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Monitoring of radiation-induced germline mutation in humans

Yuri E. Dubrova

Department of Genetics, University of Leicester, United Kingdom

Summary

Estimating the genetic hazards of radiation and other mutagens in humans depends on extrapolation from experimental systems. Recent data have shown that minisatellite loci provide a useful and sensitive experimental approach for monitoring radiation-induced mutation in humans. This review describes the progress made in validating this approach and presents the results of recent publications on the analysis of minisatellite mutation rates in the irradiated families.

Key words: germline mutation; minisatellite loci; ionising radiation; Chernobyl; Semipalatinsk

Introduction

The detection of radiation-induced changes in germline mutation rate in human populations still remains extremely difficult [1]. In previous studies on the children of atomic bomb survivors and radiotherapy patients [2, 3], germline mutation rates were indirectly estimated by analysing the incidence of mortality and the occurrence of genetic diseases among the offspring of irradiated parents. It should be stressed that the sensitivity of these approaches for monitoring radiation-induced mutations remains highly uncertain [4]. That is why germline mutation induction in mice is still used as the main source of experimental data for evaluating the genetic risk of human exposure to ionising radiation [1, 5]. Such an extrapolation has not been experimentally validated and is consequently not proven. It therefore remains increasingly clear that the reliable estimates of the genetic risk of human exposure to ionising radiation can only be derived from the relevant experimental data on germline mutation induction in human populations which, in turn, requires new experimental approaches for monitoring radiation-induced germline mutation.

We have recently developed a novel system for monitoring radiation-induced mutation in mammalian germline, which is based on a set of hypervariable tandem repeat DNA loci [6–8]. The results of our studies have shown that changes in mutation rate in laboratory mice exposed to ionising radiation or chemical mutagens can be detected in very small population samples and at doses far lower than had been possible using standard approaches for monitoring germline mutation in mice [8–10]. These data raised the possibility that tandem repeat DNA loci can also provide a useful tool for monitoring radiation-induced mutation in humans. Here I present the summary of recent publications analysing mutation induction at human tandem repeat minisatellite loci.

Tandem repeat minisatellite loci

Tandem repeat loci in the human genome are represented by relatively short microsatellites (<500 bp) with repeat size of 1–4 bp and minisatellites (0.5–20 kb) with repeat size of 10–60 bp [11, 12]. Minisatellites are the most unstable loci in human genome. They consist of repeats, showing considerable sequence variation along the array [13–19]. Mutation at these loci is almost completely restricted to the germline, with very rare and simple mutational events occurring in the somatic cells [14, 16, 17, 19–21]. Germline mutation at human minisatellites is attributed to complex gene-conversion like events altering repeat unit copy number. Previous studies have shown very high spontaneous germline mutation rates for some minisatellite loci, ranging from 0.5 up to

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13% per gamete [18, 22], which potentially make these loci useful markers for monitoring germline mutation in humans.

To date, using the pedigree approach, the frequency of minisatellite mutation has been analysed in families exposed to several different types of ionising radiation [7, 23–28]. In these studies, DNA samples were purified from blood collected from both parents and their offspring. DNA samples were digested to completion with enzymes that do not cut within the minisatellite repeats, separated on agarose gel and minisatellite loci were detected by hybridisation with minisatellite probes. Mutants were identified as novel DNA fragments present in the offspring, and mutation rates in the germline of non-exposed and irradiated parents were estimated by dividing the total number of mutations scored in the offspring by the total number of minisatellite bands.

Minisatellite mutation rates after the Chernobyl accident

The Chernobyl accident resulted in a largest accidental release of radioactive materials [29]. Many regions within the European part of the former USSR were heavily contaminated by radioactive fall-out. The highest doses of exposure to ionising radiation following the Chernobyl accident were received by the workers involved in the accident, either during the emergency period or during the clean-up phase, and by the inhabitants of heavily contaminated areas of Belarus, Ukraine and Russia [29]. To date, three groups have studied the effects of post-Chernobyl exposure on minisatellite mutation rates in human families [7, 24–27].

In the first study, the frequency of minisatellite mutation was estimated in human families inhabiting the heavily polluted rural areas of the Mogilev district of Belarus following the Chernobyl accident and compared it to mutation frequency in a control data set from the United Kingdom [7, 24]. The exposed cohort consisted of children for whom both parents were continuously resident in the Mogilev district from the time of the Chernobyl accident.

Minisatellite germline mutations were scored using two multi-locus minisatellite probes 33.6 and 33.15 and eight hypervariable single-locus minisatellite probes B6.7, CEB1, CEB15, CEB25, CEB36, MS1, MS31 and MS32, chosen for their high spontaneous mutation rate [17, 18, 22, 30]. Scoring of mutations by three independent sets of minisatellite loci detected separately by 33.15, 33.6 and eight 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 (Figure 1A). We therefore concluded that the most probable cause of increased mutation rate in the exposed group is the influence of environmental mutagens.

However the results of this pilot study, where mutation rates in the exposed group were compared with those in the non-exposed Caucasian families of different ethnical origin, did not provide enough evidence for radiation-induction of germline mutation. To verify the results of our previous study and to determine whether mutation rate in the germline of other post-Chernobyl cohorts is also elevated, we have extended this analysis to the group of exposed families inhabiting rural areas of the Kiev and Zhitomir regions of Ukraine [25].

In contrast to our previous study, the control group was composed of children born in the Kiev and Zhitomir regions of Ukraine and conceived before the Chernobyl accident. The exposed group contained children conceived after the Chernobyl accident. The control and exposed groups from Ukraine were matched by many confounding factors, including ethnicity and occupation, which provided a much better control population than used for the previous study of the Be-

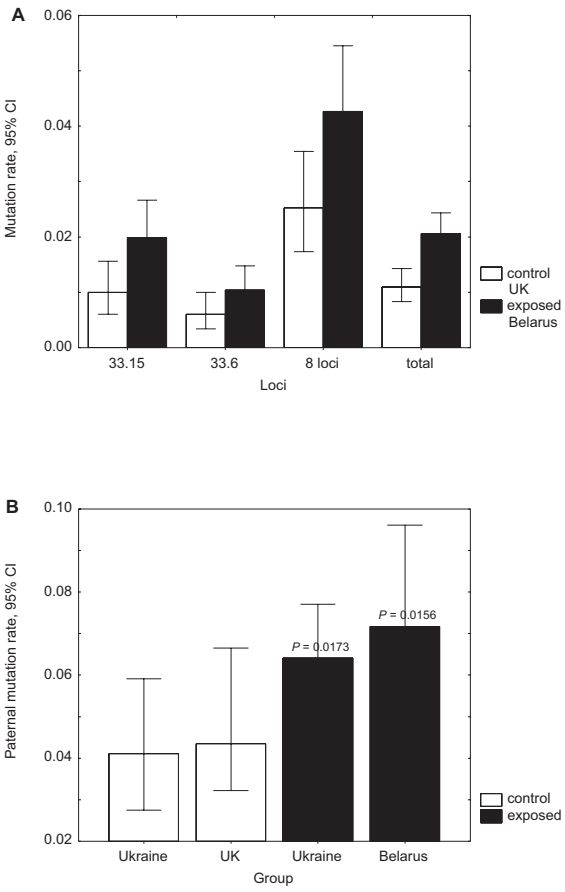

Figure 1

Germline minisatellite mutation rates in the families exposed to the post-Chernobyl radioactive contamination.
A. Mutation rates in the Belarus families.
B. Paternal mutation rates in the Ukrainian and Belarus families. Data from [7, 24, 25]. The probabilities of difference from the control group (Fisher's exact test, two-tailed) are shown.

larus families. Importantly, the Ukrainian families did not significantly differ by parental age and occupation from the Belarus families.

Minisatellite germline mutations were scored using the same eight hypervariable minisatellite probes, which were previously used for the analysis of the Belarus families. A statistically significant 1.6-fold increase in the paternal mutation rate was found in the exposed families from Ukraine, whereas maternal mutation rate in this cohort was not elevated. The same results were also obtained for the exposed group from Belarus (Figure 1B). For this set of loci, paternal mutation rate in the exposed families from Belarus was significantly higher than in the control group from Ukraine. Our data therefore show that the elevated mutation rate in the germline of exposed fathers is most likely radiation-induced and raise the issue of human exposure after the Chernobyl accident.

The Chernobyl accident resulted in a release of a wide spectrum of short-lived isotopes. During the first days after the Chernobyl accident, their contribution to the absorbed dose in air was extremely high [31, 32], which, given the short half-life of unstable radionuclides, can no longer be evaluated by means of physical dosimetry. However, retrospective dosimetry provides an alternative approach for the evaluation of the doses for the populations of contaminated territories. The analysis of stable and unstable chromosome aberrations has provided an estimate of the mean doses of exposure for the residents of heavily contaminated areas of Ukraine and Belarus ranging between 0.2 and 0.4 Gy [33–35]. Similar data were obtained by measuring the thermoluminescence of the bricks collected within contaminated Gomel-Bryansk area [36]. These estimates are markedly higher than those obtained by physical dosimetry [37] and reflect the initial external and internal exposure to the short-lived radionuclides. Assuming that the doubling dose for germline mutation in humans is 1 Gy [1, 5], an exposure to 0.2–0.4 Gy can lead to a 1.6-fold increase in minisatellite mutation rate as found in the families from Ukraine and Belarus. These data therefore provide strong evidence that the elevated minisatellite mutation rates in the Ukrainian and Belarus families can be attributed to post-Chernobyl radioactive exposure.

Another two studies of germline minisatellite mutation rate were carried out on the offspring of Chernobyl clean-up workers [26, 27]. The doses for this group of workers are thought to be extremely heterogeneous, and most of the participants involved in the decontamination work around the Chernobyl nuclear power plant, sarcophagus construction and other clean-up operations received doses less than 0.25 Gy [38]. Importantly, the group of Chernobyl clean-up workers was exposed to repeated small daily doses of ionising radiation. Previous studies in male mice have clearly shown that the yield of germline mutations after such an acute external fractionated exposure was less than when the same dose was given in a single exposure [39]. Given that the maximum reported dose to the Chernobyl clean-up workers of 0.25 Gy is below any known estimates of the doubling dose for mice [5, 8] and assuming the dose-fractionation effects, the expected increase in mutation rate in this group may be too small to be realistically detected.

Minisatellite mutation rates in the families affected by nuclear bomb tests

To verify the Chernobyl results we have recently extended this analysis to another cohort of irradiated families living in the vicinity of the Semipalatinsk nuclear test site in Kazakhstan [28]. The Semipalatinsk nuclear test site has been the site for 470 nuclear tests performed by the Soviet Union during the period 1949–89, including atmospheric and surface explosions (1949–63), and underground tests (1963–89) [40]. The surrounding population was mainly exposed to the fresh radioactive fallout from four surface explosions conducted between 1949 and 1956, and currently the radioactive contamination outside the test zone is low [40, 41].

In this study we have analysed families inhabiting the rural areas of the Semipalatinsk district of Kazakhstan around the Semipalatinsk nuclear test site. These areas are characterised by the highest effective doses of exposure to ionising radiation from the nuclear weapons tests conducted at the Semipalatinsk nuclear test site [41]. The control group was composed of non-irradiated families from the rural area of the former Taldy Kurgan district of Kazakhstan, which was not contaminated by nuclear tests. Both groups were matched by the year of birth, parental age, occupation and smoking. As in our previous studies on the Chernobyl families, minisatellite mutation rates in these families were established by using the same eight minisatellite probes.

A statistically significant 1.7-fold increase in mutation rate was found in the exposed families from the Semipalatinsk district. In contrast to the control group, mutation rates did not show homogeneity across the cohorts of exposed parents grouped according to year of birth (Figure 2). Thus, for all parents born between 1926–60, the germline mutation rate remained stable and significantly exceeded that of the control group, whereas in the later cohorts it showed a negative correlation with the parental year of birth. This heterogeneity in the mutation rate could be ex-

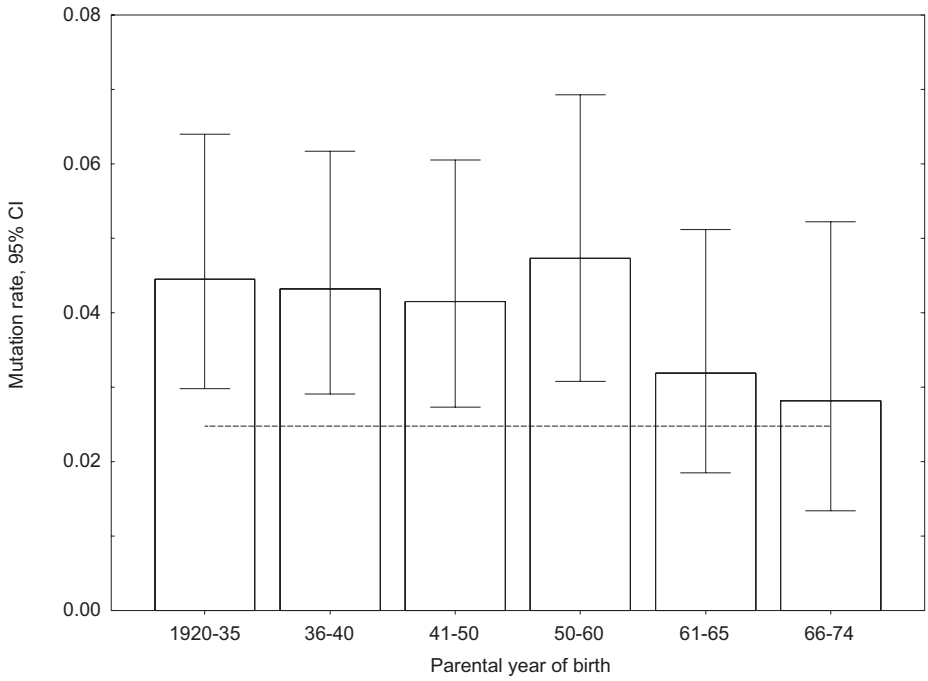

Figure 2

Germline minisatellite mutation rate in the exposed parents from the Semipalatinsk district grouped according to year of birth. The dashed line represents mutation rate in the control group. Data from [28].

plained by the differential exposure to ionising radiation within the Semipalatinsk group. It is well established that up to 85% of the collective effective dose to this population was attributed to just four surface explosions carried out in 1949, 1951, 1953 and 1956 [40, 41]. All parents born between 1926–60 were therefore directly exposed to relatively high levels of ionising radiation immediately after these tests and to the environmental contamination afterwards, whilst those born later were likely to receive considerably smaller doses as a result of the following underground explosions, which did not cause high-level contamination of the surrounding territories. If correct, then the negative correlation between mutation rate and the parental year of birth reflects the decreased exposure following the decay of radioisotopes in the late 1950s and after the cessation of surface and atmospheric nuclear tests, thus suggesting that an elevated mutation rate in the affected families is indeed radiation-induced. Most importantly, this correlation provides the first experimental evidence for change in human germline mutation rate with declining exposure to ionising radiation and therefore shows that the Moscow treaty banning nuclear weapon tests in the atmosphere (August, 1963) has been effective in reducing genetic risk to the affected population.

Conclusions

To date our studies have established that minisatellite loci are sensitive reporters of radiation-induced mutation in the human germline, and have provided the first experimental evidence for radiation-induced increases in human germline mutation rate. However, the results of other studies on children of the Hiroshima and Nagasaki atomic bomb survivors [23], the offspring of Chernobyl clean-up workers [26, 27] and cancer patients exposed to radiotherapy [42] have failed to find any statistically significant differences in minisatellite mutation rate between exposed and control groups. It should be stressed that all data on minisatellite mutation induction in humans were derived from relatively small numbers of people exposed to different sources of ionising radiation. This variety of sources currently prevents any reliable comparisons between the results of these pilot studies. Additional surveys are therefore clearly needed to evaluate in detail mutation induction at human minisatellite loci.

Correspondence:
Dr Yuri E. Dubrova
Department of Genetics
University of Leicester
University Road
Leicester LE1 7RH, UK
E-Mail: yed2@le.ac.uk

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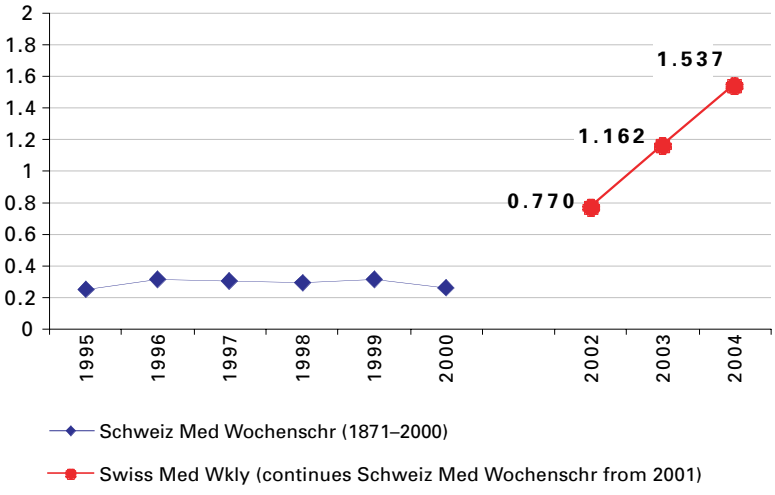

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