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Genetic Polymorphisms in *RAD23B* and *XPC* Modulate DNA Repair Capacity and Breast Cancer Risk in Puerto Rican Women

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Abstract

Studies have shown that DNA repair capacity is significantly decreased in breast cancer patients, but the molecular causes of inter-individual variations in DNA repair capacity are unknown. We hypothesized that genetic variations in the nucleotide excision repair pathway genes can modulate DNA repair capacity (DRC) and breast cancer risk in Puerto Rican women. A total of 228 breast cancer cases and 418 controls were recruited throughout Puerto Rico. For all study participants, eight SNPs in the genes *XPC*, *XPD* and *RAD23B* were genotyped using a TaqMan PCR, and the DRC levels of UV induced-DNA damage was measured in peripheral lymphocytes using a host cell reactivation assay. After adjustment for confounders, *RAD23B* rs1805329 (Ala249Val) was found to be significantly associated with breast cancer risk under all models tested ($P < 0.001$). There was also a significant association between breast cancer risk and *RAD23B* rs10739234 (intronic) under the recessive model ($P = 0.003$, OR: 2.72, 95% CI: 1.40–5.30). In cases, there was a statistically significant difference in mean DRC per genotype for *RAD23B* rs1805329 ($P < 0.001$) and *XPC* rs2607775 ($P = 0.002$). When we modeled the combined effect of multiple SNPs that each independently affected DRC on cancer risk, we observed incremental augmentations in risk with increasing number of risk genotypes at those loci (P overall model < 0.001). The increase in adverse genotypes was also correlated with a progressive decrease in DRC values. Our data indicate an additive effect of the NER SNPs on DRC and breast cancer risk in Puerto Rican women.

Keywords

Breast cancer genetics; DNA repair; nucleotide excision repair

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Introduction

Breast cancer is the most common female cancer, accounting for 28% of all malignancies affecting women in the United States (US).[1] Despite an overall decrease in cancer mortality rates in the US, racial and ethnic disparities have been observed in the prognosis of certain cancers. While African Americans, Hispanics and Native American women have lower incidences of breast cancer when compared to non-Hispanic whites, they are more likely to be diagnosed at advanced stages and have a poorer survival rate.[2–4] In Puerto Rico (PR), breast cancer is the most commonly diagnosed cancer in women and is the leading cause of female cancer death.[5] The incidence of breast cancer in PR is lower when compared to most ethnic groups in the US.[6] Racial and ethnic disparities in breast cancer incidence and mortality have been attributed to differences in risk factors and tumor biology[7] and variations in the genetic background[8].

Epidemiological studies have established a number of risk factors that are known to modify the likelihood of developing breast cancer. These risk factors include age, geographic location, socioeconomic status, reproductive events (menarche, menopause, pregnancy, and breastfeeding), exogenous hormones (hormone replacement therapy and oral contraceptives), lifestyle risk factors (alcohol, diet, obesity and physical activity) and family history of breast cancer among other factors.[9–11] Approximately 5–10% of all breast cancers are caused by mutations in highly penetrant susceptibility genes, such as *BRCA1* and *BRCA2*. [12] Deleterious mutations in genes such as *PTEN*, *p53*, *CHEK2*, *ATM*, *NBS1*, *RAD50*, *BRIP1* and *PALB2* also confer moderate to high risk of breast cancer, and together with *BRCA1* and *BRCA2*, they may explain up to 50% of breast cancer risk.[13] Complex interaction between common genetic variants with moderate effect on the phenotype together with environmental factors are expected to underlie the remaining inter-individual variability in breast cancer risk.[14–17]

In response to constant exposure to mutagenic agents from endogenous and exogenous sources, the cell protects the genome's integrity through DNA repair pathways and regulation of the cell cycle checkpoints. Approximately 170 DNA repair genes have been reported in the human genome.[18] Efficient DNA repair is critical, as accumulation of DNA damage is a major step toward tumorigenesis.[19,20] Previous studies have shown that DNA Repair Capacity (DRC) is decreased in a number of cancers, including nonmelanoma skin cancer[21], head and neck cancer[22], lung cancer[23] and breast cancer[24–26]. However, the molecular causes underlying variability in DNA repair capacity and its association with cancer risk remain poorly understood. Interestingly, the known genes for inherited BC all play a role in the maintenance of genomic integrity.[13]

The nucleotide excision repair (NER) repair pathway is highly complex and versatile. It specializes in repairing a wide variety of DNA helix distorting lesions ranging from cyclobutane pyrimidine dimers and photoproducts caused by sunlight to various bulky chemical adducts.[27] The rare hereditary syndrome *Xeroderma pigmentosum* (XP) exemplifies the link between inherited deficiencies in the NER pathway and cancer risk. This syndrome is characterized by severe skin sensitivity to sun, as well as drastic increases in the risk of skin cancer and other cancers.[28,29] Recently, a decrease in the expression

levels of a subset of genes from the NER pathway was observed in sporadic stage I breast cancer tumors[30], suggesting that NER deficiencies may also be implicated in carcinogenesis of internal tumors. This is supported by the observation that aromatic DNA adducts are found in higher concentrations in tissues adjacent to breast tumors when compared to non-cancer controls.[31] Polycyclic aromatic hydrocarbons (PAHs), which are produced by the incomplete combustion of organic materials, are metabolized into DNA adducts that are mainly repaired by the NER pathway.[32] Epidemiological studies have reported an association between PAH induced DNA adducts levels and breast cancer risk. [33,34]

Individuals differ in their capacity to repair DNA damage caused by exogenous and endogenous sources. Several studies have pointed to an association between single nucleotide polymorphisms (SNPs) in genes of the nucleotide excision repair pathway and sporadic breast cancer risk[35–41] but the evidence has been conflicting[42,43]. This is not surprising since it is expected that the genetic architecture of complex diseases vary in different populations, and experimental design discrepancies may impair reproducibility across studies. Hence, a few studies have reported differences in NER associations with cancer risk across various ethnic groups.[44–46] We hypothesized that the in DNA repair capacity associated with breast cancer risk in Puerto Rico[24,25] might be explained, at least partially, by inherited genetic variations in DNA repair genes. In the current study, we evaluated the role of 8 SNPs located in 3 candidate genes (*RAD23B*, *XPC* and *XPB*) that participate in the NER pathway in determining the DNA repair capacity and in breast cancer risk. To our knowledge, this is the first large-scale study examining the genetic factors involved in breast cancer etiology in an entirely Puerto Rican population.

Materials and Methods

Study Population

This study was approved by the Institutional Review Board (IRB) of the Ponce School of Medicine & Health Sciences, and participating hospitals. All participants signed a written Informed Consent for interviewing, drawing blood samples and, for cases, to obtain tumor material and pathology reports. This study utilized an incident cases case-control design. Cases were recently diagnosed through histopathologically confirmed breast carcinoma, prior to initiation of chemotherapy, radiotherapy or any other form of therapy. Controls were recruited from gynecological and primary care medical offices. The participants were recruited primarily through clinicians from the cities of Ponce, San Juan and Yauco and other selected collaborating cities throughout Puerto Rico representing approximately 68 out of the 78 municipalities (counties) in the island. Participants completed a standard questionnaire regarding their nutritional habits, clinical history and family history of cancer. Additionally, for breast cancer patients a tumor pathology report was obtained. Women 21 years or older, whose 3 out of 4 grandparents were born in Puerto Rico, are included in the study. All individuals recruited as controls had a negative mammography and breast examination within the last 6 months.

SNP Genotyping

Genomic DNA from lymphocytes of 228 breast cancer patients and 421 controls was extracted using QIAmp DNA Mini Kit (Qiagen, Valencia, CA), following the manufacturer's protocol. A total of 8 SNPs distributed in 3 NER candidate genes (*RAD23B*, *XPC* and *ERCC2/XPD*) were selected based on previously published association studies in various populations. Genotyping was conducted using a standard TaqMan Allelic Discrimination Assay (Applied Biosystems, Foster City, CA). Each assay consists of two primers for amplifying the sequence of interest and two TaqMan MBG probes for detecting alleles. Each TaqMan MBG probe is linked to a reporter dye in 5' (VIC is linked to allele 1 and FAM is linked to allele 2) and to a nonfluorescent quencher in 3'. For the present study we used the following genotyping assays: for rs10739234 C_31954728_20, rs10521083 C_29764369_20, rs1805329 C_11493966_10, rs13181 C_3145033_10, rs1799793 C_3145050_10, rs238407 C_8714008_10, rs2228000 C_16018061_10, and rs2607775 C_25474511_10. PCR were performed in 5 µl reaction volumes under the following conditions: initial denaturation at 95°C for 10 min, followed by 40 cycles of 95°C for 15 sec and 60°C for 60 sec.

Measure of DNA repair capacity

The DNA repair capacity (DRC) was measured using a modified host cell reactivation assay (HCR), as described previously.[24,25] This assay measures the total sum of the DRC of lymphocytes, which is a reflection of the repair capacity of the donor. In brief, a plasmid construct containing the luciferase gene is damaged by UVC exposure (0, 350, and 700 J/m²) in a controlled and quantitative manner (dose-response curve). The treated plasmid is transiently transfected in peripheral lymphocytes from patients or controls. The DNA repair capacity (DRC) is measured as the proportion of *luciferase* activity relative to an untreated plasmid control). The level of luciferase expression is a direct measure of the total repair capacity of the host mammalian cell. In an assessment of the reliability and repeatability of this assay, there was a correlation of 0.97–0.99 (95% confidence interval 0.95–1.00) in duplicates of 50 cases, 50 controls and 90 samples of three commercial cell lines. In addition, any inconsistencies between duplicates invalidate the results and the whole assay is repeated. The measurement of DRC has a coefficient of variation of less than 10% both, in triplicate and in repeated sample tests. With this precision, it has been demonstrated that this assay can distinguish the intra-assay variation (assay repeated on the same sample) and inter-assay variation (assay repeated on separate samples) by being able to maintain the ranks of samples measured in triplicate from multiple patients.

Statistical Analysis

All statistical analyses were conducted using Systat 13 (Systat Software, Inc., Chicago, IL), and Plink[47]. Differences in demographic and clinical characteristics between cases and controls were calculated by Student's 2-sided t-test or Pearson Chi-Square χ^2 test for frequencies. For each SNP locus, deviation from Hardy Weinberg Equilibrium (HWE) was tested by comparing the expected and observed genotype frequencies in the control group using the χ^2 test and Plink. Each SNP was modeled assuming an additive, recessive or dominant effect. The impact of risk/protective genotype was quantified by an Odds Ratio

(OR) and by calculating the 95% Confidence Interval (CI). The association between SNP genotype frequencies and breast cancer was analyzed by multiple logistic regression analysis using confounding factors as covariates. Comparison of DRC levels per genotype was performed using a general linear model corrected for confounding factors. If significant, a post hoc Tukey pairwise comparison test was performed.

Results

Characteristics of the study population

The characteristics of the study population grouped as breast cancer cases (n=228) and controls (n=418) are presented in Table 1. Overall, both groups were similar in body mass index (BMI) ($P=0.2$) and lifestyle risk factors such as alcohol ($P=0.3$) and tobacco use ($P=0.1$). There was a significant difference between cases and controls in age ($P<0.001$), civil status ($P<0.001$) and education level ($P<0.001$). Controls were younger, more likely to be married and less likely to be widows than cases. There was a higher proportion of controls that did not obtain a degree after high school, while controls were more likely to have obtained a bachelor diploma. When comparing the reproductive risk factors, there were no significant differences in age at menarche ($P=0.9$), prevalence of an irregular menstrual cycle ($P=0.9$), oophorectomy history (frequency and age of oophorectomy: $P=0.6$ and $P=0.8$, respectively), frequency of pregnancy ($P=0.3$), age at first child ($P=0.1$) number of children ($P=0.05$), and breastfeeding history (frequency of breastfeeding $P=0.3$, number of children breastfed $P=0.6$, and cumulative breastfeeding duration $P=0.5$). In controls, a higher proportion reported having used oral contraceptives ($P=0.03$). The same proportion of cases and controls had undergone menopause ($P=0.2$) and reported using hormonal replacement therapy (HRT) ($P=0.1$). The age at the onset of menopause and the age at the beginning of the hormonal replacement therapy were not statistically different between cases and controls ($P=0.2$ and $P>0.9$, respectively). There was no significant difference in the proportion of cases and controls that reported a family history (first- and second-degree relatives) of breast cancer and of other cancers ($P=0.1$ and $P=0.7$, respectively).

DNA repair gene polymorphisms, DRC and breast cancer risk

The distribution of the SNPs tested within the structure of the candidate genes (*RAD23B*, *XPC* and *XPB*), and the pairwise correlation coefficient (r^2) values for each pair of SNPs in the study population are illustrated in Figure 1. The distributions of the genotype frequencies in all 8 SNPs tested did not significantly deviate from those expected under the Hardy-Weinberg Equilibrium (HWE) law. For each SNP studied, the distribution of genotypes was compared between cases and controls under an additive, dominant or recessive model (Table 2). In order to correct for multiple comparison, a $P<0.006$ (Bonferroni correction: 0.05 divided by 8 SNPs tested) was considered significant. After adjustment for cofounders (age, civil status, education level, and use of oral contraceptives), the genotype at the *RAD23B* rs1805329 SNP was found to be significantly associated with breast cancer risk under all models tested ($P<0.001$). Individuals bearing the TT genotype had a 3.14-fold increased risk of breast cancer (95% CI: 1.65–5.97). There was also a significant association between breast cancer risk and the *RAD23B* intronic SNP rs10739234 under the recessive model

($P=0.003$, OR: 2.72, 95% CI: 1.40–5.30). After correction for multiple testing, the remaining SNPs tested in *RAD23B*, *XPC* and *XPB* did not reach statistical significance.

A phenotypic, quantitative assessment of the DNA repair capacity (DRC) was obtained in the 228 cases and 421 controls genotyped by means of a host-cell reactivation assay. For controls, DRC values ranged from 0.98% to 22.26%, with an average of 6.14%. In cases, DRC values ranged from 0.14% to 14.25% with an average of 2.42%. On an average, women with breast cancer had a 60% reduction in DRC when compared with controls. For cases and controls, DRC was found to decline with age, but a statistically significant difference remained between controls DRC and cases DRC after age-adjustment (*data not shown*). Next, the association between the genotype at each DNA repair candidate SNPs and DRC levels was assessed in cases and controls (Table 3). In cases, there was a statistically significant difference in mean DRC per genotype for the *RAD23B* rs1805329, and *XPC* rs2607775 ($P<0.001$, and $P=0.002$, respectively, corrected for age and education levels). Those individuals that carry the low-risk CC genotype at *RAD23B* rs1805329 had a higher DRC when compared to those carrying the CT or TT genotype, which is consistent with the association of this SNP with breast cancer risk.

DNA repair gene polymorphisms at multiple loci, DRC and breast cancer risk

After having identified 2 SNPs in *RAD23B* and *XPC* that were associated with changes in DRC levels, the combined effect of these loci on breast cancer risk was modeled through regression analysis. Cases and controls were grouped based on the number of risk genotypes carried at rs1805329 and rs2607775, ranging from 0 (individuals that did not carry any risk genotype at any of the two loci) to 2 (individuals carrying the CT or TT genotype at rs1805329, and the CG or GG genotype at rs2607775). As presented in table 4, there was an increment in breast cancer risk associated with increasing number of adverse genotypes.

After correcting for confounders (age, civil status, education level and use of oral contraceptives), there was a 1.40 fold increase in breast cancer risk in individuals carrying a single risk genotype (95% CI: 0.65–3.04). However, it did not reach statistical significance ($P=0.4$). The breast cancer risk was increased by a factor of 5.81 (95% CI: 2.64–12.79, $P<0.001$) in individuals carrying risk genotypes at both loci. The overall fit of the model did reach statistical significance ($P<0.001$). Likewise, increasing numbers of risk genotypes were associated with progressive decreases in DRC values in cases (table 4). No significant changes in DRC were associated with the number of risk genotypes in controls (table 4).

Next, the effect of the combined risk genotypes at *RAD23B* and *XPC* on the tumor characteristics was assessed in cases. The number of risk genotypes was not associated with an early onset (<55 years) ($P=0.7$), tumor size ($P=0.3$), tumor grade ($P=0.5$) and presence of lymph node invasion ($P=0.6$) (*data not shown*). The prevalence of tumors negative for the estrogen (ER), progesterone (PR) and Human Epidermal Growth Factor Receptor 2 (HER2) receptors (triple negative tumors) was not significantly different ($P=0.4$) in cases with increasing number of risk genotypes at *RAD23B* and *XPC* (*data not shown*).

Discussion

In the current study, the effect of inherited genetic variants in candidate NER genes on DRC and breast cancer risk was examined in an entirely Puerto Rican population. After a Bonferroni correction for multiple comparisons, the *RAD23B* SNPs rs10739234 (intronic) and rs1805329 (Ala249Val) were found to be significantly associated with breast cancer risk in our study population. The rs1805329 (Ala249Val) was significantly associated with breast cancer risk under all model tested (allelic, additive, recessive and dominant), with the T allele being associated with increased breast cancer risk.

Individual susceptibility to cancer development is influenced by a number of factors, including DNA repair capacity. Previous studies have shown that DRC is decreased in many types of cancer including, head and neck cancer[22], and lung cancer[23] and breast cancer[26]. In the Puerto Rican population, decreased DRC has been observed in non-melanoma skin cancer[21] and breast cancer [24,25]. A key finding of this study is that two SNPs in *XPC* (rs2607775) and *RAD23B* (rs1805329) were associated with significant changes in DNA repair capacity. Our data are in agreement with previous studies which have shown a genotype-phenotype correlation between sensitivity to benzo(a)pyrene diol epoxide (BPDE) and SNPs in *RAD23B* (including rs1805329) and/or *XPC* in healthy subjects[53], in lung[54] and breast[37] cancer patients.

For the *RAD23B* SNP rs1805329, it is noteworthy that we observed drastic differences in genotype frequencies between cases and controls. For instance, the CC and CT frequencies are 25.7% and 57.9% in cases compared to is 62.2% and 31.6% in controls. The observed genotype frequencies in our controls are consistent with those reported by the 1000Genomes project for the Puerto Rico population (PUR) at this variant: CC 61.8%, CT 30.9%, and TT 7.3% [55]. Furthermore, our group has recently showed that every 1% decrease in DRC is associated with an increase in breast cancer risk of 64% [25], which is consistent with the magnitude of the odds ratio observed for rs1805329 in the current study. Previously, the association of rs1805329 with cancer risk has been assessed with inconsistent results. In non-Hispanic whites, individuals with at least one copy of the T allele were reported to have an increased risk of esophageal cancer[48]. The T allele was also associated with the risk of lung cancer in a Chinese population[49], but these findings have not been replicated in an Eastern European population[50]. There was no association between rs1805329 and glioblastoma[51] or renal cell cancer[52]. In a study of Latinos from the San Francisco Bay area, there was no significant association between *RAD23B* rs1805329 and lung cancer risk. [44] These discrepancies may be explained by differences in the cancer type studied, environmental exposure, or population-specific genetic backgrounds. Interestingly Mechanic *et al.* [45] have observed stronger associations of NER genotypes with breast cancer in African American women when compared with white women. Nevertheless, it will be necessary to validate these results in future studies.

The variant *RAD23B* rs1805329 results in a change in amino acid (Ala249Val) that may have an impact on protein function. In contrast, rs2607775 is located in the 5' regulatory region of *XPC*, and its molecular impact on protein levels or function remains to be determined. The SNP rs10739234 was also significantly associated with breast cancer risk,

but did not result in changes in DRC. Genetic association studies do not differentiate between causative SNPs and those that are in linkage disequilibrium (LD) with causative SNPs. Therefore, we cannot exclude the possibility that other variants located in close proximity to the associated SNPs are causally responsible for this association. Apart for *RAD23B* rs1805329, the effects of genotype on DRC in the *XPC* SNPs were restricted to cases and were not significant in the control group. It is likely that the controls harbor a combination of variants in NER genes that favors higher DRC values, hence compensating for the effect of the deleterious variants. Spitz *et al.* also reported that DRC variations associated with NER polymorphisms tend to be of reduced amplitude in controls than in cases.[56] These observations are in agreement with an expected multigenic and multifactorial inheritance of most cases of breast cancer.

We found no significant association between breast cancer risk or DNA repair capacity and the remaining SNPs tested at the *RAD23B*, *XPC* or *XPB* loci. The majority of the previous studies assessing the functional significance of NER variant on DNA sensitivity to mutagens have focused on the *XPB* variants, and yielded mixed results. In human lymphoblastoid cell lines, homozygosity for the Asn allele at codon 312 was associated with a significant increase in UV-induced apoptotic response, but the polymorphism at codon 751 did not influence response to DNA damage.[57] No detectable variations on NER and basal transcription activity were observed in a baculovirus expression system expressing recombinant TFIIH complexes with the His201Tyr, Asp312Asn, and Lys751Gln polymorphisms.[58] NER-induced repair of DNA damage was previously shown to vary with the *XPB* genotype in peripheral lymphocytes of lung cancer patients[56,59], breast cancer patients[35,60], and healthy subjects[61]. However, several groups also reported negative results.[37,54,62] In Puerto Rico, polymorphisms in the *XPB* gene have been associated with the risk of non-melanoma skin cancer, but did not result in changes in DRC levels.[63] These studies vary widely in sample size, in the source of peripheral lymphocytes (for example, cancer patient versus population based volunteers), in the type of mutagen assessed and methods to measure DRC levels. A meta-analysis reviewing a total of 56 studies on the association between *XPB* variants and cancer risk concluded that there was a weak (OR 1.10; 95% CI: 1.03–1.16) but significant association between Lys751Gln and the risk of all cancers included in the analysis.[64] The statistical power to detect alleles with modest effects on risk is limited by the sample size available to the current study. Assuming an autosomal dominant mode of inheritance, the current sample size would allow detecting an OR of 1.6 or higher with a power of at least 80% for a risk allele with a frequency between 0.2 and 0.4. This power is decreased for a recessive mode of inheritance, especially for low frequency risk alleles. These factors may explain some of the observed inconsistencies in genotype-phenotype correlations across studies.

Environmental exposure to certain mutagens might also be required to observe an association between genetic variants in DNA repair genes and DNA repair capacity or breast cancer risk. It was previously demonstrated that the interaction between the Gln allele of codon 751 in *XPB* and breast cancer risk was limited to individuals with high levels of aromatic hydrocarbon (PAH)-DNA adducts and current smokers.[39] Our study design would not allow for assessing the impact of smoking on NER polymorphisms and DRC or

cancer risk because only a small fraction of the study population (14.1% of cases and 9.7% of controls) reported being a current smoker or having ever smoked. Due to the limited size of these subgroups, additional analysis stratifying the population by smoking status (or other environmental factor) would lack statistical power. The interaction with detoxifying genes may also confound the analysis of the relationship between NER genes and cancer risk. Pastorrelli *et al.* discovered that the effect of *XPB* genotypes on BPDE-DNA damage levels were restricted to individuals with lower detoxification capacity as a result of an interaction with polymorphisms in the xenobiotic metabolizing gene *GSTM1*. [65]

The majority of breast cancer cases are expected to be the result of complex interactions between common genetic variants with moderate effect on the phenotype together with environmental factor. [14–17] When the effect of two loci that each independently affected DRC was combined (*RAD23B* rs1805329, and *XPC* rs2607775), we observed incremental augmentations in risk with increasing number of risk genotypes at those loci. Using individuals that did not carry any risk genotype as a reference, the OR for breast cancer risk was 1.40 (95%CI: 0.65–3.04, P=0.4) for individuals carrying 1 risk genotype, and 5.81 (95%CI: 2.64–12.79, P<0.001) for individuals bearing a risk genotype at both loci. Lin *et al.* also observed a progressive increase in the ORs for the risk of renal cell carcinoma in individuals with increasing numbers of adverse alleles in NER pathway genes. [52] In a study of lung cancer risk, the case/control status was best predicted by the genotype at multiple risk NER pathway loci. [44] The incremental increase in cancer risk may be explained by dose-response relationships between the number of risk alleles and DRC levels. In lymphocytes of healthy subjects, Lin *et al.* reported a stronger correlation between sensitivity to BPDE-induced chromatid breaks and genotype for the combinations of NER than for individual NER polymorphisms in lymphocytes of healthy subjects. [53] In the current study, in comparison to cases that did not have any risk genotype, the DRC levels decreased by 1.86% and 2.66% in cases with 1 or 2 risk genotypes, respectively. The enhanced effect of combination of risk alleles extends beyond the genes within the NER pathway as the deleterious effects of combination of SNPs have been reported between genes of the NER pathway and genes of other DNA repair pathways in breast cancer [36], lung cancer [66,67], and adult glioma [68]. In our population, cases that are not carrying any risk genotype at the 2 loci studied had a lower DRC levels than control, suggesting that additional susceptibility variants remain to be discovered. These observations highlight the advantages of a multigenic approach when studying complex biological responses such as mutagenesis, DNA repair and breast cancer risk.

In the last years, an overall decrease in cancer mortality rates has been reported in the US. [1] However, this decline in cancer death rate is associated with racial disparities in prognosis for certain cancers. African Americans, Hispanics and Native American women living in the US have lower incidences of breast cancer, but are more likely to be diagnosed at advanced stages and have a poorer survival rate when compared to non-Hispanic whites. [1,3] Even after correcting for confounding factors such as disease stage at diagnosis and patient age, several studies have identified race as an independent predictor of breast cancer survival. [69–72] Our data provide evidence for an additive role of *RAD23B* and *XPC* in DRC levels regulation and genetic susceptibility to breast cancer in Puerto Rican women. Whether this

association is population- or cancer type- specific remains to be determined. Nevertheless, the inclusion of different races/ethnicities in cancer genetics research is necessary to acquire the knowledge required to address this health disparity gap. To the best of our knowledge, this is the first study that links inherited variations in DNA repair genes to DNA repair capacity phenotype and breast cancer risk among Puerto Rican women.

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Figure 1.

Gene structure of the candidate DNA repair genes. Gene structures (Entrez), gene and SNPs positions were obtained from NCBI reference sequence assembly NCBI36/hg18 release. The correlation coefficients between each pair of SNPs (r^2) were generated by Haploview 4.2. Darker colors indicate higher r^2 values. a- *RAD23B* UV excision repair protein RAD23 homolog B. Positions of the *RAD23B* SNPs are as follow: rs10739234 (109,087,137), rs10521083 (109,112,961) and rs1805329 (109,124,149). b- *XPC* xeroderma pigmentosum, copmplementation group C. Positions of the *XPC* SNPs are as follow: rs2228000 (14,174,889) and rs2607775 (14,195,099). c- *XPD* xeroderma pigmentosum, complementation group D. Positions of the *XPD* SNPs are as follow: rs13181 (50,546,759), rs1799793 (50,559,099) and rs238407 (50,560,318).

Table 1

Characteristics of the study participants grouped by breast cancer status.

	Controls (n=418)	Cases (n=228)	P
<i>Demographic information & lifestyle risk factors</i>			
Age (yrs)	52.1 (12.5)	57.0 (12.6)	<0.001
BMI (kg/m ²)	27.1 (5.0)	27.7 (5.4)	0.2
Civil status			
Married (%)	66.3	53.1	<0.001
Single (%)	19.0	21.5	
Divorced (%)	12.3	15.8	
Widow (%)	2.4	9.6	
Education			
Up to high school (%)	22.4	41.2	<0.001
Associate (%)	16.4	14.2	
Bachelor (%)	40.1	27.5	
Other (%)	21.1	17.1	
Smoking			
Ever smoked (%) [*]	9.7	14.1	0.1
Number of years smoking (yrs)	18.2 (8.6)	15.5 (9.3)	0.3
Drinking			
Ever drinks (%) [†]	20.0	16.7	0.3
Age started drinking (yrs)	20.1 (3.0)	23.4 (9.3)	0.08
Monthly alcohol consumption (cups)	7.1 (8.6)	9.4 (11.1)	0.2
<i>Hormonal & pregnancy history</i>			
Age at menarche			
12 yrs (%)	56.5	57.1	0.9
>12 yrs (%)	43.5	42.9	
Irregular menstrual cycle (%)	39.5	39.5	0.9
Ovaries			
Have both ovaries (%)	77.4	77.9	
One ovary removed (%)	4.7	6.2	
Both ovaries removed (%)	17.9	15.9	0.6
Age at which ovary (ies) were removed (yrs)	43.1 (9.1)	42.7 (7.6)	0.8
Pregnancy history			
Ever been pregnant (%)	82.4	85.4	0.3
Age at first child (yrs)	24.4 (4.9)	25.2 (6.1)	0.1
Number of children	2.3 (1.2)	2.6 (1.4)	0.05
Ever breastfed (%)	56.3	51.9	0.3
Number of children breastfed	2.0 (1.1)	2.1 (1.3)	0.6
Cumulative breastfeeding (wks)	9.9 (12.6)	9.0 (9.9)	0.5
Menopausal status			
Premenopausal (%)	39.9	34.4	

	Controls (n=418)	Cases (n=228)	P
Postmenopausal (%)	60.1	65.6	0.2
Age at menopause (yrs)	46.4 (6.0)	47.2 (6.5)	0.2
Contraceptive			
Ever used (%)	58.5	49.3	0.03
Age started (yrs)	24.1 (5.4)	25.1 (5.3)	0.1
HRT			
Ever used (%)	54.3	46.2	0.1
Age started (yrs)	44.0 (8.6)	44.1 (10.2)	>0.9
<i>Family history of breast and other cancers</i>			
Family history of breast cancer (%)	41.9	40.4	0.7
Family history of cancer (%)	55.0	60.5	0.2

Values are mean (standard deviation (SD)) or frequencies expressed as percentages. *P*-values were calculated by Student's 2-sided t-test for continuous variables or Pearson's Chi-Square for frequency data. Abbreviations: BMI body mass index, HRT hormone replacement therapy.

* 'Ever smoked' is defined as individuals who have smoked 100 cigarettes or more in their lifetime;

[†] 'ever drinks' refers to individuals who drink in a recurrent basis (daily, weekly, monthly). 'HRT' refers to the percentage of postmenopausal women that ever used HRT.

Table 2

Association between NER SNPs and breast cancer risk.

		Controls n (%)	Case n (%)	Adjusted OR*	95% CI	P
RAD23B	rs10521083 (intronic)					
	CC	17 (4.3)	12 (6.6)	Reference		
	AC	140 (35.6)	64 (35.0)	0.64	0.27–1.50	0.3
	AA	236 (60.1)	107 (58.5)	0.64	0.28–1.46	0.3
	CC vs AC+AA			1.57	0.69–3.56	0.3
	AA vs AC+CC			0.94	0.64–1.39	0.8
	rs10739234 (intronic)					
	AA	22 (6.0)	23 (12.8)	Reference		
	AG	169 (46.1)	68 (37.8)	0.31	0.15–0.63	0.001 [†]
	GG	176 (48.0)	89 (49.4)	0.42	0.21–0.85	0.02
	AA vs (AG+GG)			2.72	1.40–5.30	0.003 [†]
	GG vs (AG+AA)			1.10	0.74–1.63	0.6
	rs1805329 Ala249Val					
	TT	23 (6.2)	30 (16.4)	Reference		
	CT	118 (31.6)	106 (57.9)	0.65	0.33–1.27	0.2
	CC	232 (62.2)	47 (25.7)	0.14	0.07–0.29	<0.001 [†]
	TT vs CT+CC			3.14	1.65–5.97	<0.001 [†]
	CC vs CT+TT			0.20	0.13–0.31	<0.001 [†]
XPC	rs2607775 5'UTR					
	GG	64 (18.9)	40 (23.1)	Reference		
	CG	175 (51.6)	83 (48.0)	0.70	0.42–1.16	0.2
	CC	100 (29.5)	50 (28.9)	0.73	0.42–1.30	0.3
	GG vs CG+CC			1.41	0.87–2.28	0.2
	CC vs CG+GG			0.95	0.61–1.48	0.8
	rs2228000 Ala499Val					
	CC	21 (5.9)	0 (0.0)	Reference		
	CT	131 (36.9)	63 (35.4)	N/a	N/a	N/a

	Controls n (%)	Case n (%)	Adjusted OR*	95% CI	P
TT	203 (57.2)	115 (64.6)	N/a	N/a	
TT vs CT+CC			1.35	0.90–2.02	0.1
rs13181 Lys751Gln					
GG	24 (7.8)	13 (7.7)	Reference		
GT	126 (41.0)	64 (37.9)	0.80	0.37–1.76	0.1
TT	157 (51.1)	92 (54.4)	1.08	0.51–2.32	0.5
GG vs GT+TT			1.05	0.50–2.19	0.9
TT vs GT+GG			1.30	0.86–1.95	0.2
rs1799793 Asp312Asn					
TT	10 (3.3)	17 (10.0)	Reference		
CT	123 (40.1)	65 (38.2)	0.35	0.14–0.89	0.03
CC	174 (56.7)	88 (51.8)	0.35	0.14–0.88	0.03
TT vs CT+CC			2.84	1.16–6.97	0.02
CC vs CT+TT			0.88	0.58–1.33	0.5
rs238407 intronic					
TT	60 (16.6)	24 (15.9)	Reference		
AT	193 (53.3)	88 (58.3)	1.12	0.63–2.01	0.4
AA	109 (30.1)	39 (25.8)	0.91	0.48–1.73	0.8
TT vs AT+AA			0.96	0.55–1.67	0.9
AA vs AT+TT			0.83	0.52–1.32	0.4

* Adjusted for age, civil status, education levels, and contraceptive use.

† Remained significant after Bonferroni correction for multiple comparisons. OR odds ratio, CI confidence interval. Total number of controls, cases: rs10521082 n=393, n=183; rs10739234 n=367, n=180; rs1805329 n=373, n=183; rs2607775 n=339, n=173; rs2228000 n=355, n=178; rs13181 n=307, n=169; rs1799793 n=307, n=170; rs238407 n=362, n=151. The total number of subjects varies across SNP because of missing values.

Table 3

Association between NER SNPs and DNA repair capacity.

		Controls	P	Cases	P
		* DRC mean (SD)		* DRC mean (SD)	
<i>All genotypes</i>		6.14 (3.03)		2.42 (1.86)	
<i>RAD23B</i>	rs10521083 (intrinsic)				
	CC	5.82 (2.20)		2.21 (1.12)	
	AC	6.43 (3.44)		2.67 (2.30)	
	AA	6.02 (2.91)	0.3	2.40 (1.84)	0.3
	rs10739234 (intrinsic)				
	GG	5.99 (2.63)		2.46 (1.73)	
	AG	6.43 (3.49)		2.50 (1.93)	
	AA	6.27 (2.50)	0.5	2.16 (1.12)	0.7
	rs1805329 Ala249Val				
	TT	5.71 (1.95)		1.92 (1.54)	
	CT	6.77 (3.78)		2.03 (1.31)	
	CC	5.89 (2.64)	0.04	3.41 (2.23)	<0.001
<i>XPC</i>	rs2607775 5'UTR				
	GG	5.84 (2.69)		1.98 (1.38)	
	CG	5.92 (2.95)		2.34 (1.76)	
	CC	6.17 (2.72)	0.6	3.14 (2.51)	0.002
	rs2228000 Ala499Val				
	CC	5.53 (2.27)		N/a	
	CT	6.21 (3.05)		1.92 (1.39)	
	TT	6.13 (3.24)	0.7	2.52 (1.70)	0.09
<i>XPD</i>	rs13181 Lys751Gln				
	GG	5.84 (2.20)		1.67 (0.72)	
	GT	6.09 (2.99)		2.59 (2.20)	
	TT	6.36 (3.37)	0.7	2.41 (1.88)	0.5
	rs1799793 Asp312Asn				
	CC	6.04 (3.02)		2.30 (1.71)	
	CT	6.29 (2.57)		2.35 (1.59)	

	Controls * DRC mean (SD)	P	Cases * DRC mean (SD)	P
rs238407 intronic				
TT	6.31 (4.11)	0.6	1.92 (1.60)	0.6
TT	6.07 (3.49)		2.05 (1.60)	
AT	6.18 (2.81)		2.31 (1.99)	
AA	5.86 (2.65)	0.7	1.99 (1.34)	0.8

* Values are mean DNA repair capacity levels (standard deviation(SD)). P-values were calculated by a general linear model and corrected for age and education levels. DRC DNA repair capacity. Total number of controls, cases: rs10521082 n=393, n=183; rs10739234 n=367, n=180; rs1805329 n=373, n=183; rs2607775 n=339, n=173; rs2228000 n=355, n=169; rs1799793 n=307, n=170; rs238407 n=362, n=151. The total number of subjects varies across SNP because of missing values.

Table 4

Association between risk genotypes at multiple loci, breast cancer risk and DRC

Number of risk genotypes	Controls n (%)	Case n (%)	Adjusted OR*	95% CI	P	Controls DRC†	P‡	Cases DRC†	P‡
0	54 (81.82)	12 (18.18)	Ref.			6.20 (2.61)		4.55 (2.41)	Ref.
1	175 (76.42)	54 (23.58)	1.40	0.65–3.04	0.4	5.83 (2.69)		2.69 (1.86)	0.2
2	78 (48.15)	84 (51.85)	5.81	2.64–12.79	<0.001	6.42 (3.45)	0.3	1.89 (1.36)	0.007
Overall model fit					<0.001				

* Adjusted for age, civil status, education level, and use of oral contraceptive.

† Values are mean (standard deviation (SD)).

‡ P values were calculated by a general linear model corrected for age and education levels, followed by a Tukey pairwise comparison test; ANOVA P value (controls) or Tukey P values in reference to 0 risk genotype (cases) are given. OR odds ratio, CI confidence interval, DRC DNA repair capacity. Total number of controls: 307, cases: 150. Individuals for which genotype data was missing at any of the 2 risk loci were not included in the analysis.