Trans-generational effects of radiation exposure from the Chernobyl Nuclear Plant Accident: A review of studies using mutation markers of repeat DNA sequences

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Introduction

The assessment of the possible radiation effects on the children who were conceived after the Chernobyl accident is needed in order to estimate the long-term health effects of the accident, including the effects on future generations. If germline cells were even exposed at the stage before conception, the health effect of children could be "trans-generational". Additionally, the health effects of children due to exposure to radiation might also be caused by exposure at successive stages after conception including the embryonic and fetal stages.

We already have definite evidence of the trans-generational effect of radiation from experimental animals. However, it has not yet been demonstrated in humans. The Radiation Effect Research Foundation (RERF) reported, "Extensive studies of the children of survivors of the atomic bombings in Hiroshima and Nagasaki have thus far yielded no statistically significant increases in genetic effects compared to control populations." They have done an on-going epidemiological study collecting mortality from all causes, and have performed molecular studies of DNA, as well as conducting a clinical health study investigating the frequency and prevalence of adult-onset life-style-related diseases.[1)] Under such circumstances, we have only the genetic risk assessment of radiation exposure, which was estimated from the data of spontaneous mutation rates in human Mendelian diseases and chronic multi-factorial diseases, and the data of the radiation-induced germline mutations of experimental mice.[2)] Studying the possible trans-generational effects induced by radiation exposure from the Chernobyl nuclear power plant accident is important in order to discuss the reassessment of the genetic risk of radiation. People who were exposed to the radiation from the Chernobyl accident form another large population, other than the atomic survivors of Hiroshima and Nagasaki where the radiation exposure was quite different.

This review focuses on the studies using mutation markers of repeat DNA sequences which is relevant to the radiation exposures that resulted from the Chernobyl accident.

Repeat DNA sequences as markers for detection of "trans-generational effects"

The choice of mutation markers is one of the critical issues in the studies of the trans-generational effects of mutagens including radiation. The markers should be sensitive and be able to effectively detect mutations, and the system should be capable of obtaining quantitative results of the dose-effect. Spontaneous mutation rates of protein-coding genes are at most in the order of 10^{-5}-10^{-6} per generation. Trying to detect trans-generational effects using markers of these coding genes requires very large number of samples. Therefore, it is not realistic to expect "significant results" using these markers in a population which is actually available for study.

There are considerable numbers of sites with repeat DNA sequences, multiple repeats of a certain sequence of bases (a repeat unit), in the genomes of various living things including human beings. The different numbers of repeat units at a site in the genomes of individuals of the same species may lead to

polymorphic phenotypes. Most of the repeat sequences are in non-coding regions. These are sites, which can spontaneously mutate in high frequencies per generation. These sites can be used as markers to detect the genetic effects of mutagens in a limited-size population. In studies of the trans-generational effects of radiation, the results of research using markers of hypervariable minisatellites and microsatellites have been reported in human studies, as well as the results of experiments using markers of Expanded Simple Tandem Repeat (ESTR) in mice have also been reported.

Hypervariable minisatellites in humans are composed of varying numbers of GC-rich core repeats of 10 - 100 base pairs (bp). The core repeats within a stretch of minisatellite are related but are not identical. The total number of base pairs in a minisatellite is up to a few tens of thousands. There are more than ten thousand minisatellite loci in the human genome and most of them are situated close to telomeres. Some minisatellites exhibit spontaneous instability in germ cells with mutation frequencies as high as 10^{-1} - 10^{-2} per gamete, however such high mutation rates can not be seen in somatic cell lines. Recombination and gene conversion at minisatellite loci during meiosis result in complex changes in the loci, while recombination and slippage during mitosis lead to minisatellite instability of somatic cells. These different mechanisms for mutations are considered to be one of the reasons for such high instability found only in the germline.[3)]

Microsatellites in humans are composed of repeat units of less than 10 bp and the total length of a microsatellite is usually less than 100 bp. There are more than a hundred thousand microsatellites in the human genome and they are dispersed widely throughout the genome. The spontaneous mutation rate varies in each locus and is 10^{-2} - 10^{-4} per generation. It is considered that replication slippage leads to microsatellite mutations. A higher mutation rate was reported in paternal loci compared to maternal loci. Larger numbers of mitotic germline cell divisions in spermatogenesis than in oogenesis might be one possible reason for the difference in mutation rates between paternal and maternal germlines.[4)]

ESTRs of mice were at first cloned for their sequence homology to human minisatellites and they were called “minisatellites of mice” at first. However, their repeat units are only 5 - 6 bp and there is no variation of repeat units within a locus. Because of these structural differences between human and mouse minisatellites, these repeat sequences in mice were renamed ESTRs.[5)] Replication slippage is thought to cause the instability of ESTR although other mechanisms are possible.[3)]

Human experimental somatic cells showed a dose-dependent increase in mutation rates at minisatellite and microsatellite loci, when they were exposed to radiation.[6)] As for human germ cells, no increase was detected in the minisatellite mutation rate of sperm from volunteer patients who were given radiotherapy for seminoma.[7)] There is no similar research examining the radiation effects on the human microsatellite mutations of germline cells using samples from volunteer patients.

Induction of germline mutations of ESTRs in male mice by acute gamma irradiation was reported independently by two research groups.[8, 9)] The observed mutation frequency induced by irradiation was two orders of magnitude higher than the frequency which could be expected from the assumption that the DNA double strand break at the ESTR locus would cause the mutation. This suggests that ESTR loci might not be a direct target of radiation, but rather radiation might induce an indirect mechanism of some sort that would increase genetic instability in a cell.[3)] Further research is needed to clarify this mechanism. As for the most sensitive stage during spermatogenesis, different results were reported from these two research groups. Niwa’s group reported that the late spermatid stage of post-meiosis was most sensitive to radiation with regard to ESTR mutations.[9)] This result was compatible with the findings previously obtained from the experiments of the specific recessive loci in mice germline. On the contrary, Dubrova’s

group reported that the pre-meiotic stages of spermatogenesis were sensitive.[10)]

Research on children living in contaminated areas of Chernobyl

In 1996, ten years after the Chernobyl accident, Dubrova et al. reported the results of studies on residents in the Chernobyl contaminated areas in Belarus, which showed that "the frequency of mutation was found to be twice as high in the exposed families as in the control group."[11)] This research drew the attention of the scientific community as it was the first "evidence" that radiation could increase germline mutations in human beings. In 1997, they published another report which included additional subjects and minisatellite probes to reinforce the results of the first report.[12)] However, these reports were criticized because they used samples of Caucasian families in the UK as the non-exposed control group which were of different ethnic origins than the Belarusian exposed families.[13)] Therefore, they conducted further research on the residents in the contaminated areas in Ukraine. In this study, they compared the children who were conceived before and after the accident, so that they could investigate the exposed subjects and unexposed controls from the same ethnic background. They analyzed the paternal and maternal mutation rates using eight single-locus hypervariable minisatellite probes and reported that "a statistically significant 1.6-fold increase in mutation rate was found in the germline of exposed fathers, whereas the maternal germline mutation rate in the exposed families was not elevated."[14)]

Dubrova et al. also investigated the populations around the Semipalatinsk nuclear test site in Kazakhstan, as well as exposed populations in rural villages along the Techa River, which were contaminated by radioactive releases from the Mayak plutonium separation combine. They used the same eight hypervariable minisatellite probes as mutation markers and again reported elevated minisatellite mutation rates in the exposed populations.[15, 16)]

However, the results reported from Dubrova's group were contrary to the findings of other groups which had also investigated human populations using minisatellite markers. Significant elevations of the minisatellite mutation rates were not detected in the reports of the study using sperm samples of volunteer patients whose testicles had been irradiated by radiotherapy for treatment of seminoma,[7)] in the research of families of atomic bomb survivors in Hiroshima and Nagasaki,[17-19)] or in the study of the families of Chernobyl clean-up workers who were called "liquidators".[20-22)] The reasons for this discrepancy are as yet unknown. Only the research group of Dubrova investigated the exposed residents in the contaminated areas. The residents in the contaminated areas were chronically exposed to low-dose-rates of radiation, while atomic bomb survivors and the Chernobyl liquidators were mainly exposed to high-dose-rates of radiation for certain limited periods. These differences in type of radiation exposure might be the reason for the discrepancy. In the case of the residents in the contaminated areas, exposure might not be limited to a specific stage of germline maturation, but could be a successive irradiation throughout the whole process of germline maturation and it could even include the stages after conception.

One of the backgrounds of these studies using minisatellite markers to detect the radiation effects on human germline was from previous studies using experimental mice. Mutation rates of ESTRs (these loci were formerly called "minisatellites of mice") of the experimental mice were significantly increased by acute gamma irradiation.[8, 9)] As mentioned above, because of the structural differences between human minisatellites and "minisatellites of mice", the latter were renamed ESTRs and different mechanisms are considered to cause mutations at these loci in mice.[5)] Radiation may possibly cause changes that increase the genetic instability in cells, which could lead to mutations of ESTRs in mice. Considering possible different mechanisms causing mutations, the experimental model of ESTR in mice cannot be directly applied to solve questions related to human minisatellite mutations. Further research for the mechanism of

human minisatellite mutations is necessary before minisatellites will be used more widely as markers to detect and monitor the trans-generational effects of radiation in human populations.

Studies of the children of Chernobyl liquidators

As for the children of Chernobyl liquidators, results of studies using minisatellites[20-22] or microsatellites[22, 23] as mutation markers have been reported. There is no study as yet which has demonstrated "significant effects" with regard to germline mutations in liquidators.

The exposure period was much shorter in liquidators as compared to residents in contaminated areas; in most cases it was a period of up to several months. The radiation dose of individual liquidators varied widely depending on location and occupation. (However, the information on individual radiation dose was quite limited and usually external exposure alone was recorded.) In the studies of liquidators, only the paternal exposure was analyzed because the liquidators were largely men. Children conceived before the accident or residents in the non-contaminated areas of the same country were used as the non-exposed control groups.

Kiuru et al. studied the offspring of Estonian liquidators using the mutation markers of eight hypervariable minisatellite probes which Dubrova had also used in their study of residents. The minisatellite mutation rate was nonsignificantly increased among children born after the accident (Odds ratio: 1.33, 95% CI: 0.80-2.20). The rate was higher in the children born to fathers with recorded doses of 0.2 Sv or above (Odds ratio: 3.00, 95% CI: 0.97-9.30) relative to their siblings born before the accident, although this increase was not statistically significant.[21]

Livshits et al. reported that children of Chernobyl workers in Ukraine did not show elevated rates of mutations in minisatellite alleles. They used the five hypervariable minisatellites as mutation markers which had also been used by Dubrova. Their control group was made up of residents in non-contaminated areas in Ukraine. Their results showed as well that the mutation rate of the residents in the contaminated areas in Belarus (4.94%) was higher than the mutation rate of their controls (3.72%) that were residents of non-contaminated areas in Ukraine. This was reasonable as it suggested an elevated mutation rate in the residents in the contaminated areas as compared to those in non-contaminated areas; both groups were of similar ethnic background.[20]

The study using microsatellite mutations as markers to investigate the trans-generational effects of radiation in humans presents a more recent challenge than studies using minisatellites. The protocol for experiments using microsatellite markers is relatively simple: amplifying a target site including a microsatellite locus with a pair of primers, one of which is fluorescence-labeled, and analyzing the differences in length of PCR (Polymerase Chain Reaction) products using an automatic genetic analyzer. One of the advantages of this method is higher sensitivity which can detect even a difference in the length of a single base in microsatellite loci. However in the experiments using minisatellite markers, the sensitivity is less than this, with bands of different lengths of DNA fragments being analyzed in Southern Blotting.

However, as is mentioned above, the spontaneous mutation rate of microsatellite loci is one order less than that of minisatellite loci. Furitsu and Ryo et al. used as many as 72 microsatellite loci as mutation markers when they investigated a population of a limited number of liquidator families in Belarus. These 72 microsatellites loci were selected from the loci which had been reported in the literature as having higher spontaneous mutation rates. Analyzing the mutation of 40 Y-linked mincrosatellite loci, a higher mutation rate (2.9×10^{-3}) was detected in the families of liquidators in Belarus as compared to the

mutation rate of the unexposed control group (2.1 x 10^{-3}), although the increase was not statistically significant.[23)]

There is not yet sufficient basic experimental data on the sensitivity to radiation with regard to microsatellite mutations in mammalian germlines. Further research on experimental mice and human populations with different radiation doses as well as various types of exposure is necessary.

Studies on living things other than human beings in Chernobyl-contaminated areas

There have been studies of mutations in DNA repeat sequences in barn swallows and wheat plants in the Chernobyl-contaminated areas. In the study of barn swallows, an increased germline mutation rate was observed at the two hypervariable microsatellite loci in populations breeding in Chernobyl- contaminated areas in Ukraine. European populations of barn swallows migrate across the Sahara desert, wintering in southern Africa. Ellegren et al. captured adult and nestling barn swallows (as “families”) in Ukraine and Italy in 1991 and 1996. The mutation rate in the two microsatellite loci was 7.2% (number of meiosis investigated: 138) for the “families” in the contaminated areas. This rate was significantly higher than the mutation rates of control groups in the non-contaminated areas in Ukraine (3.8%, meiosis: 131) and Italy (2.0%, meiosis: 2,002).[24)]

In the study of wheat plants, two genetically identified populations derived from the same homogeneous parental line were compared. One population was grown in heavily contaminated plots of land near the Chernobyl nuclear power plant, the other was sown in a clean (<1 Ci/km^2) control area 30 km away with comparable agrochemical characteristics. They analyzed the 13 microsatellite loci in the offspring plants and estimated that the spontaneous mutation rate was 1.03 x 10^{-3} per locus (95% IC: 0.44 - 2.03 x 10^{-3}), whereas the mutation rate in the exposed group was 6.63 x 10^{-3} (95% IC: 4.28 - 9.70 x 10^{-3}).[25)] They also analyzed the complex pattern of microsatellite mutations in the germline of wheat and concluded that a simple model of replication slippage could not account for mutation events at these loci in wheat.[26)]

A study on the variation in the hypervariable portion of the mitochondrial control-region haplotypes in a population of the bank vole in northern Ukraine has been carried out, although it is not a study on the repeat sequences of nuclear DNA. Specimens of the bank vole exhibit the highest internal doses of radiocesium among small mammals inhabiting the most contaminated sites in Chernobyl. The rate of variation in the Chernobyl population was higher than in the control group living in less contaminated sites. This suggested that exposure to ionizing radiation in the contaminated environments increased the maternal mutation rate and has consequently increased mitochondrial DNA diversity.[27)] However, this increased diversity might be a function of asymmetric migration from a genetically diverse source population existing before the Chernobyl accident. The longitudinal genetic monitoring project is also on-going.[28)]

Plants and small animals usually alternate generations more rapidly than humans and they cannot intentionally avoid the environmental contamination. Therefore, it is very important to monitor the genetic changes of these living things in the contaminated areas to estimate the long-term consequences of the Chernobyl accident in the whole ecosystem.

Conclusion

In the studies of the children of Chernobyl victims so far, only Dubrova’s study showed a significant increase in the mutation rate of repeat sequences of DNA, through research done on the residents in the

contaminated areas using the markers of minisatellite loci. Other studies investigating the Chernobyl liquidators did not show any significant increase in the mutation rate even using the same hypervariable minisatellite probes as mutation markers and the same experimental method as Dubrova's group. This discrepancy might be due to the different types of exposure to radiation between residents and liquidators.

Further investigation is required to solve the question: to what extent the increase in mutations in repeat sequences would reflect in actual human health through its phenotype. Most of the repeat sequences in the human genome are in non-coding regions. However, some of them are known to cause diseases, or increase the severity of diseases, as they are in coding or modulator regions in DNA, or near them. The sensitivity to radiation in the repeat sequences, which relate to certain diseases, should be further examined and discussed concretely with respect to each site. If some radiation-induced mechanism, which leads to increased genetic instability in cells, would affect various regions in the genome other than in regions with repeat sequences, it could also influence the coding or modulator regions and induce a number of deleterious expressions of genes which might lead to the ill health of individuals.

Further research is needed on the mechanism and sensitivity for the induction of mutations through radiation exposure in DNA repeat sequences in the human germline, in each stage of germ cell maturation and also in the stages after conception.

As for the studies using microsatellite loci as mutation markers, the studies on barn swallows and wheat plants showed a significant increase in mutation rates, while studies on liquidators have so far failed to show a significant increase in mutation rates. The residents in the contaminated areas have not yet been investigated using the mutation markers of microsatellites. Basic data using microsatellite loci in experimental mice should be obtained. Further research using microsatellite markers with other human populations exposed to radiation in various ways should also be carried out.

In the studies which demonstrated significant increases of mutation rates in DNA repeat sequences, the observed mutation rate is much higher than the rate which could be expected from the assumption that the locus itself is the direct target of radiation and a double strand break at the locus can lead to mutation. This suggests that the loci might not have been directly targeted by radiation, but radiation might have induced some sort of mechanism to increase the genetic instability of a cell. The mechanisms, possibly including an epigenetic process, might be at work here and they could conceivably be common mechanisms for other biological effects of radiation, especially at low doses and through chronic exposure.

As mentioned above, there are still many unsolved problems regarding radiation effects on mutations of DNA repeat sequences. However, these results, including the significantly elevated mutation rate in mice ESTR, suggest that the genetic instability is actually increased by radiation to the germline. Therefore, it should not be ignored when we assess the risk of trans-generational effects of radiation. It is especially important in the genetic risk assessment of low dose and chronic exposure to radiation.

It is critical to carry out a long-range scientific monitoring of the trans-generational effect of the populations exposed to radiation from the Chernobyl accident. For that purpose, it is vital to establish an international research system in cooperation with the researchers in the affected countries.

Not only the environmental contamination by the Chernobyl accident but also other social and economic factors have affected the health of people in the Chernobyl areas. This complicated situation makes it more difficult to investigate the radiation health effects from the Chernobyl accident. It is not easy to study the radiation health effect separately from other risk factors. The exact radiation dose estimation is critical in assessing the health effects. However, estimating the exact individual radiation

dose from both external and internal exposures can be problematic as it depends on multiple factors such as individuals' working conditions, life styles and so on. Therefore, analyzing health effects according to doses received by individuals who may be ill is not a simple process.

Many problems are still unsolved, even though more than 20 years have passed since the Chernobyl accident. Challenges in the scientific assessment of the various radiation effects of the accident, including trans-generational effects, remain with us. However, we should not just wait for the results of further research, which could provide us with scientific information about radiation, mutations and their relation to health effects in people, without taking any action right now. We must take action, if we seriously consider the wellbeing of future generations and remedies for the Chernobyl victims as well as the problem of nuclear energy in our society.

Table 1 and 2 are the summaries of reports so far on "Trans-generational effects" of human populations using mutation markers of minisatellites and microsatellite, respectively.

Table 1. Reports on the "Trans-generational effects" of human populations using mutation markers of minisatellites

A. Chernobyl Nuclear Power Plant Accident

(1) Dubrova et al. (1996) [11]

➢ Exposed group	● Residents in the heavily contaminated rural areas of the Mogilev province in Belarus: 79 families with children born between February and September 1994; both parents had resided continuously in the contaminated areas.
➢ Non-exposed control group	● Caucasian families in the UK: 105 families; sex was matched with the offspring in the exposed group.
➢ Estimated radiation dose	● The level of Cs137 contamination was 1-15Ci/km^2. Individual radiation dose for external and internal chronic exposure to Cs137 was estimated to be less than 5mSv/year.
➢ Minisatellite probes	● A multi-locus probe: 33.15(MS1, MS31), single-locus probes: MS32, CEB1.
➢ Main results	● The overall mutation rate was 1.97-fold as high in the exposed group as it was in the control group. ● Number of mutations / Total number of bands in offspring: exposed group [49/1615], control group[23/1491] ● Mutation rate was correlated with the level of Cs 137 surface contamination. ● Mutation spectrum (ratio between male and female germline mutations, gain or loss of repeat units, size distribution) was similar in both groups.
➢ Radiation Effects	● Positive

(2) Dubrova et al. (1997)[12]

➢ Exposed group	● Residents in the heavily contaminated rural areas of the Mogilev province in Belarus: 127 families with children born between February and September 1994 (male: 60, female: 67) ; both parents had resided continuously in the contaminated areas. (They added samples to the previous study reported in 1996.)
➢ Non-exposed control group	● Caucasian families in the UK: 120 families; sex ratio of children was matched with the offspring in the exposed group (male: 53, female: 57). (They added samples to the previous study reported in 1996.)

➢ Estimated radiation dose	● The mean dose over all families for parental external and internal chronic exposure to Cs137 was 27.6±3.3mSv.
➢ Minisatellite probes	● Multi-locus probes: 33.15, 33.6, single-locus probes: MS32, CEB1, B6.7, CEB15, CEB25, CEB36.
➢ Main results	● A 1.87-fold increase in the mutation rate was found in the exposed group. ● Number of mutations / Total number of bands in offspring: exposed group [136/6616], control group [56/5099] ● Mutation rate was correlated with the estimated radiation dose of the parents. ● Mutation spectrum (ratio between male and female germline mutations, gain or loss of repeat units, size distribution) was similar in both groups.
➢ Radiation effects	● Positive

(3) Dubrova et al.(2002)[14]

➢ Exposed group	● Ukrainian families inhabiting in the contaminated areas with 240 children conceived after the Chernobyl accident and born between 1987 and 1996; number of families [171+27], 27 families with children conceived before and after the accident.
➢ Non-exposed control group	● Ukrainian families inhabiting in the contaminated areas with 98 children conceived before the Chernobyl accident and born between 1976 and 1986; number of families [54+27], 27 families with children conceived before and after the accident. Ethnic background, maternal age, parental occupations and number of smokers of parents were similar for both exposed and control groups. The paternal age in the exposed group exceeded that for the control group.
➢ Estimated radiation dose	● The level of Cs137 contamination was >2Ci/km^2 and estimated dose from chromosome aberrations was 0.2-0.4Gy.
➢ Minisatellite probes	● Single-locus probes: B6.7, CEB1, CEB15, CEB25, CEB36, MS1, MS31, MS32.
➢ Main results	● A 1.56-fold increase in the paternal mutation rate was found in the exposed families. ● Number of mutations / Total number of bands in offspring: ∗ Paternal mutation: exposed group [112/178], control group [29/706] ∗ Maternal mutation: exposed group [25/1727], control group [10/701]
➢ Radiation effects	● Paternal: positive, maternal:negative

(4) Livshits et al. (2001)[20]

➢ Exposed group	● Liquidators families from Kiev in Ukraine; fathers had worked on the Chernobyl site during the period 1986-1987; 161 families with 183 children; 88 children were conceived while the fathers were working on the Chernobyl site or within 2 months after the fathers stopped working on the site and 95 children were conceived at least 4 months after the fathers had stopped working on the site.
➢ Non-exposed control group	● Families from southern Ukraine (non-contaminated areas); 163 families with 163 children.
➢ Estimated radiation dose	● 0.048 - 1.2 Sv. Data on individual dose estimate were provided for only 28% of liquidators.

➢ Minisatellite probes	● Single-locus probes: B6.7, CEB1, CEB15, CEB25, CEB36, CEB42, CEB72.
➢ Main results	● Only paternal mutations were analyzed and mutation rates per locus in the children of liquidators did not differ significantly from the control group. ● Number of mutations / Total number of bands in offspring (analyzing the result of paternal mutations using the seven single-locus probes): exposed group [53/1154], control group [51/1036]. ● Analyzing the result of mutation rates using the five single-locus probes, which were common to Dubrova's group: * The mutation rate of the families in the contaminated areas of Belarus (4.94%, Dubrova's data) was 1.33-fold higher than the mutation rate of the families from non-contaminated areas in Ukraine (3.72%, Livshits's data). * The mutation rate of the families from non-contaminated areas in Ukraine was 1.35-fold higher than the mutation rate of Caucasian families in the UK (2.75%, Dubrova's data).
➢ Radiation effects	● Negative

(5) Kiuru et al. (2003)[21]

➢ Exposed group	● Estonian liquidator families; father had been worked at Chernobyl over a median period of 3 months between 1986 and 1991; 147 families with 155 children born within 33 months after fathers were exposed at Chernobyl (post-Chernobyl children).
➢ Non-exposed control group	● The same 147 liquidator families with 148 children born before the Chernobyl accident (pre-Chernobyl children).
➢ Estimated dose of exposed group	● Mean radiation dose was 0.11±0.06 Sv with less than 1.4% of the cohort receiving more than 0.25 Sv.
➢ Minisatellite probes	● Single-locus probes: B6.7, CEB1, CEB15, CEB25, CEB36, MS1, MS31, MS32.
➢ Main results	● Mutation rate was higher in post-Chernobyl children than in pre-Chernobyl children but the elevation was not statistically significant (Odds ratio: 1.33, 95% CI: 0.80-2.20). ● Number of mutations / Total number of bands in offspring: exposed group [52/1238], control group [42/1182] ● Post-Chernobyl children of fathers with radiation dose of ≧0.2Sv had a threefold higher mutation rate (Odds ratio: 3.00, 95% CI: 0.97-9.30).
➢ Radiation effects	● Negative (There was some indication in the liquidator exposed group ≧0.2Sv.)

(6) Slebos et al. (2004)[22]

➢ Exposed group	● Liquidator families from Kiev and Chernigov in Ukraine; father had served in the cleanup operation of the Chernobyl accident between 1986 and 1990; 75 families with 75 children conceived after the start of the cleanup operation (within them, 39 families with 39 children did not have children before the start of the cleanup operation).
➢ Non-exposed control group	● 41 liquidators families from Kiev and Chernigov in Ukraine, from the same registry as the exposed group, with 41 children conceived before the start of the cleanup operation (within them, 5 families with 5 children did not have children after the start of the cleanup operation). Both "before" (control) and "after" (exposed) children in

	a family were conceived by the same mother.
➢ Estimated radiation dose	● Median accumulated dose was 152mSv.
➢ Minisatellite probes	● Multi-locus minisatellite probes: 33.5, 33.15
➢ Main results	● No statistically significant difference in mutation rates between exposed and control groups was observed. (Small sample size limited statistical power.) ● Number of mutations / Total number of bands in offspring: exposed group [9/82], control group [9/472]
➢ Radiation effects	● Negative (Small sample size limited statistical power.)

B. Nuclear tests

(7) Dubrova et al. (2002)[15]

➢ Exposed group	● Three-generations of families inhabiting the rural areas of the Beskaragai district of Kazakhstan around the Semipalatinsk nuclear test site: those in the first generation [P0] were born between 1920 and 1950, those in the second generation [F1] were born between 1951 and 1974 and those in the third generation [F2] were children of F1. 40 families with 135 of F1 and 97 of F2.
➢ Non-exposed control group	● Three-generations of families from the geographically similar non-contaminated rural area of Kazakhstan; matched with the exposed group by ethnicity, year of birth, parental age, occupation, whether or not they were smokers. 28 families with 83 of F1 and 65 of F2.
➢ Estimated radiation dose	● >1Sv
➢ Minisatellite probes	● Single-locus probes: B6.7, CEB1, CEB15, CEB25, CEB36, MS1, MS31, MS32.
➢ Main results	● A statistically significant 1.8-fold increase in mutation rate was found in the P0 generation and a less marked 1.5-fold increase was found in the F1 generation.
➢ Radiation effects	● Positive

C. Mayak plutonium separation combine

(8) Dubrova et al. (2006)[16]

➢ Exposed group	● Populations in rural villages along the Techa River, which were contaminated by radioactive releases from the Mayak plutonium separation combine; children conceived between 1950 and 1972; both parental exposure [53 families with 101 children], paternal exposure [10 families with 18 children] and maternal exposure [22 families with 40 children].
➢ Non-exposed control group	● 53 families of non-irradiated parents with 110 children: 49 families from the non-contaminated areas with children conceived between 1946 and 1977, and 4 families from the village along Techa River with all children born between 1950 and 1952 before the discharge; sex ratio, paternal age, parental occupation and number of smokers were matched with the exposed group, but maternal age in the control group was higher than that of the exposed group.
➢ Estimated radiation dose	● Mean paternal dose was l02±12mSv and mean maternal dose was 86±9mSv. Over 80% of the total parental dose was attributed to the internal exposure of the radionuclide.
➢ Minisatellite probes	● Single-locus probes: B6.7, CEB1, CEB15, CEB25, CEB36, MS1, MS31, MS32.

➢ Main results	● A statistically significant 1.67-fold increase in mutation rate was found in the paternal mutations, whereas maternal mutation rate was not elevated in the exposed families. ● Number of mutations / Total number of bands in offspring: ∗ Paternal mutation: exposed group [42/861], control group [31/1044] ∗ Maternal mutation: exposed group [6/980], control group [10/885]
➢ Radiation effects	● Paternal: positive, maternal: negative

D. Atomic bombings

(9) Kodaira, Satoh et al. (1995, 1996, 2004)[17,18,19]

➢ Exposed group	● Atomic bomb survivor families in Hiroshima and Nagasaki in which one or both parents received A-bomb radiation of > 0.01Sv (gonadal dose). Most of the children were born more than 10 years after the bombing. Within a total of 48 families with 61 children, 1 child had parents both of whom were exposed, 29 children had exposure only through the father and 31 children had exposure only through the mother.
➢ Non-exposed control group	● Atomic bomb survivor families in Hiroshima and Nagasaki in which one or both parents were exposed to less than 0.01Sv or the families which were not in Hiroshima or Nagasaki at the time of the bombing; 49 families with 58 children.
➢ Estimated radiation dose	● More than 75% of the exposed parents had an estimated dose of >1Sv (using a neutron RBE value of 10).
➢ Minisatellite probes	● Single-locus minisatellite probes: B6.7, CEB1, CEB15, CEB25, CEB36, MS1, MS31, MS32.
➢ Main results	● No evidence that the mutation clustered in individual offspring was found. ● Number of mutations / Total number of bands in offspring: ∗ Paternal mutation: exposed group [11/240], control group [33/709] ∗ Maternal mutation: exposed group [2/256], control group [6/694]
➢ Radiation effects	● Negative

E. Radiotherapy

(10) May, Tamaki et al. [7](2000)

➢ Exposed group	● Three seminoma patients aged 33-49 years donated semen samples after radiotherapy. The post-treatment samples represented sperm derived from cells irradiated at different stages of spermatogenesis, the sperms having been taken for several times on different days after irradiation.
➢ Non-exposed control group	● Sperm samples donated from the same three patients before having radiotherapy.
➢ Estimated radiation dose	● Total testicular X-ray doses were 0.75, 0.82 and 0.38 Gy for each patient.
➢ Minisatellite probes	● Single-locus minisatellite probes : B6.7, CEB1.
➢ Main results	● No significant difference in pre- and post-irradiation mutation rates was observed at any stages.
➢ Radiation effects	● Negative

Table 2. Reports on the "Trans-generational effects" of human populations using mutation markers of microsatellites

A. Chernobyl nuclear power plant accident

(1) Slebos et al. (2004)[22]

➢ Exposed group	● Liquidator families from Kiev and Chernigov in Ukraine; father had served in the cleanup operation of the Chernobyl accident between 1986 and 1990; 75 families with 75 children conceived after the start of the cleanup operation (within them, 39 families with 39 children did not have children before the start of the cleanup operation).
➢ Non-exposed control group	● 41 liquidator families from Kiev and Chernigov in Ukraine, from the same registry as the exposed group, with 41 children conceived before the start of the cleanup operation (within them, 5 families with 5 children, that did not have children after the start of the cleanup operation). Both "before" (control) and "after" (exposed) children in a family were conceived by the same mother.
➢ Estimated radiation dose	● Median accumulated dose was 152mSv.
➢ Microsatellite loci	● Autosomal loci: 4, X-linked locus: 1
➢ Main results	● More paternal mutations were seen in "after" children than in "before" children, although differences were minimal. ● Total number of mutations at the 5 microsatellite loci / Total number of microsatellite loci examined: exposed group [6/325], control group [2/174] ● D7S1482 demonstrated germline hypermutability.
➢ Radiation effects	● Negative (Small sample size limited statistical power.)

(2) Furitsu, Ryo et al. (2005)[23]

➢ Exposed group	● Belarusian liquidator families, in which either or both of the parents were involved in cleanup operations in the contaminated areas of Chernobyl between April 1986 and July 1987 during the periods from several weeks to several months; Total number of families examined was 64 with 73 children; among them, 61 children, whose fathers were exposed before conception, were analyzed.
➢ Non-exposed control group	● Belarusian families living in the non-contaminated areas in Belarus, in which none of the parents were involved in cleanup operations in the contaminated areas; sex ratio of the children were matched to the exposed group; 66 families with 69 children.
➢ Estimated radiation dose	● Very little information of the recorded individual radiation dose was available for this cohort. UNSCEAR (2000) reported that the mean effective dose was 39mSv for the liquidators in Belarus during 1986-1987 according to the national registry.
➢ Microsatellite loci	● Autosomal loci: 31, Y-linked loci: 40, X-linked locus: 1.
➢ Main results	● A higher mutation rate (2.9×10^{-3}) of Y-linked loci was detected in the families of liquidators as compared to that of the control group (2.1×10^{-3}), although the increase was not statistically significant. ● Number of mutations / Total number of microsatellite loci examined: ∗ 40 Y-linked loci: exposed group [4/1392], control group [3/1458] ∗ 31autosomal loci: exposed group [11/1852], control group [18/2108] ∗ 1 X-linked locus: no mutations were detected in either exposed or control groups
➢ Radiation effects	● Negative

(3) Satoh et al. (1996)[18]

➢ Exposed group	● Atomic bomb survivor families in Hiroshima and Nagasaki in which one or both parents received A-bomb radiation of > 0.01Sv (gonadal dose). Most of the children were born more than 10 years after the bombing. Within the totally 50 families with 64 children, all children were exposed through either the mother or the father except one child where both parents had been exposed.
➢ Non-exposed control group	● Atomic bomb survivor families in Hiroshima and Nagasaki in which one or both parents were exposed to less than 0.01Sv or the families that were not in Hiroshima or Nagasaki at the time of the bombing; 50 families with 60 children.
➢ Estimated radiation dose	● Mean doses for gametes: maternal 1.7Sv, paternal 2.1Sv (using a neutron RBE value of 20).
➢ Microsatellite loci	● Autosomal loci: 3, X-linked loci: 2.
➢ Main results	● There was no significant difference in the mean mutation rate between the children of the exposed and control groups. ● Total number of mutations in the 5 microsatellite loci / Total number of microsatellite loci examined: exposed group [0/307], control group [4/809]
➢ Radiation effects	● Negative

References

1) Nakamura N., Genetic effects of radiation in atomic-bomb survivors and their children: past, present and future, J. Rad. Res., 47, Sup. B.67-73, 2006.

2) Committee to Assess Health Risks from Exposure to Low Levels of Ionizing Radiation, NRC, Health Risks from Exposure to Low Levels of Ionizing Radiation: BEIR VII – Phase 2, 4 HERITABLE GENETIC EFFECTS OF RADIATION IN HUMAN POPULATIONS, pp. 91-131, Washington, D.C., National Academies Press, 2006.

3) Niwa O, Indirect mechanisms of genomic instability and the biological significance of mutations at tandem repeat loci, Mut. Res., 598, 61-72, 2006.

4) Ellegren H, Microsatellite mutations in the germline: implications for evolutionary inference. Trends Genet., 16 (12), 551-8, 2000.

5) Bois P, Bois P, Stead JD, Bakshi S, Williamson J, Neumann R, Moghadaszadeh B, Jeffreys AJ. Isolation and characterization of mouse minisatellites, Genomics, 50 (3), 317-30, 1998.

6) Boyd M, Livingstone A, Wilson LE, Marshall EM, McCluskey AG, Mairs RJ, Wheldon TE, Dose-response relationship for radiation-induced mutations at micro- and minisatellite loci in human somatic cells in culture, Int. J. Radiat. Biol., 76 (2), 169-176, 2000.

7) May CA, Tamaki K, N May CA, Tamaki K, Neumann R, Wilson G, Zagars G, Pollack A, Dubrova YE, Jeffreys AJ, Meistrich ML, Minisatellite mutation frequency in human sperm following radiotherapy, Mutat Res., 453 (1), 67-75, 2000.

8) Dubrova YE, Jeffreys AJ, Malashenko AM, Mouse minisatellite mutations induced by ionizing radiation, Nature Genet., 5 (1), 92-4, 1993.

9) Sadamoto S, Suzuki S, Kamiya K, Kominami R, Dohi K, Niwa O, Radiation induction of germline mutation at a hypervariable mouse minisatellite locus, Int. J. Radiat. Biol., 65 (5), 549-57, 1994.

10) Dubrova YE, Plumb M, Brown J, Fennelly J, Bois P, Goodhead D, Jeffreys AJ, Stage specificity, dose response, and doubling dose for mouse minisatellite germ-line mutation induced by acute radiation, Proc. Natl. Acad. Sci. USA, 95 (11), 6251-5, 1998.

11) Dubrova YE, Nesterov VN, Krouchinsky NG, Ostapenko VA, Neumann R, Neil DL, Jeffreys AJ, Human minisatellite mutation rate after the Chernobyl accident, Nature, 380 (6576), 683-6, 1996.
12) Dubrova YE, Nesterov VN, Krouchinsky NG, Ostapenko VA, Vergnaud G, Giraudeau F, Buard J, Jeffreys AJ, Further evidence for elevated human minisatellite mutation rate in Belarus eight years after the Chernobyl accident, Mutat Res., 381 (2), 267-78, 1997.
13) Neel JV, Genetic studies at the atomic bomb Casualty Commission-Radiation Effects Research Foundation: 1946-1997, Proc. Natl. Acad. Sci. USA, 95, 5432-5436, 1998.
14) Dubrova YE, Grant G, Chumak AA, Stezhka VA, Karakasian AN, Elevated minisatellite mutation rate in the post-Chernobyl families from Ukraine, Am J Hum Genet., 71 (4), 801-9, 2002.
15) Dubrova YE, Bersimbaev RI, Djansugurova LB, Tankimanova MK, Mamyrbaeva ZZh, Mustonen R, Lindholm C, Hulten M, Salomaa S, Nuclear weapons tests and human germline mutation rate, Science, 295 (5557), 1037, 2002.
16) Dubrova YE, Ploshchanskaya OG, Kozionova OS, Akleyev AV, Minisatellite germline mutation rate in the Techa River population. Mutat Res., 602 (1-2), 74-82, 2006.
17) Kodaira M, Satoh C, Hiyama K, Toyama K, Lack of effects of atomic bomb radiation on genetic instability of tandem-repetitive elements in human germ cells, Am J Hum Genet., 57 (6), 1275-83, 1995.
18) Satoh C, Takahashi N, Asakawa J, Kodaira M, Kuick R, Hanash SM, Neel JV, Genetic analysis of children of atomic bomb survivors, Environ Health Perspect., 104 Suppl 3, 511-9, 1996.
19) Kodaira M, Izumi S, Takahashi N, Nakamura N, No evidence of radiation effect on mutation rates at hypervariable minisatellite loci in the germ cells of atomic bomb survivors, Radiat Res.,162 (4), 350-6, 2004.
20) Livshits LA, Malyarchuk SG, Kravchenko SA, Matsuka GH, Lukyanova EM, Antipkin YG, Arabskaya LP, Petit E, Giraudeau F, Gourmelon P, Vergnaud G, Le Guen B, Children of Chernobyl cleanup workers do not show elevated rates of mutations in minisatellite alleles, Radiat Res., 155, 74-80, 2001.
21) Kiuru A, Auvinen A, Luokkamaki M, Makkonen K, Veidebaum T, Tekkel M, Rahu M, Hakulinen T, Servomaa K, Rytomaa T, Mustonen R, Hereditary minisatellite mutations among the offspring of Estonian Chernobyl cleanup workers, Radiat Res., 159 (5), 651-5, 2003.
22) Slebos RJ, Little RE, Umbach DM, Antipkin Y, Zadaorozhnaja TD, Mendel NA, Sommer CA, Conway K, Parrish E, Gulino S, Taylor JA, Mini-and microsatellite mutations in children from Chernobyl accident cleanup workers, Mutat Res., 559 (1-2), 143-51, 2004.
23) Furitsu K, Ryo H, Yeliseeva KG, Thuy le TT, Kawabata H, Krupnova EV, Trusova VD, Rzheutsky VA, Nakajima H, Kartel N, Nomura T, Microsatellite mutations show no increases in the children of the Chernobyl liquidators, Mutat. Res., 581 (1-2), 69-82, 2005.
24) Ellegren H, Lindgren G, Primmer CR, Moller AP, Fitness loss and germline mutations in barn swallows breeding in Chernobyl, Nature, 389 (6651), 593-6, 1997.
25) Kovalchuk O, Dubrova YE, Arkhipov A, Hohn B, Kovalchuk I, Wheat mutation rate after Chernobyl, Nature, 407 (6804), 583-4, 2000.
26) Kovalchuk O, Kovalchuk I, Arkhipov A, Hohn B, Dubrova YE, Extremely complex pattern of microsatellite mutation in the germline of wheat exposed to the post-Chernobyl radioactive contamination, Mutat Res., 525 (1-2), 93-101, 2003.
27) Baker RJ, Van Den Bussche RA, Wright AJ, Wiggins LE, Hamilton MJ, Reat EP, Smith MH, Lomakin MD, Chesser RK, High levels of genetic change in rodents of Chernobyl, Nature, 390 (6655) 100, 1997.
28) Wickliffe JK, Dunina-Barkovskaya YV, Gaschak SP, Rodgers BE, Chesser RK, Bondarkov M, Baker RJ, Variation in mitochondrial DNA control region haplotypes in populations of the bank vole, Clethrionomys glareolus, living in the Chernobyl environment, Ukraine, Environ Toxicol Chem., 25 (2), 503-8, 2006.