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Chromosome Aberrations of Clonal Origin in Irradiated and Unexposed Individuals: Assessment and Implications

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Chromosome painting has proven useful for the detection of chromosomal rearrangements, although the presence of cells containing clonal aberrations can have an effect on the outcome of cytogenetic analyses (e.g. aberration frequency and chromosomal distribution studies). Cells with clonal chromosomal changes have been found in studies of both radiation-exposed Chernobyl cleanup workers ("liquidators") and healthy unexposed human subjects. We have used a simple statistical method to aid in the identification of individuals from distinct Chernobyl radiation-exposed and unexposed control populations who may possess cells containing clonal rearrangements. A χ^2 value determined from the observed number of aberrations and the expected number based on chromosome length that corresponds to a probability less than 0.005 appears to be an indicator of clonality. These selected individuals can be analyzed further for clonality, thereby sparing detailed examination of the entire population. Here we present an analysis of individuals possessing clonal aberrations to assess the influence of clonality on the results of cytogenetic studies. Our results show that the subtraction of clonal events from the χ^2 calculation for the "outliers" results in nearly all of these values losing their statistically significant deviation from proportionality. These adjustments can also be made to prevent the overestimation of frequencies of chromosome aberrations for biodosimetry. The frequency of clonal aberrations appears to increase as a function of age in control subjects, whereas an age effect was not evident in Chernobyl liquidators. This suggests that spontaneous and radiation-induced clonal expansion are occurring in control subjects and liquidators, respectively.

INTRODUCTION

Chromosome painting enables detection of chromosomal rearrangements, thereby permitting identification of cells with putative clonal aberrations. The presence of clonal changes can influence measurements of aberration frequency in both unexposed control and radiation-exposed humans (*1, 2*). Cells with clonal aberrations may contribute to an artificially high estimate of mutation frequency or may result in an apparently skewed distribution of chromosome damage relative to DNA content. Therefore, it is important to identify accurately those cells containing clonal rearrangements. Clonal expansion may occur *in vitro* during cell culture procedures, but effects should be negligible if the duration of the culture is short enough to ensure a preponderance of first-division cells. Clonal expansion *in vivo* is indicated by the presence of the same change in multiple cell cultures or by a large number of cells in one culture with identical rearrangements.

Individuals possessing cells with clonal aberrations have been found in several cytogenetic studies of radiation exposure. Early work demonstrated that the formation of clones of cells with chromosomal abnormalities occurred in individuals injected with the contrast medium Thorotrast (*3, 4*). More recently, Natarajan *et al.* (*5*) reported a high incidence of specific translocations involving chromosome 2 from one subject exposed at the Goiania radiation accident. The authors suggested a clonal origin for these cells, although they could not verify this conclusion. Lucas *et al.* (*6*) showed that 2 of 20 Hiroshima A-bomb survivors appeared to possess cells with clonal aberrations as detected by chromosome painting and G-banding. Cells with clonal rearrangements have also been found in persons exposed to radiation as a result of the Chernobyl nuclear power plant accident (*2, 7*).

Cells possessing clonal aberrations have also been observed in lymphocytes from healthy, unexposed persons (*1, 7, 8*), predominantly in individuals over 55 years of age. Mertens *et al.* (*9*) described clonal chromosome changes in non-neoplastic cells of the skin and upper aerodigestive tract mucosa, while Jin *et al.* (*10*) showed an age-dependent increase in clonal aberrations in upper aerodigestive tract mucosa. The overall frequency of cytogenetically abnormal clones appears to be relatively low, especially in younger individuals. However, clonal aberrations may be more com-

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mon in elderly subjects or those with deleterious environmental exposures.

We have applied a χ^2 test to determine the individuals most likely to possess clonal aberrations from two distinct populations; healthy, unexposed Californians, and radiation-exposed Chernobyl cleanup workers plus control subjects. We have included an analysis of the individuals possessing clonal aberrations to assess the possible effects of clonality on the outcome of cytogenetic studies (e.g. for radiation biodosimetry purposes) and have determined the relationship between the frequency of clones and age.

MATERIALS AND METHODS

Study Population

The California population consisted of 24 healthy, unexposed adults and three newborns who were analyzed in a previous cytogenetic study (*8*) and were selected from a larger population surveyed for the effect of age and lifestyle factors on the frequency of chromosome aberrations (*11*). This subpopulation included individuals over age 50 with high or low chromosome aberration frequencies, randomly selected individuals between ages 18 and 50, and fetal cord blood samples with high, medium and low aberration frequencies. Peripheral blood (adults) and umbilical cord blood (newborns) were collected and cultured (52 h, PHA-stimulated); then chromosome preparations were made according to established procedures (*1*).

The Russian population consisted of 126 Chernobyl liquidators and 72 control subjects matched to the liquidators with respect to age, sex and lifestyle (*7*). Peripheral blood was collected and cultured; then chromosome preparations were made as in the California population. These individuals were part of a larger ongoing study to determine the effects of radiation exposure on multiple end points, where a statistically significant increase has been reported in the frequencies of chromosome aberrations in the Chernobyl liquidators (9 cGy average exposure) compared to controls (*12*). All control subjects were included and liquidators were selected from a list of over 200 individuals using a random number generator.

Chromosome Analysis

In the California population, two sets of chromosomes were analyzed (*8*). One set, consisting of chromosomes 3, 5 and 6, was analyzed with each chromosome painted in a unique color. This painting combination detected 31.7% of all observable rearrangements. The second set, consisting of chromosomes 1, 2 and 4 painted in a single color, was analyzed by reviewing photographs of cells containing aberrant chromosomes and determining the chromosomes involved in the exchanges. This chromosome combination detected 34.4% of all visible exchanges. Over 3,000 cells from each donor were scored for each chromosome set.

In the Russian population, one set of chromosomes was analyzed (*7*). This set, consisting of chromosomes 1, 2 and 4 painted in a single color, was analyzed by reviewing photographs of cells containing aberrant chromosomes and determining the chromosomes involved in the exchanges. Over 1,400 cells from each donor were scored.

Criteria for Clones

An individual was identified as bearing a clonal aberration if an identical rearrangement involving a painted and an unpainted chromosome or involving two chromosomes painted in unique colors was observed in three or more cells from an individual. If only two cells from one individual were found to have the same rearrangement, this event could have arisen *in vitro* and was not considered to be a clone. At least two trained cytogeneticists identified and verified clonality in the abnormal cells.

Statistical Methods

A χ^2 analysis was used to determine whether the observed exchanges were proportional to chromosome size (*13*). In the California population, the expected numbers of exchanges were determined by multiplying the number of observed exchanges for each set of three chromosomes analyzed (i.e. 1, 2 and 4 or 3, 5 and 6) by the fraction of the potentially detectable exchanges (assuming a random distribution) represented by each chromosome in that set (*8*). In the Russian population, the same calculation was applied to the set of chromosomes 1, 2 and 4 (*7*). In both populations, χ^2 values were calculated from the observed number of aberrations and the expected number based on DNA content for each chromosome individually, then summed across all chromosomes analyzed per donor. A χ^2 value corresponding to a probability (P value) of <0.005 was used as an indicator of clonality in a donor. The cutoff of 0.005 was selected over other common probabilities (e.g. 0.05, 0.01) because it provided the best separation between the χ^2 values of clone-bearing and non-clone-bearing donors while ensuring adoption of a high degree of significance.

Analysis of Clonal Aberrations

In addition to pertinent information included from subject questionnaires (i.e. age, gender and exposure status), the percentages of abnormal cells and clonal cells were determined by dividing the number of these cells by the total number of scored cells. The percentage adjusted for the presence of clonal aberrations was determined by subtracting the percentage of clonal cells from the percentage of total abnormal cells. Since the difference in the percentage of detectable exchanges for each of the two chromosome sets is similar (34.4% compared to 31.7% for each set of chromosomes 1, 2 and 4 and 3, 5 and 6, respectively), all data have been compared directly without adjustment for DNA content.

Clonal Aberrations and Age

The percentages of control individuals and Chernobyl liquidators bearing clonal aberrations were determined and categorized into the following age groups: $\leq$19, 20–29, 30–39, 40–49, 50–59 and $\geq$60 years.

RESULTS

The χ^2 values calculated from the observed number of translocations plus insertions and the expected number based on chromosome size for each chromosome studied were totaled for each subject in both populations and plotted as a function of age. Figure 1 shows the results of the analysis of chromosomes 1 through 6 in the California population and chromosomes 1, 2 and 4 in the Russian population. The individuals with the most skewed nonproportional cytogenetic damage distributions have the highest χ^2 values, with the proposed marker of potential clonality being $P < 0.005$.

Table 1 shows the analysis of the nine donors from both populations who were identified as possessing clonal chromosomal changes. Their ages varied from 25 to 71 years, and clones were detected in five of six chromosomes studied (chromosomes 1, 2, 4, 5 and 6).

In the healthy, unexposed California population, the three individuals with χ^2 values corresponding to $P < 0.005$ ($\chi^2 > 16.7$) were found to possess clonal aberrations. None of the 24 individuals whose χ^2 values correspond to $P > 0.005$ possessed clones. Clones from two of the donors (ages 70 and 71) have been identified as translocations (*6*), while the clone in the third donor (age 60) is an insertion (*1*).

In the Russian population, the three individuals with the highest χ^2 values ($\chi^2 > 29$) possessed clones ($P \ll 0.005$); one was from the 72 control individuals (age 59) and two were from the 126 Chernobyl liquidators (ages 46 and 50).

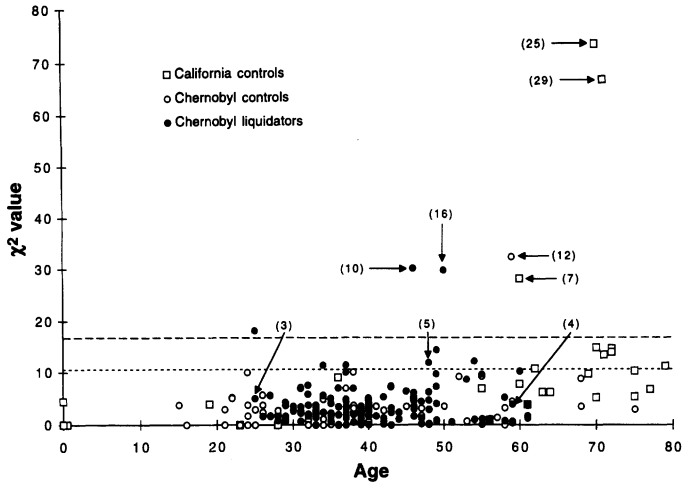

FIG. 1. χ^2 determined from the observed number of aberrations and the expected number based on chromosome size as a function of age. The dashed and dotted lines indicate χ^2 values associated with $P = 0.005$ in the California population and Russian population, respectively. Individuals possessing clonal aberrations are indicated by arrows. The numbers of clonal cells observed for each individual are indicated in parentheses.

One other liquidator (age 48) with $P < 0.005$ ($\chi^2 = 11.9$) possessed a clonal aberration. Two liquidators (ages 25 and 59) with $P > 0.005$ had clones; however, no other donors with $P > 0.005$ had clones. All clonal aberrations in the Russians have been identified as translocations (*5*).

Table 1 also shows the change in the χ^2 values after the subtraction of clonal events from the χ^2 calculation. All values from donors bearing clonal translocations are reduced to a nonstatistically significant deviation from proportionality after subtraction of the clones ($P > 0.005$). The only value which remains associated with a probability that deviates significantly from proportionality after adjustment is that for the donor bearing a clonal insertion ($P < 0.005$). Table 2 shows the percentage of control subjects and Chernobyl liquidators bearing clonal aberrations categorized by age. Clones were found in 8.3 and 13.6% of controls aged 50–59 and ≥60 years, respectively. Among the liquidators, clones were found in subjects aged 20–29, 40–49 and 50–59 at a frequency of 7.7, 4.5 and 14.3%, respectively.

DISCUSSION

Chromosome aberrations of clonal origin have been found in cytogenetic studies of both radiation-exposed and healthy unexposed humans. These clonal aberrations artificially elevate measurements of aberration frequency and skew the distribution of aberrations compared to DNA content unless such clones are identified and taken into account. The frequency of aberrations can be adjusted by considering each clone as a single mutational event. This adjustment is necessary to obtain accurate dose estimates when using translocation frequencies for biodosimetry to prevent overestimation of absorbed dose. A simple statistical test can be performed to indicate which individuals are likely to possess clonal aberrations. This is accomplished using χ^2 values determined from the observed number of aberrations and the expected number based on chromosomal DNA content. The results of this test suggest that χ^2 values above a predetermined level may be useful for identifying individuals with clones, thereby avoiding detailed analysis of abnormal cells in the majority of individuals.

In the California population, every donor found to possess a clonal aberration was identified accurately using the χ^2 test. Three donors were shown to have χ^2 values with $P < 0.005$. Two possessed clonal translocations and the third had a clonal insertion. In the Russian population, the three donors with the highest χ^2 values were found to possess clonal aberrations. One other subject possessing a clone had a χ^2 value of $P < 0.005$. Overall, nine individuals had a χ^2 value above the preset level ($P < 0.005$) and four of these bore clones (44%). Of the 189 individuals whose χ^2 values corresponded to $P > 0.005$, two were found to possess clones (1%). The latter would not be identified using this

TABLE 1
Individuals Possessing Clonal Aberrations

Population	Age	Sex	Exposure status	Cells analyzed[a]	Chromosome set analyzed[a]	χ^2 value[b]	Adjusted χ^2 value[c]	Abnormal cells	Clonal cells	Clonal aberration type	Percentages: Abnormal cells	Clonal cells	Abnormal − clonal
California	60	F	Control	3084	1,**2**,4	28.1	22.1	45	7	Insertion	1.46	0.23	1.23
	70	M	Control	3388	3,**5**,6	73.3	7.1	55	25	Translocation	1.62	0.74	0.89
	71	M	Control	3493	3,5,**6**	66.7	6.6	52	29	Translocation	1.49	0.83	0.66
Russia	59	M	Control	1599	**1**,2,4	32.3	2.7	26	12	Translocation	1.63	0.75	0.88
	25	M	Liquidator	1865	1,**2**,4	5.0	1.3	16	3	Translocation	0.86	0.16	0.70
	59	M	Liquidator	1517	1,2,**4**	3.9	1.7	20	4	Translocation	1.32	0.26	1.05
	48	M	Liquidator	1465	1,2,**4**	11.9	4.1	15	5	Translocation	1.02	0.34	0.68
	46	M	Liquidator	1475	1,2,**4**	31.1	5.0	15	10	Translocation	1.02	0.68	0.34
	50	M	Liquidator	1474	1,2,**4**	29.7	6.4	22	16	Translocation	1.49	1.09	0.41

[a] Painted chromosome involved in clonal aberration indicated in bold.
[b] χ^2 values of 16.7 and 10.6 correspond to $P = 0.005$ in California and Russian populations, respectively.
[c] Values calculated after subtraction of clonal aberrations.

TABLE 2
Frequency of Clonal Aberrations with Age

	Age (years)					
	≤19	20–29	30–39	40–49	50–59	≥60
Total number of controls	6	22	26	11	12	22
Percentage of controls with clones	0	0	0	0	8.3	13.6
Total number of liquidators	0	13	52	44	14	3
Percentage of liquidators with clones	0	7.7	0	4.5	14.3	0

statistical method. However, it is important to note that these two donors had the two lowest percentages of clonal cells among cleanup workers (0.16 and 0.26%), and the adjusted aberration frequencies were affected the least (see Table 1). The individuals with the greatest percentage of clonal aberrations (i.e. those whose aberration frequencies and distributions were affected the most by clones) were identified accurately with this method.

The three control subjects with clonal translocations had similar percentages of abnormal cells (1.6, 1.5 and 1.6%) and clonal cells (0.7, 0.8 and 0.8%), and similar adjusted percentages (0.9, 0.7 and 0.9%). Since the clonal translocations had a similar effect on the adjusted percentages in all three subjects, it appears that the influence of these clones in controls can be quantified easily. In this case, the percentage of cells with clones with translocations is approximately equal to the percentage of abnormal cells without apparent clones. In the control donor found to possess a clonal insertion, the percentage of abnormal cells was similar to that of the other controls (1.5%). However, the adjusted percentage of abnormal cells was affected less than in the case of the clones with translocations (i.e. reduced to only 1.2%). This is due to the smaller number of clonal insertions (7 clonal of 3084 total cells) compared to clonal translocations (12/1599, 25/3388 and 29/3493).

The influence of clonal aberrations appears to be greater in the radiation-exposed Chernobyl liquidators than in the control donors. For example, two liquidators with identical percentages of abnormal cells (1.02%) were found to have a twofold difference when the percentages were adjusted for the presence of clonal aberrations (0.68 and 0.34%). That is, for these two cases an initial identical estimate of absorbed dose for purposes of biodosimetry would be reduced by approximately one-third and two-thirds, respectively. This indicates the importance of both detecting and taking into consideration clonally derived chromosomal changes in scored cell samples. In addition, the highest percentage of abnormal cells among the liquidators with clones (1.49%) was reduced to the second lowest percentage when adjusted for the presence of clones (0.41%). In such cases, the clones appear to be elevating the percentages of aberrant cells more variably than in control donors. This variability suggests that the presence of clonal aberrations may be influential in cytogenetic analyses of individuals exposed to radiation. The statistical method presented here offers the potential for identification of radiation-induced clones so that their effect on measurements of aberration frequency can be quantified more accurately, and hence leads to better biodosimetric estimates of absorbed dose.

In addition to the effect of clonal aberrations on measurements of aberration frequency, clones may also skew the results of analyses of distributions of damage relative to DNA content by contributing to an apparently higher number of aberrations in that particular chromosome. This would result in a greater nonproportional distribution of aberrations based on DNA content. Our results show that this is indeed the case. With the exception of the two Chernobyl liquidators with the lowest frequency of clonal aberrations, clone-bearing individuals had χ^2 values that deviated significantly from proportionality ($P < 0.005$). When the χ^2 values were corrected for the presence of clones, all of the values corresponding to individuals with clonal translocation were reduced to a level that does not deviate significantly from proportionality ($P > 0.005$). This shows that the presence of clones does have an effect on the apparent distribution of aberrations relative to chromosome length. The only case where this effect is negligible is the individual bearing a clonal insertion. Similar to the effect of clones on measurements of aberration frequency, this may be due to the smaller number of clonal insertions compared to clonal translocations.

The frequency of clonal aberrations appears to increase with age in healthy, unexposed control individuals, with clones found only in subjects older than 55 years. This supports the conclusion of Jin *et al.* (*10*), who showed an age-dependent frequency of clones in upper aerodigestive tract mucosa. Haglund *et al.* (*14*) found a clonal abnormality in one of eight control subjects, an elderly male, when fibroblasts from hairy cell leukemia patients were assessed for chromosomal instability. Clonal aberrations appear to be rare among healthy, young, unexposed individuals, but may be more prevalent among older individuals. Among the Chernobyl liquidators, clones were found in those under 55 years of age, including ages 25, 46, 48 and 50, as well as over age 55. The observation of clonal aberrations in radiation-exposed individuals has been made by others (*2*). The frequency of clones appears to increase as a function of age in controls, whereas an age effect was not evident in Chernobyl liquidators. This suggests that spontaneous and radiation-induced clonal expansion is occurring in control donors and liquidators, respectively.

Clonal cells, as described here, are those that contain a

single chromosomal aberration (e.g. a reciprocal translocation) that was induced in a precursor cell and expanded through cell proliferation. These clones of karyotypically abnormal cells may or may not have deleterious effects on the health of an individual. It is possible that a clone with a chromosome change of this type in peripheral blood lymphocytes is merely a consequence of the growth of cell populations from precursor cells. However, since specific chromosomal rearrangements have been shown to be associated with many types of cancer (*15–17*), the observation of clones may be useful in the detection of malignancy if the alteration is of consequence to a specific tissue type for the development of a tumor. Indeed, clones with chromosome changes similar in morphology to the Philadelphia chromosome have been reported in lymphocytes from highly exposed reactor personnel of the Chernobyl power plant (*18*), although typical clinical symptoms of leukemia were not observed in these individuals. Detailed analyses of clonal aberrations, including quantification of their frequencies over time and mapping of breakpoint locations, may help to elucidate the importance of clones in human health.

Reliable estimates of translocation frequencies for both radiation biodosimetry and monitoring purposes should benefit from incorporation of an adjustment for the presence of clonal abnormalities, and the statistical method applied in this study appears to be a useful way to identify subjects with clonal aberrations. Our analyses show that clones may have a profound effect on study outcomes, justifying their careful evaluation when using cytogenetic methods for exposure assessments.

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