Screening of germline mutations in young Rwandan patients with breast cancers

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Abstract

Background: In Sub-Saharan Africa breast cancer is commonly detected at younger age and the profile is more aggressive with a high mortality rate compared to the European countries. It is suggested that African-specific genetic background plays a key role in this matter. The present study aimed at understanding the role of genetic factors in breast cancer development in young Rwandan.

Methods: We performed a massive parallel sequencing on Illumina MiSeq NGS system for the screening of 26 genes associated with hereditary breast cancer from 40 patients under 35 years old from two University Teaching Hospitals in Kigali, Rwanda. Sanger sequencing was used to confirm pathogenic and likely pathogenic mutations.

Results: Five patients out of 40 (12.5%) presented with pathogenic mutations including four patients (10%) carrying BRCA1 or BRCA2 pathogenic variants. One patient showed a missense likely pathogenic TP53 variant. We have also detected additional missense, intronic, and 3’UTR variants of unknown significance in all study participants.

Conclusion: This preliminary study suggests that the frequency of germline mutations in young Rwandan patients with breast cancer is similar to the observations made in Caucasians. However, further large studies including patients and controls are needed to better understand the impact of genetic factors as well as the environmental risk factors in the development of breast cancer in young Rwandans.

KEYWORDS
BRCA, breast cancer, NGS, Rwanda, young patients
1 | INTRODUCTION

Breast cancer (BC) is a common cause of mortality among women worldwide. In individuals under 40 years old, it is however considered to be rare as it affects less than 7% of patients (Brinton, Sherman, Carreon, & Anderson, 2008).

Although its incidence in African ancestry individuals is still lower compared to other ethnic groups, the mortality is rather higher. Indeed, breast cancer in sub-Saharan Africa is characterized by a younger age at diagnosis (Adebamowo et al., 2003; Fregene et al., 2005; Adesunkanmi, Lawal, Adelusola, & Durosimi, 2006). Compared to other age groups, BC in young people (YBC) has a worse prognosis due its advanced stage at diagnosis and a high proportion of hormone negativity subtype (Anders et al., 2008; Bharat, Aft, Gao, & Margenthaler, 2009; Colzani et al., 2011).

About 5%–10% of BC are caused by germline mutations. To date, inherited mutations associated with breast cancer risk have been identified in several genes. Those genes have been associated with different levels of risk of breast cancer ranging from high, moderate to low risk. High-risk genes include BRCA1 (OMIM: 113705), BRCA2 (OMIM: 600185), and TP53 (OMIM: 191170) and confer a lifetime relative risk of more than five. BRCA1 and BRCA2 are two major genes associated with a lifetime risk of 50%–80% of breast cancer. Other genes have been associated with a two to fivefold increase risk of breast cancer. These include genes that are involved in DNA breaks repair by homologous recombination such as PALB2 (OMIM: 610355), ATM (OMIM: 607585), and CHEK2 (OMIM: 604373; Wittersheim, Büttner, & Markiefka, 2015).

The cause of breast cancer associated with a high mortality rate in young African are still not well understood and remain understudied. A common hypothesis is that the YBC may be linked to African-specific genetic characteristics (Haffty et al., 2009; Rummel, Lovejoy, Shriver, & Ellsworth, 2017).

Few studies have been conducted in Africa to determine the role of genetic factors in development of BC in general, and in young patients in particular. The majority of those studies restricted their investigations to the screening of mutations in BRCA1 and BRCA2 genes (Abbad et al., 2018).

In Rwanda, genetic risk factors, incidence, and mortality rate of BC are not known. A recent study conducted at Butaro cancer center of excellence, it was reported that the median age at diagnosis of breast cancer was 49 and 32/144(22%) patients were below the age of 40 (Pace et al., 2015).

The determination of genetic variations associated with the occurrence of BC as well as genetic modifiers leading to the disease variability are necessary for accurate detection, prevention, and treatment.

We undertook this study to determine the germline mutations associated with BC disease in young Rwandan patients.

2 | MATERIALS AND METHODS

2.1 | Ethical compliance

This study was conducted in accordance with the Declaration of Helsinki and the approval of the Institutional Review Board (IRB) of the College of Medicine and Health Sciences (CMHS) at University of Rwanda (No: 156/CMHS IRB/2016) as well as the ethical committee of each of hospitals: Kigali University Teaching Hospital (CHUK) Clinical Research Ethical Committee (Ref.: EC/CHUK/089/2016) and Rwanda Military Hospital (RMH) Research Ethical Committee (Ref.: EC/RMH/051/2016).

All adult patients and parents of minor patients (under 21 years old) signed a written informed consent prior to enrollment.

2.2 | Study participants

Patients were recruited between April 2016 and March 2018 from the two main public Hospitals in Kigali CHUK and RMH. These two hospitals receive patients from all parts of the country. To be eligible for this study, patients had to be diagnosed with BC before the age of 35. Forty patients consented to participate in the study. Clinical information as well as data on family history of cancer were collected from patient’s medical records. Venous blood samples were collected during their routine hospital visits.

2.3 | DNA extraction

Whole blood samples were collected in EDTA tubes and stored at –20°C until use. Genomic DNA was extracted from 200 μL of whole blood samples using the QIAamp® Blood DNA Mini Kit (Qiagen) according to the manufacturer's manual. The quality of the isolated DNA was assessed using NanoDrop Spectrophotometry ensuring the ratio of $A_{260\ nm}/A_{280\ nm} \approx 2$.

2.4 | Next-generation sequencing and variant call

Libraries for next-generation DNA sequencing were constructed from 75 ng of the isolated DNA for each sample using BRCA Hereditary Cancer MASTR Plus kit (BRCA HC MASTR Plus; MR-0320.024, Agilent) following the manufacturer’s instructions. This kit is designed for the identification of single nucleotide variants (SNVs), insertions and deletions (indels), and copy number variations (CNVs) within the 26 following genes: BRCA1, BRCA2,
Briefly, targeted DNA regions made up by all coding exons and flanking intronic regions of the 26 genes, were first amplified in multiplex polymerase chain reaction (PCR)—fiveplexes per patient—using BRCA HC MASTR Plus (Agilent), then barcoded with specific molecular identifiers (MIDs) and ligated with adaptors using MID Dx (MID ML-2208.240) for Illumina Miseq® sequencer according to the manufacturer's instructions. Amplicons were pooled together and purified using AMPure XP Agencourt beads (Beckman Coulter Inc, USA) prior to sequencing. Sequencing was performed on Illumina Miseq NGS system using MiSeq Reagent Kit v3 (paired-end; 600-cycles; 2x 300 ++pb; MS-102-3003-Illumina) according to the manufacturer's instructions. Data were analyzed using MASTR reporter software v 1.0.2 (Agilent) and verified using SeqPilot (Module SeqNext) software v.4.3.1. Both softwares align to hg19 reference genome: BRCA1 (NM_007294.4), BRCA2 (NM_000059.4), CHEK2 (NM_00105735.2), BARD1 (NM_000465.4), BRI1 (NM_032043.3), RAD51C (NM_058216.3), RAD51D (NM_001142571.2), MRE11A (NM_005591.4), RAD50 (NM_005732.4), NBN (NM_001024688.3), FAM175A (NM_139076.3), ATM (NM_000051.4), PALB2 (NM_024675.4), STK11 (NM_000455.5), MEN1 (NM_130802.2), PTEN (NM_000314.8), CDH1 (NM_004360.5), MUTYH (NM_001128425.2), BLM (NM_001287248.2), XRCC2 (NM_005431.2), MLH1 (NM_000249.4), MSH6 (NM_001179.3), PMS2 (NM_000357.7), MSH2: (NM_000251.3), and EPCAM(3′UTR); NM_002354.3. At least 98% of all amplicons were covered with a minimum depth of coverage of 40x. For each retained variant, the MASTR reporter software provided annotations including its genomic position, the nucleotide change and the predicted protein change. The impact and consequence on the protein product were predicted by the Ensembl Variant Effect Predictor (VEP) tool v.83.

Sanger sequencing on ABI 3130 using standard dideoxy termination procedure was performed for the validation of plausible pathogenic variants.

2.5 Variants classification and report

As there is no specific database for mutations in African population, the pathogenicity of variants was evaluated using two mutation databases: ClinVar (www.clinvar.com) and dbSNPs (www.ncbi.nlm.nih.gov/snp). The pathogenicity effect of the VUS were evaluated using different in silico mutation interpretation softwares such as SIFT (Sorting intolerant form tolerant); http://sift-dna.org; which predicts where an amino acid substitution is deleterious to protein function; PROVEAN (Protein variation effect analyzer) www.provean.jcvi.org which predicts the functional effect on protein sequence variations; and Mutation Taster www.mutationtaster.org which evaluate the disease-causing potential of DNA variants sequences.

Variants were classified into pathogenic, likely pathogenic, VUS, or likely benign/benign following the recommendation of Association of Molecular Pathology, American Society of clinical Oncology, and College of American Pathologists (AMP-ASCO-CAP; Li et al., 2017) and annotated according to the Human Genome Variation Society (HGVS) recommendations (den Dunnen et al., 2016).

3 RESULTS

3.1 Clinical characteristics of patients

Forty Rwandan breast cancer patients were enrolled in this study. They were selected based on the early age onset independently of family history of cancer. The mean age at diagnosis was 31.2 ± 3.6 years; ranging from 17 to 34 years. Histologically, 33 patients (82.5%) presented with invasive ductal carcinoma (IDC) tumors. Invasive carcinoma of both ductal and lobular and a sarcoma of the breast were present in one and two patients, respectively. Twenty-one patients (52.5%) were at stage three of tumor development. Twenty-three patients in our cohort (58.9%) had ER negative tumors and 14 (38.9%) had Her2 positive tumors. Seven patients (17.5%) had a triple negative subtype. Nineteen patients (22.5%) had a family history with the first or second-degree relatives (FDR or SDR) with ovarian or breast cancer (HBOC). The characteristics of patients, family history, and others risk factors are summarized in Table 1.

3.2 Mutational status

3.2.1 Pathogenic or likely pathogenic variants.

Data analysis revealed plausible pathogenic variants in five patients among 40 participants (12.5%) in three genes (BRCA1, BRCA2, and TP53) out of 26 contained in the
Four patients (10%) carried *BRCA1* or *BRCA2* pathogenic mutations: one had a *BRCA2:c.1300_1303del* p.(Lys434Glufs*25) mutation, the second had a *BRCA2:c.3720_3723del* p.(Phe1241Valfs*17) mutation, the third had a *BRCA1:c.9097dupA* p.(Thr3033Asnfs*11) while the fourth carried a *BRCA1:c.4065_4068del* p.(Asn1355Lysfs*10) mutation. A fifth patient had a missense likely pathogenic *TP53*: c.726C>G (p.Cys242Trp) mutation (Table 2). Sanger sequencing confirmed the five mutations (Supplementary file S1: mutation *BRCA2:c.1300_1303del*; Supplementary file S2: mutation *BRCA2:c.3720_3723del*; Supplementary file S3: mutation *BRCA1:c.9097dupA*).

**TABLE 1** Characteristics of 40 young patients.

| N = 40 |  |
|---|---|
| **Age at diagnostic** | **Mean±SD** |
| | 31.15 ± 3.6 |
| **Age at first menarche** | **Mean±SD** |
| | 14 ± 1.56 |
| **Parity** | **Number (n) %** |
| Laterality | Left | 18 | 45.0% |
| | Right | 21 | 52.5% |
| | Bilateral | 1 | 2.5% |
| **Histology** | **IDC** | 33 | 82.5% |
| | **ILC** | 2 | 5.0% |
| | **IDC&ILC** | 1 | 2.5% |
| | **Sarcoma** | 2 | 5.0% |
| | **Metaplastic** | 1 | 2.5% |
| | **Phyllodes** | 1 | 2.5% |
| **Stage** | I | 1 | 2.5% |
| | II | 15 | 37.5% |
| | III | 21 | 52.5% |
| | IV | 1 | 2.5% |
| **Lymph nodes involvement** | Yes | 24 | 64.86% |
| | No | 13 | 35.14% |
| | Missing | 3 |  |
| **ER status** | ER- | 23 | 58.97% |
| | ER+ | 16 | 41.03% |
| | Missing | 1 |  |
| **PR status** | PR- | 13 | 92.86% |
| | PR+ | 1 | 7.14% |
| | Missing | 26 |  |
| **Her2 status** | Her2- | 22 | 61.1% |
| | Her2+ | 14 | 38.9% |
| | Missing | 4 |  |
| **Triple negative subtype (TN)** | 7 | 17.5% |
| **FDR / SDR with HBOC** | 7 | 27% |
| **FDR / SDR with another type of cancer** | 3 | 12% |
| **FDR / SDR with both HBOC and another type cancer** | 2 | 8% |
| **No familial history of cancer** | 14 | 54% |

Abbreviations: DCIS, Ductal carcinoma in situ; ER, Estrogen receptor; FDR, First degree relative; HBOC, History of breast and ovarian cancer; Her2, human growth factor 2; IDC, invasive ductal carcinoma; ILC, Invasive lobular carcinoma; Med, median; P, Percentile; SDR, second degree relative.
3.2.2 Variants with unknown significance (VUS)

In total, 33 VUS were identified. Each patient carried at least one VUS and five patients had more than two VUS in the same or different genes. Twenty-nine VUS among them (87.8%) were unique in this cohort. Eight of those VUS were predicted by different prediction tools (SIFT, Provean, and MutationTaster) to have a damaging effect on protein and 13 variants were very rare and not previously observed in African population while five variants were simply novel (Table 3).

4 DISCUSSION

In the present study, we have sequenced genomic DNA from 40 Rwandan patients aged below 35 at the time of breast cancer diagnostic. Young age at diagnosis and African origin are both well known to be associated with an advanced stage of the disease at diagnostic, a high proportion of hormone receptor negative tumors, and a worse prognosis. The reasons of this different severity when compared to Caucasian populations are still poorly understood but are thought to be related to African-specific genetic characteristics (Adesunkanmi et al., 2006), and/or environmental factors (Fregene et al., 2005). We conducted this study to gain insights into relevant genetic variations in young Rwandan with breast cancer.

We observed an overall frequency of 5/40 (12.5%) pathogenic germline variants. Among them, 10% variants were detected in BRCA1 and BRCA2. This frequency was comparable with other high frequencies of pathogenic BRCA1/2 variants reported in other studies conducted in young Africans (Awadelkarim et al., 2007; Cherbal et al., 2010), Caucasians (Copson et al., 2018; De Sanjosé et al., 2003; Tonin et al., 2001), or African American women with breast cancers (Haffty et al., 2009; Malone et al., 2006; Table 4). The four pathogenic variants in BRCA1 and BRCA2 were well known in other populations (Heramb et al., 2018), or reported by ENIGMA.

https://clinvarminer.genetics.utah.edu/variants-by-submitter/504863/gene/BRCA2/pathogenic. In Africa, the pathogenic variant BRCA1: c.4065_4068del, observed in one patient of our cohort (33 aged), was previously observed in a 38-aged Algerian (Cherbal et al., 2010) and a 28-aged Sudanese (Awadelkarim et al., 2007) breast cancer patients. The patient of our cohort had a triple negative (TN) breast cancer subtype.

We identified less BRCA1 pathogenic variants compared to BRCA2 (25% vs. 75%) in this cohort. This is in consistence with results from other studies on breast cancer in patients of African ancestry where plausible causal variants in BRCA2

| Patient ID | Patient age | Tumor stage | Tumor subtype | Family history | Gene | Nucleotide change | Protein effect | Protein impact | rsID |
|------------|-------------|-------------|---------------|----------------|------|------------------|---------------|---------------|------|
| BC01       | 27          | II          | ER+PR+Her2-   | Two Aunts, 38 and 46 years, BC | BRCA2 | c.1300_1303del | p.(Lys434Glufs*25) | Frameshift | rs397507577 |
| BC05       | 34          | III         | ER+Her2-      | Sister, 42 years, BC | BRCA2 | c.3720_3723del | p.(Phe1241Valfs*17) | Frameshift | rs886038983 |
| BC40       | 34          | III         | ER-PR-Her2+   | Unknown | BRCA2 | C.9097dupA | p.(Thr3033Asnfs*11) | Frameshift | rs8075794719 |
| BC22       | 33          | II          | ER-PR-Her2-   | Unknown | BRCA1 | c.4065_4068del | p.(Asn1355Lysfs*10) | Frameshift | rs8085794598 |
| BC23       | 31          | III         | ER-PR-Her2+   | unknown | TP53 | c.726C>G | p.Cys242Trp | Missense | rs7557874539 |

Reference sequence BRCA1 (NM_007294.4).
Reference sequence BRCA2 (NM_000059.4).
Reference sequence: TP53 (NM_000546.6).
| Gene       | Reference sequence | Variant       | Protein effect | Coding impact | rsID          | No. carriers | GMAF       | MAF—Africa |
|------------|--------------------|---------------|----------------|---------------|---------------|--------------|------------|------------|
| ATM        | NM_000051.4        | c.4339A>C     | p.(Ser1447Arg) | missense      | NA            | 1            | NA         | NA         |
| ATM        | NM_000051.4        | c.2289 T > A  | p.(Phe763Leu)  | missense      | rs34231402    | 1            | 0.0005     | 0.002      |
| ATM        | NM_000051.4        | c.131A>G      | p.(Asp44Gly)   | missense      | rs150143957   | 1            | 0.00003    | 0.0005     |
| BARD1      | NM_000465.4        | c.1148 T > A  | p.(Met383Lys)  | missense      | rs763596413   | 1            | 0.000008   | 0          |
| BARD1      | NM_000465.4        | c.421C>T      | p.(Pro281Leu)  | missense      | NA            | 1            | NA         | NA         |
| BLM        | NM_001287248.2     | c.3879A>G     | p.(Glu1293=)   | synonymous    | rs28377085    | 1            | 0.00031    | 0.00314    |
| BLM        | NM_001287248.2     | c.1881 T > C  | p.Thr627       | synonymous    | rs14867829    | 1            | 0.00003    | 0.0002     |
| BRCA1      | NM_007294.4        | c.5411 T > C  | p.(Met1804 Thr)| missense      | rs55808233    | 1            | 0.0002     | 0.0018     |
| BRCA1      | NM_007294.4        | c._−16A>G     | 5'UTR Substitution | rs777262055 | 1 | 1.00E−05 | 3.00E−05 |
| BRCA2      | NM_000059.4        | c.7502A>G     | p.(Gln2501Arg) | missense      | NA            | 1            | NA         | NA         |
| BRIP1      | NM_032043.3        | c.778A>G      | p.(Thr260Ala)  | missense      | rs138743097   | 1            | 0.0004     | 0.002      |
| BRIP1      | NM_032043.3        | c.854A>G      | p.(His285Arg)  | missense      | rs141055990   | 1            | 0.0004     | 0.0002     |
| CDH1       | NM_004360.5        | c.1004G>A     | p.(Arg335Gln)  | missense      | rs73364873    | 1            | 0.00003    | NA         |
| CHEK2      | NM_001005735.2     | c.1298A>G     | p.(Ty413Cys)   | missense      | rs209928781   | 11           | 0.0000     | NA         |
| CHEK2      | NM_001005735.2     | c.1270A>G     | p.(Met424Val)  | missense      | rs375130261   | 3            | 0.00003    | NA         |
| EPCAM (3'UTR) | NM_002354.3     | c.78A>T       | 3'UTR substitution | rs56865134    | 1            | 0.0002     | 0.001      |
| MLH1       | NM_000249.4        | c.380+16C>G   | intronic       | rs121909452   | 1            | NA         | NA         |
| MLH1       | NM_000249.4        | c.1730C>T     | p.(Ser577Leu)  | missense      | rs56185292    | 1            | 0.00006    | 0          |
| MRE11A     | NM_005591.4        | c.256G>A      | p.(Asp86Asn)   | missense      | rs763902512   | 1            | 0.00001    | 0.00002    |
| MRE11A     | NM_005591.4        | c.2080-23A>G  | intronic substitution | rs142331797   | 1            | 0.0008     | 0.0002     |
| MSH2       | NM_000251.3        | c.1C>G        | 3'UTR substitution | rs114545543   | 1            | 0.0004     | 0.0009     |
| MSH2       | NM_000251.3        | c.301G>C      | p.(Glu101Gln)  | missense      | NA            | 1            | NA         | NA         |
| MUTYH      | NM_001128425.2     | c.217G>A      | p.(Glu73Lys)   | missense      | rs1064794128  | 1            | NA         | NA         |
| NBN        | NM_001024688.3     | c.1711A>G     | p.(Lys571Glu)  | missense      | rs587780090   | 1            | 0.00001    | 0          |
| NBN        | NM_001024688.3     | c.1354A>C     | p.(Thr452Pro)  | missense      | rs141137543   | 1            | 0.0005     | 0.0009     |
| PMS2       | NM_000535.7        | c.924G>C      | p.(Glu308Asp)  | missense      | rs14185660    | 1            | 0.0004     | 0.001      |
| PMS2       | NM_000535.7        | c.1004A>G     | p.(Asn335Ser)  | missense      | rs200513014   | 1            | 0.00004    | 0          |
| PMS2       | NM_000535.7        | c.2350G>A     | p.(Asp784Asn)  | missense      | rs143340522   | 3            | 0.0013     | 0.00695    |
| PMS2       | NM_000535.7        | c.130_131delinsCT | p.(Leu458Ser) | missense      | rs58778615    | 10           | NA         | NA         |
| RAD51C     | NM_058216.3        | c.956+28C>T   | intronic substitution | NA | 1 | NA | NA |
| RAD51D     | NM_001142571.2     | c.322C>T      | p.(Arg108Cys)  | missense      | rs142387263   | 1            | 0.0004     | 0.001      |
| TP53       | NM_000546.4        | c.993+165_993+166dup | intronic insertion | rs75788764    | 1            | 0          | 0          |
| XRCC2      | NM_005431.2        | c.*3 T > C    | 3'UTR Substitution | rs754786665   | 1            | 8.89E−06   | 0          |

Note: In bold: Very rare variants (with GMAF ≈ 0); In italic: Novel variants; a Variants predicted by SIFT, Provean, Polyphen-2 and MutationTaster to be damaging on the protein.
gene were predominant compared to BRCA1 (Gao et al., 2000; Panguluri et al., 1999). Contrarily, studies in Caucasians (Krainer et al., 1997) report more BRCA1 mutations in early onset breast cancers than BRCA2. Unfortunately, we cannot make a final conclusion because of a small sample size of our cohort.

In our cohort, only one patient (2.5%) harbored a likely pathogenic TP53: c.726C>G variant. This result is similar to previous studies (Bougeard et al., 2015; Hauke et al., 2018), where germline pathogenic TP53 mutations were found in up to 5% of young breast cancer patients.

Surprisingly, in the youngest patient in our cohort, who had familial history of breast cancer and presented with a sarcoma of the breast cancer and a TN tumor, did not have a plausible TP53 or BRCA1/2 variants, as one would expect. She did not harbor a plausible variant in the other genes of the tested panel neither.

A high number of VUS (n = 33) was observed in our study; which is in consistent with other studies in black women of African ancestry where NGS panel were evaluated (Awadelkarim et al., 2007; Fackenthal et al., 2012). These variants may have no functional implication in hereditary breast cancer, but their clinical significance remains to be elucidated. Our analysis indicated that eight among these VUS were predicted by SIFT, Provean, and Mutation Taster to have a damaging effect on protein (Table 3). Those predicted damaging VUS include one variant in CDH1 and BRIP1 genes, respectively, and two variants in each of the following three genes: ATM, CHEK2, and PMS2. The four genes namely CDH1, BRIP1, ATM, and CHEK2 are known to be associated with a high or moderate risk of breast cancer. However, the association of PMS2 gene with breast cancer is still unclear. In Caucasians, contradictory reports were published on the association of PMS2 gene mutations in breast cancer (Bernstein et al., 2019), (Roberts et al., 2018). The impact of this gene in the development of breast cancer in Africa needs further investigation.

Some variants identified in this study were classified as VUS because they are not, at the time of the redaction of this manuscript, found in mutation databases or clinical reports. Thus, the final frequency of germline mutations in our cohort is pending upon further evidences and reports from the literature and databases.

We found 13 variants that had never been observed before in African population (Ensembl: GMAF=0) and five variants that had never been observed before in any population. The lack of African reference and diseases databases are still preventing the full interpretation of NGS data from African individuals. This causes possible underestimation of the role of germline mutations in development of genetic diseases such as breast cancer in African population in general and in young Rwandan in particular. The development of such databases will allow more reliable determination of genetic contribution to breast cancer development in young Africans.

This study is among very few cohort-based studies in Sub-Saharan Africa investigating the contribution of germline

| TABLE 4 | BRCA1/2 mutations frequencies in young women of Caucasians, African American, and Africans. |
|---------|------------------------------------------------------------------------------------------|
| Study   | Mutation frequencies | Sample size (n) | Age limit       | Country               |
|---------|----------------------|-----------------|-----------------|-----------------------|
| Africans|                      |                 |                 |                       |
| This study | 10.0%                | 40              | <35 years old   | Rwanda                |
| Francies et al., (2015) | 7.7%                | 78              | <50 years old   | South Africa (Francies et al., 2015) |
| Fackenthal et al., (2012) | 11.0%               | 265             | <50 years old   | Nigeria (Ibadan; Fackenthal et al., 2012) |
| Tazzite et al., (2012) | 12.5%                | 72              | <50 years old   | Morocco (Tazzite et al., 2012) |
| Cherbal et al., (2010) | 14.0%                | 49              | ≤ 40 years old  | Algeria (Cherbal et al., 2010) |
| Troudi et al., (2007) | 18.0%                | 36              | ≤ 40 years old  | Tunisia (Troudi et al., 2007) |
| Awadelkarim et al., (2007) | 12.0%               | 34              | ≤ 40 years old  | Sudan (Awadelkarim et al., 2007) |
| Fackenthal et al., (2005) | 2.5%                | 39              | ≤ 40 years old  | Nigeria (Fackenthal et al., 2005) |
| Gao et al., (2000) | 4.0%                | 70              | ≤ 40 years old  | Nigeria (Gao et al., 2000) |
| Caucasians |                      |                 |                 |                       |
| Copson et al., (2018) | 12.0%                | 2733            | ≤ 40 years old  | UK (Copson et al., 2018) |
| de Sanjosé et al., (2003) | 11.6%               | 136             | ≤ 40 years old  | Spain (De Sanjosé et al., 2003) |
| Tonin et al., (2001) | 13.0%                | 61              | ≤ 40 years old  | Canada (Montreal; Tonin et al., 2001) |
| African American |                      |                 |                 |                       |
| Haffty et al. (2009) | 14.0%                | 39              | <45 years       | USA (New Jersey; Haffty et al., 2009) |
| John et al. (2007) | 17.0%                | 30              | <35 years old   | USA (North California; John, et al., 2007) |
| Malone et al., (2006) | 10.3%                | 80              | <45 years       | USA (Seattle; Malone et al., 2006) |
mutations to breast cancer within a large panel of breast cancer susceptibility genes. The majority of previous studies conducted in Africa were limited to the assessment of mutations in \textit{BRCA1} and \textit{BRCA2} only. However, we did not detect any clear relevant variant in 23 genes out of 26, indicating that \textit{BRCA1} and \textit{BRCA2} are probably the most commonly mutated genes associated with breast cancer predisposition in African women.

Our study had limitations related to the small size of our cohort, as well as the lack of reference mutation database for African population. These may lead to a false estimation of the frequency of genetic mutations. Additionally, we have only sequenced the coding sequences and their flanking intronic regions, and interrogated SNVs and small Indels. We may have missed the deep intronic variants or CNVs that would be associated with a risk of breast cancer.

5 | CONCLUSION

Our preliminary results showed that in young Rwandan patients with breast cancers, \textit{BRCA} genes were the most mutated with a predominance of \textit{BRCA2} variants. The frequency of overall mutations was similar to the results observed in Caucasians. Further large studies including both large families and controls and interrogating more types and locations of variants would be interesting to better understand the impact of germline mutations and environmental risk factors in the development of breast cancer in young Rwandans.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHOR’S CONTRIBUTION

J.P.U, V.B, and L.M conceived the study; J.P.U, C.J, K.S, P.M, B.B, and C.F performed all laboratory tests and analyses; J.P.U, K.S, and A. Z. L. analyzed and interpreted results; V.B and L.M supervised the study; J.P.U wrote the paper with the contribution of all authors; All authors reviewed the manuscript and approved the final manuscript’s content.

DATA AVAILABILITY STATEMENT

The data generated and/or analyzed during the current study are available from the corresponding authors on reasonable request.

REFERENCES

Abbad, A., Baba, H., Dehbi, H., Elmessaudi-Ilridissi, M., Elyazghi, Z., Abidi, O., & Radouani, F. (2018). Genetics of breast cancer in African populations: a literature review. \textit{Global Health, Epidemiology and Genomics}, 3, e8. https://doi.org/10.1017/ghge.2018.8

Adebamowo, C., Ogundiran, T., Adenipekun, A., Oyesegun, R., Campbell, O., Akang, E., & Olopade, O. (2003). Waist-hip ratio and breast cancer risk in urbanized Nigerian women. \textit{Breast Cancer Research}, 5(2):R18. https://doi.org/10.1186/bcr567

Adesunkanmi, A. R. K., Lawal, O. O., Adelusola, K. A., & Durosimi, M. A. (2006). The severity, outcome and challenges of breast cancer in Nigeria. \textit{Breast}, 15(3), 399–409. https://doi.org/10.1016/j.breast.2005.06.008

Anders, C. K., Hsu, D. S., Broadwater, G., Acharya, C. R., Foekens, J. A., Zhang, Y. I., … Blackwell, K. L. (2008). Young age at diagnosis correlates with worse prognosis and defines a subset of breast cancers with shared patterns of gene expression. \textit{Journal of Clinical Oncology}, 26(20), 3324–3330. https://doi.org/10.1200/JCO.2007.14.2471

Awadelkarim, K. D., Aceto, G., Veschi, S., Elhaj, A., Morgano, A., Mohamedani, A. A., … Mariani-Costantini, R. (2007). \textit{BRCA1} and \textit{BRCA2} status in a central Sudanese series of breast cancer patients: Interactions with genetic, ethnic and reproductive factors. \textit{Breast Cancer Research and Treatment}, 102(2), 189–199. https://doi.org/10.1007/s10549-006-9303-z

Bernstein, I., Munar, G. C., Garcia, E. G., Hoogerbrugge, N., Letteboer, T. G. W., Redeker, B. J. W., & Wagner, A. (2019). Lynch syndrome caused by germline PMS2 mutations: Delineating the cancer. \textit{Risk}, 3(4), 319–325. https://doi.org/10.1002/jco.2014.57.8088

Bharat, A., Aft, R. L., Gao, F., & Margenthaler, J. A. (2009). Patient and tumor characteristics associated with increased mortality in young women (≤40 years) with breast cancer. \textit{Journal of Surgical Oncology}, 100(3), 248–251. https://doi.org/10.1002/jso.21208

Bouguerd, G., Renaux-Petel, M., Flaman, J. M., Charbonnier, C., Ferrery, P., Belotti, M., & Frebourg, T. (2015). Revisiting Li-Fraumeni syndrome from TP53 mutation carriers. \textit{Journal of Clinical Oncology}, 33(21), 2345–2352. https://doi.org/10.1200/JCO.2014.59.5728

Brinton, L. A., Sherman, M. E., Carreon, J. D., & Anderson, W. F. (2008). Recent trends in breast cancer among younger women in the United States. \textit{Journal of the National Cancer Institute}, 100(22), 1643–1648. https://doi.org/10.1093/jnci/djn344

Cherbal, F., Bakour, R., Adane, S., Boualga, K., Benais-Pont, G., & Maillot, P. (2010a). \textit{BRCA1} and \textit{BRCA2} germline mutations screening in Algerian breast/ovarian cancer families. \textit{Disease Markers}, 28(6), 377–384. https://doi.org/10.3233/DMA-2010-0718

Colzani, E., Liljegren, A., Johansson, A. L. V., Adolfsson, J., Hellborg, H., Hall, P. F. L., & Czene, K. (2011). Prognosis of patients with
breast cancer: Causes of death and effects of time since diagnosis, age, and tumor characteristics. *Journal of Clinical Oncology*, 29(30), 4014–4021. https://doi.org/10.1200/JCO.2010.32.6462

Copson, E. R., Maishman, T. C., Tapper, W. J., Cutress, R. I., Greville-Hegate, S., Altman, D. G., … Eccles, D. M. (2018). Germline BRCA1 mutation and outcome in young-onset breast cancer (POSH): A prospective cohort study. *The Lancet Oncology*, 19(2), 169–180. https://doi.org/10.1016/S1470-2045(17)30891-4

De Sanjosé, S., Léoné, M., Bérez, V., Izquierdo, A., Font, R., Brunet, J. M., & Sinilnikova, O. M. (2003). Prevalence of BRCA1 and BRCA2 germline mutations in young breast cancer patients: A population-based study. *International Journal of Cancer*, 106(4), 588–593. https://doi.org/10.1002/ijc.11271

den Dunnen, J. T., Dalgleish, R., Maglott, D. R., Hart, R. K., Greenblatt, M. S., McGowan-Jordan, J., … Taschner, P. E. M. (2016). HGVS recommendations for the description of sequence variants: 2016 update. *Human Mutation*, 37(6), 564–569. https://doi.org/10.1002/humu.22981

John, E. M., Miron, A., Gong, G., Phipps, A. I., Felberg, A., Li, F. P., West, D. W., & Whittemore, A. S. (2007). Prevalence of pathogenic BRCA1 mutation carriers in 5 US racial/ethnic groups. *JAMA*, 298(24), 2869–2876. https://doi.org/10.1001/jama.298.24.286

Fackenthal, J. D., Sveen, L., Gao, Q., Kohlmeir, E. K., Adebamowo, C., Ogundiran, T. O., & Olopade, O. I. (2005). Complete allelic analysis of BRCA1 and BRCA2 variants in young Nigerian breast cancer patients. *Journal of Medical Genetics*, 42(3), 276–281. https://doi.org/10.1136/jmg.2004.020446

Fackenthal, J. D., Zhang, J., Zhang, B., Zheng, Y., Hagos, F., Burrill, D. R., … Olopade, O. I. (2012). High prevalence of BRCA1 and BRCA2 mutations in unselected Nigerian breast cancer patients. *International Journal of Cancer*, 131(5), 1114–1123. https://doi.org/10.1002/ijc.27326

Francies, F. Z., Wainstein, T., Leeceer, K. D., Cairns, A., Murdoch, M., Nietz, S., & Poppe, B. (2015). CHEK2 c. 1100delC in different South African ethnic groups diagnosed with premenopausal or triple negative breast cancer. *BMC Cancer*, 1–10. https://doi.org/10.1186/s12885-015-1913-6

Fregene, A., & Newman, L.A., (2005). Breast cancer in sub-Saharan Africa: How does it relate to breast cancer in African-American women? *Cancer*, 103(8), 1540–1550. https://doi.org/10.1159/000084245

Gao, Q., Adebamowo, C. A., Fackenthal, J., Das, S., Sveen, L., Falusi, A. G., & Olopade, O. I. (2000). Protein truncating BRCA1 and BRCA2 mutations in African women with pre-menopausal breast cancer. *Human Genetics*, 107(2), 192–194. https://doi.org/10.1007/s00439000342

Haffty, B. G., Choi, D. H., Goyal, S., Silber, A., Ranieri, K., Matloff, E., … Moran, M. S. (2009). Breast cancer in young women (YBC): Prevalence of BRCA1/2 mutations and risk of secondary malignancies across diverse racial groups. *Annals of Oncology*, 20(10), 1653–1659. https://doi.org/10.1093/annonc/mdp051

Hauke, J., Horvath, J., Groß, E., Gehrig, A., Honisch, E., Hackmann, K., & Hahnen, E. (2018). Gene panel testing of 5589 BRCA1/2-negative index patients with breast cancer in a routine diagnostic setting: results of the German Consortium for Hereditary Breast and Ovarian Cancer. *Cancer Medicine*, 7(4), 1349–1358. https://doi.org/10.1002/cam4.1376

Heramb, C., Wangensteen, T., Grindedal, E. M., Ariansen, S. L., & Lothe, S. (2018). BRCA1 and BRCA2 mutation spectrum – an update on mutation distribution in a large cancer genetics clinic in Norway. *Hereditary Cancer in Clinical Practice*, 16(1), 3. https://doi.org/10.1186/s13053-017-0085-6

Krainer, M., Silva-Arietti, S., FitzGerald, M. G., Shimada, A., Ishioka, C., Kanamaru, R., & Haber, D. A. (1997). Differential contributions of BRCA1 and BRCA2 to early-onset breast cancer. *New England Journal of Medicine*, 336(20), 1416–1422. https://doi.org/10.1056/NEJM199705153362003

Li, M. M., Datto, M., Duncavage, E. J., Kulkarni, S., Lindeman, N. I., Roy, S., … Nikiforova, M. N. (2017). Standards and Guidelines for the Interpretation and Reporting of Sequence Variants in Cancer: A Joint Consensus Recommendation of the Association for Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists. *The Journal of Molecular Diagnostics*, 19(1), 4–23. https://doi.org/10.1016/j.jmoldx.2016.10.002

Malone, K. E., Dalig, R. J., Doody, D. R., Hsu, L., Bernstein, L., Coates, R. J., & Ostrander, E. A. (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 to 64 years. *Cancer Research*, 66(16), 8297–8308. https://doi.org/10.1158/0008-5472.CAN-06-0503

Pace, L. E., Mpunga, T., Hategukimana, V., Dusengimana, J.-M., Habineza, H., Bigirimana, J. B., … Keating, N. L. (2015). Delays in breast cancer presentation and diagnosis at two rural cancer referral centers in Rwanda. *The Oncologist*, 20(7), 780–788. https://doi.org/10.1634/theoncologist.2014-0493

Panguluri, R., Brody, L. C., Modali, R., Utley, K., Adams-Campbell, L., Day, A. A., … Dunston, G. M. (1999). BRCA1 mutations in African Americans. *Human Genetics*, 105(1-2), 28–31. https://doi.org/10.1007/s004390051059

Roberts, M. E., Jackson, S. A., Susswein, L. R., Zeinomar, N., Ma, X., Marshall, M. L., … Chung, W. K. (2018). MSH6 and PMS2 germline pathogenic variants implicated in Lynch syndrome are associated with breast cancer. *Genetics in Medicine*, 20(10), 1167–1174. https://doi.org/10.1038/s41436-017-0138-4

Rummel, S. K., Lovejoy, L., Shriver, C. D., & Ellsworth, R. E. (2017). Contribution of germline mutations in cancer predisposition genes to tumor etiology in young women diagnosed with invasive breast cancer. *Breast Cancer Research and Treatment*, 164(3), 593–601. https://doi.org/10.1007/s10549-017-4291-8

Tazzite, A., Jouhadi, H., Nadifi, S., Aretini, P., Falaschi, E., Collavoli, A., … Caligo, M. A. (2012). BRCA1 and BRCA2 germline mutations in Moroccan breast/ovarian cancer families: Novel mutations and unclassified variants. *Gynecologic Oncology*, 125(3), 687–692. https://doi.org/10.1016/j.ygyno.2012.03.007

Tonin, P. N., Perret, C., Lambert, J. A., Paradis, A. J., Kantemiroff, T., Benoît, M. H., & Ghadirian, P. (2001). Founder BRCA1 and BRCA2 mutations in early-onset French Canadian breast cancer cases unselected for family history. *International Journal of Cancer*, 95(3), 189–193. https://doi.org/10.1002/1097-0215(20010520)95:3<189:AID-IJC1032>3.0.CO;2-N

Troudi, W., Uhrhammer, N., Sibille, C., Dahan, C., Mahfoudh, W., Bouchlaka Souissi, C., … Ben Ammar Elgaaied, A. (2007). Contribution of the BRCA1 and BRCA2 mutations to breast cancer in Tunisia. *Journal of Human Genetics*, 52(11), 915–920. https://doi.org/10.1038/sj.humgen.2007.019-5
SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section.
Supplementary Material

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