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DNA double-strand break repair genotype and phenotype and breast cancer risk within sisters from the New York site of the Breast Cancer Family Registry (BCFR)

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Abstract

Purpose—We previously observed that poor DNA repair phenotype is associated with increased breast cancer (BC) risk within families. Here, we examined whether genetic variation in double strand break repair (DSBR) genes is associated with BC risk and if genotypes are related to phenotype in unaffected women.

Methods—Using data from the New York site of the Breast Cancer Family Registry, we investigated 25 SNPs involved in DSBR using biospecimens from 337 BC cases and 410 unaffected sister controls.

Results—Genotypes in *XRCC4* were associated with BC risk, with ORs of 1.67 (95% CI = 1.01–2.76) for the combined GA/AA of rs1805377 and 1.69 (95%CI=1.03–2.77) for rs1056503 TG/GG; these associations were no longer statistically significant in multivariable conditional logistic regression models. When examining the association of SNPs with phenotype, we found that genotypes of *XRCC5* rs3834 and rs1051685, which were highly correlated with each other, were associated with end joining (EJ) capacity; women with the *XRCC5* rs3834 GA genotype had better DNA repair as measured by higher levels of EJ capacity (37.8±14.1% for GA versus 27.9±11.8% for GG carriers (p=0.0006). Women with the AA genotype of *BRCA1* rs799917 also had higher EJ capacity (35.1±9.2%) than those with GG (26.4±10.1%, p=0.02).

Conclusions—Overall we found that selected DSBR genotypes were associated with phenotype, although they were not associated with BC risk itself, suggesting that phenotypic measures are influenced by endogenous and exogenous factors across the lifecourse and may be better markers than genotypic measures for ascertaining BC risk.

Keywords

breast cancer; double-strand break; end-joining capacity; polymorphism

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Introduction

DNA double-stranded breaks (DSB), induced directly from exposure to ionizing radiation or the result of endogenous or exogenous exposures, play an important role in tumorigenesis [1, 2]. In humans, two main repair pathways are responsible for repairing DSB: (1) non-homologous end joining (NHEJ), which involves direct DNA end-joining and requires no sequence homology [3], and (2) homologous recombination repair (HRR) [4], which relies on extensive nucleotide sequence complementarity between the intact and the damaged DNA strands as the basis for strand exchange and repair. DSB repair (DSBR) mechanisms are important to breast cancer (BC) as evidenced by the key major BC susceptibility genes, *BRCA1* and *BRCA2* [5], as well as supported by increased chromatid damage in x-irradiation-treated blood lymphocytes of BC patients and their first degree relatives [6]. In addition, a study of genome-wide loss of heterozygosity suggested that BC pathogenesis is driven by DSB-initiated chromosome instability [7]. Thus, it is plausible that polymorphisms in DSBR genes may alter normal protein activity and/or binding and thus affect DSBR efficiency, leading to genomic instability, and increased BC risk.

Although some studies have suggested that SNPs in DSBR genes are related to modest increases in BC risk [8–20], much of the evidence has been mixed and primarily examined in women at average risk and largely without integration of DNA repair phenotype data. Within families at higher risk of BC, we previously reported an association of impaired in DSBR capacity and increased BC risk [21]. Our results suggested that suboptimal DSBR capacity was associated with 2–4 times the risk of BC compared to women with optimal DSBR capacity [21]. Using data from the New York site of the Breast Cancer Family Registry (BCFR), we investigated 25 SNPs in 11 genes involved in DSBR using biospecimens from 337 BC cases and 410 sister controls unaffected by BC from 304 families. We also investigated the role of these SNPs in DNA end-joining (EJ) capacity in a subset 91 unaffected individuals for which EJ capacity data was available using blunt and sticky end substrates [21].

Materials and methods

Study participants

We conducted our study using data from families participating in the New York site of the BCFR, a six-sites international registry (California, New York, Philadelphia and Utah in the US, Ontario in Canada and Melbourne and Sydney in Australia) [22]. The New York site recruited high risk breast and/or ovarian cancer families from clinical and community settings within the metropolitan New York area. We recruited families if they met one of the following criteria: 1) have a female relative with breast or ovarian cancer diagnosed before age 45 years, 2) have a female relative with both breast and ovarian cancer regardless of age at diagnosis, 3) have two or more relatives with breast or ovarian cancer diagnosed after age 45 year, 4) be a male with breast cancer diagnosed at any age, or 5) have a family member with a known *BRCA* mutation. Family members completed an epidemiologic questionnaire to provide information on demographics, family history of cancer, ethnicity, smoking, alcohol consumption, reproductive history, hormone use, weight, height, and physical activity, and a self-administered food frequency questionnaire to provide information on diet.

We collected blood from participants at the time of recruitment to permit the isolation of plasma and white blood cells (WBC) fractions. For this study, we used a family-based study design with sister sets discordant for BC [21, 23–27]. Specifically, we used information from a total of 337 affected sisters with BC and 410 unaffected sisters with blood DNA available for genotyping. Among them, DNA EJ capacity in lymphoblastoid cell lines was

available for a total of 91 unaffected sisters [21]. The study was approved by Columbia University's Institutional Review Board.

SNP selection and Genotyping

We selected 25 SNPs from 11 DSB repair genes (*ATM* (rs1801516, and rs664677), *BRCA1* (rs1799966, and rs799917), *BRCA2* (rs766173, and rs144848), *NBS1* (rs1805794, and rs1805787), *XRCC3* (rs1799794), *RAD51* (rs1801321), *RAD52* (rs11226), *LIG4* (rs1805388), *XRCC4* (rs1805377, rs2075685, rs1056503, rs1478485, rs13180316, and rs963248), *XRCC5* (rs2440, rs1051677, rs3835, rs3834, rs828699, and rs1051685), and *XRCC6* (rs132793)). Our selection of DSB repair SNPs was based on the following criteria: (a) associated with cancer risk at any site in epidemiologic studies, (b) a minor allele frequency of at least 5%, or (c) coded for missense changes. Although the biological effect of most SNPs selected for study is not known, we expected that these criteria would maximize the likelihood of choosing SNPs with functional significance and with a possible association with BC.

We extracted genomic DNA from WBC by salting out procedures and genotyped in a combination of BioTrove OpenArray™ system (Applied Biosystems, Foster City, CA) with TaqMan assays and 384 well TaqMan assays. For the BioTrove platform, we used 200ng of DNA (2ul) in an array with 3,072 through-holes arranged in a pattern of 96 sub-arrays. The PCR mixtures included 1x (144ul) TaqMan Universal PCR Master Mix (Applied Biosystems), 1% (14.4ul) Pluronic F38 (BioTrove, Woburn, MA), 0.5% (9.6ul) glycerol (Sigma-Aldrich, St. Louis, MO), 0.05 mg/ml (1.5ul) BSA (Sigma-Aldrich) and 5.0ul PCR grade water (Sigma-Aldrich). The OpenArray™ AutoLoader enables loading of the mixed solution (DNA and PCR mixture) onto an OpenArray™ plate from a 384-well plate. The PCR thermal cycling protocol consisted of 92°C for 10 min followed by 50 cycles of 92°C for 15s, 55°C for 1 min and 72°C for 1 min (imaging step). Following amplification, amplicon dissociation was measured by cooling the PCR array to 65°C then slowly heating to 92°C for 1°C per min, with images collected every 0.25°C.

We used the “Image” program to obtain the three genotyping clusters in the scatter plot. Samples that failed to amplify on the array were genotyping using the TaqMan assays in 384 well assays. Samples that failed on both platforms were indicated as missing. We verified genotype reproducibility by randomly duplicating 5 percent of DNA samples (100% consistency was found).

DNA end-joining capacity assay

We evaluated DNA repair capacity in the DSB repair pathway by fluorescence measurement of the end-joined products using two different substrates with blunt (*HincII* cut) or sticky (*EcoRI* cut) ends as described previously [21]. Each EJ reaction included a negative control, also run in duplicate of one of that day's test samples after heat denaturing. T4 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. The percentage of EJ was expressed as (intensity of EJ products/total substrate)*100%. We performed all assays blinded to the subject's case-control status.

Statistical methods

We tested Hardy-Weinberg equilibrium (HWE) by the 2 test in unaffected sisters. Although six (rs1805388, rs1805377, rs1056503, rs2440, rs3834, and rs1051685) did not pass the HWE test at $p=0.05$, we did not exclude these SNPs from the data analysis. We used

ANOVA test to compare differences in EJ capacity across the various genotypes among unaffected sisters.

We used conditional logistic regression models to estimate within-family odd ratios (ORs) and 95% confidence intervals (CIs) for the main effect of genotype on BC risk. To determine the independent effects of SNPs on BC risk, we then estimated the ORs for each genotype adjusting for all SNPs simultaneously in multivariable conditional logistic regression models. In these models adjusting for age, we included all the SNPs except *XRCC4* (rs1805377) and *XRCC5* (rs1051685). These SNPs were excluded in the full model because of their strong correlation with another SNPs at the same locus. To determine the collinearity between SNPs, we used the Spearman correlation test to examine the correlation between SNPs. *XRCC4* rs1056503 and rs1805377 and *XRCC5* rs3834 and rs1051685 were significantly correlated with each other with spearman correlation coefficients of 0.994 ($p < 0.0001$) and 0.989 ($p < 0.0001$), respectively. In Model 2, we only retained SNPs significant at the 0.2 level in the full model. In Model 3, we only retained SNPs significant at the 0.2 level in Model 2. All models were adjusted for age.

In addition to the conditional logistic regression models which examined within family differences in genotype and BC risk, we also assessed population average effects of the genotype on BC risk through the use of generalized estimating equations (GEE) [28]. We were able to include women who did not have sibling controls in the GEE analyses. All analyses were performed with SAS software 9.2 (SAS Institute, Cary, NC).

Results

The distributions of demographic characteristics between cases and their unaffected sister controls were reported in the previously studies [23, 25]. In brief, the distributions of race and age at blood draw were similar between cases and their unaffected sister controls. *BRCA* mutation frequencies were higher in cases than in controls (8.2 versus 3.7% for *BRCA1* and 4.3 versus 2.6% for *BRCA2*). There were no differences in age at menarche and hormone replacement therapy use between cases and their sister controls.

Breast cancer risk and genetic variation in DSB repair genes

The overall effects of the single SNPs in genes in the NHEJ pathway on BC risk are given in Table 1. The combined OR for GA/AA genotypes of *XRCC4* rs1805377 was 1.67 (95%CI=1.01–2.76). We also observed a statistically significant effect of the combined TG/GG *XRCC4* rs1056503 genotypes with BC with an OR of 1.69 (95%CI=1.03–2.77) when compared to subjects with the TT genotype. Variant genotypes in *XRCC5* rs1051677 were inversely associated with BC. The OR for the combined TC/CC genotypes was 0.61 (95%CI=0.33–1.22). Eleven SNPs in genes involved in HRR were analyzed and results are reported in Table 2. We found modest associations of BC risk with SNPs in *ATM* rs1801516, *RAD52* rs11226, and *BRCA2* rs766173, but these associations were not statistically significant.

We performed an analysis adjusting simultaneously for all SNPs except *XRCC4* rs1805377 and *XRCC5* rs1051685 because of their strong correlation with other SNPs (see Statistical Methods). Table 3 shows the adjusted conditional logistic regression models as well as GEE models. Overall, there were elevated associations for *RAD52* rs11226 GA/AA genotypes (OR=1.58, 95%CI=0.93–2.70), *XRCC4* rs1056503 GT/GG genotypes (OR=1.52, 95%CI=0.91–2.55) and *XRCC5* rs3834 GA/AA genotypes (OR=1.59, 95%CI=0.88–2.90), however, these associations were not statistically significant. Similar results were obtained using GEE models. Only the *RAD52* GA/AA genotype was of borderline statistical significance in the final model with an OR of 1.26 (95%CI=0.97–1.64).

End-joining capacity and SNPs in DSBR genes

Associations of EJ capacity with SNPs in the NHEJ pathway are given in Table 4 among sisters unaffected by BC. *XRCC5* rs3834 and rs1051685 were significantly associated with EJ capacity. Carriers of the *XRCC5* rs3834 homozygous GG genotype have lower EJ capacity than variant carriers. Heterozygous GA and homozygous AA subjects have EJ levels of $37.8 \pm 14.1\%$ and $33.0 \pm 10.6\%$, respectively, versus $27.9 \pm 11.8\%$ for the GG subjects ($p=0.0006$) when using the blunt end substrate. The differences between mean values of repair capacity were similar for the sticky end substrate. We also found that carriers of the *XRCC5* rs1051685 AG heterozygous genotype have EJ capacity levels of $38.0 \pm 14.4\%$ while the other genotypes have lower values ($27.8 \pm 11.8\%$ for AA and $32.5 \pm 12.2\%$ for GG, $p=0.006$) when assaying the blunt end substrate. These results were consistent with the highly correlation between genotypes of *XRCC5* rs3834 and rs1051685. The mean EJ values for the sticky and blunt end substrates were also similar.

Table 5 shows the EJ capacity by HRR SNP genotypes among sisters unaffected by BC. While most HRR genotypes tested showed no significant effect on EJ capacity, we found a statistically significant effect for *BRCA1* rs799917 genotype with the sticky end substrate (Table 5). EJ capacity was significantly higher for carriers of the AA genotype ($35.1 \pm 9.2\%$) than for carriers of the GA ($29.9 \pm 10.9\%$) and GG ($26.4 \pm 10.1\%$) genotypes. This increase in EJ capacity corresponded with a statistically significant dose-response trend ($p=0.02$). While a similar increase was observed with the blunt end substrate, the difference was not statistically significant.

Discussion

We screened 25 SNPs in 11 DSBR genes and found that SNPs in *XRCC5*, *XRCC4* and *BRCA1* were associated with BC and/or had an effect in EJ capacity. However, the associations with BC risk were no longer significant in multivariable conditional logistic regression models. A role for the DSBR pathway in breast tumorigenesis has been suggested by both genetic association [8–20] and phenotypic studies [21, 29, 30]. However, there are limited data on genotype and DSBR phenotype within higher risk women. We found significantly associations with BC for *XRCC5* rs1051677, *XRCC4* rs1056503, and *XRCC4* rs1805377 variants, however these associations were no longer present in the adjusted logistic regression and GEE models. We found six SNPs did not pass the HWE test in unaffected sisters. The SNPs that did not pass in *XRCC4* and *XRCC5* were highly correlated. The strong correlation may be related to differences in genotype inheritance in higher risk families rather than genotyping error. We found statistically significant differences in EJ capacity for carriers of different variants of *XRCC5* rs3834 and its correlated SNP, rs1051685, and one *BRCA1* variant, rs799917. In this study, only variants of *XRCC5* rs3834 were associated with altered EJ capacity and there was a borderline association with increased BC risk.

XRCC5 encodes Ku80, one of the two partners of the DSB binding complex that initiates NHEJ [31]. This complex recruits additional proteins to the DSB for further processing and functions to prevent degradation of the DSB ends to conserve genetic information and to allow for efficient repair [32]. In support of this enzyme's requirement for NHEJ, *in vitro* studies with *XRCC5* mutant cell lines have shown that the frequency of DNA end-joining decreases 1000-fold in the absence of this protein [33]. In this study we found that *XRCC5* rs1051677 CC genotype was associated with decreased BC risk. In addition, we found there is a high correlation between genotypes in *XRCC5* rs3834 and rs1051685, and both heterozygous carriers of *XRCC5* rs3834 and rs1051685 individuals had higher EJ capacity when compared to carriers of the homozygous variant and wild type alleles. This unexpected association between cancer susceptibility and increased DSBR capacity suggests that we

should be cautious when interpreting results obtained from phenotypic biomarker studies. The NHEJ process is error-prone as it does not use an undamaged template for the repair of a DSB [34]. Increased end-joining might indicate not a more efficient repair but a less accurate resealing of breaks, and possibly increased degradation and DNA sequence loss, a phenotype that could lead to increased cancer susceptibility.

The BRCA1 protein plays an important role during homologous recombination [35]. Additional roles have also been described for BRCA1 during NHEJ and as a regulator of the expression of other DNA repair genes. Cell-free extracts from *BRCA1*-null fibroblasts have deficient NHEJ activity and an *in vivo* host cell end-joining assay has shown that human cells heterozygous for *BRCA1* mutations exhibit impaired fidelity of DSB end joining [36, 37]. These data suggest that BRCA1 is necessary for faithful rejoining of broken DNA ends. Whole-cell extracts from BRCA1-deficient BC cell lines compared with BRCA1-proficient ones had decreased EJ capacity [10]. Although the precise role of BRCA1 in NHEJ remains unclear, previous work has suggested that it is likely to participate in NHEJ by virtue of its interaction with the Rad50/Mre11/Nbs1 complex [38]. We investigated two SNPs in *BRCA1* and observed that for one of them, *BRCA1* rs799917, the variant AA genotype was associated with statistically significant higher EJ compared with the GG wild type genotype. This variant was also related to a non-significant decreased in BC, which might indicate a direct genotype-phenotype association. The *BRCA1* rs799917 SNP encodes a proline to leucine substitution at codon 871 in a highly conserved region of the protein [39]. While no functional analysis has been performed to date, this non conservative amino acid substitution can potentially lead to altered protein function. To predict amino acid changes that might affect protein function, we performed bioinformatics analysis using SIFT (www.blocks.fhcrc.org/sift/SIFT.html) for *BRCA1* rs799917. SIFT analysis indicates that this SNP results in an amino acid change L871P, which predicted to affect protein function with a score of 0.03. Our finding of an association of a variant in *BRCA1*, a BC susceptibility gene, with NHEJ activity, not only suggests a role for BRCA1 in NHEJ, but also provides support for the importance of the NHEJ pathway to BC development.

Several studies have investigated *XRCC4* genetic variation and BC risk. *XRCC4* forms a complex with Ligase IV which functions during NHEJ to reseat the break [40]. A study conducted in Taiwan among Asian women found that carrying an increasing number of variant alleles in *XRCC4* was associated with higher disease risk [8]. Another study found associations of *XRCC4* haplotypes with age at diagnosis of BC and risk in non-BRCA1/2 heritable individuals [16]. In the current study, we examined six SNPs in *XRCC4*. For two of these SNPs, *XRCC4* rs1056503 and rs1805377, the distributions of their genotypes were linked and we performed our analysis excluding one of them, *XRCC4* rs1805377. We found *XRCC4* rs1056503 to be associated with increased BC, but the association did not remain after adjusting for other SNPs. One previous population-based study in the US and Poland found no significant effect of *XRCC4* rs1805377 on BC risk [9].

DSBs are converted to homologous joint molecules by invasion of a homologous duplex by single-strand DNA. Normal formation of homologous joint molecules and recombination products during meiosis requires several mitotic repair genes including *RAD52* [41]. In a single SNP model, the *RAD52* rs11226 GA/AA genotypes were associated with BC with an OR of 1.53 (95% CI=0.90–2.59). In the model section process adjusted for multiple variables, the effect of genotypes of *RAD52* rs11226 remains of borderline significance in both conditional regression and GEE models, suggesting that variation in *RAD52* might be important for familial BC risk.

Family history is well-recognized as a strong risk factor for female BC [42]. Only about 4–5% of breast cancers are caused by known mutations in cancer susceptibility genes, such as

BRCA1 and *BRCA2* [43], suggesting that additional low-penetrance genetic variants might contribute to BC, particularly among higher risk women. Our study has the advantage of having higher risk women in the study to examine whether DNA repair phenotype and genotype are important predictors within these families. Within family comparisons can reduce confounding from population admixture for genetic association studies as well family-level factors confounding factors that may affect phenotypic differences[44]. In addition to the within-family analyses, which are likely underpowered, we were able to estimate our associations with genotype using generalized estimating equations. The overall inferences from these two approaches were very similar suggesting that there was minimal unmeasured fixed family-level confounding of these associations. Our SNPs selection criteria were strong and based on important requirements. Our inability to detect differences in DSB repair capacity by and BC risk and genotype was not surprising because we only included about 25% of the core genes in the DSB pathways [45] and some SNPs such as *RAD51L1* rs999737 consistently related to BC in several genome wide association studies were not examined in our study [46–48].

Studies of phenotypic biomarkers are considered to capture more of the pathway effects than studies of genetic variation alone, as phenotype measures help account for multiple enzymatic activities and protein interactions. However, these studies are difficult to perform, and our inability of accurately mimic *in vivo* conditions limits the interpretability of the data. Our results of genotype-phenotype association analysis support the importance of *XRCC5* and *BRCA1* in familial BC risk. These findings further highlight the importance of DNA repair phenotype in explaining breast cancer risk and suggest that genetic variation may explain only a small percent of DSB repair capacity and there are other factors that may contribute to individual differences in EJ capacity. Methylation in the promoter regions of gene resulting in silencing gene expression is one of the common epigenetic alterations observed in BC (reviewed in [49]). Methylation in the promoter regions of DNA repair genes such as *BRCA1* was associated with BC risk [50]. Differences in the methylation of DNA repair genes might explain some of the variation in the DNA repair capacity between sisters and future studies are need to examine both genetic and epigenetic effects on phenotype.

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Abbreviations

BC	breast cancer
BCFR	Breast Cancer Family Registry
CI	confidence interval
DSB	double-strand break
DSBR	double-strand break repair
EJ	end joining
HWE	Hardy-Weinberg equilibrium

HR	homologous recombination
NER	nucleotide excision repair
NHEJ	non-homologous end joining
OR	odds ratio
SNP	single-nucleotide polymorphism

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Age-adjusted associations for single SNPs of NHEJ pathway genes and breast cancer risk, the New York site of BCFR

Table 1

SNP	Genotype	Affected sisters No. (%)	Unaffected sisters No. (%)	OR(95%CI)	P value
<i>LIG4</i> rs1805388	GG	259(79.9)	300(75.7)	1.0(ref.)	
	GA	59(18.2)	79(20.0)	1.03(0.56–1.89)	0.88
	AA	6(1.9)	17(4.3)	0.42(0.12–1.56)	0.20
	GA/AA	65(20.1)	96(24.3)	0.92(0.51–1.65)	0.82
	Freq(A)	0.11	0.14		
<i>XRCC4</i> rs1805377	GG	200(64.1)	248(65.4)	1.0(ref.)	
	GA	93(29.8)	97(25.6)	1.78(1.06–3.00)	0.03
	AA	19(6.1)	34(9.0)	0.96(0.34–2.69)	0.93
	GA/AA	112(35.9)	131(34.6)	1.67(1.01–2.76)	0.01
	Freq(A)	0.21	0.21		
<i>XRCC4</i> rs2075685	GG	104(32.2)	127(32.2)	1.0(ref.)	
	GT	157(48.6)	182(46.2)	1.02(0.61–1.70)	0.95
	TT	62(19.2)	85(21.6)	0.88(0.41–1.76)	0.71
	GT/TT	219(67.8)	267(67.8)	0.99(0.60–1.66)	0.99
	Freq(T)	0.43	0.45		
<i>XRCC4</i> rs1056503	TT	200(61.7)	255(64.6)	1.0(ref.)	
	TG	102(31.3)	103(26.1)	1.79(1.08–2.96)	0.03
	GG	22(6.8)	37(9.3)	0.88(0.33–2.33)	0.80
	TG/GG	124(38.3)	140(35.4)	1.69(1.03–2.77)	0.04
	Freq(G)	0.22	0.22		
<i>XRCC4</i> rs1478485	GG	93(29.5)	119(30.9)	1.0(ref.)	
	GA	163(51.8)	189(49.1)	1.08(0.64–1.82)	0.78
	AA	59(18.7)	77(20.0)	1.11(0.53–2.33)	0.78
	GA/AA	222(70.5)	266(69.1)	1.08(0.64–1.82)	0.77
	Freq(A)	0.42	0.46		
<i>XRCC4</i> rs13180316	GG	209(66.4)	252(65.8)	1.0(ref.)	
	GA	96(30.5)	115(30.0)	0.85(0.51–1.42)	0.53
	AA	10(3.2)	16(4.2)	0.68(0.22–2.16)	0.52
	GA/AA	106(33.7)	131(34.2)	0.84(0.51–1.39)	0.50
	Freq(A)				

SNP	Genotype	Affected sisters No. (%)	Unaffected sisters No. (%)	OR(95%CI)	P value
XRCC4 rs963248	Freq(A)	0.19	0.19		
	TT	189(61.6)	214(58.6)	1.0(ref.)	
	TC	98(31.9)	128(35.1)	0.90(0.53–1.53)	0.70
	CC	20(6.5)	23(6.3)	1.29(0.46–3.59)	0.63
	TC/CC	118(38.4)	151(41.4)	0.92(0.54–1.54)	0.78
XRCC5 rs2440	Freq(C)	0.23	0.24		
	GG	113(35.4)	139(35.2)	1.0(ref.)	
	GA	145(45.5)	173(43.8)	1.42(0.84–2.38)	0.19
	AA	61(19.1)	83(21.0)	1.37(0.65–2.87)	0.41
	GA/AA	206(64.6)	256(64.8)	1.41(0.84–2.36)	0.19
XRCC5 rs1051677	Freq(A)	0.42	0.43		
	TT	258(79.1)	310(78.5)	1.0(ref.)	
	TC	65(19.0)	74(18.7)	0.65(0.35–1.20)	0.17
	CC	3(0.9)	11(2.8)	0.07(0.01–0.58)	0.01
	TC/CC	68(20.9)	85(20.5)	0.61(0.33–1.12)	0.11
XRCC5 rs3835	Freq(C)	0.11	0.12		
	GG	204(62.2)	246(62.4)	1.0(ref.)	
	GA	107(32.6)	125(31.7)	1.14(0.70–1.85)	0.61
	AA	17(5.2)	23(5.8)	1.03(0.41–2.58)	0.96
	GA/AA	124(37.8)	148(37.6)	1.12(0.70–1.80)	0.64
XRCC5 rs3834	Freq(A)	0.22	0.22		
	GG	236(73.1)	293(74.2)	1.0(ref.)	
	GA	77(23.8)	88(22.3)	1.40(0.79–2.46)	0.25
	AA	10(3.1)	14(3.5)	1.14(0.35–3.71)	0.82
	GA/AA	87(26.9)	102(25.8)	1.37(0.79–2.37)	0.27
XRCC5 rs828699	Freq(A)	0.15	0.15		
	CC	77(24.2)	84(21.8)	1.0(ref.)	
	CA	164(51.6)	204(52.9)	0.98(0.56–1.71)	0.93
	AA	77(24.4)	98(25.4)	0.94(0.47–1.89)	0.86
	CA/AA	241(75.8)	302(78.2)	0.97(0.56–1.68)	0.91
	Freq(A)	0.5	0.52		

SNP	Genotype	Affected sisters No. (%)	Unaffected sisters No. (%)	OR(95%CI)	P value
XRCC5 rs1051685	AA	229(72.9)	285(74.6)	1.0(ref.)	
	AG	73(23.3)	83(21.7)	1.53(0.85–2.74)	0.15
	GG	12(3.8)	14(3.7)	1.14(0.35–3.69)	0.83
	AG/GG	85(27.1)	97(25.4)	1.48(0.84–2.60)	0.18
	Freq(G)	0.16	0.14		
XRCC6 rs132793	GG	212(65.4)	260(66.5)	1.0(ref.)	
	GA	98(30.3)	116(29.7)	0.83(0.49–1.40)	0.49
	AA	14(4.3)	15(3.8)	0.78(0.26–2.36)	0.66
	GA/AA	112(34.6)	131(33.5)	0.83(0.49–1.38)	0.47
	Freq(A)	0.19	0.19		

OR adjusted for age

Age-adjusted association of overall effect for single SNPs of checkpoint and homologous recombination repair genes and breast cancer risk, the New York site of BCFR

Table 2

SNP	Genotype	Affected sisters No. (%)	Unaffected sisters No. (%)	OR(95%CI)	P value
<i>ATM</i> rs1801516	GG	259(79.0)	318(79.9)	1.0(ref.)	
	GA	62(18.9)	75(18.8)	1.31(0.71–2.43)	0.42
	AA	7(2.1)	5(1.3)	3.52(0.72–17.32)	0.13
	GA/AA	69(21.0)	80(20.0)	1.36(0.73–2.51)	0.36
	Freq(A)	0.12	0.11		
<i>ATM</i> rs664677	TT	124(39.6)	154(40.0)	1.0(ref.)	
	TC	140(44.7)	175(45.4)	0.94(0.56–1.57)	0.83
	CC	49(15.7)	56(14.6)	1.26(0.59–2.69)	0.54
	TC/CC	189(60.4)	231(60.0)	0.98(0.59–1.62)	0.96
	Freq(C)	0.38	0.37		
<i>BRCA1</i> rs1799966	TT	132(41.6)	162(42.0)	1.0(ref.)	
	TC	143(45.1)	182(47.2)	0.87(0.53–1.44)	0.59
	CC	42(13.3)	42(10.9)	0.95(0.46–1.99)	0.90
	TC/CC	185(58.4)	224(58.0)	0.88(0.54–1.45)	0.62
	Freq(C)	0.36	0.34		
<i>BRCA1</i> rs799917	GG	108(32.2)	120(29.4)	1.0(ref.)	
	GA	164(49.0)	211(51.7)	0.73(0.44–1.21)	0.22
	AA	63(18.8)	77(18.9)	0.74(0.37–1.45)	0.38
	GA/AA	227 (67.8)	288(70.6)	0.73(0.44–1.20)	0.22
	Freq(A)	0.43	0.45		
<i>BRCA2</i> rs766173	AA	280(85.9)	346(87.8)	1.0(ref.)	
	AC	45(13.8)	46(11.7)	1.75(0.89–3.45)	0.11
	CC	1(0.3)	2(0.5)		
	AC/CC	46(14.1)	48(12.2)	1.62(0.84–3.16)	0.15
	Freq(C)	0.07	0.06		
<i>BRCA2</i> rs144848	AA	164(49.1)	200(49.0)	1.0(ref.)	
	AC	138(41.3)	170(41.7)	1.01(0.64–1.59)	0.98
	CC	32(9.6)	38(9.3)	0.83(0.37–1.85)	0.65

SNP	Genotype	Affected sisters No. (%)	Unaffected sisters No. (%)	OR(95%CI)	P value
<i>NBS1</i> rs1805794	AC/CC	170(50.0)	208(51.0)	0.99(0.63–1.55)	0.95
	Freq(C)	0.30	0.30		
	CC	167(50.8)	207(52.1)	1.0(ref.)	
	CG	130(39.5)	152(38.3)	1.09(0.68–1.74)	0.73
	GG	32(9.7)	38(9.7)	1.26(0.53–3.01)	0.65
	CG/GG	162(49.2)	190(47.9)	1.10(0.69–1.75)	0.95
<i>NBS1</i> rs1805787	Freq(G)	0.29	0.29		
	CC	203(61.9)	228(57.4)	1.0(ref.)	
	CG	101(30.8)	141(35.5)	0.95(0.59–1.54)	0.85
	GG	24(7.3)	28(7.1)	1.06(0.41–2.76)	0.92
	CG/GG	125(38.1)	169(42.6)	0.96(0.60–1.54)	0.87
	Freq(G)	0.23	0.25		
<i>RAD51</i> rs1801321	GG	136(40.8)	171(42.3)	1.0(ref.)	
	GT	142(42.6)	169(41.8)	0.91(0.56–1.48)	0.71
	TT	55(16.6)	64(15.9)	1.12(0.56–2.23)	0.75
	GT/TT	197(59.2)	233(57.7)	0.94(0.59–1.52)	0.81
	Freq(T)	0.38	0.37		
	GG	100(31.9)	145(37.3)	1.0(ref.)	
<i>RAD52</i> rs11226	GA	172(54.8)	177(45.8)	1.57(0.92–2.68)	0.10
	AA	42(13.4)	66(17.0)	1.16(0.51–2.65)	0.72
	GA/AA	214(68.2)	244(62.7)	1.53(0.90–2.59)	0.12
	Freq(A)	0.41	0.40		
	TT	190(56.7)	222(54.8)	1.0(ref.)	
	TC	119(35.5)	163(40.3)	0.69(0.43–1.10)	0.12
<i>XRCC3</i> rs1799794	CC	26(7.8)	20(4.9)	1.87(0.66–5.28)	0.24
	TC/CC	145(43.3)	183(45.2)	0.75(0.47–1.19)	0.22
	Freq(C)	0.25	0.25		

OR adjusted for age

Table 3

Multiple variables model for breast cancer risk, New York site of the BCFR by conditional logistic regression and GEE models

Variable			Full Model	Model 2*	Model 3*
			OR (95% CI)	OR (95% CI)	OR (95% CI)
Conditional logistic regression model					
<i>RAD52</i> rs11226	GA/AA v.s. GG		2.01(1.01–4.00)	1.56(0.91–2.68)	1.58(0.93–2.70)
<i>XRCC4</i> rs1056503	GT/GG v.s. TT		2.21(1.04–4.71)	1.51(0.90–2.54)	1.52(0.91–2.55)
<i>XRCC5</i> rs3834	GA/AA v.s. GG		2.52(0.78–8.19)	1.52(0.83–2.79)	1.59(0.88–2.90)
<i>XRCC5</i> rs1051677	TC/CC v.s. TT		0.55(0.23–1.57)	0.75(0.40–1.41)	
GEE model					
<i>RAD52</i> rs11226	GA/AA v.s. GG		1.33(0.97–1.80)	1.24(0.94–1.62)	1.26(0.97–1.64)
<i>XRCC4</i> rs1056503	GT/GG v.s. TT		1.41(0.98–2.02)	1.08(0.80–1.42)	
<i>XRCC5</i> rs3834	GA/AA v.s. GG		1.67(0.93–2.97)	1.05(0.79–1.41)	
<i>XRCC5</i> rs1051677	TC/CC v.s. TT		0.90(0.57–1.40)	0.94(0.66–1.33)	

ORs in full model were adjusted for age at interview for controls or breast cancer diagnosis for cases, and SNPs (*ATM* rs1801516 and rs664677, *BRCA1* rs1799966 and rs799917, *BRCA2* rs766173 and rs144848, *NBS1* rs1805794 and rs1805787, *RAD51* rs1801321, *XRCC3* rs1799794, *LIG4* rs1805388, *XRCC4* rs963248, rs1478485, rs2075685, and rs13180316, *XRCC5* rs2440, rs3835, and rs828699 and *XRCC6* rs132793).

* Model 2 retained SNPs with significance at the 0.2 level in the full model

* Model 3 retained SNPs with significance at the 0.2 level in model 2.

Table 4
End joining capacity by genotype of nonhomologous end joining pathway genes in sisters unaffected by breast cancer

Genes	SNP	Genotype	No.	Sticky end substrate Mean(SD)	P	Blunt end substrate Mean(SD)	P
<i>LIG4</i>	rs1805388	GG	64	29.2(10.0)	0.41	30.7(12.1)	0.97
		GA	20	32.4(13.2)		31.4(15.5)	
		AA	7	27.1(9.3)		30.6(14.6)	
<i>XRCC4</i>	rs1805377	GG	61	29.1(10.6)	0.48	31.0(12.8)	0.98
		GA	19	31.4(12.6)		31.4(14.8)	
		AA	6	33.8(8.1)		30.2(13.5)	
<i>XRCC4</i>	rs2075685	GG	27	32.6(12.4)	0.11	35.4(13.0)	0.09
		GT	43	29.8(10.3)		29.7(12.4)	
		TT	20	26.0(8.5)		27.5(13.3)	
<i>XRCC4</i>	rs1056503	TT	66	28.9(10.4)	0.41	30.8(12.5)	0.99
		TG	20	31.4(12.2)		31.1(14.5)	
		GG	6	33.8(8.1)		30.2(13.5)	
<i>XRCC4</i>	rs1478485	GG	24	31.1(12.1)	0.48	34.6(14.0)	0.18
		GA	42	29.9(10.3)		29.6(11.8)	
		AA	21	27.3(10.4)		27.9(13.4)	
<i>XRCC4</i>	rs13180316	GG	55	30.3(10.6)	0.1	30.5(13.4)	0.28
		GA	29	27.1(10.5)		29.8(12.3)	
		AA	5	37.7(10.8)		39.8(15.6)	
<i>XRCC4</i>	rs963248	TT	57	29.9(11.3)	0.24	30.6(13.1)	0.39
		TC	31	27.7(9.2)		29.1(13.0)	
		CC	4	36.7(9.3)		38.5(7.2)	
<i>XRCC5</i>	rs2440	GG	34	31.6(10.3)	0.39	32.8(14.0)	0.31
		GA	40	28.8(10.5)		30.3(12.8)	
		AA	19	27.8(11.9)		27.0(10.8)	
<i>XRCC5</i>	rs1051677	TT	78	29.2(10.8)	0.33	30.7(13.0)	0.89
		TC	15	30.9(10.1)		30.2(13.3)	
		CC	0				
<i>XRCC5</i>	rs3835	GG	46	28.9(10.2)	0.7	29.3(12.1)	0.45
		GA	34	30.0(12.5)		32.8(15.3)	

Genes	SNP	Genotype	No.	Sticky end substrate Mean(SD)	P	Blunt end substrate Mean(SD)	P
XRCC5	rs3834	AA	9	32.2(8.0)		33.0(9.6)	
		GG	63	27.7(9.9)	0.03	27.9(11.8)	0.0006
		GA	24	34.4(12.1)		37.8(14.1)	
XRCC5	rs828699	AA	5	30.0(8.2)		33.0(10.6)	
		CC	22	30.5(9.4)	0.8	31.1(14.1)	0.78
		CA	42	29.0(12.1)		30.0(13.3)	
XRCC5	rs1051685	AA	26	30.6(9.8)		32.3(11.9)	
		AA	64	27.8(9.9)	0.02	27.8(11.8)	0.006
		AG	23	35.0(12.1)		38.0(14.4)	
XRCC6	rs132793	GG	4	29.4(9.3)		32.5(12.2)	
		GG	61	29.0(11.3)	0.73	30.8(14.5)	0.88
		GA	24	31.1(10.0)		30.9(10.5)	
		AA	7	29.9(9.5)		28.3(6.9)	

End joining capacity by genotype of checkpoint and homologous recombination repair genes in sisters unaffected by breast cancer

Table 5

Gene	SNP	Genotype	No.	Sticky end substrate	Mean(SD)	P	Blunt end substrate	Mean(SD)	P
ATM	rs1801516	GG	73	29.9(10.2)		0.48	30.4(12.6)		0.98
		GA	19	27.9(12.3)			30.5(14.4)		
		AA	0						
ATM	rs664677	TT	39	28.7(9.2)		0.72	31.3(11.7)		0.58
		TC	43	30.4(12.4)			29.7(14.7)		
		CC	9	31.1(8.9)			34.7(11.9)		
BRCA1	rs1799966	TT	37	27.0(10.7)		0.09	29.3(13.9)		0.64
		TC	41	30.8(10.6)			31.8(12.6)		
		CC	10	34.7(10.5)			32.5(11.8)		
BRCA1	rs799917	GG	32	26.4(10.1)		0.02	29.3(13.4)		0.4
		GA	44	29.9(10.9)			30.0(13.2)		
		AA	17	35.1(9.2)			34.4(11.4)		
BRCA2	rs766173	AA	72	30.8(11.1)		0.09	31.6(13.5)		0.19
		AC	19	26.0(8.5)			27.1(11.0)		
		CC	0						
BRCA2	rs144848	AA	35	29.5(10.0)		0.92	31.0(13.8)		0.97
		AC	47	29.5(10.9)			30.4(12.9)		
		CC	9	31.0(13.1)			30.3(11.4)		
NBS1	rs1805794	CC	51	30.6(10.9)		0.46	30.8(12.7)		0.93
		CG	32	27.6(10.2)			30.2(13.8)		
		GG	9	29.8(11.2)			29.0(12.8)		
NBS1	rs1805787	CC	46	30.8(9.4)		0.47	30.3(11.3)		0.98
		CG	39	28.3(11.3)			30.4(13.9)		
		GG	7	27.2(14.3)			31.4(19.0)		
RAD51	rs1801321	GG	38	30.4(12.3)		0.6	30.2(13.2)		0.77
		GT	42	29.5(9.1)			29.8(12.2)		
		TT	11	26.7(10.8)			33.1(14.2)		
RAD52	rs11226	GG	38	29.5(8.6)		0.48	31.7(11.1)		0.6
		GA	39	28.7(12.2)			28.8(13.7)		

Gene	SNP	Genotype	No.	Sticky end substrate	Mean(SD)	P	Blunt end substrate	Mean(SD)	P
XRCC3	rs1799794	AA	11	33.2(13.3)			31.6(16.7)		
		TT	53	28.9(11.9)		0.53	31.1(13.5)		0.87
		TC	36	30.9(9.1)			30.3(12.8)		
		CC	3	25.0(6.1)			27.0(10.2)		