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Discovery of Mutations in Homologous Recombination Genes in African-American Women with Breast Cancer

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Abstract

African-American women are more likely to develop aggressive breast cancer at younger ages and experience poorer cancer prognoses than non-Hispanic Caucasians. Deficiency in repair of DNA by homologous recombination (HR) is associated with cancer development, suggesting that mutations in genes that affect this process may cause breast cancer. Inherited pathogenic mutations have been identified in genes involved in repairing DNA damage, but few studies have focused on African Americans. We screened for germline mutations in seven HR repair pathway genes in DNA of 181 African-American women with breast cancer, evaluated the potential effects of identified missense variants using *in silico* prediction software, and functionally characterized a set of missense variants by yeast two-hybrid assays. We identified five likely-damaging variants, including two *PALB2* truncating variants (Q151X and W1038X) and three novel missense variants (*RAD51C* C135R, and *XRCC3* L297P and V337E) that abolish protein-protein interactions in yeast two-hybrid assays. Our results add to evidence that HR gene mutations account for a proportion of the genetic risk for developing breast cancer in African Americans. Identifying additional mutations that diminish HR may provide a tool for better assessing breast cancer risk and improving approaches for targeted treatment.

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Conflict of interest

The authors declare that they have no conflicts of interest.

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent

Informed consent was obtained from all individual participants included in the study.

Keywords

homologous recombination; germline mutations; breast cancer; African Americans; loss of protein function

Introduction

Breast cancer is the most commonly diagnosed cancer among women in the United States, and it is estimated that 12% of women will develop breast cancer during their lifetime [1]. African-American women are experiencing increased rates of breast cancer that are converging with those of non-Hispanic Caucasian women [1], with a high incidence among women younger than 40 years old [2]. Breast cancer mortality rates were also 41% higher for African-American women than non-Hispanic Caucasian women [3], with nearly double the incidence of triple-negative breast cancers (TNBCs), which may partially explain the more aggressive disease and higher mortality [3].

Because many TNBCs display profound defects in homologous recombination (HR) [4], germline defects in HR genes may explain a proportion of breast cancers in African Americans. In fact, *BRCA1* and *BRCA2*, the first genes identified as predisposition genes for hereditary breast cancer in 1995 and 1996, are involved in HR [5] and pathogenic mutations have been identified in African-American breast cancer cases [6]. In addition to BRCA1 and BRCA2, other proteins involved in repairing chromosomal double strand breaks (DSBs) by HR include PALB2, RAD51, RAD51B, RAD51C, RAD51D, XRCC2, and XRCC3 [7–12]. DSBs can arise in a developmentally programmed manner, following replication arrest or stalling, from endogenously arising DNA damage, and from exogenous DNA-damaging agents such as ionizing radiation. Unrepaired or mis-repaired DSBs can cause genome rearrangements that eventually can lead to tumorigenesis through inactivation of tumor suppressor genes or activation of oncogenes [13]. HR most often utilizes the undamaged sister chromatid as a template for repair and is generally considered an error-free mechanism.

Although there are many studies of HR gene mutations in breast cancer, the majority of mutation screening has been conducted in Caucasians of European descent, with only a few studies in African Americans [6, 14–16]. To fill this gap, we sequenced the seven HR genes, *PALB2*, *RAD51*, *RAD51B, RAD51C, RAD51D, XRCC2*, and *XRCC3* in germline DNA of 181 self-reported African-American women with breast cancer who previously tested negative for mutations in the HR genes, *BRCA1* and *BRCA2*. We identified several missense mutations specific to African Americans and evaluated their potential impact on gene function using *in silico* prediction tools. Mutations that were predicted to confer a loss of function were further characterized by yeast two-hybrid (Y2H) assays to assess their effects on specific protein-protein interactions. Effects ranged from no detectable change to complete ablation of interaction, the latter consistent with loss of function. These results support the view that defects in HR play a role in the development of breast cancer in African-American women, and that increased surveillance of HR genes is warranted.

Methods

Breast cancer cases

A total of 181 self-reported African-American women with breast cancer were recruited at Parkland Hospital, affiliated with the University of Texas Southwestern Medical Center in Dallas, TX. All women provided informed consent prior to participation in the study. The study was approved by the University of Texas Southwestern Medical Center Institutional Review Board in accordance with the Declaration of Helsinki. Criteria for inclusion were women of African-American descent with a diagnosis of breast cancer under age 50 years and/or family history of breast and/or ovarian cancers. A blood sample for DNA and demographic data were collected from each participant. Information on family history of breast and ovarian cancers, histopathologic type, TNM classification of malignant tumors (tumor/node/metastasis stage), tumor size, and histopathology grade were extracted from medical records, pathology reports, and questionnaires. All samples had been previously screened for mutations in *BRCA1* and *BRCA2*, including screening for large insertions/deletions using multiplex ligation-dependent probe amplification assays (MRC-Holland), and were negative. In addition, 139 of the samples previously had been tested for mutations in *PALB2* [14].

Mutation screening

We focused on *PALB2, RAD51*, and the *RAD51* paralogs because the proteins they code for have been directly implicated in DSB repair by HR. Amplicons of coding exons and exon-intron boundary regions of *PALB2*, *RAD51*, *RAD51B*, *RAD51C*, *RAD51D*, *XRCC2*, and *XRCC3* were screened for mutations by high-resolution melt (HRM) analysis [17] of small exons (less than 200 bp) or direct Sanger sequencing of larger exons. For *PALB2*, 139 of 181 samples had been previously screened [14]. The HRM reaction was performed using 10 ng DNA, 2x Meltdoctor® HRM Master Mix (Applied Biosystems Inc; ABI), and appropriate primers. A 7900HT Fast Real-Time PCR system was used for PCR amplification and dissociation-curve generation. Dissociation curves were analyzed with HRM software 2.0 (ABI). Variant calls were based on slopes of the dissociation curves. For each variant identified by HRM, one or more samples were sequenced to identify the actual variant responsible for the anomaly in the dissociation curve. Methods for PCR and direct sequencing have been described elsewhere [14]. PCR primers and amplification programs for each exon are listed in Supplementary Table 1S.

In-silico assessment of mutations

Variants were annotated using ANNOVAR to obtain minor allele frequencies (MAFs) from the Exome Aggregation Consortium (ExAC) database [18]. ANNOVAR also provides the output from several *in-silico* prediction algorithms. First, we removed any variants with a MAF >0.02 in the African-American ExAC dataset. Next, we used SIFT [19], PolyPhen2 [20], LRT [21], PhyloP46 [22], Mutation Taster [23], and GERP++ [24] to predict pathogenicity of missense mutations. Variants with SIFT scores =0 or predicted to be probably damaging by PolyPhen2 along with deleterious predictions in at least three of the remaining four algorithms (LRT, PhylopP46, Mutation Taster, and GERP++) were selected

for testing in Y2H assays. PhyloP46 was predicted to be deleterious with scores ≥1.3 and GERP++ was predicted to be deleterious with scores ≥4.

The structure of human RAD51 (PDB 1B22) [25] was used to model the potential effects of mutations in the *RAD51* gene on protein stability. No suitable protein structures were available for modeling the effects of *RAD51B*, *RAD51C*, *RAD51D*, *XRCC2* and *XRCC3* mutations. *RAD51* variants A55V and A79D were modeled using SYBYLx2.0 (Tripos-Certara, Inc.). The variant structures were refined through energy minimization steps using Tripos and MMFF94 force fields with the cutoff for non-bonded interactions set at 8.0Å and the distance-dependent dielectric constant set at 4.0r, following the gradient termination of the Powell method [26] with a root mean square (RMS) of 0.05 kcal/mol*Å or a maximum 1000 iterations. Structural presentations of wild-type and variant RAD51 molecules were generated using SYBYLx1.3.

Y2H analysis

The Y2H assay is based on the principle that alteration of amino acids important for interaction between proteins will affect that interaction when the proteins are separately fused to the DNA binding domain (BD) or transcriptional activation domain (AD) of the yeast transcription activator protein, Gal4. Change in the interaction between fusion proteins affects expression of an *E. coli lacZ* gene driven by the promoter of the yeast *GAL1* gene, and thus alters β-gal activity in the yeast cell. Of note, a limitation of this assay is that not all interactions of higher eukaryotic proteins can be replicated in yeast. Y2H assays have been previously used to measure interactions between the following pairs of proteins: RAD51C-RAD51B, RAD51C-XRCC3, RAD51-RAD54L, RAD51-XRCC3, and XRCC2-RAD51D, and self-association of RAD51 [27–31]. We therefore measured the effects of putative deleterious mutations on these interactions. We were not able to develop a Y2H assay to test the *PALB2* missense variants, even though it has been shown that PALB2 binds to RAD51C, RAD51, and BRCA2 through co-immunoprecipitation assays in mammalian cells [32].

Plasmids—Y2H constructs containing the full-length human cDNAs of *XRCC3*, *XRCC2*, and *RAD51D* were provided by Dr. David Schild (pDS136, pDS138, pDS160, pDS181, and pDS182) (Lawrence Berkeley National Laboratory). Y2H constructs for human *RAD51C*, *RAD51B*, *RAD51*, and *RAD54L* were created by PCR to amplify the genes with the addition of an *EcoRI* or *MclI* site on the 5′ end and a *BamHI* or *BclI* site on the 3′ end. PCR products were digested and ligated into the *EcoRI* and *BamHI* sites down-stream of the transcriptional-activation domain vector pGAD424 and Gal4 DNA-binding domain vector pGBT9 to create N-terminal fusions (Clontech)[30, 31]. Plasmids containing genes used as templates for PCR were: human *RAD51C* (pDS157), *RAD51B* (pDS151) (provided by D. Schild); human *RAD51* (JS83) (provided by Jeremy Stark, Beckman Research Institute of City of Hope); and human *RAD54L* (Open Biosystems, clone ID 40118437). Site-directed mutations were made using the Phusion Site-Directed Mutagenesis Kit (Thermo Scientific), and each mutated construct was sequenced across the insertion to confirm the mutation.

Functional characterization of missense variants by Y2H—Matchmaker Y2H kits were used according to manufacturer's instructions (Clontech). For all interactions, yeast

strain Y187 (genotype *MATα, ura3-52, his3-200, ade2-101, trp1-901, leu2-3, 112, gal4Δ, met–, gal80Δ, URA3::GAL1$_{UAS}$-GAL1$_{TATA}$-lacZ*) was co-transformed with a plasmid containing the Gal4 DNA binding domain fused to the coding sequence of a HR gene and a plasmid containing the Gal4 activation domain fused to the coding sequence of another HR gene and plated on minimal media lacking leucine and tryptophan. To quantify the interactions between selected pairs of proteins, liquid β-galactosidase (β-gal) assays were performed on extracts of co-transformed yeast cultures, using *O*-nitrophenol-β-D-galactopyranoside as substrate. Reported β-gal activities (Miller units) for each plasmid combination represent the average from five to eight independent determinations with separate independent co-transformants, and include standard deviation of the mean. P-values were determined using Student's T-test.

Results

In order to identify mutations that may play a role in the development of breast cancer in African-American women, we sequenced the PALB2, RAD51, RAD51B, RAD51C, RAD51D, XRCC2, and XRCC3 genes, that encode key components of the HR pathway, in genomic DNA of 181 African-American women diagnosed with breast cancer between the ages of 23 and 69 years. These women had previously tested negative for germline *BRCA1/2* mutations, enabling us to assess the potential contribution to breast cancer susceptibility of mutations in other HR genes. In total, we sequenced 55 exons and exon-intron boundary regions that comprised 21,227 bp of genomic DNA. We detected 125 variants (Supplementary Table 2S), of which 116 were single nucleotide variants (SNVs) and 9 were small insertions or deletions (INDELs). The list of variants includes those detected in our previous screening of *PALB2* (139 of 181 cases) [14]. Of the 125 variants, 37 (29.6%; 34 SNVs and 3 INDELs) were each found in a single breast cancer case; 17 (13.6%; 15 SNVs and 2 INDELs) were each found in two breast cancer cases, and the remaining 71 variants were observed in three or more breast cancer cases. Of the 66 coding variants, 2 were nonsense mutations, 42 were missense mutations, and 22 led to no change in amino acid sequence.

All further analysis was restricted to low-frequency (MAF < 0.02) mutations, using allele frequencies from 5200 African-ancestry samples in the ExAC database, as these mutations are more likely to be pathogenic and confer a higher risk of developing cancer than more common SNVs [33–36](Supplementary Table 3S). One nonsense mutation and 12 of the missense variants were not observed in the 5200 African ancestry ExAC database samples and 25 missense variants had MAFs less than 0.005. The two nonsense mutations, *PALB2* Q151X and W1038X had not been identified in our previous study [14].

We modeled the structures of the RAD51 variant proteins to predict how their higher order structures might be affected by the changes in amino acid sequence. The amino acid changes A55V and A79D in RAD51 alter a helix-hairpin-helix motif within the N-terminal domain. This motif is responsible for binding to the sugar phosphate backbone of DNA. We predicted that the A to V change at residue 55 in RAD51 might result in steric hindrance with Phe46, changing protein conformation and interfering with the interaction between subunits (Supplementary Figure S1–A). The change of A to D at position 79 in RAD51 could

facilitate hydrogen bonding with Gln23, putting constraints on the two neighboring helices that make the N-terminal loop less flexible during DNA binding (Supplementary Figure S1–B). The *XRCC3* L297P variant alters the sixth internal β sheet within the C-terminal domain, which Shin et al. [37] suggest is critical for maintaining appropriate folding of the RAD51 paralogs.

Using Y2H assays, four missense variants, selected based on predictions of deleterious effects on protein function by a combination of outputs from SIFT, Polyphen2, LRT, PhyloP46, Mutation Taster, and GERP++ as detailed in the Methods section (Table 1), and three variants, selected based on putative effects on protein structure and/or function as described above (Supplementary Figure S1), were tested for alterations in protein-protein interactions. We did not analyze *PALB2* missense variants because we were unable to develop a Y2H assay. The *RAD51* R150Q variant was not tested because it previously was shown to alter the physical properties of RAD51 nucleoprotein filaments and markedly lowers the catalytic efficiency for adenosine triphosphate hydrolysis [38, 39].

We created plasmid constructs containing fusions of the Gal4 AD and BD domains to the seven selected variant and corresponding wildtype (WT) HR genes (Table 2) and used them to examine the following pairwise interactions; RAD51C-RAD51B, RAD51C-XRCC3, RAD51-RAD51, RAD51-RAD54L, RAD51-XRCC3, and XRCC2-RAD51D. All of these interactions have previously been characterized by Y2H [28–31] and co-immunoprecipitation assays with extracts from human cells [30, 40–44]. The effects of the mutations on the pairwise interactions are presented as the percent of activity observed with WT proteins (Table 2). β-gal activities for all Y2H assays are shown in Supplementary Table 4S. Effects ranged from no detectable change to complete ablation of interaction, the latter consistent with loss of function. The *RAD51C* C135R mutation resulted in complete loss of observable interaction between RAD51C and both RAD51B and XRCC3 (Table 2; Supplementary Table 4S). Similarly, the *XRCC3* L297P and V337E mutations conferred complete loss of observable interactions between XRCC3 and both RAD51C and RAD51. Interestingly, *RAD51C* C135R, *XRCC3* L297P, *XRCC3* V337E are all novel variants to the best of our knowledge at the time of manuscript submission. One possible explanation for the decreased levels of interaction conferred by *RAD51C* C135R, *XRCC3* L297P, and *XRCC3* V337E is that these mutations result in reduced steady-state levels of RAD51C and XRCC3. This could be caused by a reduction in gene expression or reduced protein stability due to improper folding. Of note, the interaction between RAD51C and XRCC3 was only examined with RAD51C fused to the Gal4-AD as the BD fusion is not functional in this context [28]. Similarly, the RAD51-RAD54L interaction was examined solely with RAD51-AD and RAD54L-BD fusions [29, 45] and the RAD51-XRCC3 interaction was examined solely with RAD51-AD and XRCC3-BD [30].

A second group of mutations attenuated protein-protein interactions to a lesser extent. The *XRCC2* F270V variant conferred statistically significant ($P<0.0005$) 59% and 28% reductions in the XRCC2-RAD51D interaction, suggesting that this mutation is mildly damaging (Table 2; Supplementary Table 4S). Interestingly, while the *RAD51*A79D mutation resulted in a substantial, statistically significant ($P = 0.001$) 78% reduction in the RAD51-XRCC3 interaction, it did not significantly ($P > 0.17$) disrupt the RAD51-RAD51

or RAD51-RAD54L interactions. The remaining two mutations, *RAD51* A55V and *RAD51D* A34V, did not significantly affect (P > 0.08) protein-protein interactions.

We examined the clinical features of the 181 women in this study in order to determine if there was a link between the HR gene variants observed in this study and disease (Table 3). Age at diagnosis at the first breast cancer ranged from 23 to 69 years, with a median age of 46 years. Twenty-three women developed a second breast cancer and three developed ovarian cancer. Of the 176 cases with family history information, 95 cases (54.0%) reported at least one first-degree relative (FDR) or second-degree relative (SDR) with breast or ovarian cancer. Of these, 23 cases (24.2%) had a FDR or SDR with ovarian cancer and 72 (75.8%) cases only had a family history of breast cancer. Of the two women carrying the pathogenic truncating mutations (*PALB2* Q151X and *PALB2* W1038X), and the three carrying the likely-damaging missense mutations (*RAD51C* C135R, *XRCC3* L297P, and *XRCC3* V337E) identified by Y2H experiments (Table 4), three (60%) were diagnosed with their first breast cancers under 40 years of age, as compared to 24.4 % of the 176 patients who did not carry damaging mutations (P < 0.1). For three of these five mutation carriers (60%) with available histology, all were poorly differentiated tumors (Grade 3); in comparison, of the 112 breast cancer cases not carrying likely-damaging mutations with histology, 59.8% were poorly differentiated (P < 0.27 by Fisher's Exact Test). Of the five cases, only two cases, one carrying the *PALB2* W1038X and the other carrying the *XRCC3* L297P variants had a known family history of breast or ovarian cancer, both with mothers diagnosed with breast cancer.

Discussion

In this project, we sequenced 7 HR genes in 181 breast cancer cases of African ancestry in order to identify pathogenic or likely-pathogenic mutations. Two nonsense and 42 missense mutations were identified. We used several *in silico* prediction methods, structural modeling, Y2H assays, and population frequencies to assess possible pathogenicity of the mutations. We identified five likely-damaging variants, including two *PALB2* truncating variants (Q151X and W1038X) and three novel missense variants (*RAD51C* C135R, *XRCC3* L297P, and *XRCC3* V337E).

The PALB2 protein serves as a scaffold that binds BRCA1, BRCA2, RAD51, RAD51C, and XRCC3, and plays a vital role in coordinating DSB repair by HR [7]. Accordingly, although mutations are generally infrequent compared with *BRCA1* and *BRCA2*, the breast cancer risk associated with *PALB2* mutations has been estimated to overlap with that of *BRCA2* mutations [46]. The *PALB2* Q151X nonsense mutation observed in our analysis results in loss of 87.3% of the PALB2 protein sequence and earlier was reported to be a loss-of-function mutation [46]. The *PALB2* W1038X nonsense mutation results in both altered splicing (removal of exon10) and a stop codon at position 1038 [47], and was previously reported to confer a high risk for breast cancer [48].

Germline mutations in all five RAD51 paralogs have previously been associated with breast and ovarian cancers [33, 36, 49–57]. While the RAD51 paralogs have been implicated in HR mediated repair of DSBs and DNA damaging signaling [8–12], their precise roles are still

under investigation. Biochemical studies show that the RAD51 paralogs exist in two major complexes, RAD51B/RAD51C/RAD51D/XRCC2 (BCDX2) and RAD51C/XRCC3 (CX3) [31, 41, 42]. In particular, the RAD51 paralogs have been implicated in the repair of collapsed replication forks by RAD51-dependent HR, and there is strong evidence that interaction between these proteins is essential for this repair mechanism [10, 58]. Intriguingly, our Y2H analysis showed that the *RAD51C* C135R, *XRCC3* L297P, and *XRCC3* V337E mutations completely disrupted all tested interactions, while the *XRCC2* F270V mutation partially reduced at least one of the tested protein-protein interactions. The *XRCC2* F270V mutation has a MAF of 0.008 in the ExAC African- ancestry dataset and was not observed in more than 20,000 ExAC European ancestry individuals, suggesting it is a rare African-ancestry specific variant. Similarly, it was previously detected in one African-American breast cancer case among 1308 breast cancer cases of any race/ethnicity, and was absent in 1120 matched controls ($P<0.02$) in a study of the Breast Cancer Family Registry (BCFR)[33]. The *RAD51C* C135R, *XRCC3* L297P, and *XRCC3* V337E mutations are novel to the best of our knowledge, lending support to the notion that these variants are pathogenic and that they may also be African-ancestry specific variants.

RAD51 is the central HR protein in mammals, and is the hub for complex formation with itself, RAD52, BRCA2, PALB2, the RAD51 paralogs, and a number of other proteins [59, 60]. Germline mutations in *RAD51* have recently been associated with a Fanconi anemia-like disorder (hFANCR) [61]. The *RAD51* A79D mutation reported here is novel to the best of our knowledge, and leads to a loss in association of RAD51 with XRCC3, but not with itself or RAD54L. The *RAD51* R150Q mutation was first reported in two unrelated Japanese women diagnosed with synchronous bilateral breast cancer at ages 44 and 52 years [62] and was detected in a woman in our study diagnosed with aggressive breast cancer. In a BCFR study, it was also reported in one breast cancer case and one control, leading the authors to conclude that it was not pathogenic [63]. It has been reported that the R150Q mutation alters the physical properties of RAD51 nucleoprotein filaments and markedly lowers the catalytic efficiency for adenosine triphosphate hydrolysis [38, 39]. This mutation was observed in 0.4% of those of African ancestry, and in none of those of European ancestry in ExAC. Although the *RAD51* R150Q mutation may confer some increase in breast cancer risk based on the findings in Japanese women [62] and its ability to alter the physical properties of RAD51 [38, 39], given the observations that the mutation is present in African-ancestry controls, we do not consider *RAD51* R150Q to be pathogenic.

A large proportion of pathogenic mutations in moderate-risk breast cancer genes appear to be rare missense variants. Tavtigian et al. [34, 35] found that more than 50% of pathogenic variants in *ATM* and *CHEK2* were rare missense mutations, and that missense mutations in some critical protein domains were associated with a higher risk of developing breast cancer than nonsense mutations. Mutation screening of BCFR samples revealed that four of five *XRCC2* mutations associated with increased risk of breast cancer were rare missense variants [33]. Osorio et al. reported that three of five deleterious mutations in *RAD51C* detected in 785 Spanish breast and ovarian cancer families were rare missense variants [36]. Interestingly, *RAD51C* mutations are primarily found in breast and ovarian cancer families, but both the Spanish study and our study found mutations in women without family histories of ovarian cancer; this could reflect small family sizes. Similar to findings of others, three of

the five likely damaging mutations identified in our study were missense mutations, which also may reflect the African ancestry of the breast cancer cases. Tennessen et al. [64] analyzed data for 1351 European Americans and 1088 African Americans from the NHLBI-ESP and found that there were more novel single nucleotide variants in African Americans and that a significantly higher portion of the missense variants were predicted to be pathogenic. Similarly, John et al. [65] reported 38% of pathogenic *BRCA1* variants were missense mutations in African Americans, whereas only 14% of pathogenic variants were missense mutations in non-Hispanic Caucasians.

Although only five of the participants in this study (2.8%) were found to carry likely damaging mutations in HR pathway genes, the actual number of pathogenic HR mutations in the tested population may be significantly higher. First, Y2H was the only assay used to functionally characterize the mutations and this assay examines only one aspect of protein function. It is likely that some of our identified missense mutations have profound effects on HR even though they have no effect on protein-protein interactions. Assays have been developed using human reporter cell lines to quantify the relative frequency of HR [66]. These assays could be modified to introduce our missense mutations into these cell lines using the CRISPR-Cas9 system, enabling us to test for effects on HR [67]. Second, we conducted mutation screening on a limited set of seven HR genes; mutations in other high- (*TP53*) and moderate-penetrance (*ATM, CHEK2*, and *NBS1*) breast cancer susceptibility genes can cause deficiencies in HR [68–71] and were not tested.

Two of the five women carrying likely deleterious mutations (*PALB2* W1038X and *XRCC3* L297P) had first- or second-degree relatives with breast or ovarian cancer. While the patient harboring the *XRCC3* V337E mutation did not have a first- or second-degree relative with breast or ovarian cancer, she had two cousins with breast cancer. In addition, three of the five cases with likely deleterious mutations were diagnosed below 40 years of age. Although the clinical-pathological features of these five cases provide only moderate support for a causal role for these mutations in the development of breast cancer, we believe our identification of five likely deleterious mutations adds to the evidence that mutations in HR genes may account for a proportion of the genetic risk for the disease in African Americans. Identifying additional mutations that affect HR may provide a tool for better assessing the risk of developing breast cancer in African-American women, and improved approaches for targeted treatment.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Missense variants selected for Y2H assays

Gene	Mutation	MAF	SIFT scores	SIFT prediction	P2	LRT	PhyloP46	MT	G++
RAD51	A55V*	0.0015	0.05	D	POD	D	D	D	D
RAD51	A79D*	.	0.41	T	B	.	D	D	D
RAD51C	C135R	.	0	D	PRD	D	D	N	D
RAD51D	A34V	.	0.29	T	PRD	D	D	D	D
XRCC2	F270V	0.0084	0	D	PRD	D	D	D	D
XRCC3	L297P*	.	0.26	T	POD	D	B	D	B
XRCC3	V337E	.	0	D	PRD	D	D	D	D

Abbreviations: MAF, allele frequency in African Americans in the ExAC database; P2, PolyPhen2; MT, Mutation Taster; G++, GERP++; D, deleterious; T, tolerated; B, benign; POD, possibly damaging; PRD, probably damaging; N, neutral.

*Variants selected for Y2H experiment based on modeling of mutation effect on protein stability. The other variants were selected based on SIFT, Polyphen2, LRT, PhloP46, Mutation Taster, and GERP++ prediction scores.

Table 2

Characterization of mutation effect on protein interaction by Y2H assays

Activation Domain	DNA-binding Domain	Y2H % of WT*	p value
RAD51C C135R	RAD51B WT	0.0±0.0	**0.001**
RAD51B WT	**RAD51C C135R**	0.0±0.0	**0.001**
RAD51C C135R	XRCC3 WT	0.0±0.0	**0.001**
XRCC3 WT	RAD51C C135R	NP [28]	
RAD51 A55V	RAD51 WT	135.2±17.7	0.023
RAD51 WT	RAD51 A55V	90.8±12.4	0.22
RAD51 A55V	RAD54L WT	111.5±8.1	0.13
RAD54L WT	RAD51 A55V	NP [29, 45]	
RAD51 A55V	XRCC3 WT	97.8±7.8	0.57
XRCC3 WT	RAD51 A55V	NP [30]	
RAD51 A79D	RAD51 WT	95.1±9.5	0.44
RAD51 WT	RAD51 A79D	109.0±16.2	0.3
RAD51 A79D	RAD54L WT	90.6±4.6	0.17
RAD54L WT	RAD51 A79D	NP [29, 45]	
RAD51 A79D	XRCC3 WT	21.5±11.1	**0.001**
XRCC3 WT	RAD51 A79D	NP [30]	
RAD51D A34V	XRCC2 WT	89.6±15.7	0.26
XRCC2 WT	RAD51D A34V	96.6±14.5	0.74
XRCC2 F270V	RAD51D WT	72.0±9.0	**0.001**
RAD51D WT	**XRCC2 F270V**	41.1±8.6	**0.001**
XRCC3 L297P	RAD51C WT	NP [28]	
RAD51C WT	**XRCC3 L297P**	0.0±0.0	**0.001**
XRCC3 L297P	RAD51 WT	NP [30]	
RAD51 WT	**XRCC3 L297P**	0.0±0.0	**0.001**
XRCC3 V337E	RAD51C WT	NP [28]	
RAD51C WT	**XRCC3 V337E**	0.0±0.0	**0.001**
XRCC3 V337E	RAD51 WT	NP [30]	
RAD51 WT	**XRCC3 V337E**	0.0±0.0	**0.001**

*Values are means and standard deviation of β-gal activities obtained from eight independent colonies; β-gal activity for each variant in the Y2H assays was normalized to the WT value, considering β-gal activity for WT to be 100. NP indicates an asymmetric interaction in Y2H assays was observed and it is not possible to measure β-gal activity when vectors are cloned in a reverse direction.

Table 3

Clinical features of 181 African American patients with breast cancer selected for young age at diagnosis or family history of breast and/or ovarian cancer

	Number	Proportion
Age at breast cancer diagnosis (years)		
< 45	72	0.4
45–59	105	0.58
60+	4	0.02
Development of second breast cancer diagnosed at any age		
Yes	23	0.13
No/Unknown	158	0.87
Personal history of ovarian cancer		
Yes	23	0.13
No	177	0.98
Unknown	1	0.01
Cancer in 1° or 2° relative		
Female breast or ovarian cancer	95	0.54
Ovarian cancer only	23	0.13
Female breast cancer only	72	0.41
Male breast cancer	1	0.01
No breast or ovarian cancer	82	0.47
Unknown	3	0.02
Tumor hormone receptor status Estrogen receptor (ER)		
Positive	69	0.38
Negative	42	0.23
Unknown	70	0.39
Progesterone receptor (PR)		
Positive	52	0.29
Negative	51	0.28
Unknown	78	0.43
Her2/neu		
Positive	22	0.12
Negative	56	0.31
Unknown	103	0.57
Triple negative (TNBC)		
Yes	25	0.14
No	78	0.43
Unknown	78	0.43
Stage at diagnosis		
in situ (non invasive)	12	0.07

	Number	Proportion
1	13	0.07
2	33	0.18
3	9	0.05
4	8	0.04
Unknown	106	0.59
Grade		
I	5	0.03
II	46	0.25
III	70	0.39
Unknown	60	0.33

Table 4

Characteristics of the breast cancer cases carrying the likely damaging mutations

Case	Mutation	Dx age	T	N	M	Grade	TNBC	Family history
1	*RAD51C* C135R	35	T4	N1	M0	G3	no	no
2	*XRCC3* L297P	52	T2	N0	M0	–	no	BC in mother
3	*XRCC3* V337E	36	T2	N0	M0	G3	yes	no
4	*PALB2* Q151X	58	T2	N3	–	G3	no	no
5	*PALB2* W1038X	33	–	–	–	–	–	BC in mother

Abbreviations: Dx age: age at diagnosis of the first breast cancer; T: primary tumor size; N: regional lymph node status; M: distant metastasis status; TNBC, triple-negative breast cancer; BC, breast cancer