) = \lambda_0(z) + \beta d$, where λ is the expected number of translocations per cell, z represents covariates affecting translocation frequency, d is cumulative occupational red bone marrow dose, $\lambda_0(z)$ is the covariate-specific background number of translocations per cell, and β is the increase in translocations per cell per unit dose. A Pearson scale factor was added to the models to account for overdispersion of the data. Age at sample collection, occupational external dose, occupational plutonium dose and smoking history were all considered as covariates in the multivariate model. In all cases the parameter estimates were computed with maximum likelihood methods, and *P* values quoted are based on two-sided tests.

RESULTS

The data are presented in six dose groups. Group data on age, smoking habit, radiation doses and chromosome aberration frequencies are shown in Table 1. In addition to the data from the current study, reassessed results for the men sampled previously are presented and a combined data set is derived. For the 26 men sampled twice the data are added together to give one result per individual for the combined analysis since these were all retired workers who had received no additional occupational radiation exposure in the intervening period. FISH analysis of bicolored aberrations detects only a proportion of the total aberrations present in a cell, and therefore translocation and dicentric frequencies are converted to whole genome equivalents using the Lucas formula (*36*). Frequencies in total genome equivalents, rather than in stable genome equivalents, are derived for simple translocations in stable cells, however, with few unstable cells observed there is little difference in the frequencies. Frequencies of total dicentrics (including dicentric equivalents) are also derived for total genome equivalents to enable comparisons with dicentric frequencies determined with solid staining. Extrapolation to the whole genome is not appropriate for complex aberrations and therefore complex stable and unstable cell frequencies are provided for total cells scored. Not all individuals contributed the same number of cells to the analysis and this has the potential to introduce bias. The means of the individual frequencies for translocations and dicentrics were derived for the different dose groups, however, as these proved to be the same as the total frequencies, they are not provided. Further analysis was restricted to translocation frequencies for the combined data set.

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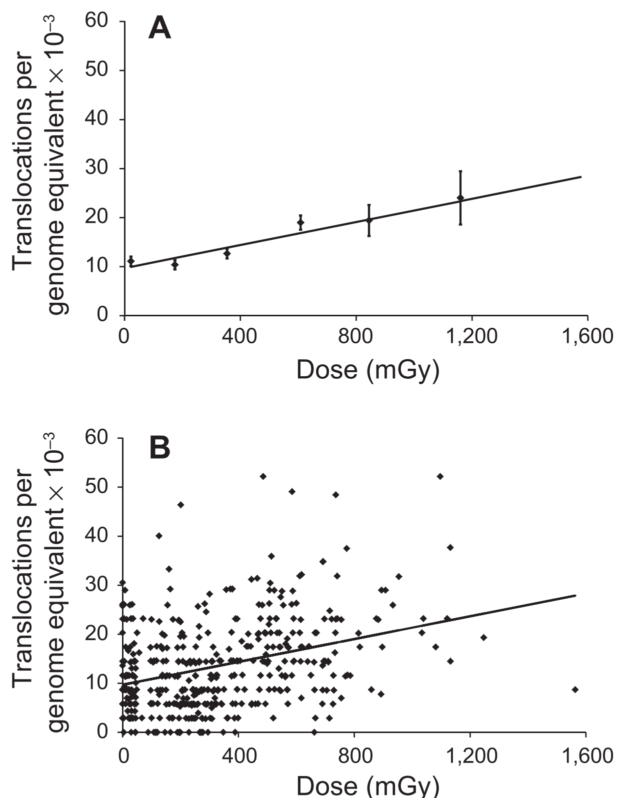

FIG. 1. Translocation frequency as a function of cumulative red bone marrow external gamma-ray dose, categorized by major dose groups (panel A) and individually (panel B).

Figure 1 shows the dose-response relationship for translocation frequencies, categorized by major dose (Fig. 1A) and individual frequency (Fig. 1B), both with the dose-response line superimposed. Univariate analysis showed a significant association between external dose and translocation frequency ($P < 0.001$) with the estimate of slope ± standard error being $1.174 \pm 0.164 \times 10^{-2}$ translocations per Gy. The multivariate analysis revealed that age at sample collection was significantly associated with the translocation frequency ($P < 0.001$) with age increasing the rate of translocations by $0.0229 \pm 0.0052 \times 10^{-2}$ per year of age. However, the impact of the age adjustment on the external dose response for translocation frequencies was minor with the new estimate of slope ± standard error being $1.163 \pm 0.161 \times 10^{-2}$ translocations per Gy. Smoking was not found to be significantly associated with translocation frequency when assessing the different rates among ever and never smokers. Workers with larger occupational plutonium exposures were excluded from this analysis and adjustment for the smaller plutonium doses received by some of the men included in this study had no material effect on the external dose response.

DISCUSSION

Translocation frequencies in peripheral blood lymphocytes are known to increase with age and the effect has been quantified in two major international studies (*33, 37*). The findings in the current study, although restricted to a limited age range, conform to expectations from these analyses. In our earlier study of retired radiation workers we reported a slope of $0.017 \pm 0.0075 \times 10^{-2}$ translocations per cell per year. The current study, which includes the data from the previous study, resulted in a similar value of $0.0229 \pm 0.0052 \times 10^{-2}$ per year of age for this age group. Smoking has been more difficult to evaluate, with the same two international studies of background translocation frequencies reporting conflicting results (*33, 37*). In the first studies of translocations in Sellafield workers we reported a significant effect of smoking (*22, 23*), however, this was not confirmed in the later larger study of retired workers (*24*). The current extension of this previous study similarly showed no impact of smoking on translocation frequencies.

The biophysics of radiation-induced chromosome aberrations has been the subject of much investigation and is well established (*1*). The formation of a simple chromosome exchange such as a dicentric or a translocation generally requires two DNA lesions and these can be formed by one single ionizing track or two separate tracks. *In vitro* studies have demonstrated that the dose-response relationship for low-LET radiation follows a linear-quadratic model. Exchanges produced by one track will have a frequency that is proportional to the linear function of dose, whereas those induced by two tracks will have a frequency proportional to the square of the dose. As the dose decreases, the linear term will predominate since the likelihood that two independent tracks will produce lesions in the same cell decreases. At doses below 0.5 Gy the probability of two tracks traversing a target is sufficiently low that simple exchanges will be produced almost exclusively by one track and at a low frequency. Decreasing the dose rate also decreases the frequency per unit dose and for chronic exposure the response is linear and is the same as the linear component of the dose-response curve for acute exposure, even for cumulative doses that may be quite high. Thus, the expectation is that for chronic low-LET exposure the predominant aberrant cell type will be a cell carrying a single translocation. This is borne out by the current study. For the dose groups <50 mGy, 50–249 mGy, 250–499 mGy, 500–749 mGy, 750–999 mGy and >1,000 mGy, the proportion of aberrant cells carrying a single translocation was 75, 81, 78, 77, 88 and 87%, respectively. Moreover, very few cells with complex aberrations were observed (Table 1), with no differences in frequencies in unstable or stable cells seen across the dose range.

While considerable dose-response data are available for dicentrics, there is much less information on the dose response for translocations and it has therefore been suggested that, for retrospective biodosimetry, the linear term for translocations be derived from that for dicentrics, which for high-energy gamma rays is ~1.5 per 100 genome equivalent cells per Gy (*4*). Fitting the data on translocations in stable cells in relationship to recorded cumulative

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external dose in the current study to a linear model results in a value of $1.163 \pm 0.162 \times 10^{-2}$ translocations per genome equivalent cells per Gy. This is in line with expectations from *in vitro* work and confirms our earlier finding on a smaller dataset (*24*). In contrast, frequencies of simple dicentrics and total dicentrics (Table 1) were similar across all groups, reflecting the fact that cells carrying dicentrics will have a finite lifetime in the peripheral blood and will not be replenished from the stem cell population.

In recent years FISH analysis for translocations in stable cells has been successfully used to assess population exposure in a range of protracted exposure situations. A reassessment of earlier data (*18*) from workers at the Mayak reprocessing plant resolved the discrepancy between translocation yield and expectations from *in vitro* work (*38*). Studies of exposure to diagnostic X rays (*39–41*) and occupational exposure received by U.S. radiologic technologists (*42*) in relationship to reconstructed cumulative doses also resulted in dose-response relationships in line with those derived from *in vitro* studies. This has led to suggestions that translocation frequency can reliably reflect cumulative exposure in low-dose protracted exposure conditions (*40, 42*). The current study confirms a linear dose response for translocations induced by chronic low-dose occupational exposure although there is wide individual variation (Fig. 1).

In an earlier study (*24*) we noted the discrepancies between the linear dose responses derived for low-dose exposures from the Japanese atomic bomb survivor data on stable aberrations (*43*) and those obtained for occupational exposure, and suggested that if chromosome aberration frequencies are indicative of cancer risk, then the lower values per unit dose derived for the Sellafield workers in comparison with the low-dose component of the Japanese dose-response curve implies that the low-dose risks derived from Japanese data will overestimate the risks associated with occupational exposure. The current study on an extended data set has resulted in a similar dose response for occupational exposure to that previously reported (*24*) and the value of $1.163 \pm 0.162 \times 10^{-2}$ translocations per cell per Gy therefore remains lower in comparison with the Japanese data. The differences between the responses for Hiroshima and Nagasaki have also been a cause for concern (*44*) and are thought to reflect uncertainties in the dosimetry and, in particular, the difficulty in assessing the weighted dose of the combined spectrum energy photons and neutrons, which was different for the two cities. *In vitro* studies on the induction of chromosome aberrations have been examined to assess the effectiveness of the different radiation profiles and used to derive a model for the relative biological effectiveness of the exposures received in the two cities (*44*). When this was applied to the chromosome data, the result was an increase in weighted dose for both cities and a reduction in the linear component of the dose response for cells with stable aberrations from 6.1 to 3.9×10^{-2} per Sv for Hiroshima and from 3.2 to 2.4×10^{-2} per Sv for Nagasaki. Moreover, it was suggested that this could have an impact on the risks deduced from the atomic bomb survivor cohort. More recently it has been suggested that the measurement of stable chromosome aberrations, namely translocations, together with a better understanding of the origin of the chromosomally aberrant descendants of irradiated cells observed in peripheral blood lymphocytes, may point to a need for new dosimetry calculations for the atomic bomb survivors (*45*). Since the currently accepted radiation risks are primarily derived from the Japanese atomic bomb survivor data (*46*), the importance of accurate dosimetry is paramount.

In this study, we have demonstrated that the dose response for simple translocations in workers with chronic exposure to primarily external gamma radiation conforms to expectations from *in vitro* studies. With the kinetics of the induction of translocations by chronic low-LET radiation now well characterized, cytogenetic analysis can play an integral role in retrospective dose reconstruction of chronic exposure in epidemiological studies of exposed populations. This will be facilitated by the introduction of automated techniques allowing more cells to be scored, thus improving the precision of individual assessments.

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