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Germline mutations in *PALB2* in African-American breast cancer cases

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Abstract

Purpose—Breast cancer incidence is lower in African Americans than in Caucasian Americans. However, African-American women have higher breast cancer mortality rates and tend to be diagnosed with earlier-onset disease. Identifying factors correlated to the racial/ethnic variation in the epidemiology of breast cancer may provide better understanding of the more aggressive disease at diagnosis. Truncating germline mutations in *PALB2* have been identified in approximately 1% of early-onset and/or familial breast cancer cases. To date, *PALB2* mutation testing has not been performed in African-American breast cancer cases.

Methods—We screened for germline mutations in *PALB2* in 139 African-American breast cases by denaturing high-performance liquid chromatography and direct sequencing.

Results—Twelve variants were identified in these cases and none caused truncation of the protein. Three missense variants, including two rare variants (P8L and T300I) and one common variant (P210L), were predicted to be pathogenic, and were located in a coiled-coil domain of *PALB2* required for RAD51- and BRCA1-binding. We investigated and found no significant association between the P210L variant and breast cancer risk in a small case-control study of African-American women.

Conclusions—This study adds to the literature that *PALB2* mutations, although rare, appear to play a role in breast cancer in all populations investigated to date.

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Keywords

germline mutations; PALB2; missense variants; breast cancer; African Americans

Introduction

Breast cancer is the most commonly diagnosed cancer in women with estimates that one in eight women in the U. S. will develop breast cancer during her lifetime [1]. African-American women tend to be diagnosed with more aggressive breast cancer at a younger age and have higher mortality from the disease than non-Hispanic White women [2]. Research has been ongoing to identify factors contributing to racial and ethnic variation in breast cancer incidence and mortality [2-7]. In a study of African-American breast cancer cases from the San Francisco Bay area, the population-based prevalence of pathogenic *BRCA1* mutations in cases younger than 35 years was high (16.7%), although the overall prevalence of *BRCA1* mutations in cases younger than 65 years was 1.3% and lower than in non-Hispanic Whites (2.2%) [6]. In a second population-based study, the prevalence of pathogenic *BRCA1* mutations was significantly lower in African-American breast cancer cases (1.4%) than in Whites (2.9%), and *BRCA2* mutations were slightly more common in African-American cases (2.6%) than in Whites (2.1%) [7]. Rare mutations in *PALB2*, *CHEK2*, *BRIP1*, and *ATM*, genes whose proteins interact with *BRCA1* and *BRCA2*, have been found to confer more than a two-fold increased risk to develop breast cancer [8]. *PALB2* was identified in 2006 as a *BRCA2*-binding protein [9], and more recently also was reported to directly interact with *BRCA1* and *RAD51* [10-12]. Since 2007, when truncating *PALB2* mutations were identified in familial breast cancer cases, there have been multiple reports of pathogenic *PALB2* mutations in breast cancer [13-20]. To date, mutation screening of *PALB2* has not been reported in African-American breast cancer cases. In this study, we tested for mutations in *PALB2* in 139 African-American breast cases diagnosed at age 55 years or younger.

Material and Methods

Samples for mutation screening

A total of 139 African-American breast cancer cases diagnosed at age 55 years or younger were screened for mutations in *PALB2*. The women were all recruited at Parkland Hospital, affiliated with the University of Texas Southwestern Medical Center in Dallas, TX under Institutional Review Board approval. A blood sample, family history and demographic data were collected from each of the participants. The age at diagnosis of the first breast cancer ranged from 23 to 55 years, with a median age of 45 years. Of the 134 cases with family history information, 52 cases (38%) and 46 cases (33%) reported at least one first-degree relative and one second-degree relative with breast cancer, respectively. All samples had been previously screened for mutations in *BRCA1* and *BRCA2* by sequencing and were negative.

PALB2 mutation screening

Sixteen primer pairs were used to amplify the 13 exons and the intron-exon boundaries of *PALB2* in DNA from peripheral blood samples [20]. The first 51 samples were screened using high throughput denaturing high-performance liquid chromatography (HT-DHPLC) on a WAVE System (Transgenomics Inc). Briefly, each PCR reaction was carried out in a total volume of 50 μ l containing 20 ng of genomic DNA, 200 pM of each primer, 100 μ M of dNTPmixture, 1 $\times$ PCR buffer (Qiagen Inc.), 2 mM $MgCl_2$, 1 unit of Taq DNA polymerase (Qiagen Inc.), and 0.1 unit of Optimase DNA Polymerase (Transgenomic Inc.). PCR

products were denatured for 5 min at 95°C and heteroduplex was formed by gradual re-annealing from 95°C to 25°C at the rate of -1.5°C per minute. Samples with aberrant chromatograph patterns were sequenced in both directions using BigDye terminator 3.1 cycle sequencing kit with sequencing performed on an 3130 Sequencer from Applied Biosystems Inc. (ABI). Sequencing traces were analyzed using SeqScape 2.5 (ABI) and by manual inspection. The remaining 88 samples were direct sequenced as described above without preliminary screening with the WAVE system.

Testing the association of *PALB2* P210L and risk of breast cancer

A set of African-American samples, including 161 invasive breast cancer cases and 224 controls from a multi-ethnic population-based case-control study of breast cancer [21] were genotyped for the P210L variant (rs57605939). Incident cases, ages 35-80 years at diagnosis, were identified through the Greater Bay Area Cancer Registry, and unrelated controls were selected through random-digit dialing and frequency matched to cases on race/ethnicity and expected 5-year age distribution. Genotyping was performed by a MGB Taqman probe assay (ABI). Briefly, reactions were carried out using 10 ng genomic DNA according to manufacturer's protocols; the fluorescence profile was read on an ABI PRISM 7900 HT instrument and the results analyzed with Sequence Detection Software (ABI). Duplicates of 19 DNA samples were genotyped for quality control. A chi-square test was used to investigate the association of carrying at least one copy of the variant allele and breast cancer risk. Statistical analysis was performed using SAS 9.1.3.

Results

The complete coding and intron-exon boundary sequences of *PALB2* were screened in DNA from 139 African-American female breast cancer cases. No truncating mutations were detected. Twelve variants were found, including eight missense variants and four silent coding variants (Table 1). Q559R (rs152451), I309V (rs3809683), and P210L (rs57605939) were reported in previous studies [18] and the remaining nine variants were not previously reported. Two programs, POLYPHEN [22] and PMUT [23], were used to predict the potential impact of the eight missense variants on protein function. Three of eight missense variants (P8L, T300L and P210L) were predicted to likely affect *PALB2* protein function by both POLYPHEN and PMUT prediction (Table 1). P8L was identified in a woman who developed breast cancer at age 30 years who had a family history of breast cancer in first- and second-degree relatives. T300L was identified in a woman with a first diagnosis of breast cancer at age 46 years and a second breast cancer at age 55 years. Both her maternal aunt and niece had ovarian cancer. P210L was relatively common in our study where 12 (8.6%) cases carried one copy and one case carried two copies of the variant. To test the association between P210L variant and breast cancer risk, we genotyped P210L in a population-based set of 161 African-American breast cancer cases and 224 African-American controls. The rare allele frequency (allele L) was 8.1% in the cases and 7.1% in the controls, thus, there was no significant association with breast cancer risk (odds ratio = 1.10; P value = 0.74).

Discussion

Previous studies have established that germline mutations in *PALB2* are associated with increased risk of breast cancer [13-20]. In the first report of mutations in *PALB2*, 5 different truncating mutations were identified in 10 of 923 familial breast cancer cases and in none of 1084 controls in the United Kingdom, conferring a 2.3-fold higher risk of breast cancer [18]. That study also identified missense mutations, some of which were predicted to be deleterious. Since that first report in 2007, there have been multiple reports of *PALB2* truncating mutations in breast cancer cases. A frameshift *PALB2* mutation (c.1592delT) was

found in 18 of 1918 Finnish female breast cancer cases and in 6 of 2501 Finnish controls. This Finnish founder mutation conferred an approximate four-fold increased risk of breast cancer [15]. Tischkowitz et al [20] identified a frameshift mutation (c.229delT) in breast cancer family of Scottish descent in Canada. Recently, novel truncating *PALB2* mutations were also detected in French-Canadian [16], Spanish [17], Italian [13], white South African [19], and Chinese [14] populations. This is the first report of *PALB2* mutations in African-American breast cancer cases. Although we identified no truncating mutations, three potentially pathogenic missense mutations (P8L, T300L and P210L) were found, as well as nine other variants that are presumed to be benign.

PALB2 plays crucial roles in the localization and stabilization of *BRCA2* in nuclear chromatin, which is essential for *BRCA2* to recruit *RAD51* to the double-strand break point in the process of homologous recombination repair [9]. Recent studies reported that *PALB2* also directly binds to *BRCA1* in the formation of a *BRCA1-PALB2-BRCA2* complex required for homologous recombination repair and that *PALB2* can directly interact with *RAD51* [10-12]. The *PALB2* N terminus (residues 1-319), containing a coiled-coil domain, is responsible for the interaction with both *BRCA1* and *RAD51* [10-12]. All three mutations (P8L, P210L and T300I) identified in this study that were predicted to be pathogenic are located in this domain and may partially abolish the interaction of *PALB2* with *BRCA1* and/or *RAD51*. P210L, which was common in our study with a frequency of 10% in screened breast cancer cases, is rare in other populations. It was observed in 1 of 1084 British controls [18], in 3 of 794 Spanish familial breast cancer cases [17], and in none of the breast cancer cases from four other ethnic groups, including Finnish [15], Italians [13], white South Africans [19], and Chinese [14]. The higher frequency of the P210L allele in the African-American breast cancer cases suggests an African and probable Yoruba origin. Based on the PMUT and POLYPHEN predictions that the variant is possibly pathogenic and the high allele frequency in African Americans, this SNP was a good candidate as a breast cancer susceptibility allele in this population. We tested it in a small population-based case-control set and found no significant association of breast cancer with P210L (odds ratio = 1.10; P-value=0.74).

In conclusion, we identified two rare missense variants and one common variant predicted to likely affect *PALB2* protein function by screening *PALB2* in 139 African-American breast cancer cases. Further functional studies are required to characterize the role of these variants. We identified no truncating mutations, unlike previous studies where truncating mutations in *PALB2* accounted for about 1% of the breast cancers in the early-onset and/or familial breast cancer cases [15-18, 20]. *PALB2* mutations, although rare, appear to play a role in breast cancer in all populations investigated to date. The detection of pathogenic mutations in *PALB2* may have therapeutic significance in the management of breast cancer, as a recent report demonstrated that *PALB2*-deficient cells, like the *BRCA2*-deficient cells, were sensitive to poly (ADP-ribose) Polymerase (PARP) inhibitors [10], which is a target therapy being tested in cancer patients carrying *BRCA1* and *BRCA2* mutations [24].

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Table 1*PALB2* sequence variants identified in 139 early-onset African-American breast cancer cases

mutation	location	frequency*	POLYPHEN	PMUT
<u>P8L</u>	Exon 1	1/278	possibly damaging	pathogenic
<u>K18R</u>	Exon 2	3/278	benign	neutral
P210L	Exon 4	14/278	possibly damaging	pathogenic
<u>N241D</u>	Exon 4	3/278	benign	neutral
<u>T300I</u>	Exon 4	1/278	possibly damaging	pathogenic
I309V	Exon 4	6/278	benign	neutral
<u>P473P</u>	Exon 4	1/278		
<u>V487V</u>	Exon 4	1/278		
<u>L536L</u>	Exon 4	2/278		
Q559R	Exon 4	35/278	benign	neutral
<u>G752G</u>	Exon 5	1/278		
<u>Q759K</u>	Exon 5	2/278	benign	neutral

* Frequency was calculated by $n/2N$ (n is the number of rare alleles, N is number of samples screened); Underlined variants have not been previously reported.