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## The heritability of G<sub>2</sub> chromosomal radiosensitivity and its association with cancer in Danish cancer survivors and their offspring

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### Abstract

**Purpose**—To investigate the relationship between chromosomal radiosensitivity and early-onset cancer under age 35 years and to examine the heritability of chromosomal radiosensitivity.

**Materials and methods**—Peripheral blood lymphocytes were cultured for 72 hours prior to being irradiated with 0.5 Gy, 300 kV X-rays. Colcemid was added to cultures 30 minutes post-irradiation. Cultures were harvested 90 minutes post-irradiation and analysed for chromatid gaps and breaks. Heritability was estimated using Sequential Oligogenic Linkage Analysis Routines (SOLAR) software and by segregation analysis.

**Results**—Elevated radiosensitivity was seen for 7 out of 29 (24.1%) cancer survivors, 3 out of 29 (10.3%) partners and 10 out of 53 (20.8%) offspring. Although the proportion of individuals displaying enhanced radiosensitivity was twice as high in both the cancer survivor and offspring groups than the partner controls, neither reached statistical significance. Heritability analysis of the radiosensitive phenotype suggested 57.9 – 78.0% of the variance could be attributed to genetic factors.

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#### Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

**Conclusions**—An association between G<sub>2</sub> chromosomal radiosensitivity and childhood and young adult cancer is suggested but was not statistically significant. In contrast, there is strong evidence for heritability of the radiosensitive phenotype. The cancer survivors included a broad range of malignancies and future studies should focus on specific cancers with known or likely faults in deoxyribonucleic acid (DNA) damage recognition and repair mechanisms.

### Keywords

Chromosomal radiosensitivity; heritability; cancer

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### Introduction

The association of G<sub>2</sub> chromosomal radiosensitivity with cancer predisposition has been demonstrated for a number of common cancers (Scott et al. 1994, Scott et al. 1996, Scott et al. 1999, Scott 2000, Baria et al. 2001, Riches et al. 2001, Baeyens et al. 2002, Baria et al. 2002, Howe et al. 2005, Lisowska et al. 2006, De Ruyck et al. 2008) with many studies concluding that G<sub>2</sub> chromosomal radiosensitivity may be a marker of inherited, low-penetrance cancer predisposition genes (Scott et al. 1999, Scott 2000, Baria et al. 2001, Scott 2004, Howe et al. 2005). Heritability of the radiosensitive phenotype has been demonstrated in healthy first-degree relatives of cancer patients (Roberts et al. 1999). Furthermore, enhanced G<sub>2</sub> chromosomal radiosensitivity has been shown to be associated with early-onset cancers (Papworth et al. 2001, Baria et al. 2002), thus supporting the premise that such cancers have a genetic inherited component. Consequently, it has been postulated that inheritance of low-penetrance genes may indeed function to predispose young individuals to cancer. We previously reported on the relationship between chromosomal radiosensitivity and early-onset cancer in a population of 23 Danish childhood and adolescent cancer survivors, their partners and offspring using the G<sub>2</sub> assay of radiation-induced chromatid breakage (Curwen et al. 2005). Failure to distinguish between the G<sub>2</sub> aberration profiles of the apparently normal partners and the cancer survivors suggested the lack of an association between chromosomal radiosensitivity and cancer predisposition, although questions were raised as to the suitability of the partner group as controls. Moreover, analysis of the Danish families did suggest that the radiosensitive phenotype was a heritable trait. In the current study, we examined an additional 30 Danish families for G<sub>2</sub> chromosomal radiosensitivity in an attempt to clarify the relationship with predisposition to early-onset cancer and to investigate heritability of the radiosensitive phenotype.

### Methods

#### Study group

Blood samples were obtained from 30 Danish survivors of childhood and young adult cancer who had been treated with radiotherapy under age 35 years, their partners and 55 out of a potential of 57 offspring born after treatment. This work forms part of an ongoing international study of trans-generational effects of cancer treatment in children and young adults (Boice et al. 2003) ([www.gcct.org](http://www.gcct.org)). Patients were invited to participate on the basis of their young age at diagnosis and having received a high gonadal radiation dose. This resulted in the group having a range of malignancies, including Hodgkin lymphoma, testicular cancer and Wilms' tumour. Selection criteria were the same as previously described (Curwen et al. 2005) with the exception that age at diagnosis was increased from under 20 years to under 35 years. To ensure anonymity, each family was assigned a study number (T29 – T58) and samples were further coded to avoid identification of cancer survivor, partner and offspring within each family group. A total of 115 blood samples were collected from the families and sent to the United Kingdom (UK) in eight shipments

between June and December 2006. In addition, blood samples were collected at every shipment from two healthy adult Danish volunteers as controls for the transportation system. Repeat blood samples from one healthy adult in-house volunteer were obtained in parallel to the eight shipments as the internal assay control. The culture failure of T36 meant that only 29 of the Danish families were available for chromosome analysis giving a total of 111 family samples. Approval for the study was obtained from the Danish Scientific Ethical Committee and the Danish Data Protection Agency.

Sample identity was confirmed using the Applied Biosystems AmpFISTR COfiler polymerase chain reaction (PCR) amplification kit (Applied Biosystems, Warrington, UK). The kit provides human identification by amplifying six tetranucleotide short tandem repeat loci (*D3S1358*, *D16S539*, *TH01*, *TPOX*, *CSF1PO*, *D7S820*) plus a segment of the sex-specific *amelogenin* locus. PCR products were analysed on a semi-automated ABI 310 genetic analyser platform (Applied Biosystems), the data files generated were then run through Genotyper 2.X (Applied Biosystems) software to automate the genotyping process. A mismatch between parent and offspring at two or more loci was considered to be a non-paternity or non-maternity. No cases of non-paternity or non-maternity were observed.

### The G<sub>2</sub> assay

The G<sub>2</sub> assay was performed as previously described (Curwen et al. 2005) and is based on a modified version of that reported by Scott et al. (1999). Peripheral blood was drawn into lithium heparin vacutainers (Fisher Scientific, Leicestershire, UK) and cultures (2ml whole blood) were set up in pre-warmed (37°C) and pre-gassed (5% CO<sub>2</sub>/95% air) Roswell Park Memorial Institute (RPMI-1640) medium (Sigma Aldrich, Dorset, UK) supplemented with 15% foetal calf serum (Invitrogen Limited, Paisley, UK), 1% L-glutamine (Invitrogen Limited) and 1% phytohaemagglutinin (M-form) (Invitrogen Limited) to a total volume of 20ml. Each culture was set up in duplicate, one flask (VWR International, Leicestershire, UK) for the determination of the spontaneous aberration frequency and one to be irradiated for the determination of the induced aberration frequency. A single foetal calf serum batch was used for the entire period of the study. Flasks were placed upright in a humidified, CO<sub>2</sub>-gassed incubator (Binder, Tuttlingen, Germany). After 48 hours, 15ml of medium was carefully removed and replaced with 15ml of fresh pre-gassed, pre-warmed medium. At 72 hours the cells were transported in a portable incubator (M-Tech Diagnostics, Warrington, UK) at 37°C and irradiated (or sham irradiated) with 0.5 Gy, 300 kV X-rays using a Siefert Isovolt 320 X-ray set (Aegleteq Ltd, Bucks, UK). Following a 30 minute recovery time, 0.2ml colcemid (10µg/ml) (Invitrogen Limited) was added and at 90 minutes after irradiation the contents of the culture flasks were transferred to 13.5ml conical base centrifuge tubes (Sterilin Ltd, Carerphilly, UK) and placed on ice. Subsequent centrifugation, hypotonic treatment (0.075M potassium chloride (KCl)) (VWR International) and fixation (methanol:glacial acetic acid, 3:1) (VWR International) were carried out at 4°C. Fixed cells were stored at -20°C overnight, or longer, prior to slide making.

Metaphase slides were made according to standard procedures and stained with Giemsa (VWR International). Slides from the family blood samples together with those from the transport and internal assay control samples were further coded prior to cytogenetic analysis. For each irradiated sample, 100 well-spread metaphases were analysed and the total number of chromatid gaps and breaks determined to give a G<sub>2</sub> aberration yield. Chromatid-type aberrations were scored according to previously outlined criteria (Smart et al. 2003), with breaks being defined as mis-aligned discontinuities and gaps as single aligned discontinuities wider than the width of a chromatid. Two scorers were used to reduce scorer bias, with each scorer analysing 50 metaphases per sample. Previous studies using the G<sub>2</sub> assay in our laboratory have shown the spontaneous aberration yield to be at a minimal level

(<1.0 aberration per 100 cells). On this basis, and due to the additional time required to score control samples, it was decided that it was not necessary to determine spontaneous aberration yields. However, control sample slides were available for each individual to investigate any spurious results. For comparisons with the previous study (Curwen et al. 2005), data were re-analysed using induced aberration yields from the irradiated samples only.

### Statistical methods

The distributions of chromatid type aberrations amongst cells in each population (cancer survivors, partner controls, offspring, internal assay control, transport control 1 and transport control 2) were analysed for approximation to the Poisson distribution using the dispersion index test of Papworth, as previously described by Savage (1970). If the observed distributions followed Poisson statistics, the variance and the mean (number of aberrations divided by the number of metaphase cells studied) of the observed distributions would be equal and a ratio value of 1.0 would be expected. If the variance is greater than the mean, this indicates that aberrations are overdispersed and a Poisson distribution does not apply. Ratios of variance to mean for each sample provided by a donor were calculated and population ratios were determined using the average value.

Chi-squared ( $\chi^2$ ) analysis was used to investigate intra-individual variation in the transport and internal assay controls, adopting the formula  $\chi^2 = (O-E)^2/E$ , where O is the observed value of aberrations per 100 cells and E is the expected value of total aberrations per 100 cells. Overdispersion of distributions of aberrations amongst metaphase cells was corrected for in each population by multiplying the expected value of total aberrations per 100 cells (E) in the denominator of the equation for chi-squared by a compensation factor (Z = average value of ratio of variance to mean) and so the formula  $\chi^2 = (O - E)^2/(EZ)$  was adopted. Chi-squared analysis was also used to assess inter-individual variation within each population with overdispersion taken into account as above.

In all cases, standard errors (SE) were calculated by adjusting for overdispersion of chromatid aberrations using the appropriate compensation factor (Z) equated for each population (Z = average value of ratio of variance to mean, calculated for each population). In addition, for the internal assay and transport controls (where repeat sampling had occurred), any additional intra-individual variation introduced was considered. This was estimated by adding all the values of chi-squared for those individuals who were sampled more than once and dividing by the total degrees of freedom and is termed Y. Standard errors were thus calculated according to the formula  $(\text{Number of aberrations} \times Z \times Y)$ , and normalised to 100 cells scored.

The non-parametric Mann-Whitney U-test was used to compare median induced aberration frequencies between controls and the other donor groups. The 90<sup>th</sup> percentile value of the aberration yield for the partner control group was determined and used as the cut-off point for determining the proportions of radiosensitive individuals in the other groups. This method was introduced by Scott et al. (1999) and, although the 90<sup>th</sup> percentile is an arbitrary value, it has resulted in good discrimination and been adopted in the majority of subsequent G<sub>2</sub> chromosomal radiosensitivity studies. Fisher's exact tests were used to compare the proportion of radiosensitive individuals in each of the three family groups.

Heritability was estimated using Sequential Oligogenic Linkage Analysis Routines (SOLAR) software, which is an extensive, flexible software package for genetic variance components analysis (Almasy and Blangero 1998). SOLAR assumes a normal distribution for the trait, given genotype. We also performed segregation analysis using a regressive model as implemented in the Statistical Analysis for Genetic Epidemiology software

(S.A.G.E. v6.0.1) as previously described (Curwen et al. 2005). In this approach, the residuals from the genotype means are assumed to be normally distributed, after Box-Cox transformation. Different disease transmission models (parameterised by  $g$ , the probability that a parent of genotype  $g$  transmits allele A to his/her offspring) and different models for the genotype means (unrestricted, dominant, additive) were compared by maximum likelihood and by use of the Akaike information criterion (AIC). Gender was included as a covariate although this was not statistically significant in any model. A parameter representing residual polygenic correlation between the family members (in addition to correlation induced by the putative major gene) was also included. For the most parsimonious model, we estimated the heritability as the ratio of the genetic variance (the variability of genotype means across the putative genotypes) to the total phenotypic variance.

## Results

### Internal assay and transport controls

Table I illustrates the characteristics and  $G_2$  aberration frequencies for the internal assay and transport control samples. The ratio of variance to mean indicated overdispersion of aberration yields compared with Poisson expectations and adjustment was made for this as described in the Methods section. The mean aberration yield for the internal assay control was  $113.6 \pm 3.4$  per 100 cells and a coefficient of variation (CV) of 20.7% was determined for intra-individual variability. Chi-squared analysis revealed a statistically significant difference between the samples ( $\chi^2_6 = 18.74, P = 0.005$ ). Analysis of the samples collected from the two individuals as controls for the transportation system also indicated significant intra-individual variation ( $\chi^2_6 = 42.95, P < 0.001$ ), ( $\chi^2_4 = 30.85, P < 0.001$ ). Mean induced aberration yields for these individuals were  $131.3 \pm 5.9$  and  $124.8 \pm 7.5$ , respectively. Moreover, sequential sampling of these individuals produced CV of 31.2% and 29.9%, respectively.

### Cancer survivors, partners and offspring

Details of the childhood and young adult cancer survivors, their partners and offspring are presented in Table II, together with their respective  $G_2$  aberration yields. Again the test for Poisson distribution indicated overdispersion in all groups. Results were considered as groups (cancer survivors, partner controls and offspring) with information on group characteristics being provided in Table III. Comparison of median aberration frequencies revealed no statistically significant difference between the partner control group and the cancer survivor group ( $P = 0.576$ ) nor between the partner control group and the offspring group ( $P = 0.497$ ). Furthermore, mean aberration frequencies for the three groups were similar.

Distributions of the  $G_2$  aberration frequencies for the three groups are illustrated in Figure 1. The cut-off point for a radiosensitive/non-radiosensitive response, as determined by the 90<sup>th</sup> percentile for the partner control group, was 167.8 aberrations per 100 cells and resulted in 24.1, 10.3 and 20.8% of the cancer survivors, partners and offspring displaying an enhanced radiosensitive response, respectively (Table III). An illustration of parentage is provided with offspring being identified on the basis of 0, 1 or 2 parents who had radiosensitivity scores greater than the 90<sup>th</sup> percentile of the partner group (Figure 1). Whilst the proportion of individuals displaying elevated sensitivity in both the cancer survivor and offspring groups was greater than that of the partner control group, neither reached statistical significance when using the partner group for comparison ( $P = 0.297, P = 0.358$ , respectively).

## Heritability analysis

Analysis of the induced G<sub>2</sub> aberration frequency data from the 29 families, performed using the SOLAR software v4.2.0, estimated heritability to be  $0.780 \pm 0.12$  ( $P < 0.001$ ). Table IV provides details of heritability of the radiosensitive phenotype as investigated by segregation analysis. There was borderline evidence to reject the non-genetic sporadic model in comparison with the general transmission model using a likelihood ratio test ( $P = 0.070$ ), indicating the general model fitted the data significantly better than the sporadic model. The Mendelian model was not rejected when compared to the general transmission model ( $P = 0.436$ ), indicating the general model did not show a significantly better fit to the data than the Mendelian model. In addition, the Mendelian model had the lowest Akaike information criterion (AIC) value compared to both the general and sporadic models. This suggested that the data were better fit by the Mendelian model, which should be accepted over both the general and sporadic models. Based on the AIC criterion, further comparison of the Mendelian model (AIC 1071.51) with an autosomal dominant model (AIC 1074.01) and an additive model (AIC 1073.77) also suggested the unrestricted Mendelian model provided the best fit for the data. The most parsimonious model was a Mendelian model with no gender covariate (AIC 1069.64). With genotype frequencies of 0.044, 0.332, 0.623 and genotype means of 131.00, 169.96 and 117.94 respectively (Table IV), the genetic variance attributable to the putative major locus under this model becomes 587.54, with a residual variance of 426.75, resulting in a heritability estimate of  $587.54 / (587.54 + 426.75) = 0.579$ , slightly lower than the estimate provided by SOLAR.

## Discussion

The G<sub>2</sub> chromosomal radiosensitivity assay requires stringent technical conditions to produce reproducible results. Problems associated with blood sample storage and transport conditions, in particular transit over long distances, have been reported (Scott et al. 1996, Scott et al. 1999, Bryant et al. 2002). In contrast, our laboratory and others have found blood storage conditions to have no effect on aberration yield (Scott et al. 1996, Scott et al. 1999, Bryant et al. 2002, Smart et al. 2003). Additionally, transportation over short distances has not been found to have an effect on reproducibility (Roberts et al. 1999, Riches et al. 2001, Smart et al. 2003). High standards of intrinsic assay reproducibility have previously been demonstrated in our laboratory (Smart et al. 2003) as well as by others using the technique (Vral et al. 2002).

The same internal control individual was used in this and the previous study (Curwen et al. 2005). However, whilst in the previous study repeated sampling indicated no significant intra-individual variability and the CV was 13.6%, repeated sampling of the same healthy adult donor throughout the present study period revealed significant variation in aberration yield and a CV of 20.7%. Nevertheless, the mean aberration yield of  $113.6 \pm 3.4$  per 100 cells was similar to that of  $108.0 \pm 2.4$  per 100 cells derived from the previous study data using the revised scoring criteria. Samples collected from two donors to monitor the transportation system showed even greater intra-individual variation with CV of 31.2% and 29.9%, respectively. Moreover, the mean aberration frequency for transport control 1 of  $131.3 \pm 5.9$  per 100 cells was significantly greater than that of  $88.9 \pm 2.4$  per 100 cells for the same individual recalculated from the previous study ( $P = 0.036$ , two-tailed student's *t*-test). Intra-individual variability was also reported for this transport control in the previous study, although it was speculated that this could be attributable to hormonal influences associated with pregnancy (Curwen et al. 2005). It is of interest that the mean aberration frequencies of all three controls were less than the radiosensitive cut-off point of 167.8 per 100 cells determined from the partner data, although one of the seven samples from transport control 1 did have a higher value. The samples were sent from Denmark at ambient temperature, by road and by air and in the possession of a personal courier at all times, to

ensure that they were not subjected to significant temperature fluctuations and thus minimise any potential transportation effect. The three repeatedly sampled controls showed no common pattern of high or low G<sub>2</sub> scores associated with specific sampling times, nor with high or low scores determined for cancer family samples taken and processed at the same time (data not shown), suggesting that factors not intrinsic to the G<sub>2</sub> assay, such as varying characteristics of the individual, are more likely than assay variability to be responsible for the intra-individual variation. Changes in hormone levels have been reported to influence *in vitro* radiosensitivity (Roberts et al. 1997, Ricoul et al. 1998), although this has been disputed in more recent studies (Baeyens et al. 2005). Nevertheless, since all three controls were female the influence of fluctuating hormonal levels cannot be ruled out.

Whilst some studies of G<sub>2</sub> chromosomal radiosensitivity have reported no significant intra-individual variability with CV in the range of 7.0 – 10.3% (Roberts et al. 1999, Scott et al. 1999, Baria et al. 2001, Papworth et al. 2001), a number of studies have experienced problems with high intra-individual variability, and some laboratories have reported levels of variation for some individuals not significantly different to inter-individual variability (Baria et al. 2002, Vral et al. 2002, Smart et al. 2003, Vral et al. 2004, Howe et al. 2005). This has led to suggestions that the G<sub>2</sub> assay is unlikely to detect any real differences in chromosomal radiosensitivity between individuals within a normal population (Vral et al. 2002, Smart et al. 2003, Vral et al. 2004, Howe et al. 2005).

Analysis of the three family groups revealed a greater proportion of radiosensitive individuals in the cancer survivor (24.1%) and offspring (20.8%) groups than in the partner control group (10.3%) but no comparisons reached statistical significance. Similarly, no statistically significant differences in median or mean aberration yields were found. In our previous study of a group of 23 childhood cancer survivors, their partners and their 38 offspring, no association between G<sub>2</sub> chromosomal radiosensitivity and predisposition to cancer was observed when using the 90<sup>th</sup> percentile value of the partner control group as the cut-off point for a radiosensitive/non-radiosensitive response. However, comparisons with an in-house control group did suggest heightened radiosensitivity profiles in the cancer survivor and offspring groups respectively (Curwen et al. 2005). A re-analysis of the previous data using the revised scoring criteria (i.e. induced score without subtraction of the spontaneous score) gave a slightly different radiosensitive cut-off point for the partners of 162.4 aberrations per 100 cells, and this is similar to that of 167.8 aberrations per 100 cells obtained in the current study. However, the current study does show a distinction in the radiosensitivity profiles between the three groups, with both cancer survivor and offspring groups demonstrating higher proportions of radiosensitive individuals than the partner control group, although these increases do not reach statistical significance.

In addition to sample size limitations, the failure to find a clear-cut relationship between G<sub>2</sub> chromosomal radiosensitivity and cancer predisposition may reflect, in part, the nature of the malignancies selected for study. Whilst early-onset cancer is often associated with inherited susceptibility, in this study there is a preponderance of haematological disorders, which may have a stronger environmental than heritable aetiology (Hemminki and Bermejo 2005, Hemminki et al. 2005). Nevertheless, a hereditary component to non-Hodgkin and Hodgkin lymphoma has been suggested within twin and family studies (Schottenfeld and Fraumeni 2006). Although this familial aggregation may relate to immune system functionality, a role for deoxyribonucleic acid (DNA) repair mechanisms seems possible at least for Hodgkin lymphoma (Schottenfeld and Fraumeni 2006) and it is noted that six of the 22 cases of haematological cancers (27.3%) did exhibit enhanced radiosensitivity. Specifically, in the current study four out of 14 survivors of Hodgkin lymphoma exhibited enhanced radiosensitivity compared to four out of ten in the previous study (Curwen et al.

2005) and three out of six reported by Baria et al. (2002). Only one out of seven cases of non-haematological cancers in the current study exhibited elevated radiosensitivity.

Direct support for the suggestion that chromosomal radiosensitivity has a heritable component has come from studies on breast cancer patients and their families (Roberts et al. 1999, Scott 2000). First-degree relatives of radiosensitive breast cancer patients were found to be more radiosensitive compared with first-degree relatives of breast cancer patients with normal responses to radiation. An indication that the trait has a heritable component can be seen in the distribution of offspring radiosensitivity scores in relation to the radiosensitivity status of their parents, using the 90<sup>th</sup> percentile of the partner distribution to distinguish those with an elevated response (Figure 1). However, although the cut-off point of the 90<sup>th</sup> percentile is an easy visual way to illustrate and test the differences in radiosensitivity between different groups, it has no real bearing on the heritability analysis which examines different models for goodness of fit to the data using the G<sub>2</sub> assay (aberration frequency per 100 cells) as a quantitative trait outcome. Segregation analysis suggested that a model with a single major gene, with two alleles combining in an additive manner to give three phenotypes, could account for 82.0% of the variability in G<sub>2</sub> sensitivity. Heritability of the phenotype was also demonstrated in our previous study of childhood and adolescent cancer survivors and their families (Curwen et al. 2005). In that study, 67.3% of the variance of G<sub>2</sub> chromosomal radiosensitivity could be attributed to a major gene locus with dominant effect. This was confirmed in the SOLAR analysis which indicated a value of 60.7%.

Heritability analysis in the current study performed using both the SOLAR software and segregation analysis indicated that the G<sub>2</sub> chromosomal radiosensitive phenotype was a heritable trait, with SOLAR suggesting strong heritability. SOLAR analysis suggested an estimate of heritability of 78.0% and segregation analysis 57.9%. However, in the current study, segregation analysis indicated that the unrestricted Mendelian model (with no gender effect) was the most parsimonious, unlike in the previous study (Curwen et al. 2005) for which the most parsimonious model was an autosomal dominant model with no residual correlation (and no gender effect). Given the complexity of segregation analysis and the large number of parameters that are estimated (using a relatively small number of individuals with relatively few different relationship types), one should beware of over-interpreting these results. However, the results are certainly consistent with an underlying genetic basis for this phenotype.

## Conclusion

In conclusion, this study has provided suggestive, albeit not conclusive, evidence of an association between chromosomal radiosensitivity and cancer predisposition. The failure to demonstrate a statistically significant difference in radiosensitive profiles between cancer survivors and their partners may reflect in part the types of malignancy studied and/or sample size limitations. The finding of significant intra-individual variation in the three repeatedly sampled controls is a concern and indicates that reliance cannot be placed on a single individual result. Nevertheless, the study provides further evidence for heritability of the G<sub>2</sub> chromosomal radiosensitive phenotype and suggests that future studies should focus on specific cancer types which are most likely to have a fault in DNA damage recognition and repair mechanisms.

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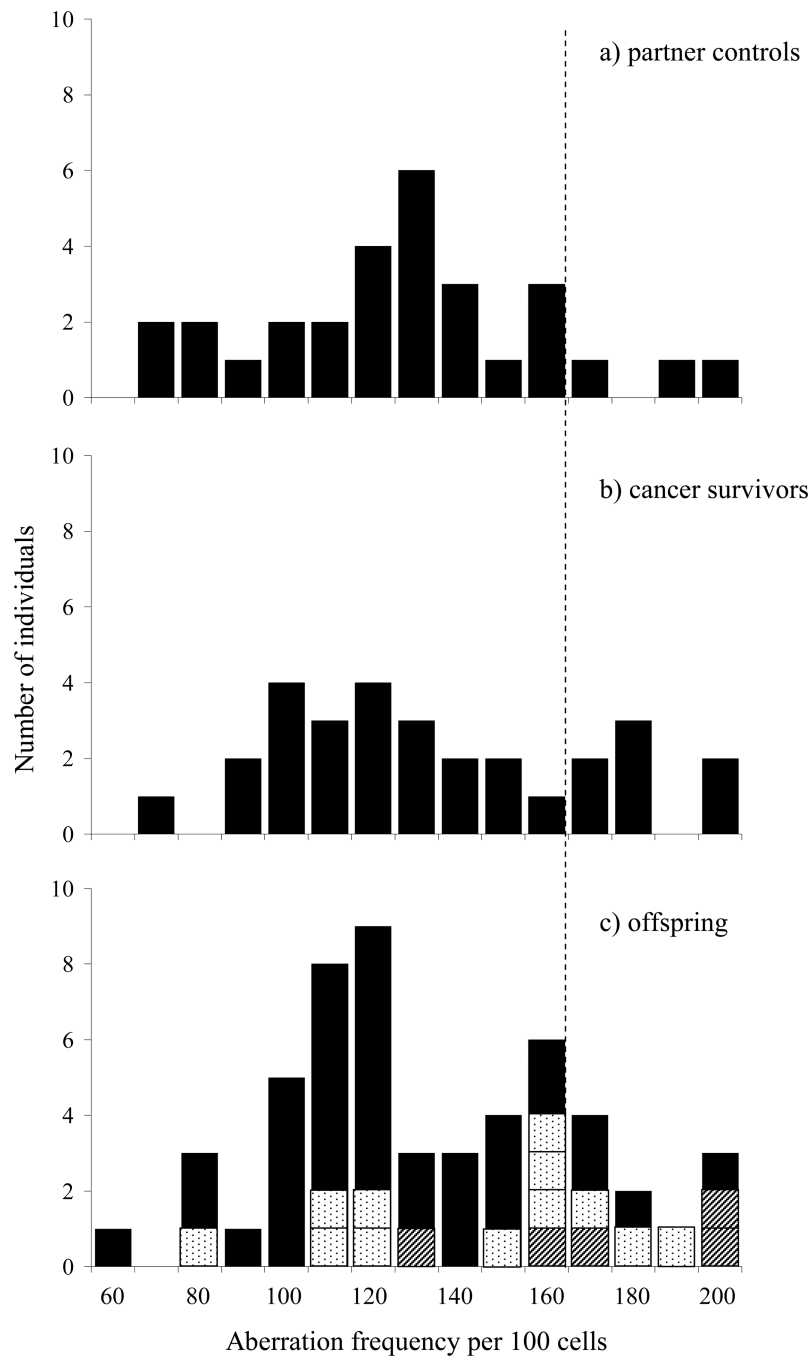


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**Figure 1.** Radiation-induced G<sub>2</sub> chromatid aberration frequencies. The dotted vertical line represents the cut-off point for a radiosensitive/non-radiosensitive response (167.8 aberrations per 100 cells) as determined by the 90<sup>th</sup> percentile of the partner control group: a) partners, b) survivors of childhood and young adult cancer, c) offspring with 0 ■ 1 ▨ 2 ▩ parents displaying elevated radiosensitivity.

Table 1

Characteristics of internal assay and transport control donors and radiation-induced G<sub>2</sub> chromatid aberration frequencies

Donor	Sex	Age at first sampling (years)	Aberrations per 100 cells at sampling shipment								Ratio of variance to mean (average value)	Mean aberration frequency $\pm$ SE per 100 cells	CV (%)
			1	2	3	4	5	6	7	8			
Internal assay control	F	41	132	126	150	85	-	97	111	94	1.55	113.6 $\pm$ 3.4	20.7
Transport control 1	F	41	149	123	-	75	160	143	82	187	1.78	131.3 $\pm$ 5.9	31.2
Transport control 2	F	56	148	152	-	61	-	-	123	140	1.45	124.8 $\pm$ 7.5	29.9

SE = standard error; CV = coefficient of variation, F = female, - = sample failed/not available.

Individual characteristics of childhood and young adult cancer survivors, their partners and offspring and radiation-induced G<sub>2</sub> aberration yields

**Table II**

Family code	Survivor					Partner			Offspring <sup>a</sup>	
	Gender	Primary cancer	Age at time of treatment (years)	Age at time of sampling (years)	Aberrations per 100 cells	Age at time of sampling (years)	Aberrations per 100 cells	Age at time of sampling (years)	Aberrations per 100 cells	
T29	M	Hodgkin	27	41	147	44	130	14	162	
T30	M	Hodgkin	14	45	102	43	131	12	202	
T31	F	Hodgkin	18	47	153	50	130	18	136	
T32	F	Neuroblastoma	2	32	123	37	103	15	125	
T33	F	Lymphosarcoma	10	62	123	75	167	23	137	
T34	F	Lymphocytic lymphosarcoma	9	41	74	40	113	19	148	
T35	F	Reticulosarcoma	9	44	110	43	143	6	171	
T37	F	Hodgkin	14	34	170	34	130	30	118	
T38	F	Hodgkin	15	41	142	53	144	12	101	
T39	F	Lymphosarcoma	10	61	95	62	95	18	82	
T40	M	Lymphosarcoma	10	43	172	41	75	14	105	
T41	F	Hodgkin	19	61	114	63	122	12	127	
T42	M	Hodgkin	27	48	135	48	166	9	167	
T43	M	Hodgkin	29	41	127	38	86	8	114	
T44	M	Reticulosarcoma	17	56	107	52	121	5	125	
T45	F	Reticulosarcoma	16	55	105	63	87	34	98	

Family code	Survivor				Partner				Offspring <sup>a</sup>	
	Gender	Primary cancer	Age at time of treatment (years)	Age at time of sampling (years)	Aberrations per 100 cells	Age at time of sampling (years)	Aberrations per 100 cells	Age at time of sampling (years)		Aberrations per 100 cells
T46	F	Wilms'	3	32	183	32	79	7	128	
T47	M	Hodgkin	31	43	98	42	101	8	160	
T48	M	Hodgkin	20	52	204	45	204	18	101	
T49	M	Hodgkin	29	47	187	40	167	11	160	
T50	M	Hodgkin	5	38	124	38	171	9	207	
T51	M	Lymphosarcoma	11	60	180	54	199	33	178	
T52	M	Hodgkin	14	50	206	47	122	26	156	
T53	M	Testis (seminoma)	33	68	101	63	137	32	180	
T54	F	Hodgkin	16	54	133	57	141	29	197	
T55	M	Testis (teratoma)	25	61	113	59	154	33	173	
T56	M	Testis (seminoma)	28	55	134	55	118	24	133	
T57	M	Testis (teratocarcinoma)	30	58	165	57	129	24	115	
T58	M	Testis (seminoma)	25	52	152	59	137	22	168	
Ratio of variance to mean (average value for each population)										
					1.73				1.78	1.62

M = male; F = female.

<sup>2</sup>Offspring born after cancer treatment of survivor in birth order.

Characteristics of childhood and young adult cancer survivor, partner and offspring groups and radiation-induced G<sub>2</sub> chromatid aberration frequencies

**Table III**

Group	Number of individuals	Male/female	Median age in years (range)	Median aberration frequency per 100 cells (range)	<i>P</i> <sup>1</sup>	Mean aberration frequency ± SE per 100 cells	Sensitive response		
							Number of individuals	%	<i>P</i> <sup>2</sup>
Cancer survivors	29	17/12	48 (32–68)	133.0 (74–206)	0.576	137.2 ± 2.9	7	24.1	0.297
Partners	29	12/17	48 (32–75)	130.0 (75–204)	-	131.1 ± 2.8	3	10.3	-
Offspring	53	29/24	18 (1–41)	128.0 (66–207)	0.497	137.4 ± 2.1	11	20.8	0.358

<sup>1</sup> Significance value obtained when using the non-parametric Mann-Whitney U-test to compare the median induced aberration frequency of the partner controls group with those of the cancer survivor and offspring groups, respectively.

<sup>2</sup> Significance value obtained when using Fisher's exact tests to compare the number of individuals displaying a radiosensitive response in the partner controls group with those of the cancer survivor and offspring groups, respectively.



Table IV

Model parameters from segregation analysis of G<sub>2</sub> chromosomal radiosensitivity in 29 Danish families of survivors of childhood and young adult cancer

Parameter	Models analysed			
	General	Equal (sporadic)	Mendelian	Mendelian (no gender effect)
$Q_A$	0.499 ± 0.083	0.217 ± 0.208	0.210 ± 0.055	0.210 ± 0.055
$AA$	0.249 ± 0.082	0.047 ± 0.090	0.044 ± 0.023	0.044 ± 0.023
$AB$	0.499 ± 0.000	0.340 ± 0.234	0.332 ± 0.064	0.332 ± 0.064
$BB$	0.250 ± 0.083	0.611 ± 0.325	0.623 ± 0.087	0.623 ± 0.087
$AA$	0.852 ± 0.120	$Q_A$	1.000	1.000
$AB$	0.000	$Q_A$	0.500	0.500
$BB$	1.000	$Q_A$	0.000	0.000
$AA$	167.29 ± 7.18	151.99 ± 53.89	131.19 ± 12.46	131.00 ± 12.53
$AB$	118.35 ± 5.53	151.99 ± 19.10	169.87 ± 6.03	169.96 ± 6.00
$BB$	124.26 ± 8.26	120.17 ± 9.12	117.89 ± 4.05	117.94 ± 4.07
Residual variance	575.12 ± 123.21	750.20 ± 220.17	425.72 ± 96.65	426.75 ± 97.32
(Box-Cox parameter)	0.90 ± 0.36	0.54 ± 0.49	1.12 ± 0.35	1.13 ± 0.36
Residual $\rho_{o=ss}$	0.539 ± 0.053	0.510 ± 0.068	0.386 ± 0.104	0.388 ± 0.104
Gender covariance	2.54 ± 4.58	-1.96 ± 5.34	-1.54 ± 4.36	-
$-2 \ln L$	1053.89	1060.96	1055.51	1055.64
AIC	1071.89	1076.96	1071.51	1069.64
$P$ -value	-	0.070 <sup><i>I</i></sup>	0.436 <sup><i>I</i></sup>	-

A = dominant allele; B = recessive allele;  $Q_A$  = frequency of dominant allele; = genotype frequencies; = transmission probabilities; = genotype dependent mean (induced aberration frequency per 100 cells);  $\rho_o$  = correlation between parent of offspring pairs,  $\rho_{ss}$  = correlation between siblings; AIC = Akaike information criterion

<sup>*I*</sup> likelihood ratio test compared with the general model