Published in final edited form as:

Environ Mol Mutagen. 2011 January ; 52(1): 72–76. doi:10.1002/em.20633.

A study of DNA damage recognition and repair gene polymorphisms in relation to cancer predisposition and G₂ chromosomal radiosensitivity

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Abstract

The previously reported association of the *APEX* Asp148Glu SNP with cancer and the suggestion of associations of the *XRCC3* Thr241Met and *hOGG1* Ser326Cys SNP sites with G₂ chromosomal radiosensitivity were investigated in a new study of 30 childhood and young adult cancer survivors, their 30 partners and 55 offspring. An additional SNP, *hOGG1* Arg46Gln, was also analysed. Data on G₂ chromosomal radiosensitivity was available on 29 of the families including 53 offspring. No significant associations of genotype with cancer or G₂ chromosomal radiosensitivity were observed.

Keywords

Chromosome aberrations; DNA repair genes; cancer susceptibility

INTRODUCTION

The association of G₂ chromosomal radiosensitivity with cancer predisposition has been demonstrated for a number of common cancers (see Curwen et al. 2010 for review). Heritability of the radiosensitive phenotype has also been reported in several family studies (Roberts et al. 1999; Scott 2000; Curwen et al. 2005, Curwen et al. 2010) indicating that variation in radiosensitivity is influenced by genetic factors. These observations suggest that G₂ chromosomal radiosensitivity may be a marker of inherited, low-penetrance cancer predisposition genes whose role is to respond to DNA damage (Bondy et al. 2001; Baria et al. 2002; Scott 2004; Howe et al. 2005).

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The exact genetic mechanisms underlying inter-individual differences in radiosensitivity remain unknown. The importance of the role of DNA repair in the protection of the genome and prevention of cancer is however recognised (Goode et al. 2002). The G₂ chromosomal radiosensitivity assay indirectly measures the ability of cells to repair DNA damage induced by ionising radiation exposure, hence polymorphic variation in DNA damage recognition and repair genes may explain the genetic basis of inter-individual variation. Indeed, several polymorphic variants in DNA repair genes have already been reported to influence the response to both *in vivo* and *in vitro* ionising radiation susceptibility (see Wilding et al. 2007 and Iarmarcovai et al. 2007 for reviews).

The aim of the current study was to extend earlier work which investigated 16 polymorphisms in nine DNA repair genes in a group of 23 Danish childhood and adolescent cancer survivors, their 23 partners and 37 offspring, who were also being studied for G₂ chromosomal radiosensitivity (Wilding et al. 2007). An additional 30 cancer survivors and their families were studied for G₂ chromosomal radiosensitivity (Curwen et al. 2010) and this enabled further investigation of the three single nucleotide polymorphisms (SNPs) which produced suggestive results in the earlier study, i.e. *APEX* Asp148Glu, *hOGG1* Ser326Cys, and *XRCC3* Thr241Met. An additional SNP, *hOGG1* Arg46Gln, was also examined.

MATERIALS AND METHODS

Study group

Blood was obtained from 30 Danish survivors of cancer who had been treated with radiotherapy, their partners and 55 of 57 offspring, giving a total of 115 samples. Patients were selected using criteria applied previously (Curwen et al. 2005) with the exception that age at diagnosis of cancer was increased to under 35 years. Blood was drawn into vacutainers for transportation to Westlakes Research Institute, UK, for the study of G₂ chromosomal radiosensitivity and genotyping. Full details of the study group have been reported (Curwen et al. 2010). For the study of polymorphism analysis, DNA was extracted using a FlexiGene DNA Purification Kit (Qiagen, Qiagen House, Fleming Way, Crawley, West Sussex, RH10 9NQ, UK). Sample identity was confirmed using the AmpFISTR COfiler PCR amplification kit (Applied Biosystems, Lingley House, 120 Birchwood Boulevard, Warrington, WA3 7QH, UK) (Curwen et al. 2010). Approval for the study was obtained from the Danish Scientific Ethical Committee and the Danish Data Protection Agency.

G₂ assay

The G₂ chromosomal radiosensitivity data used in this report come from Curwen et al. (2010). Briefly, whole blood samples were cultured for 72 hours, irradiated with 0.5 Gy 300kV X-rays and, following a further 1.5 hours incubation, harvested for chromosome analysis. To determine the G₂ radiosensitivity score, 100 well-spread metaphases were analysed for chromatid-type aberrations according to standard criteria (Sanford et al. 1989; Scott et al. 1999).

Molecular analysis

Information on the four SNPs studied is provided in Table I. All SNPs were examined using polymerase chain reaction (PCR) restriction fragment length polymorphism (RFLP) analysis, with genotypes determined by agarose gel electrophoresis and quality control measures as reported previously (Wilding et al. 2007).

Statistical analysis

Conformity of genotype frequencies with their respective Hardy-Weinberg expectations (HWEs) was tested at each locus (Wilding et al. 2007). Genotype data were analysed for population differences using the Genepop (4.0.10) software (Raymond and Rousset, 1995; Rousset, 2008) (<http://genepop.curtin.edu.au/>). Association of genetic polymorphism with G₂ radiosensitivity was measured by pooling all data into a single sample and analysis of variance (ANOVA) was performed to test if the mean G₂ radiosensitivity scores were significantly different across genotypes for each SNP site.

RESULTS

Genotype and allele frequencies plus HWEs for the loci examined are presented in Table II. Analyses show no deviations from HWEs in any population examined. The *hOGG1* Arg46Gln site lacked any homozygote mutants and, therefore, the exact test could not be performed.

Table III shows a summary of the results obtained from heterogeneity testing at each of the loci examined. *P*-values indicate that genotype frequencies did not differ between survivors, partners and offspring, or between survivors and partners or parents and their offspring. A combined analysis with the results of the previous study (Wilding et al. 2007) also failed to reveal any differences.

G₂ chromosomal radiosensitivity data for one family was unavailable due to laboratory failure, leaving 29 families with a total of 53 offspring available for ANOVA tests (Table IV). No statistically significant differences in G₂ radiosensitivity scores were observed among the genotypes. In contrast to the analysis of genotype with respect to family subgroups, a combined analysis of the present G₂ chromosomal radiosensitivity results with those of Wilding et al. (2007) was considered inappropriate because of the possibility of different experimental conditions influencing G₂ scores. In a further analysis, the genotypic profile of the subgroup of 21 individuals with heightened chromosomal radiosensitivity as determined by the 90th percentile cut-off for the partner control group G₂ scores (Curwen et al. 2010) was compared with that for the group of 90 non-sensitive individuals (Table V). No differences were observed. Moreover, a combined analysis with results from the study group reported by Wilding et al. (2007) (which used the chromosome data and associated 90th percentile cut-off point of Curwen et al. 2005), revealed no differences in genotypic profile between the total group of 27 radiosensitive individuals and the total group of 167 non-sensitive individuals.

DISCUSSION

The literature detailing studies investigating the association of SNPs within DNA damage recognition and repair genes with cancer is extensive (Kiyohara et al. 2002; Benhamou and Sarasin 2005). Whilst many studies fail to find significant associations, others have reported conflicting results. In an earlier study of 23 childhood and adolescent cancer survivors and their families (Wilding et al. 2007), we reported an analysis of 16 polymorphisms (13 SNPs and three microsatellite) in 9 DNA repair genes. A significant association of genotype with cancer at the *APEX* Asp148Glu SNP site was observed, this being due to the enhanced frequency of the Asp148 allele among cancer survivors in comparison with their partners. Statistical significance remained after correction for multiple testing. However, the present study of 29 families of childhood and young adult cancer survivors failed to confirm this association as did a combined analysis of the 53 families from both studies (Table III). The earlier study (Wilding et al. 2007) also reported an association of genotype with G₂ chromosomal radiosensitivity for the *XRCC3* Thr241Met site. Individuals with the wild-

type homozygous genotype displayed a higher mean G₂ score, suggesting that the Met241 mutant variant might have a protective role upon the genome. In addition, the *hOGG1* Ser326Cys site revealed a weak association with G₂ score. However, neither association with G₂ chromosomal radiosensitivity remained significant following multiple test adjustment. The current study has found neither association between genotype and G₂ score (Table IV) nor were there any differences in genotype profile when those with heightened chromosomal radiosensitivity were compared with the rest of the group (Table V). This study has therefore failed to confirm the previously reported associations.

The childhood and young adulthood cancer survivors who participated in this and the earlier study (Wilding et al. 2007) were treated for a range of cancer types with a preponderance of haematological and lymphopoietic disorders. Such malignancies have a stronger environmental aetiology than a genetic aetiology with any heritable influence predominantly related to immune function (Schottenfeld and Fraumeni 2006). This might argue against a strong association with inherited variation in DNA repair mechanisms or impact on radiosensitivity. Future studies should focus on specific cancer types which are more likely to have a fault in DNA damage recognition and repair mechanisms.

The exact mechanisms underlying inter-individual variation in susceptibility to radiation exposure and its association with cancer susceptibility are likely to be dependent on many polymorphic genetic variants that have a combined effect on multiple cellular pathways. The major challenge will be to identify the correct combination of SNPs to investigate in the many low-penetrance genes that are the cause of such variation. This study illustrates the limitations of reporting sporadic associations in small populations and the value of replication studies, and highlights the need for large-scale studies using high throughput multiple array technology on populations with well-defined specific conditions.

Acknowledgments

The authors thank the cancer survivors and their families for participating in this study, Brian Møllgren, Rigshospitalet, for collection of blood samples from the families and Professor Per Guldberg, Institute of Cancer Biology, Danish Cancer Society, who is in charge of the Danish Biobank. 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 and [H-KF] 01 – 150/01). We are grateful for the support provided by the Danish Cancer Society. This work was funded by a grant from the National Institutes of Health, U.S.A. (Grant Number 1 RO1 CA 104666) through Vanderbilt Medical Centre, USA and a student internship from the University of Central Lancashire, UK.

REFERENCES

- Baria K, Warren C, Eden OB, Roberts SA, West CM, Scott D. Chromosomal radiosensitivity in young cancer patients: possible evidence of genetic predisposition. *Int J Radiat Biol.* 2002; 78:341–346. [PubMed: 12020425]
- Benhamou S, Sarasin A. ERCC2 / XPD gene polymorphisms and lung cancer: a HuGE review. *Am J Epidemiol.* 2005; 161:1–14. [PubMed: 15615908]
- Bondy ML, Wang LE, El-Zein R, de Andrade M, Selvan MS, Bruner JM, Levin VA, Alfred Yung WK, Adatto P, Wei Q. Gamma-radiation sensitivity and risk of glioma. *J Natl Cancer Inst.* 2001; 93:1553–1557. [PubMed: 11604478]
- Curwen GB, Cadwell KK, Winther JF, Tawn EJ, Rees GS, Olsen JH, Rechnitzer C, Schroder H, Guldberg P, Cordell HJ, Boice JD. The heritability of G₂ chromosomal radiosensitivity and its association with cancer in Danish childhood and young adult cancer survivors and their offspring. *Int J Radiat Biol.* 2010 in press.
- Curwen GB, Winther JF, Tawn EJ, Smart V, Whitehouse CA, Rees GS, Olsen JH, Guldberg P, Rechnitzer C, Schroder H, Bryant PE, Sheng X, Lee HS, Chakraborty R, Boice JD. G₂ chromosomal radiosensitivity in Danish survivors of childhood and adolescent cancer and their offspring. *Br J Cancer.* 2005; 93:1038–1045. [PubMed: 16234827]

- Goode EL, Ulrich CM, Potter JD. Polymorphisms in DNA repair genes and associations with cancer risk. *Cancer Epidemiol Biomarkers Prev.* 2002; 11:1513–1530. [PubMed: 12496039]
- Howe O, Daly P, Seymour C, Ormiston W, Nolan C, Mothersill C. Elevated G₂ chromosomal radiosensitivity in Irish breast cancer patients: a comparison with other studies. *Int J Radiat Biol.* 2005; 81:373–378. [PubMed: 16076752]
- Hu JJ, Smith TR, Miller MS, Mohrenweiser HW, Golden A, Case LD. Amino acid substitution variants of APE1 and XRCC1 genes associated with ionizing radiation sensitivity. *Carcinogenesis.* 2001; 22:917–922. [PubMed: 11375899]
- Iarmarcovai G, Bonassi S, Botta A, Baan RA, Orsiere T. Genetic polymorphisms and micronucleus formation: A review of the literature. *Mutat Res.* 2007; 658:215–233. [PubMed: 18037339]
- Kiyohara C, Otsu A, Shirakawa T, Fukuda S, Hopkin JM. Genetic polymorphisms and lung cancer susceptibility: a review. *Lung Cancer.* 2002; 37:241–256. [PubMed: 12234692]
- Raymond M, Rousset F. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *J Heredity.* 1995; 86:248–249.
- Roberts SA, Spreadborough AR, Bulman B, Barber JB, Evans DG, Scott D. Heritability of cellular radiosensitivity: a marker of low-penetrance predisposition genes in breast cancer? *Am J Hum Genet.* 1999; 65:784–794. [PubMed: 10441587]
- Rousset F. Genepop'007: a complete reimplement of the Genepop software for Windows and Linux. *Mol Ecol Resources.* 2008; 8:103–106.
- Sanford KK, Parshad R, Gantt R, Tarone RE, Jones GM, Price FM. Factors affecting and significance of G₂ chromatin radiosensitivity in predisposition to cancer. *Int J Radiat Biol.* 1989; 55:963–981. [PubMed: 2567334]
- Schottenfeld, D.; Fraumeni, JF, Jr. *Cancer epidemiology and prevention.* 3rd ed. UK: Oxford University Press; 2006.
- Scott D. Chromosomal radiosensitivity, cancer predisposition and response to radiotherapy. *Strahlentherapie Onkologie.* 2000; 176:229–234.
- Scott D. Chromosomal radiosensitivity and low penetrance predisposition to cancer. *Cytogenet Gen Res.* 2004; 104:365–370.
- Scott D, Barber JB, Spreadborough AR, Burrill W, Roberts SA. Increased chromosomal radiosensitivity in breast cancer patients: a comparison of two assays. *Int J Radiat Biol.* 1999; 75:1–10. [PubMed: 9972785]
- Shen MR, Jones IM, Mohrenweiser H. Nonconservative amino acid substitution variants exist at polymorphic frequency in DNA repair genes in healthy humans. *Cancer Res.* 1998; 58:604–608. [PubMed: 9485007]
- Smart V, Curwen GB, Whitehouse CA, Edwards A, Tawn EJ. Chromosomal radiosensitivity: a study of the chromosomal G₂ assay in human blood lymphocytes indicating significant inter-individual variability. *Mutat Res.* 2003; 528:105–110. [PubMed: 12873728]
- Wikman H, Risch A, Klimek F, Schmezer P, Spiegelhalter B, Dienemann H, Kayser K, Schulz V, Drings P, Bartsch H. hOGG1 polymorphism and loss of heterozygosity (LOH): significance for lung cancer susceptibility in a caucasian population. *Int J Cancer.* 2000; 88:932–937. [PubMed: 11093817]
- Wilding CS, Curwen GB, Tawn EJ, Sheng X, Winther JF, Chakraborty R, Boice JD Jr. Influence of polymorphisms at loci encoding DNA repair proteins on cancer susceptibility and G₂ chromosomal radiosensitivity. *Environ Mol Mutagen.* 2007; 48:48–57. [PubMed: 17177211]

TABLE I

Details of the SNPs and references to the primers used.

Pathway	Gene	Base change	dbSNP reference	Amino acid change	Method
BER ^a	<i>APEX</i>	T G	Rs17858508	Asp148Glu	(Hu et al. 2001)
	<i>hOGG1</i>	C G	Rs1052133	Ser326Cys	(Wikman et al. 2000)
	<i>hOGG1</i>	G A	n/a	Arg46Gln	(Wikman et al. 2000)
HRR ^b	<i>XRCC3</i>	C T	rs861539	Thr241Met	(Shen et al. 1998)

^aBER = base excision repair,
^bHRR = homologous recombinational repair.

Observed genotype frequencies and allele frequencies in cancer survivors, partners and offspring.

TABLE II

Gene	WT/WT ^a	WT/M ^a	M/M ^a	f(WT) ^b	f(M) ^b	P _{HWE} ^c
<i>APEX</i> Asp148Glu	Genotype	T/T	T/G	G/G	f(G)	
	Survivors (n = 30)	4	20	6	0.46	0.53
	Partners (n = 30)	7	18	5	0.53	0.46
<i>hOGG1</i> Ser326Cys	Offspring (n = 55)	13	26	16	0.49	0.51
	Genotype	C/C	C/G	G/G	f(G)	
	Survivors (n = 30)	18	11	1	0.78	0.22
<i>hOGG1</i> Arg46Gln	Partners (n = 30)	23	6	1	0.87	0.13
	Offspring (n = 55)	39	13	3	0.83	0.17
	Genotype	G/G	G/A	A/A	f(A)	
<i>XRCC3</i> Thr241Met	Survivors (n = 30)	27	3	0	0.95	0.05
	Partners (n = 30)	28	2	0	0.97	0.03
	Offspring (n = 55)	52	3	0	0.97	0.03
<i>XRCC3</i> Thr241Met	Genotype	C/C	C/T	T/T	f(T)	
	Survivors (n = 30)	14	15	1	0.72	0.28
	Partners (n = 30)	9	17	4	0.58	0.42
	Offspring (n = 55)	29	22	4	0.73	0.27

^aWT = wild type, M = mutant;

^bf(WT) = wild type allele frequency, f(M) = mutant allele frequency;

^cProbability of observed genotype using exact method (Hardy-Weinberg expectations).

TABLE III

Heterogeneity test of genotype frequencies among cancer survivors, partners and offspring.

Gene	<i>P</i> -values (exact test)		
	Survivors, partners, and offspring	Survivors vs. partners	Parents vs. offspring
<i>APEX</i> Asp148Glu	0.727 (0.241)	0.585 (0.171)	0.699 (0.484)
<i>hOGG1</i> Ser326Cys	0.475 (0.125)	0.339 (0.081)	1.000 (0.707)
<i>hOGG1</i> Arg46Gln	0.895 (1.000)	1.000 (1.000)	0.727 (0.758)
<i>XRCC3</i> Thr241Met	0.144 (0.125)	0.184 (0.195)	0.263 (0.157)

Figures in brackets () indicate results from combined analyses of current and earlier (Wilding et al. 2007) study data sets.

TABLE IV

Mean G₂ radiosensitivity scores^a ± standard deviations (SD), and ANOVA results of G₂ radiosensitivity scores and SNPs.

Gene	WT/WT ^b	WT/M ^b	M/M ^b	ANOVA result	
				R ² (%)	P - value
<i>APEX</i> Asp148Glu	Genotype	T/T	T/G	G/G	
	Number of individuals	24	60	27	0.319
	Mean G ₂ score ± SD	141.92 ± 34.75	131.33 ± 30.23	139.96 ± 38.34	
<i>hOGG1</i> Ser326Cys	Genotype	C/C	C/G	G/G	
	Number of individuals	76	30	5	0.198
	Mean G ₂ score ± SD	137.61 ± 35.97	128.00 ± 26.41	153.40 ± 19.91	
<i>hOGG1</i> Arg46Gln	Genotype	G/G	G/A	A/A	
	Number of individuals	103	8	0	0.898
	Mean G ₂ score ± SD	135.83 ± 34.40	134.25 ± 15.73	-	
<i>XRCC3</i> Thr241Met	Genotype	C/C	C/T	T/T	
	Number of individuals	50	52	9	0.388
	Mean G ₂ score ± SD	137.20 ± 32.91	136.85 ± 33.65	121.00 ± 34.47	

^a chromatid aberrations per 100 cells;

^b WT = wild type, M = mutant.

TABLE V

Heterogeneity test of genotype frequencies amongst radiosensitive and non-sensitive individuals.

Gene	<i>P</i> -values (exact test)
	Radiosensitive v non-sensitive ^a
<i>APEX</i> Asp148Glu	1.000 (0.884)
<i>hOGG1</i> Ser326Cys	0.127 (0.369)
<i>hOGG1</i> Arg46Gln	0.227 (0.258)
<i>XRCC3</i> Thr241Met	1.000 (1.000)

^aRadiosensitive individuals are defined as those with chromatid aberration scores the 90th percentile cut-off point for the partner control group.

Figures in brackets () indicate results from combined analyses of current and earlier (Wilding et al. 2007) study data sets.