Prevalence of Inherited Mutations in Breast Cancer Predisposition Genes among Women in Uganda and Cameroon

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ABSTRACT

Background: Sub-Saharan Africa (SSA) has a high proportion of premenopausal hormone receptor negative breast cancer. Previous studies reported a strikingly high prevalence of germline mutations in *BRCA1* and *BRCA2* among Nigerian patients with breast cancer. It is unknown if this exists in other SSA countries.

Methods: Breast cancer cases, unselected for age at diagnosis and family history, were recruited from tertiary hospitals in Kampala, Uganda and Yaoundé, Cameroon. Controls were women without breast cancer recruited from the same hospitals and age-matched to cases. A multigene sequencing panel was used to test for germline mutations.

Results: There were 196 cases and 185 controls with a mean age of 46.2 and 46.6 years for cases and controls, respectively. Among cases, 15.8% carried a pathogenic or likely pathogenic mutation in a

breast cancer susceptibility gene: 5.6% in *BRCA1*, 5.6% in *BRCA2*, 1.5% in *ATM*, 1% in *PALB2*, 0.5% in *BARD1*, 0.5% in *CDH1*, and 0.5% in *TP53*. Among controls, 1.6% carried a mutation in one of these genes. Cases were 11-fold more likely to carry a mutation compared with controls (OR = 11.34; 95% confidence interval, 3.44–59.06; $P < 0.001$). The mean age of cases with *BRCA1* mutations was 38.3 years compared with 46.7 years among other cases without such mutations ($P = 0.03$).

Conclusions: Our findings replicate the earlier report of a high proportion of mutations in *BRCA1/2* among patients with symptomatic breast cancer in SSA.

Impact: Given the high burden of inherited breast cancer in SSA countries, genetic risk assessment could be integrated into national cancer control plans.

Introduction

The discovery of susceptibility genes for common cancers has remarkably advanced the care of individuals with hereditary cancers and their families. Perhaps the most studied and most clearly understood are the mutational profile of the *BRCA1* and *BRCA2* genes and their role in the management of breast cancer. The lifetime risk of breast and ovarian cancer among *BRCA1* mutation carriers is 57% to 65% and 20% to 50%, respectively; whereas for *BRCA2*, the risks are 35% to 57% and 5% to 23%, respectively (1, 2). Healthy carriers of damaging mutations in high penetrance genes such as *BRCA1* and *BRCA2* genes now have the opportunity for more intensive surveil-

lance for early detection, and could potentially benefit from interventions for primary prevention such as risk reducing surgeries or chemoprevention with tamoxifen (3, 4). Those diagnosed with cancer also benefit from personalized management of their cancer and interventions to reduce second primary cancers (5, 6).

The prevalence of damaging mutations in *BRCA1* and *BRCA2* in patients with breast cancer varies by study design and the composition of early-onset cases, cases with strong family history, or a particular cancer subtype, such as triple-negative breast cancer (TNBC). Mutation frequency is relatively high (15%–55%) among breast cancer cases and families evaluated in cancer risk clinic settings where patients with strong family history are more likely to be referred for risk assessment (7–13). A case series of young patients with breast cancer (but unselected for family history) found 5.9% for *BRCA* mutation prevalence among women younger than 36 years (14), whereas a prevalence of 11.2% was recently reported in women with TNBC (15) and a 23% mutation frequency was reported in young Mexican women with TNBC (16).

Population-based studies, in which breast cancer patients were recruited regardless of age at diagnosis or family history, gave estimates of *BRCA* mutation prevalence lower than those reported in cancer-risk clinic settings. For example, Malone and colleagues reported a 4.7% *BRCA1/2* mutation prevalence in patients ages 35 to 64 years (17), Newman and colleagues reported a 3.3% *BRCA1* mutation frequency in patients younger than 75 years (18), and John and colleagues reported a 2.2% *BRCA1* mutation frequency in non-Hispanic white patients younger than 65 years (19). It is well-documented that Ashkenazi Jews have high frequencies of deleterious founder mutations in *BRCA1* and *BRCA2*, with >9% mutation frequency in unselected breast cancer cases (17, 19, 20). In cancer-free individuals, Ashkenazi Jews had *BRCA1/2* combined frequencies above 2% (20, 21), in contrast to 0.6% in the general population (22, 23).

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It is reported that African Americans had slightly lower proportion of *BRCA1/2* mutation compared with European Americans. Malone and colleagues (17) reported that 4.0% of African American patients had a mutation in *BRCA1* or *BRCA2*, compared with 5.0% in European Americans. John and colleagues (19) found a 1.3% *BRCA1* mutation frequency among African Americans compared with 2.2% among non-Hispanic whites, but young (<35 years) African Americans had a high *BRCA1* mutation frequency (16.7%).

Breast cancer mortality rate is highest in Sub-Saharan Africa (SSA) in part due to early onset and aggressive disease, poor health infrastructure, and lack of access to diagnostics and modern cancer medicines (24–26). The recent advances in cancer genetics and genomics hold great promise for global oncology and could be harnessed to improve cancer outcomes among indigenous Africans. Yet, to date, little is known about the genetic susceptibility for breast cancer among native African women. We have previously reported high proportions of *BRCA1* (7.1%) and *BRCA2* (3.9%) mutations among indigenous Nigerian women with breast cancer unselected for age of cancer onset and family history of the disease (27). Recently, we expanded the study using a multi-gene panel on 1,136 cases and 997 controls and found similarly high frequencies of 7.0% and 4.1% for deleterious mutations in *BRCA1* and *BRCA2*, respectively (28). It is unknown if this finding also holds in other SSA countries. Therefore, we examined the burden of inherited breast cancer and the spectrum of germline mutations in breast cancer susceptibility genes using a case–control study in Cameroon and Uganda.

Materials and Methods

Study participants

This study is part of the African Breast Cancer Study—a multi-country epidemiological study on breast cancer risk factors among indigenous African women that began in Nigeria in 1998 and was expanded to Cameroon and Uganda in 2011. Details of the study design and procedures have been reported in previous publications (29, 30). Breast cancer cases ages 18 years or older were recruited at the breast and endocrine unit in the department of surgery of the Mulago Hospital in Kampala, Uganda, and the department of medical oncology of Yaounde General Hospital in Yaounde, Cameroon. All consecutive cases between 2011 and 2015 were approached and enrolled, regardless of family history and age at onset of disease. In Cameroon, controls were women randomly recruited from the clinics of general medicine and obstetrics and gynecology departments at Yaounde General Hospital, frequency-matched to cases for age (within 5-year-age category) and ethnicity. In Uganda, female controls were randomly recruited from the general outpatient clinics and surgical ward admissions at Mulago Hospital, frequency-matched to cases for age (within 5-year-age category) and ethnicity. At both sites, controls were unselected for their medical conditions (except that no clinically known breast cancer) and they were not relatives of cases. The study protocol was reviewed by the institutional review boards of the two study sites and the University of Chicago. All study participants provided written informed consent prior to their interview.

Gene selection and panel sequencing

A 30-gene hereditary cancer risk panel developed by Color Genomics was used for variant detection. Twelve known and candidate breast cancer genes in the panel were included in this study: *ATM, BARD1, BRCA1, BRCA2, BRIP1, CDH1, CHEK2, NBN, PALB2, PTEN,* *STK11,* and *TP53*. These genes were assessed for variants within coding exons and noncanonical splice regions. High molecular weight genomic DNA was extracted from whole blood and rigorous quality control was conducted. Next-generation sequencing (NGS) procedures were performed at the Color laboratory under Clinical Laboratory Improvements Amendments (no. 05D2081492) and College of American Pathologists (no. 8975161) compliance. NGS library preparation was performed using the Kapa HyperPlus Library Preparation Kit (Kapa Biosciences), and target enrichment was performed using Agilent SureSelect XT probes (Agilent). Sequencing was performed on an Illumina NextSeq 500/550 instrument (Illumina) for 150 bp paired-end sequencing (31).

NGS variant calling

Sequence reads were aligned against human reference genome GRCh37.p12 with the Burrows-Wheeler Aligner (BWA-MEM; ref. 32), and duplicate and low-quality reads were removed. Single-nucleotide variants (SNV) and small (2–50 bp) insertions and deletions (indels) were called using the GATK HaplotypeCaller module (33), and large (>50 bp) structural variants (SV) were detected on the basis of read-depth and using dedicated split-read based algorithms (34) at the Color laboratory. A no template control and two positive controls containing a set of known variants were concurrently run within every batch of samples (31). The NGS coverage requirements for reporting were ≥20× for each base of the reportable range and ≥50× for 99% of the reportable range. Median coverage was achieved at 200 to 300×. In parallel, FASTQ files were transferred to University of Chicago (UChicago) through Globus Online (35, 36) and germline variants were identified using the ConVarCal analysis toolkit in Globus genomics platform (37, 38). The consensus candidate variants were independently called by the Color and UChicago teams that were blinded to

Table 1. Characteristics of women with breast cancer and controls in Uganda and Cameroon.

Variable	Cases (*n* = 196) Mean (SD)	Controls (*n* = 185) Mean (SD)
Age (years)	46.2 (12.4)	46.6 (11.5)
Height (cm)	162.6 (7.6)	163.2 (7.3)
Weight (kg)	68.8 (14.2)	72.4 (13.5)
Body mass index (kg/m²)	25.9 (5.2)	27.2 (5.0)
Lifetime breastfeeding duration (months)	70.8 (62.3)	62.3 (45.9)
Age at menarche (years)	14.4 (1.5)	14.4(1.6)
Number of live births	4.2 (2.8)	4.3 (2.7)
Age at first live birth (years)	22.4 (5.6)	22.1 (5.6)
	n (%)	
Country and ethnicity[a]		
Cameroon	91 (46.4)	101 (54.6)
Cameroonian Bantu	49 (25.5)	56 (30.4)
Cameroonian Semi-Bantu	34 (17.7)	43 (23.4)
Other Cameroon	8 (4.2)	2 (1.1)
Uganda	101 (53.6)	83 (45.1)
Bagandan	43 (22.4)	41 (22.3)
Other Ugandan	58 (30.2)	42 (22.8)
Family history of breast cancer	13 (6.6)	16 (8.7)
Lifetime alcohol use	96 (49.0)	100 (58.4)
Hormonal contraceptive use	73 (38.0)	87 (48.3)
Premenopausal	131 (66.8)	124 (67.0)
Benign breast disease	27 (13.8)	12 (6.5)

[a]Five respondents gave "Don't know" responses and were excluded.

the phenotypes of the subjects. The variants were reviewed, discussed, and later classified as variants of uncertain significance (VUS), likely pathogenic, or pathogenic according to the American College of Medical Genetics and Genomics 2015 guidelines, based on criteria that evaluate molecular structural effect, computational prediction, experimental functional study, clinical findings, and population data (39). All variant classifications were approved by an American Board of Medical Genetics and Genomics board-certified medical geneticist at the Color laboratory.

Statistical analysis

Data were analyzed using frequencies and Chi-square tests. OR and exact 95% confidence interval (CI) were calculated to indicate the strength of association between germline mutation and breast cancer risk. The t test was used to compare age at breast cancer diagnosis between patients with and without a mutation, and the Fisher exact test was used to compare mutation frequency between patients with and without family history of breast cancer. Two-sided P value <0.05 was considered statistically significant.

Results

The study included 381 study participants with 196 breast cancer cases and 185 controls. Of these, 187 were enrolled in Uganda and 194 in Cameroon. The mean age of cases and controls was 46.2 and 46.6 years, respectively. Summary statistics for breast cancer risk factors are shown in **Table 1**. Of all 135 variants (34 P/LP mutations and 101 VUS) identified in the 12 genes, the majority were SNVs (119, found in 104 women), 15 indels (one per woman), and 1 SV. Thirty-four P/LP mutations were found in 34 women (one mutation per woman), including 31 cases (15.8%) and 3 controls (1.6%). Of the 34 P/LP mutations, there were 18 SNVs, 15 indels, and 1 SV; among them 13 and 11 mutations were found in *BRCA1* and *BRCA2*, respectively (**Fig. 1**).

Among breast cancer cases, most P/LP mutations were found in *BRCA1* ($n = 11$, 5.6%) and *BRCA2* ($n = 11$, 5.6%), followed by three (1.5%) in *ATM*, two (1%) in *PALB2*, and one each in *BARD1, CDH1, TP53,* and *CHEK2*. Three controls had P/LP mutations in *BRCA1* ($n = 2$, 1.1%) and *BARD1* ($n = 1$, 0.5%; **Table 2**). There was a strong
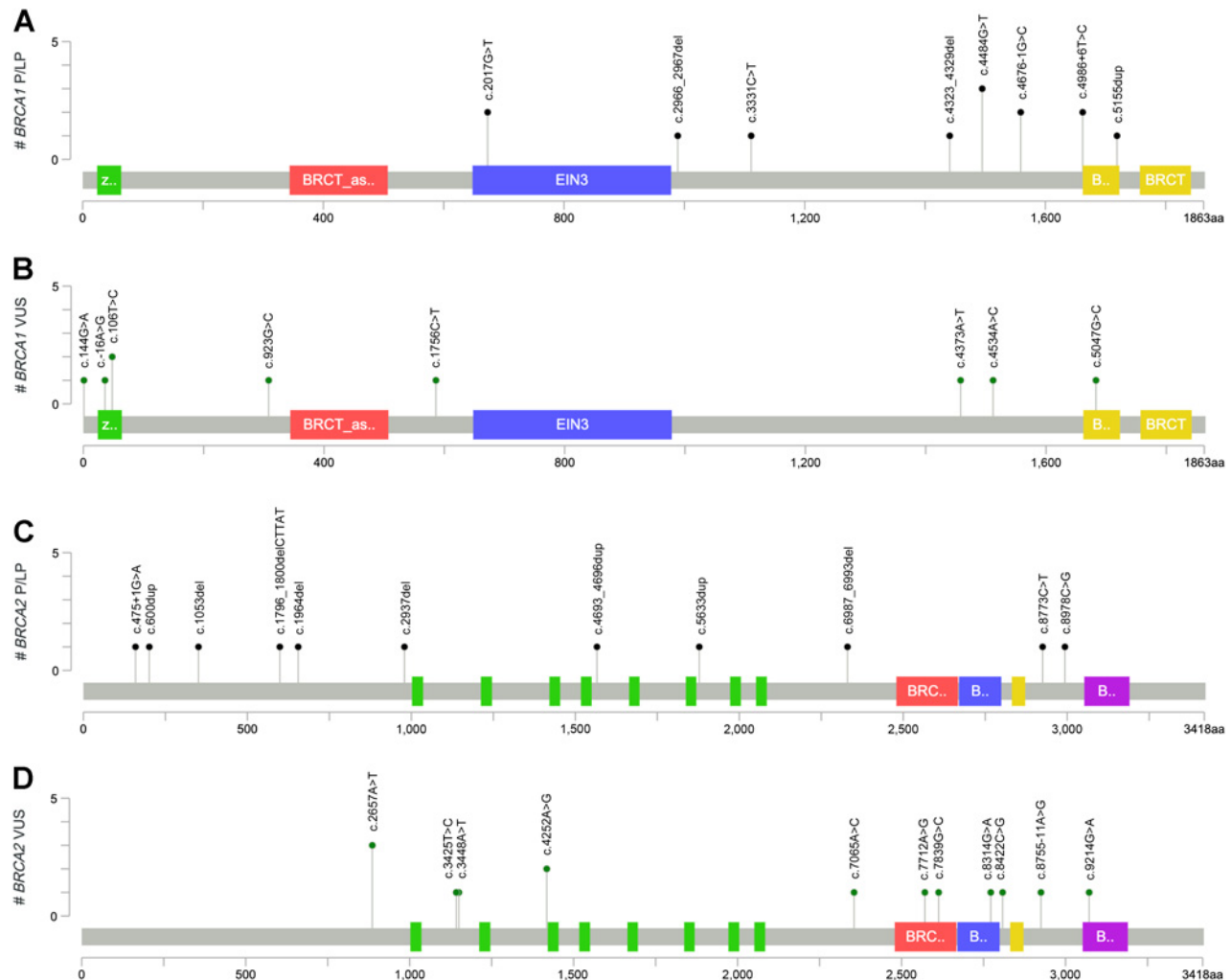

Figure 1.
Deleterious mutations and VUS in *BRCA1* and *BRCA2* genes. Distribution of *BRCA1* P/LP (**A**), *BRCA1* VUS (**B**), *BRCA2* P/LP (**C**), and *BRCA2* VUS (**D**). Variants are displayed along the protein. Length of vertical lines reflects the number of events.

Table 2. Frequency of deleterious mutations in genes among women in Uganda and Cameroon.

Gene	Cases, frequency (%)	Controls, frequency (%)
Overall	**(n = 196)**	**(n = 185)**
BRCA1	11 (5.6)	2 (1.1)
BRCA2	11 (5.6)	0
PALB2	2 (1.0)	0
TP53	1 (0.5)	0
ATM	3 (1.5)	0
CDH1	1 (0.5)	0
CHEK2	1 (0.5)	0
BARD1	1 (0.5)	1 (0.5)
Total	**31 (15.8)**	**3 (1.6)**
Cameroon	**(n = 93)**	**(n = 101)**
BRCA1	8 (8.6)	2 (2.0)
BRCA2	4 (4.3)	0
PALB2	1 (1.1)	0
TP53	0	0
ATM	2 (2.2)	0
CDH1	1 (1.1)	0
CHEK2	1 (1.1)	0
BARD1	0	1 (1.0)
Total	**17 (18.3)**	**3 (3.0)**
Uganda	**(n = 103)**	**(n = 84)**
BRCA1	3 (2.9)	0
BRCA2	7 (6.8)	0
PALB2	1 (1.0)	0
TP53	1 (1.0)	0
ATM	1 (1.0)	0
CDH1	0	0
CHEK2	0	0
BARD1	1 (1.0)	0
Total	**14 (13.6)**	**0**

Table 3. Associations between carrying pathogenic or likely pathogenic mutations with breast cancer risk, family history of breast cancer, and age at diagnosis.

	Mutation, frequency (%)	OR (95% CI)	P value
P/LP mutation in BRCA1/2			
Cases (n = 196)	22 (11.2)	11.6 (2.8–102.5)	<0.001
Controls (n = 185)	2 (1.1)	1.0 (ref.)	
P/LP mutation in any breast cancer gene			
Cases (n = 196)	31 (15.8)	11.4 (3.4–59.0)	<0.001
Controls (n = 185)	3 (1.6)	1.0 (ref.)	
P/LP mutation in any breast cancer gene			
Cases with FH of breast cancer (n = 13)	4 (30.8)		0.13
Cases without FH of breast cancer (n = 183)	27 (14.8)		
	Mean (SD)		***P* value**
Age at breast cancer diagnosis			
Cases with BRCA1 P/LP mutation (n = 11)	38.3 (10.6)		0.03
Cases without BRCA1 P/LP mutation (n = 185)	46.7 (12.4)		

Abbreviations: FH, family history; LP, likely pathogenic; P, pathogenic.

association between carrying a P/LP mutation in any breast cancer gene and breast cancer risk (OR = 11.4; 95% CI, 3.4–59.0; P < 0.001), and also for mutations in either BRCA1 or BRCA2 (OR = 11.6; 95% CI, 2.8–102.5; P < 0.001; **Table 3**). The mean age of breast cancer cases with P/LP BRCA1 mutations was 38.3 years compared with 46.7 years among other cases without such mutations (P = 0.03). Of the 13 cases with a positive family history, four (30.8%) had a mutation in breast cancer susceptibility genes, compared with 27 of 183 (14.8%) cases without a family history of breast cancer (P = 0.13).

Table 4 shows the spectrum of pathogenic or likely pathogenic mutations. There was a SV (deletion of exon 2) in BARD1. Recurrent mutations were found in BRCA1 (c.4484G>T, three cases; c.2017G>T, one case and one control; c.4676-1G>C, one case and one control; c.4986+6T>C, two cases), and ATM (c.7913G>A, two cases). Novel mutations in BRCA1 among our sample were c.2966_2967del, c.4323_4329del, and in BRCA2 were c.1053del, c.1964del, c.2937del, c.4693_4694dup, c.5633dup, c600dup, and c.6987_6993del. The BRCA1 mutation c.1796_1800delCTTAT was reported in our most recent study among Nigerian women (28). The TP53 variant, c.818G>A, had been reported in the 1,000 Genomes database among European populations (Supplementary Table S1).

As would be expected, there were 101 VUS in breast cancer genes found in 96 individuals (25.2%), of which 53 were cases and 43 were controls. Nine VUS in BRCA1 were found in seven cases (3.6%) and two controls (1.1%), whereas in BRCA2, 14 VUS were found in seven cases (3.6%) and seven controls (3.8%). The 72 unique VUS

in breast cancer genes and their frequency of occurrence are shown in **Table 5**. VUS found among women in both Cameroon and Uganda were ATM (c.4082A>G, c.131A>G), BARD1 (c.1067A>T), and PALB2 (c.365A>G). There were no scenarios where individuals with VUS in BRCA1 or BRCA2 also had pathogenic mutations in these genes. However, women with some VUS in ATM, BARD1, CDH1, CHEK2, and NBN also had deleterious mutations in the same or other genes (**Table 5**). Most of the VUS were not reported in the 1,000 Genomes database, except the VUS in BRCA1 (c.923G>C), BRCA2 (c.7712A>G), ATM (c.4082A>G, c.131A>G), BARD1 (c.1067A>T, c.764A>G, c.155G>A) were found in African populations, ATM c.8071C>T was found only in European populations and BARD1 c.155G>A was found only in American populations of the same database (Supplementary Table S1).

Discussion

This study has shown a high prevalence (11.2%) of mutations in the BRCA1 (5.6%) and BRCA2 genes (5.6%) among women with breast cancer in Uganda and Cameroon, which is similar to our previous report of 11.1% (7.0% in BRCA1 and 4.1% in BRCA2) among Nigerian women (28). In addition, we found mutations in other breast cancer susceptibility genes, giving an overall mutation frequency of 15.8% among patients with breast cancer in Uganda and Cameroon. In addition, there was a high VUS rate which underscores the need for expanded research to resolve the clinical significance of these variants.

The comparable high mutation frequency found among women across three SSA countries suggests a significant burden of heritable risk factors across these countries. Population-based studies such as Malone and colleagues (17) reported mutation frequencies of 2.4% and 2.3% in BRCA1 and BRCA2, respectively, in African Americans, whereas John and colleagues (2007) found a mutation frequency of 1.3% in BRCA1 among African American breast cancer cases. Possibly,

Table 4. Spectrum of pathogenic or likely pathogenic mutations in breast cancer susceptibility genes among women in Uganda and Cameroon[a].

Gene	Nucleotide change (number of occurrences)	Protein change	Age[b]	Ethnicity	Family history	Status	Previous reports[c]
BRCA1	c.2017G>T (2)	p.Glu673*	20	Semi-Bantu	No	Case	USA
			47	Semi-Bantu	Yes	Control	
	c.2966_2967del (1)	p.Phe989Cysfs*2	27	Baganda	No	Case	None
	c.3331C>T (1)	p.Gln1111*	34	Other Uganda	No	Case	Singapore, USA
	c.4323_4329del (1)	p.Asp1441Glufs*13	45	Baganda	No	Case	None
	c.4484G>T (3)	p.Arg1495Met	35	Bantu	No	Case	France, Brazil, Canada, Italy, Portugal, USA
			36	Bantu	No	Case	
			37	Bantu	No	Case	
	c.4676-1G>C (2)		40	Bantu	No	Case	Clinvar
			46	Bantu	No	Control	
	c.4986+6T>C (2)		33	Other Cameroon	No	Case	Peru, Germany, USA, Canada, France
			56	Other Cameroon	Yes	Case	
	c.5155dup (1)	p.Val1719Glyfs*6	52	Semi-Bantu	No	Case	Germany
BRCA2	c.1053del (1)	p.Lys351Asnfs*16	51	Other Uganda	No	Case	None
	c.1796_1800delCTTAT (1)	p.Ser599*	28	Other Uganda	No	Case	Italy, Holland, Sweden, UK, USA, Australia, Canada, France, Germany
	c.1964del (1)	p.Pro655Glnfs*5	44	Bantu	No	Case	Clinvar
	c.2937del (1)	p.Ile979Metfs*12	69	Semi-Bantu	Yes	Case	None
	c.4693_4696dup (1)	p.Thr1566Lysfs*10	55	Semi-Bantu	No	Case	None
	c.475+1G>A (1)		54	Other Uganda	No	Case	Italy, USA
	c.5633dup (1)	p.Asn1878Lysfs*4	60	Other Uganda	No	Case	None
	c.600dup (1)	p.Pro201Thrfs*5	52	Other Uganda	No	Case	None
	c.6987_6993del (1)	p.Ile2330Valfs*35	41	Other Uganda	No	Case	None
	c.8773C>T (1)	p.Gln2925*	26	Bantu	Yes	Case	Clinvar
	c.8978C>G (1)	p.Ser2993*	61	Other Uganda	No	Case	Clinvar
CDH1	c.2296-1G>A (1)		30	Bantu	Yes	Case	Clinvar
CHEK2	c.470T>C (1)	p.Ile157Thr	72	Cameroon, ethnicity missing	No	Case	Clinvar
PALB2	c.419del (1)	p.Lys140Serfs*37	31	Bantu	No	Case	None
	c.886dup (1)	p.Met296Asnfs*7	40	Baganda	No	Case	Clinvar
TP53	c.818G>A (1)	p.Arg273His	36	Baganda	No	Case	Clinvar
ATM	c.7913G>A (2)	p.Trp2638*	44	Semi-Bantu	No	Case	Clinvar
			49	Semi-Bantu	No	Case	
	c.8833_8834delCT (1)	p.Leu2945Valfs*10	35	Other Uganda	No	Case	Clinvar
BARD1	c.1573dup (1)	p.Ile525Asnfs*12	37	Bantu	No	Control	None
	deletion of exon 2 (1)		58	Other Uganda	No	Case	None

[a]Three mutations (c.4676-1G>C in BRCA1, c.475+1G>A in BRCA2, c.2296-1G>A in CDH1) are considered likely pathogenic, whereas others are considered pathogenic.
[b]Age at diagnosis (cases) or interview (controls) in years.
[c]Checked using three recent publications (10, 28, 52) and ClinVar database (http://www.clinvar.com/).

the relatively low prevalence of nongenetic risk factors for breast cancer in SSA could explain the higher mutation frequencies among sub-Saharan women. Indigenous African women are younger at the onset of breast cancer, and have a higher prevalence of nongenetic protective factors such as longer breastfeeding duration, late menarche, early onset of childbearing, and higher number of live births compared with women in developed countries (**Table 1**). This enrichment of heritable breast cancer provides a unique opportunity to develop genetic risk prediction models for breast cancer unique to African ancestry groups and to identify new causal variants for breast cancer that may be targeted for interventions to reduce risk among women of African ancestry.

The consistently high BRCA1 and BRCA2 mutation frequencies found among SSA women with breast cancer has significant implications for cancer interventions. The first is the introduction of low-cost genetic testing among women in low resource settings. DNA sequencing cost is significantly reduced, and genetic counseling and testing services are now a feasible option in low resource settings such as SSA.

However, to our knowledge, there are no guidelines in SSA concerning when BRCA testing should be offered and healthy high-risk women continue to die from preventable cancers. Replication of our data from Nigeria in Uganda and Cameroon makes our results more generalizable for Africans than all studies previously primarily conducted among women of European ancestry. Ongoing efforts to integrate genomic testing for population risk stratification as a way to accelerate progress in eradicating breast and ovarian cancers as causes of premature mortality in SSA women should be supported. Improved access to genetic counseling and testing services and interventions to reduce risk are clearly warranted. National governments in SSA can leap frog by adopting technological advances in cancer genetics and genomics to develop demand and market for cancer prevention services. Many of breast cancers in SSA are TNBC, a cancer subtype that is curable when optimal chemotherapy is used in the early stages but become highly resistant and refractory to treatment in advanced stages. Expanding global access to life saving cancer medicines and clinical trials of PARP inhibitors and immunotherapy-based therapies

Table 5. Variants of unknown significance in breast cancer susceptibility genes among women in Uganda and Cameroon.

Gene	Nucleotide position	Country	Number of occurrence	Protein change	Co-occurrence with pathogenic allele
BRCA1	c.4373A>T	Cameroon	1	p.Gln1458Leu	
	c.1756C>T	Cameroon	1	p.Pro586Ser	
	c.144G>A	Both in Uganda	2	p.Met48Ile	
	c.923G>C	Uganda	1	p.Ser308Thr	
	c.106T>C	Uganda	1	p.Ser36Pro	
	c.4534A>C	Uganda	1	p.Ser1512Arg	
	c.5047G>C	Uganda	1	p.Glu1683Gln	
	c.-16A>G	Uganda	1		
BRCA2	c.7839G>C	Cameroon	1	p.Lys2613Asn	
	c.7712A>G	Uganda	1	p.Glu2571Gly	
	c.7065A>C	Cameroon	1	p.Glu2355Asp	
	c.2657A>T	All in Uganda	3	p.Asn886Ile	
	c.4252A>G	Both in Uganda	2	p.Ile1418Val	
	c.3448A>T	Uganda	1	p.Thr1150Ser	
	c.8422C>G	Uganda	1	p.Leu2808Val	
	c.8314G>A	Uganda	1	p.Glu2772Lys	
	c.8755-11A>G	Uganda	1	IVS21-11A>G	
	c.9214G>A	Uganda	1	p.Val3072Met	
	c.3425T>C	Uganda	1	p.Phe1142Ser	
ATM	c.3743A>G	Cameroon	1	p.Tyr1248Cys	
	c.4082A>G	4 in Cameroon, 1 in Uganda	5	p.Gln1361Arg	
	c.6343G>A	Cameroon	1	p.Val2115Ile	
	c.5660C>T	Both in Cameroon	2	p.Ala1887Val	
	c.3560C>G	Cameroon	1	p.Pro1187Arg	BRCA2 c.8773C>T
	c.131A>G	1 in Cameroon, 7 in Uganda	8	p.Asp44Gly	
	c.3978C>A	Cameroon	1	p.Asn1326Lys	
	c.7552C>T	Cameroon	1	p.Pro2518Ser	
	c.3031A>G	Cameroon	1	p.Thr1011Ala	
	c.3035G>A	Uganda	1	p.Arg1012Lys	
	c.5972A>G	Both in Uganda	2	p.Glu1991Gly	
	c.2150G>A	Uganda	1	p.Arg717Gln	
	c.8071C>T	Uganda	1	p.Arg2691Cys	
	c.2771G>A	Uganda	1	p.Arg924Gln	
	c.4329C>A	Uganda	1	p.His1443Gln	
	c.492G>T	Uganda	1	p.Trp164Cys	
	c.4894A>G	Uganda	1	p.Met1632Val	
	c.268A>G	Uganda	1	p.Arg90Gly	
	c.6543G>T	Cameroon	1	p.Glu2181Asp	
BARD1	c.1718T>C	Cameroon	1	p.Ile573Thr	
	c.2296T>C	Cameroon	1	p.Cys766Arg	
	c.1993G>A	Cameroon	1	p.Glu665Lys	
	c.1067A>T	1 in Cameroon, 1 in Uganda	2	p.Asn356Ile	
	c.764A>G	Cameroon	1	p.Asn255Ser	
	c.155G>A	Cameroon	1	p.Arg52His	
	c.1148T>A	Both in Uganda	2	p.Met383Lys	BRCA2 c.8978C>G
	c.1439T>C	Uganda	1	p.Leu480Ser	
	c.188T>C	Uganda	1	p.Leu63Ser	
BRIP1	c.2803G>T	Cameroon	1	p.Val935Leu	
	c.2867C>T	Both in Cameroon	2	p.Ser956Leu	
	c.628C>T	Uganda	1	p.Pro210Ser	
	c.854A>G	Both in Uganda	2	p.His285Arg	
CDH1	c.377C>T	Cameroon	1	p.Pro126Leu	
	c.225C>G	Both in Cameroon	2	p.Phe75Leu	
	c.1996A>C	Cameroon	1	p.Asn666His	CDH1 c.2296-1G>A
	c.1961C>T	Cameroon	1	p.Pro654Leu	
	c.1136C>T	Uganda	1	p.Thr379Met	
	c.2254G>A	Uganda	1	p.Val752Ile	
	c.865G>A	Uganda	1	p.Ala289Thr	
CHEK2	c.164C>T	Cameroon	1	p.Ser55Phe	BRCA2 c.4693_4696dup
	c.1169A>G	Uganda	1	p.Tyr390Cys	

(Continued on the following page)

Table 5. Variants of unknown significance in breast cancer susceptibility genes among women in Uganda and Cameroon. (Cont'd)

Gene	Nucleotide position	Country	Number of occurrence	Protein change	Co-occurrence with pathogenic allele
NBN	c.1481A>C	Cameroon	1	p.Gln494Pro	
	c.1711A>G	All in Uganda	3	p.Lys571Glu	ATM c.8833_8834delCT
PALB2	c.949A>C	Both in Cameroon	2	p.Thr317Pro	
	c.3211T>C	Cameroon	1	p.Phe1071Leu	
	c.365A>G	2 in Cameroon, 2 in Uganda	4	p.Asp122Gly	
	c.821C>T	Uganda	1	p.Thr274Ile	
	c.610T>A	Uganda	1	p.Ser204Thr	
PTEN	c.*13C>T	Uganda	1		
	c.-19C>G	Uganda	1		
STK11	c.1229C>T	Cameroon	1	p.Ala410Val	
	c.1253G>C	Uganda	1	p.Cys418Ser	
TP53	c.1120G>C	Both in Uganda	2	p.Gly374Arg	

that have shown considerable promise among patients with aggressive young onset breast cancer would promote health equity and accelerate research to understand the genomic basis of treatment resistance in diverse populations (6, 40).

Notwithstanding the high mutation prevalence in *BRCA1/2*, the penetrances of *BRCA1/2* in SSA populations are unknown. Previous studies have shown variations in *BRCA1/2* penetrance based on geographic location (41), so it is equally important to estimate penetrances of *BRCA1/2* for better risk assessment and counseling in SSA populations. In addition, the psychosocial consequences and social implications of *BRCA1/2* mutations in the African context have not been studied. More work is needed to develop culturally tailored interventions that promote adoption of genomic testing for comprehensive risk assessment and prevention.

Our finding that the majority of women with deleterious mutations in *BRCA* genes had no family history of breast cancer has been previously reported (27, 28, 42). Importantly, clinicians should be aware that the absence of a family history of breast cancer does not preclude the presence of deleterious *BRCA* mutations (41). At the same time, it is noteworthy that family history reports in SSA may be less reliable than in developed countries given the low literacy rate, low cancer awareness, and poor utilization of health care services resulting in underreporting. Other explanations for low family history reports include death from other causes at earlier ages due to lower life expectancies and poor ascertainment of cancer as a cause of death. It is also conceivable that there are other genetic and nongenetic modifiers of risk that modulate the penetrance of pathogenic mutations and VUS identified in this study.

An appreciable number of mutations in *BRCA1* and *BRCA2* found in this study had not been previously reported, whereas recurrent mutations were only found in a few women. This finding is consistent with previous studies among SSA women (27, 43), suggesting that targeting selected *BRCA1/2* mutations with founder effect may not be a good strategy for genetic testing.

We performed panel testing and found deleterious mutations in other breast cancer susceptibility genes in 4.5% of women, supporting the use of panel testing of multiple genes. Although this is an efficient strategy in well-established laboratories, the penetrance of pathogenic mutations in moderate susceptibility genes such as *ATM, CHEK2*, and *BARD1*, the spectrum of cancer risk, and clinical utility of testing for these genes are less well understood (44). More rigorous evaluation will be needed before clinical guidelines for mutation carriers in these increasingly important moderate susceptibility genes can be devel-

oped. Also, the relatively high VUS frequencies found in this study represent a major clinical conundrum (45) because of the diversity or normal variations in African Genomes that have been understudied. VUS has been reported by several studies among African ancestry women focusing predominantly on early onset or TNBC (27, 46–50), although lower frequencies have been reported in others (51). This underscores the need for larger genomic sequencing studies in Africans.

The limitations of this study include the relatively small sample size and the lack of data on hormone receptor status that would have allowed the evaluation of mutation prevalence by breast cancer subtype. It is noteworthy that the wide confidence intervals around the OR estimates are a consequence of the small sample size and low mutation frequency among controls and thus they should be interpreted with caution.

In conclusion, our findings confirm the earlier report of a high proportion of deleterious mutations in *BRCA1* and *BRCA2* among patients with breast cancer in SSA. As most of these women present with advanced breast cancer, there is an urgent need to improve access to genetic testing in national cancer control plans in SSA.

Disclosure of Potential Conflicts of Interest

A.Y. Zhou is Head of Research at Color Genomics. R.K. Madduri is Chief Technology Officer at and has ownership interest (including patents) in Navipoint Genomics LLC. O.I. Olopade reports receiving other commercial research support from Color Genomics Foundation, has ownership interest (including patents) in CancerIQ and Tempus, and is a consultant/advisory board member for Healthy Life For All Foundation. No potential conflicts of interest were disclosed by the other authors.

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