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Germline minisatellite mutations in survivors of childhood and young adult cancer treated with radiation

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Abstract

Purpose—To investigate minisatellite germline mutation rates in survivors of childhood and young adult cancer who received radiotherapy.

Materials and Methods—DNA samples from 100 families, where one parent was a cancer survivor, were analysed for mutations at eight hypervariable minisatellite loci (*B6.7*, *CEB1*, *CEB15*, *CEB25*, *CEB36*, *MS1*, *MS31*, *MS32*) by Southern hybridisation.

Results—No significant difference was observed between the paternal mutation rate of 5.6% in exposed fathers with a mean preconceptional testicular dose of 1.23 Gy (56 mutations in 998 informative alleles) and that of 5.8% in unexposed fathers (17 in 295 informative alleles). Subgrouping the exposed fathers into dose groups of <0.10 Gy, 0.10 – 0.99 Gy, 1.00 – 1.99 Gy, ≥ 2.00 Gy revealed no significant differences in paternal mutation rate in comparison with the unexposed fathers. Maternal mutation rates of 1.6% in cancer survivor mothers with a mean preconceptional ovarian dose of 0.58 Gy (five mutations in 304 informative alleles) and 2.1% in unexposed mothers (21 in 987 informative alleles) were not significantly different. There were no differences in minisatellite mutation rates associated with treatment with chemotherapeutic agents.

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Conclusions—This study provides evidence that preconception radiotherapy for childhood or early adulthood cancer does not increase the germline minisatellite mutation rate.

Keywords

Minisatellite; germline mutation; ionising radiation; childhood and young adult cancer

Introduction

Although there is considerable evidence for radiation-induced germline mutation in animal experimental models, to date, radiation has not been confirmed as a germinal mutagen causing genetic disease in humans (United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR) 2001, Biological Effects of Ionizing Radiation (BEIR) VII 2006, International Commission on Radiological Protection (ICRP) 2007, Wyrobek et al. 2007). Epidemiological studies on a range of adverse pregnancy outcomes have shown no evidence of germline mutagenesis in the atomic bomb survivors (Neel et al. 1990) or in cancer survivors treated with radiotherapy (Boice et al. 2003). In addition, a recent review of studies of radiation workers concludes that there is no increased risk of leukaemia or other childhood cancers associated with occupational paternal preconception exposure (Draper 2008). Consequently, in the absence of direct data, risks of radiation-induced genetic disease in humans have been derived by applying mouse data on radiation-induced mutation rates to human data on spontaneous frequencies of genetic diseases (UNSCEAR 2001, BEIR VII 2006, ICRP 2007).

Spontaneous mutation rates at loci associated with human genetic diseases are very low and technological limitations in identifying such low frequency events are likely to have hampered their detection, rather than a human resistance to their induction (Wyrobek et al. 2007). This has led to a search for other genetic markers of radiation-induced germline mutation, which might allow a quantification of radiation genetic risk. Minisatellite mutations at hypervariable loci have been suggested as a model for the study of radiation-induced human germline mutation (Dubrova 2003a, Dubrova 2006), as mutations in minisatellite regions are approximately 1000 times more common than mutations in genes that code for proteins (Debrauwere et al. 1997). Minisatellites are tandemly repeated regions of DNA which occur at a high frequency throughout the genome and some of these repeat DNA sequences exhibit high frequencies of spontaneous germline mutations to new allele lengths. Screening for length changes provides the opportunity to detect induced germline mutations using relatively small population samples.

Initial studies of populations contaminated by the Chernobyl accident living in Belarus (Dubrova et al. 1996, 1997) and Ukraine (Dubrova et al. 2002a) reported statistically significant increases in paternal, but not maternal, minisatellite mutation rates in the offspring of those exposed, which correlated with estimated environmental contamination with radionuclides. In a similar study, a significantly increased rate of paternal minisatellite mutation, which extended to a second generation, was observed in a population living around the Semipalatinsk nuclear test site in Kazakhstan (Dubrova et al. 2002b). A population living along the Techa river, contaminated by discharges from the Russian Mayak plutonium facility, also demonstrated a statistically significant increase in the germline mutation rate of exposed fathers (Dubrova et al. 2006).

In contrast, other studies of radiation-exposed populations have failed to demonstrate increases in the rate of minisatellite mutations. No statistical differences in minisatellite mutation rates were observed among children of Ukrainian (Livshits et al. 2001) and Estonian (Kiuru et al. 2003) Chernobyl cleanup workers conceived before the accident

compared with children born after the accident. Two further studies of Chernobyl cleanup 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). A study of Japanese atomic bomb survivors and their offspring, using the same eight hypervariable minisatellite loci analysed in studies by Dubrova and colleagues, failed to detect an increase in the germline mutation rate (Kodaira et al. 1995, 2004). An analysis of sperm DNA from seminoma patients taken both before and after radiotherapy treatment also failed to find an increase in minisatellite mutations (May et al. 2000). Similarly, in a forerunner to the present work, no increase in minisatellite mutations was observed in a pilot study of childhood cancer survivors and their families, which compared those survivors treated with radiotherapy with their unexposed partners (Rees et al. 2006). Finally, the hypothesis that a proportion of childhood leukaemia cases might be associated with an increase in minisatellite germline mutations resulting from parental exposure could not be sustained, when no increase in inherited germline minisatellite mutations was found in children with leukaemia (Davies et al. 2007).

The failure to find an increase in maternal minisatellite mutations in previous studies is thought to reflect the difference in gametogenesis between the two sexes (Dubrova et al. 2002a, 2006). In males the stem cell spermatogonia continue to undergo meiosis to produce mature sperm throughout adult life, whereas in females oocytes are formed during embryogenesis and remain arrested until the onset of puberty. Thus it is postulated that radiation induction of minisatellite mutations in the maternal germline will only be detected in females irradiated during the early stages of gestation. The present study has, therefore, concentrated on male cancer survivors, and extends from 24 families to 100 families an earlier pilot study (Rees et al. 2006), in order to obtain a more precise assessment of the impact of pre-conception gonadal exposure to radiation on minisatellite mutation. The work is part of an ongoing international study investigating adverse reproductive outcomes in childhood and young adult cancer survivors (Boice et al. 2003, www.gcct.org).

Materials and Methods

Study Cohort

Blood samples were collected from Danish survivors of childhood and young adult cancer treated with radiation, their partners and offspring. Patients were identified from the Danish Childhood Cancer Survivor Cohort as previously described (Rees et al. 2006), although for the present study age at cancer diagnosis was increased from 20 to 35 years or under. All participating families gave informed consent. In the pilot study, samples were collected from 28 families and 24 families were successfully screened for minisatellite mutations (Rees et al. 2006). Samples from a further 76 families were obtained in two separate collections of 30 and 46 families. Whole blood was frozen within three hours of collection and shipped to Westlakes Research Institute, UK, for DNA extraction using standard techniques (FlexiGene, Qiagen, West Sussex, UK).

To ensure anonymity, all family samples were coded at the time of collection. Each of the 76 families in this study extension was assigned a study number (T29 – T106), and each sample given an additional suffix -01 and -02 for parents and -03, -04, -05 and so on for offspring. Two families, T69 and T88, who were initially identified, failed to provide samples. All samples were analysed blinded as to which parent was the cancer survivor. Approval for the study was obtained from the Danish Scientific Ethical Committee and the Danish Data Protection Agency.

A different method was adopted for confirmation of maternity, paternity and sample identity to that used previously. Briefly, for families T01 – T28 in the pilot study (Rees et al. 2006) a

polymerase chain reaction (PCR) based assay utilising four stable minisatellite loci, 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) was implemented. However, for families T29 – T106, the Applied Biosystems (Warrington, UK) AmpFlSTR COfiler PCR amplification kit, which amplifies six tetranucleotide short tandem repeat loci (*D3S1358*, *D16S539*, *TH01*, *TPOX*, *CSF1PO*, *D7S820*) plus a segment of the sex-specific *amelogenin* locus was utilised. Semi-automated analysis of PCR products was carried out on an ABI 310 genetic analyser platform, the data files generated were then analysed using Genotyper 2.X software (Applied Biosystems). For both procedures, a mismatch between parent and offspring at two or more loci was considered to be a non-paternity or non-maternity. No mismatches were found.

In the final collection of 46 families, where all the cancer survivors were male, identification analysis was carried out after minisatellite mutation analysis to avoid any bias when scoring mutations. There were no exclusions based on non-paternity or non-maternity and all 76 families were included. The final study population, including the 24 families from the pilot study, comprised 100 families with 170 offspring: 75 fathers were cancer survivors with 129 offspring and 25 mothers were cancer survivors with 41 offspring. Family T92 consisted of a cancer survivor father who had two offspring with his first wife and two offspring with his second wife. This is reflected in the final data set as 75 male cancer survivors with 76 unexposed partners.

Testicular or ovarian 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 modeling (Stovall et al. 2004).

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 provided by Professor Yuri Dubrova, University of Leicester, UK. PCR products were purified and ligated into the cloning vector pGEM-T easy (Promega, Southampton, 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, West Sussex, UK) and probe purified by restriction digest with *Eco* RI (New England Biolabs, Hitchin, UK) followed by gel extraction using a QiaQuick gel extraction kit (Qiagen). MS probes were a gift from Professor Alec Jeffreys, University of Leicester, UK.

Minisatellite mutation analysis

Five micrograms of genomic DNA was digested with restriction enzyme *Alu* I (New England Biolabs) and electrophoresed on a 30 cm 0.8% agarose gel in 1x Tris-Borate-EDTA (ethylenediaminetetraacetic acid) (TBE) buffer (Sigma-Aldrich Company Ltd, Dorset, UK) containing 0.5 μg/ml ethidium bromide (Sigma-Aldrich) overnight at 110V to separate. The DNA was then denatured, neutralised and transferred to a nylon membrane (Magnacharge, Genetic Research Instrumentation, Braintree, UK) where it was fixed by ultra-violet (UV) cross-linking. Minisatellite probes were random prime labelled using a BioPrime DNA labelling system (Invitrogen, Paisley,UK) and hybridised to the immobilised DNA/nylon membrane during an overnight incubation at 42°C. Detection and visualisation of the

resulting DNA bands was achieved using a KPL Detector AP Chemiluminescent Blotting Kit (Insight Biotechnology Limited, Wembley, UK) and exposing the membranes to Hyperfilm (Amersham Biosciences, Little Chalfont, UK) for exposure times of 5 minutes to 6 hours. Blots were independently scored by two different assessors and also digitally using Phoretix 1D software (Non-Linear Dynamics, Newcastle upon Tyne, UK) using a 1 Kb ladder (Promega, Southampton, UK) for size reference across the well resolved 1 – 23 Kb region. Following exposure, membranes were stripped of the hybridised probe in 1 litre of boiling 0.1% sodium dodecyl sulphate (SDS) (Sigma-Aldrich) and stored in 0.03 M sodium citrate, 0.3 M sodium chloride, pH 7 (2xSSC) (Sigma-Aldrich).

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. Any small suspected mutations were run on a second gel for a longer time period to resolve the size difference between parental and offspring bands.

Statistical Analysis

The distribution of mutations was tested for evidence against a Poisson distribution using a χ^2 test. 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, and then dividing by the total number of loci analysed. Individual locus and total mutation rates were compared using Fisher's exact test (two-tailed).

Results

Data on cancer type, age at treatment, radiation dose to the gonads, presence or absence of chemotherapy, number of offspring analysed per family and time from end of treatment to birth of offspring, are presented for exposed fathers in Table I and exposed mothers in Table II. All offspring in this study were conceived after cancer treatment had finished and therefore the dose to the gonads represents the pre-conception gonadal dose. Time from end of treatment to birth of offspring is given to the nearest year. Offspring conceived within the first six months following paternal radiation treatment, and therefore born within 15 months of treatment ending, will be associated with post-spermatogonial irradiation, whereas those born later will be the product of sperm derived from stem cell spermatogonial exposure. A more detailed analysis identified three families of male cancer survivors where one child was born within 15 months of end of treatment: family T29 had a child born 9 months after the end of the father's first session of treatment, family T84 a child born 14 months after treatment and family T87 a child born 9 months after treatment. The first child in family T29 had a paternal mutation as did the later child born three years after completion of all treatment, but the mutations were different. The offspring from families T84 and T87 had no mutations. The remaining 126 offspring born to male cancer survivors were the products of sperm which had developed following spermatogonial irradiation.

Mean parental ages at birth of offspring were similar for the exposed and unexposed parents. Paternal ages were 33 years (range 23 – 51 years) for male cancer survivors and 30 years (range 22 – 47 years) for the unexposed partners of female cancer survivors. Maternal ages were 29 years (range 19 – 36 years) for exposed female cancer survivors and 30 years (range 31 – 42 years) for the unexposed partners of male survivors.

Minisatellite mutation results for 170 offspring from 100 families are presented in Table III. In a few families not all loci were informative and this is reflected in differences in the total number of alleles analysed for each loci. In the study group as a whole, 99 mutations were identified in 170 offspring. Seventy-three mutations were of paternal origin and 26 of maternal origin, resulting in a paternal mutation rate of 5.6% (73 mutations in 1293 alleles) and a maternal mutation rate of 2.0% (26 mutations in 1291 alleles). Of the 73 mutations in paternal alleles, 56 occurred in offspring of fathers exposed to radiation compared to 17 in offspring of unexposed fathers (Table III). Comparison of paternal mutation rates of 5.6% in the exposed fathers (56 mutations in 998 alleles) and 5.8% in the unexposed fathers (17 mutations in 295 alleles) revealed a difference of 0.2% with 95% confidence limits for the difference of −2.7% and 3.9%. The overall rate ratio was 0.97 (95% CI 0.51–1.68). No statistically significant difference was found between the groups, either overall ($p = 0.89$) or at any single locus (Table III). A mutation rate of 1.6% was observed in the exposed mothers (5 mutations in 304 alleles) compared with a mutation rate of 2.1% (21 mutations in 987 alleles) in the unexposed mothers (Table III). The difference was 0.5% with 95% confidence limits of −2.0% and 2.0%. The overall rate ratio was 0.77 (95% CI 0.29–2.05). Again, there was no significant difference between the two groups ($p = 0.82$).

The 129 offspring of exposed fathers were divided into four dose groups of roughly equal numbers with increasing testicular doses (<0.10 Gy, 0.10 – 0.99 Gy, 1.00 – 1.99 Gy, ≥ 2.00 Gy), and the paternal minisatellite mutation rates for the four groups were compared with that in 41 offspring of unexposed fathers (Table IV). No significant differences from control offspring were found with p values of 1.00, 0.85, 0.54 and 1.00 respectively. No indication of a dose-response pattern was seen, i.e., the trend in mutation rates over categories of testicular dose was negative and the p-value for trend was 0.17. Similarly no difference was found between the paternal mutation rate in the offspring of unexposed fathers and the rates in offspring of fathers with additional treatment with chemotherapeutic agents ($p = 0.46$) or those whose fathers had only received radiotherapy ($p = 0.88$) (Table IV). Because the maternally exposed offspring comprised a much smaller group, no subgrouping into dose categories was possible. However, a breakdown of maternal mutations in offspring of mothers who had and had not also received chemotherapy revealed no difference in mutation rates in comparison with offspring of the control group of unexposed mothers ($p = 0.23$ and 0.53 respectively) (Table V).

The distribution of mutations between individual offspring is presented in Table VI. For total mutations there was no deviation from Poisson expectations for the group as a whole ($p = 0.72$) nor for offspring of exposed fathers ($p = 0.72$) or mothers ($p = 0.73$). Subdivision into paternal and maternal mutations revealed no deviation from expectations for paternal mutations for the whole group ($p = 0.81$) nor was there any difference between the distributions in offspring of exposed fathers ($p = 0.66$) and nonexposed fathers (i.e. partners of exposed mothers) ($p = 0.66$). However, maternal mutations were overdispersed in the group as a whole ($p = 0.004$). For the small group of 41 offspring of exposed mothers, there were only 5 maternal mutations in total and the test for Poisson distribution did not reach significance ($p = 0.12$). However, the distribution of mutations in the larger group of 129 offspring of unexposed mothers (i.e. exposed fathers) did suggest overdispersion ($p = 0.016$). Table VII presents the distribution of offspring with paternal mutations in families with two or more offspring. Applying the binomial distribution to derive expected values indicates no deviation from expectations.

Discussion

In a recent review of available information on the association between radiation exposure and germline minisatellite mutations in humans, Bouffler et al. (2006) concluded that the

data are inconsistent and no firm conclusions can be drawn. It is noted that not all studies of exposed human populations have exhibited raised mutation rates. Those populations with elevated rates were living in areas contaminated with radionuclides where the accuracy of dosimetry and the influence of potential confounders are an issue, whereas populations with predominantly external radiation exposure, with robust dose reconstruction methods applied to individuals, have not demonstrated raised rates. Moreover, no reliable dose-response relationships have been demonstrated.

Bouffler et al. (2006) also note that germline mutation at human minisatellite loci is often the result of multiple mutational changes and that no single model can account for the different types of mutation. Whilst initiation by DNA double strand breaks has been postulated, and may therefore indicate a role for radiation exposure, it has also been suggested that the mutation process is driven by staggered nicks into the repeat array leading to intra-allelic exchange, or by meiotic recombination.

Studies of hereditary effects in the offspring of cancer survivors can provide meaningful information on radiation risks (Boice et al. 2003). Survivors of childhood and young adult cancer have detailed dosimetry records that enable accurate assessments of gonadal doses. Thus, in this study, the mean preconception dose to the testes of 1.23 Gy (range 0.00 – 6.40 Gy) for the male survivors and the mean preconception dose to the ovaries for female survivors of 0.58 Gy (range 0.01 – 9.20 Gy) (Tables I and II) can be stated with considerable certainty. However, in an analysis of the mutation rates at the eight most frequently studied hypervariable minisatellite loci, no increase in germline mutation rate was observed in parents who had received radiotherapy as part of their treatment for childhood or young adult cancer. Moreover, detailed analysis of male survivors in four dose groups revealed no evidence of a dose response, i.e., the trend was negative, with all dose groups having similar mutation frequencies which did not differ significantly from the control group.

The negative findings are in agreement with those found in other studies of populations which have been exposed acutely, or within a short time period, and for which the dosimetry is reasonably well defined (May et al. 2000, Livshits et al. 2001, Kiuru et al. 2003, Kodaira et al. 1995, 2004, Furitsu et al. 2005). Moreover, the mean frequencies for paternal mutations in the present study are similar to those obtained in the same laboratory, using the same technique, in a preliminary analysis of minisatellite mutation rates associated with paternal occupational exposure to radiation (Rees et al. 2008). The mean mutation rate for the same eight loci was 5.7% for the control group with a mean occupational preconception dose of 7.5 mSv (range 0.0 – 40.7 mSv) and 5.1% for the exposed workers with a mean occupational preconception dose of 192.6 mSv (range 50.8 – 739.1 mSv). In addition to radiation treatment, some cancer survivors also received treatment with chemotherapeutic agents (Tables I and II) but this was not found to influence mutation frequencies (Tables IV and V).

It has been suggested that minisatellite mutation induction, rather than being the result of a directly targeted insult, may be influenced by a more general genomic instability, which affects meiosis (Dubrova 2003b). Thus some individuals might be resistant to minisatellite mutation induction whereas others are more susceptible. If so, it might be expected that the distribution of mutations between individual offspring may be skewed, with more individuals having two or more mutations than if the mutations were distributed according to chance. Consideration of the distribution of all mutations in all offspring revealed no deviation from Poisson expectations (Table VI). Neither was there any suggestion of overdispersion when paternal mutations were examined in all offspring nor when offspring of exposed and nonexposed fathers were considered separately. Our findings for paternal mutations are in agreement with those of Kodaira et al. (2004) who reported no deviation

from randomness in the distribution of both paternal and maternal mutations in the offspring of atomic bomb survivors. In contrast, in the current study, maternal minisatellite mutations in the group as a whole were significantly overdispersed (Table VI). This deviation from Poisson expectations was also observed in the offspring of unexposed mothers but the number of maternal mutations in the group of offspring of exposed mothers was too small to draw conclusions. The overdispersion observed in maternal mutations between individuals should be viewed with some caution since it is driven by only a few individuals. However, it might be productive to explore this further since it could be postulated that this overdispersion is indicative of individual variation in the stability of the genome during maternal meiosis. Any influences on this would have had to occur whilst the mother was *in utero* since oogenesis occurs during embryogenesis.

Since some families had two or more offspring, the distribution of mutations between families could be examined, and thus the possibility of familial minisatellite mutations or the influence of differences in familial genomic stability could be evaluated. The number of maternal mutations was low and in no family did more than one offspring carry maternal mutations. Of 58 families with two or more offspring, eight families had two or more offspring who carried paternal mutations, seven of these had exposed fathers and one had an exposed mother. However, in no family was the same mutation seen in more than one offspring and the distribution of offspring with paternal mutations between these 58 families (Table VII) did not deviate from random expectations. The one outlier was family T56 which comprised four offspring, all with paternal mutations, born to a male cancer survivor who had been treated for testicular seminoma and received a testicular dose of 0.45 Gy. Overall, however, there was no suggestion of any familial genomic instability.

In summary, this study found no evidence of an increase in germline minisatellite mutations associated with radiation treatment for childhood and young adult cancer. The distribution of paternal mutations between individuals and families did not deviate from random expectations, suggesting that a more general inherited instability affecting mutation induction was unlikely. The finding of overdispersion in the distribution of maternal mutations between individuals, however, may be indicative of a more generalised effect during female meiosis. In light of the small number of maternal mutations, this finding should be viewed with caution and needs to be verified in a larger population. In the search for a measure of radiation-induced germline mutation in man, it is important to develop an understanding of the mechanisms of mutation induction and how these mechanisms might be influenced by radiation exposure. Future studies might be better to focus on molecular genetic changes known to be or suspected as being more specifically associated with disease, which would give a more direct measure of health risk.

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Table I

Male Cancer Survivors. Data on cancer type, age at treatment, testicular dose, presence or absence of chemotherapy, number of offspring analysed per family, time from end of treatment to birth of offspring.

Patient ID	Cancer type	Age at treatment (years)	Dose to testes (Gy)	Chemotherapy (yes/no)	Number of offspring analysed	Time from end of treatment to offspring birth (years)
T03	Rhabdomyosarcoma	9	0.25	yes	2	17, 20
T05	Hodgkin's disease	10	1.20	no	2	16, 18
T08	Neuroblastoma	<1	0.21	no	1	29
T09	Wilms' tumour	7	0.17	yes	2	21, 22
T14	Ewing's sarcoma	7	0.30	no	2	28, 31
T15	Pineocytoma	19	0.23	no	1	9
T17	Germinoma	17	0.17	yes	1	5
T18	Malignant schwannoma	19	<0.01	no	2	13, 15
T19	Hodgkin's disease	17	0.04	no	2	6, 7
T22	Wilms' tumour	1	0.21	yes	1	27
T23	Wilms' tumour	5	0.20	yes	2	21, 26
T29	Hodgkin's disease*	27	0.04, 0.15	no	2	<1, 3
T30	Hodgkin's disease	14	0.34	yes	2	13, 16
T36	Malignant lymphoma	18	0.04	yes	2	10, 16
T40	Malignant lymphoma	10	0.05	yes	2	17, 22
T42	Hodgkin's disease	27	0.03	no	2	6, 8
T43	Hodgkin's disease	29	0.07	yes	1	10
T44	Malignant lymphoma	17	0.08	no	2	10, 12
T47	Hodgkin's disease	31	0.03	yes	1	4
T48	Hodgkin's disease	20	0.04	yes	3	14, 17, 22
T49	Hodgkin's disease	29	0.03	no	2	7, 9
T50	Hodgkin's disease	5	0.08	yes	2	24, 31
T51	Malignant lymphoma	11	0.02	no	2	16, 18
T52	Hodgkin's disease	14	0.04	no	2	10, 12
T53	Testis (seminoma)	33	1.50	no	2	4, 6
T55	Testis (teratoma)	25	1.70	no	1	3
T56	Testis (seminoma)	28	0.45	no	4	2, 5, 7, 11
T57	Testis (teratocarcinoma)	30	2.40	yes	1	4
T58	Testis (seminoma)	25	4.10	no	2	5, 12
T59	Testis (seminoma)	29	2.00	no	1	2
T60	Testis (seminoma)	28	1.30	no	1	4
T61	Testis (embryonal carcinoma)	29	6.40	no	1	13
T62	Testis (seminoma)	26	1.30	no	2	3, 4
T63	Testis (embryonal carcinoma)	23	2.10	no	3	7, 9, 11
T64	Testis (seminoma)	26	1.80	no	2	2, 5
T65	Hodgkin's disease	17	0.03	no	1	16

Patient ID	Cancer type	Age at treatment (years)	Dose to testes (Gy)	Chemotherapy (yes/no)	Number of offspring analysed	Time from end of treatment to offspring birth (years)
T66	Testis (seminoma)	22	2.00	no	2	3, 6
T67	Hodgkin's disease	21	1.50	no	3	7, 11, 16
T68	Testis (seminoma)	29	1.80	no	1	17
T70	Testis (seminoma)	24	1.80	no	2	4, 6
T71	Malignant lymphoma	7	2.40	no	1	18
T72	Testis (seminoma)	24	2.50	no	1	7
T73	Testis (embryonal carcinoma)	23	2.40	yes	3	4, 6, 9
T74	Testis (seminoma)	27	2.00	no	2	4, 7
T75	Testis (seminoma)	30	1.80	no	2	3, 8
T76	Testis (embryonal carcinoma with choriocarcinoma)	27	2.20	yes	1	5
T77	Testis (seminoma)	30	4.90	no	1	7
T78	Testis (teratoma)	26	2.20	no	3	4, 8, 10
T79	Testis (seminoma)	26	1.30	no	2	3, 7
T80	Testis (seminoma)	32	0.47	no	2	4, 10
T81	Hodgkin's disease	30	0.02	yes	1	4
T82	Testis (seminoma)	30	1.80	no	1	8
T83	Hodgkin's disease	31	0.07	yes	1	2
T84	Malignant lymphoma	29	0	no	1	1
T85	Testis (seminoma)	27	5.40	no	3	4, 7, 9
T86	Testis (seminoma)	22	High	no	2	19, 21
T87	Testis (seminoma)	33	0.52	no	1	<1
T89	Hodgkin's disease	22	0.10	yes	1	6
T90	Testis (seminoma)	32	4.00	no	1	5
T91	Hodgkin's disease	22	0.03	yes	1	12
T92	Hodgkin's disease	28	0.04	no	4**	2, 5, 11, 14
T93	Testis (seminoma)	28	0.45	no	2	2, 4
T94	Testis (seminoma)	31	1.80	no	2	7, 11
T95	Testis (teratocarcinoma)	25	2.50	yes	2	9, 11
T96	Testis (seminoma)	28	4.20	no	1	9
T97	Testis (embryonal carcinoma and teratoma)	27	0.51	yes	1	4
T98	Testis (embryonal carcinoma)	32	2.40	yes	2	15, 18
T99	Testis (seminoma)	28	1.80	no	2	3, 8
T100	Hodgkin's disease	30	0.02	yes	2	4, 6
T101	Testis (embryonal carcinoma)	25	2.30	yes	2	10, 14
T102	Testis (embryonal carcinoma and teratoma)	24	2.00	no	1	5
T103	Testis (embryonal carcinoma)	28	2.40	no	1	17
T104	Testis (seminoma)	29	1.80	no	1	3
T105	Hodgkin's disease	23	0.23	no	2	4, 9

Patient ID	Cancer type	Age at treatment (years)	Dose to testes (Gy)	Chemotherapy (yes/no)	Number of offspring analysed	Time from end of treatment to offspring birth (years)
T106	Testis (seminoma)	30	1.80	no	1	8
Mean age at treatment		**23**				
Mean preconceptional testicular dose for all offspring (excluding T86)			**1.23**			
Number offspring with parental chemotherapy yes/no				**39/90**		
Total number of offspring					**129**	
Mean time from treatment to birth of offspring						**10**

* First offspring conceived at end of first treatment period, second offspring conceived after all treatment completed.

** Family 92 consisted of two offspring from each of two mothers.

Table II

Female Cancer Survivors. Data on cancer type, age at treatment, ovarian dose, presence or absence of chemotherapy, number of offspring analysed per family, time from end of treatment to birth of offspring.

Patient ID	Cancer type	Age at treatment (years)	Dose to ovaries (Gy)	Chemotherapy (yes/no)	Number of offspring analysed	Time from end of treatment to offspring birth (years)
T01	Hodgkin's disease	15	0.28	yes	2	18, 19
T02	Hodgkin's disease	11	0.11	no	2	21, 23
T04	Hodgkin's disease/Thyroid cancer	15/30	0.31 *	yes/no	1	2
T06	Teratoma	<1	<0.01	no	3	18, 24, 27
T07	Hodgkin's disease	19	0.08	yes	1	7
T13	Malignant lymphoma **	20	0.05	no	1	10
T16	Hodgkin's disease	20	0.29	yes	1	8
T20	Hodgkin's disease	17	0.29	yes	2	12, 12 ***
T21	Hodgkin's disease	19	0.09	yes	1	6
T24	Malignant lymphoma **	14	0.01	yes	2	10, 13
T25	Neuroblastoma	1	9.20	yes	1	26
T26	Hodgkin's disease	19	0.08	yes	2	4, 7
T28	Wilms' tumour	2	1.70	yes	2	28, 28 ***
T31	Hodgkin's disease	18	0.74	no	2	6, 10
T32	Neuroblastoma	2	0.72	yes	1	25
T33	Malignant lymphoma	10	0.03	no	1	22
T34	Malignant lymphoma	9	3.00	yes	1	20
T35	Malignant lymphoma	9	0.13	no	2	16, 21
T37	Hodgkin's disease	14	0.09	yes	2	9, 11
T38	Hodgkin's disease	15	0.09	yes	2	18, 21
T39	Malignant lymphoma	10	0.03	no	1	17
T41	Hodgkin's disease	19	0.48	no	2	4, 7
T45	Malignant lymphoma	16	0.10	no	1	16
T46	Wilms' tumour	3	0.63	yes	2	22, 28
T54	Hodgkin's disease	16	0.24	no	3	8, 10, 18
Mean age at treatment		**13**				
Mean preconceptional ovarian dose for all offspring			**0.58**			
Number of offspring with parental chemotherapy yes/no				**23/18**		
Total number of offspring					**41**	
Mean time from treatment to birth of offspring						**15**

* Dose to gonads associated with treatment for both malignancies;

** Diagnoses updated since their inclusion in the pilot study (Rees et al. 2006).

*** Offspring were dizygotic twins.

Table III

Minisatellite mutations detected at eight hypervariable minisatellite loci for 170 offspring from 100 cancer survivor families. Is this table somewhat confusing in that the top starts with exposed fathers whereas the bottom starts with the unexposed mothers? Reverse one?

	Exposed Fathers (n = 75) (Male Cancer Survivors)			Unexposed Fathers (n = 25) (Partners of Female Survivors)			*p*
Locus	**No. of Mutations**	**No. of Alleles**	**Mutation Rate (%)**	**No. of Mutations**	**No. of Alleles**	**Mutation Rate (%)**	
B6.7	9	115	7.8	2	36	5.6	1.00
CEB1	25	124	20.2	5	38	13.2	0.47
CEB15	4	125	3.2	2	38	5.3	0.62
CEB25	5	128	3.9	3	37	8.1	0.38
CEB36	3	122	2.5	0	32	0.0	1.00
MS1	6	128	4.7	4	38	10.5	0.24
MS31	4	127	3.1	1	37	2.7	1.00
MS32	0	129	0.0	0	39	0.0	1.00
Total	**56**	**998**	**5.6**	**17**	**295**	**5.8**	**0.89**
Mean Mutation Rate			**5.7**			**5.7**	
	Unexposed Mothers (n = 76 *) (Partners of Male Survivors)			Exposed Mothers (n = 25) (Female Cancer Survivors)			
B6.7	4	117	3.4	1	36	2.8	1.00
CEB1	3	118	2.5	0	38	0.0	1.00
CEB15	2	124	1.6	0	39	0.0	1.00
CEB25	3	126	2.4	0	39	0.0	1.00
CEB36	0	117	0.0	2	38	5.3	0.06
MS1	8	129	6.2	2	39	5.1	1.00
MS31	0	127	0.0	0	37	0.0	1.00
MS32	1	129	0.8	0	38	0.0	1.00
Total	**21**	**987**	**2.1**	**5**	**304**	**1.6**	**0.82**
Mean Mutation Rate			**2.1**			**1.6**	

p = probability Fisher's exact test, two-tailed;

* Family 92 consisted of four offspring from two different mothers

Table IV

Paternal minisatellite mutation rates in offspring of exposed fathers compared to that in offspring of the control group of unexposed fathers, by testicular radiation dose and chemotherapy.

Group	No. offspring	Mean paternal testicular dose (range) (Gy)	Paternal alleles	Paternal mutations	Paternal mutation rate (%)	*p*
Control *	41	0.00	295	17	5.8	-
Total paternal exposed	129	1.23 (0.00 – 6.40) **	998	56	5.6	0.89
Chemotherapy						
+ chemotherapy	39	0.76 (0.02 – 2.50)	304	13	4.3	0.46
− chemotherapy	90	1.43 (0.00 – 6.40) **	694	43	6.2	0.88
Testicular dose						
<0.10 Gy	37	0.04 (0.00 – 0.08)	285	17	6.0	1.00
0.10 – 0.99 Gy	28	0.31 (0.10 – 0.52)	215	14	6.5	0.85
1.00 – 1.99 Gy	27	1.60 (1.20 – 1.80)	208	9	4.3	0.54
≥2.0 Gy	37	2.90 (2.00 – 6.40) **	290	16	5.5	1.00

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

* Offspring of male partners of exposed mothers;

** Mean dose calculated excluded two offspring because of the uncertainty in the testicular dose for one male survivor;

Table V

Maternal minisatellite mutation rates in offspring of exposed mothers compared to that in offspring of the control group of unexposed mothers.

Group	No. offspring	Mean maternal ovarian dose (range) (Gy)	Maternal alleles	Maternal mutations	Maternal mutation rate (%)	*p*
Control *	129	0.00	987	21	2.1	-
Total maternal exposed	41	0.58 (0.01 – 9.20)	304	5	1.6	0.82
Chemotherapy						
+ chemotherapy	23	0.87 (0.01 – 9.20	168	1	0.6	0.23
− chemotherapy	18	0.22 (0.01 – 0.74)	136	4	2.9	0.53

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

* Offspring of female partners of exposed fathers;

Table VI

Distribution of minisatellite mutations between individual offspring.

Study group	No. of mutations					*p*
	0	**1**	**2**	**3**	**4**	
Total offspring						
all mutations	98	51	16	4	1	0.72
paternal mutations	112	45	11	2		0.81
maternal mutations	149	16	5			0.004
Offspring of male cancer survivors						
all mutations	73	40	12	3	1	0.72
paternal mutations	85	34	8	2		0.66
maternal mutations	112	13	4			0.016
Offspring of female cancer survivors						
all mutations	25	11	4	1		0.73
paternal mutations	27	11	3			0.66
maternal mutations	37	3	1			0.12

p = probability of conforming to Poisson expectations using χ^2test.

Table VII

Numbers of offspring with paternal minisatellite mutations in families with two or more offspring.

Family size	Number of families	Number of offspring with mutations (expected*)				
		0	1	2	3	4
2 offspring	48	24 (20.9)	18 (21.5)	6 (5.5)		
3 offspring	8	4 (2.3)	3 (3.6)	1 (0.3)		
4 offspring	2	0 (0.37)	1 (0.78)	0 (0.6)	0 (0.21)	1 (0.03)

* expected values based on binomial distribution