Double-strand breaks repair in lymphoblastoid cell lines from sisters discordant for breast cancer from the New York site of the BCFR

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Unrepaired DNA double-strand breaks (DSBs) may have serious consequences for cells by inducing chromosomal aberrations, thereby increasing genetic instability and cancer risk. One’s capacity to repair DSB is therefore an important factor to consider when estimating cancer risk. We assessed DNA end-joining (EJ) capacity in cell lines derived from sisters discordant for breast cancer to determine if individual differences in DSB repair are a significant risk factor. We used an in vitro phenotypic assay on nuclear extracts from lymphoblasts of 179 subjects including 86 cases and 93 controls. EJ activity was functionally estimated as the ability of extracts to join together monomers of the plasmid pUC18 linearized either with sticky (EcoRI) or blunt ends (HincII). Mean percentage of EJ capacity was slightly lower in cases than controls, both for EcoRI (cases 27.9 ± 11.1; controls 29.6 ± 10.7, P = 0.28) and HincII substrates (cases 28.5 ± 12.2; controls 30.6 ± 13.0, P = 0.36); however, no significant differences were observed. Categorizing EJ capacity into tertiles and using the highest activity as the referent, we observed elevated associations for each tertile of decreased repair [Odds ratio (OR) = 2.20, 95% confidence interval (CI) = 0.77–6.22 and OR = 4.22, 95% CI thins; p = 1.22–14.0, P = 0.02], respectively, for EcoRI. Results were not statistically significant for HincII (OR = 1.37, 95% CI = 0.51–3.70 and OR = 2.32, 95% CI = 0.57–9.38, P = 0.24). These data suggest that individual differences in EJ capacity may represent a risk factor predisposing women to breast cancer.

Introduction

Unrepaired DNA double-strand breaks (DSBs) can easily lead to gross chromosomal aberrations, increased genetic instability and ultimately to cancer development. The capacity to repair these DNA lesions is therefore of paramount importance to prevent the accumulation of tumor-causing mutations (1–3). Two main pathways remove DSB, homologous recombination (HR) and non-homologous end-joining (NHEJ) repair. HR uses the second intact strand of DNA as a template for restoring sequences spanning the breaks, so that the overall process has a high fidelity rate and is error free (1–3). Micro-homologies among sequences flanking the breaks can also be used for DNA repair in a non-conservative process indicated as single-strand annealing (1–3). Nevertheless in mammals, NHEJ is the predominant pathway repairing DSB. While NHEJ is more efficient, it is also potentially error prone since it might reseal the two broken termini with extensive end modifications in a process that may result in sequence insertions or deletions (1–3).

In mammals the conserved complex Mre11–Rad50–Nbs1 activates the DSB repair machinery for both the HR and NHEJ pathways.

Abbreviations: CI, confidence interval; DSB, double-strand break; EJ, end joining; HR, homologous recombination; NER, nucleotide excision repair; NHEJ, non-homologous end joining; OR, odds ratio; SNP, single-nucleotide polymorphism.

NHEJ repair is initiated by the end-binding proteins Ku70 and Ku80 as a Ku heterodimer, which subsequently recruits the DNA-dependent protein kinase catalytic subunit (DNA-PKcs). Bound to the broken ends, DNA-dependent protein kinase is activated, phosphorylating itself together with other targets (i.e. RPA, WRN, Artemis). DNA termini are processed and the break is resealed by the complex ligase IV/XRCC4.

High levels of DNA damage and deficiency in the repair of chromatin breaks induced by radiation in blood cells of women with sporadic and familial breast cancer compared with controls suggest that impaired DSB repair is a predisposing risk factor (4–15). Down-regulation and mutations affecting mediator genes in the DNA damage surveillance network (i.e. ATM, CHEK2, BRCA1, BRCA2 and TP53), that coordinates cell cycle progression, DNA repair and cell survival, provide further data to support the hypothesis that breast cancer pathogenesis is driven by DSB-induced genomic instability (1).

It is intriguing that single-nucleotide polymorphisms (SNPs) might also play a role in breast cancer susceptibility, but to date only a few polymorphisms in HR and NHEJ have been associated with small increases in risk (16–20). However, when SNPs in the NHEJ pathway were studied in breast cancer cases together with breast cancer-associated BRCA1 variants, the risk associated with different genotypes of NHEJ genes was more significant in women bearing at least one variant allele of BRCA1 (21). Recently, an increased breast cancer risk was observed in women harboring putative high-risk genotypes of the genes encoding proteins of the Mre11–Rad50–Nbs1 complex (22).

Although SNPs in key genes are easier to measure in large epidemiological studies than DNA repair phenotype, they are also often poor predictors of the integrity of an entire repair pathway (23). Even multiple SNPs in several genes may not be really informative for the complex molecular events that impact DNA repair capacity; it is, therefore, more important to establish the function of the entire repair pathway. Since associations between DNA repair gene SNPs and cancer risk are fairly weak or non-existent, functional data on DNA repair capacity and cancer risk may be more informative (23). Thus, a key parameter to assess the association between DSB repair and breast cancer risk is measurement of the phenotypic activity.

In this study, we assessed the EJ capacity of nuclear extracts prepared from lymphoblastoid cells derived from sisters discordant for breast cancer. Participants are from the New York Family Registry, one of six collaborating sites of the Breast Cancer Family Registry (http://epi.grants.cancer.gov/BCFR/) (24). EJ capacity was measured using an in vitro fluorescent end-joining (EJ) assay that tested the ability of nuclear proteins to join together monomers of a linearized plasmid substrate, either with sticky (EcoRI) or blunt ends (HincII). The goal was to determine whether altered and/or reduced DNA repair activity is a risk factor predisposing women to breast cancer. A previous study in the same subjects demonstrated a lower efficiency of removal of in vitro-induced benzo[a]pyrene diol epoxide–DNA adducts in cases compared with controls, suggesting that decreased nucleotide excision repair (NER) is a risk factor for breast cancer development (25). We hypothesized that in addition to the NER, deficiencies in other DNA repair pathways, such as DSB, might contribute to altered breast cancer susceptibility and account for interindividual differences in cancer risk (26).

Materials and methods

Study design/population

The study population was selected from families participating in the New York site of the Breast Cancer Family Registry, an international registry of families with breast and ovarian cancers supported by the National Cancer Institute. The study was approved by Columbia University’s Institutional Review Board; written informed consent was obtained from all subjects, and strict quality assurance measures were implemented to prevent bias in the data collection process.
controls and safeguards were used to protect confidentiality. The description of the resources, the recruitment and data collection methods of the Breast Cancer Family Registry are detailed elsewhere (24). Families participating in the New York site have been recruited since 1995 from health clinics, hospitals and community organizations in the New York metropolitan area. Recruitment was initially not based on a family history of breast cancer, whose family history was defined as one of the following criteria: (i) one first- or second-degree relative with breast or ovarian cancer diagnosed before age 45 years; (ii) one first- or second-degree relative with both breast and ovarian cancer regardless of age at diagnosis; (iii) two first- or second-degree relatives with either breast or ovarian cancer after age 45 years; (iv) one male family member with a history of breast cancer and (v) a family member with a known BRCA mutation. The present study included all families for whom lymphoblastoid cell lines were available for at least two sisters discordant for breast cancer who had at least five vials of frozen cells available. Sister sets consisted of 86 case patients and 91 control subjects from 85 families of the total of 1336 families. At enrollment, each consenting participant was asked to complete several questionnaires including a family history questionnaire on age and site of cancers diagnosed among blood relatives; an epidemiology questionnaire that collected information on demographics, ethnicity, smoking, alcohol consumption, reproductive history, hormone use, weight, height and physical activity and a self-administered food frequency questionnaire. Blood was collected from all participating family members at the time of recruitment. For case patients, blood was collected 5 years after diagnosis, on average.

**Laboratory methods**

**Cell culture** All cell lines from the sisters in the study were developed from frozen lymphocytes, with some transformations carried out at Coriell Institute and others in our laboratory. Cells were transformed using Epstein–Barr virus isolated from the marmoset line B95-8 and the same procedure was used by both locations (27). DSB repair Cesium chloride gradient-purified pUC18 (generous gift of Dr Greg Freyer, Columbia University) was used as the DNA substrate for the EJ reactions (28). pUC18 is a commonly used cloning vector isolated from Escherichia coli, a double-stranded circle, 2686 base pairs in length (L09136 GenBank/EMBL accession number) and contains a multiple cloning site with several unique restriction sites. pUC18 was incubated separately with EcoRI or HincII that cut the plasmid vector within the multiple cloning site to produce a linear DNA with either sticky or blunt ends. Restriction endonucleases were purchased from New England BioLabs® (Ipswich, MA) and digestions performed as indicated by the manufacturer. Nuclear extracts were prepared using the NE-PER® Nuclear and Cytoplasmic Extraction Reagent kit; Halt® Protease Inhibitor Cocktail (PIERCE Biotechnology, Rockford, IL) was added according to the manufacturer’s instructions. Nuclear protein extractions were performed considering the cell count in order to have an adequate yield of protein for each sample. Nuclear proteins were quantitated and supplemented with 20% glycerol for storage at −80°C until use. EJ reactions were developed and performed according to the protocol detailed by Perrault et al. (29) with minor modifications. The EJ assay batched discordant sisters for simultaneous testing in order to prevent confounding effects due to assay conditions but with the laboratory blinded to case–control status. Reactions used 20 mM HEPES–KOH (pH 7.5), 80 mM KCl, 10 mM MgCl₂, 1 mg/ml bovine serum albumin, 1 mM adenosine triphosphate and 1 mM dithiothreitol. Bovine serum albumin, adenosine triphosphate and dithiothreitol were added into the reaction mixture just before the assay was initiated. One microgram of nuclear proteins was incubated for 60 min at 25°C with 40 ng of substrate DNA in a reaction volume of 20 μl. Reactions were stopped with addition of RNase (1 μg/ml final concentration) for 20 min at 37°C followed by proteinase K (1 μg/ml final concentration), 20 min at 37°C. One half of the reaction was then loaded onto a 0.7% agarose gel and run at 1 V/cm for 140 min. Gels were stained with 10,000 SYBR® Green (Invitrogen, Carlsbad, CA) and scanned with a FluorChem™ SP imaging assay. The sister-set samples were run in duplicate and loaded onto the same gel; DSB repair capacity was evaluated by measuring the fluorescence emissions of the end-joined products. Time course experiments were run on five randomly selected samples and dimers, trimers and higher forms were measured in the linear range of the EJ reaction. The percentage of EJ was expressed as (intensity of EJ products/total substrate intensity) × 100%. Each EJ reaction included a negative control, also run in duplicate of one of that day’s test samples after heat denaturing at 74°C ligase was run as a positive control in each experiment. As an additional quality control, a large batch of nuclear proteins were isolated from a randomly selected cell line and assayed in each EJ reaction and gel run. The products of controls were run in every gel together with the actual samples.

**Statistical methods**

Differences between sister sets were examined using conditional logistic regression (30). In these regression models, the association between DRC and case–control status was estimated while simultaneously adjusting for age at blood donation, current body mass index [(weight in kg)/(height in m)²] and smoking. Odds ratios (ORs) and P _trend_ values were calculated using maximum likelihood methods (30). In addition to treating DNA repair as a continuous variable, we also categorized DNA repair based on the median value and then by tertiles of the unaffected sisters. In addition to the primary analyses, we compared models for individuals whose blood specimen was collected within 2 years of diagnosis for the affected sister and those individuals whose blood sample was collected >2 years after diagnosis of the affected sister.

**Results**

To examine the association between altered DNA repair capacity and breast cancer risk, we measured the EJ activity in lymphoblastoid cells from sisters discordant for breast cancer with a fluorescent in _vitro_ assay. The EJ capacity was measured in the linear range of the assay using the plasmid pUC18 linearized with EcoRI and HincII (sticky or blunt termini) and the specificity of the reaction was checked with inactive nuclear proteins. Although deficient repair can be linked to any of the genes involved in the NHEJ pathway, ligation is the last key step carried out by ligase IV in human cells. We therefore included as a positive control the EJ products of T4 ligase. Figure 1 shows the increase in percentage EJ in reactions ranging from 10 to 90 min. Also shown is a representative gel with test samples. The day-to-day variability of the assay was addressed by including as an internal control extract from a randomly selected sample which was run together with the test samples in every experiment. With the EcoRI substrate, the mean percent repair of 18 repeat assays was 32.1 ± 6.2 (coefficient of variability = 19.4%), whereas with the HincII substrate, it was 34.7 ± 9.8 (coefficient of variability = 28.4%). The capacity to rejoin the two DNA substrates with cohesive and non-cohesive termini was correlated with Pearson coefficients of 0.677 (P < 0.0001) in cases and 0.810 (P < 0.0001) in controls. For both the EcoRI- and HincII-linearized pUC18 DNA substrates, EJ activity was slightly higher in control subjects compared with cases (29.6 ± 10.7 versus 27.9 ± 11.1 and 30.6 ± 13.0 versus 28.8 ± 12.2, respectively); however, there was no statistically significant difference (Table I). We analyzed case–control differences in DSB repair capacity after stratification of subjects by age, ethnicity, body mass index and smoking status. For both repair substrates, all the subgroups showed a slight, non-significant reduced DNA repair in cases compared with controls, with the exception of the subjects older than 60 years at the time of blood donation and those smoking <10 cigarettes per day. The largest differences in the mean value of EJ repair between cases and controls were observed in women younger than 40 years. However, these differences also were not statistically significant (Table I). There was no difference in repair by body mass index and smoking status. For both substrates, control subjects smoking >10 cigarettes per day had significantly higher EJ activity compared with those smoking <10 cigarettes per day but the number of subjects in each group was small. While case patients had a statistically higher repair of blunt-ended DNA substrate than patients of other ethnicities but not of the sticky-ended substrate. Cases younger than 40 years had lower DNA repair capacity than older case, with a slightly linear age-related trend with both the substrates; however, the trend was not significant.

Using conditional logistic regression and treating EJ capacity as a continuous variable, the adjusted ORs of breast cancer were 1.07 [95% CI = 1.01–1.13] and 1.08 (95% CI = 1.01–1.15), respectively, for the EcoRI and HincII substrates (Table II). Dichotomizing values using the median in control subjects suggested a 10–20% increase in risk with poorer repair capacity but this result was not significant. Categorizing EJ capacity into tertiles and using the highest capacity as the referent, we observed elevated associations with OR = 2.20, 95% CI = 0.77–6.22 for Tertile 2 and OR = 4.22, 95% CI = 1.22–14.6 for Tertile 3 for EcoRI (P _trend_ = 0.02). Results were
not statistically significant for HincII (OR = 1.37, 95% CI = 0.51–3.70 for Tertile 2 and OR = 2.32, 95% CI = 0.57–9.38 for Tertile 3 compared with Tertile 1 \( P_{\text{trend}} = 0.24 \)]. Similar trends were observed when stratifying these models by time between blood collection and age at diagnosis of the affected sister (<2 years and ≥2 years), though the point estimates were higher among the subset with blood collected within 2 years of diagnosis (data not shown).

All the subjects had previously been analyzed for NER phenotype (25). These data were combined with the DSB repair activity data using median values among controls for both assays (27.6% for DSB and 35.4% for NER) to categorize subjects into high and low activity. Using subjects with above the median repair for both assays as the reference group, the adjusted OR was 1.16 (95% CI = 0.42–3.17) for subjects with one assay below the median and 4.92 (95% CI = 1.36–17.8) for those with both assays below the median.

Discussion

We used EJ capacity as marker of DSB repair capacity in lymphoblastoid cell lines from sisters discordant for breast cancer. Different substrates, either with sticky or blunt ends, were tested with an in vitro assay to rapidly visualize on agarose gels the products of rejoining activity (dimers, trimers and high molecular weight multimers). The aim was to determine if reduced and/or altered DSB repair is associated with increased breast cancer risk. We used a non-radioactive method with the fluorescent dye SYBR® Green rather than a previously used radioactive probe (31). Although we believe that SYBR® Green is a less sensitive detection system than \(^{32}\)P, fluorescent quantification of EJ reactions using 20 ng of substrate allowed the assessment of gel DNA in amounts as little as 0.5 ng (32,33).

The mean repair activity was non-significantly lower in breast cancer cases than controls with both substrates. When using EJ values as a continuous variable, the adjusted ORs indicated an increased risk of 1.07-fold (1.01–1.13) and 1.08-fold (1.01–1.15) for reduced repair capacity with EcoRI- and HincII-linearized pUC18, respectively. Stratifying data into tertiles based on the controls, we observed a 4-fold increased breast cancer risk (OR = 4.22, 95% CI = 1.22–14.6) for those with the poorest repair capacity using the EcoRI substrate. To date, only Bau et al. (34) used an in vitro EJ assay within the epidemiological setting of a case–control study. They provided evidence that lymphoblastoid cells from breast cancer patients had lower capacity to religate a DNA substrate bearing cohesive ends than those from healthy controls (34). They had also demonstrated previously that whole-cell extracts from BRCA1-deficient breast cell lines compared with BRCA-proficient ones had decreased EJ capacity (21). Our observations with EcoRI-linearized pUC18 (cohesive ends) are in agreement with their results, although we used a different substrate that was not radioactively labeled.

Bau et al. (34) also used a luciferase-containing plasmid with blunt ends due to cutting with HindIII to detect the EJ fidelity in transfected mononuclear cells of 22 breast cancer cases and 20 controls. We instead assayed in vitro DNA substrates with two different types of termini, confirming the reduced EJ capacity observed with EcoRI-linearized pUC18. Blunt DNA termini are the easiest type to be repaired by NHEJ, usually with an accurate ligation. However, it has also been shown that different types of DNA termini are processed by different enzymatic complexes recruited by the NHEJ machinery, depending upon the type of DSB (35). There was a correlation between activity measured with both substrates in cases and controls (0.677 and 0.810, \( P < 0.0001 \)) respectively. The comparable extent of EJ repair observed with the two different types of substrate supports the result of a general impairment of DSB repair and not the result of a single NHEJ pathway.

The lack of an increased breast cancer risk in the tertile with the poorest EJ activity, between cases and controls, observed with the HincII substrate might be accounted for a higher day-to-day variability (28.4%) compared with the EcoRI substrate (19.4%).

These same subjects were included in a larger prior study in which we observed increased risk associated with decreased NER capacity with an OR of 2.99 (95% CI = 1.45–6.17) for those in the quartile with the poorest repair. NER was also lowest in the youngest cases and oldest controls. Similarly for EJ capacity, we observed that the youngest cases had the lowest DNA repair capacity, although the trend with age was not significant. When looking at the combination of repair capacity in the two pathways, NER and EJ, those with decreased repair in both had an OR of 4.92 (95% CI = 1.36–17.8), compared with the ORs of 2.44 (1.46–4.11) for those with NER below the median (25) and 1.23 (0.46–3.30) for those with EJ below the median. This is in contrast to studies of upper aerodigestive tract cancer patients in which the mutagen sensitivity assay was carried out using bleomycin and benzo[a]pyrene diol epoxide as test mutagens (36). Subjects with sensitivity to both agents were at a more than additive 19.2-fold increased risk compared with those not sensitive to either agent.

Several other studies have reported on DNA repair capacity as a risk factor for breast cancer development. Using the comet assay, Smith et al. (15) found decreased capacity to repair DNA damage induced by ionizing radiation in peripheral lymphocytes from breast cancer cases compared with controls. Using the host cell reactivation assay,
Ramos et al. (37,38) showed that younger breast cancer cases less efficiently repaired UV-induced DNA damage than older cases or age-matched control subjects. Studies of non-melanoma skin cancer provide additional evidence on age effects; younger patients affected by basal cell carcinoma had a lower capability to repair DNA compared with their matched control subjects and repair capacity was also lower compared with older cases (37,39,40). In our study, after age stratification, mean values of EJ capacity among controls decreased with age.

### Table I. Percentage of DNA EJ capacity in case patients and control subjects in a population of sisters discordant for breast cancer

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>EcoRI</th>
<th></th>
<th>HincII</th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>Case patients</td>
<td>Control subjects</td>
<td></td>
<td>Case patients</td>
</tr>
<tr>
<td>n</td>
<td>Mean ± SD</td>
<td>n</td>
<td>Mean ± SD</td>
<td>n</td>
</tr>
<tr>
<td>Total</td>
<td>86</td>
<td>27.9 ± 11.1</td>
<td>93</td>
<td>29.6 ± 10.7</td>
</tr>
<tr>
<td>Age at diagnosis, years</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;50</td>
<td>73</td>
<td>27.9 ± 11.7</td>
<td>13</td>
<td>27.7 ± 7.7</td>
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<tr>
<td>≥50</td>
<td>16</td>
<td>25.5 ± 11.8</td>
<td>17</td>
<td>30.5 ± 13.0</td>
</tr>
<tr>
<td>P</td>
<td>0.4</td>
<td>0.5</td>
<td>0.37</td>
<td>0.73</td>
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<tr>
<td>Interval between age at diagnosis and age at interview, years</td>
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<td></td>
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<td>&lt;40</td>
<td>46</td>
<td>26.1 ± 11.5</td>
<td>40</td>
<td>29.9 ± 10.5</td>
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<td>&gt;40</td>
<td>16</td>
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<td>P</td>
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<td>0.73</td>
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<td>Age at blood donation, years</td>
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<tr>
<td>&lt;25</td>
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<td>26.2 ± 11.9</td>
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<td>≥25</td>
<td>23</td>
<td>26.2 ± 11.9</td>
<td>28</td>
<td>29.4 ± 10.2</td>
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<td>BMI, kg/m²</td>
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<td>45</td>
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<tr>
<td>≥25</td>
<td>38</td>
<td>26.9 ± 10.3</td>
<td>48</td>
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<tr>
<td>P</td>
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<td>51</td>
<td>27.6 ± 11.4</td>
<td>35</td>
<td>28.2 ± 10.9</td>
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<td>11</td>
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<td>32.5 ± 10.9</td>
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<tr>
<td>P</td>
<td>0.09</td>
<td>0.01</td>
<td>0.08</td>
<td>0.02</td>
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### Table II. Conditional logistic regression analysis of the relationship between DNA EJ capacity and breast cancer risk among sisters discordant for breast cancer

<table>
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<th>HincII</th>
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<tbody>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>Adjusted OR (95% CI)</td>
<td></td>
<td>OR (95% CI)</td>
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<td>Continuous (decrease by 1%)</td>
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<tr>
<td>% by median</td>
<td>1.07 (1.01–1.13)</td>
<td>1.07 (1.01–1.13)</td>
<td></td>
<td>1.09 (1.02–1.16)</td>
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<td>&gt;27.6</td>
<td>1.00 (referent)</td>
<td>1.00 (referent)</td>
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<td>1.00 (referent)</td>
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<tr>
<td>≤27.6</td>
<td>1.29 (0.52–3.21)</td>
<td>1.23 (0.46–3.30)</td>
<td></td>
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<td>% by tertile</td>
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<tr>
<td>&gt;33.3</td>
<td>1.00 (referent)</td>
<td>1.00 (referent)</td>
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<td>23.3–33.7</td>
<td>1.90 (0.70–5.17)</td>
<td>2.20 (0.77–6.22)</td>
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<td>1.37 (0.51–3.66)</td>
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<td>≤23.3</td>
<td>4.00 (1.22–13.1)</td>
<td>4.22 (1.22–14.6)</td>
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<td>2.99 (0.76–11.8)</td>
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<td>P trend</td>
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<td>0.12</td>
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### Notes

- Cell lines were obtained from 85 families in the New York Breast Cancer Family Registry. Medians were used to categorize the intervals between age of diagnosis and age at the interview, BMI and smoking level.
- P value using two-tailed Student’s t-test.
- P value using two-sided one-way analysis of variance.

### References

N. Machella et al. (37,38) showed that younger breast cancer cases less efficiently repaired UV-induced DNA damage than older cases or age-matched control subjects. Studies of non-melanoma skin cancer provide additional evidence on age effects; younger patients affected by basal cell carcinoma had a lower capability to repair DNA compared with their matched control subjects and repair capacity was also lower compared with older cases (37,39,40). In our study, after age stratification, mean values of EJ capacity among controls decreased with age.
until age >60 years for both substrates (Table 1). As DSB repair is expected to become less efficient during normal aging (41), the lack of a decrease in the repair of DNA in the oldest controls may be due to the small number older than 60 years. In addition to a reduced efficiency of EJ activity, age-related genomic instability might be also be induced by the infidelity of NHEJ (42). Pre-senescent and senescent fibroblasts have a compromised fidelity of NHEJ and, in particular, older cells do not accurately ligate cohesive ends and tend to generate large deletions at the junctions (43).

Similar to Bau et al. (34), we used lymphoblastoid lines derived from breast cancer patients and healthy women. This provides outcomes with more biological significance compared with previous studies that used embryonic fibroblasts and breast cancer cell lines to investigate DSB repair (44). However, in contrast to Bau et al. (34), we used a family-based design of at least one affected sister with breast cancer and one unaffected sister. This partially removes potential confounding related to population admixture and reduces confounding by family-level factors. The sister-set design has been recently discussed in terms of its usefulness compared with other study types (25,45,46).

The present study also has limitations. Because the DNA repair tests are quite time consuming, our sample size is relatively small. Another constraint faced by most DNA repair phenotyping studies is the use of a surrogate tissue; in our case, transformed lymphoblastoid cells that might not reflect the actual EJ capacity of the target tissue, the mammary epithelium. Nevertheless, due to the difficulty of sampling target tissues, most DNA repair phenotyping studies have been performed on lymphocytes (6). In addition, frozen viable cells are much more readily available than target tissues and, for lymphoblastoid cells, have the advantage of an almost unlimited source of material for molecular studies. Bleomycin sensitivity was found to be similar for mononuclear cells compared with lymphoblastoid cell lines (47).

Another potential limitation is that blood samples were collected after diagnosis and our breast cancer cases were prevalent; blood samples were collected on average 5 years after diagnosis. To address these concerns, we stratified the main model by years from breast cancer diagnosis to evaluate the potential impact of disease or treatment on the association between EJ capacity and breast cancer risk. We found that the relationship between EJ capacity and breast cancer risk was similar for women diagnosed within 2 years of blood draw compared with women diagnosed >2 years from blood draw. Without conducting a prospective study, we cannot entirely exclude an effect of treatment or disease. However, a recent study, using the COMET assay, demonstrated no difference in DNA damage of lymphoblastoid cells developed from blood samples collected before or after breast cancer diagnosis (48). Given the complexities of DNA repair phenotyping assays, there are currently no prospective studies of DNA repair capacity and breast cancer risk.

In summary, the present data add support to the hypothesis that inefficient DNA repair is associated with breast cancer susceptibility. The possibility to non-invasively detect biomarkers indicating compromised DNA repair capacity can greatly impact the determination of breast cancer risk and prognosis in high-risk subjects, especially in breast cancer families.

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References


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