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Heterogeneity in the clastogenic response to X-rays in lymphocytes from ataxia-telangiectasia heterozygotes and controls

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A coded analysis of X-ray-induced chromatid aberrations in lymphocyte cultures from 45 control individuals and 19 ataxia-telangiectasia (A-T) heterozygotes was performed. The distribution of chromatid breaks induced in the late G_2 portion of the cell cycle by 60 cGy of X-rays appeared bimodal in the study population. In six controls (13 percent) and in 12 of 19 (63 percent) A-T heterozygotes, the yields of X-ray-induced breaks observed were within the higher mode of the distribution. However, lymphocytes from A-T heterozygotes sensitive to the induction of chromatid breaks by 60 cGy did not contain increased numbers of aberrations following exposure to 20 cGy. The radio-resistant inhibition of DNA synthesis that occurs in A-T homozygotes was not observed in heterozygotes. Co-cultivation experiments showed an increased G_2 delay in lymphocytes from an A-T heterozygote whose lymphocytes contained increased X-ray-induced chromatid breaks. The results show a significant association of A-T heterozygosity with G_2 chromosomal sensitivity ($P < 0.001$; Wilcoxon rank sum test). The measurement of X-ray-induced breaks, however, failed to identify 37 percent of A-T heterozygotes tested. The predicted prevalence of increased sensitivity to X-rays in controls is approximately three- to 30-fold greater than the estimated frequency of A-T heterozygotes in the general population. Therefore, although the increased sensitivity to X-ray-induced chromatid breaks appears to be associated with the A-T-gene, it is not a reliable indicator of A-T heterozygosity. Genetic or environmental factors other than the A-T gene also must be involved in the increased clastogenic response.

Key words: Ataxia telangiectasia, chromosome breakage, lymphocytes, X-rays.

Introduction

Ataxia-telangiectasia (A-T) is a rare autosomal recessive human disorder in which individuals exhibit cerebellar ataxia, oculocutaneous telangiectasia, immunological defects, abnormal radiation sensitivity and predisposition to cancer.[1-3] Carriers of the A-T gene (A-T heterozygotes), although phenotypically normal, have been reported to be at increased risk for the development of cancer;[4-6] female carriers may have

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a sixfold increased relative risk of breast cancer.[7,8] Because A-T heterozygotes may represent 0.68-7.7 percent of the general population[6] and are cancer-prone, there is a need for a rapid and simple method for detecting carriers of the A-T gene. Most laboratory methods for the detection of A-T heterozygotes have used cultured cell lines and rely on the differential effects of X- or gamma-irradiation on cell cycle progression,[9-11] cell survival,[12-17] or the formation of chromosomal aberrations.[18-24] Furthermore, the ability of these methods to detect A-T heterozygotes reliably from the broad range of responses observed in cells from normal controls is not clear.

The development of an assay based on short-term culture of peripheral blood lymphocytes would allow rapid identification of A-T carriers and would be especially useful in the study of cancer risk associated with the A-T gene in the general population.

To test whether A-T heterozygotes can be detected by cytogenetic analysis of short-term lymphocyte cultures, we examined the number of chromatid aberrations induced in peripheral blood lymphocytes by low doses of X-rays in obligate A-T heterozygotes and in persons with no family history of the disease. Cytogenetic analysis was carried out on lymphocytes that were treated with colcemid immediately after irradiation in order to maximize differences in aberration yields due to either intrinsic radiosensitivity or variation in cell cycle delay. To test whether the results correlated with other possible markers of an abnormal response to radiation in A-T heterozygotes, studies of cell cycle progression, and inhibition of DNA synthesis also were carried out.

Materials and methods

The study group consisted of obligate A-T heterozygotes (*i.e.*, parents of patients diagnosed with A-T) and unrelated individuals with no family history of the disease. Nineteen A-T heterozygotes and 45 control subjects were studied. Data on participants' age, gender, and smoking history were collected. Repeat studies were carried out on seven subjects (five controls and two A-T heterozygotes), approximately one year apart. A-T heterozygotes and A-T probands who were studied were drawn from cases diagnosed or followed at Moffitt Hospital, San Francisco, California or at the Brigham and Women's Hospital, Boston, Massachusetts over the time period, May 1986 to June 1988. Controls, who were roughly matched for age and gender, consisted of friends of A-T heterozygotes and laboratory or support staff. All blood samples were coded prior to laboratory analyses; in all cases, samples were analyzed in groups including both controls and A-T heterozygotes and in some groups A-T homozygotes were included as well.

Cell culture and cytogenetics

Venous blood was drawn into 15 ml heparinized vacutainers. Whole blood (0.5 ml) was added to 4.5 ml of RPMI 1640 medium containing 10 percent fetal calf serum, 2 mM glutamine, 100 units/ml penicillin, 100 μg/ml streptomycin, and two percent phytohaemagglutinin M (PHA-Gibco). The blood was cultured at 37°C in one-ounce glass prescription bottles. The same lots of vacutainers, serum and prescription bottles were used for all experiments. For studies of X-ray-induced chromatid breaks in the late G_2 portion of the cell cycle, cultures were irradiated 48 hours after incubation with PHA. Colcemid (final concentration = 2×10^{-7} M) was added immediately after radiation treatments and cells harvested 3 h later. Fixation was performed by standard cytologic procedures, *i.e.*, the cells were exposed to a 0.075 M KCl solution for 8 min to spread chromosomes and then fixed in methanol-acetic acid solution (3:1). Cytologic preparations were made by placing cells on wet slides and staining with Giemsa. The slides were scored for chromatid and isochromatid breaks, gaps and chromatid exchanges. Chromatid gaps were defined as achromatic lesions less than the width of the chromatid. The blood samples in all experiments were received, coded, and the cells were scored blindly.

Co-cultivation experiments

To study the relative progression of cells into metaphase following X-ray treatments, PHA-stimulated blood cultures from a male A-T heterozygote were mixed in equal volumes with parallel blood cultures from a normal female blood-donor immediately prior to irradiation (*i.e.*, 48 h). Mixed male and female cultures, along with the appropriate controls, were treated with 60 cGy of X-rays and the cells harvested 0.5, 1.0, 2.0, 3.0, and 6.0 h later. In these experiments, colcemid was added 0.5 h prior to harvest. The effect of irradiation on the mitotic index of each population was determined by examining the proportion of male (XY) and female (XX) metaphases observed on the slides. Only cells with 46 chromosomes were analyzed. Mitotic indices are based on counts of at least 3,000 interphase cells from the same cultures used for male: female ratios.

X-irradiation

Cells were irradiated at room temperature with 250 kVp X-rays (Phillips model 250 therapeutic unit, 15

mA; half value layer, 2.06 mm Cu) at a dose rate of 100 cGy/min for cytogenetic studies and 400 cGy/min for studies of X-ray-induced inhibition of DNA synthesis.

X-ray inhibition of DNA synthesis

The method developed to measure inhibition of DNA synthesis in fibroblasts[25] was modified for the analysis of peripheral blood lymphocytes. DNA from PHA-stimulated lymphocytes was prelabeled with [^{14}C] thymidine (0.01 μCi/ml) from 24 to 48 h of culture. At 48 h, cultures were exposed to 0, 500, 1,000, and 2,000 cGy X-rays, incubated at 37°C for 45 min, treated with [^{3}H]thymidine, 5 μCi/ml (specific activity 6.6 Ci/mmol) for 15 min and then harvested. Red blood cells were lyzed with hypotonic solution. The rate of DNA synthesis was estimated from the ratio of ^{3}H to ^{14}C acid-precipitable radioactivity. Results are expressed as a percentage of the ratios in nonirradiated control cultures for each individual.

Statistical analysis

The number of chromatid aberrations in control and A-T heterozygotes was non-normally distributed, including log transformed data. Consequently, data from controls on A-T heterozygotes were compared using the Wilcoxon rank sum test. The chi-square test was used to compare the proportion of male and female cells. Nonparametric analysis of variance was used to assess the effects of various factors (*e.g.*, age, smoking, sex) on aberration yields.

Results

Dose-response studies

Lymphocyte cultures from three A-T heterozygotes and three controls were irradiated with 20, 40, and 60 cGy of X-rays, and the number of chromatid aberrations was measured three hours later. At 20 cGy (Figure 1), the number of chromatid breaks induced in cells from A-T heterozygotes was not different from the yields observed in lymphocytes from controls cultured and irradiated at the same time. At the intermediate 40 cGy dose, lymphocytes from A-T heterozygotes showed a small increase in the number of chromatid breaks relative to controls. When lymphocytes were irradiated with 60 cGy of X-rays, the observed yields of chromatid breaks in lymphocytes from A-T heterozygotes were approximately twice those observed in controls ($P<0.01$; Wilcoxon rank sum test). These results indicate that increased sensitivity to the induction of chromatid breaks by X-rays could be associated with A-T heterozygosity, and that the effect may be dependent on X-ray dose.

Co-cultivation experiments

When cells are irradiated in the late G_2 portion of the cell cycle and mitotic cells are collected over the following 3 h for cytogenetic studies, as in the present experiments, the cells analyzed are a mixture of two populations of cells, *i.e.*, those which progress directly into mitosis following irradiation and those that undergo a delay before entering mitosis. Cells which do not undergo delay are highly damaged by the X-ray exposure and contain numerous chromosomal aberrations, whereas cells undergoing delay contain relatively few such aberrations owing to chromosomal repair processes that take place during the delay period. The relative proportions of these two populations are dependent on X-ray dose, the extent of mitotic delay, and the length of time after irradiation that cells are collected for analysis.[26]

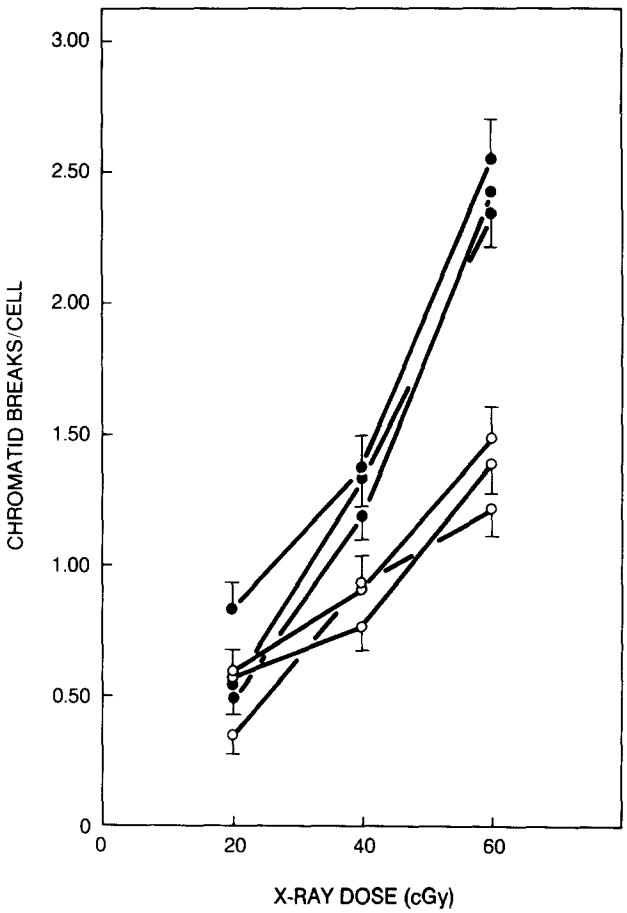

Figure 1. Induction of chromatid breaks by 20, 40, and 60 cGy of X-rays in lymphocyte cultures from three A-T heterozygotes (●) and three controls (○). Error bars indicate standard error of the mean.

To test whether the increased yields of X-ray-induced chromatid breaks in lymphocytes from A-T heterozygotes are associated with a different rate of cell progression into mitosis after irradiation, co-cultivation studies were carried out. Blood cells from a male A-T heterozygote that showed increased sensitivity to chromatid break induction by X-rays in dose-response studies were studied (Figure 1).

The subject's affected son was also studied to confirm increased G_2-chromosomal radiosensitivity; lymphocytes from the son that were treated with 60 cGy X-rays contained 438 chromatid breaks per 100 cells. In the control experiment, in which normal non-irradiated female lymphocytes were mixed with non-irradiated lymphocytes from the male A-T heterozygote, we observed a ratio of 1.8 female:male mitotic cells (Table 1). In this experiment, the normal female cells appeared to have a higher baseline mitotic activity.

When lymphocyte mitoses were examined 0.5 h after treatment with 60 cGy of X-ray, the ratio of female to male cells was 1.95. This did not differ significantly from the nonirradiated control culture. At 0.5 h, the mitotic indices were also similar to the nonirradiated cultures. These data indicate that lymphocytes harvested 0.5 h following radiation exposures have progressed beyond the 'transition point' for mitotic delay. At later times following irradiation, however, the mitotic yields of both normal female and male A-T heterozygote lymphocytes decreased dramatically. Furthermore, the ratio of female:male mitotic cells at 2.0 and 3.0 h after irradiation was significantly greater than the ratio of the nonirradiated control ($P < 0.005$; chi-square test). The relative decrease in the numbers of male cells detected at these points in time suggest that the A-T heterozygote lymphocyte population undergoes a relatively longer mitotic delay following the X-ray treatment. That the ratio of female:male mitotic cells returned to control values at 4 h following irradiation suggests that the observed increase in mitotic delay in A-T heterozygote lymphocytes is transient.

Survey of 19 A-T heterozygotes and 45 controls

Figure 1 shows significantly increased yields of X-ray-induced chromatid breaks in lymphocytes from some A-T heterozygotes irradiated with 60 cGy X-rays. The results of studies on cultures from 19 obligate A-T heterozygotes and 45 control individuals exposed to 60 cGy are shown in Table 2. Neither the yields of induced breaks nor their log transformed values were

Table 1. X-ray-induced chromosomal aberrations in lymphocyte cultures from A-T heterozygotes and controls

	A-T heterozygotes (No. = 19)			Controls (No. = 45)		
	Mean	Median	Range	Mean	Median	Range
Chromatid breaks per 100 cells	198	217	112-336[a]	138	130	74-269
Chromatid exchanges per 100 cells	32	28	12-74[b]	24	20	8-56
Total aberrations per 100 cells	230	250	128-368	162	150	93-296
>190 Breaks/100 cells	12			6		

[a] Significantly greater than control; $P < 0.005$; Wilcoxin rank sum test.
[b] Significantly greater than control; $P < 0.05$; Wilcoxin rank sum test.

Table 2. Relative numbers of co-cultivated normal female and male A-T heterozygote lymphocytes recovered at mitosis after X-irradiation

Treatment	Number of mitotic cells		Ratio female:male	Mitotic cells/1,000[a]	χ^2
	Female cells	Male cells			
Control (no irradiation)	131	73	1.80	14	—
60 cGy, 0.5 h	144	74	1.95	11	0.51
60 cGy, 1 h	n.d.[b]	n.d.[b]	n.d.[b]	0.6	—
60 cGy, 2 h	89	26	3.42	2	8.53[c]
60 cGy, 3 h	181	46	3.94	8	24.75[d]
60 cGy, 4 h	102	50	2.04	20	0.71
60 cGy, 6 h	100	47	2.13	15	1.06

[a] Based on 3,000 cells.
[b] n.d. = no data.
[c] Statistically significant at $P < 0.005$.
[d] Statistically significant at $P < 0.0005$.

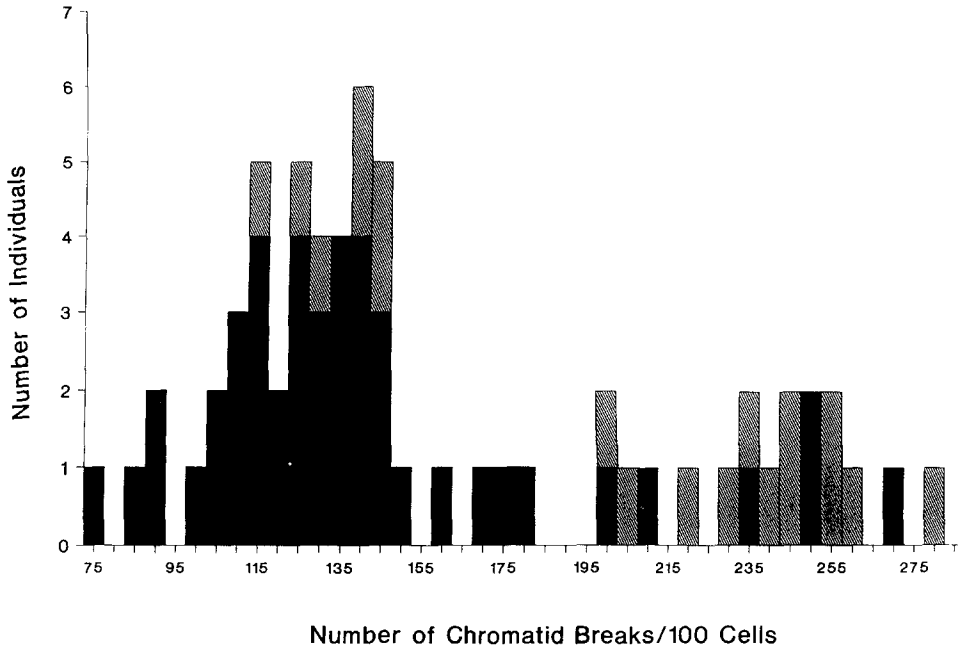

Figure 2. Frequency histogram of chromatid breaks induced by 60 cGy X-rays in lymphocyte cultures from 19 A-T heterozygotes (hatched bars) and 45 control subjects (solid bars). Where the hatched and solid bars are shown at the same chromatid break value, the hatched bars should be read as beginning at the top of the black bars.

normally distributed in both controls and A-T heterozygotes. The distribution appeared bimodal (Figure 2). We therefore arbitrarily chose a cutoff value of 190 breaks/100 cells to define individual scores in the higher and lower modes of the distribution. Six of 45 controls (13 percent, 95 percent confidence interval [CI] = 3-23 percent) and 12/19 A-T heterozygotes (63 percent, CI = 41-85 percent) had breakage scores of greater than 190 breaks/100 cells. Lymphocytes from A-T heterozygotes were found to be 11 times more likely to have increased breakages rates following exposure to 60 cGy X-rays. The CI of the proportion of controls sensitive to X-ray induction of chromatid breaks overlaps with previous prevalence estimates of A-T heterozygotes (*i.e.*, 0.68-7.7 percent). After adjusting for the sensitivity of the current procedure for detecting A-T heterozygotes (*i.e.*, 63 percent), the current results suggest a 21 percent prevalence of A-T heterozygotes in the present control population, approximately three- to 30-fold higher than previous estimates. Analysis of variance indicated no significant association of age, sex, or smoking status on aberration yield.

Because sparsely ionizing radiation, such as energetic X-rays, randomly deposits energy within the cell nucleus, the resultant distribution of chromosomal breaks within the cell population also is distributed randomly and follows a Poisson probability distribution. If the cell population is not homogeneous in its response to radiation, then deviations from the Poisson distribution are expected. Our co-cultivation experiments indicate that lymphocytes from A-T heterozygotes that have increased yields of X-ray-induced chromatid damage undergo a more prolonged mitotic delay than those from control individuals following irradiation. Variations in the duration of the mitotic delay in cells from A-T heterozygotes that have not reached the transition point at the time of irradiation could lead to different proportions of highly damaged cells in the cellular distribution of chromosomal breaks. Consequently, for A-T-heterozygotes and controls we tested whether the distribution of chromatid breaks within cells followed a Poisson distribution, and compared the results in persons with normal sensitivity with results from those with increased yields of radiation-induced breaks.

In 42 of 46 (91 percent) individuals (A-T heterozygotes or controls) in which an individual's yield of aberrations was less than 190 breaks/cell the observed distribution of chromatid breaks was consistent with Poisson probabilities ($P > 0.05$, chi-square test). However, the distribution of chromatid breaks deviated from the Poisson distribution in all experiments with individuals whose yield of aberrations was increased (*i.e.*, 190 or more chromatid breaks/cell). The analysis indicated over-dispersion in the frequency of cells with

Table 3. Repeat studies of X-ray-induced chromatid breaks in lymphocytes from normal subjects and A-T heterozygotes

Subject	Sample	No. of chromosomal aberrations per 100 cells				Percent difference in breaks
		Cd[a]	Iso[b]	Chromatid exchange	Total breaks	
Normal						
1	1	127	4	19	131	−37
	2	94	0	15	94	
2	1	133	8	16	141	−20
	2	110	2	12	112	
3	1	110	3	9	113	+19
	2	126	9	5	135	
4	1	119	1	14	120	+12
	2	124	2	8	134	
5	1	138	2	22	140	−6
	2	129	2	48	131	
A-T heterozygote						
6	1	124	0	12	124	−48
	2	82	2	19	84	
7	1	240	3	26	243	−14
	2	204	5	31	209	

[a] Cd = chromatid deletion.
[b] Iso = isochromatid deletion.

greater than five chromatid breaks per cell. There were more than five high-frequency cells (HFCs) in 14/19 (74 percent) A-T heterozygotes compared with 11 of 45 (24 percent) in the controls. Increased HGCs and over dispersion was also found in the A-T heterozygote whose lymphocytes were studied in co-cultivation experiments. These results are consistent with the hypothesis that increased yields of chromatid breaks are associated with disproportionate increases in the frequency of highly damaged cells that might result from the presence of two cell populations with different cell cycle delay characteristics.

Repeat analyses

Repeat blind analyses on recoded samples (Table 3) show that the measurement of X-ray-induced G_2-chromosomal breaks is reproducible. Individuals classified as sensitive or resistant to X-irradiation (*i.e.*, sensitive: 190 or more breaks/100 cells; resistant: less than 190 breaks/100 cells) were consistently identified. Of special interest were Subjects 6 and 7—the father and mother of a son with A-T. On both occasions, the mother, but not the father, had an increased number of X-ray-induced aberrations.

Inhibition of DNA synthesis by X-rays

To test whether the variable response of lymphocytes from A-T heterozygotes to the induction of chromatid breaks correlated with the inhibition of DNA synthesis by X-rays, we carried out studies in which both endpoints were measured in the same experiment. In one A-T family, lymphocytes from the mother, father, and affected son were studied. In this family, the affected son was found to be highly sensitive to the induction of chromatid breaks by X-rays (data not shown). The mother (Subject 7, Table 3) had an increased yield of chromatid breaks, but the father (Subject 6) had nor-

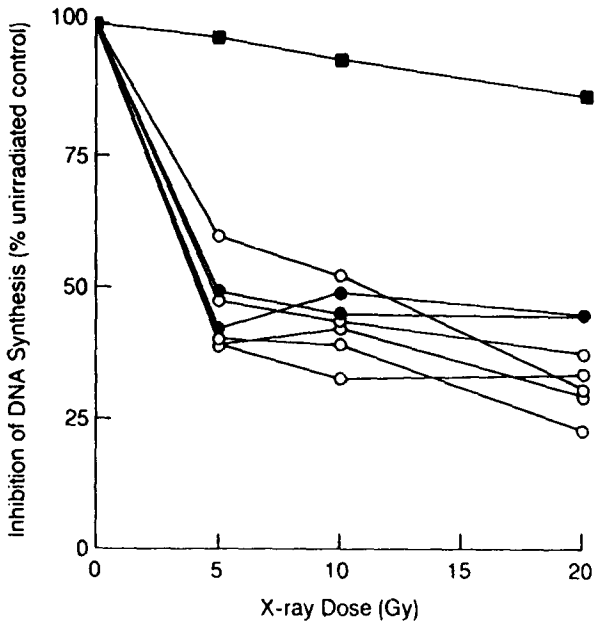

Figure 3. Inhibition of DNA synthesis by X-rays in lymphocyte cultures from a patient with ataxia-telangiectasia (■), the patient's parents (A-T heterozygotes; ●), and six control subjects (○).

mal levels of chromatid damage following irradiation.

The inhibition of DNA synthesis by different doses of X-rays in this family and in six concurrent controls is shown in Figure 3. The proband shows a typical radio-resistant inhibition of DNA synthesis. The extent of inhibition of DNA synthesis in both parents, however, fell within the range observed in the concurrent control experiments. Although lymphocytes from the mother showed increased sensitivity to the induction of chromatid breaks, her cells had a normal response to the inhibitory effects of X-rays on DNA synthesis. Thus, there appears to be no correlation between G_2 chromosomal radiosensitivity and the inhibition of DNA synthesis in A-T heterozygotes showing different sensitivities to the induction of chromatid breaks by X-rays.

Discussion

Human tumor cells[27-29] and fibroblasts from persons with genetic disorders predisposing to high risk of cancer, including A-T,[30,31] Fanconi's anemia, Bloom's syndrome, dysplastic nevus syndrome, and Gardner's syndrome, exhibit an enhanced G_2-chromosomal radiosensitivity to X-rays.[31-34] Apparently normal fibroblasts from individuals with familial cancer, including breast cancer, also exhibit increased sensitivity to X-ray-induced chromosomal aberrations.[31] These observations have led to the proposal that the measurement of G_2-chromosomal sensitivity could be a rapid assay for detecting genetic susceptibility to cancer in the general population.[31,34] A test such as this could have far-reaching effects on cancer prevention and early diagnosis.

Recent work, however, raises important questions concerning the validity of this approach for identifying high-cancer-risk individuals. For example, Bender *et al*[35] observed normal G_2-chromosomal radiosensitivity in cells from a familial cancer syndrome kindred. No increase in the chromosomal radiosensitivity in cell lines from A-T heterozygotes has been reported.[21,36,37] Other studies have reported broad variability in the response of cells from presumably normal individuals[38] and suggested that fibroblast cell strains derived from up to 30 percent of apparently normal individuals may show increased G_2 chromosomal sensitivity. Our study of 45 normal individuals emphasizes the fact that the specificity (*i.e.*, the rate of false positives) of the assay for G_2-chromosomal radiosensitivity is still unknown. Similarly, induction of chromosomal aberrations by radiomimetic chemicals is highly variable in normal blood donors. Increased sensitivity to the induction of chromosomal aberrations by bleomycin (a radiomimetic antitumor agent) was observed in 23.6 percent of control individuals.[39] These results underscore the need for expanded studies of the prevalence of increased sensitivity to radiation and radiomimetic chemicals in the general population, including analyses of the role of environmental and genetic factors in the induction of chromosomal aberrations.

On the other hand, our observation that lymphocytes from 63 percent of A-T heterozygotes contained increased yields of X-ray-induced chromatid breaks compared with 13 percent in controls suggests a significant association of X-ray sensitivity with the A-T gene in A-T carriers. The results of repeated blood samplings and cytogenetic analyses show that the assay can identify, reproducibly, persons with high yields of X-ray-induced chromatid breaks. Thus, these results support the association of increased sensitivity to the clastogenic effects of X-rays with A-T heterozygotes although the response is heterogeneous in both A-T carriers and controls. Increased sensitivity to radiation-induced chromatid breakage, however, was not associated with radioresistant inhibition of DNA synthesis in lymphocytes from A-T heterozygotes. A similar observation has been made using cultured fibroblasts.[40]

This apparent heterogeneity in the response of A-T gene carriers is consistent with the results of several other studies. The survival of G_0 human lymphocytes irradiated with gamma rays showed a slight increase in radiosensitivity in A-T heterozygotes but considerable overlap with the normal range.[41] An increased frequency of exfoliated oral epithelial cells containing micronuclei was reported in 16 of 26 A-T heterozygotes,[42] whereas mutations at the glycophorin locus in peripheral red blood cells was increased in some but not all A-T heterozygotes examined.[43] On the other hand, a blinded study of the sensitivity of cell killing of fibroblast cell strains derived from A-T heterozygotes fell well within the broad range of the normal response.[44] Similarly, a study of X-ray-induced chromosomal aberrations in lymphocytes reported no increase in the number of chromatid aberrations induced in the late G_2 portion of the cell cycle in six A-T heterozygotes compared with five controls.[36] Methodologic differences such as the radiation doses employed in this and in the present study could account for the disparate results. Consistent with our results on the G_2 delay, a more pronounced inhibition of cell cycle progression has been reported previously in lymphoblasts from A-T patients.[45]

The failure of the present assay of X-ray-induced chromosomal damage to identify 37 percent of the A-T heterozygotes studied indicates that this measurement cannot be used to identify A-T heterozygotes in the general population or in clinical practice. Chromoso-

mal localization and mapping of the gene responsible for A-T[46] may provide gene probes that will be valuable for further studies of the relationship between X-ray sensitivity in A-T heterozygotes and cancer risk.

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References

1. Taylor AMR, Harnden OG, Arlett CG, *et al.* Ataxia-telangiectasia: a human mutation with abnormal radiation sensitivity. *Nature* (Lond) 1975; **258:** 427-9.
2. Spector BD, Filipovich AH, Perry GS III, Kersey JH. Epidemiology of cancer in ataxia-telangiectasia. In: Bridges BA, Harnden DG, eds. *Ataxia Telangiectasia: A Cellular and Molecular Link Between Cancer, Neuropathology and Immune Deficiency.* New York: Wiley, 1982: 103-38.
3. Boder E. Ataxia telangiectasia: an overview. In: Gatti RA, Swift M, eds. *Ataxia Telangiectasia: Genetics, Neuropathology and Immunology of a Degenerative Disease of Childhood.* New York: Alan R. Liss, 1985: 1-63.
4. Swift M, Sholman L, Perry M, Chase C. Malignant neoplasms in the families of patients with ataxia-telangiectasia. *Cancer Res* 1976; **36:** 209-15.
5. Swift M. Genetics and epidemiology of ataxia-telangiectasia. In: Gatti RA, Swift M, eds. *Ataxia Telangiectasia: Genetics, Neuropathology, and Immunology of a Degenerative Disease of Childhood.* New York: Alan R. Liss, 1985: 133-46.
6. Swift M, Morrell D, Cromartie E, Chamberlin AR, Skolnick MH, Bishop DT. The incidence and gene frequency of ataxia-telangiectasia in the United States. *Am J Hum Genet* 1986; **39:** 573-83.
7. Swift M, Reitnauer PJ, Morrell D, Chase CL. Breast and other cancers in families with ataxia-telangiectasia. *N Engl J Med* 1987; **316:** 1289-94.
8. Swift M, Morrell D, Massey RB, Chase CL. Incidence of cancer in 161 families affected by ataxia-telangiectasia. *N Engl J Med* 1991; **325:** 1831-6.
9. Nagasawa H, Kraemer KH, Shiloh Y, Little JB. Detection of ataxia-telangiectasia heterozygous cell lines by post-irradiation cumulative labeling index: measurements with coded samples. *Cancer Res* 1987; **47:** 398-402.
10. Little JB, Nagasawa H. Effect of confluent holding on potentiallly lethal damage repair, cell cycle progression, and chromosomal aberrations in human normal and ataxia-telangiectasis fibroblast. *Radiat Res* 1985; **101:** 81-93.
11. Rudolph NS, Nagasawa H, Little JB, Latt SA. Identification of ataxia telangiectasia heterozygotes by flow cytometric analysis of X-ray damage. *Mut Res* 1989; **211:** 19-29.
12. Chen PC, Lavin MF, Kidson C, Moss D. Identification of ataxia-telangiectasia heterozygotes—a cancer prone population. *Nature* (Lond) 1978; **274:** 484-6.
13. Paterson MC, Anderson AK, Smith BP, Smith PJ. Enhanced radiosensitivity of cultured fibroblasts from ataxia-telangiectasia heterozygotes manifested by defective colony-forming ability and reduced DNA repair replication after hypoxic X-irradiation. *Cancer Res* 1979; **39:** 3725-34.
14. Kinsella TJ, Mitchell JB, McPherson S, Russa A, Tretze F. *In vitro* X-ray sensitivity in ataxia-telangiectasia homozygote and heterozygote skin fibroblasts under oxic and hypoxic condition. *Cancer Res* 1982; **42:** 3950-6.
15. Arlett CF, Green MHL, Priestley A, Harcourt SA, Mayne LV. Comparative human cellular radiosensitivity. I. The effect of SV40 transformation and immortalisation on the gamma-irradiation survival of skin derived fibroblasts from normal individuals and from ataxia-telangiectasia patients and heterozygotes. *Int J Radiat Biol* 1988; **44:** 911-28.
16. Arlett CF, Priestley A. An assessment of the radiosensitivity of ataxia-telangiectasia heterozygotes. In: Gatti RA, Swift M, eds. *Ataxia Telangiectasia: Genetics, Neuropathology and Immunology of a Degenerative Disease of Childhood.* New York: Alan R. Liss, 1985: 101-9.
17. Weeks DE, Paterson MC, Lange K, *et al.* Assessment of chronic radiosensitivity as an *in vitro* assay for heterozygote identification of ataxia-telangiectasia. *Radiat Res* 1991; **128:** 90-9.
18. Waghray M, Al-Sedairy S, Ozand PT, Hannan MA. Cytogenetic characterization of ataxia-telangiectasia (A-T) heterozygotes using lymphoblastoid cell lines and chronic x-irradiation. *Hum Genet* 1990; **84:** 532-4.
19. Shiloh Y, Parshad R, Frydman M, *et al.* G_2 chromosomal radiosensitivity in families with ataxia-telangiectasia. *Hum Genet* 1989; **84:** 15-8.
20. Kohn PH, Kraemer KH, Buchanan JK. Influence of ataxia-telangiectasia gene dosage on bleomycin-induced chromosome breakage and inhibition of replication in human lymphoblastoid cell lines. *Exp Cell Res* 1982; **137:** 387-95.
21. Natarajan AT, Meyers M, van Zealand AA, Simons JWIM. Attempts to detect ataxia-telangiectasia (A-T) heterozygotes by cytogenetical techniques. *Cytogenet Cell Genet* 1982; **33:** 145-51.
22. Rosin MP, Ochs HD. *In vivo* chromosomal instability in ataxia-telangiectasia homozygotes and heterozygotes. *Hum Genet* 1986; **74:** 335-40.
23. Parshad R, Sanford KK, Jones GM, Tarone RE. G_2 chromosomal radiosensitivity of ataxia-telangiectasia heterozygotes. *Cancer Genet Cytogenet* 1985; **14:** 163-8.
24. Sanford KK, Parshad R, Price FM, *et al.* Enhanced chromatid damage in blood lymphocytes after G2 phase X-irradiation, a marker of the ataxia-telangiectasia gene. *JNCI* 1990; **82:** 1050-4.
25. Painter RB. Radioresistant DNA synthesis: an intrinsic feature of ataxia-telangiectasia. *Mutat Res* 1981; **84:** 183-90.
26. Okada S. Radiation effects on cell progress through the life cycle. In: Altman KI, Gerber GB, Okada S, eds. *Radiation Biochemistry.* New York: Academic Press, 1970: 190-239.
27. Parshad R, Gantt R, Sanford KK, Jones GM. Chromosomal radiosensitivity of human tumor cells during the G_2

cell cycle period,. *Cancer Res* 1984; **44:** 5577-82.
28. Parshad R, Gantt R, Sanford KK, Jones GM, Tarone RE. Repair of chromosome damage induced by X-irradiation during G_2 phase in a line of normal human fibroblasts and its malignant derivative. *JNCI* 1982; **69:** 409-14.
29. Sanford K, Parshad R, Stanbridge EJ, *et al.* Chromosome radiosensitivity during the G_2 cell cycle period and cytopathology of human normal X tumor cell hybrids. *Cancer Res* 1986; **46:** 2045-9.
30. Natarajan AT, Meyers M. Chromosomal radiosensitivity of ataxia-telangiectasia cells at different cell cycle stages. *Hum Genet* 1979; **52:** 127-32.
31. Parshad R, Sanford KK, Jones GM. Chromosomal radiosensitivity during the G_2 cell-cycle period of skin fibroblasts from individuals with familial cancer. *Proc Natl Acad Sci USA* 1985; **82:** 5400-3.
32. Parshad R, Sanford KK, Jones GM. Chromatid damage after G_2 phase X-irradiation of cells from cancer-prone individuals implicates deficiency in DNA repair. *Proc Natl Acad Sci USA* 1983; **80:** 5612-6.
33. Bigelow SB, Rary JM, Bender MA. G_2 chromosomal radiosensitivity in Fanconi's anemia. *Mutat Res* 1979; **63:** 1899-99.
34. Sanford KK, Tarone RE, Parshad R, Tucker MA, Greene MH, Jones GM. Hypersensitivity to G_2 chromatid radiation damage in familial dysplastic naevus syndrome. *Lancet* 1987; **ii** 1111-6.
35. Bender MA, Viola MV, Fiore J, Thompson MH, Leonard RC. Normal G_2 chromosomal radiosensitivity and cell survival in the cancer family syndrome. *Cancer Res* 1988; **48:** 2579-84.
36. Bender MA, Rary JM, Kale RP. G_2 chromosomal radiosensitivity in ataxia-telangiectasia lymphocytes. *Mutat Res* 1985; **152:** 39-47.
37. Nagasawa H, Latt SA, Lalande ME, Little JB. Effects of X-irradiation on cell cycle progression induction of chromosomal aberrations and cell killing in ataxia-telangiectasia (A-T) fibroblasts. *Mutat Res* 1985; **148:** 71-82.
38. Nagasawa H, Little JB. Radiosensitivities of ten apparently normal human diploid fibroblast strains to cell killing, G_2-phase chromosomal aberrations, and cell cycle delay. *Cancer Res* 1988; **48:** 4535-8.
39. Spitz MR, Fueger JJ, Beddingfield NA, *et al.* Chromosome sensitivity to bleomycin-induced mutagenesis, an independent risk factor for upper aerodigestive tract cancers. *Cancer Res* 1989; **49:** 4626-8.
40. Young BR, Painter RB. Radioresistant DNA synthesis and human genetic diseases. *Hum Genet* 1989: **82:** 113-7.
41. Cole J, Arlett CF, Green MHL, *et al.* Comparative human cellular radiosensitivity. II. The survival following gamma-irradiation of unstimulated (G_0) T-lymphocytes, T-lymphocyte lines, lymphoblastoid cell lines and fibroblasts from normal donors, from ataxia-telangiectasia patients and from ataxia telangiectasia-heterozygotes. *Int J Radiat Biol* 1988; **54:** 929-43.
42. Rosin MP, Ochs HD, Gatti RA, Boder E. Heterogeneity of chromosomal breakage levels in epithelial tissues of ataxia-telangiectasia homozygotes and heterozygotes. *Hum Genet* 1989; **83:** 133-8.
43. Bigbee WL, Langlois RG, Swift M, Jensen RH. Evidence for an elevated frequency of *in vivo* somatic cell mutations in ataxia-telangiectasia. *Am J Hum Genet* 1989; **44:** 402-8.
44. Little JB, Nove J. Sensitivity of human diploid fibroblast cells strains from various genetic disorders to acute and protracted radiation exposure. *Radiation Res* 1990; **123:** 87-92.
45. Ford MD, Martin L, Lavin MF. The effects of ionizing radiation on cell cycle progression in ataxia-telangiectasia. *Mutat Res* 1984; **125:** 115-22.
46. Gatti RA, Berkel I, Boder E, *et al.* Localization of an ataxia-telangiectasia gene to chromosome 11q22-23. *Nature* 1989; **336:** 577-80.