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Influence of Polymorphisms at Loci Encoding DNA Repair Proteins on Cancer Susceptibility and G₂ Chromosomal Radiosensitivity

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Abstract

Sixteen candidate polymorphisms (13 SNPs and 3 microsatellites) in nine genes from four DNA repair pathways were examined in 83 subjects, comprising 23 survivors of childhood cancer, their 23 partners, and 37 offspring, all of whom had previously been studied for G₂ chromosomal radiosensitivity. Genotype at the Asp148Glu SNP site in the APEX gene of the base excision repair (BER) pathway was associated with childhood cancer in survivors ($P = 0.001$, significant even after multiple test adjustment), due to the enhanced frequency of the APEX Asp148 allele among survivors in comparison to that of their partners. Analysis of variance (ANOVA) of G₂ radiosensitivity in the pooled sample, as well as family-based association test (FBAT) of the family-wise data, showed sporadic suggestions of associations between G₂ radiosensitivity and polymorphisms at two sites (the Thr241-Met SNP site in the *XRCC3* gene of the homologous recombination pathway by ANOVA, and the Ser326Cys site in the *hOGG1* gene of the BER pathway by FBAT analysis), but neither of these remained significant after multiple-test adjustment. This pilot study provides an intriguing indication that DNA repair gene polymorphisms may underlie cancer susceptibility and variation in radiosensitivity. *Environ. Mol. Mutagen.* 48:48–57, 2007.

Keywords

chromosomal radiosensitivity; DNA repair genes; cancer susceptibility

INTRODUCTION

In vitro chromosomal radiosensitivity assays measure individual variation in sensitivity to ionizing radiation. The cell cycle based G₂ chromosomal radiosensitivity assay, which provides the best discrimination, uses irradiation in the G₂ phase of the cell cycle to induce DNA damage. Unrepaired damage is seen at the subsequent metaphase as chromatid gaps and breaks. The assay was originally developed in fibroblasts as a possible measure of cancer predisposition

[Parshad et al., 1983; Sanford et al., 1989] and subsequently adapted for lymphocytes [Scott et al., 1996]. Enhanced G₂ chromosomal radiosensitivity has been detected in several well-defined cancer susceptibility syndromes, most notably ataxia telangiectasia [Parshad and Sanford, 2001]. Increased G₂ chromosomal radiosensitivity also has been reported in a high proportion of patients with a diverse range of cancers [Scott et al., 1994a; Parshad et al., 1996; Patel et al., 1997; Scott et al., 1999; Terzoudi et al., 2000; Bondy et al., 2001; Riches et al., 2001; Baeyens et al., 2002; Baria et al., 2002] leading to suggestions that it could be a marker of cancer predisposing genes of low penetrance whose role is to respond to DNA damage [Scott et al., 1999; Bondy et al., 2001; Baria et al., 2002]. Support for the heritability of chromosomal radiosensitivity across generations can be inferred from family studies [Roberts et al., 1999; Scott, 2000; Curwen et al., 2005]. Heritability of sensitivity to the radiomimetic bleomycin also has been demonstrated [Cloos et al., 1999].

Polymorphisms of genes encoding components of DNA repair pathways represent strong candidates to explain the genetic basis of chromosomal radiosensitivity. Ionizing radiation can induce a variety of lesions in the DNA, and to protect the integrity of the genome, cells can invoke a number of DNA repair pathways. Single strand breaks are repaired chiefly through the base excision repair pathway (BER) whilst double strand breaks are dealt with through either homologous recombinational repair (HRR), where the undamaged copy in the homologous chromosome or sister chromatid is used as a template for repair, or via nonhomologous end joining (NHEJ) [Hoeijmakers, 2001; Jackson, 2002; Slupphaug et al., 2003]. Polymorphic variation in DNA repair genes has been reported to influence the response to in vitro irradiation as measured by cell cycle delay [Hu et al., 2001], cytogenetic damage [Lunn et al., 2000; Au et al., 2003; Marcon et al., 2003], and DNA single strand break induction and repair using the Comet assay [Aka et al., 2004; Godderis et al., 2004; Rzeszowska-Wolny et al., 2005]. However, whilst variation in DNA repair gene polymorphisms has been reported to influence the in vivo frequencies of micronuclei [Angelini et al., 2005] and unstable chromosome aberrations [Kiuru et al., 2005] following low dose irradiation, no such effect was found when stable translocations were used as the endpoint [Kiuru et al., 2005; Wilding et al., 2005].

Using a variety of endpoints, variant DNA repair genes have been shown to influence baseline and smoking-related levels of DNA damage (for review see [Au et al., 2004]) and to be implicated in cancer predisposition (for review see [Goode et al., 2002]). Approximately 150 human DNA repair genes have been identified to date [Wood et al., 2005], and a large number of polymorphisms within these genes have been discovered through resequencing [Mohrenweiser et al., 2003]. The DNA double strand break is the recognized principal initiating lesion leading to chromosome and chromatid aberrations but inadequate single strand break repair can also act as a precursor to double strand breaks. Deficiencies in a variety of DNA repair pathways are therefore potential contributors to elevated radiosensitivity as measured with the G₂ chromosomal radiosensitivity assay and it is likely that variation at DNA repair gene loci may explain some of the interindividual variation in radiosensitivity. In this report we describe variation at 13 single nucleotide polymorphisms (SNPs) and 3 microsatellite repeat regions in 9 genes involved in DNA repair in a group of survivors of childhood cancer, their partners, and their offspring. These subjects have previously been studied for G₂ chromosomal radiosensitivity [Curwen et al., 2005].

Specifically, we have studied four polymorphic genes in the BER pathway coding for XRCC1, ADPRT, APEX and hOGG1. XRCC1 interacts with ADPRT (PARP) to form a complex which binds to, and is activated by, single strand breaks [Hoeijmakers, 2001] and APEX is involved in the excision of abasic sites [Hoeijmakers, 2001], which are then repaired by an XRCC1/DNA polymerase β complex. hOGG1 is specifically concerned with the removal of 8-hydroxyguanine resulting from the action of reactive oxygen species (formed via ionizing

radiation-induced hydrolysis). Variation at two genes, *XRCC4* and *XRCC5*, within the NHEJ pathway also has been analyzed. *XRCC4* forms a complex with DNA ligase IV which binds to, and links, the ends of DNA, whilst *XRCC5* (Ku 86) contributes to NHEJ as part of the Ku p70/p86 dimer which acts to stabilize and bring together broken DNA ends [Mohrenweiser et al., 2003]. In the HRR pathway, we studied the genes encoding *XRCC2* and *XRCC3* which complex with *RAD51c* and *RAD51b* in double strand break and crosslink repair [Mohrenweiser et al., 2003]. Polymorphic variation of the *XPD* gene, which is involved in transcription and the nuclear excision repair (NER) pathway, also was examined since variation at codon 751 has previously been reported to influence G₂ chromosomal radiosensitivity [Lunn et al., 2000]. This work forms part of a pilot study for the investigation of a range of genetic endpoints associated with germ cell mutagenesis and cancer susceptibility [Boice et al., 2003].

MATERIALS AND METHODS

Study Group

Blood samples were obtained from Danish childhood and adolescent cancer survivors treated with radiotherapy, their partners, and their offspring. Selection criteria for patients have been described previously [Curwen et al., 2005]. In total, blood samples were received from 100 individuals (28 cancer survivors, 28 partners, and 44 offspring). Blood was drawn into two lithium heparin vacutainers for transportation to Westlakes Research Institute for the G₂ assay. DNA was extracted from an additional blood sample in Denmark using a Puregene kit (Gentra Systems, Minneapolis, MN) and transported by courier to Westlakes Research Institute.

Approval for the study was obtained from the Danish Scientific Ethical Committee and the Danish Data Protection Agency. Informed consent was obtained from each family. To ensure anonymity, each family was assigned a study number and subject number to avoid identification of the cancer survivor, partner, and offspring within each family group.

G₂ Assay

The methodology for the G₂ chromosomal radiosensitivity assay has been described previously [Smart et al., 2003; Curwen et al., 2005]. Briefly, 2 ml whole blood were added to 18 ml prewarmed and pregassed (5% CO₂, 95% air) RPMI 1640 medium (Sigma, Dorset, UK) supplemented with 15% fetal bovine serum (Invitrogen, Paisley, UK), 1% L-glutamine (Invitrogen) and 1% phytohemagglutinin (M-form) (Invitrogen). The medium was changed after 48 hr. At 72 hr, cultures were irradiated with 0.5 Gy 300 kV X-rays or were sham-irradiated (controls). After a further 0.5 hr incubation, 0.2 ml colcemid (10 µg/ml) (Invitrogen) was added and the incubation continued for 1 hr. At 1.5 hr postirradiation, the cultures were plunged into ice and the cells were treated with a hypotonic solution (0.075 M KCl), and fixed (3:1 methanol:acetic acid). Slides were prepared according to standard procedures and Giemsa stained [Rooney, 2001]. Chromatid-type aberrations (gaps and breaks) were scored from 100 well-spread metaphases according to criteria outlined by [Sandford et al., 1989] and [Scott et al., 1999].

Molecular Analysis

Thirteen SNPs and 3 microsatellite repeats in 9 DNA repair genes were studied. SNPs (including dbSNP reference numbers) and references to primers (MWG, London, UK) and methods employed for their analysis are given in Table I, by grouping the sites examined by their genes and pathways. All SNPs except *XRCC2* Pro36Ser were examined using polymerase chain reaction (PCR) restriction fragment length polymorphism (RFLP) analysis, with genotypes determined by agarose gel electrophoresis (Sigma) or genotyping on an ABI Prism 310 platform (Applied Biosystems, Warrington, UK). Real-time PCR and allelic

discrimination using an ABI Prism 7000 plate reader was used to investigate XRCC2 Pro36Ser (Applied Biosystems). Typing of an $[AC]_n$ repeat in the 3' UTR of *XRCC1*, an $[AC]_n$ repeat in intron 3 of *XRCC3* and a $[GAPyA]_n$ repeat 120 kb 5' of *XRCC5* utilized the primers of Price et al. [1997] (MWG), although microsatellite sizing was undertaken via a multiplex PCR using fluorescently labeled primers followed by size discrimination of PCR products on an ABI Prism 310 platform as described by Wilding et al. [2005]. The silent substitution in *XRCC4* was amplified with the primer pair XRCC4T921GF 5'-TCT CTA AAC CAA TTT GAA ACA GGA-3' and XRCC4 T921GR 5'-CAG ACA GGA TGT TGG ACA GC-3' (based on Outside reverse primer of Ford et al. [2000]) (MWG).

For quality control purposes, positive and negative controls were used in all assays. Positive controls were DNA samples from a newborn cohort that had been genotyped previously for the same range of SNPs [Wilding et al., 2006]. Genotyping was undertaken blind and 10% of all samples were repeated. On completion of genotyping of the cohort, for QA purposes all assigned genotypes were checked and 10% checked again by an independent third party.

Statistical Analysis

Genotype data from 16 polymorphic sites (13 SNPs and 3 microsatellites) were first analyzed for genotype/allele frequency differences among: (i) survivors of childhood cancers (survivors), their partners, and their offspring; (ii) survivors versus their partners; and (iii) parents (pooled data on survivors and their partners) and their offspring. These analyses were performed by considering the genotype data in the relevant groups in the form of $r \times c$ contingency tables, for each of which the empirical levels of significance (P -values) were computed by the permutation test using the algorithm described in Roff and Bentzen [1989]. For each test, P -values were obtained from 10,000 replications of permutations. For the SNP loci, these tests were conducted with genotype data, but since a large number of possible genotypes for the three microsatellite loci remained unobserved for the sample size of our study, such heterogeneity tests for the three microsatellite loci were conducted with allele count data alone. Because of codominant alleles at all loci, allele frequencies were computed by the gene count method from the genotype data [Li, 1976]. Conformity of genotype frequencies with their respective Hardy-Weinberg expectations (HWEs) was tested for each locus by the exact test method as described by Guo and Thompson [1992] and as implemented in the Arlequin software v.3 (<http://cmpg.unibe.ch/software/arlequin3>).

Association of genetic polymorphism with G_2 radiosensitivity was measured in two ways. First, data on all individuals (survivors, partners, and offspring) were pooled to form a single sample, for which analysis of variance (ANOVA) was used to test if the mean values of G_2 sensitivity were significantly different across genotypes for each polymorphic site. The one-way ANOVA routine (SAS version 8.1 for Windows; SAS Institute, Cary, NC) yielded R^2 values (i.e., proportion of variance explained by genotypic difference) and P -values (for ANOVA), indicating any possible signature of association. Second, noting that the genotype as well as G_2 sensitivity scores for the children are correlated with the same of their parents (both survivors and partners), tests of no-association and no-linkage were performed by using the family-based association statistic of the FBAT software (version 1.5.5), the theoretical rationale for which is described in Horvath et al. [2001].

As done in our earlier segregation analyses of G_2 sensitivity data [Curwen et al., 2005], an individual with an outlier G_2 sensitivity score (404 chromatid aberrations per 100 cells) was excluded from the ANOVA as well as FBAT analysis. FBAT analyses with multiallelic tests (providing a χ^2 value for the sites as a whole, with its P -value) were performed using an additive genetic model, as it is known to perform well even when the true genetic model is not additive (see e.g., [Tu et al., 2000; Horvath et al., 2001]). Finally, since several of the polymorphic sites are linked, the FBAT analyses also were repeated at the haplotype level for all five genes

(*hOGG1*, *XRCC1*, *XRCC4*, *XRCC3*, and *XPB*) for which the FBAT routine internally constructs the haplotypes based on multiple tightly linked markers. The test statistic and *P*-value were computed treating the haplotypes as multiple alleles (described in FBAT-toolkit user manual).

RESULTS

Three families were excluded from the study due to failure to culture in the G_2 assay, one family was excluded through lack of offspring samples, and one sample was excluded due to cancer being removed from the final diagnosis. Thus, G_2 results were available for 23 family units including 38 children (37 of which were used in the present analyses, because of the presence of one outlier, described above). Full details of the 23 families together with individual and group results have been reported previously [Curwen et al., 2005]. Briefly the cancer survivor group comprised 10 cases of Hodgkin's disease, 6 cases of Wilm's tumor, 2 cases of neuroblastoma and 1 case each of rhabdomyosarcoma, teratoma, lymphoepithelioma, pineocytoma, and lymphoblastic lymphoma. Details of age, sex, and aberration frequencies for the three groups are provided in Table II.

Genotype and allele frequencies (based on gene count estimates) at the 13 SNP loci examined are given in Table III. Three PCRs (all *XRCC2* Pro36Ser) failed; thus for this SNP, genotypes for 22 partners and 35 offspring were available. Of the 13 SNPs, five showed very skewed allele frequencies (with mutant alleles having frequencies <10%). Of these, the *hOGG1* Arg46Gln site was nearly monomorphic, while the other four (*XRCC1* Arg194Trp, *XRCC1* Arg280His, *XRCC4* Ile134Thr, and *XRCC4* Ser307Ser) lacked any mutant homozygotes in our sample. As a consequence, these sites exhibited only a limited number of informative families for the family based association test (FBAT) analysis.

The allele counts for the three microsatellite loci (for the *XRCC1*, *XRCC3*, and *XRCC5* genes) are shown in Table IV, for the survivors, their partners, and their offspring separately. Each of these microsatellites was sufficiently polymorphic, exhibiting 8, 9, and 8 segregating alleles, respectively, even with the small sample size of this pilot study.

Table V presents summary results of the test of heterogeneity of genotype/allele frequencies at the 16 sites of polymorphism among survivors, their partners, and their offspring. The exact *P*-values (based on 10,000 replications of permutations) suggest that, except for the *APEX* Asp148Glu SNP site, genotype and/or allele frequencies at the sites did not differ between the survivors and their partners, nor between parents and offspring. The *APEX* Asp148Glu site showed significant genotype frequency differences between survivors and their partners ($P = 0.001$), but not between parents and offspring. The level of significance ($P = 0.008$) for the 3×3 contingency table test, however, did not meet the requirement of significance (at 5% level) after correction for multiple testing (e.g., Bonferroni adjustment for 16 tests), but the survivor versus partner differences of genotype frequencies at this site remained significant ($P = 0.016$ even with Bonferroni adjustment for 16 tests). The heterogeneity tests for the three microsatellite loci, conducted at the level of allele frequency only, did not show any significant differences of allele frequencies for any of the three types of contrasts tested.

Analyses of Table III genotype frequencies also revealed that both the parental and offspring generations showed no departure from HWE expectations of genotype frequencies (results not shown). However, the exact test of HWE could not be done for the five SNP sites (*hOGG1* Arg46Gln, *XRCC1* Arg194Trp, *XRCC1* Arg280His, *XRCC4* Ile134Thr, and *XRCC4* Ser307Ser) that showed limited variation (since the presence of all three genotypes at a biallelic locus would be needed to have any power for rejecting the HWE hypothesis).

Table VI shows summary results of the tests of association between the G₂ radiosensitivity scores and the polymorphisms studied. Of the total of 16 ANOVA test results, only the *XRCC3* Thr241Met site showed a significant ($P = 0.030$) G₂ radiosensitivity score difference among genotypes (with the CC genotype showing a significantly higher G₂ score, 128.77 ± 4.67 , than the other two genotypes together, 112.61 ± 3.82). The proportion of variance (of G₂ score) explained by this polymorphic site (R^2) was 8.4%. In contrast, the FBAT analyses results showed a significant departure from the no-linkage and no-association hypothesis only for the *hOGG1* Ser326Cys site ($\chi^2 = 4.254$ with 2 d.f., $P = 0.039$). None of these P -values, however, remained significant (at the 5% level) after Bonferroni adjustment for multiple tests.

Since other tightly linked markers also were typed for these two genes (*hOGG1* Arg46Gln for the *hOGG1* gene, and a microsatellite locus at the *XRCC3* gene), we used haplotype-based FBAT analysis to investigate if the apparent association of G₂ radiosensitivity scores with polymorphisms at these loci could be detected at the haplotype level as well for these two genes. Table VII shows the summary results of such haplotype-based FBAT analyses (statistic called HBAT for this option) for all five genes studied here. A signature for association/linkage was not detected for any gene, as the P -value for each test exceeds the nominal level of 5%, although the *hOGG1* haplotype-based result just failed to reach significance ($\chi^2 = 5.254$, $P = 0.072$).

DISCUSSION

DNA damage repair, as assessed through the use of in vitro assays, is influenced by a number of polymorphisms in genes encoding DNA repair enzymes [Au et al., 2004]. However, the assayed endpoints are multifarious and are often analyzed in cells in varying phases of the cell cycle. The G₂ chromosomal radiosensitivity assay examines a defined endpoint resulting from irradiation of cells in G₂ and reflects residual chromosome damage following a period of repair after the radiation insult. This pilot study, although of limited sample size, is the first attempt to examine the association of candidate polymorphisms in multiple DNA repair pathways with G₂ sensitivity score. Our observations failed to detect any such association unequivocally.

Out of the 16 polymorphic sites examined, we detected only a single SNP site, *APEX* Asp148Glu on the *APEX* gene of the BER pathway, that showed significant differences in genotype frequencies between the survivors of childhood cancer and their partners (Table V). At this site, the significant genotype frequency difference is induced by an enhanced frequency of the *APEX* Asp148 allele among the survivors compared with that in their partners (65% vs. 33%, Table III). The fact that this result remains significant even after the multiple-test adjustment makes this SNP worthy of further investigation. None of the other six polymorphic sites of the BER pathway genes showed such a trend (Table III and Table V). Since the sites examined in this study did not include any polymorphism linked to the *APEX* Asp148Glu site, we could not verify this suspected association at the haplotype level. Previously Hu et al. [2001,2002] showed that delay at the G₂ checkpoint is higher in individuals carrying *APEX* Glu148 alleles and also that increased radiation-induced cell cycle delay is associated with breast cancer predisposition. In contrast, in our study it is the *APEX* Asp148 allele which is at enhanced frequency in the cancer survivors, which on the basis of the findings of [Hu et al., 2001,2002] would imply less cell cycle delay. It is, therefore, of interest that Scott [2003] reported decreased G₂ cell cycle delay in cells from breast cancer patients and a similar effect is observed for ataxia telangiectasia [Scott et al., 1994b; Terzoudi et al., 2005].

ANOVA testing indicated a significantly higher G₂ score in *XRCC3* Met/Met241 individuals (128.77 ± 4.7 aberrations per 100 cells) compared with individuals carrying at least one Thr241 allele (112.61 ± 3.82). Individuals carrying *XRCC3* Met241 have previously been shown to be deficient in DNA repair as attested by an increase in chromosome deletions using an in vitro

cytogenetic challenge assay [Au et al., 2003] and by elevated levels of chromosomal translocations [Kiuru et al., 2005] and micronuclei [Angelini et al., 2005] following in vivo radiation exposures. However, although the present finding would appear to provide further support for an influence on DNA repair, the association between the *XRCC3* Thr241Met SNP and G₂ radiosensitivity was not confirmed with FBAT analysis. In this analysis, a single sporadic deviation from the null hypothesis of no-association and no-linkage for the *hOGG1* Ser326Cys site was revealed, but this result failed to meet the stringency of significance after multiple test adjustment. Nevertheless, individuals with at least one *hOGG1* Cys326 allele exhibit significantly slower DNA repair rates, as measured with the Comet assay [Aka et al., 2004], and *hOGG1* Cys326Cys individuals showed higher levels of oxidative damage following exposure to Cr(VI) [Lee et al., 2005]. Carriers of this genotype are also at increased risk of orolaryngeal cancer [Elahi et al., 2002].

To our knowledge, the only previous study of the influence of DNA repair gene variants specifically on G₂ chromosomal radiosensitivity examined just two polymorphisms within the *XPB* gene [Lunn et al., 2000] and individuals with the *XPB* Lys751Lys genotype exhibited significantly more chromatid breaks than genotypes homozygous or heterozygous for the *XPB* Gln751 allele. In the present study, neither analysis method confirmed the previous findings [Lunn et al., 2000], with no indication that genotypes at the *XPB* Lys751Gln site affect G₂ chromosomal radiosensitivity.

Although no definitive association between DNA repair genotype and chromosomal radiosensitivity as measured with the G₂ assay has been found, four observations imply that this preliminary study should be extended further, both in terms of larger sample sizes as well as through analysis of additional polymorphisms within DNA repair genes. First, the sample size of our study was rather limited and hence the prospect of detecting associations was small (power <30% for a relative risk ratio not exceeding 1.5). Second, in terms of the association of polymorphisms in DNA repair pathway genes with cancer predisposition, the significance of the association found at the *APEX* Asp148Glu site is worth further investigation. The cancer types of the survivors in this sample are not homogeneous and therefore this result needs to be replicated with a detailed classification of cancer type. In addition, further linked polymorphisms should be examined to detect any causal mechanism underlying this association. Third, we also examined the possible effect of misspecification of the genetic model for susceptibility to G₂ radiosensitivity. The FBAT analysis results reported here were under the additive genetic model of G₂ radiosensitivity score. As our previous segregation analysis of these families implied a putative major gene effect with dominance [Curwen et al., 2005], we repeated the FBAT analyses with the dominant model of inheritance of G₂ sensitivity score, for which the results were almost identical to the results presented in Table V and Table VI. In other words, the sporadic indications of association are not due to a misspecified genetic model of susceptibility of the G₂ sensitivity scores. Fourth, the haplotype-based FBAT results for the *hOGG1* gene and the ANOVA result for *XRCC3* are suggestive and worth further investigation, since a larger sample size is likely to show significance if this association is indeed valid.

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REFERENCES

- Aka P, Mateuca R, Buchet JP, Thierens H, Kirsch-Volders M. Are genetic polymorphisms in *OGG1*, *XRCC1* and *XRCC3* genes predictive for the DNA strand break repair phenotype and genotoxicity in workers exposed to low dose ionising radiations? *Mutat Res* 2004;556:169–181. [PubMed: 15491645]
- Angelini S, Kumar R, Carbone F, Maffei F, Forti GC, Violante FS, Lodi V, Curti S, Hemminki K, Hrelia P. Micronuclei in humans induced by exposure to low level of ionizing radiation: Influence of polymorphisms in DNA repair genes. *Mutat Res* 2005;570:105–117. [PubMed: 15680408]
- Au WW, Salama SA, Sierra-Torres CH. Functional characterization of polymorphisms in DNA repair genes using cytogenetic challenge assays. *Environ Health Perspect* 2003;111:1843–1850. [PubMed: 14630517]
- Au WW, Navasumrit P, Ruchirawat M. Use of biomarkers to characterize functions of polymorphic DNA repair genotypes. *Int J Hyg Environ Health* 2004;207:301–313. [PubMed: 15471094]
- Baeyens A, Thierens H, Claes K, Poppe B, Messiaen L, De Ridder L, Vral A. Chromosomal radiosensitivity in breast cancer patients with a known or putative genetic predisposition. *Br J Cancer* 2002;87:1379–1385. [PubMed: 12454765]
- 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]
- Boice JD Jr, Tawn EJ, Winther JF, Donaldson SS, Green DM, Mertens AC, Mulvihill JJ, Olsen JH, Robison LL, Stovall M. Genetic effects of radiotherapy for childhood cancer. *Health Phys* 2003;85:65–80. [PubMed: 12852473]
- Bondy ML, Wang LE, El-Zein R, de Andrade M, Selvan MS, Bruner JM, Levin VA, Alfred Yung WK, Adatto P, Wei Q. γ -Radiation sensitivity and risk of glioma. *J Natl Cancer Inst* 2001;93:1553–1557. [PubMed: 11604478]
- Cloos J, Nieuwenhuis EJ, Boomsma DI, Kuik DJ, van der Sterre ML, Arwert F, Snow GB, Braakhuis BJ. Inherited susceptibility to bleomycin-induced chromatid breaks in cultured peripheral blood lymphocytes. *J Natl Cancer Inst* 1999;91:1125–1130. [PubMed: 10393720]
- Cottet F, Blanche H, Verasdonck P, Le Gall I, Schachter F, Burkle A, Muir ML. New polymorphisms in the human poly(ADP-ribose) polymerase-1 coding sequence: Lack of association with longevity or with increased cellular poly(ADP-ribosyl)ation capacity. *J Mol Med* 2000;78:431–440. [PubMed: 11097112]
- 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 Jr. G_2 chromosomal radiosensitivity in Danish survivors of childhood and adolescent cancer and their offspring. *Br J Cancer* 2005;93:1038–1045. [PubMed: 16234827]
- Elahi A, Zheng Z, Park J, Eyring K, McCaffrey T, Lazarus P. The human OGG1 DNA repair enzyme and its association with orolaryngeal cancer risk. *Carcinogenesis* 2002;23:1229–1234. [PubMed: 12117782]
- Ford BN, Ruttan CC, Kyle VL, Brackley ME, Glickman BW. Identification of single nucleotide polymorphisms in human DNA repair genes. *Carcinogenesis* 2000;21:1977–1981. [PubMed: 11062157]
- Godderis L, De Boeck M, Haufroid V, Emmery M, Mateuca R, Gardinal S, Kirsch-Volders M, Veulemans H, Lison D. Influence of genetic polymorphisms on biomarkers of exposure and genotoxic effects in styrene-exposed workers. *Environ Mol Mutagen* 2004;44:293–303. [PubMed: 15468052]
- 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]
- Guo SW, Thompson EA. Performing the exact test of Hardy-Weinberg proportion for multiple alleles. *Biometrics* 1992;48:361–372. [PubMed: 1637966] Abstract only.
- Hoeijmakers JH. Genome maintenance mechanisms for preventing cancer. *Nature* 2001;411:366–374. [PubMed: 11357144]
- Horvath S, Xu X, Laird NM. The family based association test method: Strategies for studying general genotype–phenotype associations. *Eur J Hum Genet* 2001;9:301–306. [PubMed: 11313775]

- 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]
- Hu JJ, Smith TR, Miller MS, Lohman K, Case LD. Genetic regulation of ionizing radiation sensitivity and breast cancer risk. *Environ Mol Mutagen* 2002;39:208–215. [PubMed: 11921191]
- Jackson SP. Sensing and repairing DNA double-strand breaks. *Carcinogenesis* 2002;23:687–696. [PubMed: 12016139]
- Kiuru A, Lindholm C, Heilimo I, Ceppi M, Koivistoinen A, Ilus T, Hirvonen A, Norppa H, Salomaa S. Influence of DNA repair gene polymorphisms on the yield of chromosomal aberrations. *Environ Mol Mutagen* 2005;46:198–205. [PubMed: 15971256]
- Lee AJ, Hodges NJ, Chipman JK. Interindividual variability in response to sodium dichromate-induced oxidative DNA damage: Role of the Ser326Cys polymorphism in the DNA-repair protein of 8-oxo-7,8-dihydro-2'-deoxyguanosine DNA glycosylase 1. *Cancer Epidemiol Biomarkers Prev* 2005;14:497–505. [PubMed: 15734978]
- Li, CC. *First Course in Population Genetics*. Pacific Grove, CA: Boxwood; 1976.
- Lunn RM, Langlois RG, Hsieh LL, Thompson CL, Bell DA. XRCC1 polymorphisms: Effects on aflatoxin B1-DNA adducts and glycophorin A variant frequency. *Cancer Res* 1999;59:2557–2561. [PubMed: 10363972]
- Lunn RM, Helzlsouer KJ, Parshad R, Umbach DM, Harris EL, Sanford KK, Bell DA. XPD polymorphisms: Effects on DNA repair proficiency. *Carcinogenesis* 2000;21:551–555. [PubMed: 10753184]
- Marcon F, Andreoli C, Rossi S, Verdina A, Galati R, Crebelli R. Assessment of individual sensitivity to ionizing radiation and DNA repair efficiency in a healthy population. *Mutat Res* 2003;541:1–8. [PubMed: 14568289]
- Mohrenweiser HW, Wilson DM, Jones IM. Challenges and complexities in estimating both the functional impact and the disease risk associated with the extensive genetic variation in human DNA repair genes. *Mutat Res* 2003;526:93–125. [PubMed: 12714187]
- Parshad R, Sanford KK. Radiation-induced chromatid breaks and deficient DNA repair in cancer predisposition. *Crit Rev Oncol Hematol* 2001;37:87–96. [PubMed: 11166582]
- Parshad R, Sanford KK, Jones GM. Chromatid damage after G₂ phase x-irradiation of cells from cancer-prone individuals implicates deficiency in DNA repair. *Proc Natl Acad Sci USA* 1983;80:5612–5616. [PubMed: 6577447]
- Parshad R, Price FM, Bohr VA, Cowans KH, Zujewski JA, Sanford KK. Deficient DNA repair capacity, a predisposing factor in breast cancer. *Br J Cancer* 1996;74:1–5. [PubMed: 8679441]
- Patel RK, Trivedi AH, Arora DC, Bhatavdekar JM, Patel DD. DNA repair proficiency in breast cancer patients and their first-degree relatives. *Int J Cancer* 1997;73:20–24. [PubMed: 9334804]
- Price EA, Bourne SL, Radbourne R, Lawton PA, Lamerdin J, Thompson LH, Arrand JE. Rare microsatellite polymorphisms in the DNA repair genes *XRCC1*, *XRCC3* and *XRCC5* associated with cancer in patients of varying radiosensitivity. *Somat Cell Mol Genet* 1997;23:237–247. [PubMed: 9542526]
- Relton CL, Daniel CP, Hammal DM, Parker L, Tawn EJ, Burn J. DNA repair gene polymorphisms, prenatal factors and the frequency of somatic mutations in the glycophorin-A gene among healthy newborns. *Mutat Res* 2004;545:49–57. [PubMed: 14698416]
- Riches AC, Bryant PE, Steel CM, Gleig A, Robertson AJ, Preece PE, Thompson AM. Chromosomal radiosensitivity in G₂-phase lymphocytes identifies breast cancer patients with distinctive tumour characteristics. *Br J Cancer* 2001;85:1157–1161. [PubMed: 11710829]
- 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]
- Roff DA, Bentzen P. The statistical analysis of mitochondrial DNA polymorphisms: χ^2 and the problem of small samples. *Mol Biol Evol* 1989;6:539–545. [PubMed: 2677600]
- Rooney, DE., editor. *Human Cytogenetics: Constitutional Analysis—A Practical Approach*. Vol. 3rd ed.. Oxford, NY: Oxford University Press; 2001.

- Rzeszowska-Wolny J, Polanska J, Pietrowska M, Palyvoda O, Jaworska J, Butkiewicz D, Hancock R. Influence of polymorphisms in DNA repair genes *XPD*, *XRCC1* and *MGMT* on DNA damage induced by γ radiation and its repair in lymphocytes in vitro. *Radiat Res* 2005;164:132–140. [PubMed: 16038584]
- 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]
- Scott D. Chromosomal radiosensitivity, cancer predisposition and response to radiotherapy. *Strahlentherapie Onkologie* 2000;176:229–234.
- Scott D, Spreadborough A, Levine E, Roberts SA. Genetic predisposition in breast cancer. *Lancet* 1994a; 344:1444. [PubMed: 7968110]
- Scott D, Spreadborough AR, Roberts SA. Radiation-induced G₂ delay and spontaneous chromosome aberrations in ataxia-telangiectasia homozygotes and heterozygotes. *Int J Radiat Biol* 1994b; 66:S157–S163. [PubMed: 7836843]
- Scott D, Spreadborough AR, Jones LA, Roberts SA, Moore CJ. Chromosomal radiosensitivity in G₂-phase lymphocytes as an indicator of cancer predisposition. *Radiat Res* 1996;145:3–16. [PubMed: 8532833]
- 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]
- Scott D, Spreadborough A, Roberts SA. Less G₂ arrest in irradiated cells of breast cancer patients than in female controls: A contribution to their enhanced chromosomal radiosensitivity? *Int J Radiat Biol* 2003;79:405–411. [PubMed: 12963542]
- 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]
- Slupphaug G, Kavli B, Krokan HE. The interacting pathways for prevention and repair of oxidative DNA damage. *Mutat Res* 2003;531:231–251. [PubMed: 14637258]
- 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 interindividual variability. *Mutat Res* 2003;528:105–110. [PubMed: 12873728]
- Spitz MR, Wu X, Wang Y, Wang LE, Shete S, Amos CI, Guo Z, Lei L, Mohrenweiser H, Wei Q. Modulation of nucleotide excision repair capacity by XPD polymorphisms in lung cancer patients. *Cancer Res* 2001;61:1354–1357. [PubMed: 11245433]
- Terzoudi GI, Jung T, Hain J, Vrouvas J, Margaritis K, Donta-Bakoyianni C, Makropoulos V, Angelakis P, Pantelias GE. Increased G2 chromosomal radiosensitivity in cancer patients: The role of cdk1/cyclin-B activity level in the mechanisms involved. *Int J Radiat Biol* 2000;76:607–615. [PubMed: 10866282]
- Terzoudi GI, Manola KN, Pantelias GE, Iliakis G. Checkpoint abrogation in G2 compromises repair of chromosomal breaks in ataxia telangiectasia cells. *Cancer Res* 2005;65:11292–11296. [PubMed: 16357135]
- Tu IP, Balise RR, Whittemore AS. Detection of disease genes by use of family data. II. Application to nuclear families *Am J Hum Genet* 2000;66:1341–1350.
- 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, Relton CL, Rees GS, Tarone RE, Whitehouse CA, Tawn EJ. DNA repair gene polymorphisms in relation to chromosome aberration frequencies in retired radiation workers. *Mutat Res* 2005;570:137–145. [PubMed: 15680411]
- Wilding CS, Rees GS, Relton CL, Tawn EJ. Genotype profiles of loci encoding DNA repair enzymes in newborn and elderly populations: No evidence of association with longevity. *Biogerontology* 2006;7:35–41. [PubMed: 16518718]
- Wood RD, Mitchell M, Lindahl T. Human DNA repair genes. *Mutat Res* 2005;577:275–283. [PubMed: 15922366]

TABLE I

Details of the SNPs and Microsatellites Analyzed^a

Pathway	Gene	Base change	dbSNP reference	Amino acid change	Method
BER	<i>ADPRT</i>	T → C	rs17853760	Val1762Ala	Cottet et al. [2000] ^b
	<i>APEX</i>	T → G	rs17858508	Asp148Glu	Hu et al. [2001]
	<i>hOGGI</i>	C → G	rs1052133	Ser326Cys	Wikman et al. [2000] ^c
	<i>hOGGI</i>	G → A	n/a	Arg46Gln	Wikman et al. [2000]
	<i>XRCCI</i>	G → A	rs25487	Arg399Gln	Lunn et al. [1999]
NHEJ	<i>XRCCI</i>	C → T	rs1799782	Arg194Trp	Lunn et al. [1999]
	<i>XRCCI</i>	G → A	rs25489	Arg280His	Lunn et al. [1999]
	<i>XRCCI</i>	Microsatellite	—	Noncoding	Wilding et al. [2005]
	<i>XRCC4</i>	T → C	rs28360135	Ile134Thr	Relton et al. [2004]
	<i>XRCC4</i>	G → T	rs1056503	Ser307Ser	See text
HRR	<i>XRCC5</i>	Microsatellite	—	Noncoding	Wilding et al. [2005]
	<i>XRCC2</i>	C → T	rs17856704	Pro36Ser	See text
	<i>XRCC3</i>	C → T	rs861539	Thr241Met	Shen et al. [1998]
NER	<i>XRCC3</i>	Microsatellite	—	Noncoding	Wilding et al. [2005]
	<i>XPB</i>	G → A	rs1799793	Asp312Asn	Spitz et al. [2001]
	<i>XPB</i>	A → C	rs13181	Lys751Gln	Spitz et al. [2001]

^aReferences to primers and methods are given. Minor modifications to published primers are detailed in the footnotes below. Novel primer sequences are given in the text.
^bReverse primer = Pex 19 of [Cottet et al. 2000]. Forward primer ADPRTT2444CF 5'-CAC CAT GAT ACC TAA GTC GG-3'.
^cPrimers with 5' *EcoRI* site removed. Forward primer with additional 3'GGCT.

TABLE II

Characteristics of Cancer Survivor, Partner and Offspring Groups With Median G₂ Radiation-Induced Aberration Frequencies

Group	Number of individuals	Male/female	Median age in years (range)	Median aberration frequency per 100 cells (range)
Cancer survivors	23	8/15	34 (25–37)	122 (73–160)
Partner controls	23	15/8	35 (26–43)	112 (79–189)
Offspring	38	22/16	4.5 (4 months–14)	123 (73–404)

Genotype Frequencies and Allele Frequencies for 13 SNPs in Loci Involved in DNA Repair in 23 Families of Survivors of Childhood Cancer

Pathway	Gene		WT/WT ^a	WT/M ^a	M/M ^a	f(WT)	f(M)
BER	<i>ADPRT</i> <i>Val762Ala</i>	Genotype	T/T	T/C	C/C	f(T)	f(C)
		Survivors (N = 23)	14	8	1	0.78	0.22
		Partners (N = 23)	14	8	1	0.78	0.22
	<i>APEX</i> <i>Asp148Glu</i>	Offspring (N = 37)	25	10	2	0.81	0.19
		Genotype	T/T	T/G	G/G	f(T)	f(G)
		Survivors (N = 23)	7	16	0	0.65	0.35
	<i>hOGG1</i> <i>Arg46Gln</i>	Partners (N = 23)	2	11	10	0.33	0.67
		Offspring (N = 37)	11	20	6	0.57	0.43
		Genotype	G/G	G/A	A/A	f(G)	f(A)
	<i>hOGG1</i> <i>Ser326Cys</i>	Survivors (N = 23)	23	0	0	1.00	0.00
		Partners (N = 23)	22	1	0	0.98	0.02
		Offspring (N = 37)	36	1	0	0.99	0.01
NHEJ	<i>XRCC1</i> <i>Arg194Trp</i>	Genotype	C/C	C/G	G/G	f(C)	f(G)
		Survivors (N = 23)	14	6	3	0.74	0.26
		Partners (N = 23)	18	4	1	0.87	0.13
	<i>XRCC1</i> <i>Arg280His</i>	Offspring (N = 37)	20	16	1	0.76	0.24
		Genotype	C/C	C/T	T/T	f(C)	f(T)
		Survivors (N = 23)	22	1	0	0.98	0.02
	<i>XRCC1</i> <i>Arg399Gln</i>	Partners (N = 23)	18	5	0	0.89	0.11
		Offspring (N = 37)	32	5	0	0.93	0.07
		Genotype	G/G	G/A	A/A	f(G)	f(A)
	<i>XRCC4</i>	Survivors (N = 23)	22	1	0	0.98	0.02
		Partners (N = 23)	17	6	0	0.87	0.13
		Offspring (N = 37)	31	6	0	0.92	0.08

Pathway	Gene	WT/WT ^a	WT/M ^a	M/M ^a	f(WT)	f(M)
HRR	<i>Ile134Thr</i>	Survivors (N = 23)	18	5	0	0.89
		Partners (N = 23)	22	1	0	0.98
		Offspring (N = 37)	33	4	0	0.95
	<i>XRCC4</i>	Genotype	G/G	G/T	T/T	f(G)
		Survivors (N = 23)	18	5	0	0.89
		Partners (N = 23)	19	4	0	0.91
	<i>Ser307Ser</i>	Offspring (N = 37)	31	6	0	0.92
		Genotype	C/C	C/T	T/T	f(C)
		Survivors (N = 23)	11	9	3	0.67
	<i>Pro365Ser</i>	Partners (N = 22) ^b	9	9	4	0.61
NER	<i>XRCC3</i>	Offspring (N = 35) ^c	15	17	3	0.67
		Genotype	C/C	C/T	T/T	f(C)
		Survivors (N = 23)	11	10	2	0.70
	<i>Thr241Met</i>	Partners (N = 23)	9	12	2	0.65
		Offspring (N = 37)	19	16	2	0.73
		Genotype	G/G	G/A	A/A	f(G)
	<i>XPB</i>	Survivors (N = 23)	16	5	2	0.80
		Partners (N = 23)	13	7	3	0.72
		Offspring (N = 37)	21	16	0	0.78
	<i>Lys751Gln</i>	Genotype	A/A	A/C	C/C	f(A)

^a WT = wild-type, M = mutant; for example, ADPRT Val762Ala, WT/WT = Val/Val, WT/M = Val/Ala, M/M = Ala/Ala.

^b 1 missing data point.

^c 2 missing data points.

TABLE IV
Allele Counts for 3 Microsatellite Loci Involved in DNA Repair in 23 Families of Survivors of Childhood Cancer

Allele size	XRCC1 microsatellite			XRCC3 microsatellite			XRCC5 microsatellite		
	Survivor	Partner	Offspring	Survivor	Partner	Offspring	Survivor	Partner	Offspring
11	1	4	4						
12				2	2	1			
13				6	6	8			
14				8	9	13	2		
14 + 2								1	
15				11	12	17	19	20	31
15 + 2							3		
16	4		3	13	12	22	17	18	33
16 + 2							1	1	2
17	16	14	26	2	2	5	4	5	6
18	15	16	24						
19	5	6	7	1	1	2		1	1
20	1	3	3	2	2	5			
21	3	3	7	1		1			
22	1								

TABLE V
Test of Heterogeneity of Genotype/Allele Frequencies for 13 SNPs and 3 Microsatellites Involved in DNA Repair Among Survivors of Childhood Cancer, Their Spouses, and Their Offspring

Pathway	Gene	Base change	Amino acid change	P-values (exact test)		
				Survivors, spouses, and offspring	Survivors vs. spouses	Parents vs. offspring
BER	<i>ADPRT</i>	T → C	Val762Ala	0.962	~1.0	0.806
	<i>APEX</i>	T → G	Asp148Glu	0.008 ^a	0.001 ^b	0.753
	<i>hOGG1</i>	C → G	Ser326Cys	0.120	0.458	0.079
		G → A	Arg46Gln	0.757	~1.0	~1.0
	<i>XRCC1</i>	G → A	Arg399Gln	0.598	0.444	0.564
		C → T	Arg194Trp	0.221	0.189	~1.0
		G → A	Arg280His	0.128	0.093	~1.0
		Microsatellite	Noncoding	0.754	0.328	0.996
NHEJ	<i>XRCC4</i>	T → C	Ile134Thr	0.193	0.182	~1.0
		G → T	Ser307Ser	0.939	~1.0	0.780
		Microsatellite	Noncoding	0.441	0.460	0.889
		C → T	Pro36Ser	0.839	0.924	0.666
HRR	<i>XRCC2</i>	C → T	Thr241Met	0.915	0.904	0.745
	<i>XRCC3</i>	C → T	Noncoding	~1.0	~1.0	0.966
		Microsatellite	Asp312Asn	0.142	0.685	0.051
		G → A	Lys751Gln	0.879	0.838	0.705
NER	<i>XPD</i>	A → C				

^a Significant at 5% level but not after multiple test adjustment.

^b Significant at 5% level even after multiple test adjustment.

TABLE VI
Analysis of Variance (ANOVA) and Family Based Association Test (FBAT) Results of G₂ Radiation Sensitivity and DNA Repair Gene Polymorphisms

Pathway	Gene	Base change	Amino acid change	ANOVA result		FBAT result	
				R ²	P-value	χ ² 0.18	P-value
BER	<i>ADPRT</i>	T → C	Val1762Ala	0.064	0.071	0.189	0.663
	<i>APEX</i>	T → G	Asp148Glu	0.011	0.641	0.016	0.898
	<i>hOGGI</i>	C → G	Ser326Cys	0.030	0.301	4.254	0.039 ^a
	<i>XRCCI</i>	G → A	Arg46Gln	0.001	0.828		
		G → A	Arg399Gln	0.002	0.917	0.136	0.712
		C → T	Arg194Trp	0.004	0.573	0.132	0.716
		G → A	Arg280His	0.010	0.378	0.333	0.564
		Microsatellite	Noncoding	0.198	0.450	2.831	0.726
		T → C	Ile134Thr	0.000	0.867	1.194	0.274
NHEJ	<i>XRCC4</i>	G → T	Ser307Ser	0.009	0.390	1.398	0.237
	<i>XRCC5</i>	Microsatellite	Noncoding	0.154	0.490	3.179	0.365
		C → T	Pro36Ser	0.002	0.942	0.004	0.950
HRR	<i>XRCC2</i>	C → T	Thr241Met	0.084	0.030 ^a	0.335	0.563
	<i>XRCC3</i>	C → T	Noncoding	0.362	0.094	1.278	0.865
		Microsatellite	Asp312Asn	0.033	0.265	0.216	0.642
NER	<i>XPB</i>	G → A	Lys751Gln	0.010	0.678	0.208	0.648
		A → C					

^a Significant at 5% level but not after multiple test adjustment.

TABLE VIIHaplotype FBAT Test for Association of DNA Repair Genes With G₂ Score

Gene	Polymorphisms involved	HBAT result	
		χ^2	<i>P</i> -value
<i>hOGG1</i>	Ser326Cys, Arg46Gln	5.254	0.072
<i>XRCC1</i>	Arg399Gln, Arg194Trp, Arg280His, and Microsatellite	2.630	0.757
<i>XRCC4</i>	Ile134Thr, Ser307Ser	2.592	0.274
<i>XRCC3</i>	Thr241Met, Microsatellite	0.508	0.917
<i>XPB</i>	Asp312Asn, Lys751Gln	1.546	0.672