

## Inherited predisposition to breast cancer among African American women

Jane E. Churpek · Tom Walsh · Yonglan Zheng · Zakiya Moton · Anne M. Thornton · Ming K. Lee · Silvia Casadei · Amanda Watts · Barbara Neistadt · Matthew M. Churpek · Dezheng Huo · Cecilia Zvosec · Fang Liu · Qun Niu · Rafael Marquez · Jing Zhang · James Fackenthal · Mary-Claire King · Olufunmilayo I. Olopade

Received: 26 June 2014 / Accepted: 31 October 2014 / Published online: 27 November 2014  
© The Author(s) 2014. This article is published with open access at Springerlink.com

**Abstract** African Americans have a disproportionate burden of aggressive young-onset breast cancer. Genomic testing for inherited predisposition to breast cancer is increasingly common in clinical practice, but comprehensive mutation profiles remain unknown for most minority populations. We evaluated 289 patients who self-identified as African American with primary invasive breast cancer and with personal or family cancer history or tumor characteristics associated with high genetic risk for all classes of germline mutations in known breast cancer susceptibility genes using a validated targeted capture and multiplex sequencing approach. Sixty-eight damaging germline mutations were identified in 65 (22 %, 95 % CI 18–28 %) of the 289 subjects. Proportions of patients with unequivocally damaging mutations in a breast cancer gene were

26 % (47/180; 95 % confident interval [CI] 20–33 %) of those with breast cancer diagnosis before age 45; 25 % (26/103; 95 % CI 17–35 %) of those with triple-negative breast cancer (TNBC); 29 % (45/156; 95 % CI 22–37 %) of those with a first or second degree relative with breast cancer before age 60 or with ovarian cancer; and 57 % (4/7; 95 % CI 18–90 %) of those with both breast and ovarian cancer. Of patients with mutations, 80 % (52/65) carried mutations in *BRCA1* and *BRCA2* genes and 20 % (13/65) carried mutations in *PALB2*, *CHEK2*, *BARD1*, *ATM*, *PTEN*, or *TP53*. The mutational allelic spectrum was highly heterogeneous, with 57 different mutations in 65 patients. Of patients meeting selection criteria other than family history (i.e., with young age at diagnosis or TNBC), 48 % (64/133) had very limited information about the history of cancer in previous generations of their families. Mutations in *BRCA1* and *BRCA2* or another breast cancer gene occur in one in four African American breast cancer patients with early onset disease, family history of breast or ovarian cancer, or TNBC. Each of these criteria defines patients who would benefit from genomic testing and novel therapies targeting DNA repair pathways.

Jane E. Churpek and Tom Walsh have contributed equally to the manuscript.

J. E. Churpek · Y. Zheng · Z. Moton · C. Zvosec · F. Liu · Q. Niu · J. Zhang · J. Fackenthal · O. I. Olopade  
Department of Medicine, Center for Clinical Cancer Genetics,  
The University of Chicago, Chicago, IL, USA

T. Walsh · A. M. Thornton · M. K. Lee · S. Casadei · A. Watts · M.-C. King (✉)  
Division of Medical Genetics, Department of Medicine,  
University of Washington, Seattle, WA, USA  
e-mail: mcking@uw.edu

B. Neistadt · M. M. Churpek · R. Marquez · O. I. Olopade (✉)  
Department of Medicine, The University of Chicago, Chicago,  
IL, USA  
e-mail: folopade@medicine.bsd.uchicago.edu

M. M. Churpek · D. Huo  
Department of Health Studies, The University of Chicago,  
Chicago, IL, USA

**Keywords** Breast cancer · Next-generation sequencing · African American · Inherited susceptibility

### Introduction

African American breast cancer patients are more likely than breast cancer patients from other populations to be affected at a young age; to develop aggressive, triple-negative breast cancers (TNBC) that lack expression of estrogen, progesterone, and HER2 receptors; and to die from their disease [1–4]. Furthermore, patients from all

populations with inherited mutations in *BRCA1* (MIM 113705) and *BRCA2* (MIM 600185) are more likely to be diagnosed at a young age, and especially among *BRCA1* carriers, to develop TNBC [5, 6]. Thus, one potential contributor to the higher incidence of early-onset aggressive breast cancer among African American patients may be a previously undefined higher burden of inherited breast cancer in this population. High frequencies of mutations in *BRCA1* and *BRCA2* have already been observed in breast cancer patients of African ancestry from Nigeria [7] and from the Bahamas [8]. In addition to *BRCA1* and *BRCA2*, other genes that harbor mutations that predispose to breast cancer include *PALB2* [9, 10], *CHEK2* [11], *ATM* [12–15], *BARD1* [16, 17], *RAD51C* [18], *RAD51D* [19], *TP53* [20], and *PTEN* [21]. The relationship between aggressive young-onset breast cancer and mutations in these other genes is not yet well characterized.

Advances in DNA sequencing technologies have yielded assays that efficiently and cost-effectively screen multiple genes at the same time. One such assay, BROCA, was designed and validated for the simultaneous detection of mutations in multiple genomic regions encompassing all known and emerging breast cancer susceptibility genes [22, 23]. BROCA detects, in a single assay, all classes of mutations: single nucleotide variants, small insertions and deletions (indels), and large genomic rearrangements.

To investigate the genomic basis of early-onset breast cancer in African American women, we examined the frequencies of inherited mutations in cancer genes in self-reported African American patients ascertained through a cancer genetics clinic at a single tertiary-care medical center.

## Patients and methods

### Patients

Participants were women who self-reported as African American, who were diagnosed with primary invasive breast cancer, and who were ascertained at The University of Chicago Medicine Breast Program and the Cancer Risk Clinic between 1993 and July 2013. The University of Chicago serves a population catchment area that is predominantly African American; approximately 35 % of ethnically identified samples in our Breast Program biospecimen bank are derived from African Americans. Implementation of dedicated consenting led to a remarkably high consent rate of nearly 95 % on our biospecimen banking protocol. If related affected persons appeared on the registry, only the first to be listed was included. Of 502 unrelated African American women with a diagnosis of invasive breast cancer, DNA was available from 370 patients. Among them, a total of 289 African American

patients were included in the present study based on meeting at least one of the following criteria: breast cancer diagnosis before age 45; personal history of both breast and ovarian cancer; TNBC; or family history of breast cancer before age 60 or of ovarian cancer at any age in a first or second degree relative. The Institutional Review Boards (IRB) at The University of Chicago and the University of Washington approved the study. Patients had been consented for use of their samples for future genomic analysis. Permission for use for this study was obtained from the IRB. BROCA testing results were returned to participants according to their preference of knowing or omitting the information.

### Mutation detection

From each subject, 3 ug of DNA were used to prepare paired-end libraries with 150-bp inserts, which were hybridized to a custom pool of oligonucleotides designed to capture 48 genomic regions, and sequenced as previously described [22, 23]. For this project, only unambiguously damaging mutations in breast cancer genes *BRCA1*, *BRCA2*, *PALB2*, *CHEK2*, *ATM*, *BARD1*, *RAD51C*, *RAD51D*, *TP53*, and *PTEN* were evaluated. Single base-pair substitutions and indels [23] and large copy number variants (CNVs) [24] were identified as previously described. Unambiguously damaging mutations were defined as truncations from any source, complete gene deletions, splice mutations shown experimentally to yield truncations, and missense mutations shown experimentally to disrupt protein function. Single base-pair substitutions and indels were validated by independent PCR amplification and Sanger sequencing. CNVs were validated by real-time PCR using TaqMan probes (Life Technologies, Carlsbad, CA, USA) targeting the deleted or duplicated region. Qualifying patients were included in the study regardless of previous genetic testing; for those with prior genetic testing, sequencing and analysis was repeated, blind to previous results.

### Statistic analysis

Proportions of mutation carriers and exact 95 % confidence intervals (CI) were estimated using binominal distribution. Fisher's exact tests were used to examine whether mutation proportions vary across age groups or among patients meeting different numbers of eligibility criteria.

## Results

A total of 289 patients qualified for the study; their clinical features are shown in Table 1. Approximately, half of

**Table 1** Clinical features of 289 African American patients with primary invasive breast cancer selected for young age at diagnosis, family history, or TNBC

	N	Prop.
Age at breast cancer diagnosis		
< 45	180	0.62
45–59	85	0.29
60+	24	0.08
Personal history of ovarian cancer		
Yes	7	0.02
No	282	0.98
Cancer in 1° or 2° relative		
Breast cancer dx < 60	144	0.50
Ovarian cancer, any age	29	0.10
Male breast cancer, any age	5	0.02
Neither	67	0.23
Unknown	64	0.22
Tumor hormone receptor status		
Estrogen receptor (ER)		
Positive	109	0.38
Negative	135	0.47
Unknown	46	0.16
Progesterone receptor (PR)		
Positive	83	0.29
Negative	153	0.53
Unknown	53	0.18
Her2/neu		
Positive	35	0.12
Negative	177	0.61
Unknown	77	0.27
Triple negative (TNBC)		
Yes	103	0.36
No	131	0.45
Unknown	55	0.19
Stage		
1	69	0.24
2	91	0.31
3	56	0.19
4	15	0.05
Unknown	58	0.20
Grade		
I	9	0.03
II	71	0.25
III	128	0.44
Unknown	81	0.28
All patients	289	1.00

qualifying patients met more than one selection criterion, as illustrated in Fig. 1a. BROCA yielded >400-fold median coverage with 94 % of targeted bases covered >50-fold and 99.8 % of bases covered >10-fold. Sixty-eight

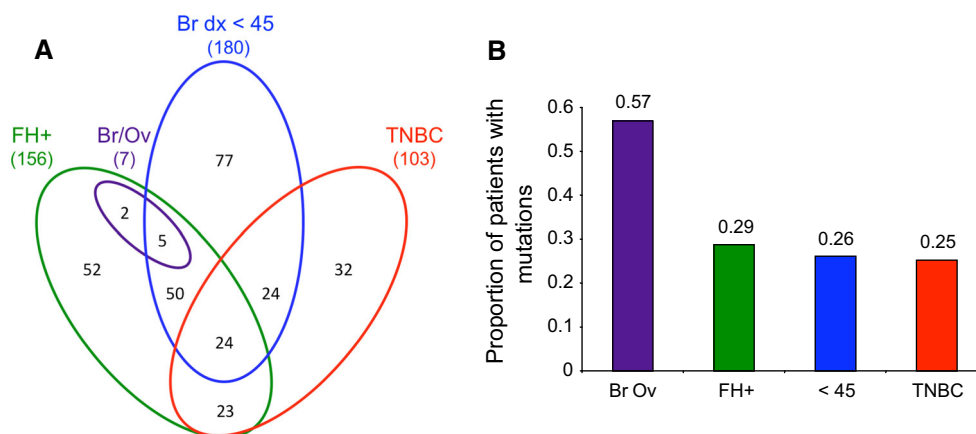
damaging mutations were identified in 65 (22 %, 95 % CI 18–28 %) of the 289 subjects. All mutations previously identified and published [25, 26] were independently identified by BROCA. The proportions of patients with unequivocally damaging mutations were 26 % (47/180; 95 % CI 20–33 %) of those with breast cancer diagnosis before age 45, 25 % (26/103; 95 % CI 17–35 %) of those with TNBC, 29 % (45/156; 95 % CI 22–37 %) of those with a qualifying family history, and 57 % (4/7; 95 % CI 18–90 %) of the small group with both breast and ovarian cancer (Fig. 1b).

The mutations were distributed among eight genes, with the most in *BRCA1* (29) and *BRCA2* (23) and the remaining in *PALB2* (3), *ATM* (5), *CHEK2* (4), *BARD1* (1), *TP53* (1), and *PTEN* (2) (Fig. 2). No truncations were present in either *RAD51C* or *RAD51D*, even among the 35 patients with personal or family history of ovarian cancer. Four mutations were large genomic deletions, three in *BRCA1* and one in *ATM*. All damaging mutations are indicated in Table 2. In addition to the 68 damaging mutations, we classified two variants as unknown significance, or “VUS”: *BRCA1\_5160A >G* (U14680) in exon 17, and *BRCA2\_8614C >T* (U43746) in exon 19, both predicted to alter splicing by disrupting exonic splice enhancer sites, but thus far without functional tests of patient RNA. These two variants were not included in subsequent analyses.

Patients meeting more than one qualifying criteria were more likely to carry a mutation ( $P = 0.001$ ). Proportions of patients with mutations were 15 % (22/144) of those meeting only one criterion, 25 % (29/116) of those meeting two criteria, and 48 % (14/29) of those meeting three criteria. No patients met all four criteria. The distribution of mutations gene-by-gene differed in that mutations in *BRCA1* were more frequent among patients diagnosed at age <45 than among patients diagnosed at age  $\geq 45$  and selected on other criteria (24/180 versus 5/109;  $P = 0.016$ ). There were no other differences in the distribution of mutations gene-by-gene among patients selected by various criteria. The prevalence of damaging mutations among familial TNBC cases was 34.0 % (16/47) versus 17.9 % (10/56) in non-familial TNBC, but this was not statistically significant ( $P = 0.071$ ).

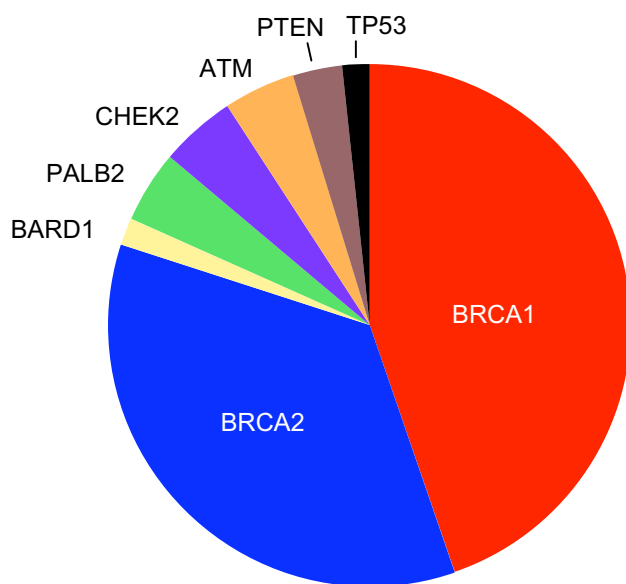
Three patients carried two mutations each. Patient 101 was homozygous for *CHEK2* p.G210R and was diagnosed with TNBC at age 33. Patient 276 carried both *BRCA2* p.R3128X and *ATM* p.R248X. She was diagnosed with receptor-positive breast cancer at age 31. Patient 312 carried both *CHEK2* p.R160G and *ATM* p.R457X. She was diagnosed with stage 3, receptor-positive breast cancer at age 34.

Three patients carried mutations in genes responsible for breast cancer in the context of syndromic disease. Patient 446 carried *TP53* p.P278A. She was diagnosed at ages 32



**Fig. 1** Frequencies of inherited mutations in breast cancer genes among African American breast cancer patients with young age at diagnosis, family history of breast or ovarian cancer, or triple negative breast cancer (TNBC). **a** Numbers of patients meeting each of the study criteria. All patients were diagnosed with primary invasive breast cancer and self-identified as African American. *FH+* indicates a first or second degree relative with breast cancer diagnosed at age

<60 or ovarian cancer at any age; *Br/Ov* indicates breast and ovarian cancer in the same subject; *Br dx <45* indicates that the participant's breast cancer was diagnosed at age <45; *TNBC* indicates that the breast cancer was triple negative; i.e., lacking estrogen and progesterone receptors and Her2. The study included 289 patients in all. **b** Proportions of patients with an unequivocally damaging mutation in a breast cancer gene, for each group from (a)



**Fig. 2** Eight genes with mutations in African American breast cancer patients. A total of 68 mutations were identified, 76 % in *BRCA1* or *BRCA2* and 24 % in other breast cancer genes

and 36 with bilateral breast cancer. Her family history is unknown. Patient 333 carried *PTEN* p.R335X. She has hyperkeratosis and was diagnosed at age 35 with bilateral breast cancer, including foci of TNBC and DCIS and LCIS, and at age 46 with renal cell cancer. Her father carried the same mutation and was previously diagnosed with Cowden syndrome (MIM 158350). He developed renal cell cancer at age 53. The family includes multiple other relatives with cancer. Patient 248 carried *PTEN* c.1027(ivs8)(-1)G>T,

which alters splicing of the last exon of *PTEN*, leading to reduced stability of the *PTEN* message. She was diagnosed with breast cancer at age 43; her family cancer history is unknown.

## Discussion

These results are based on the largest clinic-based cohort of prospectively ascertained African American breast cancer patients to undergo genomic testing of cancer genes known to be associated with inherited breast cancer. Using a more cost-effective and innovative high-throughput sequencing technology, we found that nearly one in four of patients meeting selection criteria carried an inherited damaging mutation in at least one gene. There were 57 different pathogenic mutations in eight different genes among 65 patients, supporting the clinical utility of simultaneous multi-gene testing, rather than relying on a limited mutation panel or a gene-by-gene approach. The majority of mutations were found in *BRCA1* and *BRCA2* genes. These results are consistent with data from clinic-based cohorts in other populations.

There are strengths and weaknesses to any single institution study. There is paucity of data on well-phenotyped patients of African ancestry. This study represents the first report of a large cohort of self-reported African American patients with integrated mutation results from next-generation sequencing, clinical characteristics of the patients, and tumor phenotype. Our results confirm that inherited mutations in the *BRCA1* and *BRCA2* tumor suppressor genes are still the strongest predictors of breast and/or

**Table 2** Germline mutations in African American patients with primary breast cancer with young age at diagnosis (Br dx <45), family history of breast cancer younger than age 60 (FH Br <60) or of ovarian cancer (FH Ov), or triple-negative breast cancer (TNBC)

Patient BROCA ID	Age dx	Gene	Mutation (BRCA1 U14680; BRCA2 U43746)	Br dx <45	FH Br <60	FH Ov	Self Br+Ov	TNBC	Breast Ca type	ER	PR	HER2	Stage	Grade
390	34	BRCA1	del exons 1–19 (>64,761 bp)	Yes	Yes	Yes	No	Yes	IDC	Neg	Neg	Neg	2	III
389	39	BRCA1	C61Y	Yes	Yes	No	No	Yes	IDC	Neg	Neg	Neg	1	II
179	49	BRCA1	C64G	No	Yes	No	No	Unk	Br nos					
372	34	BRCA1	943ins10	Yes	Yes	No	No	No	IDC	Pos	Neg	Neg	1	III
227	34	BRCA1	943ins10	Yes	Unk	Unk	No	Yes	IDC	Neg	Neg	Neg	1	III
391	33	BRCA1	Q491X	Yes	Yes	Yes	No	Yes	IDC	Neg	Neg	Neg	1	
285	37	BRCA1	1759delATAA	Yes	No	No	No	Yes	IDC	Neg	Neg	Neg	2	II
347	34	BRCA1	1832delAGAAT	Yes	Yes	No	Ov 46	No	IDC	Pos	Neg		2	III
343	43	BRCA1	1832delAGAAT	Yes	Yes	No	No	Unk	Br nos					
393	48	BRCA1	3347delAG	No	Yes	No	No	Yes	IDC	Neg	Neg	Neg	2	III
201	42	BRCA1	S1212X	Yes	Yes	No	No	Unk	Br nos					
411	38	BRCA1	E1222X	Yes	Yes	No	No	Yes	IDC	Neg	Neg	Neg	1	III
427	37	BRCA1	K1290X	Yes	Unk	Unk	No	Unk	Br nos					
338	28	BRCA1	3883insA	Yes	Yes	No	No	No	IDC	Pos	Pos	Neg	2	III
452	37	BRCA1	3890delGGinsC	Yes	No	No	No	Yes	IDC	Neg	Neg	Neg	3	III
141	34	BRCA1	Q1604X	Yes	Unk	Unk	No	Unk	Br nos	Neg	Neg		2	III
255	46	BRCA1	del ex17 (3,118 bp)	No	Yes	No	No	Yes	IDC	Neg	Neg	Neg	1	III
251	43	BRCA1	5296delGAAA	Yes	Yes	Yes	No	Unk	Br nos					
345	38	BRCA1	5296delGAAA	Yes	Yes	Yes	No	Unk	IDC				3	III
380	29	BRCA1	5296delGAAA	Yes	Yes	No	Ov 49	Unk	Br nos					
409	24	BRCA1	5296delGAAA	Yes	Yes	No	Ov 45	Unk	IDC				1	
234	29	BRCA1	5296delGAAA	Yes	No	No	No	No	IDC	Pos	Neg	Neg	2	III
123	40	BRCA1	5312(ivs19)(+1)G>A	Yes	No	Yes	No	Yes	IDC	Neg	Neg	Neg	2	III
180	35	BRCA1	R1751X	Yes	Yes	No	No	Unk	Br nos					
344	29	BRCA1	R1751X	Yes	Yes	No	No	Yes	IDC	Neg	Neg	Neg	3	III
186	39	BRCA1	M1775R	Yes	Yes	Yes	No	Unk	Br nos					
473	60	BRCA1	IVS23+1G>A	No	Yes	No	No	Yes	IDC	Neg	Neg	Neg	1	II
476	31	BRCA1	IVS23+1G>A	Yes	No	No	No	No	IDC	Pos	Neg	Neg	1	III
487	59	BRCA1	del (ex 24+3UTR)	No	Yes	No	No	No	IDC	Neg	Neg	Pos	3	III
386	46	BRCA2	746delG	No	Yes	No	No	No	IDC	Pos				
407	38	BRCA2	886delGT	Yes	Yes	Yes	No	Unk	Br nos					
455	53	BRCA2	886delGT	No	Yes	No	No	Yes	Br nos					
340	40	BRCA2	1433delG	Yes	No	No	No	Yes	IDC	Neg	Neg	Neg	3	II
272	54	BRCA2	2808delA	No	Yes	No	No	Yes	IDC	Neg	Neg	Neg	1	III
260	44	BRCA2	2808delAAAC	Yes	Unk	Unk	No	Yes	IDC	Neg	Neg	Neg	4	III
335	33	BRCA2	2816insA	Yes	Yes	Yes	No	No	IDC	Pos	Pos		2	III
273	35	BRCA2	3034delAAAC	Yes	yes	No	No	Yes	IDC	Neg	Neg	Neg	3	III
397	42	BRCA2	3036del4	Yes	Yes	Yes	No	No	IDC	Pos	Pos	Neg	2	II
157	21	BRCA2	K944X	Yes	No	No	No	Unk	IDC					
441	66	BRCA2	4355del4	No	Yes	No	No	No	IDC	Pos	Pos	Neg	1	III
447	38	BRCA2	4682del4	Yes	Yes	No	No	Yes	Br nos	Neg	Neg	Neg		
432	34	BRCA2	4959delA	Yes	Yes	Yes	no	Yes	IDC	Neg	Neg	Neg	1	III
600	51	BRCA2	5579insA	No	Yes	No	No	Unk	Br nos					
504	43	BRCA2	5844del5	Yes	Yes	No	No	Yes	IDC	Neg	Neg	Neg	2	III
454	36	BRCA2	5844del5	Yes	Unk	Unk	No	No	IDC	Pos	Neg	Neg	2	II

**Table 2** continued

Patient BROCA ID	Age dx	Gene	Mutation (BRCA1 U14680; BRCA2 U43746)	Br dx <45	FH Br <60	FH Ov	Self Br+Ov	TNBC	Breast Ca type	ER	PR	HER2	Stage	Grade
497	52	BRCA2	5920delG	No	Yes	Yes	No	No	IDC	Pos	Pos	Neg		
110	35	BRCA2	5946delCT	Yes	Yes	No	No	Yes	IDC	Neg	Neg	Neg	3	III
290	40	BRCA2	7297delCT	Yes	Unk	Unk	No	Yes	IDC	Neg	Neg	Neg	1	III
450	39	BRCA2	R2520X	Yes	Yes	No	No	No	IDC	Pos	Pos	Neg	2	III
342	51	BRCA2	7795delCT	No	Yes	No	No	No	Br nos	Pos	Pos		1	
439	54	BRCA2	8529delT	No	Yes	No	Ov 48	Unk	Br nos	Neg				
276	31	BRCA2	R3128X*	Yes	No	No	No	No	IDC	Pos	Pos	Pos		
185	51	ATM	c.6404insTT	No	Unk	Unk	No	Yes	IDC	Neg	Neg	Neg	1	II
318	29	ATM	Y2755X	Yes	No	No	No	No	IDC	Pos	Pos		3	
307	58	ATM	del ex62–63 (9,769 bp)	No	Yes	No	No	No	IDC	Pos	Pos	Pos	1	III
448	52	BARD1	S551X	No	Yes	No	No	No	IDC	Pos	Pos	Neg	2	III
312	34	CHEK2	R160G***	Yes	Yes	No	No	No	IDC	Pos	Pos		3	III
262	35	CHEK2	H186R	Yes	No	No	No	No	IDC	Pos	Pos	Neg	1	II
101	33	CHEK2	G210R homozygous	Yes	Unk	Unk	No	Yes	IDC	Neg	Neg	Neg	3	III
187	45	PALB2	758insT	No	Yes	No	no	No	IDC	Pos	Pos	Neg	2	II
155	36	PALB2	1479delC	Yes	Yes	No	No	No	IDC	Pos	Pos	Neg	2	III
191	60	PALB2	3048delT	No	No	No	No	Yes	IDC	Neg	Neg	Neg	3	II
333	35	PTEN	R335X	Yes	No	No	No	Yes	IDC	Neg	Neg	Neg	2	II
248	43	PTEN	c.1027(ivs8)(-1)G>T	Yes	Yes	No	No	No	IDC	Pos			1	
446	32	TP53	P278A	Yes	Unk	Unk	No	No	IDC	Neg	Neg	Pos	2	II

\* This patient also carried an ATM (p.R248X) mutation; \*\* This patient also carried a ATM p.R457X mutation

ovarian cancer risk in women of African ancestry. It is well recognized that prevalence of mutations in both genes among breast cancer patients vary by ethnicity, study inclusion criteria, and mutation detection techniques [27]. In this study, we found 10.0 % (29 out of 289) of cases carried pathogenic *BRCA1* mutations and 8.0 % (23 out of 289) of cases are *BRCA2*-positive. The prevalence of recurrent *BRCA1* and *BRCA2* mutations among selected breast cancer patients are higher in founder populations such as Ashkenazi Jewish populations where a relatively cost-effective strategy of testing for three common mutations in *BRCA1* and *BRCA2* genes currently exists [25, 28, 29]. In a study examining *BRCA1*, *BRCA2*, *CHEK2*, and *TP53* in US high-risk families (95 % were of European ancestry), 9.6 and 6.5 % of probands were found to be *BRCA1* and *BRCA2* mutation carriers, and, mutation prevalence increased to 13.2 % (*BRCA1*) and 7.4 % (*BRCA2*, *CHEK2* and *TP53*) among patients <49 years old, respectively [30]. We have previously reported high mutation rates among clinic-based cohorts of women of African ancestry with early-onset breast cancer (<45 years) [25]. In a population-based study from North California Breast Cancer Family Registry, *BRCA1* mutation prevalence were 1.3, 2.2 and 3.5 % in African American, non-Hispanic and Hispanic patients overall but

16.7 % in African American cases diagnosed under the age of 35 years old [28]. Among 46,276 subjects (78.3 % Western European ancestry and 3.8 % African ancestry) tested by Myriad Genetics, Inc., *BRCA1* and *BRCA2* mutation prevalence was 10.2 and 5.7 % in the African ancestry group, respectively versus 6.9 and 5.2 % in populations of Western European ancestry [31]. In a genetic counseling TNBC cohort, *BRCA1* and *BRCA2* mutation prevalence has been reported to differ by populations [32]; 50 % in Ashkenazi Jewish women, 33.3 % in Caucasian women, and 20.4 % in African American women which is consistent with what we found in this study. A limitation of our study is that there is significant genetic diversity in the African Diaspora and results from our single institution study are not generalizable to the general population. Larger studies using next-generation sequencing in diverse populations are needed to derive true estimates of the burden of inherited breast cancer in underserved and understudied populations.

Next-generation sequencing assays promise to accelerate progress in screening for somatic as well as inherited genetic mutations among cancer patients. Several laboratories now offer genomic testing with reasonable turn around times, but the clinical utility of these cancer gene panels remain in question. BRCAplus, a high-risk breast

cancer diagnostic assay, includes six breast cancer genes: *BRCA1*, *BRCA2*, *TP53*, *PTEN*, *STK11*, and *CDH1* [33] and has been evaluated in more than 3,000 clinical samples referred to Ambry Genetics for mutation detection. The result showed that *BRCA1* and *BRCA2* mutations were found in 5.7 % of the patients and contributed 85 % of all the mutations identified. Another 27 gene targeted sequencing panel has also been evaluated in 708 hereditary breast and/or ovarian cancer patients [34]. In total, 109 germline mutations were detected, among them, 37 in *BRCA1*, 32 in *BRCA2*, 4 in *TP53*, and 36 in other genes. Similarly, *BRCA1* and *BRCA2* mutations accounted for around 60 % of the positive test results. Using BROCA in this study, 80 % of the cases had mutations in *BRCA1* and *BRCA2* while mutations in *PALB2*, *CHEK2*, *BARD1*, *ATM*, *PTEN*, and *TP53* were found in the remaining 20 %. Given the diversity in ethnic populations, mutation detection methods, breast cancer genes included, degree of disease risk, sample size, and so on, the estimate of the prevalence of mutations in the rapidly expanding number of cancer susceptibility genes will also vary. Nonetheless, these massively parallel sequencing panels indeed prove their useful implementation in clinics and confirm that genes involved in DNA repair pathways, including *BRCA1* and *BRCA2* are the major genes contributing to inherited breast and ovarian cancer in African American populations [10].

The limited information about family history of cancer in several of these patients reflects a general problem. Of patients meeting selection criteria other than family history (i.e., selected for young age at diagnosis or TNBC), 48 % (64/133) had very limited information about cancers in previous generations of their families. This problem likely reflects historical disparities in access to medical care and strongly suggests that criteria other than family history should be widely recognized by clinicians and sufficient for referral for genomic testing. As has been demonstrated in Ashkenazi Jewish women and women of European ancestry [32], our results suggest that African American women with young age at onset, family history of breast or ovarian cancer, or TNBC could benefit very substantially from genomic testing. Identifying inherited mutations in breast cancer patients is important for at least three reasons. Patients with inherited mutations in genes involved in DNA repair pathways may be treated with synthetic lethal therapeutic approaches based on poly-ADP-ribose polymerase inhibitors (PARPi) [35–37]. In addition, women with inherited mutations in *BRCA1*, *BRCA2*, and *PALB2* should be advised about risk-reducing salpingo-oophorectomy [38]. Furthermore, each breast cancer patient with inherited disease represents a family including as-yet-unaffected sisters and daughters and nieces for whom genetic knowledge can empower cancer prevention. Increased focus on cancer control and

prevention to improve population health has the potential to reduce cost of care for advanced disease in underserved populations.

Despite its limitation as a single institution study, this study speaks to a fundamental problem of disparities in breast cancer outcomes among African American women. African American breast cancer patients are more likely than breast cancer patients of other ancestries to be affected at a young age; to develop aggressive breast cancers; and to die from their disease [1, 3, 4]. It is also known that patients with inherited mutations in *BRCA1* are more likely to be diagnosed at a young age [5, 6]. The distribution of genetic and non-genetic risk factors for breast cancer is not uniform across populations and there is significant heterogeneity in breast cancer. It is possible that one potential contributor to the higher incidence of aggressive early-onset breast cancer among African American patients is a higher burden of previously understudied inherited mutations in breast cancer susceptibility genes. In order to test this hypothesis rigorously, taking into consideration the significant genetic diversity in the African Diaspora, it would be necessary to evaluate large, population-based cohorts of breast cancer patients of African and other ancestries, including both comprehensive genomic analysis and thorough characterization of patient history, tumor characteristics, treatment, and survival. Our study addressed the converse question: the frequency of mutations given family history or young-onset aggressive breast cancer. We suggest that the mutation frequency of approximately 25 % among African American patients with these features underscores the need for larger studies among women from diverse populations.

Heretofore, high cost and insurance coverage has been a major barrier to incorporating genomic testing into clinical cancer care. Targeted capture and multiplexed sequencing offers the capacity to sequence multiple relevant genes at once, in a single test, rather than having to order sequential genetic tests at a cost of thousands of dollars per gene. The cost of genetic testing for breast cancer predisposition should drop quickly in a competitive marketplace, allowing more equitable access. Diffusion of genetic technologies in diverse populations has the potential to accelerate precision medicine, improve cancer prevention, lower costs of cancer care, and reduce disparities in health outcomes.

**Acknowledgments** We thank the patients for their participation in this study. This work was supported by the National Institutes of Health grants R01CA157744, R01CA89085, P50CA125183, and K12CA139160; and by the Breast Cancer Research Foundation, the Ralph and Marion Falk Medical Research Trust, and Susan G. Komen for the Cure. M.C.K and O.I.O. are American Cancer Society Professors.

**Conflict of interest** The authors declare that they have no conflicts of interest.

**Open Access** This article is distributed under the terms of the Creative Commons Attribution Noncommercial License which permits any noncommercial use, distribution, and reproduction in any medium, provided the original author(s) and the source are credited.

## References

- Carey LA, Perou CM, Livasy CA, Dressler LG, Cowan D, Conway K, Karaca G, Troester MA, Tse CK, Edmiston S, Deming SL, Geradts J, Cheang MC, Nielsen TO, Moorman PG, Earp HS, Millikan RC (2006) Race, breast cancer subtypes, and survival in the Carolina breast cancer Study. *JAMA* 295(21):2492–2502
- Cheang MC, Voduc D, Bajdik C, Leung S, McKinney S, Chia SK, Perou CM, Nielsen TO (2008) Basal-like breast cancer defined by five biomarkers has superior prognostic value than triple-negative phenotype. *Clin Cancer Res* 14(5):1368–1376
- DeSantis C, Ma J, Bryan L, Jemal A (2014) Breast cancer statistics, 2013. *CA Cancer J Clin* 64(1):52–62
- Menashe I, Anderson WF, Jatoi I, Rosenberg PS (2009) Underlying causes of the black-white racial disparity in breast cancer mortality: a population-based analysis. *J Natl Cancer Inst* 101(14):993–1000
- Atchley DP, Albarracín CT, Lopez A, Valero V, Amos CI, Gonzalez-Angulo AM, Hortobagyi GN, Arun BK (2008) Clinical and pathologic characteristics of patients with *BRCA*-positive and *BRCA*-negative breast cancer. *J Clin Oncol* 26(26):4282–4288
- Foulkes WD, Smith IE, Reis-Filho JS (2010) Triple-negative breast cancer. *N Engl J Med* 363(20):1938–1948
- Fackenthal JD, Zhang J, Zhang B, Zheng Y, Hagos F, Burrill DR, Niu Q, Huo D, Sveen WE, Ogundiran T, Adebamowo C, Odetunde A, Falusi AG, Olopade OI (2012) High prevalence of *BRCA1* and *BRCA2* mutations in unselected Nigerian breast cancer patients. *Int J Cancer* 131(5):1114–1123
- Akbari MR, Donenberg T, Lunn J, Curling D, Turnquest T, Krill-Jackson E, Zhang S, Narod SA, Hurley J (2014) The spectrum of *BRCA1* and *BRCA2* mutations in breast cancer patients in the Bahamas. *Clin Genet* 85(1):64–67. doi:10.1111/cge.12132
- Tischkowitz M, Capanu M, Sabbaghian N, Li L, Liang X, Vallee MP, Tavtigian SV, Concannon P, Foulkes WD, Bernstein L, Bernstein JL, Begg CB (2012) Rare germline mutations in *PALB2* and breast cancer risk: a population-based study. *Hum Mutat* 33(4):674–680
- Antoniou AC, Casadei S, Heikkinen T, Barrowdale D, Pylkas K, Roberts J, Lee A, Subramanian D, De Leeneer K, Fostira F, Tomiak E, Neuhausen SL, Teo ZL, Khan S, Aittomaki K, Moilanen JS, Turnbull C, Seal S, Mannermaa A, Kallioniemi A, Lindeman GJ, Buys SS, Andrulis IL, Radice P, Tondini C, Manoukian S, Toland AE, Miron P, Weitzel JN, Domchek SM, Poppe B, Claes KB, Yannoukakis D, Concannon P, Bernstein JL, James PA, Easton DF, Goldgar DE, Hopper JL, Rahman N, Peterlongo P, Nevanlinna H, King MC, Couch FJ, Southey MC, Winqvist R, Foulkes WD, Tischkowitz M (2014) Breast-cancer risk in families with mutations in *PALB2*. *N Engl J Med* 371(6):497–506
- Weischer M, Nordestgaard BG, Pharoah P, Bolla MK, Nevanlinna H, Van't Veer LJ, Garcia-Closas M, Hopper JL, Hall P, Andrulis IL, Devilee P, Fasching PA, Anton-Culver H, Lambrechts D, Hoening M, Cox A, Giles GG, Burwinkel B, Lindblom A, Couch FJ, Mannermaa A, Alnaes GG, John EM, Dork T, Flyger H, Dunning AM, Wang Q, Muranen TA, van Hien R, Figueroa J, Southey MC, Czene K, Knight JA, Tollenaar RA, Beckmann MW, Ziogas A, Christiaens MR, Collee JM, Reed MW, Severi G, Marme F, Margolin S, Olson JE, Kosma VM, Kristensen VN, Miron A, Bogdanova N, Shah M, Blomqvist C, Broeks A, Sherman M, Phillips KA, Li J, Liu J, Glendon G, Seynaeve C, Ekici AB, Leunen K, Krieger M, Cross SS, Baglietto L, Sohn C, Wang X, Kataja V, Borresen-Dale AL, Meyer A, Easton DF, Schmidt MK, Bojesen SE (2012) *CHEK2*\*1100delC heterozygosity in women with breast cancer associated with early death, breast cancer-specific death, and increased risk of a second breast cancer. *J Clin Oncol* 30(35):4308–4316
- Goldgar DE, Healey S, Dowty JG, Da Silva L, Chen X, Spurdle AB, Terry MB, Daly MJ, Buys SM, Southey MC, Andrulis I, John EM, Khanna KK, Hopper JL, Oefner PJ, Lakhani S, Chenevix-Trench G (2011) Rare variants in the *ATM* gene and risk of breast cancer. *Breast Cancer Res* 13(4):R73
- Renwick A, Thompson D, Seal S, Kelly P, Chagtai T, Ahmed M, North B, Jayatilake H, Barfoot R, Spanova K, McGuffog L, Evans DG, Eccles D, Easton DF, Stratton MR, Rahman N (2006) *ATM* mutations that cause ataxia-telangiectasia are breast cancer susceptibility alleles. *Nat Genet* 38(8):873–875
- Tavtigian SV, Oefner PJ, Babikyan D, Hartmann A, Healey S, Le Calvez-Kelm F, Lesueur F, Byrnes GB, Chuang SC, Forey N, Feuchtinger C, Gioia L, Hall J, Hashibe M, Herte B, McKay-Chopin S, Thomas A, Vallee MP, Voegelé C, Webb PM, Whiteman DC, Sangrajrang S, Hopper JL, Southey MC, Andrulis IL, John EM, Chenevix-Trench G (2009) Rare, evolutionarily unlikely missense substitutions in *ATM* confer increased risk of breast cancer. *Am J Hum Genet* 85(4):427–446
- Thompson D, Duedal S, Kirner J, McGuffog L, Last J, Reiman A, Byrd P, Taylor M, Easton DF (2005) Cancer risks and mortality in heterozygous *ATM* mutation carriers. *J Natl Cancer Inst* 97(11):813–822
- Baer R (2013) Luring *BRCA1* to the scene of the crime. *Cancer Cell* 23(5):565–567
- Brzovic PS, Keffe JR, Nishikawa H, Miyamoto K, Fox D 3rd, Fukuda M, Ohta T, Klevit R (2003) Binding and recognition in the assembly of an active *BRCA1/BARD1* ubiquitin-ligase complex. *Proc Natl Acad Sci USA* 100(10):5646–5651
- Meindl A, Hellebrand H, Wiek C, Erven V, Wappenschmidt B, Niederacher D, Freund M, Lichtner P, Hartmann L, Schaal H, Ramser J, Honisch E, Kubisch C, Wichmann HE, Kast K, Deissler H, Engel C, Müller-Myhsok B, Neveling K, Kiechle M, Mathew CG, Schindler D, Schmutzler RK, Hanenberg H (2010) Germline mutations in breast and ovarian cancer pedigrees establish *RAD51C* as a human cancer susceptibility gene. *Nat Genet* 42(5):410–414
- Loveday C, Turnbull C, Ramsay E, Hughes D, Ruark E, Frankum JR, Bowden G, Kalmyrzaev B, Warren-Perry M, Snape K, Adlard JW, Barwell J, Berg J, Brady AF, Brewer C, Brice G, Chapman C, Cook J, Davidson R, Donaldson A, Douglas F, Greenhalgh L, Henderson A, Izatt L, Kumar A, Lalloo F, Miedzybrodzka Z, Morrison PJ, Paterson J, Porteous M, Rogers MT, Shanley S, Walker L, Eccles D, Evans DG, Renwick A, Seal S, Lord CJ, Ashworth A, Reis-Filho JS, Antoniou AC, Rahman N (2011) Germline mutations in *RAD51D* confer susceptibility to ovarian cancer. *Nat Genet* 43(9):879–882
- Gonzalez KD, Noltner KA, Buzin CH, Gu D, Wen-Fong CY, Nguyen VQ, Han JH, Lowstuter K, Longmate J, Sommer SS, Weitzel JN (2009) Beyond Li Fraumeni syndrome: clinical characteristics of families with p53 germline mutations. *J Clin Oncol* 27(8):1250–1256
- Tan MH, Mester JL, Ngeow J, Rybicki LA, Orloff MS, Eng C (2012) Lifetime cancer risks in individuals with germline *PTEN* mutations. *Clin Cancer Res* 18(2):400–407
- Walsh T, Casadei S, Lee MK, Pennil CC, Nord AS, Thornton AM, Roeb W, Agnew KJ, Stray SM, Wickramanayake A, Norquist B, Pennington KP, Garcia RL, King MC, Swisher EM (2011) Mutations in 12 genes for inherited ovarian, fallopian



- tube, and peritoneal carcinoma identified by massively parallel sequencing. *Proc Natl Acad Sci USA* 108(44):18032–18037
23. Walsh T, Lee MK, Casadei S, Thornton AM, Stray SM, Pennil C, Nord AS, Mandell JB, Swisher EM, King MC (2010) Detection of inherited mutations for breast and ovarian cancer using genomic capture and massively parallel sequencing. *Proc Natl Acad Sci USA* 107(28):12629–12633
  24. Nord AS, Lee M, King MC, Walsh T (2011) Accurate and exact CNV identification from targeted high-throughput sequence data. *BMC Genom* 12:184
  25. Nanda R, Schumm LP, Cummings S, Fackenthal JD, Sveen L, Ademuyiwa F, Cobleigh M, Esserman L, Lindor NM, Neuhausen SL, Olopade OI (2005) Genetic testing in an ethnically diverse cohort of high-risk women: a comparative analysis of *BRCA1* and *BRCA2* mutations in American families of European and African ancestry. *JAMA* 294(15):1925–1933
  26. Zheng Y, Zhang J, Niu Q, Huo D, Olopade OI (2012) Novel germline *PALB2* truncating mutations in African American breast cancer patients. *Cancer* 118(5):1362–1370
  27. Fackenthal JD, Olopade OI (2007) Breast cancer risk associated with *BRCA1* and *BRCA2* in diverse populations. *Nat Rev Cancer* 7(12):937–948
  28. John EM, Miron A, Gong G, Phipps AI, Felberg A, Li FP, West DW, Whittemore AS (2007) Prevalence of pathogenic *BRCA1* mutation carriers in 5 US racial/ethnic groups. *JAMA* 298(24):2869–2876
  29. Malone KE, Daling JR, Doody DR, Hsu L, Bernstein L, Coates RJ, Marchbanks PA, Simon MS, McDonald JA, Norman SA, Strom BL, Burkman RT, Ursin G, Deapen D, Weiss LK, Folger S, Madeoy JJ, Friedrichsen DM, Suter NM, Humphrey MC, Spirtas R, Ostrander EA (2006) Prevalence and predictors of *BRCA1* and *BRCA2* mutations in a population-based study of breast cancer in white and black American women ages 35–64 years. *Cancer Res* 66(16):8297–8308
  30. Walsh T, Casadei S, Coats KH, Swisher E, Stray SM, Higgins J, Roach KC, Mandell J, Lee MK, Ciernikova S, Foretova L, Soucek P, King MC (2006) Spectrum of mutations in *BRCA1*, *BRCA2*, *CHEK2*, and *TP53* in families at high risk of breast cancer. *JAMA* 295(12):1379–1388
  31. Hall MJ, Reid JE, Burbidge LA, Pruss D, Deffenbaugh AM, Frye C, Wenstrup RJ, Ward BE, Scholl TA, Noll WW (2009) *BRCA1* and *BRCA2* mutations in women of different ethnicities undergoing testing for hereditary breast-ovarian cancer. *Cancer* 115(10):2222–2233
  32. Greenup R, Buchanan A, Lorizio W, Rhoads K, Chan S, Leedom T, King R, McLennan J, Crawford B, Marcom PK, Hwang ES (2013) Prevalence of *BRCA* mutations among women with triple-negative breast cancer (TNBC) in a genetic counseling cohort. *Ann Surg Oncol* 20(10):3254–3258
  33. Chong HK, Wang T, Lu HM, Seidler S, Lu H, Keiles S, Chao EC, Stuenkel AJ, Li X, Elliott AM (2014) The validation and clinical implementation of BRCAplus: a comprehensive high-risk breast cancer diagnostic assay. *PLOS One* 9(5):e97408
  34. Castera L, Krieger S, Rousselin A, Legros A, Baumann JJ, Bruet O, Brault B, Fouillet R, Goardon N, Letac O, Baert-Desurmont S, Tinat J, Bera O, Dugast C, Berthet P, Polycarpe F, Layet V, Hardouin A, Frebourg T, Vaur D (2014) Next-generation sequencing for the diagnosis of hereditary breast and ovarian cancer using genomic capture targeting multiple candidate genes. *Eur J Hum Genet*. doi:10.1038/ejhg.2014.16
  35. Ashworth A (2008) A synthetic lethal therapeutic approach: poly(ADP-ribose) polymerase inhibitors for the treatment of cancers deficient in DNA double-strand break repair. *J Clin Oncol* 26(22):3785–3790
  36. Fong PC, Boss DS, Yap TA, Tutt A, Wu P, Mergui-Roelvink M, Mortimer P, Swaisland H, Lau A, O'Connor MJ, Ashworth A, Carmichael J, Kaye SB, Schellens JH, de Bono JS (2009) Inhibition of poly(ADP-ribose) polymerase in tumors from *BRCA* mutation carriers. *N Engl J Med* 361(2):123–134
  37. Tutt A, Robson M, Garber JE, Domchek SM, Audeh MW, Weitzel JN, Friedlander M, Arun B, Loman N, Schmutzler RK, Wardley A, Mitchell G, Earl H, Wickens M, Carmichael J (2010) Oral poly(ADP-ribose) polymerase inhibitor olaparib in patients with *BRCA1* or *BRCA2* mutations and advanced breast cancer: a proof-of-concept trial. *Lancet* 376(9737):235–244
  38. Finch AP, Lubinski J, Moller P, Singer CF, Karlan B, Senter L, Rosen B, Maehle L, Ghadirian P, Cybulski C, Huzarski T, Eisen A, Foulkes WD, Kim-Sing C, Ainsworth P, Tung N, Lynch HT, Neuhausen S, Metcalfe KA, Thompson I, Murphy J, Sun P, Narod SA (2014) Impact of oophorectomy on cancer incidence and mortality in women with a *BRCA1* or *BRCA2* mutation. *J Clin Oncol* 32(15):1547–1553