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PRINCIPAL INVESTIGATOR: Kathy J. Helzlsouer, M.D., MHS

CONTRACTING ORGANIZATION: Johns Hopkins University
Baltimore, Maryland 21205

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13. ABSTRACT (Maximum 200 Words) Investigations of potential gene-environment interactions may improve our understanding of the etiology of breast cancer, a disease where less than 40% of cases can be attributed to known risk factors. The objectives of this proposal are to examine the association between DNA repair proficiency and breast cancer risk, and the contribution of this factor to the familial clustering of breast cancer. We hypothesize that mechanisms leading to suboptimal repair of DNA damage are susceptibility factors predisposing women to breast cancer through increased sensitivity to carcinogenic damage from environmental exposures such as ionizing radiation. To evaluate this hypothesis a case-control study and a family study are being conducted. Women with a personal history of breast cancer and women at increased breast cancer risk will be compared to control women for the presence of Lys/Lys 751 XPD genotype. We observed a strong association between presence of the Lys/Lys 751 XPD genotype and higher number of chromatid aberrations. Thus, we plan to assess the XPD genotype, in place of the DNA repair proficiency cytogenetic assay which we originally planned to do. We will also evaluate the possible interaction between Lys/Lys 751 XPD genotype and ionizing radiation exposure, by stratifying the case-control data on exposure status, and assessing the relation between breast cancer risk and the genotype. The family study will be used to confirm the results of the case-control study.				
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ANNUAL REPORT YEAR 3: DNA REPAIR AND BREAST CANCER RISK STUDY

1. INTRODUCTION

The purpose of the study is to assess whether suboptimal repair of DNA damage is associated with increased breast cancer risk, to assess the possible interaction between DNA repair proficiency and ionizing radiation exposure, and to evaluate the inheritance pattern of suboptimal DNA repair proficiency. The study consists of two parts: a case-control study and a family study. Because of delay introduced by difficulties in assay development we have requested a one year no-cost extension to complete the study.

2. BODY

Case-control and Family studies, Tasks 1-6

Task 1: Develop and produce study brochure. Finalize questionnaire and family history form.

We developed study brochures for recruiting participants and finalized the questionnaire and family history form in year 1.

Task 2: Identify and recruit eligible participants (300 cases and controls).

To date we have recruited 68 incident breast cancer cases, 126 women at increased risk of breast cancer due to family history, 20 women at increased risk due to results of breast biopsies, and 131 controls, for a total of 345 cases and controls.

We continue to recruit incident breast cancer cases among women attending clinics at the Breast Center at the Johns Hopkins Outpatient Center, and Medical Oncology and Radiation Oncology, at the Johns Hopkins Oncology Center, and will complete recruitment in November. Incident cases are women diagnosed with breast cancer in the previous 12 months, and without metastatic disease. Our goal is to have at least 100 incident cases.

We recruited cases with increased risk of breast cancer among women attending clinics at the Breast Center at the Johns Hopkins Outpatient Center, women having mammograms at the the Johns Hopkins Outpatient Center, and among employees and students of the Johns Hopkins East Baltimore Campus. Employees and students tend to receive their care at the Outpatient center, and therefore are similar to clinic patients. Women at increased risk have at least one first-degree relative, or at least two second-degree relatives on the same side of the family, with breast cancer, or have had a breast biopsy which showed atypia, hyperplasia, proliferative disease, fibroadenoma, or lobular carcinoma in-situ. Cases with increased risk have no previous diagnosis of cancer other than nonmelanoma skin cancer.

We recruited controls among women attending clinics at the Breast Center at the Johns Hopkins Outpatient Center, women having mammograms at the Johns Hopkins Outpatient Center,

and among employees and students of the Johns Hopkins East Baltimore Campus. Employees and students were recruited because many women visiting the Breast Center or mammography were ineligible as controls due to a family history of cancer. The majority of our employee and student participants seek care at outpatient clinics of the Johns Hopkins Medical Institutions, and therefore are similar to clinic patients. Controls have no history of cancer in themselves or in their first-degree family members, other than nonmelanoma skin cancer; breast or ovarian cancer occurring in at most one second-degree relative on each side of the family; and no history of breast biopsy, other than aspiration of a fluid-filled cyst.

We recruited 17 family members of 15 women diagnosed with breast cancer, and women with a family history of breast cancer. Pending results of the case-control study, we will recruit additional families and family members as funding is available.

Task 3: Collect questionnaire data and blood samples.

Blood samples have been collected for all participants, and 92% of questionnaires have been completed and returned. We are in the process of calling participants to retrieve outstanding questionnaires, and will complete questionnaires by phone interview as necessary.

Task 4: Perform DNA repair assays.

Efforts to develop the DNA repair cytogenetic assay in our laboratory

We originally planned to measure DNA repair proficiency using the cytogenetic assay developed at NIH by Sanford and Parshad and used in our pilot assay in 1993-5 (1). Due to problems with repeatability of this assay in our laboratory, we stopped doing the assay in November of 1999. The difficulties in reproducing the assay have been extensively detailed in previous reports and are outlined briefly below.

To transfer the assay to our laboratory, we had to adapt the assay to a gamma (cesium chloride) radiation source, because an ionizing source was not available. We found that 25cGy radiation, and 1.5 hours of repair time with colcemid added at 0.5 hour (the repair time used at NIH), at first appeared optimal for repair in our standard for good repair, a donor who had good repair eleven times in our pilot study at NIH in 1993-5. Repeatability was good for this standard, and for a donor who had poor repair at NIH and in our laboratory. We started recruiting patients to the study. Shortly thereafter, we found that we were not able to reproduce results previously obtained at the NIH laboratory. To optimize conditions for good repair, we started using a 37°C warm room instead of a waterbath, for repair time, when the warm room became available to us in September of 1998. We tried to minimize the risk of bacterial contamination, which can lead to few metaphase cells, poor chromosome spread, and inconsistent results. Contamination, probably through nutrient depletion or metabolic products, which may enhance susceptibility to radiation damage, hinder repair processes, protect against damage, or facilitate repair (2). Other modifications we tried in late 1998 included growing cells in 10% CO₂ instead 5% CO₂ to better maintain a pH near 7 during the assay; growing cells in a portable tissue culture chamber to reduce the risk of contamination; and using an ice-water bath to stop repair after all repair time. We

continued to grow cells in 10% CO₂, without the portable chamber, and to use an ice-water bath to stop repair, for the remainder of the assays.

In April of 1999 we incorporated modifications that Scott et al. made to decrease variability in this assay (3,4), including: adding fresh media to cells one hour before irradiating; not centrifuging cells before irradiating, because centrifuging can slow progression of cells into metaphase (3); and lysing cells at 4⁰C, instead of at ambient temperature, because repair may continue to varying extents at ambient temperature (3). These changes did not solve our problems with the assay. In an assay we did in April of 1999, we found inconsistent results using our quality control standard for good repair, with and without Scott's modifications.

In September of 1999, we compared letting cells repair for 0.5 and one hour before adding colcemid for one hour, and irradiated cells with the dose that Scott used, 50cGy radiation, instead of our standard 25cGy radiation. We resumed letting cells repair in a 37⁰C waterbath, because the warm room may have been a source of bacterial contamination. With these modifications, a donor with poor repair at NIH had 1.4 times as many breaks and gaps as our quality control standard for good repair, when colcemid was added 0.5 hour after irradiation; and two times as many when colcemid was added one hour after irradiation. These results were promising and consistent, and we resumed the assay, irradiating cells with 50cGy radiation, and letting cells repair for two hours, with colcemid added at one hour after after irradiation. More inconsistent results followed in October and November of 1999, and we stopped doing the cytogenetic assay, because the problems with the assay appeared to be unresolvable within the timeframe of the study.

In the interim we conducted a genotype-phenotype study using DNA from 31 women whose blood was assayed for DNA repair proficiency by the cytogenetic assay in our pilot study at NIH (5) (Appendix 1). We observed a strong association between presence of the Lys/Lys 751 XPD genotype, and higher number of chromatid aberrations in the cytogenetics assay (Table 1). Thus, we plan to assess the XPD genotype, in place of the DNA repair proficiency cytogenetic assay which we originally planned to do.

Task 5: Enter questionnaire data.

Questionnaire data entry is ongoing.

Task 6: Analyze data and write report.

As mentioned above we have requested a one year no-cost extension. Data analysis and writing of the final report will be done pending completion of recruitment and assay of the XPD genotype.

3. **KEY RESEARCH ACCOMPLISHMENTS**

- The results of our research were presented by Dr. Harris (co-investigator) at the meeting this past year in Atlanta. A copy of the poster is appended (Appendix 2).
- A genotype-phenotype study was conducted using DNA from 31 women whose blood was assayed for DNA repair proficiency by the cytogenetic assay in our pilot study at NIH (5). XPD participates in nucleotide excision repair and transcription-coupled repair. Transcription-coupled repair affects repair of different types of DNA damage, including that due to ionizing radiation. There is genetic variation in XPD that results in amino acid substitutions: Asp312Asn, and Lys751Gln (Table 1).

Table 1. Association of XPD genotypes with DNA repair proficiency

XPD Polymorphism	DNA Repair Proficiency ¹		
	Adequate	Suboptimal ²	OR (95% CI) ³
ASP312Asn			
Asp/Asn or Asn/Asn	46% (N=6)	31% (N=5)	1 (ref)
Asp/Asp	54% (N=7)	69% (N=11)	1.8 (0.3-11.0)
Lys751Gln			
Lys/Gln or Gln/Gln	73% (N=11)	25% (N=4)	1 (ref)
Lys/Lys	27% (N=4)	75% (N=12)	7.2 (1.01-87.7)

¹Measured by examination of metaphase cells for chromatid breaks and gaps.

²Suboptimal repair: More than a total of 60 breaks and gaps per 100 metaphase cells. Distribution of breaks and gaps is bimodal, with two distinct and non-overlapping distributions.

³Age-adjusted

Adapted from Lunn et al., *Carcinogenesis* 21:551-555, 2000

Conclusions from genotype-phenotype study:

The association between the Asp312Asn polymorphism and DNA repair proficiency is not statistically significant. The association between the Lys751Gln polymorphism and DNA repair proficiency is statistically significant. Individuals with the “wild-type” genotype are more likely to have suboptimal DNA repair.

Genetic variation in XPD may be associated with DNA repair proficiency. We plan to study the association of XPD genotype variation with breast cancer risk; rather than the association of DNA repair proficiency by the cytogenetic assay with breast cancer risk.

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APPENDICES

- Appendix 1: 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.
- Appendix 2: DNA Repair and Breast Cancer Risk: Poster presented by Dr. Emily Harris (co-investigator) at the meeting this past year in Atlanta.

ACCELERATED PAPER

XPD polymorphisms: effects on DNA repair proficiency

Ruth M.Lunn¹, Kathy J.Helzlsouer², Ram Parshad³,
David M.Umbach⁴, Emily L.Harris^{2,5},
Katherine K.Sanford⁶ and Douglas A.Bell^{1,7}

¹Laboratory of Computational Biology and Risk Analysis, National Institute of Environmental Health Sciences, National Institutes of Health, MD C3-03, PO Box 12233, Research Triangle Park, NC 27709, ²Department of Epidemiology, The Johns Hopkins University School of Hygiene and Public Health, Baltimore, MD, ³Department of Pathology, Howard University College of Medicine, Washington, DC, ⁴Biostatistics Branch, National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, NC and ⁶Laboratory of Cellular and Molecular Biology, Division of Cancer Etiology, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA

⁵Present address: Kaiser Permanente Center for Health Research, Portland, OR, USA

⁷To whom correspondence should be addressed
Email: bell1@niehs.nih.gov

XPD codes for a DNA helicase involved in transcription and nucleotide excision repair. Rare XPD mutations diminish nucleotide excision repair resulting in hypersensitivity to UV light and increased risk of skin cancer. Several polymorphisms in this gene have been identified but their impact on DNA repair is not known. We compared XPD genotypes at codons 312 and 751 with DNA repair proficiency in 31 women. XPD genotypes were measured by PCR-RFLP. DNA repair proficiency was assessed using a cytogenetic assay that detects X-ray induced chromatid aberrations (breaks and gaps). Chromatid aberrations were scored per 100 metaphase cells following incubation at 37°C (1.5 h after irradiation) to allow for repair of DNA damage. Individuals with the Lys/Lys codon 751 XPD genotype had a higher number of chromatid aberrations (132/100 metaphase cells) than those having a 751Gln allele (34/100 metaphase cells). Individuals having greater than 60 chromatid breaks plus gaps were categorized as having sub-optimal repair. Possessing a Lys/Lys751 genotype increased the risk of sub-optimal DNA repair (odds ratio = 7.2, 95% confidence interval = 1.01-87.7). The Asp312Asn XPD polymorphism did not appear to affect DNA repair proficiency. These results suggest that the Lys751 (common) allele may alter the XPD protein product resulting in sub-optimal repair of X-ray-induced DNA damage.

Introduction

Hereditary genetic defects in DNA repair lead to increased risk of cancer. Individuals with xeroderma pigmentosum (XP), a rare autosomal recessive disease resulting from a defect in nucleotide excision repair (NER) of UV-damaged DNA, have

a >1000-fold increased risk of skin cancer (1). These individuals are extremely hypersensitive to sunlight and have pigmentation abnormalities. Cell fusion analyses have identified seven genetic complementation groups (XPA to XPG) that encode for proteins participating in different steps of the NER pathway (2,3). NER deficiencies are also responsible for two other genetic diseases, Cockayne syndrome (CS), characterized by growth and mental retardation and neurological degeneration, and trichothiodystrophy (TTD), characterized by sulfur-deficient brittle hair and impaired mental and physical development (4,5).

Although CS, TTD and XP exhibit different clinical manifestations, they all have NER deficiencies. Moreover, all of these diseases can result from deficient XPD or XPB proteins. Recent elucidation of the functions of the various repair proteins encoded by the complementation groups has provided insight into these disease processes. NER is composed of two sub-pathways, global genome repair and transcription-coupled repair. Transcription-coupled repair occurs rapidly because it repairs damage to the transcribed strand of active genes, whereas global repair is much slower and repairs damage to inactive genes (6,7). XP results from defects in both transcription-coupled repair and global repair depending on the complementation group, and TTD and CS are caused by defective transcription-coupled repair and probably alterations in transcription (8). Transcription and repair are linked via the TFIIF complex, a basal transcription factor that participates in NER and transcription initiation. Moreover, XPB and XPD are components of the transcription factor, TFIIF, thus explaining their involvement in diseases with different phenotypes (9).

XPD protein possesses both single-strand DNA-dependent ATPase and 5'-3' DNA helicase activities and is thought to participate in DNA unwinding during NER and transcription (9,10). NER repairs DNA damage induced by UV radiation and bulky DNA adducts. However, because XPD is involved in both transcription and NER, it may contribute to repair of other types of damage, such as ionizing radiation. Studies using lymphocytes containing mutant XP genes have an elevated chromatid aberration frequency after exposure to ionizing radiation, suggesting a role for NER proteins in the repair of ionizing radiation-induced damage (11). Ionizing radiation induces oxidative damage and several studies suggest XP proteins may participate in the repair of this type of damage (12,13).

Because XPD is important in multiple cellular tasks and rare XPD mutations result in genetic diseases, XPD polymorphisms may operate as genetic susceptibility factors. As a preliminary test of functionality, we studied the association of three XPD polymorphisms located at codons 199 (Ile→Met), 312 (Asp→Asn) and 751 (Lys→Gln) with proficiency for repair of X-ray-induced chromatid breaks and gaps. Reduced

Abbreviations: 95% CI, 95% confidence interval; CS, cockayne syndrome; NER, nucleotide excision repair; OR, odds ratio; TTD, trichothiodystrophy; XP, xeroderma pigmentosum.

Table I. PCR-RFLP: primers restriction enzymes and fragment sizes

	XPD (199) ^a	XPD (312) ^a	XPD (751)
PCR primers			
Forward	22872F: ctg ttg gtg ggt gcc cgt atc tgt tgg tct	22872F: ctg ttg gtg ggt gcc cgt atc tgt tgg tct	35844F: cct ctc cct ttc ctc tgt tc
Reverse	23952R: (mutant) taa tat cgg ggc tca ccc tgc agc act tcc t	23952: (mutant) taa tat cgg ggc tca ccc tgc agc act tcc t ^b	36560R: cag gtg agg ggg aca tct
PCR fragment (bp)	757	757	734
RFLP			
Restriction enzyme	<i>DpnII</i> ^c	<i>StyI</i> ^c	<i>MboII</i>
Fragment sizes (bp)			
Control cut	357	357	131
Wild-type homozygote	73, 176	151	98, 505
Heterozygote	73, 176, 243	34, 117, 151	98, 505, 603
Variant homozygote	243	34, 117	603
Agarose gel	3% 3:1 (NuSieve)	3% 3:1 (NuSieve)	2% 3:1 (NuSieve)

^aAmino acids 199 and 312 amplified on same PCR fragment.

^btaa tat added to the 5' end of the primer; primer is mutated (g→c, underlined) at bp 23593.

^cPCR fragment containing amino acids 199 and 312 was double digested with *DpnII* and *StyI*.

DNA repair as measured in this assay has been associated with a high degree of cancer incidence in family members (14–16). We found that the Lys/Lys751 was associated with reduced repair of X-ray-induced DNA damage.

Materials and methods

Subjects

We identified *XPD* genotypes and examined their association with DNA repair proficiency as measured previously in 31 Caucasian women from the Breast Surveillance Service at The Johns Hopkins Medical Institutions and female employees at the same institution (14). One woman had breast cancer, whereas the other women had no previous diagnosis of cancer and were categorized as either high risk ($n = 15$), defined as having at least one first-degree relative or two second-degree relatives on the same side of the family with breast cancer, or low risk ($n = 15$) for breast cancer.

DNA repair proficiency

DNA repair proficiency was assessed previously in a masked fashion using the assay developed by Sanford and co-workers (17,18). This assay measures unrepaired DNA (breaks and gaps) in cytogenetic preparations of metaphase lymphocytes isolated from freshly drawn blood of the subjects. The lymphocytes were exposed to X-rays and incubated to allow for repair. Colcemid was added after 0.5 h, the cells were then incubated for another 1 h and then lysed. Since the distribution of breaks and gaps is bimodal, individuals can be categorized as having normal (less than 60) and sub-optimal (greater than 60) repair proficiency based on the sum of breaks and gaps per 100 metaphase cells (14). Helzlsouer *et al.* (14) reported that sub-optimal DNA repair was more prevalent among women with a family history of breast cancer in this study population.

Genotyping

XPD genotypes were determined using a PCR-RFLP technique. Polymorphisms located at amino acids 199, 312 and 751, were amplified from 50 ng DNA using 200 μ M of each dNTP, 0.5 U *Taq* (Promega, Madison, WI) + *TaqStart* Antibody (Sigma, St Louis, MO), 0.8 μ M primer (Table I) and either 1.5 mM (codons 199 and 312) or 2.0 mM (codon 751) $MgCl_2$ in 1 \times PCR buffer (Promega). Codons 199 and 312 were amplified together using 5% DMSO as an additive, whereas codon 751 was amplified in a separate PCR reaction. Both reactions used the same PCR program which consisted of a 4 min denaturation step at 94°C followed by 30 cycles of 30 s at 94°C, 30 s at 60°C and 60 s at 72°C. The PCR amplicons were digested for 2 h at 37°C with a discriminating restriction enzyme, and the digestion products were separated by agarose gels. Table I delineates these conditions.

Statistical analysis

We compared the number of chromatid aberrations (per 100 metaphase cells) for each *XPD* genotype using the Mann-Whitney Rank Sum test. We also categorized individuals as having either normal or sub-optimal DNA repair proficiency as defined earlier and examined whether the risk of sub-optimal repair proficiency was associated with *XPD* genotype. We stratified by age (dichotomized at the mean age of 42 years for our subjects). Exact odds ratios (ORs) and 95% confidence intervals (95% CIs) were calculated via conditional

maximum likelihood methods for 2 \times 2 \times 2 tables using LogXact software (Cytel Software Corporation, Cambridge, MA).

Results

We found no variants at codon 199 in our study population, precluding further analysis of that locus. The *XPD* 312 variant (Asn) allele occurred with a frequency of 0.42, similar to that reported by Shen *et al.* (19). The 751 variant (Gln) allele occurred with a frequency of 0.26 which is also comparable with previous reports (19,20) and to an independent North Carolina genotyping study conducted in our laboratory (unpublished results).

Mean and/or median chromatid breaks, gaps and totals (breaks + gaps) were calculated for each *XPD* genotype (Table II). Because variant homozygotes were rare ($n = 2$ and $n = 1$ for *XPD* 312 and 751, respectively), we combined them with heterozygotes and compared that pooled genotype with the more common homozygote genotype. Individuals homozygous for the Lys751 (common) allele had significantly ($P = 0.01$) higher chromatid aberrations (median = 132) than those having a Gln751 allele (median = 34). The median number of chromatid aberrations was also higher in individuals homozygous for the more common Asp312 allele than those having at least one variant Asn312 allele (132 and 50 breaks and gaps, respectively). However, this difference was not statistically significant ($P = 0.22$).

Figure 1 depicts chromatid aberrations plotted for each Lys751Gln genotype group and stratified by familial breast cancer risk status. Women having the Lys/Lys751 genotype had higher median chromatid aberrations than those with genotypes containing the variant Gln allele whether they came from the low-risk group (89 versus 34, respectively; $P = 0.34$) or from the high-risk group (136 versus 38, respectively; $P = 0.05$). While the difference is statistically significant only in the high-risk group, the number of individuals in each group is small. Interestingly, all the women ($n = 7$) having both the Lys/Lys751 genotype and categorized as having high risk for breast cancer had sub-optimal repair.

To assess the risk of sub-optimal repair due to different *XPD* alleles, we categorized individuals into two repair proficiency groups (sub-optimal and normal repair) based on the number of chromatid aberrations (Table III). In our study population, age was related to the Lys751Gln polymorphism ($P = 0.01$)

Table II. XPD genotypes and chromatid aberrations

XPD genotypes	n	Breaks [median (mean \pm SEM)]	Gaps [median (mean \pm SEM)]	Total (breaks+gaps) [median (mean \pm SEM)]
Asp312Asn				
Asp/Asp	18	81 (62 \pm 8.8)	46 (39 \pm 5.7)	132 ^a (100 \pm 13.6)
Asp/Asn + Asn/Asn	12	34 (49 \pm 9.41)	16 (28 \pm 5.0)	50 ^a (77 \pm 14.9)
Lys751Gln				
Lys/Lys	16	82 (72 \pm 8.0)	46 (42 \pm 4.8)	132 ^c (114 \pm 12.5)
Lys/Gln + Gln/Gln	15	22 (36 \pm 7.7)	14 (23 \pm 4.7)	34 ^b (59 \pm 12.3)

^a $P = 0.22$ by Mann-Whitney Rank Sum test.

^b $P = 0.01$ by Mann-Whitney Rank Sum test.

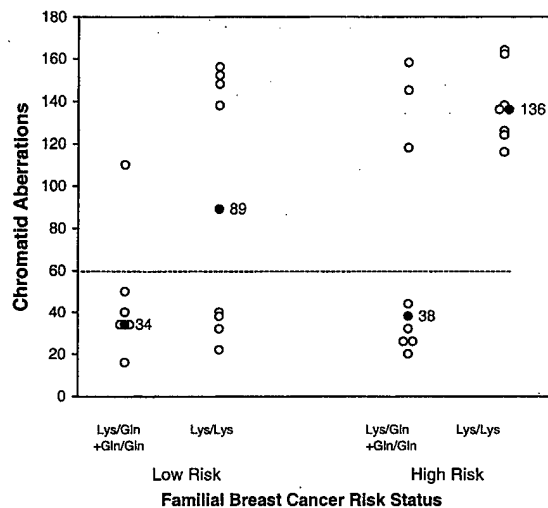


Fig. 1. XPD Lys751Gln genotypes in low and high breast cancer risk groups. Total chromatid aberrations (breaks and gaps) are plotted for XPD Lys751Gln genotypes (Lys/Lys and Lys/Gln + Gln/Gln) stratified according to familial breast cancer risk status. Open circles, data points for each individual; closed circles, median values for each group. The difference in chromatid aberrations between XPD genotypes, Lys/Lys versus Lys/Gln + Gln/Gln, stratified according to familial breast cancer risk was tested using the rank sum test ($P = 0.34$ and $P = 0.05$ for the low- and high-risk groups, respectively). The dotted line represents the bimodal division between good repair and sub-optimal repair (60 chromatid aberrations).

but not to risk status ($P = 0.6$) or to the Asn312Asp polymorphism ($P = 0.53$). We observed a significant increased risk of sub-optimal DNA repair for women having the Lys/Lys genotype (OR = 7.2, 95% CI = 1.01–87.7; $P = 0.035$), but not for the Asp/Asp312 genotype (OR = 1.8, 95% CI = 0.3–11.0, $P = 0.47$).

Discussion

In this study, the Lys/Lys751 genotype was associated with sub-optimal repair of DNA damage induced by X-irradiation. DNA repair was assessed using a cytogenetic assay that measures chromatid aberrations 0.5–1.5 h after treatment with X-rays. Chromatid aberrations result from unrepaired DNA strand breaks caused directly by X-irradiation, or indirectly as a result of repair of other X-ray-induced damage such as base damage (16). Persistence of these breaks suggests a deficiency in DNA repair. DNA repair proficiency, as measured by this cytogenetic assay, has been associated with predisposition to cancer (14–16). Parshad *et al.* (21) reported the sensitivity of the chromatid aberration assay was sufficient to detect XP carriers (heterozygotes) who were clinically normal. While the cytogenetic assay is well documented to detect deficient DNA

Table III. XPD genotypes and risk of sub-optimal DNA repair

XPD allele	Repair (good/sub-optimal)	OR; CI ^a
Asp312Asn		
Asp/Asn + Asn/Asn	6/5	1.0 (ref.)
Asp/Asp	7/11	1.8 (0.3–11.0); $P = 0.47$
Lys751Gln		
Lys/Gln + Gln/Gln	11/4	1.0 (ref.)
Lys/Lys	4/12	7.2 (1.01–87.7); $P = 0.035$

^aAdjusted for age.

repair, we need to address three points concerning our findings: (i) the relationship between X-ray irradiation and XP; (ii) possible discordance between the present work and results from previous studies of rare mutant XPD genotypes; and (iii) the high frequency of the 751 Lys allele (Lys/Lys751 is the most common genotype).

Clinically, XP is due to deficient NER repair of UV-induced damage. Nevertheless, XP mutant cell lines are deficient in the repair of ionizing radiation as measured by chromatid aberrations (11). XP proteins probably participate in the repair of oxidative damage induced by ionizing radiation. Satoh *et al.* (12) reported that extracts from XP cells were unable to repair a specific class of oxygen free radical induced base lesions. Oxidative damage induced by ionizing radiation is preferentially repaired on the transcribed strand, suggesting a role for enzymes such as XPD which participate in transcription-coupled repair (13). Thus, there is a plausible role for the XPD protein in the repair of some types of radiation-induced damage.

With regard to the second point, we observed an increased chromatid aberration frequency in cells from individuals having the Lys/Lys751 XPD polymorphism; however, Sanford *et al.* (22), using the same cytogenetic assay, reported that the chromatid aberration frequency was not elevated in cells containing a rare XPD mutation (obtained from an XP patient). This discrepancy may be due to the different location of the polymorphism and the mutation; the polymorphism occurs at codon 751 whereas most XP individuals in complementation group D have a mutation at codon 683, the putative nuclear location signal which is believed to be responsible for XP symptoms (8,23–25). XPD is part of the TFIIH transcription factor, which is a multi-protein complex involved in many different functions, including transcription, NER, transcription-coupled repair, apoptosis and cell cycle regulation (13). Thus, XPD interacts with many different proteins as part of this complex. Amino acid variants in different domains, such as 683 and 751, of XPD may affect different protein interactions, and result in the expression of different phenotypes. While we

have no additional data to support this hypothesis, it is possible that the 751 Lys allele could have different effects in different DNA repair pathways (as assessed using other DNA repair assays).

Thirdly, we unexpectedly found the more common allele (Lys751) was associated with higher levels of chromatid aberrations than the variant allele (Gln751). Dybdahl *et al.* (20) also reported that individuals with the common allele (Lys751) had an elevated, but not significantly so, risk of basal cell carcinoma (OR = 4.2, 95% CI = 0.8–24). While one might expect the common allele to confer protection rather than risk, putative 'at risk' genotypes of metabolism genes such as glutathione *S*-transferase M1 have frequencies in excess of 60% in some populations (26). In addition, the effect of a given allele on repair may depend on the exposure and interaction with other genes participating in DNA damage recognition, repair and cell cycle regulation.

We found that all of the individuals with both the Lys/Lys751 genotype and a family history of breast cancer had sub-optimal repair (data grouped in the upper right corner of Figure 1), as defined by an increase in chromosomal aberrations induced by G₂ X-irradiation. This chromosomal radiosensitivity has been shown to be associated with individuals reporting cancer, including breast cancer, in their family. Roberts *et al.* (27) studied cellular radiosensitivity, using a cytogenetic assay similar to ours, in family members of radiosensitive (breast cancer patients) and non-sensitive individuals. Segregation analysis suggested that the radiosensitivity was heritable with a single major gene accounting for 82% of the variance among family members. The addition of a second, rarer gene to the model resulted in a better fit of the data. The authors postulated that these cancer-predisposing genes were common, low penetrance alleles found in normal populations (27), such as polymorphisms in DNA repair genes. The present finding is consistent with the hypothesis that multiple low-penetrance alleles could be involved in heritable radiosensitivity. The XPD Lys/Lys751 polymorphisms, specifically, and underlying genetic determinants of familial risk, in general, would be possibilities. Because of the small study population ($n = 31$), however, further statistical analysis of possible inter-relationships among XPD genotype and familial risk status using the data available to us would not be informative. Also, the confidence intervals around the risk estimates are wide, indicating a need to confirm both the qualitative and quantitative results of this work by extending it to a much larger study population. Nevertheless, the finding that the Lys/Lys751 genotype is associated with sub-optimal repair provides sufficient evidence to justify further phenotype/genotype studies as well as determining the impact of the Lys751Gln polymorphism on cancer risk.

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DNA Repair and Breast Cancer

Kathy J. Helzlsouer¹, Nancy Davidson¹, Emily L. Harris, Eileen Perry¹, Kim Butash¹, Dawn Phillips¹, Ruth Lum³, Ram Parshad⁴
 The Johns Hopkins University Schools of Public Health and Medicine, ²Kaiser Permanente Center for Health Research,
³National Institute of Environmental Health Sciences, ⁴Howard University College of Medicine

A B S T R A C T

The objective of this study was to examine the association between DNA repair proficiency and the familial clustering of breast cancer. We hypothesize that mechanisms leading to suboptimal repair of DNA damage are susceptibility factors predisposing to environmental exposures such as ionizing radiation.

Screening for an association study is ongoing. Women with a personal history of breast cancer (N=100) were compared to control women (N=100). We had planned to compare the groups with respect to their ability to repair ionizing radiation-induced DNA damage in lymphocytes leading to suboptimal repair of DNA damage are susceptibility factors predisposing to environmental exposures such as ionizing radiation.

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B A C K G R O U N D

Ionizing radiation is a risk factor for breast cancer. There are differences among individuals in proficiency of repairing ionizing radiation-induced DNA damage. Preliminary data indicate that DNA repair proficiency is associated with breast cancer risk.

Table 1 Association of suboptimal DNA repair proficiency with increased breast cancer risk

	Women with increased breast cancer risk	Control women
Adequate DNA repair	0% (N=0)	28% (N=3)
Suboptimal DNA repair	100% (N=4)	72% (N=12)
OR (95% CI)*	Undefined	5.2 (1.04-26.6) 1 (reference)

*Age-adjusted
 Adapted from Helzlsouer et al. *J Natl Cancer Inst* 88:734-735, 1996.

S T U D Y D E S I G N

Study groups:

- 100 women newly diagnosed with breast cancer
- 100 women at average breast cancer risk
- 100 women with a personal history of breast cancer

Primary study risk factor:

- DNA repair proficiency: cytogenetic assay that measures X-ray induced chromosomal alterations

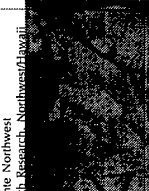
Co-factors:

- Breast cancer risk factors
- Medically related ionizing radiation exposure

Based on family history of breast cancer; at least one first-degree relative with two second-degree relatives on the same side of the family

No history of breast cancer; does not meet criteria above for being at increased breast cancer risk

Correspondence:
 Emily L. Harris, Ph.D.,
 Kaiser Permanente Northwest
 Center for Health Research, Northwest Hawaii
 3800 N. Interchange Blvd.,
 Portland, OR 97218
 Phone: 503-333-3333
 Fax: 503-333-3333
 E-mail: emily.harris@kp.org

Please visit our


DNA REPAIR PROFICIENCY ASSAY

(Sanford et al. *J Natl Cancer Inst* 82:1036-1034, 1990; Parshad et al. *Proc Natl Acad Sci USA* 89:5612-5616, 1992)

Description of assay:

- At room temperature, add two ml of freshly drawn whole blood to a sterile glass tube with open end of 100 µl of RPMI 1640 with 15% precooled fetal bovine serum with 10 µl/ml of heparin and 0.1 mg/ml of gentamicin.
- Centrifuge cell suspension, and resuspend in 20 ml of medium in which PHA is added.
- Increase culture upright for 72 hours before X-irradiation, and invert 4 times.
- After 72 hours, withdraw and discard 10-15 ml of the supernatant.
- Determine the appropriate cell density for X-irradiation, and irradiate the cells (100 R in air; 58 R in the tube because of absorption by the Pyrex) with a Torex 150 X-ray fitted with a 0.5mm aluminum filter and operated at 140 kilovolts and 5 milliamperes at a dose rate of 109 rads per minute.
- Arrest cells in metaphase by colcemid 0.5 hour to 1.5 hours after X-irradiation.
- Examine metaphase cells for chromatid damage (breaks and gaps). Chromatid breaks show displacement of the broken segment. Gaps show discontinuity but no displacement and are scored only if the broken segment is displaced at least 10% of the chromatid width; these are sometimes referred to as nondisplaced breaks.

DNA repair proficiency: Number of breaks and gaps, representing unrepaired DNA strand breaks, observed at 0.5 to 1.5 h after X-irradiation.

Intrasay coefficient of variation: was 9% in NIH laboratory.

Reporting results:

- Suboptimal DNA repair: breaks + gaps is bimodal, with two distinct and overlapping distributions.
- Suboptimal repair: More than a total of 60 breaks + gaps per 100 metaphase cells.

Assay problems & possible solutions

- Transfer of assay to the Johns Hopkins Oncology Center
- Required change in radiation source to cesium chloride (gamma radiation) using 25 cGy radiation
 - Quality control samples showed inconsistent results. Attempts were made to:
 - Re-evaluate 25 cGy vs. 50 cGy radiation dose
 - Optimize temperature control
 - Optimize 5% vs. 10% CO₂ during incubation
 - Optimize 50 vs. 100 RPMI 1640 (50 RPMI 1640)
 - Add modifications by Scott et al. (*Blood Res* 14:52-16, 1996; *Int J Rad Biol* 75:1-10, 1999)
 - None of the modifications improved repeatability.

XPD POLYMORPHISMS AND DNA REPAIR PROFICIENCY

From Lum et al., *Carcinogenesis* 21:551-553, 2000
 Supported by the American Cancer Society through a Johns Hopkins Oncology Center institutional grant, and NIEHS

Rationale

- XPD participates in nucleotide excision repair and transcription-coupled repair.
- Transcription-coupled repair affects repair of different types of DNA damage, including that due to ionizing radiation.
- There is genetic variation in XPD that results in amino acid substitutions, *ex. A3122A>G, Lys75 Gln*.

Results

Table 2 Association of XPD genotypes with DNA repair proficiency

XPD polymorphism	DNA repair proficiency	OR (95% CI)*
A3122A/Gln	Adequate	31%
A3122A/Gln	Suboptimal	1 (ref)
A3122A/Gln	Adequate	54% (N=7)
A3122A/Gln	Suboptimal	69% (N=11)
Lys75 Gln	Adequate	73% (N=1)
Lys75 Gln	Suboptimal	23% (N=4)
Lys75 Gln	Adequate	27% (N=4)
Lys75 Gln	Suboptimal	7.2 (1.01-87.7)

*Age-adjusted
 Adapted from Lum et al., *Carcinogenesis* 21:551-553, 2000

The association between the A3122A/Gln polymorphism and DNA repair proficiency is not statistically significant.

The association between the Lys75Gln polymorphism and DNA repair proficiency is statistically significant. Individuals with the "wild-type" genotype are more likely to have suboptimal DNA repair.

CONCLUSIONS & IMPLICATIONS FOR THIS STUDY

- The problems with the DNA repair proficiency assay that we originally planned to use appear to be unresolvable within the timeframe of the study.
- Genetic variation in XPD may be associated with DNA repair proficiency and breast cancer risk.
- We plan to study the association of XPD variation with breast cancer risk, rather than studying the association between DNA repair proficiency and breast cancer risk.

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