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Three Somatic Genetic Biomarkers and Covariates in Radiation-Exposed Russian Cleanup Workers of the Chernobyl Nuclear Reactor 6–13 Years after Exposure

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Three somatic mutation assays were evaluated in men exposed to low-dose, whole-body, ionizing radiation. Blood samples were obtained between 1992 and 1999 from 625 Russian Chernobyl cleanup workers and 182 Russian controls. The assays were chromosome translocations in lymphocytes detected by FISH, hypoxanthine phosphoribosyltransferase (*HPRT*) mutant frequency in lymphocytes by cloning, and flow cytometic assay for glycophorin A (GPA) variant frequency of both deletion (N/Ø) and recombination (N/N) events detected in erythrocytes. Over 30 exposure and lifestyle covariates were available from questionnaires. Among the covariates evaluated, some increased (e.g. age, smoking) and others decreased (e.g. date of sample) biomarker responses at a magnitude comparable to Chernobyl exposure. When adjusted for covariates, exposure at Chernobyl was a statistically significant factor for translocation frequency (increase of 30%, 95% CI of 10%–53%, $P = 0.002$) and *HPRT* mutant frequency (increase of 41%, 95% CI of 19%–66%, $P < 0.001$), but not for either GPA assay. The estimated average dose for the cleanup workers based on the average increase in translocations was 9.5 cGy. Translocation analysis is the preferred biomarker for low-dose radiation dosimetry given its sensitivity, relatively few covariates, and dose–response data. Based on this estimated dose, the risk of exposure-related cancer is expected to be low.

INTRODUCTION

A critical element in assessing the potential for health risk after exposure to radiation is an estimate of the dose and the biological effectiveness of the exposure. This need is especially difficult to meet when the exposure is low, time has passed since the exposure, and physical dosimetry is limited or unavailable. The exposure of hundreds of thousands of people during the containment and cleanup effort after the Chernobyl nuclear power accident poses a challenge and an opportunity. This report presents an extensive comparison of the ability of three somatic genetic biomarkers to detect and estimate effects of radiation exposure and other covariates in Russian Chernobyl cleanup workers.

Exposure to radiation causes many types of damage to the genetic material. Failure of cells to repair this damage completely and accurately results in chromosome and gene alterations. The extent of these consequences is related to the dose, to the individual's inherited capacity to repair each type of damage, and to the error-prone nature of some repair processes. An underlying assumption of somatic genetic biomarker studies is that risk of cancer rises with increasing genetic alterations in somatic cells. Prospective studies associating frequency of chromosomal alterations in peripheral blood lymphocytes with cancer risk have recently provided support for this assumption (*1–4*).

Biomarkers that monitor the frequency of somatic cell gene mutations and chromosome aberrations, the two major types of genetic alterations associated with progression toward cancer, have been developed and used in a number of population studies. Several excellent overviews of the genetic alterations assayed, the cell types used, and examples of assay usage are available (*5–9*).

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A brief summary of studies of somatic genetic biomarkers in radiation-exposed populations places the current study in context. Studies of atomic bomb survivors provided data on responses measured decades after exposure. The availability of reconstructed doses for atomic bomb survivors has been invaluable for the interpretation of biomarker studies. Extensive studies of chromosome aberrations, by a range of methodologies applied over the years, indicate that this end point has a characteristic dose response and high sensitivity as a reporter, even decades after exposure (*10, 11*). Assays of HLA, T-cell receptor and hypoxanthine phosphopribosyltransferase (*HPRT*) mutation in atomic bomb survivors' lymphocytes tended to have low signal when measured decades after exposure [reviewed in refs. (*8, 12*)]. The assay for glycophorin A (GPA) variant cell frequency using erythrocytes detected a dose response in atomic bomb survivors (*13, 14*) similar to that seen after more recent exposures [cf. Chernobyl (*15*); Goiânia ^{137}Cs accident (*16*)], but interindividual variation in atomic bomb survivor GPA variant frequency was substantial. Although the data sets for the specific gene mutation assays in atomic bomb survivors are limited, they have provided proof-of-principle and highlight the limitations of these assays when substantial time has passed since an acute exposure.

Studies of biomarkers in people exposed to radiation as a result of the Chernobyl nuclear power plant accident in April 1986 provide a contrast to the atomic bomb survivor studies. An estimated 600,000 to 800,000 people were involved in the cleanup. Accepting that the registered doses for the 119,000 cleanup workers with confirmed doses have many shortcomings, those people who were at Chernobyl in 1986 and 1987 are estimated to have received a mean whole-body exposure of 16 cGy and 9 cGy, respectively (*17*). The dose estimates are based only on external exposure, with ^{131}I (for those at Chernobyl in the earliest times), ^{134}Cs and primarily ^{137}Cs being the key radionuclides. The earliest biomarker studies emphasizing the more highly exposed individuals largely confirmed the relationship between biomarker responses, physical dosimetry, and clinical status [among many others, see refs. (*15, 18, 19*)]. Recent studies of workers whose work assignments were intended to limit exposure to 25 cGy or less have assessed the biological effect of low-dose, low-dose-rate exposure, and the reliability of available dosimetry. Over 700 cleanup workers from Estonia and Latvia and 51 local controls were assayed for GPA variant frequency in an effort to prescreen the population for sufficient exposure to warrant epidemiological studies of cancer incidence (*20*). The GPA results were interpreted to be consistent with an exposure that did ''not greatly exceed 10 cGy''. Estimates of doses based on physical dosimetry in this population ranged from 0.02 to 28 cGy. Chromosome translocation studies by fluorescence *in situ* hybridization (FISH) of 118 Estonian cleanup workers in this population found no increase in translocations (*21*), ruling out an exposure of 10 cGy or more and suggesting that the dosimetry available may have overestimated exposures for these cleanup workers. In contrast, a FISH translocation study of 34 Russian cleanup workers estimated an exposure of 25 cGy, compared to 26 cGy as their documented dose (*22*). The difference between these two studies most likely is due to the heterogeneity of the exposures associated with different work assignments and selection of a group with higher exposures in the latter study. It should be noted that the distribution of registered doses suggests that relatively few cleanup workers received doses in excess of 25 cGy (*17*).

In an earlier report (*23*), we described initial results of our studies of Chernobyl cleanup workers. This data set revealed statistically significant increases in the translocation frequency and *HPRT* mutant frequency in the exposed subjects, translocations having the larger increase. There was no increase in GPA variant frequency. Using an *in vitro* cytogenetic dose–response curve, the average dose to the cleanup workers studied was estimated to be 9 cGy. Data were limited for comparisons of the biomarkers within the same subject, or over time. Key questions that remained were: What is the relative sensitivity of these somatic genetic biomarkers for detecting exposures in populations with low-dose, low-dose-rate radiation exposures? As time passes after exposure, are biomarker responses affected? What is the accuracy of an estimate of exposure for the population? Are there optimal ways to use these assays? The results presented here extend through 1999 the study begun in 1991. The goal was to accumulate additional data using each biomarker over a longer period, to enhance comparisons of the biomarkers, and to study the effect of passage of time on results, and thereby gain more insight into the answers to these key questions.

MATERIALS AND METHODS

Recruitment of Subjects and Blood Samples

The recruitment of subjects, obtaining of samples, and completion of questionnaires were reviewed by Institutional Review Boards at all institutions involved and by the Office for Protection from Research Risk of the U.S. Public Health Service. All subjects gave informed consent prior to participation in this study.

Subjects from Russia. All individuals were males 18 years of age or older. Subjects were primarily selected at health care clinics in St. Petersburg or Tula, Russia. In St. Petersburg there were four clinics: at the Central Research Institute for Roentgenology and Radiology, Ministry of Health of Russia; at the Consulting Diagnostic Center of St. Petersburg Pediatric Institute; at the Division of Occupational Pathology in Territorial Medical Unit N 20; and at the St. Petersburg Institute of Pulmonology, Ministry of Health of Russia. In Tula the selection site was the Tula Institute of New Medical Technologies. Some subjects were recruited from clinics in Moscow. Individuals from the exposed cleanup worker population attended one of these clinics periodically to monitor their health status. Individuals included in the control population were selected from friends and relatives of the cleanup workers and other attendees of the clinics, with the goal of group matching the cleanup worker population with respect to age, sex and lifestyle. All subjects donated a venous blood samples of ~40 ml and were asked to complete a questionnaire (see below). Blood samples were collected into acid citrate dextrose anticoagulant and kept at low temperature (4–15°C) during short-term stor-

age and shipment. All samples were shipped to the Lawrence Livermore National Laboratory (LLNL) by courier service as rapidly as possible after collection. Shipping usually took about 3 days. Visual inspections of samples showed variation in their condition but no obvious patterns over time.

Subjects from Livermore. Blood samples for the *in vitro* dose–response studies were obtained from healthy, local male subjects, age 20 to 46. Information on age, smoking history, diet and lifestyle factors was obtained with a questionnaire. Samples were irradiated and cultures established the same day as venipuncture, as described previously (*24*).

Questionnaire

Each donor was asked to complete a questionnaire. The questionnaire was printed in Russian and information and instruction were provided in that language. For those subjects enrolled in the study prior to March 1997, the questionnaire was 12 pages long, as described previously (*23*). Subjects enrolled after that date were asked to complete a 6-page questionnaire designed to capture similar information in a more user-friendly manner, with fewer free text responses requiring translation and recoding. Included were marital status, current occupation and employment status, potential occupational exposure to carcinogens and radiation, medical history including medications and dental and medical radiation exposures, smoking history, and caffeine and alcohol consumption. In addition, for cleanup workers, official dose estimate, time(s) at Chernobyl, site of work, and work assignment when at Chernobyl were queried. Some self-reported dose estimates were based on thermoluminescence detectors (TLDs); most were based on interviews conducted by the Russians. It is unknown which were based on TLDs and which were based on interview information. Copies of the original Russian questionnaires (either on paper or on computer diskettes) were sent to LLNL where they were translated onto an identical form in English. All information provided in the questionnaires was entered into a computerized database (Sybase). Data from the first phase of the study were exported from the earlier database, with recoding as necessary to bring data on all subjects into the same data fields.

Handling of Biomarker Assays

On arrival at LLNL, blood samples were divided into aliquots for each assay, depending on the sample volume. In general the volume of blood required for each assay was as follows: cytogenetics, 3 ml; *HPRT*, $\geq$10 ml with a few exceptions of 6 to 10 ml; glycophorin A, 1 ml, N/M blood type only. Priority was given to cytogenetic over *HPRT* analysis when blood volume was limiting. Each laboratory that performed the bioassays had ongoing quality control to ensure comparability of results over time. To prevent bias, all assays were scored with sample classification (cleanup worker or control) blinded.

Chromosome Aberration Assays

Lymphocyte cultures. Whole blood (0.8 ml) was added to 10 ml of RPMI 1640 medium (Gibco/BRL) supplemented with 15% fetal bovine serum, 100 IU/ml penicillin, 100 μg/ml streptomycin, 1% sodium heparin, 2% phytohemagglutinin and 2 m*M* L-glutamine. Multiple cultures were prepared for each subject. Cultures were incubated at 37°C in a 95% air/5% CO_2 environment for 52 h, the last 4 h with 0.1 μg/ml Colcemid. Cells were harvested by swelling in 0.075 *M* KCl, then fixed a minimum of three times in 3:1 (v/v) methanol:glacial acetic acid. Well-spread metaphase cells, with little overlying cytoplasm, were prepared for chromosome painting as has been described in detail (*25*). Slides were stored at −20°C in sealed plastic bags in the presence of a desiccant and N_2 gas until needed for hybridization. Slides made from samples received up until 1995 were hybridized with Spectrum Orange-conjugated chromosome specific DNA probes (Gibco/BRL) for chromosomes 1, 2 and 4 simultaneously according to the manufacturer's instructions with minor modifications, essentially as described previously (*26, 27*). Slides made from samples received in 1996 and thereafter were hybridized with whole-chromosome painting probes (Cytocell, Ltd) for six chromosomes simultaneously: 1, 2 and 4 were labeled in red, and 3, 5 and 6 were labeled in green (*28*). All slides were mounted in a DAPI antifade solution (*29*).

Cell scoring. All cells were visualized with dual- or triple-band pass filters (Vysis, Inc.) for one- or two-color painting, respectively, which allowed simultaneous viewing of the painted and the DAPI-counterstained chromosomes. Metaphase cells were considered scorable if they met the following criteria: (a) The cells appeared to be intact, (b) the centromeres of the chromosomes were morphologically detectable as primary constrictions, (c) the centromeres from all painted chromosomes were present, and (d) the label was sufficiently bright to detect exchanges between painted and unpainted chromosomes or between chromosomes labeled in different colors. Every abnormal metaphase cell was photographed with Kodak Ektachrome 400 film or recorded with a CCD-based image capture system. These images provide a permanent record of each aberrant cell and were used to resolve ambiguous aberrations. All aberrations were initially scored according to the Protocol for Aberration Identification and Nomenclature Terminology (PAINT) (*30*). For statistical analysis all translocations were subsequently recategorized so that reciprocal and nonreciprocal translocations were each counted as a single translocation. This approach is based on the rationale that the low doses and low dose rates encountered by the cleanup workers generally did not result in complex chromosome rearrangements involving multiple color junctions which are often associated with nonreciprocal exchanges. There is also evidence that translocations that appear nonreciprocal at the cytological level usually contain telomere signals (*31*), suggesting that many of these exchanges may actually be reciprocal at the molecular level. In this study, translocation frequencies are reported as the number of translocations per 100 cells scored, with the number of cells scored converted to genome equivalents (see below).

Assays for generating in vitro dose–response curves. To estimate radiation doses to exposed subjects, we conducted *in vitro* dose–response studies. Lymphocytes from four healthy U.S. donors were exposed to a ^{137}Cs source *in vitro* at dose rates of 5 to 60 cGy per minute for doses ranging from 5 cGy to 4 Gy. For most data sets, doses from 0 to 1 Gy (in steps of 20 cGy) were emphasized. The number of cell equivalents scored ranged from 3670 at dose 0 to 670 at the highest dose. The acute exposures in this calibration study are not expected to model the chronic exposures experienced by the cleanup workers. However, in the absence of specific knowledge about the dose rate for each worker, and because of uncertainties about the magnitude of the dose-rate reduction effect, the dose estimates reported here are based on the acute dose–response curve generated from these data.

Conversion of metaphase cells scored to whole-genome equivalents. The fraction of all translocations detected by hybridization (F_h) was determined from the fraction of the genome painted in each color and was compared to the fraction observable by G-banding (F_b) using the equation

$$F_h = p^2 + 2pq + q^2 + 2pr + 2qr + r^2,$$

where p and q represent the fraction of the genome painted red (chromosomes 1, 2 and 4) and green (chromosomes 3, 5 and 6), and r represents the unpainted fraction of the genome. The terms p^2 and q^2 represent chromosome exchanges between two chromosomes painted in the same color, and the term r^2 represents exchanges between two unpainted chromosomes. The remaining terms represent exchanges between chromosomes labeled in different colors. Thus the fraction of all chromosome exchanges detected is $2pq + 2pr + 2qr$. For single-color painting, all terms involving q drop out, and the equation reduces to

$$F_h = p^2 + 2pr + r^2.$$

When chromosomes 1–6 are painted in two colors, 56% of the exchanges are detected; when chromosomes 1, 2 and 4 are painted, 34.4% are detected (*32*). In this study, approximately 1500 metaphase cells were scored per subject. This is equivalent to 840 (1500 × 0.56) metaphase cells if the full genome were scored (defined as cell equivalents) when

six chromosome pairs were painted, or 500 cell equivalents (1500 × 0.344) when three chromosome pairs were painted.

HPRT Mutant Frequency Assays

Lymphocytes were separated and the *HPRT* mutant frequency assays were performed as described previously (*33*). Mononuclear cells were isolated from 6- to 40-ml aliquots of blood samples using Lymphocyte Separation Medium (ICN Biomedicals INC, Aurora, OH). The isolated cells in most samples were cultured immediately. Mononuclear cells of some samples were cryopreserved for up to 16 months prior to culture. Cryopreservation was in RPMI 1640 medium supplemented with 8% dimethylsulfoxide and 20% fetal bovine serum and was performed in a controlled freezing chamber. Cells then were stored in liquid nitrogen. Cells were cultured at 37°C in 5% CO_2 for up to 40 h at 1×10^6 cells per milliliter with the mitogen phytohemagglutinin (PHA; 1 μg/ml; type HA16, Murex Biotech, Kent, England), then counted and plated in round-bottomed wells with 5% (v/v) LAK supernatant (lymphokine-activated killer cell supernatant containing 15,000 U/ml human interleukin 2, serum-free medium and any factors present in the supernatant after the 3–4-day activation of peripheral blood monocytes), 5% T-Stim Culture Supplement (Collaborative Biomedical Products, Bedford, MA), reduced PHA (0.1 μg/ml), irradiated lymphoblastoid feeder cells (20,000 viable irradiated TK-6 or 91x-C4 cells per well; irradiated with 50 Gy ^{137}Cs delivered at 4.2 Gy per min), and β-mercaptoethanol (50 m*M*), with or without thioguanine (1μg/ml) selection, essentially as per O'Neill *et al.* (*34, 35*). Throughout this work the basal medium was RPMI 1640 supplemented with 5% fetal bovine serum, 20% HL-1 (Bio-Whittaker, Walkersville, MD), penicillin (100 U/ml) and streptomycin (100 μg/ml). Donor cells were plated at 1 and 2 or 2, 5 and 10 cells/well in the absence of thioguanine (96 wells for each cell density) to determine the nonselective cloning efficiency (CE), and at 1×10^4 cells/well in 96 wells and at 2×10^4 cells/well using the rest of the lymphocytes in the presence of thioguanine to determine the cloning efficiency in the presence of thioguanine (mutant efficiency). Mutant frequency was calculated from individual cloning efficiency and mutant efficiency values as described in the *Statistical Methods* below. The number of plates at each cell density with thioguanine was dependent on the number of cells available for analysis. Plates were incubated at 37°C in 95% air/5% CO_2 and scored for growth in individual wells using an inverted microscope 15–18 days of incubation.

Assays of Glycophorin A Variant Frequency

Since the GPA variant frequency assay can be performed only on individuals with heterozygous N/M blood type (*36*), each sample was immunotyped using commercial antisera (Ortho Diagnostics, Raritan, NJ). This blood type comprises 50% of the human population.

The BR6 version of the GPA assay was performed as described previously (*36*). Briefly, blood samples were treated using sodium dodecyl sulfate (SDS) to produce spherical cells; 0.1 ml whole blood was mixed with 1.0 ml of Isolyte-S multi-electrolyte solution (Kendall McGraw Laboratories, Irvine, CA) containing 50 μg/ml SDS and 1 mg/ml bovine serum albumin (BSA). After 1 min, the sphered cells were added to 10 ml fixative solution [9.7 ml Isolyte S, 0.3 ml formalin (37% formaldehyde), 10 μg/ml SDS] and held in fixative overnight at room temperature. These fixed cells were rinsed twice with buffer [10 m*M* sodium phosphate (pH 7.2), 0.15 *M* NaCl, 5 mg/ml BSA, 0.01% Nonidet P-40, and 100 μg/ml NaN_3], then refrigerated in this buffer until staining. Fixed cells were immunolabeled with fluorescein-labeled monoclonal antibody BRIC 157, specific for the N-form of GPA, and biotinylated 6A7, specific for the M-form of GPA, followed by streptavidin-phycoerythrin (Caltag Laboratories, South San Francisco, CA). All cells were then counterstained with propidium iodide. A FACScan flow cytometer (Becton Dickinson, Mountain View, CA) was used to determine the frequency of variant erythrocytes. Analysis was limited to erythrocyte singlets based on forward scatter and log side scatter distributions. Two variant cell phenotypes were measured simultaneously in the GPA assay; hemizygous phenotype cells (N/Ø) lack expression of the M allele and express the N allele normally, and homozygous phenotype cells (N/N) lack expression of the M allele and express the N allele at twice the heterozygote level. The GPA assay was not affected by blood transit times up to 2 weeks because cell viability is not required (*36*). Duplicate fixations and analyses were performed on each sample with a total of 5×10^6 erythrocytes measured for each analysis. Mean values of these duplicate analyses were used to determine the frequencies of N/Ø and N/N variant cells per million erythrocytes.

Statistical Methods

All analyses were performed using S-PLUS (*37*).

1. Significance testing

All *P* values are two-sided. Tests of homogeneity of discrete variables across multiple groups were performed using χ^2 tests. Tests of homogeneity of continuous variables across two groups were performed using a Wilcoxon rank-sum test, while tests for continuous variables across more than two groups were performed using a Kruskal-Wallace test. No *P* values were calculated when any cell in a table had a count less than five, or when any group had fewer than five unique values.

Tests of significance for regression coefficients are asymptotic tests using standard errors derived from the observed information matrix.

2. General approach to the analysis of biomarker data

Each of the three biomarkers required a slightly different type of model to describe its relationship with covariates of interest. For chromosome aberration assays, the outcome is the number of translocations observed upon viewing some number of metaphase cells. Because of the discrete nature of translocations and the presence of variation beyond what would be expected using Poisson models, negative binomial maximum likelihood models were used to analyze translocation frequencies. For *HPRT* mutant frequencies, the outcome to be analyzed is a maximum-likelihood estimate of log mutant frequency, as well as an estimate of the precision with which the mutant frequency was estimated. For this outcome, weighted linear models were used on the log-transformed mutant frequencies obtained by maximum likelihood. Finally, for GPA variant frequencies, where no estimate of relative precision was available, ordinary linear models were used on log-transformed variant frequencies.

Two analyses were performed for each biomarker. The first analysis attempted to determine the overall ''average'' effect associated with being a cleanup worker. This analysis did *not* involve any variables specific to being a cleanup worker, e.g., the job performed at Chernobyl or the length of time in the 30-km zone. For each biomarker, the result of this analysis was an estimate of the average increase in the biomarker due to being at Chernobyl. The second analysis for each biomarker was performed for cleanup workers only. This analysis attempted to further dissect any observed average increase and discover if there were any Chernobyl-specific exposure variables that were associated with biomarker levels. The result of this analysis was a set of exposure variables that further partition the variation seen in cleanup worker biomarker values. For both analyses, adjustments for covariates were also produced.

For the first analysis, the relationship between each individual biomarker and being at Chernobyl was assessed by the following procedure. First, a backwards elimination procedure was used on an initial set of variables to remove variables that did not affect the outcome from further analysis. The initial set of variables included most of the variables from the questionnaire. Exceptions were due to intentional redundancy in the questionnaire information solicited for the smoking questions, which were recoded to produce a more orthogonal set of questions. At each stage of this procedure, likelihood ratio tests were performed on each variable currently in the model to compare the current model with one that eliminated that variable. The variable with the largest *P* value was eliminated. The backwards elimination procedure was terminated when the *P* values for all variables tested were less than 0.05, producing a final ''marginal'' model for each outcome.

Next, for *HPRT* and GPA outcomes, outliers among the observations

with respect to this final marginal model were tested for using the method described in Hadi and Simonoff (*38*). ''Method 1'' of the Hadi and Simonoff procedure was used to obtain the initial ''clean'' set of observations. For chromosome aberrations, no outlier-detection algorithm was available, so the distribution of crude aberration frequencies was examined for obviously influential observations. One observation had a value two orders of magnitude larger than the rest of the data set and was eliminated.

Finally, a regression was performed for each outcome to estimate the overall average effect due to being a cleanup worker. This regression used the final set of observations and the final set of variables for each outcome.

The second analysis on Chernobyl cleanup workers was done in a similar fashion. Only Chernobyl workers not eliminated as outliers in the first analysis were used. First, a backwards elimination procedure was performed on an initial set of variables. These variables included all of the covariates identified as significant in the first analysis, along with all of the variables associated with being a cleanup worker. The same likelihood ratio approach to variable elimination was used. After the variable elimination step, a regression was performed on the remaining variables. No outlier detection was performed on the second analysis.

3. Regressions with translocations as an outcome

Regressions involving translocations as an outcome were performed using an extended form of a negative-binomial maximum generalized linear model in which the dispersion term is also estimated by maximum likelihood (*39*). That is, the number of translocations counted in each subject was assumed to follow a negative binomial distribution with an unknown common dispersion term. For biomarker data, the logarithm of the underlying translocation frequency per cell scored was assumed to be a linear function of covariates. For dose–response data, the frequency itself was assumed to be a quadratic function of dose, but with a different background level for each subject. For negative binomial models, the ''percentage of variation accounted for by a model'' was estimated by fitting a corresponding linear model to the square-root-transformed outcome and using the resulting R^2.

4. Computing HPRT mutant frequencies

The methods and rationale for data analysis have been described in detail (*40*). Briefly, for each study subject, the natural logarithms of cloning efficiency and mutant frequency were estimated directly from the counts of positive and negative wells by the method of maximum likelihood, using a binomial generalized linear model with a complementary log-log link. Standard errors (SEs) were estimated using the asymptotic standard errors provided as a by-product of the fit. That is, the standard errors are the square root of the diagonal elements of the inverse of the Hessian matrix at the maximum likelihood estimate.

The distributions of cloning efficiency and mutant frequency were skewed, as is common with these kinds of data. Consequently, the natural logarithm of mutant frequency was used in regressions to reduce the effect of skewness. In addition, the precision of the estimates of log mutant frequency differed greatly among study subjects, due in part to a reduced number of cells plated under selective conditions and/or low cloning efficiency. To account for differences in precision, estimates of the relationships between log mutant frequency outcomes and predictors of interest (and their interactions) were calculated using weighted linear regression. The weight used for any given subject was the inverse of the variance of the estimated mutant frequency for that subject, estimated by the square of 1/SE, where SE corresponds to the standard error in estimating the mutant frequency for that subject.

5. Estimating average dose

The dose–response relationship was estimated by regression on *in vitro* dose–response data, using a negative-binomial maximum likelihood regression with an identity link, as described above. Insufficient data were available to estimate the variation in α and β between individuals. Consequently, a common dose–response curve was assumed for all subjects. Standard errors used for α and β were those obtained as a by-product of the fit. That is, the standard errors are the square root of the diagonal elements of the inverse of the Hessian matrix at the maximum likelihood estimate. The increase in translocation frequency for any specific cleanup worker due to being at Chernobyl was estimated as follows. First, the worker's predicted translocation frequency R_1 was calculated from the final marginal model for translocations and that individual's age, smoking status, and retirement status (covariates found to be significant in the population). The deviance residuals from the marginal fit were examined using a quantile-quantile plot, and they did not indicate any unusual deviation from a normal distribution (data not shown). Next, the worker's predicted translocation frequency R_0 were that person to have been a control was also calculated from the final marginal model. The increase due to being at Chernobyl was then estimated as $R_1 - R_0$. The standard error of the increase was estimated by adding the square of the standard error for R_1 to the square of the prediction error for R_0 and taking the square root of the resulting sum. The average increase over all of the cleanup workers in the sample was estimated by averaging the individual $R_1 - R_0$ values. The standard error of the average increase was found by summing the squares of the individual standard errors, dividing by the square of the number of cleanup workers, and taking the square root. The average dose was then calculated by solving for dose in the equation

$$\text{Average increase} = \alpha(\text{dose}) + \beta(\text{dose})^2.$$

Finally, the standard error for the estimated dose was calculated by the ''Delta Method'' (i.e. a Taylor expansion), incorporating the error estimates for the average increase, α, and β.

RESULTS

The Study Subjects

The study sample is presented in Table 1. Only samples obtained after August 24, 1992 are included. On this date consistent use of a questionnaire and of sample acquisition and handling practices for studies with multiple end points were instituted. The last samples included were received on December 12, 1999. Subjects were excluded if they had had chemotherapy, radiotherapy or a blood transfusion in the past year. Subjects recruited at clinics in three cities in Russia entered the study from 1992 to 1999 with the receipt of the first blood sample they provided (Table 2). Analyses of the data for each biomarker were performed to determine whether the sampling date affected the value of the biomarker (see below). The potential for differences between subjects from different cities was assessed for each biomarker (see below). This report contains results that extend the report of Moore *et al.* (*23*) to include 107 more control subjects and 419 more cleanup workers who were enrolled in the study over an additional period of approximately 4 years.

General Demographic Information

For this report, information collected by the original questionnaire (*23*) and the revised questionnaire (see the Materials and Methods) was used. Although the majority of controls and cleanup workers provided questionnaires (78 and 79%, respectively), not all questions were answered by all subjects. As a result, differences between the groups may not have been detected. For merging of information from the two questionnaires and for analytical purposes, summary attributes were developed, e.g. coding medical radiation exposure other than radiotherapy as ''any X

TABLE 1
The Study Sample

Study group attributes	Exposure status: Controls (N = 187)	Exposure status: Cleanup workers (N = 744)	Overall (N = 931)
Reasons for exclusion from study:			
Acquired before August 24, 1992			
No	185	627	812
Yes	2	117	119
Blood transfusion			
No	140	467	607
Yes	1	10	11
No answer	46	267	313
Radiation treatment			
No	141	518	659
Yes	1	2	3
No answer	45	224	269
Chemotherapy			
No	63	278	341
Yes	0	4	4
No answer	124	462	586
Final study subjects			
No	7[a,b]	133[b]	140[b]
Yes	180	611	791
Questionnaire available[c]			
Yes	140	485	625
No	40	126	166

Note. The initial all-male study sample and numbers of subjects that did not meet the criteria for inclusion are presented.

[a] Three individuals whose samples were received in 2000 were excluded.

[b] Individuals not included in the final study set include those with any of the four exclusion attributes.

[c] Absence of a questionnaire was not used as an exclusion criterion.

rays'', yes/no, to summarize a variety of such exposures. Summary variables for alcohol consumption and smoking were developed in addition to detailed information. Vitamin usage was coded yes/no. Data were coded as ''no answer'' when a questionnaire response was not provided, enabling detection of variation due to the nonresponding subjects. This practice has the advantage of increasing power by including more subjects, but it has the potential to introduce bias if nonresponders and responders are not randomly distributed. The above procedure for data with missing values was applied consistently throughout the analyses. Some of the primary characteristics of controls and cleanup workers who provided a questionnaire are presented in Table 3. Controls and cleanup workers differed significantly in 8 of these 17 attributes. In addition to the variables in Table 3, all information available for controls and cleanup workers from the questionnaires on employment status, occupational exposures, medicines taken, and diet was analyzed to identify which of these fractions affected the biomarker outcomes. Results of the univariate analyses for the three biomarkers for 34 potential risk factors used to identify potential covariates are not presented.

Exposure Information for Cleanup Workers

Characteristics of individual exposure histories at Chernobyl are presented in Table 4 as a function of the first year at Chernobyl. Data on self-reported (''official'') dose, length of time worked (longer in later years presumably due to lower dose rates), and working in block 4 (the site of the damaged reactor; fewer cleanup workers at Chernobyl in later years) are consistent with lower exposure rates for those at Chernobyl in the later years. Age when first at Chernobyl was fairly constant. There is a slight decline in the time elapsed between first work at Chernobyl and providing a sample for those who were at Chernobyl in the later years. However, this is a relatively small group. Due to the preferential recruitment of subjects who were first at Chernobyl in 1986, there are few subjects who first worked at Chernobyl in years 1989 and later. The Pearson correlation between the date entered into study and the years elapsed since first at Chernobyl was 0.94. Occupation at Chernobyl was summarized in nine categories (listed in

TABLE 2
Subject Entry into Study over Time by Exposure Status and Clinic Site

Year entered study[a]	Moscow (N = 36): Controls (N = 6)	Moscow (N = 36): Cleanup workers (N = 30)	St. Petersburg (N = 461): Controls (N = 129)	St. Petersburg (N = 461): Cleanup workers (N = 332)	Tula (N = 294): Controls (N = 45)	Tula (N = 294): Cleanup workers (N = 249)	Exposure status (all cities): Controls (180)	Exposure status (all cities): Cleanup workers (611)	Total (791)
1992	0	0	1	29	0	0	1	29	30
1993	0	0	0	39	0	8	0	47	47
1994	0	5	3	40	7	45	10	90	100
1995	0	0	69	20	8	50	77	70	147
1996	0	0	11	71	2	26	13	97	110
1997	1	12	7	42	5	48	13	102	115
1998	5	13	24	51	2	58	31	122	153
1999	0	0	14	40	21	14	35	54	89

[a] Year of first sample from subject.

TABLE 3
Characteristics of Those Controls and Cleanup Workers Studied for whom a Questionnaire was Available

Characteristics	Controls (N = 140)	Cleanup workers (N = 485)	P value[c]
Number (%)	140 (22%)	485 (78%)	
Age			0.02
Median	39.7	43.7	
Marital status			<0.001
Divorced	18 (13.0%)	54 (11.00%)	
Married	91 (65.0%)	422 (87.00%)	
Never married	28 (20.0%)	3 (0.62%)	
No answer	3 (2.1%)	6 (1.20%)	
Smoking status			0.77
Never smoked	35 (25.0%)	116 (24.0%)	
Ex-smoker	21 (15.0%)	59 (12.0%)	
Current smoker	81 (58.0%)	296 (61.0%)	
No answer	3 (2.1%)	14 (2.9%)	
Smoked cigarettes or popirossi			0.22
Nothing	35 (25.0%)	116 (24.0%)	
Cigarettes only	71 (51.0%)	266 (55.0%)	
Popirossi only	13 (9.3%)	25 (5.2%)	
Cigarettes and popirossi	18 (13.0%)	54 (11.0%)	
No answer	3 (2.1%)	24 (4.9%)	
Years smoked cigarettes[a]			0.01
Median	19	24	
No answer	58	186	
Pack-years of cigarettes[a]			0.34
Median	14.9	14.8	
No answer	60	213	
Years smoked popirossi[b]			0.83
Median	23.8	22.3	
No answer	112	418	
Pack-years of popirossi[b]			0.24
Median	13.8	22.5	
No answer	113	431	
Currently employed			0.001
Yes	98 (70.0%)	255 (53.0%)	
No	41 (29.0%)	222 (46.0%)	
No answer	1 (0.7%)	8 (1.6%)	
Retired			0.12
Yes	8 (5.7%)	37 (7.6%)	
No	44 (31.0%)	191 (39.0%)	
No answer	88 (63.0%)	257 (53.0%)	
Beer consumed (bottles/week)			0.39
None	24 (17.0%)	105 (22.0%)	
1 or 2	9 (6.4%)	42 (8.7%)	
3 or 4	6 (4.3%)	32 (6.6%)	
More than 4	6 (4.3%)	16 (3.3%)	
No answer	95 (68.0%)	290 (60.0%)	
Wine consumed (bottles/month)			0.67
None	29 (21.0%)	128 (26.0%)	
1 or 2	13 (9.3%)	49 (10.0%)	
3 or 4	3 (2.1%)	7 (1.4%)	
More than 4	1 (0.7%)	3 (0.62%)	
No answer	94 (67.0%)	298 (61.0%)	
Other alcohol (bottles/month)			0.59
None	15 (11.0%)	69 (14.0%)	
1 or 2	25 (18.0%)	109 (22.0%)	
3 or 4	4 (2.9%)	15 (3.1%)	
More than 4	1 (0.7%)	5 (1.0%)	
No answer	95 (68.0%)	287 (59.0%)	
Coffee consumed (cups/day)			0.74
None	13 (9.3%)	59 (12.0%)	
1 or 2	72 (51.0%)	258 (53.0%)	
3 or 4	8 (5.7%)	30 (6.2%)	
More than 4	5 (3.6%)	17 (3.5%)	
No answer	42 (30.0%)	121 (25.0%)	
Vitamins taken regularly			0.05
No	100 (71.0%)	299 (62.0%)	
Yes	39 (28.0%)	171 (35.0%)	
No answer	1 (0.7%)	15 (3.1%)	
Take nerve medicines			0.004
No	117 (84.0%)	337 (69.0%)	
Yes	23 (16.0%)	147 (30.0%)	
No answer	0 (0%)	1 (0.21%)	
Exposure to chemicals or solvents			0.005
No	45 (32.0%)	230 (47.0%)	
Yes	17 (12.0%)	50 (10%)	
No answer	78 (56.0%)	205 (42%)	
Any diagnostic X rays[d]			<0.001
No	12 (8.6%)	131 (27.0%)	
Yes	128 (91.0%)	331 (68.0%)	
No answer	0 (0.0%)	23 (4.7%)	

[a] Among the 409 self-reported cigarette smokers only.
[b] Among the 110 self-reported popirossi smokers only.
[c] P values reflect differences between characteristics of controls and cleanup workers. ''No answer'' individuals were included. P values were calculated using non-parametric rank tests for homogeneity between controls and cleanup workers.
[d] Includes all who had any diagnostic X rays, dental, bone, chest, special.

Table 8, discussed below). The first year at Chernobyl and date first in 30-km zone (an area of restricted access surrounding the reactor) are synonymous. Self-reported dose did not vary systematically as a function of date entered in the study (Table 5), suggesting consistency in recruitment over the span of the study.

Biomarker Assays Performed

Table 6 summarizes the assays performed. When blood volume was limited, priority was given to translocation analysis over *HPRT*. Translocation analyses were completed on all possible samples; about one quarter of cultures did not provide sufficient metaphases to complete translocation frequency analyses. Culture failures tended to occur for all samples of a given shipment, suggesting that problems encountered in transit were a likely cause. These failures showed no pattern over time. *HPRT* analyses of cleanup worker samples were performed preferentially on GPA heterozygotes. GPA assays were performed on essentially all N/M heterozygotes, i.e. 58% of the samples received. The 1.2-fold enrichment over the expected 50% occurrence of N/M heterozygotes among the cleanup workers was the

TABLE 4
Summary of Exposure History of Cleanup Workers by First Year at Chernobyl

Characteristics	First year at Chernobyl					All cleanup workers (N = 611)
	1986	1987	1988	1989 and 1990	Unknown	
Number of cleanup workers	346	74	36	6	149	611
Self-reported dose (cGy)[a]						
Minimum	0	1	0	—	1	0
25 percentile	18	9	2	—	10	10
Median	22	10	4	—	20	20
75 percentile	25	15	5	—	24	24
Maximum	340	25	30	—	56	340
Days in 30-km zone[b]						
Minimum	1	1	1	31	—	0
25 percentile	30	62	64	103	—	1
Median	61	91	106	167	—	45
75 percentile	91	122	152	183	—	91
Maximum	1230	1250	372	930	—	1250
Worked in Block 4[c]						
No	125	25	14	4	3	171
Yes	212	47	19	2	9	289
Age at Chernobyl (years)[d]						
Minimum	17.6	20.4	24.1	25.1	—	17.6
25 percentile	26.9	29.7	31.0	31.5	—	27.8
Median	33.2	34.4	32.9	32.9	—	33.2
75 percentile	38.1	38.1	37.2	53.4	—	38.0
Maximum	58.0	51.9	45.1	53.4	—	58.0
Years between work at Chernobyl and sample[e]						
Minimum	5.9	5.4	5.4	3.4	—	3.4
25 percentile	8.8	8.7	7.5	7.3	—	8.5
Median	10.9	10.8	9.5	8.6	—	10.6
75 percentile	11.8	11.4	10.0	9.3	—	11.7
Maximum	13.2	12.4	11.1	9.8	—	13.2

[a] Information not available for 259 cleanup workers.
[b] Information not available for 149 cleanup workers.
[c] Information not available for 153 cleanup workers.
[d] Information not available for 149 cleanup workers.
[e] Information not available for 149 cleanup workers.

result of occasional prescreening of subjects for blood type in Russia.

Analysis of Translocation Results

Five factors were identified as contributing to the best model for chromosome translocation frequencies for controls and cleanup workers in the sense that removing any one of them reduced the fit of the model in a statistically significant way. Together these factors account for approximately 28% of the variation in translocation frequencies. The percentage increases in translocation frequency for these factors are given in Table 7. Three factors that can be easily understood as affecting translocation frequency, exposure status, i.e. whether a person was a control or a cleanup worker, smoking history, and age, were statistically significant, as has been noted in other studies [for example, refs. (*41–43*) and references cited therein]. The other two variables are less easily understood relative to genetic damage. For both controls and cleanup workers, translocation frequencies for samples obtained in 1998 were 51% lower than samples obtained in 1995. When the other years were tested relative to 1995 using a multiple testing procedure (Sidak's method), only the 1998 values were statistically significantly different from those for 1995 at the 0.05 level. No explanation is available for the lower values in 1998. It does not correlate with any known difference in methodology (painting of six chromosomes rather than three began in 1996) or any known difference in the subjects studied. The 1998 effect may be a random finding or may be a surrogate for otherwise unascertained differences in lifestyle or environmental exposures of the subjects that resulted in lower genetic damage, such as altered diet, reduced pollution, or immunological/health status. An analysis of translocation frequency as a function of the year of the sample, adjusted for other covariates, detected a trend over time to lower values ($P = 0.001$). There is no evidence that the trend differs for cleanup workers and controls.

Additional analyses were performed to assess the impact

TABLE 5
Self-Reported Dose by Year Sample Acquired

	Year sample acquired								
	1992 (N = 29)	1993 (N = 47)	1994 (N = 90)	1995 (N = 70)	1996 (N = 97)	1997 (N = 102)[a]	1998 (N = 122)	1999 (N = 54)	All (N = 611)
Self-reported dose, cGy									
Minimum	2.0	1.0	4.0	0.0	1.0	2.0	0.0	1.0	0.0
25 percentile	10.5	16.0	10.5	4.7	6.5	16.0	10.0	9.0	10.0
Median	20.0	22.0	20.0	17.5	18.0	24.0	20.0	19.0	20.0
75 percentile	24.0	25.0	23.0	24.3	23.3	50.0	24.3	22.0	24.0
Maximum	56.0	54.0	51.0	60.0	28.0	340.0	120.0	25.0	340.0
No answer, no.	7	10	59	50	45	37	38	13	261

[a] P value for 1997 dose being different was 0.07.

of cleanup workers' exposure histories on translocation frequencies. When covariates identified in the first analysis of translocation results (Table 7) and the five Chernobyl exposure variables listed in Table 4 plus the first year an individual worked at Chernobyl after the accident were tested for inclusion in a best-fit model for cleanup workers alone, four variables were identified, one of which was an exposure-related risk factor. The percentage increases in translocation frequency for these variables from the regression analysis are given in Table 8. These four variables account for approximately 25% of the variation in translocation frequencies of cleanup workers. The primary new result in this analysis is the association of translocation frequency with occupation at Chernobyl. Relative to those with unknown tasks at Chernobyl, those who were either construction workers or mechanics had translocation frequencies increased by ~60%.

Analysis of HPRT Mutant Frequency Results

Nine factors were identified as contributing to the best model for *HPRT* mutant frequencies of controls and cleanup workers. The percentage increases in *HPRT* mutant frequency for these factors are given in Table 9. These nine variables account for approximately 26% of the variation in *HPRT* mutant frequencies (multiple R^2 for the regression). Cleanup worker status and age were significant factors, as in previous studies (*7, 23, 33, 44*). The other seven variables are less easily understood relative to genetic damage. There was a downward trend with increased wine consumption (P = 0.01 for any differences) but an upward trend with increased consumption of alcohol other than beer

TABLE 6
Number of Assays Completed for each Biomarker and Combination of Biomarkers

	Exposure status		
Biomarker combination	Controls (N = 180)	Cleanup workers (N = 611)	Overall (N = 791)
Each biomarker			
Translocations	110	341	451
HPRT mutant frequency	129	319	448
GPA variant frequency	88	370	458
Two biomarkers[a]			
Translocations and *HPRT* mutant frequency	87	231	318
Translocations and GPA variant frequency	55	245	300
HPRT mutant frequency and GPA variant frequency	67	254	321
All three biomarkers	45	189	234

[a] All instances in which two or more biomarkers were measured.

TABLE 7
Percentage Increase in Translocation Frequency for Important Risk Factors, Adjusting for Other Covariates

		95% confidence interval		
Risk factors	Value	Low	High	P value
(Intercept)	−6.46	−6.93	−5.98	<0.001
Exposure status				
Control	Reference			
Cleanup workers	29.9%	10.3%	53.0%	0.002
Age (per year)	4.0%	3.2%	4.8%	<0.001
Year of sample				
1992	26.7%	−11.1%	80.5%	0.19
1993	13.2%	−22.5%	65.4%	0.52
1994	21.1%	−3.3%	51.6%	0.10
1995	Reference[a]			
1996	22.5%	−3.4%	55.2%	0.09
1997	−25.9%	−42.0%	−5.4%	0.02
1998	−51.3%	−64.7%	−32.8%	<0.001
1999	−26.5%	−48.9%	5.5%	0.09
Smoking status				
Non-smoker	Reference			
Ex-smoker	33.6	5.8	68.7	0.01
Current smoker	28.6	7.9	53.2	0.005
No answer	14.2	−7.4	40.8	0.21
Retired				
No	Reference			
Yes	−27.3	−47.9	1.6	0.06
No answer	−23.0	−40.9	0.31	0.05

[a] Chosen as reference due to being the year with the largest number of samples.

TABLE 8
Percentage Increase in Translocation Frequency for Important Exposure Risk Factors among Cleanup Workers, Adjusting for Other Covariates

Risk factors	Value	95% confidence interval Low	High	*P* value
(Intercept)	−6.22	−6.68	−5.75	<0.001
Age (per year)	3.2%	2.3%	4.2%	<0.001
Year of sample				
1992	16.4%	−18.3%	66.0%	0.40
1993	0.98%	−31.0%	47.8%	0.96
1994	6.5%	−17.1%	36.7%	0.62
1995	Reference[a]			
1996	20.4%	−8.3%	58.2%	0.18
1997	−20.3%	−37.9%	2.2%	0.74
1998	−45.2%	−57.8%	−29.0%	<0.001
1999	9.1%	64.7%	64.7%	0.68
Smoking status				
Non-smoker	Reference			
Ex-smoker	32.8	1.8	73.4	0.04
Current smoker	39.6	15.1	69.4	<0.001
No answer	28.5	−2.7	69.6	0.08
Chernobyl job				
Unknown	Reference			
Administrator	20.2	−25.6	94.3	0.45
Construction worker	64.5	14.1	137.0	0.008
Digger/sacrcophagus work	13.1	−15.0	50.6	0.40
Driver	−23.5	−48.4	13.5	0.18
Decontamination worker	1.0	−19.2	26.4	0.93
Mechanic	62.9	6.7	149.0	0.02
Medical/radiation specialist	27.1	−7.2	74.1	0.13
Other[b]	−10.9	−43.5	40.6	0.62

[a] Chosen as reference due to being the year with the largest number of samples.
[b] Includes small numbers of multiple other tasks.

TABLE 9
Percentage Increase in *HPRT* Mutant Frequencies for Important Risk Factors, Adjusting for Other Covariates

Risk factors	Value	95% confidence interval Low	High	*P* value
(Intercept)	−12.2	−12.7	−11.80	<0.001
Exposure status				
Control	Reference			
Cleanup worker	40.5	18.9	66.1	<0.001
Year of sample				
1992	−8.1%	−63.2%	129.0%	0.86
1993	−1.5%	−32.5%	43.0%	0.94
1994	−29.4%	−44.8%	−9.7%	0.006
1995	Reference[a]			
1996	−22.5%	−40.6%	1.1%	0.06
1997	−28.7%	−47.4%	−3.2%	0.03
1998	−23.0%	−45.7%	8.9%	0.14
1999	7.2%	−25.1%	53.5%	0.70
Age (pear year)	2.1	1.4	2.7	<0.001
Retired				
No	Reference			
Yes	41.6	6.2	88.8	0.02
No answer	29.6	−0.29	68.3	0.05
Exposure to chemicals or solvents				
No	Reference			
Yes	2.5	−17.6	27.7	0.82
No answer	−35.0	−53.8	−8.6	0.01
Take nerve medicines				
No	Reference			
Yes	−19.2	−31.7	−4.4	0.01
No answer	4.5	−15.9	29.8	0.69
Wine (bottles/month)				
None	Reference			
1–2	11.5	−10.6	39.0	0.33
3–4	−45.8	−75.5	20.0	0.13
More than 4	−63.1	−89.6	30.4	0.12
No answer	118.0	17.9	305.0	0.01
Other alcohol (bottles/month)				
None	Reference			
1–2	8.6	−11.8	33.8	0.44
3–4	41.6	−7.6	117.0	0.01
More than 4	104.0	−5.9	341.0	0.07
No answer	−29.6	−63.3	34.9	0.29
Clinic city				
St. Petersburg	Reference			
Tula	−17.3	−28.2	−4.7	0.009
Moscow	3.0	−29.5	50.6	0.88

[a] Chosen as reference due to being the year with the largest number of samples.

or wine ($P = 0.005$ for any differences), and there were statistically significant differences ($P = 0.03$) between clinic cities. For exposure to chemicals or solvents and for wine consumption, the category of people for whom information was not available had statistically significantly different mutant frequency effects than those reporting no exposure. Interestingly, no smoking effect was detected with *HPRT* mutant frequency in this data set, unlike earlier studies of this and other populations (*7, 33, 40, 44*). An analysis of *HPRT* mutant frequency as a function of the year of the sampling, adjusted for other covariates, detected no trend over time ($P = 0.41$).

Additional analyses were performed to assess the impact of cleanup workers' exposure histories on *HPRT* mutant frequency. When covariates identified in the first analysis of *HPRT* mutant frequency (above, Table 10) and the Chernobyl exposure variables in Table 4 were tested for inclusion in a best-fit model for cleanup workers alone, two exposure variables were identified as statistically significant: first year at Chernobyl and years between first year at Chernobyl ($P = 0.02$) and date of blood sampling ($P = 0.03$). Unexpectedly, those who were at Chernobyl first in 1987 or 1988 had a statistically significantly increased *HPRT* mutant frequency relative to those there first in 1986. This difference, whose cause is unknown, may account for the apparent increase in *HPRT* mutant frequency with time

TABLE 10
Percentage Increase in *HPRT* Mutant Frequencies for Important Exposure Risk Factors among Cleanup Workers, Adjusting for Other Covariates

		95% confidence interval		
Risk factors	Value	Low	High	*P* value
(Intercept)	−13.60	−15.10	−12.00	<0.001
Year of sample				
1992	−25.4%	−71.4%	94.8%	0.55
1993	−4.2%	−42.3%	59.3%	0.87
1994	−37.0%	−56.4%	−9.0%	0.01
1995	Reference[a]			
1996	−40.7%	−59.2%	−13.7%	0.007
1997	−46.3%	−63.9%	−20.1%	0.002
1998	−47.2%	−68.9%	−10.4%	0.02
1999	−39.1%	−68.0%	15.6%	0.13
Age (per year)	2.5%	1.5%	3.4%	<0.001
Retired				
No	Reference			
Yes	55.5%	13.1%	114.0%	0.007
No answer	38.3%	0.08%	91.4%	0.05
Wine (bottles/month)				
None	Reference			
1 or 2	19.3%	−7.7%	54.2%	0.17
3 or 4	−48.2%	−85.8%	89.2%	0.32
More than 4	−38.5%	−71.4%	32.6%	0.21
No answer	140.0%	11.4%	415.0%	0.02
Other alcohol (bottles/month)				
None	Reference			
1–2	18.2%	−38.3%	127%	0.61
3–4	82.5%	−22.9%	332%	0.17
More than 4	−27.9%	−38.3%	−15.8%	<0.001
No answer	13.1%	−9.8%	42.0%	0.29
Clinic city				
St. Petersburg	Reference			
Tula	−13.4%	−43.7%	33.2%	0.51
Moscow	4.9%	−17.5%	33.4%	0.69
First year at Chernobyl				
1986	Reference			
1987	77.8%	21.6%	160%	0.003
1988	199%	38.1%	548%	0.006
1989	58.6%	−50.5%	408%	0.44
1990	−16.0%	−33.7%	6.4%	0.15
Years between work at Chernobyl and sample (per year)	15.8%	1.6%	31.8%	0.03

[a] Chosen as reference due to being the year with the largest number of samples.

TABLE 11
Percentage Increase in GPA N/Ø Variant Frequency for Important Risk Factors, Adjusting for other Covariates

		95% confidence interval		
Risk factors	Value	Low	High	*P* value
(Intercept)	2.3	2.07	2.60	<0.001
Year of sample				
1992	47.2%	5.3%	106%	0.02
1993	−28.4%	−46.6%	−3.8%	0.03
1994	−21.2%	−36.5%	−2.1%	0.03
1995	Reference[a]			
1996	8.0%	−12.7%	33.7%	0.48
1997	−9.7%	−26.5%	10.9%	0.33
1998	−16.9%	−32.5%	2.4%	0.08
1999	−38.8%	−23.1%	−23.1%	
Coffee (cups/day)				
None	Reference			
1 or 2	−19.0%	−33.4%	−1.6%	0.03
3 or 4	−39.3%	−54.6%	−18.4%	<0.001
4+	−33.0%	−56.3%	2.80	0.07
No answer	−18.8%	−33.6%	−0.62%	0.04
Wine (bottles/month)				
None	Reference			
1 or 2	5.6%	−15.6%	32.1%	0.63
3 or 4	294%	98.1%	683%	<0.001
More than 4	21.6%	−39.0%	142%	0.58
No answer	7.1%	−8.5%	25.4%	0.39

[a] Chosen as reference due to being the year with the largest number of samples.

elapsed since exposure. When considering cleanup workers only, all of the covariates identified for both controls and cleanup workers (Table 9) remained significant with the exception of taking nerve medicines. The eight variables account for approximately 28% of the variation in *HPRT* mutant frequencies in cleanup workers (multiple R^2 for the regression). As above, the year of the sample affected the *HPRT* mutant frequency of cleanup workers, with estimates of all of the years in the study being lower than the reference year of 1995. These results suggest that for some unknown reason cleanup workers sampled in 1995 had higher *HPRT* mutant frequencies. In comparison, translocation frequencies were reduced only for cleanup workers sampled in 1998 (Table 8).

Analysis of GPA N/Ø Variant Frequency Results

For GPA N/Ø variant frequency, exposure status (control compared to cleanup worker) was not a significant factor, unlike translocations and *HPRT*. Three factors were identified in the best-fit model for *GPA* N/Ø variant frequency. The percentage increases in GPA N/Ø variant frequency for the three variables are given in Table 11. These three variables account for approximately 15% of the variation in *GPA* N/Ø variant frequency (multiple R^2 for the regression). There was a consistently lower GPA N/Ø variant frequency for all levels of coffee consumption and a consistently higher variant frequency for all levels of wine consumption ($P = 0.016$ and $P = 0.004$ for any differences, respectively). As with translocation frequencies and *HPRT* mutant frequency, GPA N/Ø variant frequency varied from year to year in a statistically significant way ($P < 0.001$ for any differences). An analysis of GPA N/Ø variant frequency as a function of the year of the sample, adjusted for other covariates, detected a trend over time to lower values ($P = 0.003$).

TABLE 12
Percentage Increase in GPA NN Variant Frequency for Important Risk Factors, Adjusting for Other Covariates

Risk factors	Value	95% confidence interval Low	High	*P* value
(Intercept)	2.18	1.68	2.69	<0.001
Year of sample				
1992	39.9%	−4.2%	104%	0.08
1993	20.3%	−13.3%	67.0%	0.27
1994	−0.43	−22.0%	27.2%	0.97
1995	Reference[a]			
1996	−3.9%	−25.7%	24.1%	0.76
1997	−19.6%	−37.7%	3.8%	0.09
1998	−39.2%	−56.7%	−14.7%	0.004
1999	−50.2%	−64.5%	−30.1%	<0.001
Age (per year)	1.6%	0.87%	2.3%	<0.001
Cigarettes or popirossi smoked				
Nothing	Reference			
Cigarettes only	4.1%	−13.1%	24.8%	0.66
Popirossi only	30.5%	−33.4%	156%	0.44
Both cigarettes and popirossi	−18.9%	−54.8%	45.5%	0.48
No answer	−35.6%	−60.8%	5.9%	0.08
Popirossi years smoked				
None	Reference			
0+ to 9	16.4%	−40.3%	127%	0.65
9+ to 20	−5.3%	−52.6%	89.3%	0.88
20+ to 31	−18.9%	−58.5%	58.3%	0.54
31+	12.7%	−45.2%	132%	0.74
No answer	72.3%	8.2%	174%	0.02
Wine (bottles/month)				
None	Reference			
1 or 2	17.6%	−8.4%	50.8%	0.20
3 or 4	223%	50.2%	595%	0.003
More than 4	75.7%	−17.9%	276%	0.15
No answer	−5.3%	−21.2%	13.7%	0.56
Retired				
No	Reference			
Yes	−30.2	−46.6%	−8.8%	0.008
No answer	−37.5	−51.5%	−19.3%	<0.001

[a] Chosen as reference due to being the year with the largest number of samples.

Analysis of GPA N/N Variant Frequency Results

In this analysis, one outlier, as measured by Cook's distance (*38*, *45*), was eliminated. Exposure status was not a significant factor for GPA N/N variant frequency, as expected based on other studies of response to radiation exposure (*14*, *15*, *46*). Six factors were identified in the best-fit model. The percentage increases in GPA N/N variant frequency for these variables are given in Table 12. These six variables account for approximately 16% of the variation in *GPA* N/N variant frequency (multiple R^2 for the regression). GPA N/N variant frequency increased about 1.6% per year, as seen in this and other populations (*20*, *23*, *47*). As with N/Ø variant frequency, all levels of wine consumption were associated with a higher GPA N/N variant frequency ($P = 0.008$ for any differences), but coffee consumption was not significantly associated. As with translocation frequencies, *HPRT* mutant frequency and GPA N/Ø variant frequency, GPA N/N variant frequency varied from year to year in a significant way ($P < 0.001$ for any differences). An analysis of GPA N/N variant frequency as a function of the year of the sample, adjusted for other covariates, detected a highly significant trend over time to lower values ($P < 0.0001$). No defined effect of smoking was observed; it is not known why those who did not answer the smoking questions would have lower GPA N/N variant frequency. Smoking has been a covariate for increased GPA N/N variant frequency in another population (*47*).

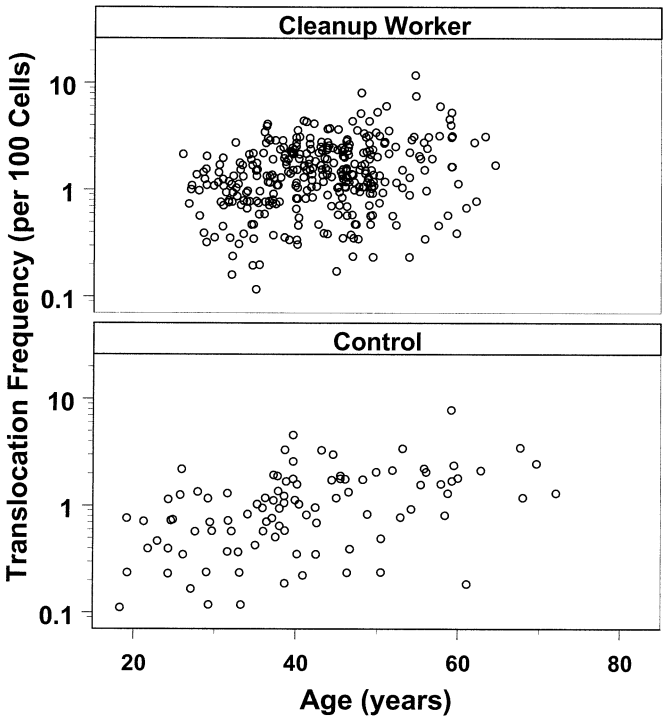

FIG. 1. The frequency of chromosome translocations as a function of age. Controls and cleanup workers are presented separately. Translocation frequencies are shown as the number of translocations per 100 cells scored, adjusted to genome equivalents.

Age Effects

Translocation frequency, *HPRT* mutant frequency, and GPA N/N variant frequency were all increased with age (Tables 7, 8 and 12). To illustrate the age effects, as well as display the data underlying the preceding analyses, the unadjusted biomarker results are plotted as a function of age in Figs. 1, 2 and 3.

Relationships between Biomarkers

A number of analyses were performed to assess whether obtaining both translocation frequency and *HPRT* mutant frequency, rather than just translocation results, added value

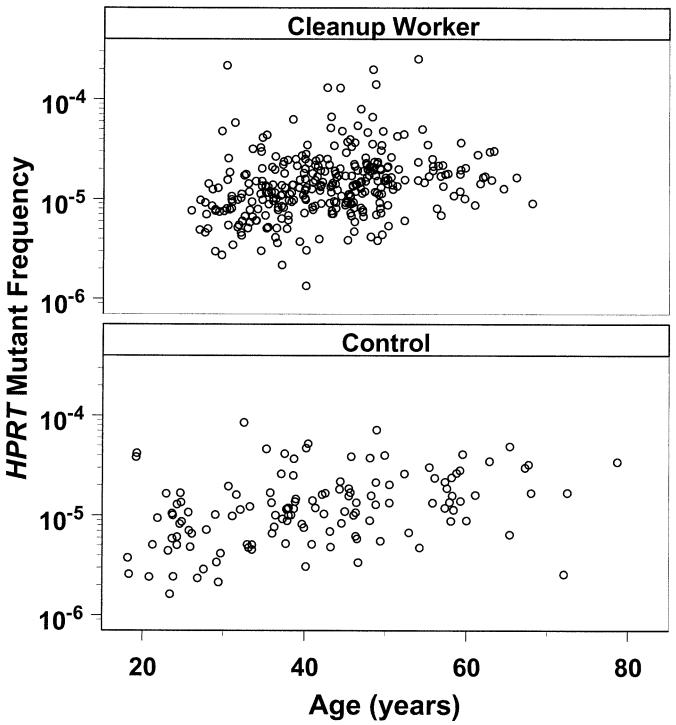

FIG. 2. *HPRT* mutant frequency as a function of age. Controls and cleanup workers are presented separately.

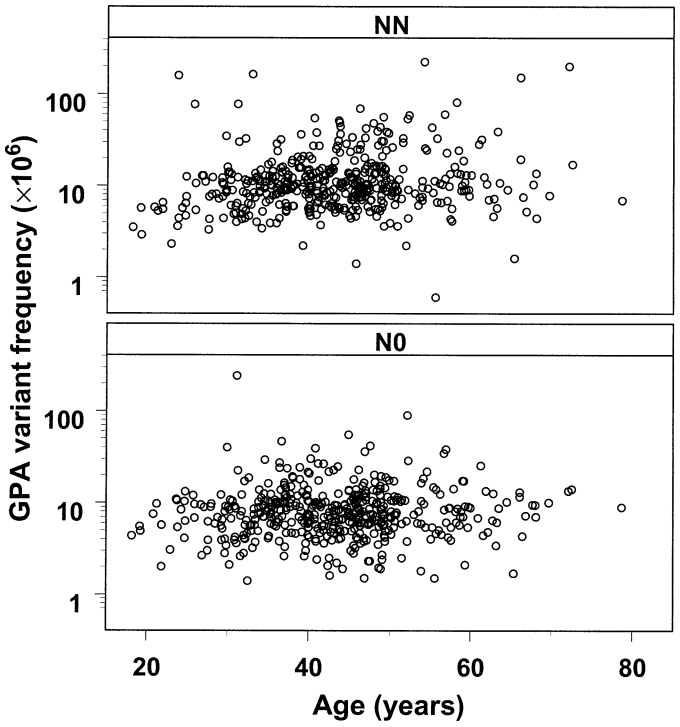

FIG. 3. The GPA N/Ø and NN variant frequencies as a function of age. Controls and cleanup workers are presented together for GPA N/Ø variant frequencies (lower panel) and NN variant frequencies (upper panel).

to assessing the Chernobyl exposure effect. GPA variant frequency results were not included in these analyses since Chernobyl exposure did not affect GPA variant frequencies. No difference was detected in translocation frequency between subjects having an *HPRT* mutant frequency and those who did not. No difference was detected in translocation frequency between subjects having GPA variant frequencies and those who did not. However, a small but statistically significant ($P = 0.006$) correlation of 0.10 (as measured by Kendall's τ) was detected between the two lymphocyte outcomes after adjusting for the variables shown in Tables 7 and 9, suggesting that including *HPRT* mutant frequency might increase the precision with which Chernobyl exposures could be measured. Including the logarithm of *HPRT* mutant frequency in the model in Table 7 did produce a statistically significantly better fit ($P = 0.01$). However, the practical effect was negligible: Including the value for log mutant frequency into the model in Table 7 did not increase the precision of the estimate for the effect of Chernobyl exposure.

Dose Estimates

An estimate of the radiation dose corresponding to the detected increase of translocations in cleanup workers was made using LLNL *in vitro* dose–response data. Data were combined from studies using lymphocytes of four LLNL subjects. There were insufficient data to determine whether the dose responses varied among the LLNL subjects. Background frequencies were subtracted, and it was assumed that age of subjects did not affect the responses of their cells *in vitro.* The majority of the data are in the range of 0.20 to 1.0 Gy (Fig. 4). A negative binomial fit to the data was made assuming that the only variation in the data was due to measurement error. This analysis defined the coefficients in the relationship

$$\text{translocation frequency} = c + \alpha d + \beta d^2$$

to be α of 0.019 translocations per genome equivalent per Gy (95% confidence interval 0.0107–0.0273), β of 0.0597 translocations per genome equivalent per Gy^2 (95% confidence interval 0.050–0.0693), where c is the background frequency and d is dose. These values of α and β are in good agreement with other studies of the effects of ionizing radiation (*48–53*). Based on the distribution of the translocation frequency increases of cleanup workers with the influence of covariates taken into account (age, smoking status, retirement status) (Fig. 5), the mean increase in translocations per 100 cell equivalents among the 611 cleanup workers was estimated to be 0.24, with a standard error of 0.04. These results lead to an estimated average dose of 9.5 cGy, with a standard error of 2.2 cGy. This error estimate does not address the poor fit of the dose–response curve to data at doses below 20 cGy (Fig. 4), the range of the estimated dose for cleanup worker, nor does it take into account any variation in dose response between individuals. The standard error above is for the estimate of the *average dose* among the 611 cleanup workers. This

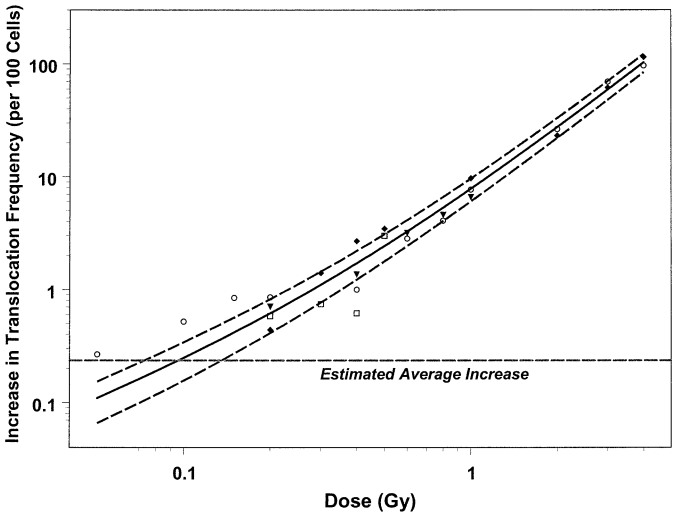

FIG. 4. Increase in translocation frequencies of human lymphocytes exposed *in vitro* to various doses of radiation from ^{137}Cs. Data for four subjects are represented by separate symbols (▼, ○, ◆, □). Each data point is the difference between the observed translocation frequency at a given dose and the observed translocation frequency when no radiation was administered. Translocation frequencies are shown as the number of translocations per 100 cells scored, adjusted to genome equivalents. Also shown are the predicted mean response and 95% confidence intervals as a function of dose produced by a negative binomial regression, as described in the Materials and Methods.

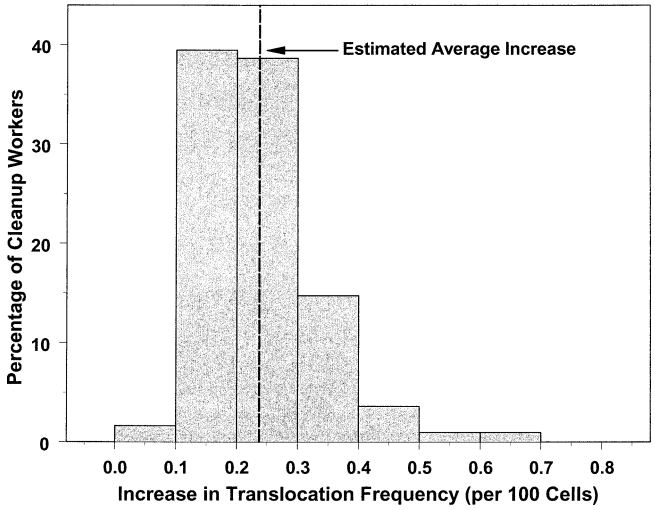

FIG. 5. Distribution of the predicted increase in translocation frequencies among cleanup workers due to being at Chernobyl. Translocation frequencies are shown as the number of translocations per 100 cells scored, adjusted to genome equivalents. The increase for each worker was calculated as described in the Materials and Methods. The mean increase per 100 cell equivalents was estimated to be 0.24 for these subjects, with a standard error of 0.06.

cytogenetically based dose estimate is lower than the median self-reported ''official'' dose of 20 cGy (for the 197 subjects with this information) but is in good agreement with our previous estimate of 9 cGy based on data from 126 cleanup workers and on dose–response data for one individual (*23*). These estimates assume no selection against cells with translocations in the years between Chernobyl exposure and translocation analysis. As discussed below, there may have been selection against some cells with translocations induced by exposures at Chernobyl prior to sampling of the cleanup workers. If this *in vivo* selection effect was greater than the selection that occurred during the short *in vitro* cell culture for the dose response, the dose estimate would undervalue exposure at Chernobyl. A further limitation of the dose estimate is that the calibration curve is based on single acute doses whereas exposures of cleanup workers were fractionated over varying periods. It is noteworthy that at the estimated average dose of 9.5 cGy of the cleanup workers, and using the values of α and β from our *in vitro* dose response, the dose-dose-rate effect reduction factor (DDREF) is 1.3.

DISCUSSION

The detailed analysis of three biomarker responses in the Russian controls and cleanup workers has addressed several key questions and raised new ones. The biomarker responses suggested in our earlier report (*23*) have been verified and extended. With the extension of the study and coding of lifestyle and exposure attributes from extensive questionnaire resources, the impact of additional lifestyle and exposure covariates has been identified. When all identified covariates were taken into account, translocation frequency and *HPRT* mutant frequency were elevated in cleanup workers by 30% and 41%, respectively, but neither GPA N/Ø nor NN variant frequency was associated with Chernobyl exposure.

The radiation dose to which these biomarker responses correspond is low and is known only imprecisely. The dose estimate of 9.5 cGy based on cytogenetic responses is consistent with success by those in charge of the Chernobyl cleanup in limiting the exposure of most cleanup workers to doses below 25 cGy. The recruitment of cleanup workers for this study was intentionally biased to those at Chernobyl first in 1986, with the goal of increasing the likely exposure (*54, 55*) and therefore the ability to compare the biomarkers. As a result, the average dose for the whole cleanup worker population would be expected to be $<$9.5 cGy. To the extent that there was selection against cells with translocations between exposure at Chernobyl and obtaining the blood sample, the dose estimate may be low. *In vitro* and *in vivo* studies suggest that up to 40% of aberrant cells may be lost over time (*28, 56–63*). Given that data acquisition began in 1992, and serial sampling of individuals was not performed, the role of *in vivo* selection in this study is unknown. The dose estimate used cleanup worker data adjusted for age and other covariates that affected translocation frequency, a need recognized at the CEC Concerted Action Programme workshop on biological dosimetry (*42, 64*). With the known uncertainties in the cytogenetically based dose estimate, it appears that the self-reported official

doses were roughly correct on average for this group of cleanup workers.

One source of uncertainty in the dose estimate is the imprecision with which the dose response was defined at low doses. Even with the use of FISH for six chromosomes identifying 56% of chromosome exchanges, detection of low frequencies of translocations is labor intensive. The estimated average dose, 9.5 cGy, is in a poorly defined region of the dose response. The few data points below 20 cGy in our dose calibration studies were all outside the 95% confidence interval for the dose–response curve used to calculate the estimated dose. It is gratifying that the observed 30% increase in translocation frequency in cleanup workers and the group dose estimate are consistent with projections based on a recent extensive calibration of *in vitro* responses. That study of responses of six U.S. subjects to X-ray doses from 0.12 to 1 Gy concluded that an increase of 40–65% relative to controls would be expected for a whole-body dose of 10–11 cGy (*21*). In our estimate of dose and its confidence limits, potential differences in the response of individuals whose cells were used in the calibration were ignored; insufficient data were generated for doses at and below 20 cGy to compare the low-dose responses of the subjects. However, exploratory analyses of the data (not presented) for two subjects suggested that there may be interindividual differences in the α coefficient of the dose–response relationship. Hence quantification of the low-dose exposure was limited not only by the small number of events scored in cells of cleanup workers and in the calibration studies but also by the ability to relate the imprecisely detected responses to as yet undefined sources of variation in radiation responses within the human population. Addressing these shortcomings will require considerable resources to score many more cells in both exposed individuals and calibration studies, either using current technology or developing a more cost-effective approach that facilitates detection of translocations. Determining a specific individual's dose response would be the most precise approach to his/her dosimetry, but the approach would rarely be practical with current technology. Future studies of variables that affect individual dose responses will be key to achieving more precise individual dosimetry.

The translocation-based dose estimate for cleanup workers provides a valuable reference point for evaluating the *HPRT* mutant frequency and GPA variant frequency responses to low-dose radiation exposure. With respect to *HPRT* mutant frequency, the considerable variation in dose–response estimates in different systems has been a topic of previous discussions (*23, 40*). Revisiting the literature, we find that the closest response to that reported here in cleanup workers was a report of the average response of 11 subjects whose non-cycling peripheral blood lymphocytes were exposed *in vitro* to 0.5 to 4 Gy X rays at 3 Gy/min (*65*). Those data suggest a response of $\sim 3–6 \times 10^{-5}$ mutants/Gy, compared to our value of approximate 5×10^{-5} mutants/Gy. This *in vitro* response was considerably higher than the 7.5×10^{-7} mutants/Gy increase seen in atomic bomb survivors 40–50 years after exposure (*66, 67*). The low recovery of HPRT-deficient mutants in atomic bomb survivors has been attributed to failure of mutant cells to persist over the decades between exposure and analysis, because of selection against mutants during proliferative responses after high radiation doses and during normal immunological responses (*67*). With respect to GPA, the lack of a GPA N/Ø variant frequency response to exposure of Chernobyl cleanup workers is consistent with our earlier studies of this population (*15, 23*), studies of cleanup workers from the Baltic states by others (*20*), and a recent report of other populations exposed to low doses (*68*). Two factors may contribute to the implied limits of the response of GPA variant frequency to low-dose radiation. One factor is the shape of the dose response, particularly at low dose rates. Relevant here is the dose-rate reduction effect of a factor of 2 for GPA N/Ø variant frequency observed in a study of thyroid cancer patients receiving high total doses at a low dose rate from ^{131}I treatment (*46*). In addition, analysis of GPA results in a study including both high- and low-dose-rate Chernobyl exposures (*15*) found that a linear-quadratic relationship fit the data better than a linear relationship. Studies of other instances of protracted exposure have also noted a reduction of induced GPA variant frequency (*69*). These results suggest that estimates of GPA N/Ø variant frequency response based on dose–response relationships at high dose rates, cf. (*14, 15, 70*), would overestimate the response expected in cleanup workers who received an average dose of 9.5 cGy from low dose rates. The other factor is the biological limitation on sensitivity. The number of target cells, bone marrow progenitors, and the number of progenitors represented among peripheral blood erythrocytes at any given time contribute biological noise to GPA variant frequency assays. Whereas stem cell killing might have contributed to noisiness in the data for atomic bomb survivor GPA variant frequency (*14*), bone marrow progenitor loss is not expected to be a factor at the low doses of the cleanup workers.

Systematic review of questionnaire information identified a number of attributes that affected each biomarker response. These analyses were critical given that the control and cleanup worker groups were not perfectly matched for some attributes, including certain attributes that had significant effects on the biomarkers. However, when a large number of comparisons are made, as done here, some might be significant due to chance. Hence those seen as affecting two or more biomarkers in a consistent manner, and those that were stable in multiple analyses, are more credible. Those factors that stand out that should be included in future population studies were age, smoking status, year the sample was acquired, and clinic city. The latter two may be idiosyncratic to this population and may reflect exposure factors not otherwise identified. Given the period involved, many changes in exposure factors could have affected these citizens of Russia over time, with the urban and more rural inhabitants of St. Petersburg and Tula, respectively, having

different experiences. In hindsight, initial questionnaire design should be optimized to reduce the challenges of coding results from translated text, to avoid having multiple questionnaires, and to reduce incomplete responses, all challenges encountered in this study. The most interesting Chernobyl exposure-related finding was that being a mechanic or construction worker at Chernobyl was associated with higher translocation frequencies. It is not known whether these occupations affect translocation frequencies in controls as well.

It is noteworthy that a number of variables other than radiation exposure had significant effects on biomarker responses. Covariates with both positive and negative effects were identified. Increases in biomarker values with age and smoking were expected, having been detected previously in multiple studies as noted earlier. However, the protective effect for GPA N/N variant frequency, and perhaps translocation frequencies, of being retired was a new observation whose mechanism is not known. This result contrasts with the protective effect of *not* being retired for *HPRT* mutant frequency. Whether the contradictory results are a spurious consequence of multiple comparisons or the biomarkers respond differently to this covariate cannot be discerned. These results require confirmation in other studies, as do the results suggesting an apparent protective effect of taking certain medications, drinking coffee, being from one clinic city rather than another, and variable effects of different alcohol consumption habits. Relative to radiation dosimetry, the results illustrate the complexity of assessing the significance on an individual's biomarker values in the absence of extensive information about variables other than the radiation exposure.

There was little indication that the Chernobyl exposure biomarker responses changed as time passed after exposure at Chernobyl. For most subjects at least 6 years had passed between their first work at Chernobyl and being sampled for this study. There is evidence of selection both *in vivo* and *in vitro* against cells with certain types of translocations (*57–63*) or HPRT-deficient cells (*40, 71, 72*). Persistence after low-dose exposures $<$20 cGy has not been assessed. The majority of any selection may have occurred prior to August 1992, when sampling of subjects began. Analysis for *HPRT* mutant frequency of a limited number of samples obtained prior to August 1992 is consistent with decline in *HPRT* mutant frequency over time in cleanup workers (*73, 74*). Complicating the issue, three biomarkers, translocation frequency, GPA N/Ø variant frequency, and GPA N/N variant frequency, appeared to decline over the course of the study in both controls and cleanup workers independent of a radiation exposure effect for unknown reasons. Lack of longitudinal sampling of subjects prevented rigorous analysis of this issue for any biomarker.

An additional goal of the study was to determine if having data on multiple biomarkers would improve low-dose dosimetry. After adjusting for covariates, the two lymphocyte biomarkers were only slightly correlated (robust correlation coefficient of 0.13). In earlier, smaller data sets, the translocation frequency response to Chernobyl-related exposure of this population was consistent and stable even when only age and smoking status were taken into account. In contrast, the response of *HPRT* mutant frequency in the absence of extensive covariate data was smaller, dependent on the date of sample, and less stable (*23, 40, 73, 74*). Although a number of analytical approaches were tested, none indicated that having *HPRT* mutant frequency in addition to translocation frequency improved the sensitivity or precision of detection of radiation exposure.

Many challenges remain for radiation biodosimetry by biomarkers, both in the technical domain and in data interpretation. The current assays are too time intensive and labor intensive to apply routinely to large populations; technical innovations are needed for epidemiological studies, more extensive low-dose calibration data, and evaluation of interindividual differences in low-dose responses. To achieve more precise dose estimates, an understanding of the variables affecting individual dose response, such as age and genetic susceptibility, is needed. To better interpret effects of past exposures, longitudinal study designs are essential to obtain knowledge of the impact of time since exposure, and perhaps age at time of exposure, on the persistence of aberrant cells. The ability to distinguish between the genetic consequences of exposure to radiation and to genotoxic chemicals remains limited; mutation spectrum analysis is not yet practical for low-dose exposures (*73*).

Finally, we owe it to subjects and to readers to estimate the potential health effects that may be associated with either the translocation rates or the 9.5-cGy mean dose we have measured in this biodosimetric study. The simplest way to make this estimate is to use the physical dose, 9.5 cGy, and ask what effect it would have based on the cancer incidence or the mortality of the atomic bomb survivors. Using the linear dose response for solid cancer incidence given by Thompson *et al.* (*75*), the relative risk for solid cancer is 1.06 at 9.5 cGy ignoring dose-rate differences. At this dose, the α and β translocation coefficients from our study of 0.019 and 0.0597, respectively, yield a DDREF of 1.30, an adjusted dose of 13 cGy, and a relative risk of 1.08. The corresponding mortality data can be taken from Pierce and Preston (*76*) and without or with dose-rate correction give relative risks of 1.05 and 1.07, respectively, with about $\pm$0.02 variation on these numbers depending on which of the models one uses from this reference. These risk estimates depend on how well the acute, single, whole-body exposure to γ rays and neutrons of the wartime male and female Japanese atomic bomb survivors of all ages can be extrapolated to the multiply exposed, mixed-radiation, and roughly whole-body exposure of the young mature male Russian cleanup workers. With these caveats, we conclude that the health risk is small and would be very difficult to detect in the scattered populations of surviving cleanup workers.

Overall, the results of these studies are consistent with

the induction of genetic damage by low-dose and low-dose-rate exposures to ionizing radiation, and with the ability to detect these effects in populations using selected biomarkers. Confidence in low-dose estimates will remain relatively low until more precise information on the low-dose responses of biomarkers is obtained and the sources of inter-individual variation in response are better defined. Although our results suggest that translocation frequency by FISH and *HPRT* mutant frequency by cloning have similar responses to these exposures, our experience over time indicates that translocation frequency of peripheral blood lymphocytes is the more sensitive and robust biomarker, particularly if information on covariates is limited. In situations in which the dose range may be quite wide, analysis of GPA variant frequency has merit for screening a population (*20*). GPA N/Ø variant frequency is expected to show a strong response for radiation doses in excess of 1 Gy (*15*) with little effect of lifestyle or age. The speed, low expense, and low blood volume requirements of the assay are attractive, as is the lack of need for special sample shipping conditions. Despite current limitations in the precision of dose estimates based on calibration studies and limited knowledge of persistence over time, translocation frequency remains the preferred biomarker for radiation dosimetry.

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