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Genetic trends and common *BRCA1/2* pathogenic sequence variants in black African and Indian breast cancer patients presenting at Inkosi Albert Luthuli Central Hospital, KwaZulu-Natal, South Africa

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Background. Hereditary breast cancer is characterised by the presence of a pathogenic sequence variant passed from one generation to the next. These cancers are aggressive, develop early, and account for 5 - 10% of all breast cancer cases. In South Africa (SA), the common variants that predispose to hereditary breast cancer have been well documented among white patients and form part of screening panels during targeted testing. For non-white patients, common variants are not well understood, and as such, all populations are offered the same test optimised for white patients. This carries a risk of misdiagnosis, the consequences of which include recurrence and increased mortality.
Objectives. To retrospectively describe genetic trends in the black African and Indian breast cancer patients from KwaZulu-Natal Province, SA.
Methods. We reviewed clinical and genetic data of breast cancer and high-risk patients who consulted at Inkosi Albert Luthuli Central Hospital between 2011 and 2021. Inclusion criteria were based on clinical and demographic characteristics as defined by SA clinical guidelines.
Results. Black African patients were young (mean 37.6 years, standard deviation 11.16) and had the majority of triple-negative tumours (37.5%). Indians represented 50% of bilateral breast cancers and of high-risk individuals. We identified 30 pathogenic *BRCA1/2* sequence variants, four large genomic rearrangements and 13 variants of unknown significance. Twenty black patients carried 12, 13 white patients carried 4, 25 Indian patients carried 16, and 3 coloured patients carried 3 pathogenic sequence variants. The most frequent variants were *BRCA2* c.5771_5774del, p.Ile1924fs among black patients, *BRCA2* c.7934del, p.Arg2645fs among white patients, and *BRCA2* c.8754+1G>A among Indian patients. None of the founder mutations common in white patients was reported in either black, Indian or coloured patients, which explains why black, Coloured and Indian SA patients consistently test negative during targeted screening.
Conclusion. This study highlights unique genetic trends for SA populations and the need for more inclusive targeted tests that are optimal for these populations.

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Breast cancer (BC) is the most commonly occurring cancer in the world, and the second leading cause of cancer mortality among women.[1] It is a multifactorial disease with genetic and environmental factors. The risk of developing BC varies with age, race, lifestyle choices, reproductive choices and genetic predisposition.[2,3] In South Africa (SA), at least 1 in 30 women is said to be at risk of developing BC by the age of 74 years, although the risk differs greatly between SA racial populations.[4] Additionally, the majority of SA BC patients are black African, mainly of luminal subtype, and many patients present at a late stage.[5-7]

Depending on the variant's origin, BC can either be sporadic (SBC) or hereditary (HBC). The SBCs form the majority of BC cases, and develop as a result of a combination of genetics and environmental, biological or physiological triggers.[2,8] The pathogenic sequence variants identified in SBC cases usually accumulate during the course of life, and are often confined to tumours in the affected tissues.[9] These cancers usually develop at an advanced age, are less aggressive and have a relatively good prognosis, and treatment of SBCs includes surgery and taxane-based chemotherapy regimens.[10,11] In contrast, HBCs are characterised by early occurrence, aggression, recurrence and high mortality. They are caused by the presence of germline pathogenic sequence variants in high-risk genes such as BRCA1 DNA repair associated (*BRCA1*) and BRCA2 DNA repair associated (*BRCA2*), which carry a cumulative lifetime risk of 72% and 69%, respectively.[2,12-15] Treatment of HBCs includes prophylactic bilateral mastectomy, oophorectomy and platinum-based chemotherapy regimens.[10,11,16,17] In countries such as the USA, HBCs account for 5 - 10% of BC cases while in SA, HBC prevalence is unknown.[1,8]

The SA population is currently estimated at 60.6 million people spread across nine provinces, and comprises four main racial classifications, which are black Africans, whites, coloureds and Asians/Indians, as introduced by apartheid.[18,19] The black Africans form 81% of the total population and include nine tribes, such as Zulu, Basotho and Venda, all of whom predominantly speak the Bantu languages and have different cultural practices.[18] The coloured population forms 8.8% of SA and comprises individuals of mixed ancestry that includes Europeans, immigrant Asians, Africans and indigenous Khoe and San.[18,19] They are known to speak Afrikaans or English. The white population forms 7.7% and comprises two main groups: Afrikaners, who descend from Dutch, German and French ancestry and speak Afrikaans, and the English who are of British, Irish and European descent, and mainly speak English.[18,19] The Asians/Indians form the minority (2.6%) and constitute descendants of South Africans and South Asia, many of

whom were taken to SA as slaves in the 1900s.[18,20] Although terms such as African or Caucasian are usually subscribed to populations similar to those in SA, the distinction is important, especially in the diagnosis of genetic conditions such as HBC, which rely on the correct ancestry and geography.

According to the 2020 national cancer incidence report, BC is predominant among SA Asian/Indian females, accounting for 41.8% of all cancer cases, and least common among white females, accounting for 21.9%.[4] Furthermore, the risk of BC development varies significantly, where 1 in 12 white females, 1 in 18 Indian females, 1 in 22 coloured females and 1 in 40 black African females are said to have a lifetime risk of developing BC by age 74 years.[4] Clinically, SA black patients are known for early-onset, high-grade tumours, high mortality and triple-negative BCs (TNBCs).[10,21,22] The incidence of BC among black patients has also been increasing steadily over the years.[23,24] Compared with other women of African ancestry, SA black patients reportedly present with fewer cases of TNBCs thanwomen from Botswana, but more cases compared with African-American patients.[25,26] This suggests that women of African descent vary from country to country. For Asians, especially Indians from KwaZulu-Natal Province, patients are known for their extensive family history of breast and/or other cancers.[5,10,22] The white SA population is characterised by late-onset BC, family history and the lowest mortality in SA.[10,22,27] Specifically, Afrikaner and Ashkenazi Jewish HBC patients are known to carry unique pathogenic sequence variants commonly referred to as founder mutations.[14,28]

Thus far, the genetic aetiology of the SA white population has been well defined, especially in BC. Three founder mutations have been identified in Afrikaners, which are *BRCA1* c.1374delC, p.Asp458fs, *BRCA1* c.2641G>T, p.Glu881Ter and *BRCA2* c.7934delG, p.Arg2645fs, and three among Ashkenazi Jews, which are *BRCA1* c.68_69delAG, p.Glu23fs, *BRCA1* c.5266dupC, p.Gln1756fs and *BRCA2* c.5946delT, p.Ser1982Afs.[28-30] Although the incidence of these variants in white SA patients is unknown, they are frequently identified in patients, and therefore have been included in the mutation panel for genetic screening.[31] Another founder mutation, *BRCA2* c.5771_5774del, p.Ile1924fs, was identified in black and coloured patients originating in Western Cape Province.[32] However, some studies reported the absence of this variant in the black population across the country,[33] which suggests that it might be more common within a specific geographical location as opposed to a specific race. In the Indian population, Combrink *et al.*[34] reported that SA Indians were extensively diverse and differed greatly from mainland Indians, so that the understanding of patterns specifically in SA-based Indians remains a challenge.

The observed genetic and demographic diversity among SA populations influences diagnostic approaches in HBC. These tests not only predict risks of disease development for carriers and relatives but also inform treatment approaches to minimise recurrence and cancer-related mortality among patients.[11] Currently, there are two main testing approaches in private and state laboratories, which are targeted and comprehensive screening. The targeted tests screen for the presence of any of the seven SA founder mutations, or a known familial mutation.[35] This option is affordable, ranging between ZAR1 500 and ZAR2 000, and is offered by the vast majority of laboratories in SA.[31,36] Although this approach is affordable and accessible, it is optimal for the white population, specifically Afrikaners and Ashkenazi Jews, while other SA populations with hereditary clinical features consistently test negative for the selected variants, and thus run the risk of misdiagnosis. In contrast, comprehensive screening scans all genes of interest to identify both known and unknown variants. These can be one or more of the high- and medium-risk genes such as *ATM*, *BARD1*, *BRIP1*, *BRCA1*, *BRCA2*, *CDH1*, *CHEK2*, *PALB2*, *PTEN*, *PIK3CA*, *STK11* and *TP53*, depending on the laboratory.[37] This is the most thorough approach, especially for black, coloured and Indian SA patients. However, it is expensive, ranging between ZAR8 000 and ZAR15 000, and is offered by a limited number of laboratories nationwide.[31,36,37] Therefore, targeted screening remains the preferred option for many patients and healthcare workers, and it is imperative to optimise the current targeted tests for all SA populations.

Currently, the HBC genetic data are scarce at both the national and provincial levels. The genetic trends have also not been well defined for individual SA racial populations. This retrospective study aimed to describe common genetic variations in KwaZulu-Natal, a SA province comprising 11.5 million people who are 86.8% black (mainly Zulu) and 7.4% Asian (mainly Indian).[19] In the study, we reviewed the genetic data of patients who consulted at the Genetics Clinic at Inkosi Albert Luthuli Central Hospital (IALCH) between 2011 and 2021. From our data, we identified 30 pathogenic sequence variants in *BRCA1* and *BRCA2*, 4 large genomic rearrangements and 13 variants of unknown significance (VUS). Most importantly, we identified variants that are unique to each population group, and that could be diagnostic targets during genetic screening.

Methods

We retrospectively reviewed the clinical records of BC and high-risk patients attending the Genetics Clinic at IALCH between 2011 and 2021. Inclusion was aligned with the clinical guidelines by the SA Department of Health for genetic screening, which were as follows: BC diagnosis before age 50, TNBC, bilateral or recurrent BC and male BC; for high-risk patients, a record of a strong family history of breast, ovarian or pancreatic cancer, or the presence of a known pathogenic sequence variant in the family.[35] We excluded all patients who did not carry a sequence variant or large genomic rearrangement regardless of pathogenicity, or those who carried variants in genes besides *BRCA1* and *BRCA2* (Fig. 1).

The data collected from files included race, gender, type of tests administered, variants identified and, where available, tumour characteristics. Race was defined as black African, white, Asian/Indian or coloured as per SA classification,[19] and was self-reported by patients. All variants were reported by a single state facility (National Health Laboratory Services (NHLS)), which utilised various molecular techniques over 11 years as technology advanced. These were polymerase chain reaction, protein truncation test, single-stranded conformation

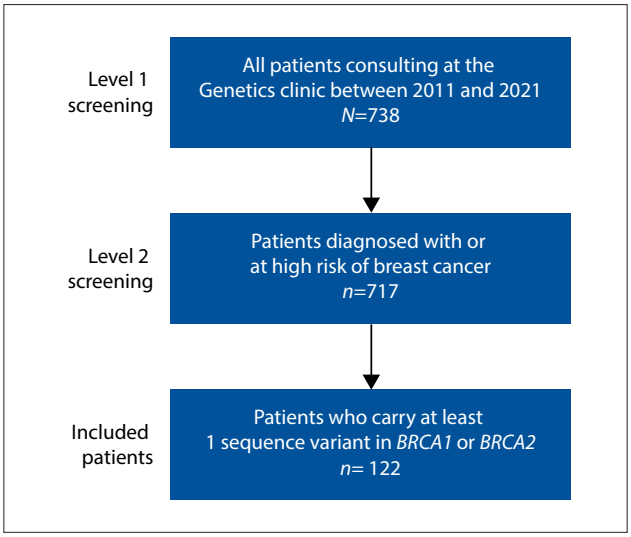

Fig. 1. Selection criteria for patients included in the study.

polymorphism, high-resolution melting analysis, multiplex ligation-dependent probe amplification and next-generation sequencing. The target for variant screening was *BRCA1* and *BRCA2* genes.

The variants' nomenclature and classification were as reported by the laboratory, and were verified using the following databases: ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/), dbSNP (https://www.ncbi.nlm.nih.gov/snp/), Varsome (https://varsome.com/), ClinGen (https://clinicalgenome.org/) and MedGen (https://www.ncbi.nlm.nih.gov/medgen//). The variants were aligned to *BRCA1* NM_007294.3 and *BRCA2* NM_000059.3 reference sequences, which served as controls. Peer-reviewed literature was also used to identify variants previously reported in SA. Ethical approval to conduct this research was granted by the Biomedical Research Ethics Committee, University of KwaZulu-Natal (ref. no. BREC/00000613/2019).

Results

Cohort and epidemiology

A total of 738 patients who presented at IALCH were screened, from whom 122 were selected for inclusion in the study (Fig. 1, Table 1). The included patients were aged between 21 and 81 years and comprised 40.7% SA Indians, 34.1% blacks (mainly Zulu), 18.7% whites and 5.7% coloureds. Males were a minority, constituting 3.3%. The majority (62.6%) were diagnosed with invasive ductal carcinoma of no special type (IDC-NST), while 4.9% were bilateral or recurrent BCs. The molecular subtypes of tumours were indicated in TNBC cases, and these constituted 19.5% of the patients. At least 69.9% of the patients reported a positive family history, of whom the majority were white and Indian. Genetically, 52.8% of patients carried at least one pathogenic sequence variant, 18.7% carried at least one VUS, 19.5% carried benign/likely-benign sequence variants and 12.3% carried novel SA variants.

Within the black population, at least 90.5% of the patients were diagnosed before age 50, 21.4% were TNBCs, 38.1% had a positive family history and 50% carried a pathogenic sequence variant. For white patients, 56.5% were diagnosed before 50 years, 26.1% were diagnosed with TNBC, 91.3% had a positive family history and 60.9% carried a pathogenic sequence variant. Among Indian patients, 57.1% were diagnosed before 50 years, 12% were TNBC cases, 83.7% had a positive family history and 55.1% carried a pathogenic sequence variant. The coloured patients comprised 75% early-onset BC, 33% were TNBC patients, all patients had a positive family history and 37.5% carried a pathogenic sequence variant.

Genomic variants spectrum

A total of 30 pathogenic *BRCA1* and *BRCA2* sequence variants were identified using either targeted or comprehensive screening (Table 1). Twenty patients carried 15 *BRCA1*, of which five were frameshift, four were nonsense, three were splice donor, one was a deletion, one was a missense and one was an intronic variant. According to ClinVar, three of the variants were classified as likely pathogenic (class 4) while 12 were pathogenic (class 5). Most variants (9/15) were identified in the Indian population, five were identified in black patients, one variant in white patients and one in coloured patients (Table 2). Only one variant was shared between black and Indian patients, where each population had one carrier. In *BRCA2*, we reported 15 pathogenic sequence variants in 31 patients, where 7 variants were reported in 15 black patients, 7 in 12 Indians, 7 in 12 whites and 2 variants in 2 coloured patients. Most *BRCA2* variants were nonsense mutations (8/15), followed by frameshift mutations (4/15), while the rest were missense (1/15), synonymous (1/15) and a splice site donor (1/15) (Table 3). All variants were pathogenic (class 5) as per ClinVar classification. One variant was identified in both black and Indian patients, and another was identified in both whites and Indians.

There were 13 VUSs reported in 19 patients comprising 11 black, 4 white and 4 Indian patients (Table 4). Four of the variants were exclusive to black patients, three variants were exclusive to white and four variants were exclusive to Indian. At least one variant was identified in both black and Indian patients, while another was in both blacks and whites. We also reported 13 novel variants in a total of 15 patients comprising 5 black, 6 Indian and 4 coloured patients (Table 5). Nine of the variants were in *BRCA1*, while four variants were in *BRCA2*. The majority were missense variants (5/13), followed by synonymous variants (3/13), and there was at least one frameshift, one intronic variant, one deletion and one insertion. Two of the variants have been reported in international databases (rs876660684 and rs11571673) by the NHLS and are currently classified as likely-benign. However, they have not been reported in any other populations across the globe. Additionally, the laboratory classified two variants as pathogenic and two as VUS.

We reported five non-pathogenic *BRCA1* variants in 20 patients comprising 2 white, 15 Indian and 3 coloured patients (Appendix 1: https://www.samedical.org/file/2214). Two of the variants were missense, two were synonymous and one was in an intron. Three of the variants were classified as benign while two were likely benign. In *BRCA2*, a total of 19 variants comprising 12 benign and 7 likely-benign variants were reported in 71 patients. The majority of patients were Indian (62%), followed by white (14.1%), then black (12.7%), and the lowest number was coloured patients (11.3%). Seven of the variants occurred in intronic regions, six were missense, four variants were synonymous, one was nonsense and one was in the 5' untranslated region (5'UTR).

Large genomic rearrangements

Four large genomic rearrangements were identified in five patients, which were *BRCA1* exon 13 duplication [NG_005905.2(LRG_292): g.(141369_141497)dup] reported in a 45-year-old white TNBC patient, *BRCA1* exon 17 deletion [NG_005905.2(LRG_292): g.(154032_154111)del] in two Indian patients: a 58 year-old high-risk and 36-year-old BC patient, *BRCA1* exon 21 deletion [NG_005905.2(LRG_292): g.(168789_168864)del] identified in a 34-year-old black TNBC patient and the *BRCA2* gene deletion [NG_012772.3(LRG_293): g(5982_882910)del] identified in an Indian patient diagnosed with bilateral BC.

Discussion

SA genetic services have relied for many years on targeted tests that screen for seven SA founder mutations. For the black, Indian and coloured populations, this option has been unreliable, as patients with hereditary clinical characteristics consistently test negative. This is because six of the seven variants are exclusively common to the white population, while one variant was reported in mainly the Western Cape Province. This indicates the risk of misdiagnosis among blacks, coloureds and Indians, which impacts treatment approaches offered to patients and an oversight on preventive measures for carrier relatives. Our study outlines variants reported over a 12-year period, which were identified using various screening methods and are potential diagnostic targets, especially for blacks, coloureds and Indians.

Patients and genetic tests

The cohort mainly comprised black and Indian populations, which was representative of KwaZulu-Natal's general population.[19] The number of males was low compared with females, which was consistent with both SA and global prevalences of male BC. For female patients, the primary reasons for referral varied between

Table 1. The number and characteristics of breast cancer patients from KwaZulu-Natal Province, South Africa, 2011 - 2020

Characteristic	Black (*n*=42)	White (*n*=23)	Coloured (*n*=7)	Indian (*n*=50)	Total (*n*=122)
Gender					
Female	41	22	7	48	118
Male	1	1	0	2	4
Age of diagnosis (years)					
Mean	37.6	47.7	42.9	47.4	43.7
Range	21 - 76	32 - 67	32 - 61	23 - 81	21 - 81
Median	36.5	45	39	48	41
Diagnosis					
IBC-NST	30	13	1	34	78
Triple-negative BC	9	6	3	6	24
Bilateral/recurrent BC	1	1	1	3	6
High-risk	2	3	2	7	14
Family history					
Present	16	21	7	41	85
Absent	26	2	0	9	37
Genomic variations in patients					
Pathogenic/likely pathogenic	20	13	3	25	61
VUS	11	4	0	5	20
Benign/likely benign	9	12	8	43	72
Novel SA variants	5	0	4	6	15
Large genomic rearrangements	1	1	0	3	5

IBC-NST = invasive breast carcinoma of no special type; BC = breast cancer; VUS = variant of unknown significance; SA = South Africa.

Table 2. Actionable *BRCA1* sequence variants identified in KwaZulu-Natal Province breast cancer patients, 2011 - 2021

				Race of carrier, *n*			
Variant	rs number	Effect	Pathogenicity	Black	White	Indian	Coloured
NC_000017.11:g.(?_43045658)_(43045822_?)del	no rs	Deletion	Pathogenic	1			
NM_007294.4(BRCA1):c.68_69del, p.Glu23fs	rs80357914	Frameshift	Pathogenic	1		1	
NM_007294.4(BRCA1):c.191G>A, p.Cys64Tyr	rs55851803	Missense	Pathogenic			3	
NM_007294.4(BRCA1):c.1360_1361del, p.Glu453_Ser454Ter	rs80357969	Frameshift	Pathogenic			3	
NM_007294.4(BRCA1):c.2641G>T, p.GLu881Ter	rs397508988	Nonsense	Pathogenic		1		
NM_007294.4(BRCA1):c.3108del, p.Phe1036fs	rs80357841	Frameshift	Pathogenic			1	
NM_007294.4(BRCA1):c.3593T>A, p.Leu1198Ter	rs397509095	Nonsense	Pathogenic			1	
NM_007294.4(BRCA1):c.3756_3759del, p.Ser1253fs	rs80357868	Frameshift	Pathogenic			1	
NM_007294.4(BRCA1):c.4309del, p.Ser1437fs	rs886040223	Frameshift	Pathogenic			1	
NM_007294.4(BRCA1):c.4327C>T, p.Arg1443Ter	rs41293455	Nonsense	Pathogenic				1
NM_007294.4(BRCA1):c.4987-5T>A	rs397509214	Intron variant	Pathogenic/likely pathogenic	1			
NM_007294.4(BRCA1):c.5332+1G>C	rs80358041	Splice donor	Pathogenic/likely pathogenic	1			
NM_007294.4(BRCA1):c.5467+2T>G	rs80358009	Splice donor	Likely pathogenic	1			
NM_007294.4(BRCA1):c.5468-1G>A	rs80358048	Splice donor	Pathogenic			1	
NM_007294.4(BRCA1):c.5484_5485del, p.Cys1828_Glu1829delinsTer	rs886038046	Nonsense	Pathogenic			1	

populations, where black patients were mainly diagnosed with early-onset disease and TNBCs, while white, Indian and coloured patients presented with a positive family history of breast and related cancers. The average age of disease onset in black patients was 37.6 years (standard deviation (SD) 11.2), which was ~10 years lower than that of Indians (mean 47.4 years, SD 13.3) and whites (mean 47.7 years, SD 10.2). In all populations, both bilateral BC and recurrent BCs were rare, while high-risk patients were predominant among Indians. The majority of mutation carriers were Indian, and they carried 17 pathogenic sequence variants.

Pathogenic sequence variants

Patients carried a total of 30 pathogenic *BRCA* variants, of which 1 was synonymous, 1 was a deletion, 2 were missense, 9 were frameshift and 12 were nonsense, all located within exons. Additionally, there were 4 variants found in the splice site and 1 in an intron. The most commonly reported variant was *BRCA2* c.7934del, p.Arg2645fs (rs80359688) reported in 9 white patients. It is a SA founder mutation common in Afrikaners, a subset of the SA white population.[30] It was previously identified in SA white and coloured patients in multiple studies,[27,30,32,38] and forms part of variant panels in targeted screening

Table 3. Actionable *BRCA2* variants identified in KwaZulu-Natal Province breast cancer patients, 2011 - 2021

				Race of carrier, *n*			
Variant	**rs number**	**Effect**	**Pathogenicity**	**Black**	**White**	**Indian**	**Coloured**
NM_000059.4(BRCA2):c.93G>A, p.Trp31Ter	rs80359214	Nonsense	Pathogenic	1			
NM_000059.4(BRCA2):c.582G>A, P.Trp194Ter	rs80358810	Nonsense	Pathogenic	4		1	
NM_000059.4(BRCA2):c.1261C>T, p.Gln421Ter	rs80358419	Nonsense	Pathogenic	2			
NM_000059.4(BRCA2):c.4003G>T, p.Glu1335Ter	rs747070579	Nonsense	Pathogenic			1	1
NM_000059.4(BRCA2):c.4936G>T, p.Glu1646Ter	rs886038111	Nonsense	Pathogenic			1	
NM_000059.4(BRCA2):c.5279C>G, p.Ser1760Ter	rs80358751	Nonsense	Pathogenic			1	
NM_000059.4(BRCA2):c.5771_5774del, p.Ile1924fs	rs80359535	Frameshift	Pathogenic	5			
NM_000059.4(BRCA2):c.5946del, p.Ser1982fs	rs80359550	Frameshift	Pathogenic		2	1	1
NM_000059.4(BRCA2):c.6761_6762del, p.Phe2254fs	rs80359624	Frameshift	Pathogenic	1			
NM_000059.4(BRCA2):c.7934del, p.Arg2645fs	rs80359688	Frameshift	Pathogenic		9		
NM_000059.4(BRCA2):c.8165C>G, p.Thr2722Arg	rs80359062	Missense	Pathogenic			1	
NM_000059.4(BRCA2):c.8754+1G>A	rs397508006	Splice donor	Pathogenic			6	
NM_000059.4(BRCA2):c.9105T>A, p.Tyr3035Ter	rs886040819	Nonsense	Pathogenic	1			
NM_000059.4(BRCA2):c.9117G>A, p.Pro3039=	rs28897756	Synonymous	Pathogenic	1			
NM_000059.4(BRCA2):c.9382C>T, p.Arg3128Ter	rs80359212	Nonsense	Pathogenic		1		

Table 4. Variants of unknown significance identified in patients from KwaZulu-Natal Province, South Africa, 2011 - 2021

			Carriers, *n*		
Variant	**rs number**	**Effect**	**Black**	**White**	**Indian**
NM_007294.4(BRCA1):c.503A>C, p.Lys168Thr	rs273901743	Missense	1		1
NM_007294.4(BRCA1):c.884A>G, p.Asp295Gly	rs772684048	Missense		1	
NM_007294.4(BRCA1):c.1724A>G, p.Glu575Gly	rs111539978	Missense	4	1	
NM_007294.4(BRCA1):c.2120G>A, p.Gly707Asp	rs80357192	Missense	1		
NM_007294.4(BRCA1):c.5005G>A, p.Ala1669Thr	rs80357087	Missense			1
NM_000059.4(BRCA2):c.467A>G, p.Asp156Gly	rs68071147	Missense	1		
NM_000059.4(BRCA2):c.2465G>A, p.Cys822Tyr	no rs	-			1
NM_000059.4(BRCA2):c.2581C>A, p.GIn861Lys	rs773356478	Missense			1
NM_000059.4(BRCA2):c.4795AAT[1], p.Asn1600del	rs276174851	Deletion	3		
NM_000059.4(BRCA2):c.7051G>A, p.Ala2351Thr	rs80358930	Missense		1	
NM_000059.4(BRCA2):c.7759C>T, p.Leu2587Phe	rs56335340	Missense		1	
NM_000059.4(BRCA2):c.7955T>G, p.Val2652Gly	rs1555286868	Missense			1
NM_007294.4(BRCA1):c.24T>C, p.Val8_Glu9=	rs2055739304	Synonymous	1		

by pathology laboratories for SA patients. In addition to variants identified among whites, an Afrikaner founder mutation *BRCA1* c.2641G>T, p.GLu881Ter (rs397508988) was reported in an early-onset TNBC patient, while Ashkenazi Jewish founder mutation *BRCA2* c.5946del, p.Ser1982fs (rs80359550) was reported in a high-risk patient, and another patient diagnosed with early-onset disease. The fourth variant among whites was *BRCA2* c.9382C>T, p.Arg3128Ter (rs80359212), which was identified in an early-onset patient. This variant had previously been reported in a coloured SA patient,[39] which did not come as a surprise because of shared ancestry. Similarly, *BRCA1* c.4327C>T, p.Arg1443Ter (rs41293455), which was diagnosed in a young TNBC coloured patient, had also previously been reported in a 34-year-old SA Indian patient, which demonstrates genetic diversity among the SA coloured population.

Among black patients, five *BRCA1* and seven *BRCA2* pathogenic sequence variants were reported in 20 patients. Of those, *BRCA2* c.5771_5774del, p.Ile1924fs (rs80359535) was the most commonly diagnosed in five patients. It was first described in SA by van der Merwe *et al.*[32] in black and coloured patients from the Western Cape Province, although contradictory results were reported in other

Table 5. Novel South African variants

Variant	Effect	Pathogenicity	ClinVar Reference	Patients, *n*		
				Black	Indian	Coloured
NM_007294.4(BRCA1):c.125dup, p.Phe43fs	Frameshift	Pathogenic			1	
NM_007294.4(BRCA1):c.212+66A>G	Missense	-		1		
NM_007294.4(BRCA1):c.414T>C, p.Lys99=	Synonymous	-			1	
NM_007294.4(BRCA1):c.663A>C, p.(=)	Synonymous	-			1	
NM_007294.4(BRCA1):c.1812A>C, p.Lys604Asn	Intron variant	VUS				1
NM_007294.4(BRCA1):c.3948C>T, p.Phel1316=	Synonymous	-		1		
NM_007294.4(BRCA1):c.4333C>G, p.Pro1445Ala	Missense	Likely benign	rs876660684		1	1
NM_007294.4(BRCA1):c.5068G>A, p.Ala1690Thr	Missense	-			1	
NM_007294.4(BRCA1):c.5075_13T>G	Missense	-			1	
NM_000059.4(BRCA2):c.6842-73T>A		Likely benign	rs11571673			2
NM_000059.4(BRCA2):c.5082_5083insA, p.Glu1695Argfs	Insertion	-		1		
NM_000059.4(BRCA2):c.5893C>G, p.Leu1965Val	Missense	VUS		1		
NM_000059.4(BRCA2):c.9833_9842del, p.Pro3278fs	Deletion	Pathogenic		1		

VUS = variant of unknown significance.

provinces. Currently, it is included in the targeted sequencing panel in some pathology laboratories while excluded by others.[31,36] Additionally, *BRCA2* c.582G>A, P.Trp194Ter (rs80358810) was reported in four black patients and one Indian patient. It was previously reported in similar populations in SA,[29,34] as well as in Fanconi anaemia cases.[40] The other 10 variants were rare and had been reported in previous SA populations, except for NC_000017.11:g.(?_43045658)_(43045822_?)del, a pathogenic *BRCA1* missense variant identified in a TNBC patient with early-onset disease. This variant has been previously reported in African-American BC patients, although very rarely.[24]

The Indian patients presented a broader spectrum of variants owing to their diversity. Overall, 16 variants were reported in 25 patients, of which *BRCA2* c.8754+1G>A (rs397508006) was the most commonly identified. It is a splice donor previously reported among SA Indians,[29,34,41] Europeans[42] and Danish patients.[43] Additionally, *BRCA1* c.191G>A, p.Cys64Tyr (rs55851803) and *BRCA1* c.1360_1361del, p. p.Glu453_ Ser454Ter (rs80357969) were each reported in three patients. These variants had previously been described in SA Indians, although with low occurrence.[34,41] Our study is the first to report this frequency in a single SA province, which suggests that the prevalence of these three variants may be higher nationwide. Perhaps more investigations may be conducted, and the variants be considered for founder candidacy. The rest of the reported variants were rare and had been described in SA, except for *BRCA1* c.125dup, p.Phe43fs, which has only been submitted to the ClinVar database but not described. It was identified in a 57-year-old patient with bilateral BC, who had no family history of breast or any other cancer.

In addition, there were 13 SA novel variants reported among black, Indian and coloured patients, which comprised five missenses, three synonymous, one insertion, one deletion, one frameshift and one intronic variant (Table 3). Two of the variants have been reported to international databases and have been classified as likely benign. To date, there have not been any submissions from other countries besides SA. There were four other variants that were described by the reporting laboratory. These were *BRCA1* c.125dup, p.Phe43fs and *BRCA2* c.9833_9842del, p.Pro3278fs, which were classified as pathogenic, and *BRCA1* c.1812A>C, p.Lys604Asn and *BRCA2* c.5893C>G, p.Leu1965Val, which were classified as VUS. They have neither been reported in international databases nor in the literature. The variants were rare in our cohort, where each had a single occurrence except for *BRCA2* c.6842-73T>A, which was reported in two coloured patients. We encourage further investigations into these variants, especially their pathogenicity, so that appropriate action may be taken in carriers.

Non-pathogenic variants

Benign and likely-benign sequence variants were more common among Indians compared with other populations. In total, we recorded 5 *BRCA1* and 19 *BRCA2* variants in 8 coloured, 9 black, 12 white and 44 Indian patients (Appendix 1). *BRCA2* c.9875C>T, p.Pro3292Leu (rs56121817) was frequently reported among black patients, while *BRCA1* c.4308T>C, p.Ser1436_Ser1437= (rs1060915) and *BRCA1* c.4837A>G, p.Ser1613Gly (rs1799966) were the most common among Indians.

Interestingly, three unrelated Indian patients carried *BRCA1* c.442-34C>T, *BRCA1* c.4308T>C, p.Ser1436=, *BRCA1* c.4837A>G, p.Ser1613Gly, *BRCA2* c.1114A>C, p.Asn372His and *BRCA2* c. 8755-66T>C variants, concurrently. Two of the patients were 38 years old and diagnosed with IDC-NST, while one was a 35-year-old patient diagnosed with TNBC. Another combination was of *BRCA1* c.4308T>C, p.Ser1436=, *BRCA1* c.4837A>G, p.Ser1613Gly, *BRCA2* -26G>A, *BRCA2* c.681+56C>T and *BRCA2* c.7242A>G, p.Ser2414=, which were reported in four Indian patients. The patients were all young, with an age range of 46 - 56 years, and were all diagnosed with IDC-NST. All seven patients reported a positive family history of BC, and while their clinical characteristics were indicative of HBC, they all tested negative for pathogenic sequence variants during comprehensive testing. While these benign and likely-benign *BRCA* sequence variants carry a very low risk of carcinogenesis, it will be interesting to investigate their effect on compound heterozygosity.

Large genomic rearrangements

We identified three *BRCA1* and one *BRCA2* large genomic rearrangement in five young patients. The *BRCA1* exon 17 deletion, which was identified in a 36-year-old Indian BC patient and her 58-year-old relative at high risk of BC, had been described in young BC patients of Dutch and Polish descent.[44,45] Similarly, we reported the deletion of *BRCA1* exon 21 in a 34-year-old black woman diagnosed with TNBC. This is a common rearrangement previously reported in populations such as the Czech, the Dutch and the Pakistani.[44,46,47] Another deletion, a whole *BRCA2* gene, was reported in a 41-year-old Indian patient presenting with bilateral BC. It is a very rare deletion mostly described in non-human species

and *in silico*.[48,49] Lastly, a 45-year-old white female diagnosed with TNBC, who had a positive family history of BC, carried a *BRCA1* exon 13 duplication. This has been described in Swedish and Italian populations where they were also reportedly rare.[50,51] In SA, these variants have been reported before with similarly low prevalence, and all carriers also presented with an early-onset and aggressive disease.[29,41]

Study limitations

The reported variants were identified using different molecular tests within different populations and over different time periods. Some tests screened for known variants, while comprehensive tests screened the entire genes. This suggests the probability of variant underreporting, especially in targeted testing.

Conclusion

This study reports numerous *BRCA1* and *BRCA2* pathogenic sequence variants identified in various SA populations. The majority of variants were exclusive to specific races, which makes them ideal candidates for the diagnosis of HBC. Considering the limitations of SA targeted screening, these variants can be included to optimise the tests, especially for black and Indian populations in KwaZulu-Natal Province. For novel variants identified in this study, we encourage further research and reporting to international databases to aid in the classification of each variant. We also encourage a nationwide study describing black and Asian patients, as our data represent the black African and Indian subpopulations from KwaZulu-Natal only.

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