# Human minisatellite mutation rate after the Chernobyl accident

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**Germline mutation at human minisatellite loci has been studied among children born in heavily polluted areas of the Mogilev district of Belarus after the Chernobyl accident and in a control population. The frequency of mutation was found to be twice as high in the exposed families as in the control group. Mutation rate in the Mogilev families was correlated with the level of caesium-137 surface contamination, consistent with radiation induction of germline mutation.**

THE accident on 26 April 1986 at reactor 4 of the Chernobyl nuclear power station resulted in the largest reported accidental release of radioactive material[1]. Many regions within the European part of the former Soviet Union were heavily contaminated by radioactive fallout. In the first three months after the accident, acute irradiation of humans occurred through external and internal exposure to iodine-131 with a half-life of 8 days[2]. Following $^{131}I$ decay, exposure to more stable isotopes, mainly $^{137}Cs$, became the main source of radiation risk for people in contaminated regions.

Despite the magnitude of the Chernobyl disaster, little is known about the effects of radiation exposure in human populations inhabiting polluted areas. A considerable increase of thyroid carcinoma in children around Chernobyl has been reported[3,4], as well as an elevated frequency of chromosome aberrations in most residents tested[5]. Additionally, the frequency of congenital malformations in newborns and human embryos has increased in heavily contaminated areas of Belarus following the accident[6,7]. In contrast, nothing is known about the effects of chronic radiation exposure on germline mutation in humans.

Tandem repeat minisatellite loci have several potential advantages for monitoring germline mutations in humans. The very high rate of spontaneous mutation altering allele length (repeat copy number)[8–10] provides a system capable in principle of detecting induced mutations in relatively small population samples. We have previously shown that acute doses of ionizing γ-radiation cause a significant increase in minisatellite germline mutation rate in mice[11], detectable by DNA fingerprint analysis of small numbers of families at doses substantially lower than can be monitored by standard genetic techniques. A similar increase in mutation rate at one unstable minisatellite locus has been found in the progeny of irradiated mice[12,13]. Here we report that minisatellite mutation rate is also unusually high in exposed human populations following the Chernobyl accident.

## Population variability and mutation scoring

Blood samples were collected from 79 families (father, mother, child) inhabiting the heavily polluted rural areas of the Mogilev district of Belarus (Bychoskii, Krasnopol'skii and Cherikovskii regions; Fig. 1). This cohort is composed of children born between February and September 1994 for whom both parents were continuously resident in the Mogilev district from the time of Chernobyl accident. The control sample consists of 105 non-irradiated caucasian families sex matched to the exposed group of offspring. Because the entire Belarus area was contaminated, and it proved logistically difficult to sample Belarus families with children born before the Chernobyl accident, the control group was from the United Kingdom.

DNA fingerprints were produced from all families by using multilocus minisatellite probe 33.15 (ref. 8) and two hypervariable single-locus minisatellite probes MS1 and MS31 (loci *D1S7*, *D7S21*)[9]. In addition, most families were DNA profiled with the minisatellite probes MS32 and CEB1 (loci *D1S8*, *D2S90*)[9,14]. These probes, chosen for their relatively high mutation rates[9,10,14], provided sufficient information to verify the parentage of all children analysed, even in the presence of mutation[10]. Mutants were identified as novel DNA fragments present in the offspring that could not be ascribed to either parent. DNA fingerprints were only scored over the well-resolved region (3.5–22 kilobases).

Examples of families with an offspring containing a mutant band are shown in Fig. 2. Using probe 33.15, $18.12 \pm 0.36$ (s.d. = 5.58) DNA fingerprint bands were scored per offspring in the control group and $17.52 \pm 0.35$ (s.d. = 3.24) bands in the offspring of irradiated parents ($t = 1.18, P > 0.05$; Bartlett test for homogeneity of group variances, $\chi^2 = 0.89$, d.f. = 1, $P > 0.05$). Furthermore, the frequency of band sharing between parents in the control and Mogilev samples was indistinguishable

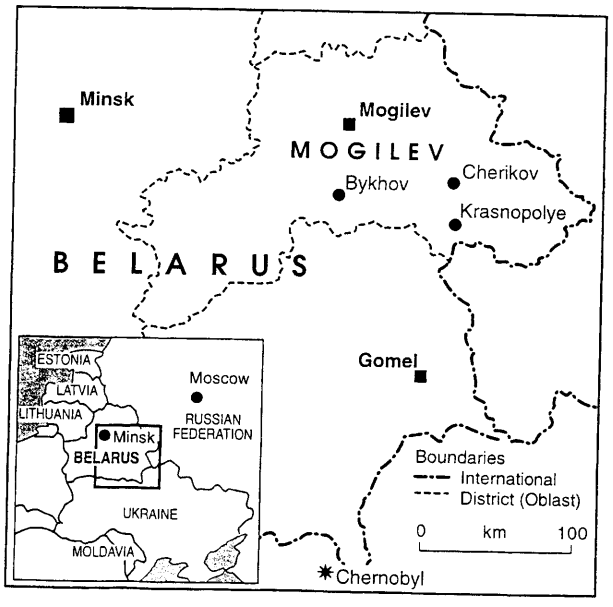


FIG. 1 Map showing the study area.

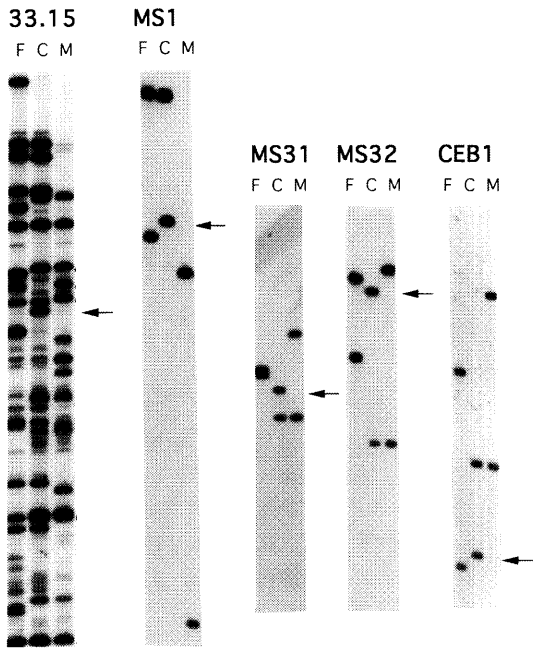


FIG. 2 Examples of minisatellite germline mutation. DNA profiles were produced for each father (F), child (C) and mother (M) using probes 33.15, MS1, MS31, MS32 and CEB1. New mutant bands are arrowed.
METHODS. Samples (4 μg) of DNA extracted from blood were digested to completion with *Alu*I, electrophoresed through a 35-cm (for probe 33.15) or 40-cm (for single-locus probes) long agarose gel (SeaKem, type LE, FMC) in 1 × TBE buffer (89 mM Tris-borate, pH 8.3, 2 mM EDTA), transferred to a nylon membrane (Hybond-Nfp, Amersham) and hybridized to $^{32}$P-labelled probes as described elsewhere[28]. All autoradiographs were scored by eye by three independent assessors. New mutant bands were identified as offspring bands present in neither parent.

($0.124 \pm 0.007$ and $0.118 \pm 0.008$, respectively, $t = 0.57$, $P > 0.05$). Parental allele sizes and allele-length frequency distributions were also determined for the four single loci tested (Fig. 3). For three loci, allele frequency distributions were indistinguishable in the control and irradiated populations; for MS32, minor but significant differences between the two groups were found. We therefore conclude that any differences in minisatellite variability between these two caucasian populations are likely to be negligible.

## Mutation rate

Spontaneous mutation rates in the control families were similar to those previously measured in caucasian populations using these five probes[9,10,14] (Table 1). In contrast, the frequency of mutant bands was elevated in the offspring of irradiated parents. By using multilocus probe 33.15, we found a statistically significant twofold increase in mutation frequency in the offspring of irradiated parents. Reprobing with four single-locus probes showed that some mutant bands detected by probe 33.15 are derived from minisatellites MS1 and MS31, whereas probes MS32 and CEB1 detect sets of bands that do not hybridize with 33.15. We therefore divided mutants scored by 33.15 into those attributable to MS1 plus MS31, and to those from other unknown loci (Table 1). An increased mutation rate in the exposed group was found for both sets of loci. A statistically significant doubling of mutation rate in Mogilev sample was also found at the highly unstable locus *D2S90* (probe CEB1). In contrast, a decrease of mutation rate in the exposed group was found at locus *D1S8* (probe MS32). However, very few mutants were found in either group, and the data do not deviate significantly from those expected for equal mutation rate in both populations (see Table 1) or for a doubled rate in the Mogilev group (Poisson approximation, $P = 0.11$). Finally, we estimated the total frequency of mutant bands in offspring analysed using all three independent probes (33.15, MS32, CEB1); the overall mutation rate was again twofold higher in the Mogilev group.

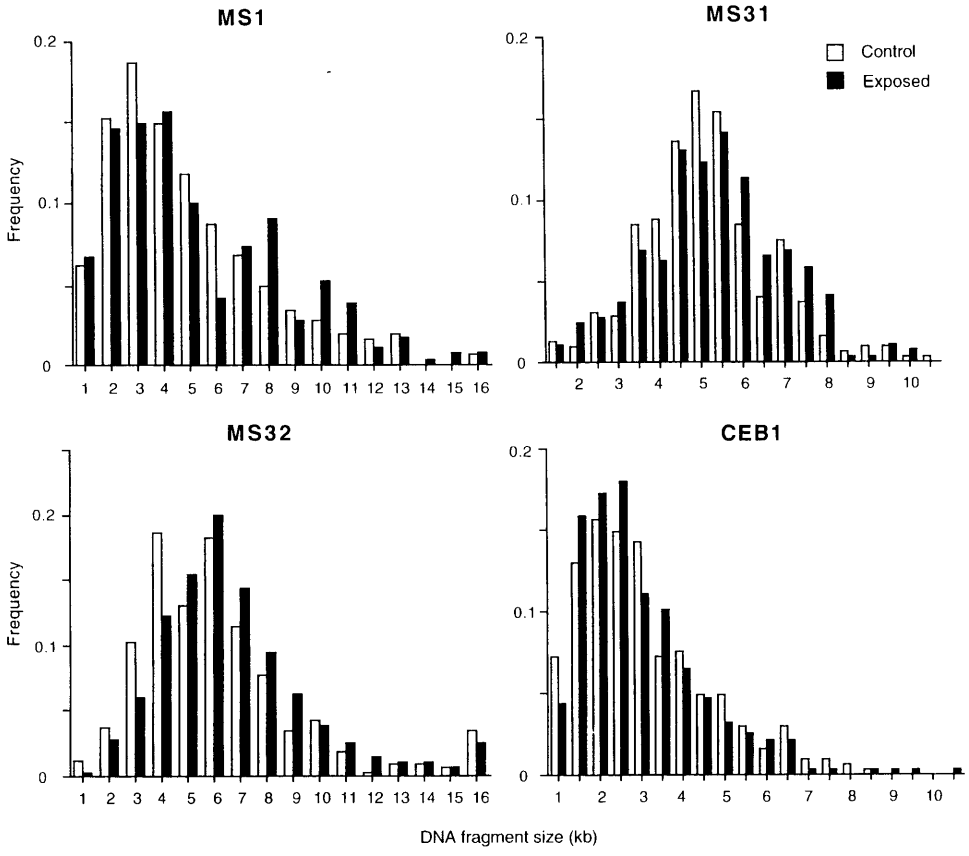


FIG. 3 Distribution of minisatellite allele sizes. DNA fragment sizes were estimated by the method of Southern[29], using a 1-kb DNA ladder (GIBCO, BRL) included on all gels, and binned into 0.5-kb (MS31, CEB1) or 1-kb (MS1, MS32) intervals. Differences between the control (white bars) and exposed (black bars) population distributions were analysed using the Kolmogorov–Smirnov two-sample test: MS1, $P > 0.05$; MS31, $P > 0.05$; MS32, $P < 0.05$; CEB1, $P > 0.05$.

TABLE 1 Mutation rates in control and exposed populations

| Probe | Locus | Control group: Total no. of bands in offspring | Control group: No. of mutations | Control group: Mutation rate per band | Exposed group: Total no. of bands in offspring | Exposed group: No. of mutations | Exposed group: Mutation rate per band | Ratio exposed to control | P* |
|---|---|---|---|---|---|---|---|---|---|
| 33.15: | | 1,903 | 19 | 0.0100 | 1,384 | 28 | 0.0202 | 2.03 | 0.0126 |
| -MS1 + MS31† | *D1S7, D7S21* | 387 | 9 (7 + 2)‡ | 0.0233 | 281 | 11 (7 + 4)‡ | 0.0392 | 1.68 | 0.1794 |
| -other bands | | 1,516 | 10 | 0.0066 | 1,103 | 17 | 0.0154 | 2.34 | 0.0288 |
| MS32† | *D1S8* | 164 | 3 | 0.0183 | 150 | 1 | 0.0067 | 0.36 | 0.5449 |
| CEB1 | *D2S90* | 164 | 11 | 0.0671 | 150 | 20 | 0.1333 | 1.99 | 0.0446 |
| 33.15 + MS32 + CEB1§ | | 1,491 | 23 | 0.0154 | 1,615 | 49 | 0.0303 | 1.97 | 0.0041 |

* Probability using Fisher's exact test of independence (two-tailed).

† All MS31 and MS32 mutants, including four showing a single repeat unit change, were verified by MVR–PCR analysis of progenitor and mutant alleles[17,18].

‡ Number of mutants scored by MS1 and MS31 are shown in brackets.

§ Data presented for the families studied by all three probes.

Blood samples from the control group were collected in the United Kingdom, and it is possible that the increased mutation rate in the Mogilev group might reflect intrinsic differences in minisatellite instability between these two caucasian populations. Indeed, profound allelic differences *in cis* in mutation rate at MS32 have already been found particularly among Africans, where they influence allelic variability[15] yet have little effect on population mutation rate as assayed here. However, increased mutation rate was seen with three groups of independent minisatellites (MS1 + MS31, other bands scored by probe 33.15, and CEB1). Further, very similar increases in mutation rate (1.7–2.3-fold) were found in the exposed group for each of these minisatellite systems, indicating that increased mutation rate cannot be attributed to a single locus that has accumulated unusually unstable alleles in the Mogilev population. We therefore conclude that the difference in mutation rate found between the two groups of families is probably caused by environmental, rather than intrinsic genetic, factors. Environmental mutagens might include industrial or agricultural pollutants as well as post-Chernobyl radioactive contamination.

## Surface contamination and mutation rate

Although the exact radiation dose received by each person in the Mogilev sample is not known, the level of surface contamination by $^{137}$Cs provides a reasonable indicator of collective doses. A significant correlation between the total radioactive fallout after the Chernobyl accident and the level of $^{137}$Cs contamination was found for most regions of Belarus[16]. Families were therefore divided according to median $^{137}$Cs surface contamination (Fig. 4*a*) into those inhabiting less contaminated (surface contamination $< 6.8\,\mathrm{Ci\,km^{-2}}$) and more contaminated ($> 6.8\,\mathrm{Ci\,km^{-2}}$) areas. The total mutation rate (probes 33.15 + CEB1 + MS32) in more-contaminated areas was 1.5 times higher than in less-contaminated areas (0.0390 versus 0.0259 per offspring band, $P = 0.0413$, Fisher's exact test of independence, one-tailed), and both were higher than in the unexposed population (0.0154 per offspring band, $P < 0.05$ for both comparisons; Fig. 4*b*). The frequency of observed mutations was greater in the more highly exposed families both for 33.15 and for CEB1 + MS32 (data not shown). This correlation of mutation rate within the exposed group with surface contamination levels is consistent with the possibility that the increased frequency of minisatellite mutations found in the exposed group is a direct consequence of irradiation following the Chernobyl accident. It is possible, however, that other non-radioactive contaminants from Chernobyl, such as heavy metals, could be responsible for the observed, apparently dose-dependent increase in mutation rate.

## Mutational spectrum

At the four single loci tested, 55 mutants were found, with most mutations at CEB1. The parental origin and germline length change were defined for each mutant band. The ratio between male and female germline mutation rate is similar in both groups (18 paternal versus 5 maternal mutations in the control group, and 28 paternal versus 4 maternal in the exposed group; $\chi^2 = 0.78$, d.f. = 1, $P > 0.05$). The incidence of mutations involving gain or loss of repeat units is similar in both groups (14 gains versus 9 losses in the control group, and 17 gains versus 15 losses in the offspring of irradiated parents; $\chi^2 = 0.32$, d.f. = 1, $P > 0.05$). Most mutation events involve the gain or loss of only a few repeat units, and the size distributions of mutations are not distinguishable between the two groups (Fig. 5*a*). The sizes of progenitor alleles are also similar in both groups (Fig. 5*b*). Mutants at MS31 and MS32 (all paternal) in the control and irradiated families were further characterized by minisatellite variant repeat mapping by polymerase chain reaction (MVR–PCR)[17,18] to determine the order of variant repeat units along progenitor and mutant alleles (data not shown). Most mutants in both groups showed features expected from previous analyses of mutants identified in pedigrees and by single-sperm analysis[18], namely mutational polarity with changes in repeat copy number restricted to the unstable end of the tandem repeat array, and with evidence in some cases for interallelic transfer of repeats during mutation (data not shown). We therefore conclude that there is no obvious difference in mutation process between the control and exposed groups.

If enhanced mutation is the result of radiation exposure, it appears highly unlikely that minisatellites themselves are the

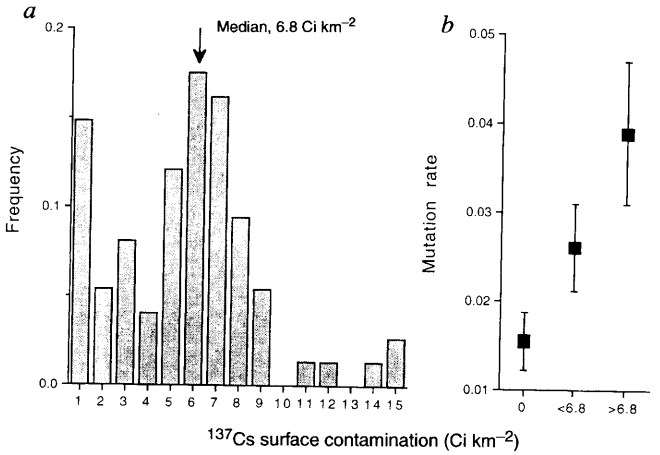


FIG. 4 $^{137}$Cs surface contamination and mutation rate. *a*, Distribution of $^{137}$Cs surface contamination in the place of residence of each Mogilev family analysed (data taken from ref. 30). *b*, Frequency of minisatellite mutation (±s.e.m., probes 33.15, MS32 and CEB1) in offspring grouped according to surface contamination (0, control population).

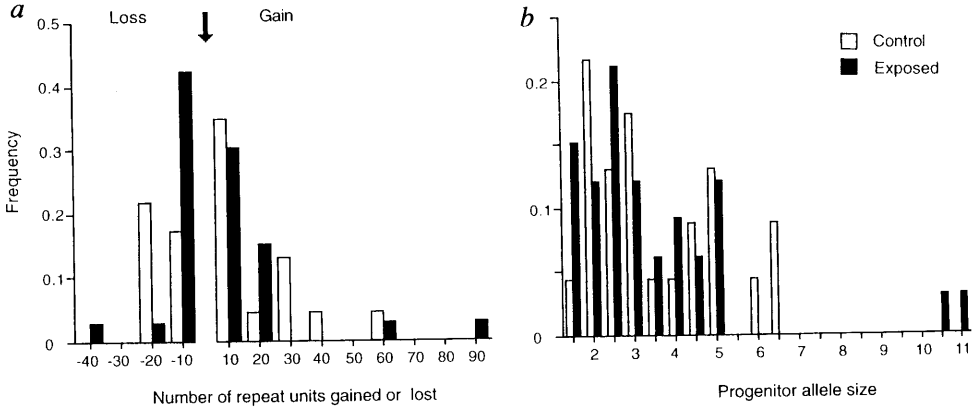


FIG. 5 Characteristics of minisatellite mutants detected by four single-locus probes. *a*, Distribution of fragment size changes (Kolmogorov–Smirnov test, $P > 0.05$). *b*, Distribution of progenitor allele sizes for mutants (Kolmogorov–Smirnov test, $P > 0.05$). The progenitor allele was assumed to be the parental allele closer in size to the mutant allele[9]; progenitors were verified for MS31 and MS32 mutants by MVR–PCR[17,18].

direct targets of irradiation[12]. The human haploid genome contains about $3 \times 10^9$ base pairs, and the average minisatellite scored by probe 33.15 has about 5,000 base pairs. The increase in mutation rate apparently caused by radiation was found to be about 0.01 per offspring band (probe 33.15; Table 1). If minisatellite mutations are initiated by double-strand breaks (DSBs)[18], then this increase would require 6,000 extra DSBs per haploid genome, assuming that minisatellite loci are random targets; no more than 70 DSBs are induced per cell per 1 Gy of irradiation[19]. This discrepancy is even greater for CEB1, which shows an increase in paternal mutation rate in the Mogilev group of 0.13 per sperm. It therefore seems that the increase in mutation rate is not caused by targeted events (DNA damage induced directly at minisatellites), but rather results form non-targeted effects caused by radiation elsewhere in the genome. One possibility is that radiation induces some system in the germline that in turn stimulates minisatellite instability, for example by interacting with a rate-limiting step in the complex gene conversion-like pathway known to be involved in mutation at human minisatellites[18]. It remains to be seen whether this system alters the mutation process itself, as seen for somatic mutations in two human protein-coding loci, which show a marked change in structural basis between spontaneous and radiation-induced mutation[20,21].

## Discussion

We have used five minisatellite probes to estimate the mutation rate after the Chernobyl accident and found a doubling in mutation frequency among offspring of irradiated parents that may be a direct consequence of radiation exposure. Data collected in Hiroshima and Nagasaki during the past 40 years from the children of atomic bomb survivors, using eight different indicators, do not provide evidence of any statistically significant differences between exposed and control families[22,23]. We therefore believe that the present study provides the first experimental evidence that germline mutation rates in humans can be increased by ionizing radiation. These data have been obtained by a new approach using hypervariable loci with spontaneous mutation rates at least 1,000 times higher than in most protein-coding loci. Evidence for germline-mutation induction was obtained from a small sample (only 552 individuals from both the control and exposed groups), which is substantially lower than the sample size needed to detect the same increase in mutation rate by standard genetic techniques[24].

The dose–response curve for radiation-induced minisatellite mutation remains unknown. Estimates of radiation dose in rural populations of the Mogilev district suggest an average thyroid exposure by $^{131}I$ of about 0.185 Gy per person[2]. In contrast, the individual radiation dose for external and internal chronic exposure to $^{137}Cs$ was estimated to be less than 5 mSv per year[2], a value far below that predicted from mouse data[11–13,25] and current estimates of the doubling dose for humans[23]. It therefore seems either that the observed increase in minisatellite mutation rate, if resulting from radiation, was caused by the initial acute $^{131}I$ exposure, or that doses of chronic irradiation by $^{137}Cs$ have been substantially underestimated. Alternatively, it is possible that low doses of chronic irradiation are more effective in mutation induction than higher doses of acute irradiation[26,27]. Additional population surveys are needed to test whether ionizing radiation does induce minisatellite mutation, and to investigate the relative impact of acute and chronic radiation exposure on germline instability.

*Note added in proof*: A similar study of minisatellite mutation in a relatively small number of families of atomic-bomb survivors from Hiroshima and Nagasaki has failed to show evidence for mutation induction following acute exposure[31]. □

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