BRCA1, BRCA2 and PALB2 mutations and CHEK2 c.1100delC in different South African ethnic groups diagnosed with premenopausal and/or triple negative breast cancer

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Abstract

Background: Current knowledge of the aetiology of hereditary breast cancer in the four main South African population groups (black, coloured, Indian and white) is limited. Risk assessments in the black, coloured and Indian population groups are challenging because of restricted information regarding the underlying genetic contributions to inherited breast cancer in these populations. We focused this study on premenopausal patients (diagnosed with breast cancer before the age of 50; n = 78) and triple negative breast cancer (TNBC) patients (n = 30) from the four South African ethnic groups. The aim of this study was to determine the frequency and spectrum of germline mutations in BRCA1, BRCA2 and PALB2 and to evaluate the presence of the CHEK2 c.1100delC allele in these patients.

Methods: In total, 108 South African breast cancer patients underwent mutation screening using a Next-Generation Sequencing (NGS) approach in combination with Multiplex Ligation-dependent Probe Amplification (MLPA) to detect large rearrangements in BRCA1 and BRCA2.

Results: In 13 (12 %) patients a deleterious mutation in BRCA1/2 was detected, three of which were novel mutations in black patients. None of the study participants was found to have an unequivocal pathogenic mutation in PALB2. Two (white) patients tested positive for the CHEK2 c.1100delC mutation, however, one of these also carried a deleterious BRCA2 mutation. Additionally, six variants of unknown clinical significance were identified (4 in BRCA2, 2 in PALB2), all in black patients. Within the group of TNBC patients, a higher mutation frequency was obtained (23.3 %; 7/30) than in the group of patients diagnosed before the age of 50 (7.7 %; 6/78).

Conclusion: This study highlights the importance of evaluating germline mutations in major breast cancer genes in all of the South African population groups. This NGS study shows that mutation analysis is warranted in South African patients with triple negative and/or in premenopausal breast cancer.

Keywords: Triple negative breast cancer, Premenopausal breast cancer, BRCA mutations, South Africa
Background

Breast cancer is the most common cancer amongst South African women with a lifetime risk of 1 in 32 [1]. South Africa is a country consisting of citizens from diverse ethnic groups. These include: black/African (79.8 %), white/Caucasian (8.7 %), mixed ancestry/coloured (9.6 %) and Indian/Asian (2.5 %) (Statistics South Africa, 2013) [2]. According to the most recent report from the National Cancer Registry of South Africa, the lifetime risk of developing breast cancer differs according to ethnicity. The lifetime risk is 1/53 in black women, 1/15 in white women, 1/21 in coloured women and 1/20 in Indian women (National Cancer registry, NHLS, 2006) [1].

Breast cancer has a strong heritable component, with approximately 15–20 % of cases exhibiting a family history of the disease [3, 4]. Mutations in genes such as BRCA1 and BRCA2 lead to autosomal dominant inherited cancer susceptibility and confer a high lifetime risk of breast cancer, as well as ovarian and other cancers. Recently it was suggested that the risk to develop breast cancer for PALB2 mutation carriers is as high as the risk for BRCA1 and BRCA2 gene, seen in European and American populations [26, 27], has not been investigated in a South African cohort. Individuals included in the study were of different ethnicities (with a majority from the understudied black population) and had been diagnosed with premenopausal breast cancer (less than 50 years) or exhibited the so-called “triple negative” histological phenotype. Approximately 15 % of breast cancers lack the expression of estrogen receptors, progesterone receptors and HER2/NEU receptors and are known as triple negative breast cancer (TNBC) [24]. This type of breast cancer is associated with an aggressive disease progression, higher histological grade, poor prognosis, high rate of recurrence and decreased survival rates. The frequent occurrence of TNBC is strongly correlated with younger patients of African descent and increased incidence has been noted among black South African breast cancer patients [16, 17, 25]. The strong association between TNBC and mutations in the BRCA1 gene, seen in European and American populations [26, 27], has not been investigated in a South African cohort.

This study aimed to evaluate the contribution of germline BRCA1, BRCA2 and PALB2 mutations and the CHEK2 c.1100delC allele to breast cancer in a high-risk South African cohort. Individuals included in the study were of different ethnicities (with a majority from the understudied black population) and had been diagnosed with premenopausal breast cancer (less than 50 years) or exhibited the “triple negative” histological phenotype. We chose to analyse BRCA1, BRCA2 and PALB2 as associated risks are well established and clinically relevant. In addition, the prevalence of CHEK2 c.1100delC was evaluated in this cohort and compared with the prevalence in individuals of limited financial and human resources, limited community knowledge of breast cancer, limited information on family history and historical difficulty accessing health care, makes it more complex to perform risk assessments in these populations [13]. Overall, the cancer incidence in sub-Saharan Africa is lower as compared to developed countries but there is evidence to suggest changes in the disease burden as the impact of communicable diseases is mitigated [14]. South African women tend to be diagnosed with breast cancer at younger ages [15–17]. However, the diagnosis only occurs at advanced stage due to the lack of awareness, access to diagnostic centres available and limited screening. Hence, the inclusion criterion for a “young” breast cancer or premenopausal (PM) breast cancer patient was set at 50 years (See Additional file 1: Table S1).

Another factor that is generally considered as an indicator of genetic susceptibility to breast cancer is the so-called “triple negative” histological phenotype. Approximately 15 % of breast cancers lack the expression of estrogen receptors, progesterone receptors and HER2/NEU receptors and are known as triple negative breast cancer (TNBC) [24]. This type of breast cancer is associated with an aggressive disease progression, higher histological grade, poor prognosis, high rate of recurrence and decreased survival rates. The frequent occurrence of TNBC is strongly correlated with younger patients of African descent and increased incidence has been noted among black South African breast cancer patients [16, 17, 25]. The strong association between TNBC and mutations in the BRCA1 gene, seen in European and American populations [26, 27], has not been investigated in a South African cohort.

A few South African studies on mutations in BRCA1, BRCA2 and PALB2 are available [7–10]. Three South African population groups exist in which the presence of BRCA1/2 founder mutations occur; these are the Ashkenazi Jewish population [11], the Afrikaans population [7] and the black Xhosa population [10]. Other family-specific mutations have also been identified, as is typical of populations elsewhere. Table 1 shows data from studies done in South Africa to date. These studies have been performed mostly in white breast cancer patient cohorts. Furthermore, African populations are known to exhibit greater genomic diversity when compared to white populations, and genetic findings in one population cannot necessarily be extrapolated to another [12]. Consequently, there is a need to establish the aetiology of inherited breast cancer in this population. The epidemiology of breast cancer in South African black populations exhibits a number of unique trends when compared to other population groups worldwide. The difference in underlying genetic architecture, family structure,
European ancestry. We applied a cost efficient next generation sequencing (NGS) approach for analysis of the complete coding regions of \textit{BRCA1}, \textit{BRCA2} and \textit{PALB2} [28]. Furthermore, large rearrangements have been reported in both \textit{BRCA1} and \textit{BRCA2} in several populations which may be missed by sequencing. We therefore complemented the sequencing approach with multiplex ligation-dependent probe amplification (MLPA), for these two genes.

### Table 1: Literature overview on \textit{BRCA1} and \textit{BRCA2} mutations detected in a South African population

| Study (Reference) | Ethnic group | Gene   | Mutation detected | Patients/families tested | Frequency (%) | Detection method                        |
|-------------------|--------------|--------|-------------------|--------------------------|---------------|---------------------------------------|
| Yawitch & Van Rensburg 2000 [51] | Black | \textit{BRCA1} | N/A | 0/206 | 0 | PTT and SSCP/HA; limited to regions with Afrikaner founder mutations |
| Reeves et al., 2004 [7] | White/Ashkenazi Jewish | \textit{BRCA1} | c.68_69delAG | 4/18 | 4.4 | PTT and SSCP/HA |
|                       | White | \textit{BRCA1} | c.329dupA | 1/18 | 1.1 | PTT and SSCP/HA |
|                       | White | \textit{BRCA1} | c.1008dupA | 1/18 | 1.1 | PTT and SSCP/HA |
|                       | White/Afrikaner | \textit{BRCA1} | c.1352C>A; p.S451* | 1/18 | 1.1 | PTT and SSCP/HA |
|                       | White/Afrikaner | \textit{BRCA1} | c.1374delC | 2/18 | 2.2 | PTT and SSCP/HA |
|                       | Indian | \textit{BRCA1} | c.4957insC | 1/18 | 1.1 | PTT and SSCP/HA |
|                       | White/Ashkenazi Jewish | \textit{BRCA1} | c.5266dupC | 3/18 | 1.1 | PTT and SSCP/HA |
| Schlebusch et al., 2010 [52] | White/Afrikaner, Ashkenazi Jewish, Black, Indian | \textit{BRCA1} | N/A | 26/129 | 20.2 | PTT and SSCP/HA and MLPA |
|                       | BRCA2 | N/A | 43/129 | 33.3 | PTT and SSCP/HA and MLPA |
| Sluiter et al., 2011 [9] | White/Afrikaner | \textit{BRCA1} + \textit{BRCA2} | N/A | 0/36 | MLPA |
|                       | White/Ashkenazi Jewish | \textit{BRCA1} | Ex23-24del | 1/30 | 3.3 | MLPA |
| Van der Merwe et al., 2012 [10] | Coloured | \textit{BRCA1} | c.1504_1508delTTAAA | 1/105 | 1.0 | PTT and SSCP/HA |
|                       | \textit{BRCA1} | c.2641G>T; p.E881* | 1/105 | 1.0 | PTT and SSCP/HA |
|                       | \textit{BRCA2} | c.2826_2829delAAATT | 1/105 | 1.0 | PTT and SSCP/HA |
|                       | \textit{BRCA2} | c.5771_5774delTTCA | 4/105 | 3.8 | PTT and SSCP/HA |
|                       | \textit{BRCA2} | c.6448dupTA | 1/105 | 1.0 | PTT and SSCP/HA |
|                       | \textit{BRCA2} | c.7934delG | 1/105 | 1.0 | PTT and SSCP/HA |
|                       | Black | \textit{BRCA2} | c.5771_5774delTTCA | 4/16 | 25.0 | MLPA |
| Schoeman et al., 2013 [13] | White, Mixed Ancestry, Black | \textit{BRCA1} | c.2641G>T; p.E881* | 7/302 | 2.3 | SSCP/HA |
|                       | \textit{BRCA1} | c.68_69delAG | 2/302 | 0.7 | SSCP/HA |
|                       | \textit{BRCA1} | c.1374delC | 2/302 | 0.7 | SSCP/HA |
|                       | \textit{BRCA1} | c.5266dupC | 1/302 | 0.3 | SSCP/HA |
|                       | \textit{BRCA2} | c.7934delG | 17/302 | 5.6 | SSCP/HA |
|                       | \textit{BRCA2} | c.5771_5774delTTCA | 7/302 | 2.3 | SSCP/HA |
|                       | \textit{BRCA1} | N/A | 4/302 | 1.3 | PTT |
|                       | \textit{BRCA2} | N/A | 5/302 | 1.7 | PTT |
|                       | \textit{BRCA1} | N/A | 2/302 | 0.7 | Sequencing |
|                       | \textit{BRCA2} | N/A | 2/302 | 0.7 | Sequencing |
|                       | \textit{BRCA1} | N/A | 18/302 | 6.0 | Sequencing |

\textit{PTT} protein truncation test, SSCP/HA PCR-single strand conformation polymorphism/heteroduplex analysis, N/A mutations were not described; * indicates the presence of a premature stop codon (cfr. nomenclature HGVS (Human Genome Variation Society)).
Methods

Patients
EDTA blood samples of 108 breast cancer patients were collected from breast clinics in two state hospitals and a private hospital in Johannesburg - Charlotte Maxeke Johannesburg Academic Hospital, Chris Hani Baragwanath Academic Hospital and Wits Donald Gordon Medical Centre respectively. Patients were selected if their tumour was triple-negative (TN), and/or their breast cancer diagnosis was premenopausal. All patients were categorized as black, white, Indian or coloured based on patients’ self-reported data from questionnaires. The cohort consisted of 85 black patients (78.7 %), 16 white patients (14.8 %), 5 Indians (4.6 %) and 2 coloureds (1.9 %). Table 2 presents the overview of the distribution of ethnicity in the cohort. All patients signed informed consent. Pathology data were obtained from the hospital files. Genetic counselling was offered to the patients, prior to obtaining their consent.

The study was approved by the Human Research Ethics Committee (Medical), University of the Witwatersrand (No. M091023; M110922; M130450).

DNA extraction
Genomic DNA was extracted from 4 - 6 ml of peripheral blood using a modified version of the standard salting out method [29].

Target enrichment, library preparation and sequencing
BRCA1, BRCA2 and PALB2 analysis was successfully conducted on 108 samples using Illumina’s MiSeq desktop sequencer. Target enrichment was achieved by high throughput PCR. Primers were designed for the complete coding region including splice site regions of BRCA1 (31 amplicons), BRCA2 (42 amplicons) and PALB2 (19 amplicons) using Primer XL (www.pxlence.com). PCR conditions according to the protocol described by De Leeneer et al. were utilised [28].

Library preparation was performed using a modified version of the Nextera XT (Illumina) protocol. Sequencing was conducted on the MiSeq v2 instrument (Illumina Inc.) according to manufacturer’s instructions. The approach is described in detail by De Leeneer et al. [28].

Table 2 Overview of distribution of ethnicity in our South African cohort

|        | Black (%) | White (%) | Indian (%) | Coloured (%) |
|--------|-----------|-----------|------------|--------------|
| Dx < 50 n = 92 TNBC | 7 (7.6)   | 4 (4.3)   | 2 (2.2)    | 1 (1.1)      |
|         | 70 (76.1) | 5 (5.4)   | 2 (2.2)    | 1 (1.1)      |
| Dx > 50 n = 16 TNBC | 8 (50.0)  | 7 (43.8)  | 1 (6.3)    | 0            |
| Total n = 108      | 85 (78.7) | 16 (14.8) | 5 (4.6)    | 2 (1.9)      |

Dx: Age at diagnosis

Sanger sequencing
All genetic variants and pathogenic mutations identified via NGS were confirmed with Sanger sequencing. For confirmation by Sanger sequencing, an independent PCR amplification step was performed. In addition, the presence of all deleterious mutations was confirmed on an independently extracted DNA sample. All fragments with a coverage of <28× were also analysed by Sanger sequencing. For an overview of the number of amplicons that required Sanger sequencing, refer to Additional file 2: Table S2.

Nucleotide positions and protein translation correspond to reference sequence and Genbank account number NM_007294.3; NP_009225.1 for BRCA1, NM_000059.3; NP_000050.2 for BRCA2, NM_024675.3; NP_078951.2 for PALB2 and NM_007194.3 for CHEK2 c.1100delC. Nucleotide numbering uses the A of the ATG translation initiation site start as nucleotide 1.

MLPA
Large genomic rearrangements and/or gene dosage alterations in both the BRCA1 and BRCA2 genes were screened for in 108 patient samples using MLPA. BRCA1 MLPA analysis was performed using the SALSA MLPA P002 probemix (version C2-1113) (MRC-Holland) and BRCA2/CHEK2 MLPA using the SALSA MLPA P045 probemix (version B3-1113) (MRC-Holland). MLPA setup was performed according to the manufacturer’s protocol. Fragment detection and sizing was conducted using capillary gel electrophoresis on the ABI 3730XL genetic analyser (Applied Biosciences). All fragments positive for the CHEK2 mutation (c.1100delC) in the MLPA analysis were confirmed with Sanger sequencing.

The screening was performed in a research setting. We used the infrastructure and the protocols supplied by a molecular diagnostic laboratory with an ISO15189 accreditation.

Data analysis
Mapping of sequencing data was performed with CLC bio Genomics Workbench v6 software (CLC bio Inc.). Various in-house scripts were used for sequence analysis [28]. The Sanger sequencing data were analysed using SeqPilot v4.1.2 build 512 and SeqSpace v2.5.0. MLPA data were analysed using Coffalyser (MRC-Holland).

Variants of unknown significance (VUS) were evaluated using in silico mutation interpretation software – Alamut. We used the computational algorithms of SIFT, AlignGVGD, Polyphen and Mutation Taster for missense variants and the splice site prediction programs SpliceSiteFinder, MaxEntScan, NNSPLICE, GeneSplicer and Human Splicing Finder for intronic, silent and missense variants. Based on these predictions and in
combination with a study of the literature and published minor allele frequencies, variants were classified in five classes. Unfortunately, due to limited availability of data, Bayesian likelihood analyses could not be performed to calculate the degree of likelihood of pathogenicity. Therefore, we applied the following rules:

- Variants with a MAF (minor allele frequency) of > 0.01 were classified as class 1 (data not shown)
- Variants were classified as class 2 if all prediction programs provided neutral scores (data not shown)
- Variants with two or more programs with deleterious predictions were allocated to class 3 (Table 5)
- All truncating and unequivocal splice site variants were considered as deleterious, in addition to missense variants in the RING domain of BRCA1 (class 4–5) (Table 3)

**Statistical analysis**

Mutation frequency was calculated with 95 % confidence intervals. The Fisher's exact test was used to compare mutation frequencies in the different groups of patients. Statistical analysis was performed with Graphpad Prism software.

**Results**

In the total study population (n = 108), 15 heterozygous pathogenic mutations in 14 patients were identified (12.9 %; 95 % CI = 7.3–20.8 %): six in BRCA1, seven in BRCA2; two patients were found to carry CHEK2 c.1100delC of which one patient also harboured a deleterious BRCA2 mutation. All mutations were identified by sequencing on Miseq, except a large deletion in BRCA1 and the CHEK2 c.1100delC mutation which were detected by MLPA. No unequivocal deleterious mutations were identified in the PALB2 gene (Table 3).

The distribution of BRCA1/2 mutations among the different subgroups (TNBC and/or PM) and based on ethnicity is presented in Table 4. A significantly higher mutation detection ratio was obtained within the group of TNBC patients (7/30; 23.3 %; 95 % CI = 9.9–42.3 %) compared to the premenopausal breast cancer group without TNBC (6/78; 7.7 %; 95 % CI = 2.9–16.0 %) (p = 0.0432). Not surprisingly, the highest mutation detection ratio was obtained within the subgroup of TNBC patients diagnosed before the age of 50 (5/14; 35.7 %; 95 % CI = 12.7–64.9 %).

The BRCA2 c.7934delG Afrikaner founder mutation was identified in 2 (white) patients, one with TNBC and one diagnosed with premenopausal breast cancer. In the black patient population, two previously unreported mutations were identified in BRCA1 (c.1155G > A and c.1953_1954insA) and one in BRCA2 (c.582G > A) (see Table 3). Six (6/85; 7.1 %; 95 % CI = 2.6–14.7 %) pathogenic BRCA1/2 mutations were observed in the black population group and five (5/16; 31.3 %; 95 % CI = 11.0–58.7 %) in the white population group. Two mutations were identified in the Indian group (2/5; 40 %; 95 % CI = 5.3–85.3 %) and no mutations were identified either in

| Table 3 BRCA1, BRCA2 and CHEK2 germline pathogenic mutations identified in triple negative and premenopausal breast cancer patients using NGS and MLPA |
| --- |
| Patient no. | Ethnicity | Category | Gene | Exon | Nucleotide change | Amino acid change | Mutation effect | Reference |
| 1 | White | TNBC/PM | BRCA1 | 4 | c.181 T > G | p.Cys61Gly | Missense | [53] |
| 2 | Black | TNBC/PM | BRCA1 | 4 | c.212G > A | p.Arg71Lys | Missense | [54] |
| 3 | Indian | TNBC/PM | BRCA1 | 10 | c.3593 T > A | p.Leu1198* | Nonsense | [55] |
| 4 | Black | PM | BRCA1 | 10 | c.1155G > A | p.Trp385* | Nonsense | Novel |
| 5 | Black | PM | BRCA1 | 10 | c.1953_1954insA | p.Lys652fs | Frameshift | Novel |
| 6 | White | TNBC | BRCA1 | 1–2 | - | - | Deletion | [30] |
| 7 | Black | PM | BRCA2 | 7 | c.582G > A | p.Trp194* | Nonsense | Novel |
| 8 | Black | TNBC | BRCA2 | 11 | c.5771_5774delTATA | p.Ile1924fs | Frameshift | [10] |
| 9 | White | PM | BRCA2 | 11 | c.5213_5216delCTTA | p.Thr1738fs | Frameshift | [56] |
| 10 | White | TNBC | BRCA2 | 17 | c.7934delG | p.Arg2645fs | Frameshift | [10] |
| 11 | White | PM | BRCA2 | 17 | c.7934delG | p.Arg2645fs | Frameshift | [10] |
| 12 | Indian | TNBC/PM | BRCA2 | 21 | c.8754 + 1G > A | Non-coding | Splice site | [57] |
| 13 | Black | PM | BRCA2 | 23 | c.9097_9098insA | p.Thr3033fs | Frameshift | [53] |
| 14 | White | PM | CHEK2 | 11 | c.1100delC | p.Thr367fs | Frameshift | [39] |

PM Premenopausal
*MLPA results
*indicates the presence of a premature stop codon (cfr. nomenclature HGVS (Human Genome Variation Society))
The CHEK2 mutation (c.1100delC) was observed in 2/108 (1.9 %) patients. Both of these patients were white, premenopausal patients. One of these patients was also positive for a deleterious BRCA2 mutation.

In addition to pathogenic mutations, several VUS were identified: 1 in BRCA1, 3 in BRCA2 and 2 in PALB2. In Table 5 we provide an overview of the variants which were classified as class 3 based on in silico prediction programs. Three of the four in silico prediction programs used classified the BRCA2 variant c.9875C > T and c.7712A > G as “probably damaging.” The BRCA2 variant c.9875C > T was
identified in two black patients. Two of the four prediction programs consulted classified the PALB2 variants c.118A > G and c.2845 T > C as “probably damaging”.

**Discussion**

The current study is the first study performing mutation analyses in BRCA1, BRCA2 and PALB2 and determining the frequency of CHEK2 c.1100delC in triple negative and/or premenopausal breast cancer patients in South Africa through both next generation sequencing and large rearrangement testing. In total we detected 13 BRCA1/2 mutations in our study cohort of 108 patients (12 %; 95 % CI = 6.6–19.7 %), thus reinforcing the important contribution of inherited breast cancer in this mixed South Africa cohort. Two patients harboured a CHEK2 c.1100delC mutation, one of them in combination with a deleterious BRCA2 mutation. Previous studies done on South African breast cancer populations reported BRCA1/2 mutation frequencies of 1 to 25 % [7–10] (for an overview: see Table 1). The prevalence of mutations in BRCA1/2 genes in these South African studies varies by inclusion criteria, ethnicity and mutation screening techniques used. None of these studies looked specifically at TNBC or premenopausal patients.

The mutation frequency was higher in the subgroup of TNBC than in the premenopausal breast cancer patients: 23.3 % (7/30) of TNBC patients harbour a pathogenic mutation in either BRCA1 or BRCA2, compared to 12.0 % (11/92) of all premenopausal breast cancer patients.

Various studies have shown the frequency of BRCA1 mutations to be higher than BRCA2 in patients exhibiting the triple negative phenotype [27, 32, 33]. In our study 13.3 % (4/30) of TNBC patients had a pathogenic mutation in BRCA1 compared to 10 % (3/30) in BRCA2.

In our premenopausal cohort, the prevalence of BRCA1 mutations were similar (5/92; 5.4 %) to BRCA2 mutations (6/92; 6.5 %). BRCA2 mutations are in general less frequent than BRCA1 in younger white women with breast cancer [19]. A relatively high number of BRCA2 mutations compared to BRCA1 has been reported in other studies of young black populations [34–36] and is contradictory to the scenario in Western populations. This could be due to the unique genetic background of African patients.

In the black population, the overall frequency of mutations identified was 7.1 % as compared to 31.3 % in the white population. Due to the presence of the BRCA2 c.7934delG Afrikaner founder mutation, BRCA2 is the most important contributor in the white population in our study cohort, while BRCA1 and BRCA2 mutations were observed in equal numbers in the black patients studied. We identified neither the Ashkenazi Jewish nor the Xhosa mutations in our study groups. Our patient cohort was recruited in the region of Johannesburg and is characterized by diverse population structure/ethnic backgrounds. Therefore we did not anticipate finding a large number of founder mutations.

The CHEK2 c.1100delC allele contributes to a moderate increased breast cancer risk. The frequency is estimated to be only 1 % in familial breast cancer and 0.5 % in early onset breast cancer [37, 38]. In the Dutch population the prevalence in the general population is 1.1 %, 2.5 % in unselected breast cancer cases, and up to 4.9 % in familial breast cancer cases [39]. Within our South African cohort we identified this allele in two white patients (2/16 = 12.5 %), but in none of the patients from other ethnicities (0/92). White Afrikaner South Africans mainly descend from Dutch immigrants which could explain the higher percentage of CHEK2 c.1100delC in this cohort.

Previous studies that aimed to clarify the prevalence of BRCA1/2 mutations in black populations from other parts of Africa and African Americans have indicated similar rates [6, 22, 27, 36, 40]; although it is difficult to compare them since eligibility criteria for study participation varies extensively. Churpek et al. [40] reported a pick-up rate of 26 % (47/180) for pathogenic mutations in a group of black patients with early onset disease (age of diagnosis <45) and 25 % pick-up rate (26/103) for pathogenic mutations in triple negative black patients. Here we report BRCA1/2 mutation frequency of 14 % (1/7) in the premenopausal triple negative black subgroup. Our overall mutation detection rate of BRCA1/2 mutations in the black premenopausal breast cancer patients was 6.5 % (5/77). This is similar to the mutation rate reported in a study by Pal et al. [22] in young black African American breast cancer patients (9 %; 13/144). Although the prevalences are similar among the studies on West African, African American breast cancers and our study, we identified 3 novel mutations in the South African black patients. Furthermore, historical evidence has shown that African Americans descend from West African ancestry and so it is not surprising that there are some differences between these two and the South African black population, who have some distinct genetic differences at the population level [12, 41].

Large genomic rearrangements in BRCA, detected with MLPA, were only observed in 0.9 % (1/108) of our cohort. No large rearrangements were identified in the black South African breast cancer patients. Generally, low frequencies for large rearrangements have been reported in black patients, e.g. Pal et al., [22], detected 2 rearrangements in 144 young African-American women with breast cancer (1.4 %), both of which were in BRCA1. Zhang et al., [42] reported one BRCA1 exon deletion (0.3 %) in a cohort of 352 Nigerian breast cancer patients.
patients. In another South African study on 52 unrelated families of European ancestry, only 1 large deletion was detected in BRCA1 [9]. The lack of detection in BRCA2 led the authors to suggest that large rearrangements in BRCA2 might not play a role in inherited breast cancer in South African patients [9]. However, to draw final conclusions on the presence of large rearrangements in both white and black South African breast cancer patients, a larger patient population should be extensively studied.

Gene sequencing techniques also resulted in the identification of several VUS. Based on in silico predictions, we assigned a class (class 1–3) to each VUS for clinical interpretation [43]. VUS with a probability of increased pathogenicity are assigned a higher class. A number of studies have presented models and performed functional assays for the classification of VUS in BRCA1/2 [43–46]. We detected six VUS in the 85 black patients of our cohort and none in the 16 white patients. Also other studies suggested that the frequency of VUS is higher in patients of African descent, for instance Nanda et al. [47].

A previous study conducted in a South African cohort revealed a pathogenic PALB2 mutation in 2 % of early onset white breast cancer patients [8]. Our cohort consisted of a small number of white patients and no unequivocal deleterious mutations in PALB2 were identified. However two missense variants with suggestive in silico predictions were identified (Table 5) that warrant further functional analyses. Until recently, the pathogenic effect of PALB2 missense variants has not been firmly proven. For some missense variants in the WD40 domain (from amino acids 853–1186) [48] altered patterns of direct binding to the RAD51C, RAD51 and BRCA2 h proteins in biochemical assays have been shown [49]. We identified a missense variant in the WD40 domain (c.2845 T>C; p.Cys949Arg). In order to elucidate the pathogenicity of missense variants in PALB2, additional (functional, segregation) analyses are required.

We focused on identifying mutations in BRCA1, BRCA2 and PALB2 and the CHEK2 c.1100delC mutation, as the risks for the development of breast and associated cancers with these genes have been determined by analysing large study populations. The search for the remaining genetic contribution towards breast and ovarian cancer has been carried out extensively, with numerous other genes being identified. However, at this time, the contribution and associated risks of mutations in most of these genes is not yet well established. As the prevalence of mutations in each of these genes is much lower than germline BRCA1/2 mutations in the large cohorts (white American) of patients investigated up until now [50], international collaborations in populations of different ethnicities will be required to gain insight into the exact risks associated with mutations in these genes.

Conclusion
This study is the first to evaluate the use of NGS technology as a diagnostic testing platform for inherited breast cancer in a South African cohort. The results presented herein are particularly relevant for inherited cancer testing in the black population of South Africa, a previously under-researched group. The NGS approach applied [28] is a cost and time effective approach; it shows great promise for BRCA1/2 screening in developing countries like South Africa. The advent of NGS allows the costs of mutation analysis to fall dramatically, which should allow testing to become more widely available, especially in countries with limited healthcare resources, like South Africa. This will create opportunities to improve patient treatment and challenges for breast cancer multidisciplinary teams. The finding of a germline deleterious mutation could alter treatment decisions; for instance, women with germline mutations might opt for more radical surgery or may consider prophylactic surgery to the contralateral breast or ovaries.

Our results have highlighted the contribution of BRCA1/2 germline mutations in South African breast cancer patients with triple negative breast tumours and/or premenopausal breast cancer of different ethnicities.

Additional files

Additional file 1: Table S1. Overview of grading and staging of breast cancer on diagnosis (DOC 30 kb)

Additional file 2: Table S2. Overview of sequencing coverage per run (DOC 29 kb)

Abbreviations
MAF: Minor allele frequency; MLPA: Multiplex ligation-dependent probe amplification; NGS: Next-generation sequencing; PM: Premenopausal; TN: Triple negative; TNBC: Triple negative breast cancer; VUS: Variants of unknown significance.

Competing interests
The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

Authors’ contributions
FZT, TW carried out the molecular work, analysed data and helped draft the manuscript. KDL, BC, IC carried out the molecular work and analysis of data. AC, MM, SN, HC, BP, TVM provided samples for this study. RK, JPS, AV, AK revising the manuscript. AB, KBMC design of the study and drafting the manuscript. All authors have read and approved the manuscript.

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