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Further evidence for elevated human minisatellite mutation rate in Belarus eight years after the Chernobyl accident

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Abstract

Analysis of germline mutation rate at human minisatellites among children born in areas of the Mogilev district of Belarus heavily polluted after the Chernobyl accident has been extended, both by recruiting more families from the affected region and by using five additional minisatellite probes, including multi-locus probe 33.6 and four hypervariable single-locus probes. These additional data confirmed a twofold higher mutation rate in exposed families compared with non-irradiated families from the United Kingdom. An elevated rate was seen at all three independent sets of minisatellites (detected separately by multi-locus probes 33.15, 33.6 and six single-locus probes), indicating a generalised increase in minisatellite germline mutation rate in the Belarus families. Within the Belarus cohort, mutation rate was significantly greater in families with higher parental radiation dose estimated for chronic external and internal exposure to caesium-137, consistent with radiation induction of germline mutation. The spectra of mutation seen in the unexposed and exposed families were indistinguishable, suggesting that increased mutation observed over multiple loci arises indirectly by some mechanism that enhances spontaneous minisatellite mutation. © 1997 Elsevier Science B.V.

Keywords: Minisatellite mutation; Radiation; Chernobyl accident; Human populations

1. Introduction

Minisatellite loci provide the only currently available system for the efficient monitoring of germline mutation in humans, and are capable in principle of detecting induced mutations in relatively small population samples. Previous studies have shown that acute doses of ionising γ-radiation cause a significant increase in minisatellite mutation rate in experimentally-irradiated mice [1–3]. We have recently extended this analysis to human populations inhabiting rural areas of the Mogilev district of Belarus heavily contaminated with radionuclides after the
Chernobyl accident [4]. The frequency of mutation was assayed both by DNA fingerprinting using one multi-locus probe and by single-locus analysis using four probes. Both monitoring systems revealed mutation rates two times higher in the offspring of exposed parents than in control unexposed families from the United Kingdom. In this pilot study, evidence for correlation between the level of caesium-137 surface contamination and minisatellite mutation rate was also found within exposed families, suggesting that increased mutation rate might be attributed to ionising radiation. If correct, these data provide the first experimental evidence for radiation-induction of human germline mutation. However, the apparently elevated mutation rate in the exposed families could also be explained by sampling error, by intrinsic genetic and environmental differences between the exposed and control families in our study, or by post-Chernobyl contamination by non-radioactive pollutants. To determine whether the Belarus families do indeed show an unusually high mutation rate, we have extended this analysis to more families from the affected area and have introduced five additional minisatellite systems selected for their high spontaneous mutation rate. In addition, dose-response analysis within the Belarus families has been refined and re-assessed by using estimates of individual parental radiation doses from chronic exposure to environmental caesium-137 as an index of overall exposure to radionuclides following the Chernobyl disaster.

2. Materials and methods

DNA was purified from frozen blood using phenol chloroform extraction. Four-μg samples of DNA were digested to completion with AluI, electrophoresed through a 35-cm (for probes 33.15 and 33.6) or 40-cm (for single-locus probes) long 0.8% 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 hybridised to 32P-labelled probes as described elsewhere [5]. DNA fingerprints were produced using two multi-locus minisatellite probes 33.6 and 33.15 [6], and eight hypervariable single-locus minisatellite probes MS1, MS31, MS32, CEB1, CEB15, CEB25, CEB36 (loci D1S7, D7S21, D1S8, D2S90, D1S172, D10S180, D10S473) and B6.7 (located in chromosome 20q13), chosen for their relatively high spontaneous mutation rate ([7–10] and G. Vergnaud, unpublished).

All autoradiographs were scored over the well-resolved regions (3.5–22 kb for multilocus probes and 1–22 kb for single-locus probes). DNA fragment sizes were estimated by the method of Southern [11], using a 1-kb DNA ladder (Gibco BRL) included on all gels.

3. Results

3.1. Population groups

Blood samples were collected from 48 additional families (father, mother, child) which, together with the 79 families studied previously [4], gave in total 127 families inhabiting the heavily polluted rural areas of the Mogilev district of Belarus (Bychovskii region, 19 families; Cherikovskii region, 40 families; Krasnopol’skii region, 38 families; Slavgorodskii region, 23 families; Krichevskii region, 4 families and Mogilevskii region, 3 families; Fig. 1). The whole cohort is composed of children born between February and September 1994 for whom both parents were

Fig. 1. Map showing the study area.
continuously resident in the Mogilev district from the time of the Chernobyl accident. For all parents, the local level of surface contamination by $^{137}$Cs exceeded 1 Ci/km$^2$. As in our previous study, the control sample consisted of 120 non-irradiated Caucasian families from the United Kingdom [4].

The control and exposed families consisted of 63 and 60 boys and 57 and 67 girls, respectively ($\chi^2 = 0.68$, df = 1, $p = 0.4096$). The maternal ages for the control group and exposed group were indistinguishable (24.4 ± 0.7 years, SD = 5.4, and 24.5 ± 0.5 years, SD = 5.6, respectively; $t = 0.14$, $p = 0.8872$). Bartlett's test for homogeneity of group variances; $\chi^2 = 0.14$, df = 1, $p = 0.7074$). The paternal ages in the control and exposed families were also similar (26.5 ± 0.9 years, SD = 7.2, and 27.7 ± 0.6 years, SD = 6.0, respectively; $t = 0.90$, $p = 0.3686$; $\chi^2 = 2.58$, df = 1, $p = 0.1082$).

3.2. Population variability

Using multi-locus probes 33.6 and 33.15, 41.86 ± 0.66 DNA fingerprint bands were scored per offspring in the control group and 40.93 ± 0.52 bands in the offspring of irradiated parents ($t = 1.13$, $p = 0.2607$, Fig. 2a). The frequency of band-sharing between parents in the control and Mogilev samples was indistinguishable (0.1380 ± 0.0065 and 0.1283 ± 0.0056, $t = 1.14$, $p = 0.2548$, Fig. 2b) and close to that previously measured by these two probes in a large set of caucasian families [8]. Both results are also similar to those reported in our previous study of 79 families from the exposed group scored by multi-locus probe 33.15 [4].

To analyse DNA fingerprint variation in more detail, band-sharing between offspring and parents was also investigated. Among 4395 DNA fingerprint bands scored in offspring from the control families 4361 (99.2%) matched with parental bands (46.4% paternal, 45.3% maternal and 7.6% shared with both parents). The same ratios were found for 5198 offspring bands in the exposed group (5123 or 98.6% parental bands, 46.2%, 45.5% and 6.9% maternal and shared bands, respectively). The number of paternal and maternal bands scored per child ranged from 8 to 32 (Fig. 2c,d).

Fig. 2. Population variability in control and exposed populations scored by multi-locus probes 33.15 and 33.6. a: distribution of number of bands in offspring (Kolmogorov–Smirnov two-sample test, $p = 0.1430$). b: distribution of band-sharing between parents (determined as $2n_{PA}/(n_p + n_M)$, where $n_{PA}$, $n_p$ and $n_M$ are number of shared bands between parents, total number of paternal and maternal bands, respectively; Kolmogorov–Smirnov two-sample test, $p = 0.6476$). c: distribution of number of paternal bands scored in child (Kolmogorov–Smirnov two-sample test, $p = 0.8749$). d: distribution of number of maternal bands scored in child (Kolmogorov–Smirnov two-sample test, $p = 0.8691$).
Table 1
Parental allele sizes in control and exposed populations

<table>
<thead>
<tr>
<th>Probe</th>
<th>Locus</th>
<th>Control mean ± SE</th>
<th>Exposed mean ± SE</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>B6.7</td>
<td>20q13</td>
<td>3920 ± 111 1908 4041 ± 116 2242 0.8097</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CEB1</td>
<td>D2S90</td>
<td>3354 ± 85 1723 3359 ± 77 1654 0.6932</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CEB15</td>
<td>D15S772</td>
<td>3940 ± 124 2326 3989 ± 119 2455 0.9979</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CEB25</td>
<td>D15S180</td>
<td>5139 ± 171 3342 5232 ± 163 3486 0.6333</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CEB36</td>
<td>D10S473</td>
<td>3548 ± 105 1882 3519 ± 107 2051 0.5676</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MS1</td>
<td>D15S7</td>
<td>5379 ± 168 2999 5815 ± 162 3480 0.1461</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MS31</td>
<td>D7S21</td>
<td>5418 ± 85 1521 5550 ± 70 1536 0.1131</td>
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<td></td>
</tr>
<tr>
<td>MS32</td>
<td>D15S8</td>
<td>6773 ± 162 3354 7415 ± 163 3581 0.0010</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Probability that the parental allele sizes are distributed identically in two populations (Kolmogorov-Smirnov two-sample test).

Parental allele sizes and allele-length frequency distributions were also determined for the eight single loci tested (Table 1). For seven loci, including the four new loci, allele frequency distributions were indistinguishable between the control and irradiated families. As reported before [4], a significant difference between the two groups was found for minisatellite MS32; the mean allele length in the Mogilev sample exceeded that of the control group, though the variances of the two distributions were indistinguishable (Table 1). Overall, however, there are no substantial differences in minisatellite variability between the two populations.

3.3. Mutation rate

To determine the extent to which multi-locus and single-locus minisatellite probes detect overlapping sets of bands, the same blots were hybridised with all ten probes. In most cases minisatellite probes detected sets of completely independent bands, whereas some bands detected by probe 33.15 are derived from minisatellites MS1 and MS31. We therefore present three different estimates of mutation rate based on independent sets of minisatellites (Table 2).

For each of the 10 probes, the spontaneous mutation rate determined from the control families (Table 2) was similar to that previously measured in Caucausian populations ([7–10] and G. Vergnaud, unpublished). In contrast, the frequency of mutant bands was elevated in the offspring of irradiated parents.

Using multi-locus probe 33.15, a statistically significant twofold increase in mutation frequency was found in the offspring of irradiated parents. A very similar increase in mutation rate was also found by profiling with multi-locus probe 33.6.

Another estimate of mutation rate was based on scoring mutants at six independent single-locus minisatelites (Table 2). For this set of loci the mutation rate in the exposed families was 1.7-times higher than in the control group. Overall, five of the six

Table 2
Mutation rates in control and exposed populations

<table>
<thead>
<tr>
<th>Probe</th>
<th>Control group</th>
<th>Exposed group</th>
<th>Ratio exposed to control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of families</td>
<td>No. of offspring bands</td>
<td>No. of mutations</td>
</tr>
<tr>
<td>33.15</td>
<td>105</td>
<td>1903</td>
<td>19 (7 + 2)*</td>
</tr>
<tr>
<td>33.6</td>
<td>105</td>
<td>2492</td>
<td>15</td>
</tr>
<tr>
<td>B6.7</td>
<td>109</td>
<td>218</td>
<td>5</td>
</tr>
<tr>
<td>CEB1</td>
<td>109</td>
<td>218</td>
<td>16</td>
</tr>
<tr>
<td>CEB15</td>
<td>109</td>
<td>218</td>
<td>4</td>
</tr>
<tr>
<td>CEB25</td>
<td>109</td>
<td>218</td>
<td>3</td>
</tr>
<tr>
<td>CEB36</td>
<td>109</td>
<td>218</td>
<td>2</td>
</tr>
<tr>
<td>MS32</td>
<td>109</td>
<td>218</td>
<td>3</td>
</tr>
<tr>
<td>Total (6 loci)</td>
<td>109</td>
<td>1308</td>
<td>33</td>
</tr>
<tr>
<td>Grand total</td>
<td>94</td>
<td>5099</td>
<td>56</td>
</tr>
</tbody>
</table>

* Probability using Fisher’s exact test of independence (two-tailed).
* Number of mutants scored by MS1 and MS31 are shown in brackets.
loci, including the four new loci, showed an increase in mutation rate in the exposed group (Wilcoxon signed ranks test, \( Z = 1.99 \), \( p = 0.0464 \)).

We also estimated the total frequency of mutant bands in offspring using all eight independent probes (33.15, 33.6, B6.7, CEB1, CEB15, CEB25, CEB36 and MS32). A highly statistically significant 1.9-fold increase in the mutation rate (\( p = 2.53 \times 10^{-5} \)) was found in the exposed group. The mutation rate for the set of 48 new families did not differ significantly from the estimate for the previous set of 79 families (0.0189 and 0.0216 per offspring band, respectively; \( p = 0.1101 \), Fisher’s exact test), and both were higher than in the unexposed population (\( p = 0.0036 \) and \( p = 0.00004 \), respectively). Furthermore, for five new independent minisatellite systems included in this study (33.6, B6.7, CEB15, CEB25 and CEB36) the mutation rate was found to be twice as high in the exposed families as in the control group (mean rate of 0.0160 and 0.0077 per offspring band, respectively; \( p = 0.0012 \)).

To determine whether the increased frequency of mutant bands in the exposed group might reflect non-paternity, the probabilities of paternal exclusion were estimated for all families. Consider a family in which \( n \) non-maternal bands are detected in a child with \( k \) bands present in the alleged father and with \( m \) unassignable bands present in neither parent and scored here as mutant bands (\( n = k + m \)). The distribution of the total number of mutant bands in offspring closely follows the Poisson distribution predicted from the mean number of mutant bands per offspring in the control and exposed families (Fig. 3a,b). If correct, then the probability of finding \( m \) mutant bands in an offspring is \( Q_m(x = m) = (\lambda^m/m!)e^{-\lambda} \), where \( \lambda \) is the mean number of mutant bands per offspring. For non-paternity, assuming full statistical independence of fingerprint bands [8], the probability that a man unrelated to the child would contain \( k \) out of \( n \) non-maternal offspring bands is approximated by the binomial distribution \( Q_m(x = k) = (n!/(m!k!))s^k(1 - s)^{n-k} \), where \( s \) is the band-sharing between individuals. Using the most conservative estimate of the mean number of mutant bands per offspring in the control group (\( \lambda = 56/94 \approx 0.5 \), Table 2), the number of fingerprint bands

![Figure 3](image-url)

**Fig. 3.** Occurrence of mutation and paternity testing. a, b: observed and expected (predicted from the Poisson distribution) distribution of number of mutant bands in offspring in control (a) and exposed (b) populations (data for eight independent probes 33.15, 33.6, B6.7, CEB1, 15, 25, 36 and MS32; Kolmogorov–Smirnov one-sample test, \( p = 0.4483 \) and \( p = 0.1334 \) for the control and exposed group, respectively). c, d: the likelihood ratio of paternity to non-paternity in families with different number of mutant bands in offspring, estimated for the frequency of band-sharing 0.15 (c) and 0.30 (d).
which show the same mobility in each alleged father and child (Fig. 2c) and the mean band-sharing between parents (Fig. 2b), we estimated the likelihood ratio of paternity to non-paternity in each family as $Q_1/Q_2$ (Fig. 3c). For all families, including those with non-parental mutant bands, this ratio ranged from $10^3$ to $10^{23}$. Even at an unrealistically high frequency of band-sharing ($s = 0.3$, Fig. 3d), which may reflect some unknown instances of consanguineous marriages in the Belarus population, the likelihood ratios still remain very high. The same results were also obtained for the likelihood ratio of maternity to non-maternity (data not shown). These data therefore provide strong evidence for the correct parentage of all children analysed, including those with the occurrence of multiple mutations.

3.4. Chronic radiation dosage and mutation rate

Our previous study showed that mutation rate in the affected area was correlated with the level of $^{137}$Cs soil contamination which was used to provide an approximate indicator of relative parental exposure [4]. While this correlation provided important evidence for radiation induction of germinal mutation, the absolute radiation dose received by each person in the Mogilev sample is more appropriate. The individual radiation doses for chronic exposure were therefore estimated for 126 families in the exposed group, using published data on the annual external and internal exposure to $^{137}$Cs in soil, milk and vegetables in different localities of the Mogilev district [12] and family histories after the Chernobyl accident. The parental radiation dose for each family was taken as the mean value of paternal and maternal doses obtained from the time of the Chernobyl accident to the conception of the child.

The mean dose over all families for parental external and internal chronic exposure to $^{137}$Cs was $27.6 \pm 3.3$ mSv ($SD = 36.8$ mSv, median = 19.7 mSv). Families within the exposed group were therefore divided according to the median of the distribution (Fig. 4a) into less exposed (< 20 mSv) and more exposed (> 20 mSv) families, and the total mutation rate (probes 33.15, 33.6, B6.7, CEB1, CEB15, CEB25, CEB36 and MS32) was estimated for both sets of families. The mutation rate in the more exposed families was 1.35-times higher than in the less exposed ($p = 0.0154$, Table 3), and both were higher than in the unexposed population ($p = 3.03 \times 10^{-6}$ and 0.0030, respectively; Fig. 4b). Moreover, a marginally significant positive correlation between the parental dose and the number of mutant bands in offspring was found within the exposed group (Kendall's $\tau = 0.1111$, $p = 0.0672$), consistent with a steady increase of mutation rate with radiation dose. This correlation of mutation rate within the exposed group with parental radiation dose suggests that the observed increase in mutation rate may be directly caused by ionising radiation. More detailed dose–response analysis by further subdivision of the Belarus families according to dose was not possible, given the limited number of families and lack of statistical power arising primarily.

**Fig. 4.** Parental radiation dose for external and internal chronic exposure to $^{137}$Cs and mutation rate. a: distribution of parental dose. b: frequency of minisatellite mutation ($\pm SE$, data pooled for probes 33.15, 33.6, B6.7, CEB1, CEB15, CEB25, CEB36 and MS32) in offspring grouped according to parental dose.
from the clustering of parental doses around the median (Fig. 4a).

3.5. Mutational spectrum

The parental origin and germline length change were defined for 119 de novo mutations found at the eight single loci tested (42 and 77 mutants in the control and exposed families, respectively). The ratio between male and female germline mutation rate and the incidence of mutations involving gain or loss of repeat units were similar in the unexposed and exposed families (Table 4). Most mutation events in-

| Table 3 |
|-----------------|----------------|----------------|-----------------|-----------------|----------------|
| Probe           | Parental dose < 20mSv | Parental dose > 20mSv | Ratio more exposed to less |
|                 | No. of families | No. of offspring bands | No. of mutations | Mutation rate per band | No. of families | No. of offspring bands | No. of mutations | Mutation rate per band |
| 33.15           | 63            | 1108             | 19 (3 + 1)       | 0.0172            | 63            | 1095             | 25 (5 + 4)       | 0.0228            | 1.33 | 0.0773 |
| 33.6            | 63            | 1474             | 14               | 0.0095            | 63            | 1483             | 17               | 0.0115            | 1.21 | 0.1251 |
| B6.7            | 62            | 124              | 3                | 0.0242            | 62            | 124              | 8                | 0.0645            | 2.67 | –     |
| CEB13           | 62            | 124              | 12               | 0.0968            | 62            | 124              | 16               | 0.1290            | 1.33 | –     |
| CEB15           | 62            | 124              | 3                | 0.0242            | 62            | 124              | 4                | 0.0322            | 1.33 | –     |
| CEB25           | 62            | 124              | 5                | 0.0403            | 62            | 124              | 7                | 0.0564            | 1.40 | –     |
| CEB36           | 62            | 124              | 2                | 0.0161            | 62            | 124              | 2                | 0.0161            | 1.00 | –     |
| MS2             | 62            | 124              | 1                | 0.0081            | 62            | 124              | 1                | 0.0081            | 1.00 | –     |
| Total (6 loci)  | 62            | 744              | 26               | 0.0349            | 62            | 744              | 38               | 0.0511            | 1.46 | 0.0317 |
| Grand total     | 62            | 3284             | 58               | 0.0177            | 62            | 3282             | 78               | 0.0238            | 1.35 | 0.0154 |

a Probability using Fisher’s exact test of independence (one-tailed).

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

| Table 4 |
|-----------------|----------------|-----------------|-----------------|-----------------|----------------|
| Probe           | Parental origin of mutants | Type of mutants | Control | Exposed |
|                 | Paternal | Maternal | Paternal | Maternal | Gains | Losses | Gains | Losses |
| B6.7            | 3        | 2        | 7        | 4        | 2     | 3      | 6     | 4      |
| CEB13           | 15       | 1        | 28       | 0        | 11    | 5      | 16    | 12     |
| CEB15           | 4        | 0        | 4        | 3        | 2     | 2      | 4     | 3      |
| CEB25           | 3        | 0        | 8        | 4        | 2     | 1      | 4     | 8      |
| CEB36           | 1        | 1        | 3        | 1        | 1     | 1      | 2     | 2      |
| MS1             | 3        | 4        | 3        | 5        | 4     | 3      | 5     | 3      |
| MS31            | 2        | 0        | 5        | 0        | 0     | 2      | 1     | 4      |
| MS32            | 3        | 0        | 1        | 1        | 3     | 0      | 1     | 1      |
| Total           | 34       | 8        | 59       | 18       | 25    | 17     | 39    | 37     |

χ² = 0.30, df = 1, p = 0.5850

χ² = 0.73, df = 1, p = 0.3915

* For one mutant in the exposed group scored by B6.7 the progenitor allele is unknown.
volved the gain or loss of only a few repeat units and the distributions of length changes were indistinguishable between the two groups (Fig. 5a). The sizes of progenitor alleles were also similar in the exposed and unexposed families (Fig. 5b). These data, as well as results of our previous study [4], show that despite an increased mutation rate in the Belarus families, there is no obvious difference in mutational spectrum between the two groups.

4. Discussion

The data presented here provide strong support for all of our previous conclusions [4], namely: differences in minisatellite variability between the control and Mogilev samples are negligible; minisatellite mutation rate is unusually high in the exposed population; mutation rate in the exposed group is elevated over multiple minisatellite loci; mutation rate within the Mogilev families is correlated with the parental exposure to radiation; there are no obvious differences in the mutation spectrum between exposed and control families.

The main result of the present study is that, despite increasing the number of families in the exposed group by more than 50%, and profiling of all families with an additional five minisatellites, the difference in the mutation rate between the two groups remains unchanged from that previously reported [4]. As already discussed [4], the increased mutation rate found in the exposed group might be explained either by genetic or environmental factors. The new evidence refutes the hypothesis that the increased mutation rate in the Mogilev group might reflect the existence in this population of minisatellite loci with unusually high spontaneous mutation rates. Scoring of mutations by three independent sets of minisatellite loci detected separately by 33.15, 33.6 and six different single-locus probes revealed an increased mutation rate in the exposed group for most of the minisatellite systems, indicating that increased mutability cannot be attributed to a single locus which has accumulated unusually unstable alleles in the Mogilev population. Thus, the most probable cause of increased mutation rate in the exposed group is the influence of environmental mutagens.

The Chernobyl accident resulted in a large release of radioactive materials throughout the Northern Hemisphere, particularly within the European part of the former Soviet Union [16,17]. In the first 2 months after the accident, high dose-rate irradiation of humans occurred through external and internal exposure to iodine-131 with a half-life of 8 days [17,18]. Following $^{131}$I decay, exposure to more stable isotopes, mainly $^{137}$Cs, became the main source of radiation risk for people in contaminated regions. Exposure to $^{131}$I has already caused a considerable increase of thyroid carcinoma in children from Belarus and Ukraine [19,20]. The total radiation exposure has also resulted in an increase in somatic mutations (chromosomal aberrations and gene muta-
A Response to the Magnox Sites RSA Consultation

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Annex 1: Radiation and Health

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As noted before, the release of $^{131}$I resulted in a considerable thyroid exposure for the residents of Belarus with an average dose per thyroid gland of 0.185 Gy per person from the Mogilev district [18]. Judging from the distribution of thyroid doses for the age-matched cohort from the population of Belarus [18], it appears that the radiation thyroid doses for some of the exposed parents could exceed 1 Gy. Again, a strong positive correlation between the surface contamination of $^{131}$I and $^{137}$Cs ($r = 0.73–0.92$) has been found within a 500-km zone around the Chernobyl power station [23]. Thus, the correlation between parental chronic exposure and minisatellite mutation rate found within the exposed group might also reflect components of parental exposure to other radionuclides, including both iodine-131 and strontium-90.

However, work on the Chernobyl power station aimed to prevent the release of radioactive materials from the damaged reactor also resulted in the discharge of non-radioactive heavy metals such as lead over radioactive-polluted territories [24]. Several in vitro and in vivo studies indicate that some heavy metals...
metals, particularly lead, are capable of producing genotoxic effects [25]. Furthermore, it has recently been suggested that pollutants such as polycyclic aromatic compounds and, probably, heavy metals may be responsible for increased minisatellite germline mutation rate in urban birds [26]. However, the post-Chernobyl level of environmental contamination by heavy metals remains unknown, and the relationship between elevated mutation rate and heavy metal contamination cannot therefore be tested. As noted above, a significant correlation between minisatellite mutation rate and parental exposure to 137Cs was found within the Mogilev district. If the post-Chernobyl discharge of heavy metals caused the elevated mutation rate, then a very high positive correlation between the environmental pollution by these metals and by caesium-137 should occur in the Mogilev district. This appears to be unlikely because the Mogilev radioactive fallout pattern was established on April 28–29, 1986, as a result of rainfall [27], whereas the main work on the Chernobyl power station started later and would therefore most likely result in a completely different pattern of environmental contamination by heavy metals.

To the best of our knowledge, these data and our previous results [4] provide the first experimental evidence for radiation induction of human germline mutation. In contrast, data collected during the past 40 years on children of the Hiroshima and Nagasaki atomic bomb survivors using standard mutation monitoring systems have not provided evidence for any statistically significant differences in mutation rate between exposed and control families [28]. The shortcoming of standard monitoring systems is the necessity to use very large numbers of individuals (often more than 100,000) to detect increases in mutation rate or indeed any de novo mutation at all [29]. In contrast, our data have been obtained using hypervariable loci with a very high mutation rate, allowing spontaneous instability and mutation induction to be monitored in much smaller numbers of families. However, a similar study of minisatellites has failed to show evidence for radiation-induced mutation in offspring of atomic bomb survivors from Hiroshima and Nagasaki with acute gonadal radiation exposure higher than for the post-Chernobyl population [30,31]. This apparent discrepancy could reflect the totally different nature of exposure in Japan following the atomic bomb explosions to that following the Chernobyl accident. The atomic bomb explosions in the Japanese cities resulted in a considerable acute exposure of residents to γ- and neutron radiation, whereas chronic exposure to 131I, 137Cs and other radionuclides was the main source of radiation hazard after the Chernobyl disaster. In addition, most of the children of atomic bomb survivors studied by minisatellite profiling were born more than 10 years after the single acute parental irradiation, which means that at least some radiation-induced DNA alterations could have been repaired over this period of time. In contrast, the population of the post-Chernobyl affected areas has been constantly irradiated following the accident. Finally, the Japanese data are derived from 50 exposed families in which mainly one parent received A-bomb radiation, whereas our current estimates of the mutation rate in the Mogilev district were obtained by analysis of 127 families with both parents exposed to chronic irradiation after the Chernobyl accident profiled by more unstable minisatellites.

Our data do not provide any evidence for significant differences in the mutational spectrum between control and exposed families, indicating that minisatellite loci themselves are not the direct targets of irradiation. Furthermore, our previous estimates suggest that if minisatellite mutations found in the exposed group are initiated by direct targeted events, then this would require an unrealistically high number of extra double-strand breaks (DSBs) per genome [4]. It therefore seems that the increase in mutation rate is not caused by DNA damage induced directly at minisatellites, but rather results from radiation-induced damage elsewhere in genome. Thus, our data may be better explained by recent analysis of mutant alleles at minisatellite MS32, which has revealed that the mutational process involves complex conversion-like events and may include gap repair as an important step in the process [14]. If so, then DNA damage induced by radiation elsewhere in the genome might result in an increase of DSB repair activity which could, in turn, influence minisatellite mutation rate. Recent data establish that mutation at human minisatellites MS32 and MS205 occurs predominantly in the germline and most likely at meiosis [14,32,33]. This raises the issue of the timing of induction of minisatellite mutation by radiation. Most
of the mutants in both groups are paternal (Table 4). Human spermatogenesis extends over 74 days with a relatively short interval for meiosis [34]. Theoretically, this would make minisatellites mainly vulnerable to brief acute radiation exposure during meiosis. For chronic radiation to influence instability, it is possible that stem cells could accumulate DNA damage over a considerable period of radiation exposure after the Chernobyl accident which later may alter the stability of minisatellites. Further work is needed to clarify in detail the structural basis of induced minisatellite mutation.

The current data provide useful information on the potential use of human minisatellites for radiation monitoring. A strong positive correlation between mutation rate in the unexposed and exposed families was found for eight independent minisatellite loci (B6.7, CEB1, 15, 25, 36, MS1, MS31 and MS32; \( r = 0.9137, \ p < 0.05 \)). It therefore appears that the increase in mutation rate found in the exposed group is attributable to all minisatellites in proportion to their spontaneous mutation rate. If correct, then minisatellites with the highest spontaneous mutation rate, such as CEB1, will be most efficient at monitoring induced mutation. Nevertheless, additional surveys are clearly needed to evaluate in detail the radiation induction of minisatellite mutation in mammals and to determine whether minisatellite mutation can be used as a bio-monitoring system for radiation-induced germine mutation. Major questions remain concerning dose–response parameters for mutation induction, the effects of different types of irradiation, and the structural basis of induced mutation. Concerning dose–response analysis, it should be stressed that all three studies on the induction of minisatellite mutation in mice have failed to produce any reliable relationships between radiation dose and mutation frequency [1–3]. Here we have obtained evidence for a positive correlation between mutation rate and individual radiation dose for external and internal chronic exposure to \(^{137}\text{Cs}\) within the Mogilev group. However, as mentioned before, the important component of gonadal radiation exposure to \(^{31}\text{P}, ^{90}\text{Sr}\) and other isotopes for these families remains unknown. Therefore, the current data are inadequate for any detailed evaluation of dose–response pattern for radiation-induced minisatellite mutation in humans. Future work should directly address dose–response analysis in mice and humans.

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References

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