Published in final edited form as:
Int J Radiat Biol. 2006 March ; 82(3): 153–160. doi:10.1080/09553000600640538.

A pilot study examining germline minisatellite mutations in the offspring of Danish childhood and adolescent cancer survivors treated with radiotherapy

GWEN S. REES[1], **MICHAEL Z. TRIKIC**[1], **JEANETTE F. WINTHER**[2], **E. JANET TAWN**[1], **MARILYN STOVALL**[3], **JØRGEN H. OLSEN**[2,4], **CATHERINE RECHNITZER**[5], **HENRIK SCHRØDER**[6], **PER GULDBERG**[7], and **JOHN D. BOICE Jr.**[4,8]

[1]Genetics Department, Westlakes Research Institute, Cumbria, UK

[2]Institute of Cancer Epidemiology, Danish Cancer Society, Copenhagen, Denmark

[3]Department of Radiation Physics, The University of Texas, M.D. Anderson Cancer Center, Houston, Texas, USA

[4]School of Medicine, Vanderbilt University, Nashville, Tennessee, USA

[5]The Late Effect Clinic, Juliane Marie Center, Rigshospitalet, Copenhagen University Hospital, Denmark

[6]Department of Pediatrics, University Hospital of Aarhus, Denmark

[7]Institute of Cancer Biology, Danish Cancer Society, Copenhagen, Denmark

[8]International Epidemiology Institute, Rockville, Maryland, USA

Abstract

Purpose—To investigate germline mutation rate at eight minisatellite loci in 24 Danish families, where one parent is the survivor of childhood or adolescent cancer treated with radiotherapy.

Materials and methods—Parents and offspring were profiled for eight hypervariable minisatellite loci (*B6.7*, *CEB1*, *CEB15*, *CEB25*, *CEB36*, *MS1*, *MS31*, *MS32*) by Southern blotting.

Results—Seven paternal mutations were observed for 130 informative alleles in 18 offspring from 11 radiation-exposed fathers (mean preconceptional dose for offspring 0.29 Gy, range <0.01 - 1.2 Gy), compared to six mutations for 146 informative alleles in 21 offspring from 13 unexposed fathers. No statistically significant difference between the total paternal mutation rates was observed (5.4% for exposed fathers and 4.1% for unexposed fathers). Three maternal mutations were observed for 148 informative alleles in 21 offspring from 13 radiation-exposed mothers (mean preconceptional dose for offspring 0.71 Gy, range <0.01 - 9.2 Gy), compared to one mutation for 130 informative alleles in 18 offspring from 11 unexposed mothers. Again, no statistically significant difference was observed between the total maternal mutation rates (2.0% for exposed mothers and 0.8% for unexposed mothers).

Conclusions—The data from this pilot study demonstrate no statistically significant increase in germline minisatellite mutation rate associated with radiotherapy for childhood and adolescent cancer.

Correspondence: Gwen S. Rees, Genetics Department, Westlakes Research Institute, Westlakes Science and Technology Park, Moor Row, Cumbria, CA24 3JY, UK. Tel: +44 1946 514157. Fax: +44 1946 514057. E-mail: gwen.rees@westlakes.ac.uk.

Keywords

Minisatellite mutation; ionizing radiation; radiotherapy; transgenerational effect; childhood; adolescent cancer

Introduction

To date, epidemiological studies have demonstrated no convincing or consistent evidence for radiation-induced germline mutation in humans and risks of radiation-induced genetic disease have had to rely on extrapolation from mouse data (United Nations Scientific Committee on the Effects of Atomic Radiation [UNSCEAR] 2001). The low frequency of spontaneous mutations at loci associated with genetic disease means that changes in the germline mutation rate are difficult to detect. Therefore, in practical terms, very large population sizes are required to determine mutations which manifest as a clinical phenotype. It has recently been suggested that detection of germline mutations at hypervariable minisatellite loci may provide a new approach to the study of radiation-induced human germline mutation, since the high spontaneous mutation rate at such loci enables mutation detection in relatively small populations (Dubrova 2003). Minisatellites are tandemly repeated regions of DNA, consisting of repeat units of 6 - 100 bp in length, which occur with a high frequency throughout the genome. Minisatellite mutations usually consist of gains or losses of one or more repeat units and mutations at hypervariable minisatellite loci are up to 1000 times more common than mutations in protein coding genes (Debrauwere et al. 1997).

Initial studies on populations living in areas of Belarus (Dubrova et al. 1996, 1997) and Ukraine (Dubrova et al. 2002a) contaminated by radioactive debris from the Chernobyl accident reported significant increases in minisatellite mutation rates which correlated with environmental contamination with radionuclides. An increased rate of minisatellite mutations, which extended to the subsequent generation, was also observed in a population living around the Semipalatinsk nuclear test site in Kazakhstan (Dubrova et al. 2002b). In contrast, two other studies using the same single locus probes failed to reveal significant increases in the rate of minisatellite mutation in offspring of Ukrainian (Livshits et al. 2001) and Estonian (Kiuru et al. 2003) Chernobyl clean-up workers. Two further studies of Chernobyl clean-up workers using related techniques also reported no significant increase in germline mutations using multi-locus minisatellite probes (Slebos et al. 2004) and microsatellite markers (Furitsu et al. 2005).

An early study of the offspring of Japanese atomic bomb survivors also failed to detect an increase in the germline mutation rate (Kodaira et al. 1995) and this was confirmed in a subsequent study (Kodaira et al. 2004) which evaluated the same eight hypervariable minisatellite loci used in the studies of populations from the former Soviet Union. Furthermore, no increase in minisatellite mutations has been observed in a study of germline cells from seminoma patients treated with radiotherapy, when pre-treatment sperm DNA was compared with post-treatment sperm DNA derived from irradiated pre-meiotic, meiotic and postmeiotic cells (May et al. 2000).

The aim of this pilot study is to investigate changes in the germline minisatellite mutation rate at the eight most commonly studied hypervariable loci in survivors of childhood and adolescent cancer treated with radiotherapy for whom accurate doses to the gonads have been modelled. The work forms part of a larger project investigating adverse reproductive outcomes in cancer survivors (Boice et al. 2003, Winther et al. 2004). Maternal and paternal mutations were identified in offspring by comparison with parental alleles and mutation frequencies in radiation exposed parents compared with those in unexposed parents.

Methods

Study cohort

Twenty-eight cancer survivors who had received radiotherapy were identified from the Danish Childhood Cancer Survivor Cohort. This cohort comprises 4,676 survivors notified to the Danish Cancer Registry with cancer at age ≤20 years between 1943 and 1996 who survived until onset of fertility (age 15 years). Survivors had to be alive on or born after April 1, 1968, when the national Central Population Register (CPR) was established and a unique personal identification number was assigned for all citizens. A search in the CPR identified offspring and partners. Blood samples were taken from 28 survivors and their families for a pilot study aimed at investigating a range of genetic endpoints associated with germ cell mutagenesis and cancer susceptibility (Boice et al. 2003, Tawn et al. 2005, Curwen et al. 2005). Information on cancer in relatives, smoking habits and medication was obtained from a short questionnaire completed by the cancer survivors and their partners. Approval for the study was obtained from the Danish Scientific Ethical Committee and the Danish Data Protection Agency.

All family samples were coded at the time of collection with the suffix -01 and -02 for parents and -03, -04, -05 for offspring. Samples were successfully taken from 28 cancer survivors, 28 partners and 44 offspring. For one family it was not possible to obtain a sample from the offspring and this family was excluded from the study group. DNA was extracted from whole blood or lymphocytes using a Nucleon BACC3 kit (Amersham Biosciences, Little Chalfont, Buckinghamshire, UK). All 27 families with offspring blood samples were analysed for minisatellite mutations, but samples from three families failed due to degraded DNA. In two families with three offspring (T05, T09) a single offspring sample failed due to degraded DNA or weak signal and was excluded. The final study population comprised 24 families with 39 offspring, where 11 fathers were cancer survivors with 18 offspring and 13 mothers were cancer survivors with 21 offspring.

Organ doses for individual patients were reconstructed based on information available in radiotherapy records. The records were submitted to The University of Texas M. D. Anderson Cancer Center, Houston, Texas, USA, for data abstraction and dose modelling (Boice et al. 2003, Winther et al. 2004, Stovall et al. 2004). Individual data on cancer type, age at treatment, radiation dose to the gonads, chemotherapy treatment and number of offspring analysed per family are presented in Table I, together with the mean preconceptional doses for the offspring of exposed fathers and exposed mothers. Mean paternal and maternal ages at birth of offspring were 29 for male cancer survivors (exposed fathers) and 28 for their partners (unexposed mothers); 28 for female cancer survivors (exposed mothers) and 29 for their partners (unexposed fathers).

Confirmation of maternity and paternity

Maternity, paternity and sample identity were confirmed using polymerase chain reaction (PCR) based assays utilizing four stable minisatellite loci selected for their low background mutation frequency. Apolipoprotein b (*apoB*) (2p23-p24) (Boerwinkle et al. 1989), *HRAS* (11p15.5) (Lindstedt et al. 1999), *MCOB19* (D19S20) (Tully et al. 1993) and *YNZ22* (D17S5) (Ugozzoli et al. 1991) were amplified using a Qiagen multiplex PCR kit (Qiagen, Crawley, West Sussex, UK) and separated on 0.8% agarose gels in 0.09 M Tris-Borate, 0.002 M EDTA (1 × TBE). PCR products were sized in comparison to a 1 Kb ladder (Promega, Southampton, Hampshire, UK) using Gene Tools software (Syngene, Cambridge, UK). Non-maternity/paternity/sample mix up was confirmed if two or more of the four minisatellite loci analysed had an allele inconsistent with the parent. No cases of non-maternity or non-paternity were identified in this study group.

Preparation of minisatellite probes

Minisatellite mutations were analysed using eight single locus probes by Southern blotting. Hypervariable loci were selected according to their high background mutation frequency. Probes used were *B6.7* (20q13), *CEB1* (D2S90), *CEB15* (D1S172), *CEB25* (D10S180), *CEB36* (D10S473), *MS1* (D1S7), *MS31* (D7S21) and *MS32* (D1S8). *B6.7* and *CEB* probes were made by PCR amplification of alleles <5kb, according to methods kindly provided by Professor Yuri Dubrova, University of Leicester, UK. PCR products were purified and ligated into the cloning vector pGEM-T easy (Promega, Southampton, Hampshire, UK) and transformed into XL-10 Gold Ultracompetent cells (Stratagene Europe, Amsterdam, The Netherlands). Plasmid DNA was extracted from 150 ml bacterial cultures using Hi-Speed Maxi Prep kits (Qiagen, Crawley, West Sussex, UK) and probe purified by restriction digest with *Eco* RI (New England Biolabs, Hitchin, Hertfordshire, UK) followed by gel extraction using a QiaQuick gel extraction kit (Qiagen, Crawley, West Sussex, UK). MS probes were a kind gift from Professor Alec Jeffreys, University of Leicester, UK.

Minisatellite mutation analysis

Five micrograms of genomic DNA was digested with *Alu* I (New England Biolabs, Hitchin, Hertfordshire, UK) and separated on 30 cm 0.8% agarose gels in 1 × TBE, 0.5 μg/ml ethidium bromide for 18 h at 125 V. DNA was denatured, neutralized and transferred to a nylon membrane (Magnacharge, Genetic Research Instrumentation, Braintree, Essex, UK) where it was fixed by UV cross-linking. DNA probes were random prime labelled with fluorescein-11-dUTP (Gene Images Random Prime, Amersham Biosciences, Little Chalfont, Buckinghamshire, UK) and hybridized to the sample DNA immobilized on the membrane, then incubated with an anti-fluorescein-horseradish peroxidase conjugate. Detection utilized the substrate CDP Star (Gene Images Detection Module with CDP Star, Amersham Biosciences, Little Chalfont, Buckinghamshire, UK) and bands were visualized by exposing the nylon membranes to Hyperfilm (Amersham Biosciences, Little Chalfont, Buckinghamshire, UK) without intensifying screens for 10 min - 48 h. After each exposure, membranes were stripped of hybridized probe in 1 litre of boiling 0.1% sodium dodecyl sulphate (SDS) and stored in 0.03 M sodium citrate, 0.3 M sodium chloride, pH 7 (2 × SSC) at 4°C for re-hybridization.

Blots were scored visually by two independent assessors and digitally using Phoretix 1D software (Non-Linear Dynamics, Newcastle upon Tyne, UK) using a 1 Kb ladder (Promega, Southampton, Hampshire, UK) for size reference across the well resolved 1 - 23 Kb region. Criteria for identification of mutations were taken from previously published studies (Dubrova et al. 1996, Kiuru et al. 2003), i.e., a mutation was considered to be a band present in the offspring that was inconsistent with bands from either parent, and was larger or smaller than the parental band by at least one band-width.

Statistical analysis

Mutation rates were calculated for each locus by dividing the number of mutant bands by the number of alleles analysed. The total mutation rate for eight loci was calculated by dividing the total number of mutations by the total alleles analysed. Mean mutation rate was calculated by adding together individual mutation rates for each locus, then dividing by the total number of loci analysed. Individual locus and total mutation rates were compared using Fisher's exact test (two-tailed). The distribution of mutations was tested against Poisson expectations using a χ^2 test.

Results

Minisatellite mutation results for 39 offspring from 24 families are presented in Table II. Analysis was not possible at all loci for all offspring due to the presence of small alleles, which migrated out of detection range (1.0 - 23 Kb). In the study group as a whole, 17 mutations were identified in 39 offspring and these were distributed amongst the offspring according to Poisson expectations ($p = 0.71$). Thirteen of the mutations were of paternal and four of maternal origin, resulting in a paternal mutation rate of 4.7% (13 mutations in 276 alleles tested), and a maternal mutation rate of 1.4% (four mutations in 278 alleles tested). Of the 13 mutations in paternal alleles, six occurred in offspring of unexposed fathers and seven in the offspring of exposed fathers (Table II). Thus there is no statistically significant difference between the mutation rate of 4.1% in the unexposed fathers and that of 5.4% in the exposed fathers, ($p = 0.78$). One mutation was observed in the offspring of unexposed mothers compared to three mutations in the offspring of exposed mothers (Table II). Hence, the mutation rates for maternal alleles of 0.8% for unexposed mothers and 2.0% for the exposed mothers were not significantly different ($p = 0.63$).

The combined mutation rate for alleles inherited from unexposed parents (seven mutations in 276 alleles = 2.5%) compared to the combined mutation rate for alleles inherited from exposed parents (10 mutations in 278 alleles = 3.6%) is not significantly different, ($p = 0.62$). Furthermore, no statistically significant differences were observed between the exposed and unexposed parents at any individual locus (Table II). Restricting the analysis to those with gonadal doses of ≥0.2 Gy similarly revealed no statistically significant difference (data not shown).

The analysis above was based on total mutation rates in all eight loci analysed. However, since different loci are known to have different mutation rates, and not all loci were informative in all families, the mean of the mutation rates for all eight loci are also presented (Table II). These did not differ significantly from the total mutation rates. Due to the small population size in this pilot study, comparison of the distribution of parental allele sizes in the offspring and changes/gains/losses in repeat numbers in mutations would not be statistically meaningful and was not undertaken.

Discussion

In this pilot study of the offspring of childhood and adolescent cancer survivors, we examined the mutation rate at the eight most frequently studied hypervariable minisatellite loci, and observed no evidence of an increased germline mutation rate in parents who had received radiotherapy as part of their treatment for cancer.

Survivors of childhood or adolescent cancer who have been treated with radiotherapy prior to having a family are a meaningful group with which to investigate germline minisatellite mutations, as detailed dosimetry records are available which enable accurate estimation of doses to the gonads. Thus, we are able to say with some certainty that the mean preconceptional dose for the offspring of fathers with testicular irradiation is 0.29 Gy (range <0.01 - 1.20 Gy) and the mean preconceptional dose for the offspring of mothers with ovarian irradiation is 0.71 Gy (range <0.01 - 9.2 Gy) (Table I). As part of their therapy regimes, some of the cancer survivors had also received chemotherapy (Table I). Detailed modelling of possible gonadal exposures to chemotherapy agents is not available. However, when the mean mutation rate of 2.8% for the eight minisatellite loci in the families in which the cancer survivor received both radiotherapy and chemotherapy ($n = 15$, mean preconceptional dose 0.71 Gy) was compared to that of 3.4% for the families with a history of radiotherapy, but not chemotherapy ($n = 9$,

mean preconceptional dose 0.24 Gy), no statistically significant difference was observed (p = 0.80, data not shown).

The results observed in our pilot study are consistent with previous studies using single locus minisatellite probes which examined the effect of radiation received over a relatively short time period and for which no increases in minisatellite mutation rate were detected (Kodaira et al. 1995, Livshits et al. 2001, Kiuru et al. 2003, Kodaira et al. 2004). These studies taken together cover a wide dose range and encompass the doses received in the present study. Although the doses received by the Chernobyl cleanup workers are subject to some uncertainty, maximum doses received by those in the Ukrainian study were thought not likely to exceed 0.15 - 0.2 Sv (Livshits et al. 2001) whilst the mean dose derived for the Estonian cleanup workers was 0.11 Sv (Littlefield et al. 1998, Kiuru et al. 2003). Both these studies (Livshits et al. 2001, Kiuru et al. 2003) report no increase in minisatellite mutations associated with radiation exposure. Similarly, testicular doses ranging from 0.38 - 0.75 Gy, received during treatment for seminoma, had no influence on the minisatellite mutation rate in sperm (May et al. 2000). Doses received by survivors of the Japanese atomic bombs studied for minisatellite mutations were considerably higher, with means of 1.61 Sv for paternal exposure and 1.34 Sv for maternal exposure but two studies using probes for a total of 10 different loci have failed to find an increase in the minisatellite mutation rate (Kodaira et al. 1995, 2004).

Despite receiving lower doses than those experienced by cancer survivors or atomic bomb survivors, statistically significant 1.6 - 2-fold increases in the minisatellite mutation rate have been reported in populations exposed to radiation as a result of fallout from the Chernobyl accident and atmospheric atomic bomb testing (Dubrova et al. 1996, 1997, 2002a, 2002b). The mean parental dose due to ^{137}Cs contamination from Chernobyl in the Belarus studies is estimated at 0.03 Sv (Dubrova et al. 1996, 1997). For the Ukrainian study, doses are thought not to exceed 0.05 Sv (Dubrova et al. 2002a), although it is noted that these doses do not take into account the contribution of short-lived radionuclides. Doses received by the study population from around Semipalatinsk nuclear test site were originally thought to be >1 Sv (Dubrova et al. 2002b), but could not be confirmed when subsequent retrospective biodosimetry conducted on the same population showed no increase in stable chromosome translocations compared with an unexposed population (Salomaa et al. 2002).

It has been postulated that the discrepancy between the negative finding in the offspring of the Japanese atomic bomb survivors and the increase in mutation rate seen in residents of areas around Chernobyl could be attributed to the different nature of the exposures (Dubrova et al. 1997). Doses received by the Japanese population came from a single acute exposure whereas those living in the vicinity of Chernobyl received lower dose chronic exposures as a result of environmental contamination. However, this theory is not supported by results from animal studies of radiation-induced minisatellite mutations, which show similar dose response relationships for acute X-irradiation and chronic gamma exposure, suggesting that the risk factors for both chronic and acute irradiation are similar (Dubrova & Plumb 2002).

Mutation rates in some of the loci analysed in our study are known to be skewed towards paternal events (Vergnaud & Denoeud 2000) and this is illustrated in the current study, since fewer maternal than paternal mutations were observed. Furthermore, a recent re-analysis of data from studies of families living in areas of Belarus and Ukraine contaminated by the Chernobyl accident has highlighted that the increase in minisatellite mutations occurred only in fathers and that the maternal mutation rate was not elevated (Dubrova 2003). Using published data for the large Centre d'Etudes du Polymorphisme Humain (CEPH, http://www.cephb.fr) reference population, a combined maternal and paternal mean mutation rate of 3.0% for the eight loci examined in this study can be derived (Jeffreys et al. 1988, Vergnaud & Denoeud 2000). A similar combined mutation rate of around 2.5% for a control population was reported

by Dubrova et al. (2002a) for the same set of loci. Values in line with these are found for the mean of maternal and paternal mutation rates in this study. Thus, the mean value for families with paternal radiation exposure is 3.1% compared with 3.1% for the families with maternal exposure. If the comparison is made between exposed parents and non-exposed parents the means of the combined maternal and paternal mutation rates for the eight loci are 3.6% and 2.5% respectively.

It is proposed to expand this study with a further 100 families. On the assumption of two children per family, a total of 250 offspring will have received 500 alleles per individual locus (250 from the irradiated cancer survivors and 250 from the unirradiated parents) and thus for eight loci there will be a total of 2000 irradiated and 2000 non-irradiated alleles. Assuming an average baseline mutation rate of 2.5% for these eight loci (Dubrova et al. 2002a) there will be sufficient power to detect a 1.6-fold increase in minisatellite mutation rate. This is consistent with the results from the study of 40 exposed families living in an area contaminated by atmospheric atomic bomb testing (Dubrova et al. 2002b) and the study of 198 families living in contaminated areas of the Ukraine, with children conceived after the Chernobyl accident (Dubrova et al. 2002a).

To conclude, from the results of this small pilot study, we have seen no statistically significant increase in the germline mutation associated with radiotherapy or chemotherapy as treatment for childhood or adolescent cancer rate using a panel of the eight most commonly studied hypervariable minisatellite loci. In the expanded study the emphasis will be on paternal irradiation, since baseline mutation rates are greater and it seems that a radiation-induced increase is more likely to be seen following paternal rather than maternal exposure (Dubrova 2003).

Acknowledgements

The authors would like to thank Professor Yuri Dubrova, Dr Ruth Barber and Ms Gemma Grant, University of Leicester, UK, for their help with protocols for making probes and setting up the Southern blotting techniques and Professor Alec Jeffreys, University of Leicester, for providing MS minisatellite probes; Dr Anne Kiuru, STUK, Finland, for helpful discussions regarding fingerprinting techniques and Southern blotting and Mrs Joan Killip for technical assistance. Dosimetry data were provided by Ms Rita Weathers and Ms Catherine Kasper, The University of Texas M. D. Anderson Cancer Center, USA. Permissions were granted from the Danish Data Protection Agency (2001-41-1113) and the Danish Scientific Ethical Committee ([KF] 01 - 150/01 & [KF] 11 - 129/02). This study was supported by British Nuclear Fuels plc, the Danish Cancer Society, the International Epidemiology Institute and Westlakes Research Institute.

References

Boerwinkle E, Xiong W, Fourest E, Chan L. Rapid typing of tandemly repeated hypervariable loci by the polymerase chain reaction: Application to the apolipoprotein B 3′ hypervariable region. Proceedings of the National Academy of Sciences, USA 1989;86:212–216.

Boice JD Jr, Tawn EJ, Winther JF, Donaldson SS, Green DM, Mertens AC, Mulvihill JJ, Olsen JH, Robison LL, Stovall M. Genetic effects of radiotherapy for childhood cancer. Health Physics 2003;85:65–80. [PubMed: 12852473]

Curwen GB, Winther JF, Tawn EJ, Smart V, Whitehouse CA, Rees GS, Olsen JH, Guldberg P, Rechnitzer C, Schrøder H, Bryant PE, Sheng X, Lee HS, Chakraborty R, Boice JD Jr. G2 chromosomal radiosensitivity in Danish survivors of childhood and adolescent cancer and their offspring. British Journal of Cancer 2005;93:1038–1045. [PubMed: 16234827]

Debrauwere H, Gendrel GC, Lechat S, Dutreix M. Differences and similarities between various tandem repeat sequences: Minisatellites and microsatellites. Biochimie 1997;79:577–586. [PubMed: 9466695]

Dubrova YE, Nesterov VN, Krouchinsky NG, Ostapenko VA, Neumann R, Neil DL, Jeffreys AJ. Human minisatellite mutation rate after the Chernobyl accident. Nature 1996;380:683–686. [PubMed: 8614461]

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. Mutation Research 1997;381:267–278. [PubMed: 9434883]

Dubrova YE, Grant G, Chumak AA, Stezhka VA, Karakasian AN. Elevated minisatellite mutation rate in the post-Chernobyl families from Ukraine. American Journal of Human Genetics 2002a;71:801–809. [PubMed: 12226793]

Dubrova YE, Bersimbaev RI, Djansugurova LB, Tankimanova MK, Mamyrbaeva Z, Mustonen R, Lindholm C, Hulten M, Salomaa S. Nuclear weapons tests and human germline mutation rate. Science 2002b;295:1037. [PubMed: 11834827]

Dubrova YE, Plumb MA. Ionising radiation and mutation induction at mouse minisatellite loci: The story of the two generations. Mutation Research 2002;499:143–150. [PubMed: 11827707]

Dubrova YE. Long-term genetic effects of radiation exposure. Mutation Research 2003;544:433–439. [PubMed: 14644346]

Furitsu K, Ryo H, Yeliseeva KG, le Thuy 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. Mutation Research 2005;581:69–82. [PubMed: 15725606]

Jeffreys AJ, Royle NJ, Wilson V, Wong Z. Spontaneous mutation rates to new length alleles at tandem-repetitive hypervariable loci in human DNA. Nature 1988;332:278–281. [PubMed: 3347271]

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. Radiation Research 2003;159:651–655. [PubMed: 12710876]

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. American Journal of Human Genetics 1995;57:1275–1283. [PubMed: 8533756]

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. Radiation Research 2004;162:350–356. [PubMed: 15447048]

Lindstedt B-A, Ryberg D, Zienolddiny S, Khan H, Haugen A. Hras1 VNTR alleles as susceptibility markers for lung cancer: Relationship to microsatellite instability in tumors. Anticancer Research 1999;19:5523–5527. [PubMed: 10697610]

Littlefield LG, McFee AF, Salomaa SI, Tucker JD, Inskip PD, Sayer AM, Lindholm C, Mäkinen S, Mustonen R, Sorensen K, Tekkel M, Veidebaum T, Auvinen A, Boice JD Jr. Do recorded doses overestimate true doses received by Chernobyl cleanup workers? Results of cytogenetic analyses of Estonian workers by fluorescence in situ hybridization. Radiation Research 1998;150:237–249. [PubMed: 9692369]

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. Radiation Research 2001;155:74–80. [PubMed: 11121218]

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. Mutation Research 2000;453:67–75. [PubMed: 11006413]

Salomaa S, Lindholm C, Tankimanova MK, Mamyrbaeva ZZ, Koivistonen A, Hulten M, Mustonen R, Dubrova YE, Bersimbaev RI. Stable chromosome aberrations in the lymphocytes of a population living in the vicinity of the Semipalatinsk nuclear test site. Radiation Research 2002;158:591–596. [PubMed: 12385636]

Slebos RJC, 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. Mutation Research 2004;559:143–151. [PubMed: 15066582]

Stovall M, Donaldson SS, Weathers RE, Robison LL, Mertens AC, Winther JF, Olsen JH, Boice JD Jr. Genetic effects of radiotherapy for childhood cancer: Gonadal dose reconstruction. International Journal of Radiation Oncology, Biology, Physics 2004;60:542–552.

Tawn EJ, Whitehouse CA, Winther JF, Curwen GB, Rees GS, Stovall M, Olsen JH, Guldberg P, Rechnitzer C, Schroder H, Boice JD Jr. Chromosome analysis in childhood cancer survivors and

their offspring - no evidence for radiotherapy-induced persistent genomic instability. Mutation Research 2005;583:198–206. [PubMed: 15914077]

Tully G, Sullivan KM, Gill P. Analysis of 6 VNTR loci by 'multiplex' PCR and automated fluorescent detection. Human Genetics 1993;92:554–562. [PubMed: 8262515]

Ugozzoli L, Yam P, Petz LD, Ferrara GB, Champlin RE, Forman SJ, Koyal D, Wallace RB. Amplification by the polymerase chain reaction of hypervariable regions of the human genome for evaluation of chimerism after bone marrow transplantation. Blood 1991;77:1607–1615. [PubMed: 1672615]

UNSCEAR. Hereditary effects of radiation. United Nations; New York: 2001.

Vergnaud G, Denoeud F. Minisatellites: Mutability and genome architecture. Genome Research 2000;10:899–907. [PubMed: 10899139]

Winther JF, Boice JD Jr, Mulvihill JJ, Stovall M, Frederiksen K, Tawn EJ, Olsen JH. Chromosomal abnormalities among offspring of childhood cancer survivors in Denmark: A population-based study. American Journal of Human Genetics 2004;74:1282–1285. [PubMed: 15106125]

Table I

Individual data on cancer type, age at treatment, radiation dose to the gonads, chemotherapy treatment, number of offspring analysed per family and mean preconceptional doses for offspring of the study groups

Patient ID	Cancer type	Age at treatment (years)	Dose to gonads (Gy)	Chemotherapy (yes/no)	Number of offspring analysed
Exposed fathers					
T03	Rhabdomyosarcoma	9	0.25	yes	2
T05	Hodgkin's disease	10	1.20	no	2
T08	Neuroblastoma	0.6	0.21	no	1
T09	Wilms' tumour	7	0.17	yes	2
T14	Ewing's sarcoma	7	0.30	no	2
T15	Pineocytoma	19	0.23	no	1
T17	Germinoma	17	0.17	yes	1
T18	Malignant schwannoma	19	<0.01	no	2
T19	Hodgkin's disease	17	0.04	no	2
T22	Wilms' tumour	1	0.21	yes	1
T23	Wilms' tumour	5	0.20	yes	2
Total number of offspring					18
Mean preconceptional dose for offspring			0.29		
Exposed mothers					
T01	Hodgkin's disease	15	0.28	yes	2
T02	Hodgkin's disease	11	0.11	no	2
T04*	Hodgkin's disease	15	0.31	yes	1
	Thyroid cancer	30			
T06	Teratoma	0.1	<0.01	no	3
T07	Hodgkin's disease	19	0.08	yes	1
T13	Lymphoepithelioma	20	0.05	no	1
T16	Hodgkin's disease	20	0.29	yes	1
T20	Hodgkin's disease	17	0.29	yes	2
T21	Hodgkin's disease	19	0.09	yes	1
T24	Lymphoblastic lymphoma	14	0.01	yes	2
T25	Neuroblastoma	1	9.20	yes	1
T26	Hodgkin's disease	19	0.08	yes	2

Patient ID	Cancer type	Age at treatment (years)	Dose to gonads (Gy)	Chemotherapy (yes/no)	Number of offspring analysed
T28	Wilms' tumour	2	1.70	yes	2
Total number of offspring					21
Mean preconceptional dose for offspring			0.71		

*Dose to gonads associated with treatment for both malignancies.

Table II

Minisatellite mutations detected at eight hypervariable minisatellite loci for 39 offspring from 24 cancer survivor families

Locus	Exposed fathers (Male cancer survivors)			Unexposed fathers (Partners of female survivors)			*p*
	No. of mutations	No. of alleles	Mutation rate (%)	No. of mutations	No. of alleles	Mutation rate (%)	
Mutations in paternal alleles							
B6.7	2	17	11.8	2	18	11.1	1.00
CEB1	1	14	7.1	1	18	5.6	1.00
CEB15	1	16	6.3	0	21	0.0	0.43
CEB25	1	17	5.9	0	17	0.0	1.00
CEB36	0	14	0.0	0	17	0.0	1.00
MS1	1	18	5.6	2	19	10.5	1.00
MS31	1	16	6.3	1	17	5.9	1.00
MS32	0	18	0.0	0	19	0.0	1.00
Total	7	130	5.4	6	146	4.1	0.78
Mean mutation rate			5.4			4.1	
	Unexposed mothers (Partners of male survivors)			Exposed mothers (Female cancer survivors)			
Mutations in maternal alleles							
B6.7	0	15	0.0	1	19	5.3	1.00
CEB1	1	17	5.9	0	18	0.0	0.49
CEB15	0	17	0.0	0	20	0.0	1.00
CEB25	0	15	0.0	0	19	0.0	1.00
CEB36	0	14	0.0	1	18	5.6	1.00
MS1	0	18	0.0	1	19	5.3	1.00
MS31	0	16	0.0	0	17	0.0	1.00
MS32	0	18	0.0	0	18	0.0	1.00
Total	1	130	0.8	3	148	2.0	0.63
Mean mutation rate			0.7			2.0	

(*p*=probability Fisher's exact test, two-tailed).