Genetic counselling, testing and management of hereditary breast cancer in South Africa

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DECLARATION

I, Tabitha Osler, declare that this thesis is of my own, unaided work. Where others have contributed and assisted, they have been duly acknowledged. It is being submitted for the Degree of Doctor of Philosophy at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.

Signed on the 11th day of August 2025

PRESENTATIONS ARISING FROM THIS RESEARCH PROJECT

Osler T. *Gene panel testing for hereditary breast cancer across South Africa's diverse ancestry groups.* Division of Human Genetics Seminar Series, Johannesburg, South Africa, 5th March 2025 (oral)

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PUBLICATIONS ARISING FROM THIS RESEARCH PROJECT

The research findings arising from this study were drafted and presented in four manuscripts. Three of these have been published in international peer-reviewed scientific journals. The signed co-author agreement/supervisor declaration for the use of each manuscript can be found in Appendix A.

1. **Osler, T. S.,** Brandenburg, J. T., Schoeman, M., Chen, W. C., Urban, M. F., & Mathew, C. G. (2024). Prevalence and Reclassification of Genetic Variants in South African Populations with Breast Cancer. *Genes, Chromosomes and Cancer*, *63*(9), e23275. https://doi.org/10.1002/gcc.23275

2. **Osler, T. S.,** Schoeman, M., Pretorius, W. J. S., Mathew, C. G., Edge, J., & Urban, M. F. (2025). Application of genetic testing criteria for hereditary breast cancer in South Africa. *Breast Cancer Research and Treatment*, 210, 477-486. https://doi.org/10.1007/s10549-024-07585-3

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4. Prevalence of variants in breast cancer susceptibility genes in Black South African women diagnosed aged 50 or younger

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ABSTRACT

Breast cancer (BC) is common in South Africa (SA), with some cases due to a pathogenic or likely pathogenic (P/LP) variant in a BC susceptibility gene. Data on hereditary BC testing and management in SA remains limited. The aim of this study was to assess the prevalence of P/LP variants and variants of uncertain significance (VUS) in BC genes in SA populations with BC, and to investigate genetic counselling and testing (GCT) and the subsequent clinical management pathway in BC probands and their family members. The study was based on records and sequence data from 534 BC patients who attended GCT in the Western Cape and 115 of their relatives, and on 272 Black SA women with BC aged ≤50 recruited by the Johannesburg Cancer Study (JCS).

P/LP prevalence was 18.1% in BC probands from the GCT group who had gene panel testing at Invitae Laboratory in the USA, and similar across ancestry groups. Broadening the gene test panel from 9 to 84 genes yielded few additional actionable variants but many more VUS which had a higher prevalence in the Black and Mixed ancestry participants. Of 611 VUS detected, 27% could be reclassified using frequency data from African genomes. P/LP variant prevalence in a population study of Black African women from the JCS aged ≤50 years with BC was 10.3%.

The SA National Department of Health (NDOH) testing guidelines were evaluated in 376 participants who met criteria, with P/LP detection rates calculated and multivariate analysis used to identify criteria most strongly associated with P/LP detection. P/LP prevalence was 19.9%, with higher detection in those meeting ≥2 criteria. Family history and Black African ancestry were associated with P/LP variants in *BRCA1/2*.

The uptake of genetic testing and risk-reducing measures, and factors influencing test uptake were examined in probands and family members from the GCT study. Test uptake was high in probands but much lower in family members, where it was associated with proband family history, being female, and being a first-degree relative. Risk-reducing mastectomy was the most accepted intervention.

The outcomes from this study have implications for future testing strategy and clinical management in the SA population. Restricting testing to genes associated with BC will

optimise the detection of clinically relevant variants and minimize uncertain results. African genomic data has the potential to resolve a significant proportion of VUS. The population study of Black women with BC indicates a substantial prevalence of P/LP variants in women diagnosed ≤ 50 years. The assessment of the NDOH guidelines demonstrated its usefulness in women of diverse ancestries. Limited uptake of cascade testing and variable uptake of risk-reducing options reduced the potential impact of the GCT program.

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TABLE OF CONTENTS

LIST OF FIGURES

Supplementary Figure

LIST OF TABLES

Supplementary Tables:

LIST OF ABBREVIATIONS

ACMG/AMP	American College of Medical Genetics and Genomics and the Association of Molecular Pathology
AF	Allele frequency
AGVP	African genome variation project
BC	Breast cancer
BCT	Breast conserving therapy
BED	Browser extensible data
BSO	Bilateral salpingo-oophorectomy
CANSA	Cancer Association of South Africa
CI	Confidence interval
ClinGen	Clinical Genome Resource
CRAM	Compressed reference-oriented alignment map file
ECM	Enterprise content management
EGA	European Genome-Phenome Archive
ER	Estrogen receptor
FDR	First-degree relative
GATK	Genome analysis toolkit
GCT	Genetic counselling and testing
GVCF	Genomic variant call format
gnomAD	Genome Aggregation Database
GRCh37	Genome Reference Consortium Human Build 37
GRCh38	Genome Reference Consortium Human Build 38
H3Africa	Human Hereditary and Health in Africa project
HGVS	Human Genome Variation Society,
HER2	Human epidermal growth factor receptor 2
HIC	High income country
HTS	high-throughput sequencing
JCS	Johannesburg Cancer Study
LMIC	Low-to-middle-income-country
MANE	Matched annotation from NCBI and EMBL-EBI
MRI	Magnetic resonance imaging
NDOH	National Department of Health (South Africa)
NCCN	National Comprehensive Cancer Network (USA)
NHLS	National Health Laboratory Service (South Africa)
NHI	National Health Insurance (South Africa)
NGS	Next-generation sequencing
OR	Odds ratio
PACS	Picture archiving and communication system
P/LP	Pathogenic or likely pathogenic
PR	Progesterone receptor
RRM	Risk-reducing mastectomy

SA	South Africa
SANAS	South African National Accreditation System
SBIMB	Sydney Brenner Institute for Molecular Bioscience
SDR	Second degree relative
SF	Secondary findings
SSA	Sub-Saharan Africa
SVI	Sequence Variant Interpretation working group (ClinVar)
TAH	Tygerberg Academic Hospital
TDR	Third degree relative
TNBC	Triple negative breast cancer
VCEP	Variant curation expert panel
VCF	Variant call format file
VEP	Variant effect predictor
VUS	Variant of uncertain significance
WC	Western Cape
WGS	Whole genome sequencing
USA	United States of America
USD	United States Dollar
UW	University of Washington

CHAPTER 1

INTRODUCTION

1.1 BREAST CANCER IN THE SOUTH AFRICAN POPULATION

The South African population is highly diverse, encompassing a wide range of cultures, origins, languages and religions. The South African National Census describes South Africa's three largest ancestry groups as Black African (80.2%), Coloured (8.8%) and White (7.7%). The Indian/Asian population, although the fourth largest group, makes up only 2.7% of the national population (StatsSA, 2022a). The term 'Coloured' (referred to as Mixed Ancestry in this study) refers to peoples with a unique cultural identity who have genetic ancestry from Khoisan, Black African, European and Asian populations (Choudhury et al., 2017; de Wit et al., 2010). The Black African population is an ethnically diverse group of Bantu-speaking peoples who migrated south from west Africa beginning some 2000 years ago (Choudhury et al., 2017). White South Africans are descendants of European settlers, predominantly from the Netherlands (Afrikaans speakers) and the United Kingdom, but also from France and Germany. White Afrikaans speakers have about 5% non-European admixture (Hollfelder et al., 2020).

Breast cancer (BC) is one of the most common cancers among South African women of all ancestry groups, with cumulative incidence estimates to age 74 of 1 in 43 for Black African women, 1 in 18 for women of Indian/Asian and Mixed Ancestry, and 1 in 10 for White women (National Cancer Registry, 2022). The cumulative incidence among White South African women aligns with figures seen in Western high-income countries (HICs) (Bray et al., 2024). Although cumulative incidence is currently lower among Black South African women, it is rising rapidly. This increase is attributed to an epidemiological shift toward a more Westernized lifestyle, which introduces BC risk factors such as changes in reproductive patterns, diet, alcohol consumption, and body weight (Joko-Fru et al., 2020). While age-standardized rates show higher BC incidence among women over 50 in both African and HIC settings, Africa's younger demographic means that most cases on the continent occur in young women. This, together with the rising BC rates across successive generations, can create an impression that younger women in African settings have a higher risk than those in HIC, but more likely reflects the ongoing epidemiological transition in the population (Joko-Fru et al., 2020).

Although incidence rates of BC in Black South African women are lower than in other population groups, there is a disproportionate number of deaths from the disease. This is predominately due to late presentation and inadequate access to treatment (McCormack et al., 2013; Yip et al., 2015), as well as the absence of a national BC screening service in the country (Lipschitz, 2018). The South African National Department of Health (NDOH) has published guidelines for the management of BC which include recommendations for surgery, radiotherapy and systemic treatments (National Department of Health, 2018). However high patient numbers, a shortage of providers and other resource limitations restrict the availability of these services (O'Neil et al., 2019). In addition, new systemic therapies that have led to increased survival in HIC are frequently unavailable in South Africa and other SSA (Yip et al., 2015).

Breast cancer is caused by a combination of both environmental and genetic factors (Momenimovahed & Salehiniya, 2019). The genetic contribution is partly through multiple common gene variants which each confer a small increased risk of the disease (Maxwell & Nathanson, 2013); or less often through rare disease-causing germline variants that confer a moderate to high risk of BC. This PhD study focused specifically on the estimated 5 to 6% of BC caused by rare pathogenic or likely pathogenic (P/LP) variants in BC susceptibility genes (Breast Cancer Association Consortium et al., 2021; C. Hu et al., 2021).

1.2 THE DISCOVERY OF HEREDITARY BREAST CANCER

Familial BC was first described in 1866 by French physician, Pierre Paul Broca, who observed that numerous individuals from different generations in his wife's family were affected with the disease (Krush, 1979). However it wasn't until 1974 that Mary Claire King began her search for the underlying genetic cause of familial BC and in 1990 her team used linkage studies to identify a high-risk region on chromosome 17q in families with early-onset BC (J. M. Hall et al., 1990). This led to the eventual cloning of *BRCA1* in 1994 (Miki et al., 1994) and soon after this, *BRCA2* on chromosome 13q, was also cloned (Wooster et al., 1995).

The first commercial diagnostic test for *BRCA1* and *BRCA2* genes was offered by Myriad Genetics in 1996, but widespread access to testing was delayed due to gene patents that Myriad obtained in the USA (Williams-Jones, 2002). In 2013 however, it was ruled by the U.S.

Supreme Court that genes, which are naturally occurring, cannot be patented, allowing other laboratories to freely offer *BRCA1/2* testing (*Assoc. For Molecular Pathology v. Myriad Genetics, Inc., 569 U.S. 576*, 2013). Since then, testing for *BRCA1/2* has become widely accessible in high-income countries due to the development of next-generation sequencing (NGS). NGS, introduced from 2004, enables high throughput DNA sequencing and has, over time, substantially reduced the cost of testing. This has changed the genetic testing landscape from testing only one or two genes to allowing the development of gene panels that can test hundreds of genes simultaneously (T. Hu et al., 2021).

1.3 RISKS CONFERRED BY BREAST CANCER SUSCEPTIBILITY GENES

P/LP variants in *BRCA1/2* are the most frequent cause of hereditary BC across all population groups (Tung et al., 2016). *BRCA1* P/LP variants confer an increase in BC risk in European populations from the population risk of 12% to between 55% and 72% by age 70, while *BRCA2* associated risks are slightly lower at 45% to 69% (Kuchenbaecker et al., 2017). In addition to the high risk of BC, *BRCA1* carriers are more likely to develop tumours with a high histologic grade that are estrogen receptor (ER) negative, progesterone receptor (PR) negative and less likely to have human epidermal growth factor receptor 2 (HER2) overexpression. This pattern of hormone receptor negativity on tumour cells, has led to these cancers being classified as triple-negative breast cancer (TNBC) (Mavaddat et al., 2011). TNBC account for 15-20% of sporadic tumours in Western populations but 63-90% of *BRCA1*-associated tumours (Honrado et al., 2006). TNBC is an aggressive BC subtype and lacks targeted treatment options resulting in it having a poor prognosis (Zagami & Carey, 2022). In contrast, *BRCA2*-associated tumours have a histological profile more similar to that of sporadic tumours (Honrado et al., 2006). Lifetime risks for other cancers associated with *BRCA1* and *BRCA2* include ovarian cancer (~40% and ~17% respectively), male BC (~1% and ~8%), prostate cancer (~30% and ~60%) and pancreatic cancer (~2% and ~4%) (Petrucelli et al., 2025).

Germline genetic variants in many other genes have been suggested to confer an increased risk of BC in different studies. This created a need for the systematic collection of evidence to determine the validity of reported associations. To this end, the Clinical Genome Resource (ClinGen) established Gene Curation Working Groups to collate available evidence and determine if associations are supported, disputed or uncertain. High-penetrance genes, *CDH1, PALB2, PTEN, STK11* and *TP53,* and moderate penetrance genes, *ATM, BARD1, CHEK2,*

NF1, RAD51C, RAD51D, have been confirmed to increase the risk of BC (ClinGen, n.d.). High-penetrance genes confer BC risks of up to 80%, while moderate penetrance genes confer risks of between 15 and 30%. Each of these genes has its own "risk profile" and may also be associated with increased risks of cancer in other organs (NCCN Guidelines, 2024a).

1.4 PREVALENCE OF PATHOGENIC VARIANTS IN BC SUSCEPTIBILITY GENES

Studies from populations of predominantly European origin have investigated the prevalence of germline P/LP variants in women with BC, selected either by age at diagnosis, positive family history of breast/ovarian cancer, and/or tumour histology. In these studies, P/LP variants in genes associated with BC have been detected in 7 to 15% of cases (Buys et al., 2017; Couch et al., 2017; Tung et al., 2015). These studies provide useful information about rare variants in high-risk women but are not representative of the prevalence of P/LP variants in the general population of women with BC. A recent USA population-based case-control study which aimed to overcome this limitation found a P/LP variant carrier rate of 5.03% in 28 genes (C. Hu et al., 2021). Of the participants included in this study, 12.3 % were reported to be Black, and no difference in the prevalence of P/LP variants was found between White, Black and Hispanic participants. (Caswell-Jin et al., 2017) also found the overall prevalence of P/LP variants to be similar between White, Asian and Hispanic participants but there was variation in the prevalence of P/LP variants in the specific genes implicated.

Research on hereditary BC within African populations is still at an early stage (Hayat et al., 2021). While some significant studies in the USA have included African American participants, their genetic backgrounds, primarily of West African origin with considerable admixture, make it challenging to generalize these findings to continental African populations (Baharian et al., 2016). Furthermore, the vast genetic diversity across African populations means that research outcomes in one region cannot be broadly applied across the entire continent (Campbell & Tishkoff, 2008; Choudhury et al., 2020).

Work done in sub-Saharan Africa (SSA), summarised in Table 1.1, includes a large study in Nigeria on an unselected sample of 1136 women with BC and 997 controls. P/LP variants were detected in 14.7 % of cases; 11.1% in *BRCA1/2*, 1.0% in *PALB2*, 0.4% in *TP53*, and 2.1% in any of 10 other genes (Zheng et al., 2018). In a similar but smaller study done in Uganda and Cameroon, (Adedokun et al., 2020) compared the prevalence of P/LP variants in 12 BC

susceptibility genes between 196 cases unselected for age or family history with BC and 185 controls. Of the participants with BC, 15.8% were found to carry a P/LP variant in one of the 12 BC susceptibility genes. The majority of P/LP variants were found in *BRCA1/2* (11.2 %) while 4.5% were found in one of the other genes tested. The high prevalence of *BRCA1/2* mutations in these two studies may be due to the young age of participants (mean ages 47.5 and 46.2 years). More recently, 11.9 % of unselected women with BC in Ghana were found to have a P/LP variant in any one of the 34 BC genes tested, while 6.1% had P/LP variants in *BRCA1/2* (Ahearn et al., 2022). Although several studies have been published on P/LP variant prevalence in South Africa, outcomes were limited by either a lack of ancestry data, the inclusion of only founder variant testing, and/or small numbers of participants (Eygelaar et al., 2022; Francies et al., 2015; Makhetha et al., 2024; Schoeman et al., 2013; D. C. Smith et al., 2020; van der Merwe, Combrink, et al., 2022). No studies on the prevalence of P/LP variants in unselected women with BC have been done in South Africa and studies to clarify the role that P/LP variants in BC play in South African and other SSA populations are clearly required.

Table 1.1: Population based studies of hereditary breast cancer prevalence in sub-Saharan Africa

Author and year	Country	No. of genes tested	Included participants	Average age of BC cases (years)	Prevalence of *BRCA1/2*	Prevalence of other genes
Rweye-mamu et al., (2023)	Tanzania	*BRCA1/2*	100 unselected BC	44.0	6.0 %	N/A
Ahearn et al. (2022)	Ghana	34	871 unselected BC	50.8	6.1%	5.8%
Uyisenga et al., (2020)	Rwanda	26	40 BC (selected for <35 yrs)	31.2	10.0%	2.5%
Adedokun et al. (2020)	Uganda & Cameroon	12	196 unselected BC	46.2	11.2%	4.6%
Zheng et al. (2018)	Nigeria	25	1136 unselected BC	47.5	11.1%	3.6%
Fackenthal et al., (2012)	Nigeria	*BRCA1/2*	434 unselected BC (8 males)	46.5	11.0%	N/A

1.5 CLASSIFICATION OF GENETIC VARIANTS IN BREAST CANCER SUSCEPTIBILITY GENES

Advances in genetic sequencing technologies have allowed testing of hundreds of BC susceptibility genes concurrently. However, a consequence of sequencing large numbers of genes is that multiple differences in the DNA sequence between the patient sample and the reference genome are detected. Each change must be classified according to its clinical impact, i.e., does it contribute to disease or not? A critical part of genetic testing is therefore the classification of detected variants according to their likely clinical impact (Cutting, 2014). By convention, variants are classified into one of five categories: benign; likely benign; variant of uncertain significance (VUS); likely pathogenic; or pathogenic (Table 1.2).

Table 1.2: Classification categories of DNA variants*

Category	Probability of being pathogenic	Management Recommendation
Benign	<0.001	None – of no clinical concern
Likely benign	0.001-0.05	None – of no clinical concern
Variant of uncertain significance (VUS)	Between 0.05 and 0.90	None
Likely pathogenic (LP)	0.90-0.99	Follow screening guidelines and offer family members testing
Pathogenic (PV)	>0.99	Follow screening guidelines and offer family members testing

*Information collated from (Garrett et al., 2020; Richards et al., 2015; Tavtigian et al., 2018)

Variants classified as LP or PV are considered "actionable", which means that if management guidelines are available, patients who carry such variants should be offered recommended screening and/or prophylactic surgery. In addition, family members should be offered genetic testing for the variant, known as cascade testing (Richards et al., 2015). VUS are variants in which the clinical impact is unknown. Most often they are missense variants, synonymous substitutions, in-frame insertions/deletions, or variants within non-coding regions where their impact on protein expression and/or function is difficult to predict (Federici & Soddu, 2020). VUS can frequently be reclassified as B/LB or P/LP if additional data for curation of the variant becomes available (Walsh et al., 2024). VUS are problematic because they can cause considerable uncertainty and anxiety to both patients and their clinicians, and in the worst case

scenario, lead to unnecessary medical procedures (Adam et al., 2023; Elsayegh et al., 2018; Lumish et al., 2017). Furthermore, VUS are commonly detected, highlighted by a study which found that the chance of detecting a VUS was significantly higher than the chance of detecting a P/LP variant when doing gene panel testing for BC susceptibility (van Marcke et al., 2018).

In order to standardise the classification of variants, the American College of Medical Genetics and Genomics and the Association of Molecular Pathology (ACMG-AMP) published a scoring system for the classification of variants in genes that cause rare Mendelian disorders (Richards et al., 2015). This system has become the most widely used and accepted system for variant classification in both research and clinical settings. In this system of classification multiple criteria, including population data, computational and predictive data, segregation data, allelic data, database information and other data are used in the scoring matrix. Each criterion is assigned a level of evidence strength termed supporting, moderate, strong or very strong. The scoring matrix is then used to combine the criteria that have been met, taking into account the strength level of the evidence.

The criteria used in the ACMG/AMP guidelines are discussed here with particular reference to classifying germline variants that cause hereditary cancer. Because hereditary BC is a condition that is not fully penetrant, is autosomal dominant, late onset and phenocopies are common, some of the ACMG/AMP guideline criteria are not applicable (Richardson et al., 2024). More recently gene-specific refinements to the ACMG/AMP guidelines have been made under the auspices of the NIH-funded ClinGen consortium (Clinical Genome Resource, n.d.; Rehm et al., 2015). When modifications are available, they are discussed below.

1.5.1 Computational and predictive data

Changes to the DNA sequence can result in several different consequences for the protein, making it an important criterion for determining pathogenicity. ACMG/AMP guidelines specify that there is strong evidence of pathogenicity for variants which can be assumed to lead to complete absence of the protein by nonsense-mediated decay of the altered transcript (null variants) or lack of transcription. These variants include nonsense, frameshift, splice site, initiation codon and single or multi-exon deletions. The guidelines do however specify certain considerations that should be born in mind before applying the null variant criterion (Richards et al., 2015). More recently the ClinGen Sequence Variant Interpretation (SVI) Workgroup

has published an improvement to the guidelines that recommends that the strength of evidence for pathogenicity is modified by issues that are specific to each of the variant types. For example, it is important to determine the position of a nonsense or frameshift variant before determining that it is pathogenic as it may occur in an exon that is alternatively spliced or remove a non-essential downstream region of the protein (Abou Tayoun et al., 2018). The ACMG/AMP guidelines also make several recommendations based on prior knowledge of the gene of interest. This can include knowledge of the type of known variants in the gene or at the particular nucleotide position and their impact, or knowledge that particular regions of the gene are critical for function (Richards et al., 2015).

1.5.2 Functional data

Functional data is collected from *in vitro* or *in vivo* assays that measure the impact of a variant on the functioning of a specific protein. To be a good predictor of protein function, the assay must closely represent the biological environment and the full biological function of the protein must be considered (Couch et al., 2008). Functional studies can be extremely powerful in determining the pathogenicity of a variant, but these studies are not yet routinely and widely performed in variant classification due to their cost and time demands. However, recent advances in deep mutational scanning, which can assess the functional effect of thousands of variants simultaneously, is likely to have a significant positive impact on variant classification in future (Wei & Li, 2023). In the context of BC, for example, use of a DNA repair assay to analyse the effect of missense variants in *BRCA2* resulted in the reclassification 86% of VUS to pathogenic or benign (Richardson et al., 2021).

1.5.3 Segregation data

Theoretically, data showing that a variant and disease phenotype are inherited together in a pedigree provides support for pathogenicity. However, the use of co-segregation analysis in genes that cause cancer are complicated by incomplete penetrance, the presence of phenocopies, and differences in disease risk by age, gender and body site (Rosenthal et al., 2017). Software packages that use a Bayes factor-based approach are available and integrate the calculated Bayes factor with other lines of evidence. However, limitations of this method include unavailable data about penetrance rates and base-line cancer incidence in many populations (Belman et al., 2020).

1.5.4 Population allele frequency data

When a variant is found to occur commonly in a population, it is strong evidence that the variant is benign and not the cause of a rare Mendelian genetic disorder. This criterion is the only stand-alone criterion in the ACMG/AMP guidelines which means that if a variant is found to have an allele frequency of more than 5% in the Genome Aggregation Database (gnomAD) (data from the Exome Sequencing Project, 1000Genomes Project, Exome Aggregation Consortium and others), it is classified as benign without the need for further evidence (Richards et al., 2015). The ACMG/AMP guidelines also note that if the allele frequency of a variant is higher than expected for the frequency of the disorder in a control population than this provides *strong* evidence that it is benign. On the other hand, when a variant is absent from a control group, this was used as *moderate* evidence of pathogenicity if the control data used included at least 1000 individuals from the general population who were ethnically matched to the patient. The ClinGen Sequence Variant Interpretation (SVI) Workgroup has since suggested that the weight of this evidence be reduced to *supporting* strength level due to the finding in the Exome Aggregation Consortium database that there is a high probability of an individual having one or more rare alleles (ClinGen SVI Working Group, 2020; Lek et al., 2016).

The ClinGen SVI Workgroup has also published recommended amendments to the stand-alone >5% allele frequency criterion. They have recommended that exceptions be compiled for this criterion as some pathogenic variants have been found to occur more commonly due to founder effects or because the condition is common. A list of these variants is curated on the ClinGen website. No variants in BC susceptibility genes are currently included in this list (ClinGen, 2018). In addition, they recommend that the dataset used to determine allele frequency in control populations can be any general continental population but individuals should be unrelated and at least 2000 alleles should be observed (Ghosh et al., 2018). The modification to allow the use of any general continental population database is critical for the accurate interpretation of variants in non-European populations because these genomic databases have historically included very limited data from other populations. As an example, only 6.5% of exome/genome data in gnomAD v3.1.1 are derived from African/African Americans (gnomAD, 2020).

African populations are therefore at a significant disadvantage when using the powerful population data criterion within ACMG/AMP (Gurdasani et al., 2015), which results in many

variants from patients of African origin being classified as VUS. This was demonstrated in a study by Ndugga-Kabuye and Issaka (2019) which found that African Americans who underwent a specific gene panel test including genes for hereditary BC and Lynch Syndrome, were more than three times as likely to have a VUS detected than European Americans (18.8% vs and 6.1%). This may be even higher in continental Africans given the large degree of genomic diversity across African populations.

1.5.4.1 Diversity and importance of sub-Saharan African genomes

Research has consistently demonstrated that populations from continental Africa exhibit the highest levels of genetic diversity (1000 Genomes Project Consortium et al., 2015; Choudhury et al., 2017, 2020; Gurdasani et al., 2015; Tishkoff et al., 2009). In 2015, a study published by the 1000 Genomes Project Consortium showed that five populations from SSA had on average 20 % more single nucleotide polymorphisms (differences to the reference genome) than either European or East Asian populations (1000 Genomes Project Consortium et al., 2015). In a more recent study, Choudhury et al., (2020) identified over three million previously undescribed variants through whole-genome sequencing of African genomes. Additionally, African populations have also been shown to be most diverse based on variance in microsatellite allele length and this diversity decreases in populations with increasing distance from Africa (Tishkoff et al., 2009).

The higher genetic diversity seen in African populations relative to non-African populations is due to the evolution of modern humans, who originated in Africa 200-300,000 years ago and diversified within Africa for millennia before migrating across the globe 70,000 years ago (Rosenberg et al., 2002). African populations have maintained relatively large population sizes and have had long periods of geographic and cultural isolation, while non-African populations have experienced significant loss of genetic diversity due to the population bottleneck that occurred during the out-of-Africa migrations. In addition, multiple back migrations of Eurasian populations into Africa have contributed in the significant genetic diversity seen in African populations (reviewed in Choudhury et al., 2018).

Studying and recording the variation in African genomes is valuable and important for a multitude of reasons. Not only does this data provide us with insight into human evolutionary history but is essential if African populations are to benefit from the development of precision

medicines (Campbell & Tishkoff, 2008; Pereira et al., 2021). African data is, however, not only beneficial for African populations but for all global populations. This is because there are individuals of African-descent living in most regions of the world who could benefit from African biomedical data and because African data can be used as a reference for other global populations to assist in the appropriate classification of variants to support or refute association with disease (Mulder et al., 2021). This was shown in a study by Choudhury et al., (2020) who found that in his study of African participants 54/262 variants had previously been classified as pathogenic or likely pathogenic but could be reclassified as benign due to a minor allele frequency of greater than 0.05 in at least one of the African populations studied.

The number of African genomes contained in global genomic databases however is not representative of their diversity or number (Rotimi et al., 2021). As evidence of this, a study published in 2021 showed that within the European-Genome Phenome Archive, which facilitates the archiving and sharing of human phenotypic and genetic data, only 0.8% of the data was from Africa. Furthermore, ClinVar, which is a widely used public archive of the relationship between genetic variants and phenotypes, had received only 0.09% of its submissions from African organizations and of these only 1% were from SSA (Mulder et al., 2021). Reasons for the inadequate number of large-scale genomics studies in Africa include the limited number of African scientists with experience in genomic research, the lack of infrastructure and government support for genomic research, and limited computational resources and expertise (C. Rotimi et al., 2014).

Scientists and funding agencies are making a concerted effort to obtain genomic data from resident African populations. Some of these initiatives include the African Genome Variation Project (AGVP), the Human Heredity and Health in Africa (H3Africa) Consortium databases and gnomAD (Gudmundsson et al., 2022; Gurdasani et al., 2015; C. Rotimi et al., 2014). The use of this data for variant curation could have a significant impact on the number of variants classified as VUS in African populations (Gurdasani et al., 2015), however the magnitude of the effect has not been previously investigated.

1.6 GENETIC COUNSELLING AND TESTING FOR HEREDITARY BREAST CANCER

Identifying P/LP variants in moderate to high-risk BC susceptibility genes is important for the clinical management of women with BC and for the initiation of cascade testing in their

relatives (discussed further in section 1.7). Since the advent of diagnostic testing for hereditary BC, genetic counselling has played a central role in the process. Genetic counselling aims to educate individuals about the genetics of hereditary cancer, explain the benefits and limitations of testing, and provide information on appropriate cancer screening and prevention options available. In addition, to enable decision-making, genetic counsellors provide an assessment of the individual's risk of developing cancer, as well as the likelihood that they carry a disease-causing variant. Addressing the impact of hereditary BC on the individual and any psychosocial concerns that arise is central to the process (Berliner & Fay, 2007). Genetic counselling and testing (GCT) are commonly provided as standard of care in HIC, but in LMIC like South Africa, these services are less accessible (Yip et al., 2019). Several factors contribute to the limited availability of GCT in LMICs. These include financial barriers resulting in a lack of resources as well as fragmented healthcare systems. In addition, most genetic services are concentrated in cities making it challenging for individuals in rural areas, who are often poor, to access them (Kromberg et al., 2013; Yip et al., 2019).

In 2021, the South African government published guidelines for genetic services, recommending that genetic testing for familial cancer syndromes be accessible and accompanied by genetic counselling (National Department of Health, 2021). The government subsidizes the costs of GCT along with other medical expenses, with the level of subsidy varying based on income, and ranging from full coverage to none (Department of Health and Wellness, 2022). Despite these recommendations, the availability of genetic counselling remains limited. Genetic counsellors are the primary providers of cancer genetic counselling in South Africa, yet their numbers are critically low, estimated at only 5% of the required workforce. Additionally, there is a stark geographic disparity, with state-funded genetic counselling services available in only three of the country's nine provinces (Gomes et al., 2024; South African Society of Human Genetics, n.d.). Since these services are based in tertiary facilities, patients must be referred from other clinics, a process that is further complicated by the fragmented health system, limited genetic literacy among both healthcare providers and the public, particularly in the public health sector (Düsterwald, 2015; van Wyk et al., 2016; Yip et al., 2019). Given these constraints, there is limited scope for unaffected family members to serve as the entry point into cancer genetics. Instead, the focus in South Africa is on assessing BC probands first and subsequently extending testing to at-risk family members.

The South African government has recently signed the National Health Insurance (NHI) Bill into law. This bill aims to address the imbalances in access to healthcare between private and public systems and provide universal healthcare to all citizens (President of the Republic of South Africa, 2024). How the NHI will be financed and implemented is still unknown and will likely take decades to be accomplished. Currently there is no consensus on how genetic services will be provided within the NHI (Gomes et al., 2024). Data on the strengths and limitations of the current service will be important to inform decisions in planning for the new system.

1.6.1 Genetic testing guidelines

Owing to the relatively low prevalence of P/LP variants in the overall population of women with BC, and the cost of genetic tests and the associated genetic counselling , guidelines have been developed in many high-income countries (HIC) countries to specify who qualifies for genetic testing (Daly et al., 2021; Marmolejo et al., 2021). These guidelines have undergone frequent modifications to reduce their stringency over time as more information on risk has become available and the cost of genetic testing has decreased (Marmolejo et al., 2021; Samadder et al., 2021).

The South African National Department of Health (NDOH) published guidelines in 2018 to set standards for BC prevention and management which included eligibility criteria for genetic testing (Department of Health, South Africa, 2018). These criteria are based on version 2.2016 of the widely used National Comprehensive Cancer Network (NCCN) guidelines from the USA (NCCN Guidelines, 2016). Criteria used to determine eligibility for testing in women with BC in the NDOH and NCCN guidelines include age at diagnosis, tumour histology and family history of breast and related cancers (Table 5.1). These two sets of guidelines are broadly the same except for the age of diagnosis criterion which is ≤45 or 50 years in NCCN v2.2016 and ≤40 years in the NDOH guidelines.

The prevalence of P/LP variants in *BRCA1/2* in women with BC in the USA meeting the 2017 and 2019 versions of the NCCN guidelines was found to be 9.2% and 6% respectively (Beck et al., 2020; Cropper et al., 2017). The lower prevalence in the more recent NCCN guideline is likely due to the lower stringency of these testing criteria (Daly et al., 2021). Although the South African Department of Health has based their hereditary BC testing guidelines on the NCCN v2.2016, it is unknown whether these are useful in the South African context.

The NCCN guidelines, on which the NDOH guidelines are based, have been developed using data primarily from White American and European populations (Yadav & Couch, 2019). Given South Africa's ethnic diversity, with the majority of the population being Black African (StatsSA, 2024), the suitability of these guidelines in our setting is uncertain. Reasons for this include BC incidence varying across populations (Bray et al., 2024), TNBC possibly being more common in African ancestry groups (Jiagge et al., 2016), and Black African women in South Africa being diagnosed on average at a younger age (Dickens et al., 2016). Evaluating how well these guidelines predict pathogenic variants in African ancestry populations could assist in guiding the refinement of the testing criteria.

1.6.2 Clinical utility of gene panel testing

In the South African context, an important consideration for optimizing detection and cost of genetic testing is the selection of genes to include on a hereditary BC gene panel. Advances in genetic sequencing technologies have allowed testing of hundreds of BC susceptibility genes concurrently and there is little doubt that testing beyond *BRCA1/2* is useful and increases the detection of individuals with a P/LP variant (Desmond et al., 2015; Kurian et al., 2014; Susswein et al., 2016). However, many genes included in large gene panel tests have limited clinical utility (Zion et al., 2019).

Clinical utility is broadly defined as the extent to which a test improves health outcomes compared to current best practice. These improvements are often determined by the health outcomes arising from the change in medical management after testing (P. M. Bossuyt et al., 2012). However, emotional, social, cognitive and behavioural effects, though more challenging to quantify, must also be considered when assessing test utility (Burke, 2014). A diagnostic test may not impact medical management but could provide resolution of uncertainty, aid in coping with an illness or benefit extended family members. On the other hand, it may also cause anxiety or lead to social stigmatization or isolation (P. M. M. Bossuyt & McCaffery, 2009; Pritchard et al., 2022). Evaluating the medical and personal impacts of a test is necessary to assess its clinical utility, which is the initial step in a cost-benefit analysis (P. M. Bossuyt et al., 2012).

A prerequisite for clinical utility is that the test must accurately identify patients with the condition (P. M. M. Bossuyt & McCaffery, 2009). Since many genes included in large panel tests are under-researched and have not been confirmed to confer quantifiable cancer risks, detecting a P/LP variant in these genes cannot be considered clinically useful (Ceyhan-Birsoy et al., 2022). In addition results of this nature can create significant anxiety in individuals who are aware they may be at increased risk of cancer but are not provided with clear risk mitigation strategies (Carlsson et al., 2022). Panels that include only genes with established management guidelines are available. However, arguments against this approach include the exclusion of genes that may become clinically relevant as more data emerges and limiting the potential for new research discoveries on genotype-phenotype correlations (Pilarski, 2021).

Large BC gene panels also frequently include genes with disputed or no evidence of an association with BC but known links to other cancer types. The detection of P/LP variants in these genes are referred to as secondary findings (SF) (Burke, 2014). Improved outcomes can arise from SF especially if guidelines for risk-reducing measures are available (O'Leary et al., 2017). However, these cases remain in the minority as most genes included in large panels lack established management guidelines (Ceyhan-Birsoy et al., 2022).

An additional negative consequence of testing large gene panels is the number of VUS detected, which increases with the number of genes tested (Adam et al., 2023; M. E. Roberts et al., 2020). VUS are discussed in detail in section 1.5.

1.6.3 Uptake of genetic testing

There is no published data on the proportion of eligible women in South Africa or other LMICs who receive genetic testing for hereditary BC. The testing process involves several key steps: first, at-risk women must be identified and informed about the availability of genetic testing; second, they must attend a consultation- preferably with a genetic counsellor- where testing is offered; and third, they must decide whether to proceed with testing. Obstacles can arise at any one of these three steps, potentially limiting access to genetic testing.

Without adequate knowledge of genetics and available services, healthcare professionals may fail to correctly identify and refer at-risk women. In South Africa, general practitioners have been shown to have poor knowledge of genetics and genetic services, but no published data

exist on the knowledge of oncologists and surgeons treating BC (Düsterwald, 2015; van Wyk et al., 2016). However, even in HIC, oncologists and other specialists often have a limited understanding of genomics (Mladenić et al., 2024; Shirdarreh et al., 2021) which has been shown to hinder appropriate referral of women at risk of hereditary BC. Additionally, Delikurt et al. (2015) identified several other barriers to referral by healthcare professionals, including failure to obtain an adequate family history, a lack of awareness of patient risk factors, poor coordination of referrals, and limited access to genetic services, particularly in rural areas. These challenges are likely amplified in LMIC due to overburdened healthcare professionals and scarcity of genetic services. Currently, no data exist in South Africa on the proportion of at-risk women referred for genetic testing.

The second obstacle to testing is that referred women may not attend their appointments. This has been identified as a significant barrier with a systematic review of studies from HIC showing the uptake of genetic counselling for hereditary cancer to vary between 19% and 88% after referral. This review found that the most frequently cited barriers to genetic counselling uptake included medical costs, logistical challenges, lack of perceived personal relevance and concerns about the impact of testing on family members (Willis et al., 2017). In South Africa, socioeconomic challenges are likely the most significant deterrent to attending medical appointments, such as for genetic counselling and testing, due to the high levels of poverty in the country. A study focusing on BC screening in rural South Africa identified transportation costs, the need to take time off work and the lack of accessible medical services as primary barriers to screening (Sarmah et al., 2024). Additionally, a lack of awareness about the purpose of genetic counselling and the potential benefits of genetic testing may further hinder appointment attendance (Morris et al., 2015). Integrating genetic counselling services into BC treatment centres may help overcome some of these barriers by enabling patients to receive these services on the same day as other healthcare appointments.

A high proportion (75%-96%) of women who attend genetic counselling for hereditary BC in HIC have been found to proceed with genetic testing, with the primary reason given for declining testing being financial concerns (Fang et al., 2023; Wehbe et al., 2022; White et al., 2018). Although, initially it was believed that African Americans were less likely to accept testing, it has since been shown that the barrier to testing in this group is more likely to be due to lower rates of referral rather than declining testing (Chapman-Davis et al., 2021; Childers et al., 2017; Reid et al., 2020). In LMIC, a Malaysian study showed the uptake of genetic testing

among those referred for genetic counselling to be 65%, lower than found in HIC. The reasons women declined testing in the study were, however, not investigated (Ehsan et al., 2022). Investigating the uptake of genetic testing among women with BC in South Africa is necessary to assess its acceptability and determine whether there is a basis for expanding this service to other regions of the country.

1.6.4 Cascade testing

After an individual has been identified to have a P/LP variant in a cancer susceptibility gene, cascade testing, the process of extending GCT to family members, can proceed. This process enables the implementation of risk-reducing strategies in those who are unaffected but at increased risk of developing cancer. Cascade testing is a cost-effective way of identifying individuals with hereditary cancer in the population (Tuffaha et al., 2018) and is recommended by clinical guidelines (Daly et al., 2021). Prevention of cancers in unaffected family members by cascade testing contributes to the clinical/societal utility of cancer genetic testing. The extent of benefit depends on a variety of factors relating to testing of both proband and family members (uptake of testing and detection rate in probands), and intervention outcomes in family members (Pritchard et al., 2022).

An assessment of the potential benefits of cascade testing demonstrated that if 70% of first-, second-, and third-degree relatives underwent testing, most individuals carrying P/LP BRCA variants could be identified within a decade (Offit et al., 2020). However, achieving this in practice has proven challenging, even in high-income countries (HICs). A meta-analysis of 87 studies from 21 countries reported that the uptake of cascade testing ranged from 40% to 62% for first degree relatives (FDR) depending on whether contact was via the patient or clinic (Frey et al., 2022). Although direct contact of relatives by a health professional increases the number of relatives tested significantly, there are several limitations to this approach. Firstly, it is time-consuming for health professional which can add to the demands on an already overburdened service and secondly there are concerns about data privacy and the management of information dissemination (Frey et al., 2022).

There are numerous factors that have been found to contribute to the low uptake of cascade testing. Some of the main barriers to probands sharing the information with family members include lack of family contact, emotionally distant relationships, difficulty with sharing

information/poor communication skills and a lack of awareness about the condition and risk factors (Finlay et al., 2008; McGivern et al., 2004; M. C. Roberts et al., 2018). Once family members have been informed of their risk, there are additional barriers to testing uptake. These include limited access and high costs of testing, logistical concerns, as well as emotional reasons, relationship dynamics and coping strategies (M. C. Roberts et al., 2018; Srinivasan et al., 2020). Demographic factors, such as socioeconomic status (Cheung et al., 2010; Fehniger et al., 2013), ethnicity (Braley et al., 2022; Fehniger et al., 2013), age (Cheung et al., 2010) and gender (Sanz et al., 2010) have also been shown to influence the uptake of cascade testing.

Currently, limited data exists on the uptake of cascade testing for hereditary cancer in LMICs (Ahsan et al., 2023; M. C. Roberts et al., 2018). Research in Malaysia showed that only 11% of the FDR of BC patients underwent cascade testing (Yoon et al., 2011), while a similar uptake of 10% was observed in India (Mittal et al., 2022). In contrast, over 70% of FDR of individuals with Lynch syndrome in South Africa accepted cascade testing. This high uptake might be attributed partly to the fact that it restricts its assessment to FDRs and partly to the study's setting within a research cohort which had intensive clinical follow-up, and likely does not reflect standard clinical practice (Bruwer et al., 2013). A second South African study found that incorporating a genetic counsellor or medical geneticist into the multidisciplinary team at their BC clinic increased the number of family members per proband with a LP/P variant who pursued testing (Schoeman et al., 2013). Information on the acceptance and uptake of cascade testing for hereditary BC in South Africa is important to assess its potential effectiveness in identifying individuals at risk for hereditary BC.

1.7 CANCER RISK-REDUCING STRATEGIES IN WOMEN WITH HEREDITARY BREAST CANCER

Identifying individuals with a P/LP variant in a BC susceptibility gene allows for the implementation of risk-reducing strategies and surveillance measures to prevent or detect cancer recurrence or the development of new cancers (Daly et al., 2021). Guidelines for risk reducing measures are developed based on the specific cancer risks associated with the gene. The recommendations are then applied according to the age of the individual and whether they have had cancer previously.

1.7.1 Risk reduction in women unaffected by cancer

The widely used USA-based National Comprehensive Cancer Network (NCCN) recommend that unaffected female carriers of a *BRCA1/2* P/LP variant begin breast screening with annual MRI or, if MRI is unavailable, mammography from between 25 and 29 years of age. From 30 years, it is recommended that both mammography and breast MRI are performed annually. It has been shown that regular use of MRI surveillance significantly reduces the risk of BC mortality in women with *BRCA1* P/LP variants (Lubinski et al., 2024), while mammography alone has limited value for high risk women (Chiarelli et al., 2020). The NCCN guidelines also suggest discussing the option of risk-reducing mastectomy (RRM) with women carrying a *BRCA1/2* P/LP variant, although whether RRM reduces BC-specific and overall mortality, compared to recommended screening, is contested in HIC (Domchek, 2019). However in LMIC, where MRI is less accessible and advanced cancer therapies often unavailable (Moukadem et al., 2022), RRM remains important, decreasing the risk of BC by at least 90% (Carbine et al., 2018; Dare et al., 2021).

The NCCN guidelines recommend bilateral salpingo-oophorectomy (BSO) from 35 years in *BRCA1* P/LP variant carriers and 40 years in *BRCA2* carriers (Daly et al., 2021), due to the high risk of ovarian cancer in carriers and the absence of effective screening methodologies. BSO has been shown to significantly reduce the risk of ovarian cancer, BC and all-cause mortality in *BRCA1/2* carriers (Li et al., 2016; Marchetti et al., 2014; Xiao et al., 2019).

Similar NCCN evidence-based guidelines are available for five high-risk BC genes (*CDH1, PALB2, PTEN, STK11, TP53*) and six moderate risk BC genes (*ATM, BARD1, CHEK2, NF1, RAD51C, RAD51D*). Guidelines for the high-risk genes recommend risk-mitigation strategies for BC similar to those for *BRCA1/2*. Moderate risk BC gene guidelines generally advise screening from an older age and do not recommend offering RRM as an option. All NCCN guidelines for genes that confer a risk of ovarian cancer above 3% (*BRIP1, PALB2, RAD51C, RAD51D*), recommend BSO (NCCN Guidelines, 2024a). Genes associated with a risk of cancer in other organs have specific recommendations for risk-reduction either by screening or surgery.

1.7.2 Risk-reduction in women with breast cancer

It is well established that women with a P/LP variant in a high-risk BC gene have a significantly higher risk of developing a second BC in either the ipsilateral or contralateral breast compared to those with sporadic BC (Graeser et al., 2009). As a result, guidelines recommend that these women consider total mastectomy of the affected breast and risk-reducing prophylactic mastectomy of the contralateral breast (NCCN Guidelines, 2024b). This decision is influenced by factors such as age at diagnosis and prognosis of the primary BC (Mainor & Isaacs, 2020). Women who opt against risk-reducing surgery are advised to follow the screening recommendations for unaffected individuals with a P/LP variant (NCCN Guidelines, 2024a).

In the South African state healthcare sector, genetic test results are frequently unavailable prior to breast surgery and therefore cannot guide surgical decision-making (personal communication – Prof Jenny Edge). In HICs, breast-conserving therapy (BCT) with radiotherapy has become standard practice in women with sporadic non-metastatic BC, as it has been shown to result in survival rates comparable to those of mastectomy (de Boniface et al., 2021). However, in South Africa, total mastectomy is more frequently performed (Amouzou et al., 2022; Cubasch et al., 2017). This is due to limited access and challenges related to treatment with radiation therapy and chemotherapies, as well as more patients presenting with advanced cancer (Sutter et al., 2017). Consequently, even women who do not carry a P/LP variant often undergo mastectomy rather than being considered for BCT. Furthermore, because a high proportion of women in South Africa are morbidly obese, contralateral reduction mastopexy is frequently offered to women having surgery for BC. This procedure reduces the size difference between breasts and also makes radiation therapy more effective (Cubasch et al., 2017).

In women with a *BRCA1/2* P/LP who have been treated for early-stage BC, BSO significantly reduces the risk of ovarian cancer and all-cause mortality as it does in those unaffected by BC (Metcalfe et al., 2015). However, for women with advanced BC, the prognosis linked to the primary BC should be considered before proceeding with BSO (Mainor & Isaacs, 2020). It is recommended that the BSO guidelines for women without BC should also be applied to women with BC (NCCN Guidelines, 2024a).

1.7.3 Uptake of risk-reducing strategies

The uptake of risk-reducing strategies in women with *BRCA1/2* P/LP variants varies widely between countries. For instance, the uptake of RRM in women without BC has been found to range from 49.9% in the USA to 4.5% in Poland (Metcalfe et al., 2019). Factors influencing RRM uptake in women without BC include having children, the death of a sister or mother from BC and having had a breast biopsy. Conversely, older women are less likely to choose RRM (Evans et al., 2021). Contralateral mastectomy is usually offered to women with BC who have a *BRCA1/2* P/LP variant to reduce their risk of a subsequent BC. A recent study showed that significantly more *BRCA1/2* positive women with BC choose to proceed with bilateral mastectomy compared to women who do not know their *BRCA1/2* status prior to surgery (58.7% vs 7.6%) (Apostolova et al., 2024).

Similarly to RRM, the uptake of BSO also varies significantly between countries ranging from 83.3% in France to 36.7% in China. In their meta-analysis, Metcalfe et al. (2019) reported that 64.7% of women without BC underwent BSO compared to 70.7% of those with a history of BC. However, the authors did not comment on whether the difference in uptake between these groups was statistically significant. The higher overall uptake of BSO compared to RRM among women without BC observed in the meta-analysis is likely because BSO has a more significant impact on mortality risk than RRM (Domchek, 2019; Xiao et al., 2019). Factors influencing BSO uptake include parity and having a first- or second-degree relative who died from breast or pelvic cancer (Singh et al., 2013). In addition, prior awareness of *BRCA1/2*, knowledge of cancer risk and life-expectancy as well as anticipatory cancer worry have also been identified as contributing factors (Gavaruzzi et al., 2017).

There is no data available from South Africa on risk-mitigation in women with hereditary BC. Findings from other LMIC are mixed, with uptakes of RRM and BSO of 14.3% and 34.7% in Iran (Vasigh et al., 2022), 50% and 57.1% in Turkey (Moukadem et al., 2022) and 27.3% for RRM in Pakistan (Mooghal et al., 2023). Reasons found for not proceeding with RRM in Pakistan included family/spousal pressure, believing there was no need for the procedure, aesthetic concerns, fear of surgical complications, and being unmarried or young (Mooghal et al., 2023). A qualitative study done in Malaysia found that inaccurate understanding and misperceptions contributed to the refusal of BSO, while support from a spouse and family played an important role in the decision to proceed with the procedure (Sa'at et al., 2022). These studies are all complicated by the inclusion of women with and without BC. Different

considerations affect the appropriateness of risk-mitigating strategies in these two groups of patients. For example, while evidence suggests that RRM reduces the risk of BC in unaffected women, there is no evidence that contralateral RRM (removal of the unaffected breast after unilateral BC) improves survival, as the risk of recurrence and mortality from the initial BC remains (Carbine et al., 2018). In addition, women with BC may have advanced cancer making them ineligible for BSO. A clearer understanding of uptake and influencing factors can therefore be gained by examining women with and without BC separately.

1.8 IMPORTANCE OF THIS STUDY

While significant progress has been made in studying hereditary BC in HICs, data from LMICs, particularly in SSA, remains limited. The implementation of the NHI in South Africa is expected to bring significant changes to the healthcare landscape. Robust data will be required to guide policy development for cancer genetic services. There is therefore an urgent need to establish the prevalence and role of P/LP variants in South Africa's increasing number of BC patients. Additionally, understanding the uptake of genetic testing among women with BC is vital to assess its acceptability and determine the relevance and feasibility of expanding access to other regions of the country. Also, investigating the acceptance and uptake of cascade testing for hereditary BC will enable an evaluation of its potential to identify at-risk individuals. Insights into the use of risk-reducing strategies by women with P/LP variants are necessary as this will speak to the overall clinical utility of GCT for hereditary BC. If underutilization is evident, studies will be needed to identify barriers and inform interventions. Evaluating the effectiveness of the NDOH testing guidelines, adapted from guidelines developed in the USA, is important to determine whether these guidelines are appropriate for the South African setting or if they need to be tailored to local factors.

Variants of uncertain significance (VUS) present a global challenge, but the issue is particularly acute in African populations due to their underrepresentation in existing genomic databases. Worldwide efforts are underway to optimize variant classification, with population allele frequency emerging as a key evidence type. Leveraging African genomic data for VUS reclassification is therefore a priority. Given the higher prevalence of VUS in African populations, the use of large versus small gene panels for testing also needs investigation. Assessing the clinical utility of these panels in the South African context is important to develop evidence-based recommendations for cancer genetic testing in the country.

1.9 STUDY AIMS AND OBJECTIVES

The over-arching aim of this study was to assess the effectiveness of the genetic counselling and testing process in BC patients and their family members, and the ensuing clinical management pathway in a South African setting.

The specific objectives were to:

1. Determine the prevalence of P/LP variants in South African women of different ancestries with BC in diagnostic and population-based settings.
2. Assess the clinical utility of using an expanded gene panel for testing in South Africa.
3. Establish the degree to which the use of African genomic data can contribute to the re-classification of VUS in African BC patients.
4. Assess the South African NDOH testing guidelines by determining the prevalence of P/LP variants in women meeting the guidelines in different ancestry groups and the factors associated with detecting a P/LP variant.
5. Determine the uptake of genetic testing and factors associated in women with BC and their family members.
6. Determine the uptake of risk-reducing measures, such as RRM and BSO, in patients testing positive for a P/LP variant in a BC susceptibility gene.

CHAPTER 2

METHODS

The methods used in each of the four publications included in this PhD project are detailed in each Results chapter. In this section, a summary of the methods used in each publication is provided, as well as additional general background information and a more detailed explanation of some of the analyses if not contained in the publications. This PhD project made use of data from two patient cohorts, one from Tygerberg Academic Hospital (TAH) in the Western Cape, South Africa, and the other from the Johannesburg Cancer Study (JCS). These cohorts are described below with a summary of the methods used in each of the four publications.

2.1 THE TYGERBERG ACADEMIC HOSPITAL COHORT

Data was collected as part of a Cancer Association of South Africa (CANSA) funded project at the TAH Clinical Genetics Unit entitled: *Genetic risk factors for breast cancer in South Africa: A discovery, testing and counselling pathway.* The clinical genetics and BC services at TAH serve approximately half of South Africa's Western Cape province. Three ancestry groups including, Black African, Mixed Ancestry and White, were represented in the study. Although not excluded, there were no Indian/Asian participants amongst study participants, likely due to this population group making up only 1.1% of the Western Cape population (StatsSA, 2022a). Of the 3.5 million people in the region, 60% live in metropolitan Cape Town and the remainder in small towns and farms up to 300km away. TAH serves as the primary tertiary care centre for the region. During the study period, BC services at the hospital were overseen by a multidisciplinary team which met regularly to coordinate patient care, with GCT conducted by either genetic counsellors or medical geneticists. Other services for BC diagnosis, treatment, and radiographic screening were performed at TAH or one of three secondary-level hospitals, depending on the required level of care. Demographic, clinical and genetic testing data from all women with BC who attended TAH Genetic Counselling Clinic to discuss testing for hereditary BC between January 2018 and 31 August 2022 was collected. The methods of the projects utilizing this data are summarized below.

2.1.1 Prevalence and reclassification of genetic variants in South African populations with breast cancer (Chapter 3)

Methods for participant recruitment, gene panel testing and variant classification are described in the Methods section of Chapter 3. In order to assess the use of African genomic data for the potential reclassification of VUS, we obtained access to five datasets containing African genomic data. We then determined the allele frequency of participant VUS in these datasets to determine if they could be reclassified as likely benign or benign. Most of the African genomic datasets were shared with us in Variant Call Format (VCF), not requiring further processing. However, files from the MalariaGen Consortium were shared as raw Compressed Reference-oriented Alignment Map (CRAM) files which required variant calling to be done. The variant calling process used for the MalariaGen dataset, and the process used to determine allele frequencies of the VUS are described in more detail here than was possible in the paper.

The MalariaGen dataset was shared with us via the European Genome-Phenome Archive (EGA) as 342 CRAM files, one for each participant (EGA Consortium, 2023). CRAM files contain participant genomic data aligned to a reference genome and require further processing to call positions where there is variation. Due to the large amount of data in the MalariaGen dataset, the Wits Computer Cluster was used to facilitate processing (Hazelhurst, 2024). The Genome Analysis Toolkit (GATK) best-practice guidelines and tools were used to process the MalariaGen data (van der Auwera & O'Connor, 2020). Haplotype Caller was run to produce GenomicVariant Call Format (GVCF) files from the CRAM files. GVCF files have the same format as VCF files but represent every site in the genome, even those without variation. A Nextflow script adapted from Gerrit Reinier Botha's GitHub page (https://github.com/grbot/varcall/tree/master/combine-gvcfs) was then used to consolidate the GVCF files into 24 files divided by chromosome. A second Nextflow script was used to genotype each of the output files with GenotypeGVCF (https://github.com/grbot/varcall/tree/master/genome-calling). The output file from GenotypeGVCF was a VCF file containing all participant samples, jointly genotyping containing only positions where variation occurred.

The variants in the output VCF file were then filtered for quality using the VariantRecalibrator and ApplyVQSR tools applied separately for single nucleotides and insertions/deletions. The VariantRecalibrator scores the quality of each variant using true variants from HapMap, dbSNP and 1000 genomes as a comparison. The result is a score which is added to the INFO field of

each variant and used in the next step. The tool, ApplyVQSR, divides the variants into "tranches" representing different sensitivities in the detection of the true variants from the databases supplied. Variants are not discarded but marked as filtered if they fail the tranche sensitivity cut-off. VCFtools was used to remove all related individuals from the files and to select the variants that passed the tranche sensitivity filter, set to detect 99% of real variants (Danecek et al., 2011).

The list of VUS available to us from Invitae was in HGVS nomenclature. In order to search for the VUS allele frequencies in the African genomic datasets, we used the Ensembl variant effect predictor (VEP) to determine their genomic positions in GR37 and GR38 (McLaren et al., 2016). A browser extensible data (BED) file containing the three columns, chromosome, genomic start position and genomic end position, was made for each reference genome using VCFtools (Danecek et al., 2011). VCFtools was then used to search each of the five African datasets for the positions in the BED file containing the genomic positions corresponding to the reference used in the dataset. The maximum allele frequency for each VUS in gnomAD was determined using the output from the Ensembl VEP.

2.1.2 Application of genetic testing criteria for hereditary breast cancer in South Africa (Chapter 5)

We assessed the South African NDOH guidelines by determining the prevalence of P/LP variants in women from different ancestry groups meeting the guideline criteria and compared the detection rates within each individual criterion. We also conducted a multivariate analysis to assess the association of *BRCA1/2* P/LP variants with demographic, clinical and tumour histological features. These methods are explained in further detail in Chapter 5.

2.1.3 Breast cancer genetic services in a South African setting: proband testing, cascading and clinical management (Chapter 6)

Records were reviewed for 534 consecutive female BC probands and 115 family members attending genetic counselling over a 5-year period to determine genetic testing uptake. Family pedigrees of probands testing positive for a P/LP variant were analysed to determine the number of family members eligible for testing. Family members proceeding with testing were counted to determine the uptake of family member testing. Demographic and clinical data were

included in multivariate testing to determine which factors impacted testing uptake for probands and family members.

Information on the uptake of risk-reducing measures, mastectomy, BSO and mammography was collected from hospital clinical notes, the Breast Surgery Clinic database, the NHLS laboratory results portal (National) and the imaging report portal. Eligibility for interventions was based on gene-specific guidelines. The number of eligible participants who had each intervention was counted to determine uptake. However, due to the small sample size, we were unable to investigate the factors influencing the uptake of risk-reducing measures.

2.2 THE JOHANNESBURG CANCER STUDY COHORT

Data from women with BC diagnosed at 50 years or younger who had been enrolled in the JCS between 2007 and 2009 was used. All individuals recruited into JCS were Black African individuals with a diagnosis of histologically confirmed BC (W. C. Chen et al., 2020).

2.2.1 Prevalence of variants in breast cancer susceptibility genes in Black South African women diagnosed aged 50 or younger (Chapter 4)

DNA extracted from blood samples collected at the time of participant enrolment into the JCS was sequenced at the University of Washington using a 21-gene panel designed to detect inherited mutations in genes associated with breast and ovarian cancer (BROCA Cancer Risk panel, Walsh et al., 2010). Excel spreadsheets containing the sites at which variation occurred, and their quality metrics were shared with us by our collaborators for annotation. VCF files created from the shared spreadsheets were annotated using Annovar as described in Chapter 4. The results of this annotation allowed us to determine the number of P/LP variants in the included genes. In addition, we investigated whether the VUS could be reclassified using African genomic data as described in Chapter 3.

CHAPTER 3

PREVALENCE AND RECLASSIFICATION OF GENETIC VARIANTS IN SOUTH AFRICAN POPULATIONS WITH BREAST CANCER

The work described in this chapter was published as an open access article in *Genes Chromosomes and Cancer* under a Creative Commons Attribution 4.0 International License. T. Osler performed the data curation, the bioinformatic and other data analysis, and writing of the paper. The author agreement (Appendix A) and the published version of the paper (Appendix C) are attached. All references for this thesis, including those for this article, are listed at the end of the document (p. 118).

Osler, T. S., Brandenburg, J. T., Schoeman, M., Chen, W. C., Urban, M. F., & Mathew, C. G. (2024). Prevalence and Reclassification of Genetic Variants in South African Populations with Breast Cancer. *Genes, Chromosomes and Cancer*, *63*(9), e23275. https://doi.org/10.1002/gcc.23275

3.1 SUMMARY

Background: Concurrent testing of numerous genes for hereditary breast cancer (BC) is available but can result in management difficulties. We evaluated use of an expanded BC gene panel in women of diverse South African ancestries and assessed use of African genomic data to reclassify variants of uncertain significance (VUS).

Methods: 331 women of White, Black African or Mixed Ancestry with BC had a 9-gene panel test, with an additional 75 genes tested in those without a pathogenic/likely pathogenic (P/LP) variant. The proportion of VUS reclassified using ClinGen gene-specific allele frequency (AF) thresholds or an AF >0.001 in non-guidelines genes in African genomic data was determined.

Results: The 9-gene panel identified 58 P/LP variants, but only two of the P/LP variants detected using the 75-gene panel were in confirmed BC genes, resulting in a total of 60 (18.1%) in all participants. P/LP variant prevalence was similar across ancestry groups, but VUS prevalence was higher in Black African and Mixed Ancestry than in White participants. In total, 611 VUS were detected, representing 324 distinct variants. 10.8% (9/83) of VUS met ClinGen AF thresholds in genomic data while 10.8% (26/240) in non-guideline genes had an

AF >0.001. Overall, 27.0% of VUS occurrences could potentially be reclassified using African genomic data.
Conclusion: Expanding the gene panel yielded few clinically actionable variants but many VUS, particularly in participants of Black African and Mixed Ancestry. However, use of African genomic data has the potential to reclassify a significant proportion of VUS.

3.2 INTRODUCTION

It is estimated that 5-15% of women with breast cancer (BC) have a clinically actionable variant, defined as a pathogenic or likely pathogenic (P/LP) variant in a moderate to high-risk BC susceptibility gene (Buys et al., 2017; Couch et al., 2017; C. Hu et al., 2021; Tung et al., 2015). Since the advent of high-throughput sequencing (HTS), concurrent testing of hundreds of genes for BC susceptibility is now possible and available. Given the option to test more genes, clinicians and patients frequently opt for a "the more the better" approach, especially when there is no additional cost (Manahan et al., 2019). However, it is important to consider the clinical utility of testing, which is a balance between achieving improved outcomes due to testing and the risks associated with the test (Burke, 2014). Negative consequences of testing using expanded panels include risk and management uncertainties for genes lacking guidelines (Axilbund, 2016; Pilarski, 2021), and an increase in the number of variants of uncertain significance (VUS) detected (Adam et al., 2023).

VUS are genetic variants for which the clinical impact is unclear. They can cause considerable uncertainty and anxiety to both patients and their clinicians, and may even lead to unnecessary interventions (Lumish et al., 2017; Shickh et al., 2023). Individuals of African ancestry have been found to be significantly more likely to have a VUS identified on genetic testing (Caswell-Jin et al., 2017; Ndugga-Kabuye & Issaka, 2019; Tatineni et al., 2022). This is due both to the increased genetic diversity found in African populations and the under-representation of African genomic data necessary for variant classification. The increased level of genetic diversity in continental African populations has been confirmed in multiple studies (1000 Genomes Project Consortium et al., 2015; Choudhury et al., 2017, 2020; Gurdasani et al., 2015; Tishkoff et al., 2009). One of these studies showed that five populations from Sub-Saharan Africa had, on average, 20% more single nucleotide polymorphisms/variants than either European or East Asian populations (1000 Genomes Project Consortium et al., 2015). More recently Choudhury et al., (2020) discovered over three million undescribed variants in their

study of genomic diversity in Africa using whole genome sequencing (Choudhury et al., 2020). However, the number of African genomes contained in global genomic databases are not representative of this diversity (Rotimi et al., 2021). This is evidenced by the fact that only 6.5 % of the exome/genome data in v3.1.1. of the largest publicly available database of population variation, the Genome Aggregation Database (gnomAD), are derived from continental African or African-American populations (gnomAD, 2020; Gudmundsson et al., 2022).

The lack of population variation data significantly impacts variant classification in individuals of African ancestry. According to the American College of Medical Genetics and Genomics and the Association of Molecular Pathology (ACMG-AMP) guidelines, the most compelling evidence type for variant classification is an allele frequency (AF) in a control population that is higher than the prevalence of the disease. The ACMG-AMP guidelines recommend that a variant with an AF of ≥0.05 in gnomAD can be designated as benign without the addition of other evidence (evidence code BA1 in the guidelines). An AF ≥0.01 is considered strong evidence that a variant is benign (code BS1), but additional evidence types are needed before a benign classification can be made (Gudmundsson et al., 2022; Richards et al., 2015).

More recently, the ClinGen Sequence Variant Interpretation working group (SVI) refined the guidelines to state that any population database of at least 2000 alleles from unrelated individuals can be used to establish control population AF in a gene without a gene- or variant-specific modification. The SVI did not specify the read depth or quality filtering that should be applied to the population database used (Ghosh et al., 2018). Since then, several ClinGen Variant Curation Expert Panels (VCEPs) under the auspices of the NIH-funded ClinGen Consortium have released modifications to the ACMG-AMP guidelines for specific genes (*About ClinGen Expert Panels*, n.d.; Rehm et al., 2015). These modifications include lower AF thresholds for both BA1 and BS1 (Supplementary Table 3.2) determined using the Whiffin calculator, which takes into account disorder prevalence, allelic and genetic heterogeneity, and penetrance of the specific gene (Whiffin et al., 2017).

The South African population consists of people with diverse origins, languages and cultures. The National Census describes South Africa's three largest ancestry groups as Black African (80.2%), Coloured (8.8%) and White (7.7%) (StatsSA, 2022b). These categories were inherited from the Apartheid era but are now used to identify and address inequalities and discrimination. The Black African populations indigenous to South Africa are an ethnically diverse group of

Bantu-speaking peoples (Choudhury et al., 2017), and in the post-Apartheid period include an increasing diversity of people originating elsewhere in Africa. White South Africans are predominantly descendants of European settlers, although their ancestry includes some degree of non-European admixture with those of white Afrikaner descent having about 5% non-European admixture (Hollfelder et al., 2020). The term 'Coloured' (referred to as Mixed Ancestry in this study) refers to peoples with a degree of shared cultural identity who have genetic ancestry from Khoisan, Black, European and Asian populations (Choudhury et al., 2017; de Wit et al., 2010).

Given the diversity of the South African population, we aimed to determine if an expanded gene panel for hereditary BC had similar utility in different ancestry groups by comparing the P/LP and VUS prevalences. In addition, we aimed to determine the proportion of VUS detected in our participants that could be reclassified with the use of additional African genomic data.

3.3 METHODS

3.3.1 Participants

Participants included South African females with invasive or *in situ* BC who attended Tygerberg Hospital in the Western Cape, South Africa for genetic counselling and proceeded with gene panel testing between January 2018 and June 2022. Participants were offered diagnostic genetic testing if they had been diagnosed with BC ≤40 years; had two primary BCs ≤60 years of age; had triple negative BC; had one or more close relatives with invasive ovarian cancer, male BC, or BC ≤50 years; or two or more close relatives with breast or pancreatic cancer with one diagnosed ≤60 years old. The genetic counsellor recorded the proband's ancestry group, which was required by the testing laboratory to assist with variant interpretation, according to the categories of the South African Census: White, Black African, or Coloured (Mixed Ancestry).

3.3.2 Gene Panel Testing

Saliva or buccal swabs were collected from participants and sent to Invitae Laboratory in the USA for gene panel testing (Invitae Corporation, 2023). Invitae performed gene sequencing and deletion/duplication analysis using HTS which covered all coding exons and 10 to 20 base pairs of intronic sequence on either side of the coding exon (*Technology & Quality Faqs | For*

Providers | Invitae, n.d.). To classify variants, Invitae used Sherloc, a variant classification framework developed to refine the rules within the ACMG-AMP guidelines (Nykamp et al., 2017). Population AF data is incorporated into the Sherloc framework using a machine-learning model that estimates variant pathogenicity based on AF data from large population databases (Population Frequency Modeling, 2022).

All study participants underwent testing of 9 genes known to be associated with BC. If no P/LP variant was found in these genes, participants were then tested for an additional 75 genes (see gene list in Supplementary Table 3.1). The prevalence of P/LP variants and VUS in the initial 9 genes tested was then compared to the prevalence found after testing the additional 75 genes. This comparison aimed to assess the utility of testing a large gene panel in demographically diverse, understudied African populations.

In view of the wide range in the number of VUS identified in different genes, we also investigated whether a relationship existed between the number of VUS and the size of the gene's coding region. The length of the coding region was determined using the number of amino acids in the gene's corresponding protein as a proxy.

3.3.3 Re-curating VUS with African genomic data

The use of African genomic data for the reclassification of VUS was assessed using five data sources which provided whole genome sequencing (WGS) data from a total of 1855 individuals of African ancestry (Table 3.1). After application to the relevant data access committees, these five data sources were shared with us in either VCF or CRAM file format. Variant calling was performed on the CRAM files from the MalariaGen dataset using GATKv4.4.0.0 best practice guidelines (van der Auwera & O'Connor, 2020). VCFtools v0.1.17 was used to search each of the five data sources individually using the appropriate genomic position (GRCh37 or GRCh38) of the VUS (Danecek et al., 2011). Subsequently, an overall AF using the information from each data source was calculated for every VUS. The online Whiffen calculator (*Frequency Filter*, n.d.) was then used to find the filtering AF (the lower bound of the 95% confidence interval (CI)), for each VUS (Whiffin et al., 2017). Two VUS, one identified in two participants, were excluded from this analysis because they were duplications with unknown breakpoints.

Using the filtering AF, the VUS were curated using the original population frequency thresholds from the ACMG-AMP guidelines and the VCEP gene-specific specifications, when available (Supplementary Table 3.2). Three of the data sources used did not meet the sequencing depth specified in some of the gene-specific guidelines, but all other criteria were observed during curation. The GroupMax AF (lower bound 95% CI AF from the genetic ancestry group with the highest AF) of the detected VUS were also determined in gnomAD v2.1.1 and v3.1.2 using the Ensembl Variant Effect Predictor (McLaren et al., 2016).

Table 3.1: African genomic data used in the analysis of VUS identified in the study

Database and Reference	Country of origin (no. of participants in brackets)	Total alleles	Reference genome	Sequencing depth	Data type received	No. VUS in dataset (n=323)
African Genome Variation Project (Gurdasani et al., 2015)	Ethiopia (120), South Africa (100), Uganda (100)	640 alleles	37	4-8x	WGS (VCF)	39
Awi-Gen SA (Ramsay et al., 2016)	South Africa (100)	200 alleles	38	30x	WGS (VCF)	64
ARK (Fabian et al., 2022)	South Africa (798)	1596 alleles	38	3.6x	WGS (VCF)	75
MalariaGen (Band et al., 2019)	Burkina Faso (57), Cameroon (38), Tanzania (247)	494-504 alleles[a]	37	10x	WGS (CRAM)	20
H3Africa Consortium WGS VCF (Choudhury et al., 2020)	Benin (41), Nigeria (49), Botswana (48), Zambia (29), Burkina Faso (31), Ghana (26), Cameroon (50), Uganda (51), Guinea (27), Ivory Coast (22), DRC (12)	772 alleles	37	30x	WGS (VCF)	46

WGS, whole genome sequencing; VCF, Variant Call Format; CRAM, Compressed Reference-oriented Alignment Map, [a]Due to areas without good coverage, the number of alleles at some sites varied.

3.3.4 Data Analysis

All statistical tests were done using RStudio Version 2023.12.0 (R Core Team, 2022). Statistical differences were calculated using a Chi-square test or Fisher Exact test for categorical variables and Kruskal Wallis for continuous variables. Bonferroni correction was

used to correct for multiple testing. Spearman's correlation was used to examine relationships between continuous variables. Odds ratios with 95% CI were calculated using the median-unbiased estimate method for contingency data (Rothman, 1998). A *p*-value less than 0.05 was considered statistically significant.

3.4 RESULTS

There were 344 female BC patients who had gene panel testing in the study period but 13 were excluded because they had previously tested negative for a founder disease-causing variant in *BRCA1/2* or had not had testing of all included 9 genes. Data from the remaining 331 participants was used in the analysis of the 9-gene panel results. Of these participants, a P/LP variant was detected in 58 and further testing was not done. 273 participants had additional testing of the same set of 75 genes and these results were compared to those from the 9-gene panel.

3.4.1 P/LP variants detected with 9 and 75 gene panels

No significant difference was found between the prevalence of P/LP variants in the three ancestries for either the 9-gene ($p = 0.56$) or the 75-gene panel ($p = 0.43$) (Table 3.2). All ancestry groups had significantly more P/LP variants detected with the 9-gene panel compared to the 75-gene panel ($p < 0.01$ for all groups). P/LP variants in *BRCA1* (5.7%) and *BRCA2* (7.6%) were the most common and there was no significant difference in their prevalence between the three ancestries. Other genes from the 9-gene panel found to have a P/LP variant in one or more of the participants were *PALB2, CHEK2, TP53* and *ATM* (Supplementary Table 3.3). A P/LP variant was detected in 4.4% (12/273) of the participants tested with the 75-gene panel. Of the 9 genes in which a P/LP variant was detected using this larger panel, two were in genes (*RAD51C* and *RAD51D)* conclusively linked to BC (Dorling et al., 2022; C. Hu et al., 2021) and 6 had management guidelines available (Table 3.3) (NCCN Guidelines, 2024a). No difference in the frequencies of P/LP variants between the three ancestry groups was found for any of the 84 genes tested (Supplementary Table 3.4). Including the results from both panels, 60 participants had a P/LP variant in a gene with an established association with BC (18.1%).

Table 3.2: Number of P/LP variants and VUS detected in each ancestry group

Gene panel size	Ancestry	Total no. participant	No. participant with P/LP	No. participant with ≥1 VUS	Total no. VUS detected[a]	Ratio of P/LP to VUS
9 genes (n = 331)	Black African	72	12 (16.7%)	17 (23.6%)	17	1:1.4
	White	53	12 (22.6%)	8 (15.1%)	10	1:0.8
	Mixed Ancestry	206	34 (16.5%)	57 (27.7%)	63	1:1.9
	All participants	331	58 (17.5%)	82 (24.8%)	90	1:1.6
75 genes (n = 273)	Black African	60	1 (1.7%)	49 (81.7%)	102	1:102
	White	41	1 (2.4%)	20 (48.8%)	39	1:39
	Mixed Ancestry	172	10 (5.8%)	149 (86.6%)	383	1:38
	All participants	273	12 (4.4%)	218 (79.9%)	524	1:44

P/LP, pathogenic/likely pathogenic; VUS, variant of uncertain significance. [a] Multiple participants had >1 VUS

Table 3.3: Information available for genes with P/LP variants detected with the 75-gene panel

Gene	No. P/LP variants detected	Established link to BC[a]	NCCN guidelines available (NCCN Guidelines, 2024a)
BAP1	1	no	Yes
BRIP1	1	no	yes
FH	2	no	no
MSH6	1	no	yes
MUTYH	2	no	yes[b]
RAD50	1	no	no
RAD51C	1	yes	yes
RAD51D	1	yes	yes
RECQL4	2	no	no

P/LP, pathogenic/likely pathogenic; BC, breast cancer; NCCN, National Comprehensive Cancer Network; [a]Link to BC as per standardized curations (ClinGen, n.d.), [b] Both individuals were heterozygous and guidelines for MUTYH do not recommend additional cancer screening for heterozygotes.

3.4.2 Prevalence of VUS

When comparing the three ancestry groups, no significant difference in the average number of VUS per participant was found in the 9-gene panel (p = 0.16). However, Black African and Mixed Ancestry participants had significantly more VUS per participant detected with the 75-gene panel than White participants (p= 3.8x10^{-6}). In addition, the ratio of P/LP variants to VUS detected was significantly lower using the 75-gene panel compared to the 9-gene panel for all ancestries, Black African (p<10^{-5}), Mixed Ancestry (p<10^{-5}) and White (p<10^{-5}) (Figure 3.1).

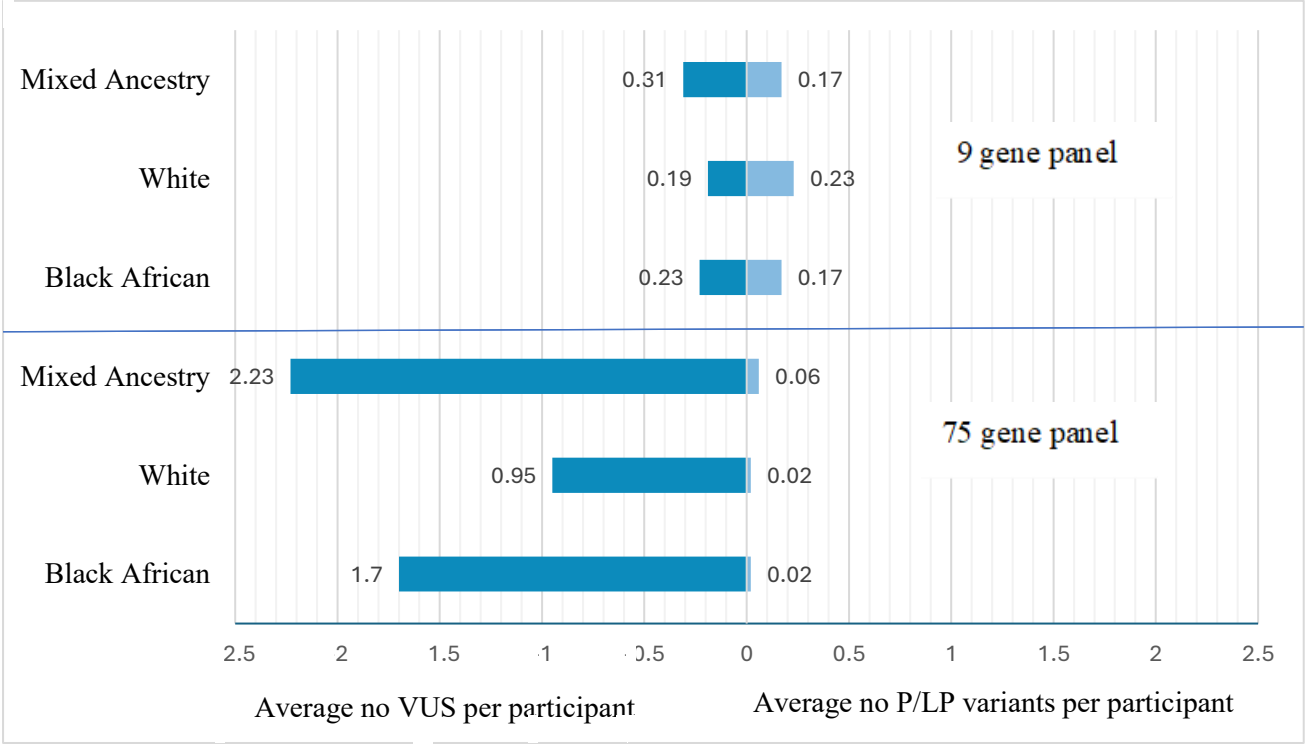

Figure 3.1: Comparison of the average number P/LP variants and VUS after initial 9 gene and then further 75 gene testing.

3.4.3 Analysis and reclassification of VUS

71.9 % (238/331) of the participants carried one or more of the 611 VUS occurrences, 323 of which were distinct variants. Nearly half (43.3%; 140/323) of the distinct VUS were found in only 10 of the 84 genes tested: *ATM* (25, 7.7%), *POLE* (25, 7.7%), *APC* (16, 5.0%), *WRN* (12, 3.7%), PALB2 (11, 3.4%), *RECQL4* (11, 3.4 %), *AXIN2* (10, 3.1%), *BARD1* (10, 3.1%), *DIS3L2* (10, 3.1%) and *MET* (10, 3.1%). A significant positive relationship was found between

the length of the gene's coding region and the number of distinct VUS detected in the gene ($p = 3.4x10^{-9}$).

35.3% (114/323) of the VUS were found to be present in the African genomes but none had a filter AF ≥0.01 and therefore did not meet the AF threshold in the original ACMG-AMP guidelines. Gene-specific AF thresholds were available for 12 of the 73 genes in which VUS were found (see Figure 3.2). Of the 83 distinct VUS in genes with guidelines, two met the gene-specific frequency thresholds for BA1 and could be reclassified as benign (Table 3.4). There were 15 additional VUS that met the gene-specific AF threshold for BS1, but of these, eight had less than five alleles, and BS1 could therefore not be applied as specified in the guidelines. Of the seven VUS that met BS1, five had additional evidence available and were reclassified as likely benign. Therefore, 7 (8.4%) of the 83 distinct VUS in guideline genes could be reclassified, and because many of these VUS occurred in more than one participant, this resulted in a total of 19.1% (26/136) of VUS occurrences in guideline genes being reclassified.

However, 61 genes in our panel did not have guidelines specifying gene-specific AF thresholds, resulting in most of the VUS in our study remaining classified as VUS. We determined the number of VUS with a filter AF ≥ 0.001, which was the most stringent threshold in any of the gene-specific guidelines used (Supplementary Table 3.2). We found that 10.8% (26/240) of distinct VUS in non-guideline genes met this threshold, accounting for 22.7% (139/611) VUS occurrences. Therefore, in total, 27.0% (165/611) of the VUS occurrences had a filter AF above either 0.001 or the gene-specific threshold in the African genomes.

On searching gnomAD, we found that 4.8% (4/83) of the distinct VUS in our participants had an AF above the stipulated gene-specific threshold, and 1.7% (4/240) had an AF >0.001 in non-guideline genes. This resulted in a total of 3.4% (21/611) of VUS occurrences having an AF ≥0.001 or above the gene-specific threshold in gnomAD. The odds of a VUS from our sample being present at an AF above the gene-specific threshold or >0.001 was 10.1 times higher (95% CI: 6.4 – 16.6, p<0.0001) in the African genomes than in gnomAD.

Table 3.4: VUS meeting gene-specific AF thresholds in African genomes

Gene	VUS (HGVS Nomenclature)	Protein prediction	No. alleles in study participants (n=331)	No. alleles in African genomes (n=1854)	Filtering AF[a] in African genomes	Groupmax filtering AF in gnomAD[b]	ACMG-AMP code[c] met with African genome AF	Other gene-specific ACMG-AMP criteria met	New classification
APC	NM_000038:c.-30530G>C	Intronic	2	3	0.0002205	0	not met	-	-
	NM_000038:c.-30582T>C	Intronic	7	3	0.0002205	0	not met	-	-
	NM_000038:c.3139G>A	p.Glu1047Lys	2	3	0.0002205	0	not met	-	-
ATM	NM_000051:c.131A>G	p.Asp44Gly	1	18	0.0031376	0.00046	BS1	none	-
	NM_000051:c.4279G>A	p.Ala1427Thr	1	14	0.0031376	0.0002042[e]	BS1	BP4	likely benign
	NM_000051:c.4082A>G	p.Gln1361Arg	1	12	0.0018673	0.000728	BS1	BP4	likely benign
	NM_000051:c.3746+18A>G	Intronic	1	9	0.0012662	0	BS1	BP7	likely benign
	NM_000051:c.2813C>T	p.Pro938Leu	1	5	0.0005313	0	BS1	BP4	likely benign
	NM_000051:c.6537T>G	p.Ile2179Met	2	5	0.0005313	0.000479	BS1	none	-
BRCA1	NM_007294:c.3076A>G	p.Ile1026Val	1	3	0.0002205	0	not met	-	-
BRCA2	NM_000059:c.4502A>G	p.Asn1501Ser	4	4	0.0003684	0	not met	-	-
	NM_000059:c.3448A>T	p.Thr1150Ser	1	3	0.0002205	0	not met	-	-
DICER1	NM_177438:c.4852T>A	p.Ser1618Thr	2	4	0.0003684	0	not met	-	-
PALB2	NM_024675:c.474G>C	p.Gln158His	6	7	0.000886	0	BS1	BP1, BP4	likely benign
RUNX1	NM_001754:c.82-84del	p.Ser28del	1	11	0.0016637	0.000149	BA1	N/A	benign
VHL	NM_000551:c.631A>C	p.Met211Leu	11	13	0.00207	0.0000789[e]	BA1	N/A	benign
	NM_000551:c.18G>T	p.Glu6Asp	2	4	0.0003684	0	not met	-	-

VUS, variant of uncertain significance; AF, allele frequency; ACMG/AMP, American College of Medical Genetics and Genomics and the Association of Molecular Pathology; [a]filtering AF refers to the lower 95% confidence interval of the allele frequency calculated using the Whiffin calculator (Whiffin et al., 2017), [b]lower bound 95% confidence interval allele frequency in gnomAD from the genetic ancestry group with the highest AF, [c]AF meets BS1 gene-specific threshold

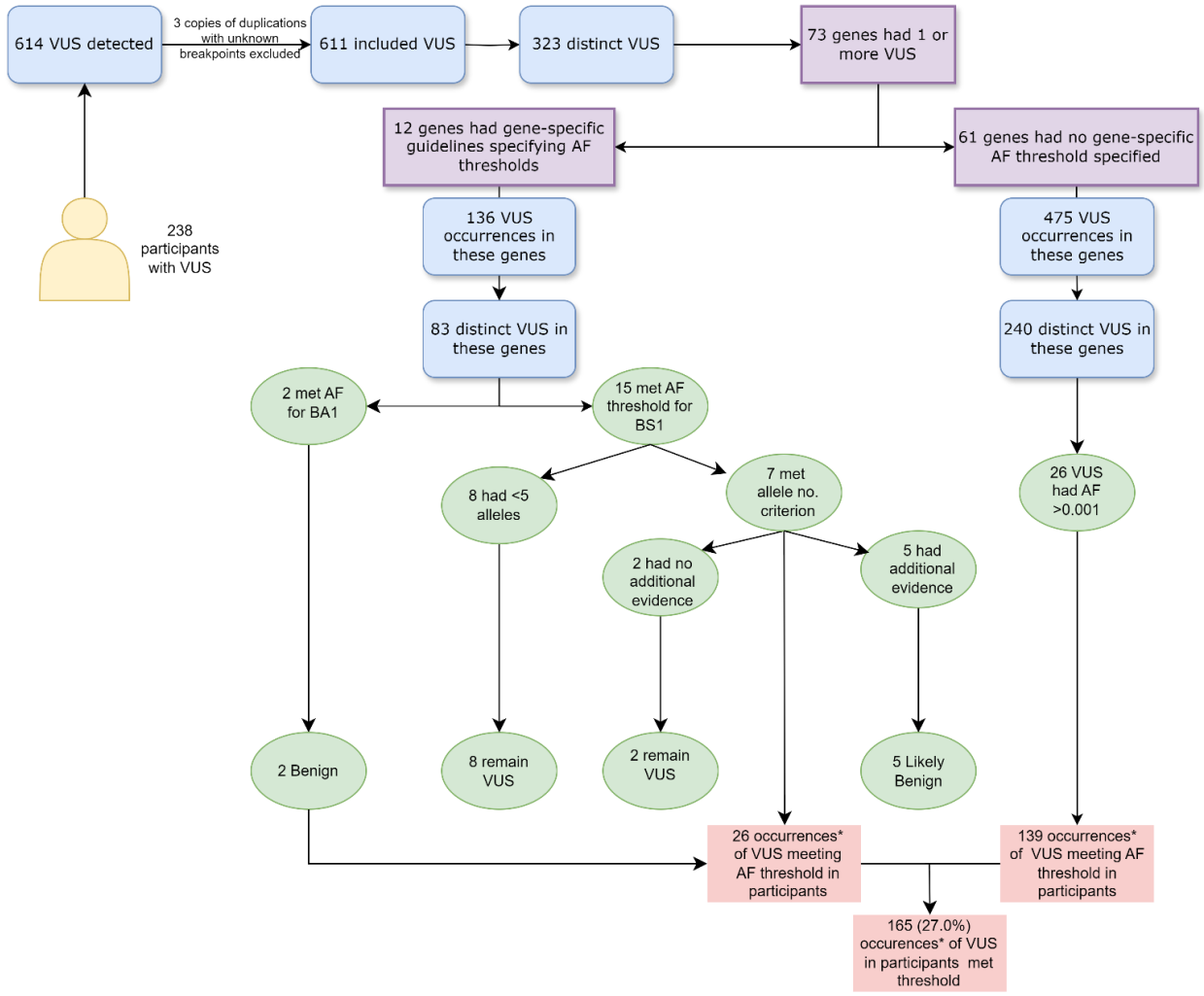

Figure 3.2: Reclassification of VUS using allele frequencies from African genomic data

*Some of the VUS occurred in multiple individuals (occurrences)

3.5 DISCUSSION

Here, we report on the prevalence of P/LP variants and VUS in BC susceptibility genes in three South African ancestries and assess the utility of expanded genetic testing. We also show the potential benefit of increasing available African genomic data for the re-curation of VUS.

We found a similar prevalence of P/LP variants in participants from all three South African population groups. This is in line with studies comparing the frequency of P/LP variants in African-American and non-Hispanic White Americans (Caswell-Jin et al., 2017; Domchek et al., 2010; Yadav et al., 2021). However, the admixture in the African-American population and their predominantly West African origins makes it challenging to draw conclusions for resident

African-ancestry populations (Baharian et al., 2016). In addition, the diversity of African populations means that genetic research findings cannot be generalized across the continent (Campbell & Tishkoff, 2008; Choudhury et al., 2020). Studies of unselected women with BC in Nigeria (Zheng et al., 2018), Uganda and Cameroon (Adedokun et al., 2020) and Ghana (Ahearn et al., 2022) have found P/LP variant prevalence in BC genes of 14.7% (11.1% in *BRCA1/2*), 15.8% (11.2% in *BRCA1/2*) and 11.9% (6.1% in *BRCA1/2*) respectively. The higher prevalence of P/LP variants associated with BC risk in our study (18.1%) is likely due to our selection criteria which included family history, young age of diagnosis or triple negative BC. Further studies are needed to determine whether the prevalence of P/LP variants is similar between African ancestry groups in both BC patients and in the general population.

There has been debate regarding the appropriate number of genes to include in a panel to test for inherited BC susceptibility (Colas et al., 2019; Thompson et al., 2016). It is widely held that testing beyond *BRCA1/2* is appropriate and increases the detection rate of P/LP variants substantially (Buys et al., 2017; Desmond et al., 2015; Kurian et al., 2018; Susswein et al., 2016). All genes included in the 9-gene panel are known to be associated with BC and have established management guidelines (NCCN Guidelines, 2024a). After excluding participants in our study with P/LP variants in *BRCA1/*2, we found that an additional 4.8 % (14/287) had P/LP variants detected with the 9-gene panel. This detection rate is consistent with findings from other studies (Susswein et al., 2016; Tung et al., 2016).

Significantly more participants had a P/LP variant detected with the 9-gene panel (17.5%) than with the 75-gene panel (4.4%). Using the 75-gene panel, only 0.7% (2/273) participants had a P/LP variant in a gene associated with BC, which was the primary indication for testing. 83.3% (10/12) of P/LP variants detected with the 75-gene panel were in genes with disputed or no evidence of an association with BC (referred to here as secondary findings (SF)), and of these, 5 (41.7%) had no current clinical utility because management guidelines for the gene were not available (Table 3.3) (Burke, 2014). Although detecting these variants leads to uncertainty (Pilarski, 2021), multiple studies have found no evidence of psychological harm caused to individuals receiving them (Hart et al., 2019; Mitchell et al., 2024; Vears et al., 2021; Wynn et al., 2018). It has however been shown that a considerable number of individuals choose not to receive SF, especially when the SF has no clinical utility (Mitchell et al., 2024; Vears et al., 2021).

SF may be of much benefit to individuals when they occur in genes with guidelines on risk-reducing measures. There were three participants in this study with SF in genes (*BAP1*, *BRIP1* and *MSH6*) with guidelines recommending interventions to mitigate other cancer types. Although detecting these P/LP variants has the potential to reduce mortality and morbidity, studies conducted in high-income settings (HIC) have found that participants with SF and their family members often do not pursue cascade testing or adopt recommended risk-reducing measures (Hart et al., 2019; Mitchell et al., 2024). This may be even more likely to be the case in our setting, where uptake of risk-reducing measures in women with hereditary BC have been shown to be lower than in HIC (Osler et al. submitted for publication).

A further issue related to expanded gene panels is the associated increase in the number of VUS detected. The detection of VUS can create uncertainty for both patients and clinicians, potentially resulting in unnecessary medical interventions (Eccles et al., 2015; Mighton et al., 2023; Welsh et al., 2017). As reported by several other studies, we confirmed that the frequency of VUS in populations not of European ancestry is significantly higher than those of European ancestry when testing a large number of genes (Caswell-Jin et al., 2017; Ndugga-Kabuye & Issaka, 2019; Tatineni et al., 2022). This may ultimately increase the risk of a negative consequence from genetic testing in populations of African ancestry compared with European populations, but this should be balanced against the limited additional detection of actionable variants. Also, such data is scarce in under-resourced settings and may have a role in clinical research.

Allele frequencies in aggregated African genomes enabled the reclassification of 8.4% of distinct VUS in genes with ClinGen gene-specific guidelines (*About ClinGen Expert Panels*, n.d.). When an AF threshold of 0.001 was applied for VUS in genes without guidelines, 10.8% of distinct VUS met this threshold in the African genomes and could potentially be reclassified if additional information was available. This was significantly more than met the same threshold in gnomAD and highlights the value of including genomes from diverse populations for variant classification. However, to realise the full potential of African genomic data, lower AF thresholds may be required, which will necessitate gene-specific thresholds to be determined and implemented for more genes. In addition, gene-specific guidelines need to be modified to allow the use of datasets other than gnomAD. Also, the sequencing depth criteria specified should be reviewed when AF is used to support a benign interpretation, as over-counting of variants is unlikely and the observed AF can be considered the minimum AF

(Fumagalli, 2013). Removing this sequencing depth requirement would make multiple other data sources available for curation.

Current limitations to using the African genome AF data are the absence of gene-specific guidelines for most genes and the relatively small number of genomes in our database. Several VUS met the AF threshold in the data, but due to the insufficient number of African genomes, they did not have the specified 5 alleles, which limited VUS reclassification. Also, future work would benefit from availability of genome data specifically from the populations being studied, such as Southern African Black and Mixed Ancestry populations. An additional limitation of this study was the limited sample size of participants (N=331); consequently, small differences in the prevalence of variants in individual genes between ancestries may not have been detected due to insufficient power.

3.5.1 Conclusions

We have shown that the prevalence of P/LP variants in commonly tested BC susceptibility genes is similar in women with BC from different ancestral populations in South Africa. Also, no apparent differences in the prevalence of P/LP variants in the genes tested were found, and thus there is no indication that gene panels need to be adjusted for different ancestral groups. Expansion of testing with the 75-gene panel produced only a small increase in the number of P/LP variants detected in BC susceptibility genes, and a significant increase in the number of VUS, especially for participants from Black African and Mixed Ancestry populations. Although secondary findings are of potential clinical relevance, further studies are needed to investigate whether feedback of such results would be welcomed by participants, and to what extent they would have clinical utility. We showed that using African genomic data for variant curation resulted in the potential reclassification of a considerable number of VUS. This indicates that increasing the number of African genomes while decreasing the AF threshold through gene-specific guidelines for more cancer susceptibility genes would be of significant benefit. In conclusion, our study indicates that expanding gene panels for hereditary breast cancer testing in South Africa offers limited clinical benefits and increases the number of VUS substantially. However, increasing the representation of African genomes and refining classification guidelines could substantially improve the reclassification of these variants.

3.6 DATA AVAILABILITY

The de-identified participant data used and analysed in this study is available from the corresponding author on reasonable request. The African genomic data were used under license for the current study. Restrictions apply to the availability of these data but applications for use can be made via the European Genome Archive at the following links:
African Genome Variation Project: https://ega-archive.org/datasets/EGAD00001001663
Awi-Gen SA: https://ega-archive.org/datasets/EGAD00001006418
MalariaGen: https://ega-archive.org/studies/EGAS00001003648
H3Africa Consortium WGS VCF: https://ega-archive.org/datasets/EGAD00001008577
ARK – the Principal Investigator can be contacted for data access at june.fabian@mweb.co.za

3.7 FUNDING

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3.8 CONFLICT OF INTEREST DISCLOSURE

The authors declare no conflict of interests.

3.9 ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was approved by the Human Research Ethics Committees of Stellenbosch University (N22/04/039) and the University of the Witwatersrand (M231195). As the study was a retrospective record review, participant consent was not required. All data were de-identified.

3.10 PATIENT CONSENT STATEMENT

Not applicable as this was a retrospective record review.

3.11 ACKNOWLEDGEMENTS

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3.12 SUPPLEMENTARY MATERIALS

Supplementary Table 3.1: Gene list for 9 gene and 75 gene panel

9 gene panel: *BRCA1, BRCA2, CDH1, PALB2, PTEN, STK11, TP53, ATM, CHEK2.*

75 gene panel*: ALK, APC, AXIN2, BAP1, BARD1, BLM, BMPR1A, BRIP1, CASR, CDC73, CDK4, CDKN1B, CDKN1C, CDKN2A (p14ARF), CDKN2A (p16INK4a), CEBPA, CTNNA1, DICER1, DIS3L2, EGFR, EPCAM, FH, FLCN, GATA2, GPC3, GREM1, HOXB13, HRAS, KIT, MAX, MEN1, MET, MITF, MLH1, MSH2, MSH3, MSH6, MUTYH, NBN, NF1, NF2, NTHL1, PDGFRA, PHOX2B, PMS2, POLD1, POLE, POT1, PRKAR1A, PTCH1, RAD50, RAD51C, RAD51D, RB1, RECQL4, RET, RUNX1, SDHA, SDHAF2, SDHB, SDHC, SDHD, SMAD4, SMARCA4, SMARCB1, SMARCE1, SUFU, TERC, TERT, TMEM127, TSC1, TSC2, VHL, WRN, WT1.*

Supplementary Table 3.2: Gene-specific modifications by ClinGen Variant Curation Expert Panels (VCEP) **to ACMG-AMP rules using population allele frequency (AF) data.**

Gene[1]	AF cut-off for BA1[2]	AF cut-off for BS1[3]	VCEP Specifications
APC	0.001	0.0001	Use the non-cancer dataset from gnomAD (v2.1.1)
ATM	0.005	0.0005	Use filtering AF
BRCA1/2	0.001	0.0001	Use maximum filter AF in gnomAD non-founder populations and consider exome and genome data separately. Do not apply if read depth <20 Do not apply to well-established pathogenic founder variants.
CDH1	0.002	0.001	Subpopulation must ≥ 2,000 alleles and have a minimum of five variant alleles present. We allow a variant to reach a likely benign classification based on BS1 alone.
DICER1	0.003	0.0003	Use gnomAD subpopulations. Subpopulations must have >2,000 alleles tested and a minimum of 5 alleles.
HRAS[4]	0.05%	0.025%	
PALB2	0.001	0.0001	Use gnomAD filtering AF
PTEN	0.00056	0.000043	Use filtering allele frequency in gnomAD.
RUNX1	0.0015	0.00015	Minor AF between 0.0015 (0.15%) in any general continental population dataset with ≥ 2,000 alleles tested and variant present in ≥ 5 alleles.
TP53	0.001	0.0003	Sub-population must have a minimum of 5 alleles present in the sub-population
VHL	0.000156	0.0000156	Follow all sequence variant interpretation working group (SVI) general guidance on applying population filters.

[1] Genes included are those with a VUS found in this study and ClinGen approved modifications.
[2] BA1 refers to the benign standalone code assigned to a variant when it occurs above a specified AF in a population database. Variants that meet BA1 can be classified as benign without further evidence.
[3] BS1 refers to the Benign Strong code assigned to a variant when it occurs above a specified AF threshold in a population database. Meeting BS1 is strong evidence the variant is benign but further evidence is required before a classification of likely benign or benign can be assigned. [4] It is not clear if the *HRAS* BA1 and BS1 criteria in gene-specific guideline refer to % or allele frequency.

Supplementary Table 3.3: P/LP variants found in the gene panels in three ancestry groups

P/LP Variants found in 9 gene panel				
Gene	**Variant**	**Mixed Ancestry (n=206)**	**White (n=53)**	**Black African (n= 72)**
ATM	NM_000051.3:c.5228C>T		2	
	NM_000051.3:c.5279dup	1		
	NM_000051.3:c.6139_6146del	1		
	NM_000051.3:c.7271T>G	1		
BRCA1	NM_007294.3:c.1016dup		1	
	NM_007294.3:c.1360_1361del	1		
	*NM_007294.3:c.1374del		1	
	NM_007294.3:c.1398del			1
	NM_007294.3:c.181T>G		1	
	NM_007294.3:c.1953_1956del	2		
	NM_007294.3:c.-232-?_4357+?del			1
	NM_007294.3:c.-232-?_4986+?del			1
	*NM_007294.3:c.2641G>T	2	2	
	NM_007294.3:c.4484G>T			1
	NM_007294.3:c.5096G>A			1
	NM_007294.3:c.5153-1G>A			1
	NM_007294.3:c.5278-?_5406+?del	1		
	NM_007294.3:c.66dup	2		
BRCA2	NM_000059.3:c.3865_3868del	1	1	
	NM_000059.3:c.3881T>A	1		
	*NM_000059.3:c.5771_5774del	2		4
	*NM_000059.3:c.582G>A	2		1
	*NM_000059.3:c.5946del	1		
	NM_000059.3:c.6447_6448dup	3		
	NM_000059.3:c.7558C>T	1		
	*NM_000059.3:c.7934del	4	3	
	NM_000059.3:c.9105T>A	1		
CHEK2	NM_007194.3:c.1100del		1	
	NM_007194.3:c.283C>T	1		
	NM_007194.3:c.779del	1		
PALB2	NM_024675.3:c.2167_2168del	1		
	NM_024675.3:c.2835-1G>C	2		1
TP53	NM_000546.5:c.831_835del	1		
	NM_000546.6:c.742C>T	1		

P/LP Variants found in additional 75 genes tested				
Gene	**Variant**	**Mixed Ancestry (n=172)**	**White (n=41)**	**Black African (n=60)**
BAP1	NM_004656.4:c.1793del	1		
BRIP1	NM_032043:c.1162C>T	1		
FH	NM_000143:c.1431_1433dup	2		
MSH6	NM_000179:c.3632T>C	1		
MUTYH	NM_001128425:c.1187G>A	1	1	
RAD50	NM_005732:c.412C>T	1		
RAD51C	NM_058216:c.491_492del	1		
RAD51D	NM_002878:c.619T>C	1		
RECQL4	NM_004260:c.2412_2420del	1		1

*Common founder P/LP variant in Afrikaans, Black or Ashkenazi Jewish populations

Supplementary Table 3.4: Number of P/LP variant per gene for each ancestry group

		Number of P/LP variant by Gene and Ancestry Group		
Gene-panel size	**Gene**	**Black (n=72)**	**White (n=53)**	**Mixed Ancestry (n=206)**
9 gene panel (n=331)	*ATM*	0	2 (3.8 %)	3 (1.5%)
	BRCA1	6 (8.3%)	5 (9.4 %)	8 (3.8%)
	BRCA2	5 (6.9%)	4 (7.5 %)	16 (7.8%)
	CHEK2	0	1 (1.9%)	2 (1.0 %)
	PALB2	1 (1.4 %)	0	3 (1.5%)
	TP53	0	0	2 (1.0 %)
	Total	12	12	34
	Gene	**Black (n=60)**	**White (n=41)**	**Mixed Ancestry (n=172)**
75 gene panel (n=273)	*BAP1*	0	0	1 (0.6%)
	BRIP1	0	0	1 (0.6 %)
	FH	0	0	2 (1.2%)
	MSH6	0	0	1 (0.6%)
	MUTYH	0	1 (2.4%)	1 (0.6%)
	RAD50	0	0	1 (0.6%)
	RAD51C	0	0	1 (0.6%)
	RAD51D	0	0	1 (0.6%)
	RECQL4	1 (1.6%)	0	1 (0.6%)
	Total	1	1	10

CHAPTER 4

PREVALENCE OF VARIANTS IN BREAST CANCER SUSCEPTIBILITY GENES IN BLACK SOUTH AFRICAN WOMEN DIAGNOSED AGED 50 OR YOUNGER

This chapter is a description of a collaborative project between the Cancer Genetics group at the Sydney Brenner Institute for Molecular Bioscience, the Division of Human Genetics at the University of Witwatersrand, and the University of Washington (UW) in Seattle USA. The patients were recruited by the Johannesburg Cancer Study, and DNA was extracted from blood samples by the Division of Human Genetics. The gene panel sequencing, calling of variants and initial classification of variants was carried out at UW, and the data was shared with the author of this thesis, T. Osler by the Division of Human Genetics. Since the initial classification of variants was carried out several years ago, a revised classification was done by T. Osler using updated reference data. The analysis of variant prevalence and breast cancer subtypes was done by T. Osler. The report provided in this chapter was written by T. Osler, with advice from her PhD supervisors. All references for this thesis, including those for this article, are listed at the end of the document (p. 118).

4.1 SUMMARY

Background: Breast cancer (BC) is one of the most common cancers among Black South African women, with a proportion of cases caused by pathogenic or likely pathogenic (P/LP) variants in BC susceptibility genes. While testing guidelines based on international data are in place, the prevalence of these variants in this population remains unknown. Additionally, although *BRCA1* P/LP variants are strongly associated with triple-negative BC (TNBC) in other populations, this association has not been confirmed in South Africa. This study aimed to determine the prevalence of P/LP variants in 12 BC susceptibility genes and assess the prevalence of TNBC among Black South African women. We also evaluated the utility of using African genomic allele frequency (AF) data in reclassifying variants of uncertain significance (VUS).

Methods: Clinical records and sequencing data from 272 Black South African women diagnosed with BC at age 50 or younger were included. The AF of VUS identified in participants was assessed using collated African genomic data to aid in classification.
Results: The overall prevalence of P/LP variants in BC susceptibility genes was 10.3%, with *BRCA1/2* accounting for 8.5%. Current guidelines recommend genetic testing for women diagnosed at age 40 or younger when there are no additional risk factors; however, we found no significant difference in P/LP variant prevalence between women diagnosed at or below age 40 and those diagnosed between 41 and 50. The prevalence of TNBC (14.5%) was lower than previously reported in South African studies, and the proportion of ER-negative tumours in participants with *BRCA1* P/LP was also lower (20.0%) than that reported previously (78.0%). Of the 65 distinct VUS identified, two had an AF above 0.01 or the gene-specific threshold in African genomic data. However, one had already been reclassified in a prior study, and the other was located in an untranslated region.
Conclusion: Our findings highlight the potential of expanding genetic testing to Black South African women diagnosed with BC between the ages of 40 and 51. They also raise the possibility that *BRCA1* P/LP variants may not be as strongly associated with ER-negative tumours in this population as in others. These insights contribute to the understanding of P/LP variant prevalence in BC susceptibility genes among Black South African women and can help inform future research and policy development.

4.2 INTRODUCTION

Breast cancer (BC) is the most common cancer in South African women and has been identified as a national priority in the country (Department of Health, South Africa, 2017; National Cancer Registry, 2022). Information on the contribution of genetic factors to BC risk is essential to allow for the provision of appropriate genetic testing, risk-reducing procedures and to determine the need for advanced cancer therapies. It is estimated, in studies of predominantly European populations, that 5 to 10% of BC cases are the result of a pathogenic/likely pathogenic (P/LP) variant in a BC susceptibility gene (Buys et al., 2017; Couch et al., 2017; C. Hu et al., 2021; Tung et al., 2015). Studies of P/LP variant prevalence in BC genes in South Africa have reported widely divergent figures. These studies are limited by relatively small numbers of participants, absence of ancestry data, significant bias through participant selection criteria or the inclusion of testing results for only founder variants which may inflate prevalence in ancestry groups with a high burden of these variants (Eygelaar et al., 2022; Francies et al.,

2015; Hayat et al., 2021; Makhetha et al., 2024; Schoeman et al., 2013; D. C. Smith et al., 2020; van der Merwe, Combrink, et al., 2022). Population-based studies investigating P/LP variant prevalence in a substantial panel of BC susceptibility genes are needed to assess mutation prevalence in a broader context.

Several population-based studies have been done in other sub-Saharan African (SSA) countries. These include two from Nigeria that recruited 434 and 1,136 unselected women with BC and both reported a *BRCA1/2* P/LP variant prevalence of about 11% (Fackenthal et al., 2012; Zheng et al., 2018). The larger study also reported the prevalence of P/LP variants in 23 other BC genes to be 3.6% (Zheng et al., 2018). A similar study in Uganda and Cameroon determined the prevalence of P/LP variants in 196 women with BC unselected for age or family history to be 15.8%. Most of the P/LP variants were found in *BRCA1/2* (11.2 %), while 4.5% were found in one of the other 10 genes tested (Adedokun et al., 2020). Two additional studies from SSA that investigated P/LP prevalence in 871 and 100 unselected women with BC, respectively were done in Ghana and Tanzania (Ahearn et al., 2022; Rweyemamu et al., 2023). Interestingly both found *BRCA1/2* P/LP prevalence to be about 6%, just more than half the prevalence found in Nigeria, Uganda and Cameroon. It is not clear whether these differences in prevalence are related to criteria for participant recruitment, technical issues such as sequencing technologies and calling algorithms, or other unknown factors.

Importantly, although a lower prevalence of P/LP variants was found in *BRCA1/2* in the Ghanaian study, it was still more than double the 2.6% prevalence reported in the BRIDGES study, which included over 60,000 women of European or Asian ancestry, (Ahearn et al., 2022; Breast Cancer Association Consortium et al., 2021). Other studies comparing the prevalence of P/LP variants in *BRCA1/2* in different population groups of unselected women with BC have mostly been done in the USA and compared African American to non-Hispanic White populations with conflicting results. In a study of 3,946 African American women and 25,287 non-Hispanic White women with BC unselected for family history or age at diagnosis, Domchek et al., (2021) reported the prevalence of *BRCA2* P/LP variants to be higher in African American women. However, after adjusting for age at diagnosis, the difference between the groups was no longer statistically significant (Domchek et al., 2021). Several studies of testing data from clinical laboratories have also been done to compare population prevalences in the USA. Participants in these studies mainly include women at increased risk of having a P/LP variant who had been offered testing. Using records from Ambry Genetics, Yadav et al., (2021)

found no difference in *BRCA1/2* prevalence between 57,003 Non-Hispanic White and 6,722 African American participants. However, data from Myriad Genetics showed African American women (15.6%) to have a higher prevalence of *BRCA1/2* P/LP variants compared to non-Hispanic White women (12.1%) (M. J. Hall et al., 2009). Findings in African American populations cannot, however, be generalized to populations in South Africa or other SSA countries owing to the extensive genetic diversity across African populations (Campbell & Tishkoff, 2008; Choudhury et al., 2020).

A strong association between and the development of triple-negative breast cancer (TNBC) and the presence of P/LP variants in *BRCA1* has been established in Western and Asian populations (Breast Cancer Association Consortium et al., 2021; H. Chen et al., 2018). These tumours, in which the cells do not express oestrogen receptors (ER), progesterone receptors (PR) or overexpress human epidermal growth factor receptor 2 (HER2), are aggressive and have a poor prognosis. The prevalence of TNBC is known to vary significantly in different population groups and to be impacted by many factors (Howard & Olopade, 2021). The proportion of TNBC tumours in Black South African women with *BRCA1* P/LP variants has not been previously established. This information is valuable to allow for appropriate risk management of Black South African women with a *BRCA1* P/LP variant.

When testing a large number of genes to detect P/LP variants, there is a considerable likelihood of identifying variants of uncertain significance (VUS). This has been shown to be significantly higher in individuals of African ancestry compared to those of European ancestry (Caswell-Jin et al., 2017; Ndugga-Kabuye & Issaka, 2019; Osler et al., 2024; Tatineni et al., 2022). We recently demonstrated that incorporating allele frequency (AF) data from African genomes provided additional information in 27% of individuals with a VUS, facilitating variant classification according to the American College of Medical Genetics and Genomics and the Association for Molecular Pathology (ACMG-AMP) guidelines (Osler et al., 2024; Richards et al., 2015).

The aim of this study was to determine the prevalence of P/LP variants in a population-based study of BC susceptibility genes in Black South African women with BC. The study was focused on women diagnosed at 50 years of age or younger to determine whether there is a higher prevalence of P/LP variants in younger women in this population, and what implications

this might have for future screening strategies. We also aimed to determine the proportion of participants with TNBC who had a *BRCA1/2* P/LP variant. In addition, we assessed the benefit of using African genome data for the re-classification of VUS detected in these genes.

4.3 METHODS

4.3.1 Study Participants

The study participants were Black South African women recruited into the Johannesburg Cancer Study (JCS) and diagnosed with primary invasive BC between 2007 and 2009 at age 50 or younger. The JCS was a population-based study that recruited participants between 1995 and 2016 to investigate risk factors associated with various cancer types. Collected data included age at diagnosis, BC subtype, and self-reported ethnolinguistic group. Blood samples were taken at recruitment into JCS and stored for later genetic testing (Chen et al., 2020). Immunohistochemistry was performed in all cases at the National Health Laboratory Service (NHLS) to determine hormone receptor status (National Health Laboratory Service, 2020) The results, including estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2), were then manually extracted from participants' histology records.

A total of 285 women from the JCS records were initially enrolled in this study; however, 12 were excluded due to factors such as insufficient DNA quality, unconfirmed diagnosis, or age over 50 years. This resulted in a final sample of 272 participants.

4.3.2 Genetic Analyses

DNA extraction from the stored samples was performed using the Qiagen Flexigene DNA kit at the Division of Human Genetics, National Health Laboratory Service in Johannesburg (W. C. Chen et al., 2018). High-throughput sequencing of DNA samples was performed at the University of Washington using the BROCA Cancer Risk panel. Sequencing and analysis were performed as previously described (Walsh et al., 2010). Although 33 genes were sequenced, we included only 12 genes in our analysis which have been confirmed by ClinGen curation expert panels to confer susceptibility to BC, namely, *ATM, BARD1, BRCA1, BRCA2, CDH1, CHEK2, PALB2, PTEN, RAD51C, RAD51D, STK11, TP53* (ClinGen, n.d.).

4.3.3 Identification of P/LP variants

We used ANNOVAR to annotate the variants detected in participants, using the Matched Annotation from NCBI and EMBL-EBI (MANE) transcript (*MANE*, n.d.; Wang et al., 2010). P/LP variants were identified by first excluding variants with an AF above 0.05 in any of the population groups in gnomAD v4 (Gudmundsson et al., 2022). We then selected variants predicted to result in a frameshift, stop-gain, or to disrupt splicing, as well as non-synonymous single nucleotide variants (SNVs) with a REVEL score threshold above 0.75 (Ioannidis et al., 2016). Finally, variants classified as likely benign or benign in ClinVar were excluded (Landrum et al., 2018). Variants with conflicting classifications in ClinVar were manually reviewed. Variants with a combination of classifications, including pathogenic and/or likely pathogenic, were retained in our study as P/LP variants. In addition, variants classified as pathogenic or likely pathogenic by at least two clinical laboratories (Ambry Genetics, SCRP, Invitae, GeneDx, Counsyl, InSiGHT), were designated as P/LP, even if another entity had entered a VUS classification in ClinVar. Variants classified with any combination of VUS, likely benign or benign were excluded.

4.3.4 Identification of VUS

We used two processes to select VUS from the list of participant variants. First, we selected those already classified as VUS in ClinVar and manually reviewed all variants with conflicting classifications in ClinVar. When there were conflicting classifications, we applied the following criteria: if one classification was significantly more frequent than the others, we adopted that classification. When different classifications were similar in number, we prioritized those curated by clinical laboratories as was done for pathogenic variants. If uncertainty remained, the variant was retained as a VUS. Secondly, when variants did not have a ClinVar classification, we selected VUS by removing those with AF greater than 0.05 in gnomAD v4. We also excluded variants that were frameshift, stop-gain or start loss as they are more likely pathogenic. Lastly, we excluded all variants in 5' and 3' untranslated regions.

As in our previous study, we determined the AF of each VUS in whole genome sequencing (WGS) data from a total of 1,865 individuals of African ancestry (Osler et al., 2024). Shared data used to create the African genomic database came from five projects: MalariaGen (252 genomes), the African Genome Variation Project (AGVP) (320 genomes), AWI-Gen SA (100 genomes), H3Africa Consortium WGS VCF (386 genomes) and ARK (798 genomes) (Band

et al., 2019; Choudhury et al., 2020; Fabian et al., 2022; Gurdasani et al., 2015; Ramsay et al., 2016). We compared the AF determined in the African genomes to gene-specific AF guidelines as specified by ClinGen (*About ClinGen Expert Panels*, n.d.). If gene-specific guidelines were not available, we compared the AF to a threshold of 0.01 as done previously. The highest sub-population AF of the detected VUS was also determined in gnomAD v4 for comparison.

VCFtools v0.1.17 was used to determine the AF of each VUS in the five data sources individually using the appropriate genomic position (GRCh37 or GRCh38) (Danecek et al., 2011). Subsequently, an overall AF using the information from each data source was calculated for every VUS.

4.3.5 Statistical Analysis

Data were collated and analysed using R Studio (R Core Team, 2022). The prevalence of variants was expressed as percentages with 95% confidence intervals. Univariate logistic regression analysis was performed to assess the association between age, both as a continuous and bivariate variable and the presence of a P/LP variant. A Fischer's Exact test was used to compare the prevalence of tumour subtypes in participants with *BRCA1/2* P/LP variants.

4.4 RESULTS

The demographic and clinical characteristics of the 272 study participants are shown in Table 4.1. The most common ethnolinguistic group in the participants was the Nguni group, which included Zulu and Xhosa speakers. Invasive ductal carcinoma (no special type) was the most frequent type of BC diagnosed in participants.

Table 4.1: Demographic and Clinical characteristics of the study sample

Demographic and Clinical Variables		Participants (n=272)
Age at diagnosis	Mean	41.2 years
	Standard Deviation	+/- 6.3 years
Home Language	Nguni	126 (46.3%)
	Sotho-Tswana	111 (40.8%)
	Tsonga	24 (8.8%)
	Tshivenda	5 (1.8%)
	Other	6 (2.2%)
Breast Cancer type	Invasive ductal carcinoma, no special type	250 (91.9%)
	Invasive lobular carcinoma	5 (1.8%)
	Invasive breast cancer, special type	17 (6.25%)

4.4.1 Pathogenic/Likely Pathogenic Variants Detected

The P/LP variants identified in the 272 participants are shown in Table 4.2. A total of 28 P/LP variants were detected, resulting in an overall prevalence of 10.3% (95% CI: 6.7% - 13.9%). Of these, seven were found in *BRCA1* and 16 in *BRCA2,* together accounting for 82.1% of the P/LP variants detected. There were seven participants found to have the known South African *BRCA2* founder variant (NM_000059:c.5771_5774del) (Oosthuizen et al., 2021), which accounted for 30.4% of the variants detected in *BRCA1/2* . The prevalence of P/LP variants in *BRCA1/2* alone was 8.5% (95% CI: 5.2% - 11.8%). No association was detected between participants' age at diagnosis and the presence of a P/LP variant. This lack of association was observed both when age was analyzed as a continuous variable (p = 0.64) and when the prevalence of P/LP variants was compared in participants aged over 40 years (18/163 (11%)) to those aged 40 years and younger (10/109 (9.2%)) (p = 0.62).

Table 4.2: Pathogenic variants detected in study participants (n=272)

Gene	HGVS nomenclature	Variant impact	ClinGen classification	dbSNP ID	No. participants with variant
ATM	NM_001351834:c.C382T (p.Q1274X)	stopgain	Pathogenic	rs1453429915	1
BRCA1	NM_007294.4:c.34C>T (p.Q12X)	stopgain	Pathogenic	rs80357134	1
	NM_007300:c.431dupA (p.N144Kfs*15)	frameshift insertion	Pathogenic	rs397509162	1
	NM_007294.4:c.1961dup (p.Y655Vfs*18)	frameshift insertion	Pathogenic	rs80357853	2
	NM_007294.4:c.2568T>G (p.Y856X)	stopgain	Pathogenic	rs80356832	1
	NM_007300:c.3227_3228del (p.G1077Afs*8)	frameshift deletion	Pathogenic	rs80357635	2
BRCA2	NM_000059:exon7:c.G582A (p.W194X)	stopgain	Pathogenic	rs80358810	1
	NM_000059:c.3790_3793del (p.K1264Vfs*11)	frameshift deletion	Pathogenic	rs80357864	1
	NM_000059:c.4709_4710del (p.E1571Gfs*3)	frameshift deletion	Not present	rs80359464	1
	NM_000059:c.C5750A (p.S1917X)	stopgain	Pathogenic	rs886040609	1
	*NM_000059:c.5771_5774del (p.I1924Rfs*38)	frameshift deletion	Pathogenic	rs80359535	7
	NM_000059:c.6146delT (p.V2049Gfs*2)	frameshift deletion	Not present	.	1
	NM_000059:c.6491_6492insGT (p.L2165Cfs*4)	frameshift insertion	Not present	.	1
	NM_000059:c.8961_8964del (p.S2988Ffs*12)	frameshift deletion	Pathogenic	rs80359734	1
	NM_000059:c.T9105A (p.Y3035X)	stopgain	Pathogenic	rs886040819	1
	NM_000059:c.9137delT (p.Q3047Rfs*15)	frameshift deletion	Not present	.	1
CHEK2	NM_007194.4:c.283C>T (p.R95X)	stopgain	Pathogenic	rs587781269	2
PALB2	NM_024675:c.2835-1G>C	splicing	Pathogenic/Likely pathogenic	rs515726099	1
TP53	NM_000546.6:c.375+1G>A	splicing	Pathogenic/Likely pathogenic	rs1567555445	1

*South African founder variant

4.4.2 Receptor Subtype Analysis

ER status was available for 220 participants, 32.3% of whom had an ER-negative tumour (Table 4.3). The number of ER-negative BC in participants with and without *BRCA1/2* P/LP was similar. Of the five participants with a *BRCA1* P/LP variant and known ER status, one

(20.0%) was ER-negative. Among the 14 participants with a *BRCA2* P/LP variant and known ER status, three (21.4%) were ER-negative. The prevalence of TNBC in the 179 participants with known ER, PR and HER2 status, was 14.5% (95%CI 9.3%-19.7%), with one of these participants having a P/LP variant in *BRCA*2.

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Table 4.3: Prevalence of pathogenic variants stratified by estrogen receptor (ER) status (n = 220)

Subtype	No. (%)	No. with P/LP detected	Gene indicated
ER-positive	149/220 (67.7%)	19/149 (12.8%)	*ATM* (1) *BRCA1* (4) *BRCA2* (11) *CHEK2* (3)
ER-negative	71/220 (32.3%)	6/71 (8.5%)	*BARD1* (1) *BRCA1*(1) *BRCA2* (3) *CHEK2* (1)

4.4.3 Variants of uncertain significance

We detected a total of 142 VUS, 65 of which were distinct. VUS were found in all 12 genes tested, with *ATM* (n =22) having the highest number of distinct VUS followed by *BRCA2* (n=11). There were 111 participants (40.8%) that had between one and four VUS. Of the 65 distinct VUS, 27 (41.5%) were present in the African genomic dataset, while 22 (33.8%) were present in gnomAD v4.

Two VUS were found to have an AF above 0.01 or the gene-specific threshold. The first was a VUS in *PTEN* (NM_001304717.5:c.90G>C), which had an AF above 0.01 in African data and was not present in ClinVar (Table 4.4). This variant is present in four of the *PTEN* transcripts but is predicted to fall within the coding sequence in only one of them. In the MANE transcript, it is located in the untranslated region (UTR) of exon 1. The second VUS which was detected in *PALB2,* had an AF exceeding the gene-specific AF threshold and more than the required five alleles in the African genomic data (Table 4.4). This VUS was reclassified as likely benign in our previously published study based on its AF in the African genomic data (Osler et al., 2024). This VUS was detected in four participants in the current study. The other 63 distinct VUS had AFs <0.01 in the African data or below the gene-specific threshold or did not have the minimum required alleles and thus could not be reclassified on the basis of variant AF.

Table 4.4: VUS found at above the gene-specific allele frequencies in African genomic data

Gene	VUS (HGVS nomenclature)	dbSNP ID	ClinVar category	No. alleles in study participants (n=272)	No. alleles in African genomes (n=1854)	AF in African genomes	Groupmax[1] AF in gnomAD	Above gene-specific AF in African genomes	Above gene-specific AF in gnomAD
PALB2	NM_024675.4:c.474G>C (p.Q158H)	rs878855119	VUS	4	7	0.0019	0.00001062	Yes	No
PTEN	NM_001304717.5:c.90G>C (p.R30S)	rs1451210152	Not present	20	42	0.011327	0.00004017	Yes	No

[1]The AF of the population group with the highest AF

4.5 DISCUSSION

Here, we provide the first population-based prevalence of P/LP variants in BC susceptibility genes in the Black South African population. Additionally, we examined the relationship between tumour receptor status and *BRCA1/2* P/LP variants and explored the potential of African genomic data in the reclassification of VUS detected in participants. This study enhances our understanding of the genetic landscape of hereditary BC in an underrepresented population, providing valuable insights into cancer genetics in South Africa.

The prevalence of P/LP variants in 12 BC susceptibility genes among Black South African women with BC aged ≤50 was found to be 10.3%. Comparing prevalence across studies is challenging due to differences in participant selection criteria and in the number of genes analysed. To address this, we first compared the prevalence of *BRCA1/2* variants across studies, before investigating variations in other BC susceptibility genes. The prevalence of P/LP variants in *BRCA1/2* in our study participants was 8.5%. In comparison, the prevalence reported for these two genes in unselected African population-based studies ranges from 6% to 11% (Zheng et al., 2018; Adedokun et al., 2020; Uyisenga et al., 2020; Ahearn et al., 2022), while figures from the general USA and European populations are much lower, ranging from 2.1% to 5.1% (Tung et al., 2016; Breast Cancer Association Consortium et al., 2021; Hu et al., 2021). While population-based studies help overcome the limitations of differing selection criteria, demographic differences between populations contribute to differences between studies. In this instance, African populations generally exhibit a lower overall incidence of BC, primarily due to the younger demographic profile in these countries (Bray et al., 2024). Consequently, the average age at BC diagnosis is younger than in high-income countries (HICs) (Lei et al., 2021). This is evident in the population prevalence studies cited above, where studies from USA and Europe report higher average ages at diagnosis (50.3 - 61.2 years) (Tung et al., 2016; Hu et al., 2021) compared to studies from African countries (44 - 50.8 years) (Zheng et al., 2018; Adedokun et al., 2020; Uyisenga et al., 2020; Ahearn et al., 2022). The latter findings are closer to the average age of 41.2 years observed in our selected study of women with BC diagnosed at or before age 50. Given the strong association between *BRCA1/2* P/LP variants and early-onset BC (Buys et al., 2017; C. Hu et al., 2021; Tung et al., 2016), this demographic difference likely contributes to the higher prevalence of these variants observed in African population studies. The influence of age on prevalence is highlighted by Tung et al.

(2016), who reported a *BRCA1/2* P/LP variant prevalence of 12.2% among BC patients aged <45 years in the USA, compared to 5.1% overall. This finding aligns with the range observed in our and other African population studies.

BRCA1/2 variants accounted for more than 80% of the P/LP variants identified in the participants from our study. Additional P/LP variants were found in *PALB2, TP53* and *ATM* (one each) and in *CHEK2* (two variants). When including only known BC susceptibility genes other than *BRCA1/2*, the combined prevalence in our study participants (1.8%) appears comparable to that reported in African populations (2.3% to 4.6%) (Adedokun et al., 2020; Ahearn et al., 2022; Zheng et al., 2018). However, given the rarity of P/LP variants in these genes, small differences in frequency may not be detectable across different population groups. Notably, in our study and other African populations, these variants account for a smaller proportion of total P/LP variants compared to large population-based studies from the USA and Europe, where their prevalence is more comparable to that of *BRCA1/2* (Breast Cancer Association Consortium et al., 2021; C. Hu et al., 2021). Since most P/LP variants in genes other than *BRCA1/2* are associated with BC at an older age, their lower prevalence in African populations is likely due to their younger average age as discussed previously.

We did not find a significant difference in the number of P/LP variants in BC susceptibility genes in women diagnosed aged 40 and below to those diagnosed between 41 and 50 years of age. This is an important finding, as the current South African National Department of Health (NDOH) guidelines for genetic testing for hereditary BC set the diagnostic age threshold for testing at age ≤40, in the absence of other risk factors (National Department of Health, 2018). Based on our findings, revision of the threshold to include genetic testing for Black South African women diagnosed with BC at ≤50 years of age should be considered.

Regarding the prevalence of receptor subtypes, the 32.3% prevalence of ER-negative tumours in our cohort appears to be consistent with other South African studies reporting rates of 33.8%, 36.7%, and 37.7% (Cubasch et al., 2018; Dickens et al., 2016; McCormack et al., 2013). The prevalence of TNBC differs markedly in different populations. A recent meta-analysis demonstrated that in Africa the highest prevalence of TNBC is in West Africa (49.4%), and the lowest in Central Africa (14.9%) (Hercules et al., 2022). We observed a lower prevalence of TNBC (14.5%) compared to the previously reported prevalence of 20.4% to 21.7% in South

African studies of Black women (Cubasch et al., 2018; Dickens et al., 2016; McCormack et al., 2013). The reason for this is unknown but provides additional evidence that the prevalence of TNBC is likely lower here than that observed in West Africa.

Only one P/LP variant (*BRCA2)* was detected in participants with TNBC resulting in a prevalence of 3.8% (1/26). This is much lower than studies of unselected participants with TNBC in Australia (n=439) and the USA (n=207) which found the prevalence of *BRCA1/2* variants to be 9.3% and 15.4% respectively (Wong-Brown et al., 2015; Sharma et al., 2014). The reason for the low prevalence of P/LP variants in TNBC participants in our study is uncertain but could be related to the significant family history of BC reported by 36 % of patients in the Sharma et al., (2014) study, as well as the small number of TNBC in our study.

BRCA1 P/LP variants, and to a lesser extent *BRCA2* P/LP variants, are well established to be associated with TNBC (H. Chen et al., 2018). However, since a substantial number of participants had incomplete hormone receptor results, we assessed the proportion of ER-negative tumours among participants with *BRCA1/2* P/LP variants. In *BRCA1* carriers, only one in five of the tumours were ER-negative. This is much lower than the 78.0% reported in a large study of 4,325 *BRCA1* carriers from the USA, Australia, and Europe, (Mavaddat et al., 2011), but the numbers are too small for a meaningful comparison. The proportion of ER-negative tumours among *BRCA2* P/LP carriers in our study was 21.4%, similar to the 23.0%, found by Mayaddat et al. (2011). The small number of participants with *BRCA1* P/LP variants and known receptor status limits the conclusions that can be drawn from these findings, further studies are needed to determine if there is an association between *BRCA1* and ER-negative tumours in the Black South African population.

Lastly, we investigated the potential for African genomic data to be used in reclassifying VUS, as demonstrated successfully in our previous study (Osler et al., 2024). In that study, the prevalence of VUS in nine genes tested was 23.6% in Black South Africans, substantially lower than the prevalence of 40.8% observed in this study. The higher prevalence here may partly be explained by the inclusion of three additional genes in the testing panel. However, even after excluding these genes, the VUS prevalence remains elevated at 36.8%, indicating that other factors are contributing to the difference. There were two VUS identified in the current study with AF above the 0.01 or gene-specific thresholds, one of which had been reclassified in our

previous study and the other of which was outside the coding region of the MANE transcript. The less successful outcome found in this study may be attributed to differences in the methods used for VUS classification across our two studies. However, the low yield of VUS reclassification in this study does not diminish the importance of incorporating additional African genomic data for variant classification as reported in our previous study (Osler et al., 2024).

This study has several limitations that should be acknowledged. Firstly, we excluded all variants located in untranslated regions. While this exclusion may have resulted in an underestimation of the prevalence of P/LP variants, it is unlikely to have had a significant impact, as P/LP variants in these regions are not commonly observed (Ellingford et al., 2022). Secondly, although this is the first population-based study examining the prevalence of P/LP variants in BC susceptibility genes in Black South African women, our inclusion criteria of women aged ≤50 years limited direct comparisons with other population-based studies, which often include broader age groups. To gain a more comprehensive understanding of the contribution of *BRCA1/2* variants to BC risk in this population, future studies should investigate a consecutive series of women with BC across all age groups. Lastly, the absence of HER2 receptor data for a subset of participants limited our ability to draw definitive conclusions about the relationship between P/LP variants and hormone receptor status.

Despite its limitations, this study offers valuable insights into the prevalence of P/LP variants in BC susceptibility genes among Black South African women, showing a prevalence of approximately 10.3% in women aged 50 and younger, consistent with findings from other studies. Furthermore, our results underscore the potential of expanding genetic testing to Black South African women diagnosed with BC between the ages of 40 and 51. These findings contribute to addressing disparities in genetic research and highlight the need for more inclusive studies to inform tailored healthcare strategies for this population.

CHAPTER 5

APPLICATION OF GENETIC TESTING CRITERIA FOR HEREDITARY BREAST CANCER IN SOUTH AFRICA

Osler, T. S., Schoeman, M., Pretorius, W. J. S., Mathew, C. G., Edge, J., & Urban, M. F. (2025). Application of genetic testing criteria for hereditary breast cancer in South Africa. *Breast Cancer Research and Treatment*, 210, 477-486.. https://doi.org/10.1007/s10549-024-07585-3

5.1 SUMMARY

Purpose Breast cancer (BC) is the commonest cancer in South African women. A proportion are associated with a pathogenic or likely pathogenic (P/LP) variant in a BC susceptibility gene. Clinical guidelines for genetic testing are used to optimise variant detection while containing costs. We assessed the detection rate in women of diverse ancestries who met the South African National Department of Health (NDOH) testing guidelines, and analysed relationships between testing criteria, participant characteristics and presence of a *BRCA1/2* P/LP variant.

Methods Records from 376 women with BC who met NDOH criteria and had genetic testing were included. Demographic, clinical and test result data were collated to describe detection rates according to criteria met, and a multivariate analysis conducted to find variables most frequently associated with a P/LP variant.

Results P/LP variant prevalence in women meeting NDOH testing criteria was 19.9% (75/376). Women meeting ≥2 guideline criteria were over twice as likely to have a P/LP variant (OR 2.27, 95%CI:1.27-4.07, p= 0.006), highlighting the guidelines' capacity to stratify risk. Family history (OR 1.97; 95%CI:1.05-3.70, p=0.03) and Black African ancestry (OR 2.58; 95%CI:1.28-5.18, p<0.01) were independently associated with having a *BRCA1/2* P/LP variant when controlling for other variables. Notably, although Black African participants were less likely to report a family history, those that did had higher odds of a P/LP variant in *BRCA1/2*.

Conclusion These results demonstrate the usefulness of the NDOH guidelines in women of diverse ancestries and provide insight into the factors associated with P/LP variants in understudied African populations.

5.2 INTRODUCTION

There were 11020 women diagnosed with breast cancer (BC) in South Africa in 2022, making it the commonest cancer in females (National Cancer Registry, 2022). A proportion are expected to be attributable to pathogenic or likely pathogenic (P/LP) variants in a germline BC susceptibility gene, such as *BRCA1/2.* Population-based studies from the USA put this proportion at 5-10% (Buys et al., 2017; Tung et al., 2015). Similar data from Africa is scarce, but West African studies give estimates of 8-14% (Adedokun et al., 2020; Ahearn et al., 2022; Zheng et al., 2018). P/LP variants in these genes are associated with lifetime risks of BC of 40% - 85% for high risk genes and 20% - 40% for moderate risk genes, causing significant morbidity and mortality (Lalloo & Evans, 2012). In addition, the risks of other cancers, such as ovarian and pancreatic cancers, are often also increased in gene carriers. Identifying individuals with these P/LP variants allows intensified surveillance, risk-reducing surgical interventions and modifications to cancer treatment to be made (Daly et al., 2021).

Given the modest proportion of BC associated with Mendelian susceptibility genes, and the historically high cost of genetic testing, clinical guidelines were developed to direct testing (Marmolejo et al., 2021; NCCN Guidelines, 2024a). Guidelines initially aimed to identify individuals with a 10% - 20% risk of carrying a germline P/LP variant in a high risk BC susceptibility gene, such as *BRCA1/2, TP53* and *PALB2* (Lalloo & Evans, 2012). Following the introduction of extended gene panel testing it has been noted that these guidelines are less successful at identifying individuals with P/LP variants in moderate penetrance genes, such as *ATM* and *CHEK2* (Beitsch et al., 2019). As a result of this, the increasing availability of targeted therapies and the decreasing cost of genetic testing over time, many high-income countries (HIC) have adjusted their policy guidelines to reduce the stringency of genetic testing criteria, with some calling for universal testing of women with BC (Marmolejo et al., 2021; Samadder et al., 2021). In low- and middle-income countries (LMIC) the successful implementation of genetic testing for hereditary cancer faces technological, health system, and patient-level barriers (Al-Sukhun et al., 2023). Clinical practice guidelines are necessary for ensuring accessible genetic testing and are recognised as a key need in some LMICs (Achatz

et al., 2020). Although guidelines have been published in a few LMICs (Malhotra et al., 2020; Xie & Wang, 2021) there is limited data available on their usefulness (Al-Sukhun et al., 2023).

In 2018, the South African National Department of Health (NDOH) published guidelines to establish standards for BC prevention and control. This included eligibility criteria for genetic testing, which remain in effect (Department of Health, South Africa, 2018). These criteria were adapted from the widely used National Comprehensive Cancer Network (NCCN) guidelines from the USA namely, "Genetic/Familial High-Risk Assessment: Breast and Ovarian, version 2.2016", which have since been updated (NCCN Guidelines, 2016). Criteria used in the NDOH and NCCN guidelines are summarised in Table 5.1 and show that the NDOH criteria were made more stringent by lowering the eligible age at diagnosis. Minimal local data was available to guide this process.

Table 5.1: A comparison of NDOH and NCCN v2.2016 criteria used to select women with breast cancer for genetic testing.

Genetic testing criteria for women with breast cancer	**NDOH[a] criteria**	**NCCN[b] v2.2016 criteria**
Known P/LP variant in a cancer predisposition gene in the family	Yes	Yes
Age of breast cancer diagnosis	≤ 40 years	Early-age onset BC (suggested ≤50y)
Triple negative breast cancer[c] <60 years	Yes	Yes
Two primary breast cancers at any age	Yes	Yes
≥1 close relatives with breast cancer < 50 years	Yes	Yes
≥1 close relative/s with invasive ovarian cancer	Yes	Yes
≥1 close relative/s with male BC	Yes	Yes
≥2 close relatives with breast/pancreatic cancer with at least one < 60 years old	Yes	Yes
From a population at increased risk	Absent	Yes

[a]NDOH – National Department of Health (South Africa) (National Department of Health, 2018), [b]NCCN – National Comprehensive Cancer Network (USA) (NCCN Guidelines, 2016), [c] Triple negative breast cancer - cancers that are estrogen and progesterone hormone receptor negative and human epidermal growth factor receptor 2 (HER2) amplification negative.

The suitability of these guidelines in our setting is uncertain for several reasons. The NCCN guidelines were developed using data from high-resource settings with predominantly European ancestry populations (Yadav & Couch, 2019). In contrast, South Africa is an upper middle income country with a majority Black African population (StatsSA, 2024). Incidence rates of BC vary considerably in different countries and populations. In South Africa, the age standardised incidence rate per 100 000 of BC in 2022 was 94.6 in White females (similar to European populations), 47.5 in Mixed Ancestry females and 21.2 in Black African females (Bray et al., 2024; National Cancer Registry, 2022). These differences raise uncertainty about whether similar genetic factors contribute to BC risk across the populations. In addition, the predictiveness of individual criteria is uncertain. For instance, one criterion is the presence of a breast tumour before the age of 60 that is estrogen and progesterone receptor negative with no overexpression of HER2, referred to as triple negative breast cancer (TNBC). Notably, populations with African ancestry may have a higher prevalence of TNBC (Jiagge et al., 2016; McCormack et al., 2013). Althoug9h TNBC has been shown to be associated with *BRCA1* P/LP variants in Nigerian patients (Zheng et al., 2018), African American women with TNBC are less likely to have a P/LP variant in *BRCA1* compared to women from other ancestry groups (Greenup et al., 2013). Consequently, the positive predictive value of TNBC for identifying P/LP variants in African populations remains unclear. Furthermore, Black African women in South Africa have on average a younger age of diagnosis than White women, (Dickens et al., 2016) which suggests that the age of BC diagnosis criterion may be less predictive of a P/LP variant in Black African women. Whether the NDOH guidelines are an effective tool for detecting P/LP variants in BC genes in South African women is unknown.

In South Africa, there is tension between optimising the detection rate of BC susceptibility genes and the cost of testing. The objectives of this study were to determine the P/LP variant prevalence in BC susceptibility genes in women meeting the NDOH guidelines in a South African setting and to compare the detection rates of individual criteria. In addition, we conducted multivariate analysis to assess the association of various demographic, clinical and tumour histological features with *BRCA1/2* detection rates.

5.3 METHODS

5.3.1 Participants

Participants included females with invasive or *in situ* BC who attended genetic counselling and testing for hereditary BC between 1 January 2018 and 1 October 2022 at Tygerberg Academic Hospital (TAH) in the Western Cape, South Africa. Participants were included if they met the NDOH testing criteria as shown in Table 5.1.

Of the 424 women who had testing during this period, 26 individuals were excluded from this study because they did not meet NDOH criteria. Additionally, 13 participants were excluded because they tested negative for common founder variants in *BRCA1/2* but were not tested for the presence of other potential P/LP variants in *BRCA1/2*. There were 9 participants with leiomyosarcoma, ovarian or pancreatic cancer who were excluded, as although these cancers are associated with P/LP variants in BC susceptibility genes, they are not included in the NDOH criteria. The remaining 376 participants were included in the study to determine P/LP variant prevalence. Three participants with a known familial P/LP variant were excluded from the multivariate analyses, as this indication is associated with a substantially higher likelihood of having a P/LP variant compared to other NDOH criteria.

5.3.2 Data Collection

Demographic, clinical, and test result data were collected for participants from TAH genetic counselling and histology records. Information on proband ancestry, required by the genetic testing laboratory to assist in variant interpretation, was inferred by the genetic counsellor in keeping with the population group categories of the South African National Census of 2022; White, Black African or Coloured. The latter descriptor is referred to as Mixed Ancestry in this study as the population has various ancestral roots, notably Khoe-San, European, Black African, and South and South-East Asian (de Wit et al., 2010). For all variables described in the study, data was available for >97% of patients.

5.3.3 Genetic testing

During the study period, genetic testing for hereditary BC was done at Invitae Corporation (Invitae Corporation, 2023) in the USA in 90.4% (340/376) of participants, with 12.8% (48/376) of participants tested locally at the National Health Laboratory Service (NHLS) (van

der Merwe et al., 2020). There were 12 participants (3.2%) who had testing at both providers. Although participants had testing of between 2 and 102 genes, we included only the results for 13 genes (*ATM, BARD1, BRCA1, BRCA2, CDH1, CHEK2, NF1, PALB2, PTEN, RAD51C, RAD51D, STK11, TP53)* known to be associated with BC and having clinical guidelines for risk-reduction. Because not all probands had testing of the same genes, with the exception of *BRCA1/2*, denominators used to calculate the prevalence of P/LP variants differed. In addition, it meant we could not include the detection of P/LP variants in genes other than *BRCA1/2* in the multivariate analysis of NDOH criteria.

The Invitae gene panel protocol used next-generation sequencing (NGS) to detect copy number and single nucleotide variants in coding exons and 10-20 flanking base pairs for most genes (Invitae Corporation, 2023; *Technology & Quality Faqs | For Providers | Invitae*, n.d.). The Sherloc modifications of the American College of Genetics and Genomics and the Association for Molecular Pathology (ACMG-AMP) guidelines were used by Invitae for variant interpretation (Nykamp et al., 2017). NHLS genetic testing was done using various methods: targeted analysis of 8 well-documented founder pathogenic variants in *BRCA1/2*; single nucleotide and copy number variant (CNV) detection within the exons and splice-site junctions of *BRCA1/2*; family variant testing; and a next generation (NGS) gene sequencing panel of 15 genes (van der Merwe, Ntaita, et al., 2022). NHLS testing was conducted in a diagnostic laboratory accredited by the South African National Accreditation System (SANAS) (*South African National Accreditation System*, n.d.), with variants interpreted using the ACMG-AMP variant interpretation guidelines (Richards et al., 2015).

5.3.4 Data Analysis

Study data were stored in a RedCap Database (Harris et al., 2009) and statistical analysis was done using RStudio (R Core Team, 2022). A *P* value <0.05 was considered statistically significant. Chi-square or Fischer's exact test were used to find group differences when variables were categorical and the Kruskal-Wallis test when data was continuous. When significant differences were found, odds ratios, 95% confidence intervals and associated p values were calculated.

Logistic regression was used to examine the relationship between detecting a P/LP variant in *BRCA1/2* and demographic and clinical variables, as well as NDOH criteria. Initially, each

variable was included in a univariate model to determine its predictive value (Table 5.2). Variables with $p < 0.2$ were included in the multivariate analysis. The absence of multicollinearity between the predictor variables was confirmed using variance inflation factors. As ancestry was comprised of three categories, the Mixed Ancestry group was used as the reference/index category with White and Black African groups compared to it. Results are reported as odds ratios (OR) with 95% confidence intervals (CI) and associated p-values.

5.4 RESULTS

The prevalence of P/LP variants found in the 376 study participants was 19.9% (75/376), with 17.0% in high-penetrance (*BRCA1/2, PALB2, TP53*) and 2.9% in moderate penetrance (*ATM, CHEK2, RAD51C, RAD51D*) genes. 15.4% (58/376) of the participants had a P/LP variant in *BRCA1/2*, with more in *BRCA2* (35/376, 9.3%) than in *BRCA1* (23/375, 6.1%) (see Supplementary Table 5.1).

To assess the NDOH criteria, we first compared the prevalence of P/LP variants for each criterion (Table 5.2). Of participants who satisfied only one of the NDOH criteria (either family history, triple negative breast cancer (TNBC), young age of cancer diagnosis or >1 primary BC), 11.7% (31/265) had a P/LP variant in *BRCA1/2* and 5.1% (12/235) in one of the other BC susceptibility genes. Individually, each of the four criteria was associated with a similar prevalence of P/LP variants in *BRCA1/2* (p=0.88) and other BC susceptibility genes (p=0.35). However, participants who met two or three of the criteria were twice as likely to have a P/LP variant detected in *BRCA1/2* (OR 2.27, 95% CI: 1.27-4.07, p= 0.006) than those meeting only one. This increased likelihood did not apply to other BC susceptibility genes, where meeting one or more criteria resulted in similar odds of having a P/LP variant (OR 1.02, 95% CI: 0.35-2.98, p=0.82).

Table 5.2: P/LP variant detection rates by NDOH testing criteria

No. NDOH criteria met (n=376)	Testing criteria	No. proband	Gene with P/LP variant detected [a]						Total detection rate in all genes[f]
			BRCA1/2	*ATM*	*CHEK2*	*PALB2*	*TP53*	*RAD51C*	
	Known variant in the family	3	2/3 (66.7%)						66.7%
One criterion	Family history	58	8/58 (13.8%)	4/56 (7.1%)	1/56 (1.8%)				22.9%
	TNBC[b] at <60 years	73	8/73 (11.0%)					1/57 (1.8%)	12.7%
	Age of diagnosis ≤40	119	14/119 (11.8%)	2/109 (1.8%)	2/109 (1.8%)	1/110 (0.9%)			16.3%
	>1 primary BC	15	1/15 (6.7%)			1/12 (8.3%)			15.0%
Two criteria[c]		90	19/89 (21.3%)			2/85 (2.4%)	2/85 (2.4%)		26.1%
Three criteria[c]		18	6/18 (33.3%)					1/15 (6.7%)	40.0%

[a] Denominators reflect the number of participants who had testing for the gene in question, [b] TNBC refers to triple negative breast cancer which is estrogen and progesterone receptor negative and HER2 amplification negative, [c] Indications include any combination of the following criteria: family history, triple negative breast cancer, young age of diagnosis and >1 primary BC. Known variant in the family was not included in this analysis., [d] Comparison of *BRCA1/2* P/LP variant prevalence in participants meeting only one of the four criteria (family history, triple negative breast cancer, age at diagnosis, >1 primary BC), [e] Comparison of prevalence of P/LP variants in other BC genes in participants meeting one of the four criteria (family history, triple negative breast cancer, age at diagnosis, >1 primary BC), [f] Calculated by summing percentages in the preceding columns.

Table 5.3 presents the univariate analysis of test indications, demographic data and clinical variables of 373 participants (three participants with known family variant were excluded) by their *BRCA1/2* P/LP variant status. Overall, age at diagnosis was the most frequently met NDOH test criterion (55.0%), followed by TNBC (35.4%) and family history (34.3%), with >1 primary BC (9.4%) being the least frequent. We assessed the influence of each of these four criteria, along with participant demographic and clinical variables, on the odds of detecting a P/LP variant in *BRCA1/2* using multivariate logistic regression. Based on the initial univariate analysis, we included ancestry, family history, >1 primary BC and cancer stage at diagnosis in the logistic regression model (Table 5.4).

The model showed that family history and ancestry were independently associated with the presence of a P/LP variant in *BRCA1/2*. Participants with a family history were almost twice as likely to have a P/LP variant in *BRCA1/2* (OR=1.97; 95% CI: 1.05-3.70, p=0.03), while participants of Black African ancestry had 2.58 (95% CI: 1.28-5.18, p<0.01) times higher odds of having a P/LP variant than the reference group (those of Mixed Ancestry). From the regression analysis, we calculated that participant of Black African ancestry reporting a family history had a 5.01 times higher chance of having a P/LP variant in *BRCA1/2* compared to the reference (Mixed Ancestry participants without a family history).

Table 5.3. Demographic and clinical data for study participants by *BRCA1/2* status

Demographic and Clinical variables		Participants (n=373)	*BRCA1/2* negative (n=317)	*BRCA1/2* positive (n=56)	*P* value
Ancestry / Population group	Mixed Ancestry	226 (60.6%)	199/226 (88.1%)	27/226 (11.9%)	0.05*
	Black African	93 (24.9%)	74/93 (79.6%)	19/93 (20.4%)	
	White	54 (14.5%)	44/54 (81.5%)	10/54 (18.5 %)	
Age at diagnosis	Median	39	39	38	0.45
	Range	18-76	18-76	24-73	
Breast cancer type[a]	Ductal carcinoma *in situ*	6/364 (1.6%)	5/6 (83.3%)	1/6 (16.7%)	0.36
	Invasive ductal carcinoma	323/364 (88.7%)	274/323 (84.8%)	49/323 (15.2%)	
	Invasive lobular carcinoma	14/364 (3.8%)	12/14 (85.7%)	2/14 (14.3%)	
	Other	21/364 (5.8%)	20/21 (95.2%)	1/21 (4.8%)	
Breast tumour receptors	Triple negative breast cancer	133/370 (35.9%)	113/133 (85.0%)	20/133 (15.0%)	0.94
	Other	237/370 (64.1%)	202/237 (85.2%)	35/237 (14.8%)	
Cancer stage[b]	*in situ*	6/368 (1.6%)	5/6 (83.3%)	1/6 (16.7%)	0.10*
	Stage 1	21/368 (5.7%)	18/21 (85.7%)	3/21 (14.3%)	
	Stage 2	127/368 (34.5%)	102/127 (80.3%)	25/127 (19.7%)	
	Stage 3	158/368 (42.9%)	140/158 (88.6%)	18/158 (11.4%)	
	Stage 4	53/368 (14.4%)	44/53 (83.0%)	9/53 (17.0%)	
	Recurrence	3/368 (0.8%)	3/3 (100%)	0	
NDOH indications					
Family history	Meets NDOH family history criterion	128/373 (34.3%)	103/128 (80.5%)	25/128 (19.5%)	0.09*
	Does not meet NDOH family history criterion	245/373 (65.7%)	214/245 (87.3%)	31/245 (12.7%)	
Triple negative breast cancer <60 years	Triple negative breast cancer	132/373 (35.4%)	112/132 (84.8%)	20/132 (15.2%)	1.0
	Other	241/373 (64.6%)	205/241 (85.1%)	36/241 (14.9%)	
Age at diagnosis	≤40 years	205/373 (55.0%)	172/205 (83.90%)	33/205 (16.1%)	0.56
	>40 years	168/373 (45.0%)	145/168 (86.3%)	23/168 (13.7%)	

>1 primary BC	Yes	35/373 (9.4%)	26/35 (74.3%)	9/35 (25.7%)	0.08*
	No	338/373 (90.6%)	291/338 (86.1%)	47/338 (13.9%)	

* Variables with p<0.2 which were included in the multivariate analysis, [a] P value calculated by comparing those with invasive ductal carcinoma to other categories combined., [b] P value calculated by comparing in situ and stages 1/2 vs stages 3, 4 and recurrence

Table 5.4: Logistic regression model predicting *BRCA1/2* P/LP variant from demographic, clinical and test indication data in study participants

Outcome: *BRCA1/2* P/LP variant	**Regression coefficient**	**Std Error**	**Z value**	**P value**	**OR (95% CI)**
Intercept	-2.17	0.32	-6.772	0	0.11 (0.06-0.21)
Family history reported	0.68	0.32	2.13	0.033*	1.97 (1.05-3.70)
>1 primary BC	0.82	0.43	1.88	0.060	2.26 (0.92-5.15)
Late cancer stage	-0.45	0.30	-1.48	0.138	0.64 (0.35-1.17)
Black African	0.95	0.35	2.67	0.008*	2.58 (1.28-5.18)
White	0.38	0.42	0.92	0.357	1.47 (0.62-3.25)

* Indicates significant values

Given that ancestry was a significant variable in the logistic regression analysis, we compared the frequency of the four testing indications by ancestry (Figure 5.1). The indications TNBC (p=0.77) and >1 primary BC (p=0.08) were found to occur at similar frequencies across the three ancestry groups. However, participants of Black African ancestry were significantly less likely to meet the NDOH family history criterion (p=2.439e-06), and more likely to meet the age at diagnosis criterion (p=2.247e-06). Participants from all three ancestry groups were equally likely to meet one (p= 0.62), two (p=0.73) or three test indications (p=0.34).

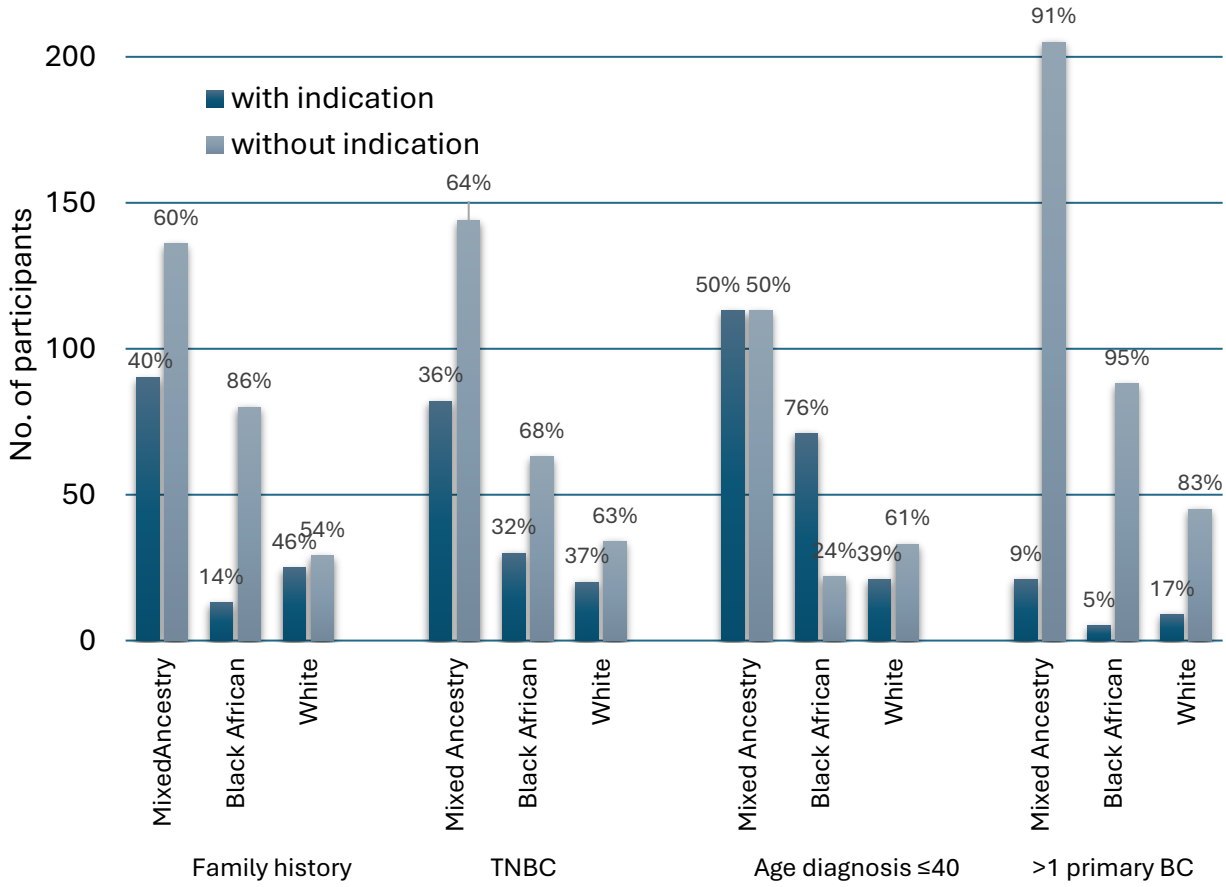

Figure 5.1 Frequency of NDOH genetic testing criteria met in South African Mixed Ancestry, Black African and White ancestry groups

5.5 DISCUSSION

The existence of a guideline for genetic testing demonstrates that the South African Department of Health acknowledges the benefit of identifying women with P/LP variants in BC susceptibility genes (National Department of Health, 2018). Our finding that almost 20% of participants meeting the recommended criteria had a P/LP variant detected indicates a high detection rate and suggests that these guidelines are useful in our setting. The higher prevalence of P/LP variants in *BRCA1/2* found in our study (15.4%) compared to other studies (9.2% and 6.0%) which investigated prevalence in women meeting different versions of the NCCN guidelines, may be due to the more stringent criteria specified in the NDOH guideline (Beck et al., 2020; Cropper et al., 2017). Although, expanding genetic testing to the entire BC population would be ideal to optimise detection, limited resources in the country make this unachievable (Kromberg et al., 2013). These resources include not only genetic testing, but also genetic counselling and changes to clinical management. Therefore, we recommend prioritizing the

expansion of genetic counselling and testing services and the development of specific guidelines tailored to local needs as an equitable way to increase identification of women with P/LP variants.

Family history of cancer, age at diagnosis, TNBC and >1 primary BC on their own were associated with a similar prevalence of P/LP variants in *BRCA1/2.* However, as has been demonstrated in other studies, participants with two or three indications were significantly more likely to have a P/LP variant in *BRCA1/2* detected (Beck et al., 2020; Cropper et al., 2017; Lertwilaiwittaya et al., 2021). This confirms that the criteria have a cumulative effect, each contributing to the likelihood of a P/LP variant, thereby making risk stratification possible. Although the NDOH guidelines were developed to detect *BRCA1/2* P/LP variants, clinically actionable variants in other BC susceptibility genes were found in an additional 5% of participants, supporting the utility of extending testing beyond *BRCA1/2* in our setting. However, the similar prevalence of P/LP variants among participants meeting one versus two or three of the criteria suggests that these criteria are ineffective at differentiating gradations of risk for variants in BC genes other than *BRCA1/2*. This finding is consistent with reports that the NCCN guidelines are less effective at identifying P/LP variants in moderate-risk BC susceptibility genes (Al-Sukhun et al., 2023; Beitsch et al., 2019; Yadav et al., 2020). Further evaluation of the guidelines is needed to determine the proportion of all P/LP variants in women with BCs that are detected by applying the NDOH criteria.

We found that when controlling for other factors, reporting a family history and being of Black African ancestry were independently associated with having a P/LP variant in *BRCA1/2.* Family history is a well-established risk factor for a *BRCA1/2* P/LP variant. The reason proportionally more participants of Black African ancestry had a *BRCA1/2* P/LP variant is uncertain. One possibility is that *BRCA1/2*-related BC is more common in Black African women in South Africa, as found in case-control studies in West African and African American populations (Adedokun et al., 2020; Ahearn et al., 2022; Zheng et al., 2018), although population studies have not been conducted locally. Alternatively, but not mutually exclusive, the proportion of *BRCA1/2*-related BC may be higher because environmental factors contribute less risk. This is compatible with South African registry data indicating that the prevalence of BC in the Black African population (lifetime risk 1 in 43) is lower than that of both Mixed Ancestry (1 in 18) and White (1 in 10) populations (National Cancer Registry, 2022). It is also consistent with evidence of increasing BC incidence in African countries, that has been

attributed to "westernization" of lifestyles (Joko-Fru et al., 2020). Population-based studies would be required to disaggregate these possibilities. A further possible cause of the disparity in *BRCA1/2* prevalence may be differences in the NDOH criteria met by Black African participants; they were less likely to meet the family history and far more likely to meet the age of diagnosis criterion. However, as age of diagnosis was not found to be associated with a P/LP variant any more strongly than the other criteria, but family history was, this seems an unlikely explanation.

The low proportion of participants of Black African ancestry meeting the family history criterion may be attributed to the above-mentioned lower incidence of BC or possibly to a lower penetrance of *BRCA1/2* P/LP variants, as was found in a case-control study in Ghana (Ahearn et al., 2022). Additionally, health-related disparities, which disproportionately affect Black South Africans (Bredenkamp et al., 2021), may contribute to shorter life expectancy, reducing the likelihood of a cancer family history, given the positive association of cancer with age (Brinton et al., 2014). The disparity in reported family history may also be the result of a lower awareness of medical family history in Black African participants. This supposition is supported by findings from a study in the USA which showed that medically under-served groups (primarily African Americans and non-English speakers) had less knowledge of their family cancer history (Chavez-Yenter et al., 2022). Likewise, a South African study found that 30% of their predominantly Black African participants with idiopathic dilated cardiomyopathy did not have knowledge of their family history. Reasons included having little recent contact with relatives living elsewhere, poor medical literacy and difficulty accessing relatives' death certificates (Bailly et al., 2019). The low frequency of family history being reported by Black African women requires exploration. However, our finding suggests that ancestry should be considered when offering genetic testing for hereditary breast cancer. We therefore recommend that in our setting Black African women with BC who have any family history of hereditary BC-associated cancers should be offered genetic testing even if they do not meet the defined NDOH criteria. In addition, awareness of the medical family history should be promoted.

Our assessment of the NDOH guidelines was limited by the relatively small numbers of P/LP variants in demographic sub-groups and in genes other than *BRCA1/2*. Consequently, we could not determine the predictive value of the different indications for each ancestry group or gene. The NDOH criteria themselves are premised on prevalence and penetrance of P/LP variants

being relatively similar to those in Western populations. Our study adds to evidence from studies elsewhere in Africa that this may not be the case. It is important to note that we assessed a clinical setting in which various genetic tests were performed depending on availability, cost and other factors. Targeted mutation analysis alone was performed in a minority of cases, and 13 were excluded because this was negative. Also, in those receiving sequencing, the number of genes sequenced varied. The quoted prevalence of hereditary breast cancer (19.9%) is therefore an estimate, but if all 13 excluded cases were included but negative, this would give a lower bound of prevalence of 19.3% (75/389).

The high prevalence of P/LP variants detected in participants as well as evidence that P/LP variant risk can be stratified by the number of criteria met, provides evidence of the value of the NDOH guidelines in a South African public health service setting. The increased detection rate of *BRCA1/2* P/LP variants in women with either a relevant family history or Black African ancestry, and especially both together is important to guide genetic testing and thereby is relevant to genetic counselling. Further research and possible refinement of the NDOH guidelines should be considered to address the fact that Black African women were less likely than other women to meet the family history criterion and more likely to meet the age of onset criterion.

5.6 ACKNOWLEDGEMENTS

None

5.7 CONFLICT OF INTEREST

The authors declare no conflicts of interest.

5.8 AUTHOR CONTRIBUTIONS

Conceptualization: Michael Urban, Tabitha Osler; Data curation: Tabitha Osler, Willem Pretorius, Mardelle Schoeman; Formal analysis: Tabitha Osler; Funding acquisition: Michael Urban, Mardelle Schoeman, Chris Mathew; Investigation: Tabitha Osler, Mardelle Schoeman, Willem Pretorius; Methodology: Michael Urban, Tabitha Osler; Project administration: Michael Urban, Willem Pretorius; Supervision: Michael Urban, Chris Mathew; Validation: Tabitha Osler, Michael Urban; Visualization: Tabitha Osler, Michael

Urban; Writing-original draft: Tabitha Osler; All authors read and approved the final manuscript.

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5.10 DATA AVAILABILITY

The datasets generated during and analysed during the current study are not publicly available but are available from the corresponding author on reasonable request.

5.11 ETHICS

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Human Research Ethics Committees of Stellenbosch University (06/02/2023, N22/04/039) and the University of the Witwatersrand (26/01/2024, M231195).

5.12 CONSENT TO PARTICIPATE

Not applicable as this was a retrospective record review.

5.13 SUPPLEMENTARY MATERIALS

Supplementary Table 5.1: P/LP variants in participants meeting the NDOH* criteria

Gene	P/LP Variant	No. in probands
ATM	c.5228C>T (p.Thr1743Ile)	1
	c.5279dup (p.Met1760Ilefs*9)	1
	c.6139_6146del (p.Val2047*)	1
	c.7271T>G (p.Val2424Gly)	2
	c.8307G>A (p.Trp2769*)	1
BRCA1	c.1016dup (p.Val340Glyfs*6)	1
	c.1360_1361del (p.Ser454*)	1
	c.1398del (p.Lys467Argfs*8)	1
	c.181T>G (p.Cys61Gly)	1
	c.1953_1956del (p.Lys653Serfs*47)	2
	c.2307_2313del (p.Ile769Metfs*21)	1
	c.2641G>T (p.Glu881*)	5
	c.4484G>T (p.Arg1495Met)	1
	c.5096G>A (p.Arg1699Gln)	1
	c.5153-1G>A (Splice acceptor)	1
	c.66_67insA (p.Glu23Valfs)	1
	c.66dup (p.Glu23Argfs*18)	1
	Deletion (Exons 1-12)	2
	Deletion (Exons 1-15)	1
	Deletion (Exons 20-21)	2
	Deletion (Exons 4-6)	1
BRCA2	c.6449_6450insTA ,p.Lys2150?fs	1
	c.3865_3868del (p.Lys1289Alafs*3)	1
	c.3881T>A (L1294*)	1
	c.5771_5774del (p.Ile1924Argfs*38)	12
	c.582G>A (p.Trp194*)	3
	c.5946del (p.Ser1982Argfs*22)	1
	c.6447_6448dup (p.Lys2150Ilefs*19)	3
	c.7558C>T (p.Arg2520*)	1
	c.7934del (p.Arg2645Asnfs*3)	10
	c.8961_8964delGAGT (p.Ser2988Phefs*12)	1
	c.9105T>A (p.Tyr3035*)	1
CHEK2	c.1100del (p.Thr367Metfs*15)	1
	c.283C>T (p.Arg95*)	1
	c.779del (p.Ser260*)	1
PALB2	c.2167_2168del (p.Met723Valfs*21)	1
	c.2835-1G>C (Splice acceptor)	3
RAD51C	c.491_492del (p.Phe164Tyrfs*3)	1
RAD51D	c.619T>C (p.Ser207Pro)	1
TP53	c.742C>T (p.Arg248Trp)	1
	c.831_835del (p.Cys277Trpfs*27)	1

*NDOH – National Department of Health (South Africa)

CHAPTER 6

BREAST CANCER GENETIC SERVICES IN A SOUTH AFRICAN SETTING: PROBAND TESTING, CASCADING AND CLINICAL MANAGEMENT.

Osler, T. S., Schoeman, M., Edge, J., Pretorius, W. J. S., Rabe, F.H., Mathew, C. G., & Urban, M. F. (2025). Breast Cancer Genetic Services in a South African Setting: Proband Testing, Cascading and Clinical Management. *Cancer Med*, 14: e70743. https://doi.org/10.1002/cam4.70743

6.1 SUMMARY

Purpose: Limited data exist on managing hereditary breast cancer in low-to-middle-income countries (LMICs). We assessed proband and cascade genetic testing, and risk-reducing measures in a South African regional breast cancer service.

Methods: We analysed records from 534 consecutive female probands receiving genetic counselling for breast cancer, and their 115 relatives who attended genetic counselling. Demographic and clinical data, family pedigrees and genetic test data were collated from hospital clinical records, regional laboratory data, screening appointments and radiological records.

Results: Test uptake in probands was high (86.9%), although cost was a deterrent in some. There were 83 (19.6%) probands who tested positive and 45.0% of them had one or more family member have testing. This resulted in 9.2% of relatives (first- to third-degree) having cascade testing. Family testing was associated with stronger family history of cancer, female gender and being a first-degree relative (uptake was 25.6% in female first-degree relatives). Risk-reducing mastectomy was accepted by 52.6% of eligible female family members, while

mammographic surveillance (30%) and bilateral salpingo-oophorectomy (15.4 %) were less frequent.

Conclusion: Genetic testing was well-accepted by probands, but uptake was low in family members. Overall, one family member carrying a pathogenic variant was identified for every 13 probands receiving genetic counselling and for every 11 probands tested. Risk reducing measures were taken up over half of those eligible. Limited uptake of cascade testing and variable uptake of risk-reducing options impacted the program. To our knowledge, this is the first study in Africa of real-world effectiveness of a breast cancer genetic service.

6.2 INTRODUCTION

Breast cancer (BC) is the commonest cancer in South African women with prevalence estimates from the National Cancer Registry of 1 in 43 for Black African women, 1 in 18 for women of Mixed Ancestry, and 1 in 10 for White women (National Cancer Registry, 2022) Internationally, 5 to 10% of unselected cases of BC are attributable to a pathogenic or likely pathogenic (P/LP) variant in a BC susceptibility gene (Buys et al., 2017; Tung et al., 2015). Identifying these individuals through genetic counselling and testing (GCT) allows implementation of recommended treatment modifications and risk-reducing strategies (Daly et al., 2021).

Although GCT is the standard of care in high-income countries (HIC), it is not widely available in low to middle income countries (LMIC) like South Africa (Hamadeh et al., 2023). Barriers to these services in LMIC include underfunded and fragmented health care services, as well as financial, geographical and logistical barriers (Kromberg et al., 2013; Yip et al., 2019). South Africa's healthcare system comprises a private sector, which primarily serves individuals with medical insurance, and a state-provided sector, on which approximately 80% of the population relies (StatsSA, 2022b). GCT services are available within the state healthcare system at tertiary facilities in only three of the country's nine provinces. Medical costs, including those for GCT, are subsidised in state facilities on an income-based sliding scale, ranging from full subsidies to none (Department of Health and Wellness, 2022). In South Africa, cancer genetic services are provided mainly by genetic counsellors – nationally there are 28 practicing genetic counsellors, the majority of whom work in private practice. This number is estimated to represent just 5% of the genetic counselling workforce needed to meet the country's demands. In addition to the workforce shortage, there is a significant disparity in geographic distribution,

with most genetic counsellors concentrated in urban centres (Gomes et al., 2024). Qualitative studies have shown that while many South Africans are unfamiliar with genetic counselling, they generally respond positively to the process after experiencing it (Malope et al., 2023; Morris et al., 2015).

Identifying individuals with a P/LP variant in a BC susceptibility gene enables the use of risk-reducing and surveillance measures for cancer recurrence and/or occurrence (Daly et al., 2021). It is well established that risk-reducing mastectomy (RRM) reduces the risk of BC by at least 90% in asymptomatic *BRCA1/2* carriers while bilateral salpingo-oophorectomy (BSO) significantly reduces morbidity and all-cause mortality (Carbine et al., 2018; Dare et al., 2021; Li et al., 2016). In addition, the National Comprehensive Cancer Network (NCCN) guidelines developed in the USA recommend surveillance for BC with MRI and/or mammogram starting from between 25 and 30 years of age. Similar evidence-based guidelines are available for other BC susceptibility genes (Daly et al., 2021). The uptake of risk-reducing strategies has been shown to vary widely between countries (Metcalfe et al., 2019), but little data is available from LMIC.

When an individual is identified to have a P/LP variant in a cancer susceptibility gene, cascade testing, the process of extending GCT to family members, can proceed. Offit et al., (2020) calculated that if 70% of first, second- and third-degree family members were to have testing, most individuals with a P/LP variant in the population would be identified within a decade (Offit et al., 2020). In practice, this has been difficult to realise even in HIC, with various barriers identified including contextual factors (Srinivasan et al., 2020), poor family communication and/or relationships (Fehniger et al., 2013; Raspa et al., 2016), and others (Braley et al., 2022; Finlay et al., 2008; Sanz et al., 2010). Currently, there is also little data on the uptake of cascade testing for hereditary cancer in LMIC with two studies, both in research cohorts, showing divergent outcomes (Bruwer et al., 2013; Yoon et al., 2011).

Considering the dearth of data on GCT for inherited BC in South Africa and other LMIC, we aimed to assess its effectiveness in facilitating the pathway from proband to cascade genetic testing and risk-reducing measures among family members, in a regional BC service.

6.3 METHODS

6.3.1 Study site

This study was conducted at Tygerberg Academic Hospital (TAH), a tertiary hospital that serves as the regional cancer genetic referral centre for ~3.5 million people, 60% living in metropolitan Cape Town and the remainder in towns and farms up to 300 km away (see Supplementary Figure 6.1). Within the region and during the study period, most aspects of BC care were provided at TAH, although breast surgery or cancer surveillance was also available at three associated secondary hospitals. Care was coordinated by a multidisciplinary team that included the breast surgery, oncology and anatomical pathology services, as well as a genetic counsellor based at TAH. Pre-test genetic counselling was provided at TAH in person by a genetic counsellor or medical geneticist. Post-test counselling including the discussion of management options for clinically relevant genetic variants was provided in-person or telephonically.

6.3.2 Participants

Records were reviewed for female probands and their family members. Initial contact with the genetic service was almost always by a proband (a woman with invasive or *in situ* BC), following referral from the BC service. They were offered testing if the genetic health-provider considered them eligible according to the South African National Department of Health (NDOH) guidelines (National Department of Health, 2018). Based on these guidelines, a woman with BC was eligible if she met any of the following criteria: diagnosis with BC at ≤40 years; two primary BCs or triple-negative BC at ≤60 years; or a family history of either ≥1 close relative with invasive ovarian cancer, male BC, or BC at ≤50 years, or ≥2 close relatives with breast or pancreatic cancer with one diagnosed ≤60 years. Probands were excluded if they had genetic testing prior to attending genetic counselling. Probands identified to have a P/LP variant in a BC susceptibility gene were asked to invite family members to attend GCT.

6.3.3 Study period

Probands were included if they were seen for pre-test GCT between 1 January 2018 and 31 August 2022. This timeframe was chosen because it was the period for which next generation sequencing (NGS) gene panel testing was available through the global testing platform of Invitae Corporation (San Francisco, USA) (Invitae Corporation, 2023). Family members were

included if they attended GCT at TAH by 31 December 2022. To allow a minimum of a full year for implementation of risk-reducing measures, medical records of both probands and family members were reviewed until 31 December 2023.

6.3.4 Genetic testing

Of the probands tested, 86.8% had NGS gene panel testing through Invitae and 19.8% received testing through the South African National Health Laboratory Service (NHLS) (Table 5.3) (Invitae Corporation, 2023; National Health Laboratory Service, 2020). Testing by the NHLS was done using various methods: targeted analysis of 8 well-documented founder mutations in *BRCA1/2* (see Supplementary Table 6.1); single nucleotide and copy number variant (CNV) detection within the exons and splice-site junctions of *BRCA1/2*; family variant testing; and a NGS panel of 15 genes. NHLS testing is conducted in an accredited diagnostic laboratory, with variants interpreted using standard guidelines (*South African National Accreditation System*, n.d.; van der Merwe et al., 2020; van der Merwe, Ntaita, et al., 2022).

The Invitae NGS gene panel protocol included assessment of single nucleotide variants and for most genes also copy number variants, in the coding and flanking DNA, with variants interpreted using their Sherloc guideline (Invitae Corporation, 2023; Nykamp et al., 2017). Family variant analysis performed by Invitae was available free of charge for 3 months via the TAH genetic counselling service to family members. By comparison, cost of family variant analysis through the NHLS depended on patient eligibility for a financial subsidy; this also applied to both Invitae and NHLS proband tests.

In this study, we included only the results for 13 genes known to be associated with BC and having clinical guidelines for risk-reduction (Table 6.1). Because not all probands had testing of the same genes, denominators used to calculate the prevalence of P/LP variants differed.

Table 6.1: Genes assessed - number of probands tested and guidelines used to assess risk-reducing interventions.

Gene	Included on NHLS[a] panel	Included on Invitae[b] panel	No. proband tested[c]	NCCN guideline starting age recommendations for risk-reducing interventions		
				Mammo-gram[d]	RRM	BSO
ATM	Yes	yes	372	40 years	no	no
BARD1	Yes	yes	313	40 years	no	no
BRCA1	Yes	yes	393	25 years	25 years	35 years
BRCA2	Yes	yes	393	25 years	25 years	40 years
CDH1	-	yes	313	30 years	30 years	no
CHEK2	yes	yes	372	40 years	no	no
NF1	-	yes	321	30 years	no	no
PALB2	yes	yes	372	30 years	30 years	45 years
PTEN	-	yes	372	30 years	30 years	no
RAD51C	-	yes	321	40 years	no	45 years
RAD51D	-	yes	321	40 years	no	45 years
STK11	-	yes	343	30 years	30 years	no
TP53	yes	yes	372	20 years	20 years	no

NHLS = National Health Laboratory Service (South Africa) , RRM = risk-reducing mastectomy, BSO = bilateral salpingo-oophorectomy, NCCN = National Comprehensive Cancer Network (USA), [a]van der Merwe *et al.*, 2022, [b] Invitae Corporation (2023), [c]Does not include probands who only had *BRCA1/2* founder variant (n=29) or known family variant testing (n=2), [d]Due to lack of access to MRI at TAH Hospital, only mammogram surveillance was analysed.

6.3.5 Data Sources and Collection

Demographic, clinical, genetic counselling and testing data were collected for probands, and more limited data for family members. Five electronic data sources were used: NHLS and Invitae online portals for laboratory data, the TAH Enterprise Content Management system (ECM) (the hospital electronic archive of scanned clinical notes) for clinical data, the Picture Archiving and Communication System (PACS) for imaging results and Clinicom for clinic appointments. Both Clinicom and PACS provided information on appointments and imaging results not only for TAH, but also for secondary hospitals in the region, enabling a comprehensive assessment of screening performed across the area. Two supplementary sources of clinical information were hard copy records held by the Genetic Counselling service and the BC service. The variables collected from each data source are summarised in Table 6.2.

Ethnicity was inferred by the genetic health professional and recorded on request forms, as required by testing laboratories, and categorized according to South African National Census groups: Black African, White, South African Coloured (Mixed Ancestry in this study) and Indian/Asian (StatsSA, 2022b) The Black African population is Bantu-speaking peoples indigenous to Southern Africa, accounting for 38.8% of the Western Cape (WC) population (Choudhury et al., 2017; StatsSA, 2022a). White South Africans, accounting for 16.4% of the WC population, are predominantly descendants of European settlers (Hollfelder et al., 2020; StatsSA, 2022a). People of Mixed Ancestry, have genetically admixed ancestry, including Khoisan, Black African, European and Asian population roots (Choudhury et al., 2017; de Wit et al., 2010). They form the largest population group in the WC accounting for 42.1%. The Indian/Asian population represents a small minority (1.1%) of the WC population (StatsSA, 2022a).

The financial subsidy provided to individuals for state healthcare, including genetic testing, is determined through a means-tested system (Department of Health and Wellness, 2022). Patients with very limited financial means receive almost full subsidies for the direct costs of healthcare, including genetic testing and cancer management, while those with greater financial means receive only partial subsidies or none at all. There are four levels of financial subsidy (H0, H1, H2, H3), as detailed in Table 6.3. However, due to the smaller number of patients in the H1, H2, and H3 categories, we combined H0 with H1, and H2 with H3 for the purposes of comparison.

Table 6.2: Types and sources of data collected for probands and their family members at Tygerberg Academic Hospital (TAH)

Data Source	Access	Data type	Collected for probands	Collected for family members
TAH Enterprise Content Management (ECM system)[a]	Electronic (TAH)	Demographic data	Date of birth, gender, ethnicity, distance from TAH, financial subsidy level	Date of birth, gender
		Clinical data	BC[d] histology, BC stage, age at BC diagnosis, any surgeries done	History of previous cancers, notes on RRM and/or BSO
		Family pedigree data	No. of FDR, SDR and TDR with cancer, family member age, family member age at cancer diagnosis	Relationship to proband
TAH Genetic Counselling Clinic records	Hard copy (Departmental)	Demographic data	To supplement data from electronic and online sources	To supplement data from electronic and online sources
		Genetic test data		
		Family pedigree data		
TAH Breast Surgery departmental records[b]	Hard copy (Departmental)	Clinical data	BC stage, breast surgery done, notes on BSO (if done)	RRM and/or BSO (if done)
Invitae online portal	Internet	Genetic test data	Result of Invitae genetic testing, ethnicity	Invitae genetic test result
NHLS reporting system (Trakcare)[c]	Internet (national)	Clinical data (histology records)	BC histology, BC stage, age at BC diagnosis, surgeries done	RRM and/or BSO (if done)
		Genetic test data	Results of genetic testing done at NHLS	Results of genetic testing done at NHLS
Picture Archiving and Communication (PACS) System	Electronic (regional reports)	Radiological procedures	Mammogram, breast ultrasound records	Mammogram, breast ultrasound records
Clinicom	Electronic (regional)	Appointment data	Attendance at screening mammogram and/or breast ultrasound	Attendance at mammogram and/or breast ultrasound

NHLS = National Health Laboratory Service, RRM = risk reducing mastectomy, BSO = bilateral salpingo-oophorectomy, BC = breast cancer, FDR=first degree relative, SDR = second degree relative, TDR = third degree relative, [a] *Enterprise content management*, 2024, [b] Available from January 2021, [c] Laboratory reports from tests done in the state system country-wide.

Probands and family members were considered eligible for interventions based on the NCCN guidelines (Table 6.1). Surgeries such as mastectomies and BSOs performed in the state healthcare system should result in both a clinical note and a NHLS histology report. Mammogram and breast ultrasound screening required an electronic appointment on the Clinicom system and were reported in the Picture Archiving and Communication System (PACS) database. Therefore, for both surgical interventions and screening procedures, two sources of evidence were assessed. In addition, screening was reviewed for all probands and family members, except in cases where an RRM had already been performed. This is because RRM is routinely preceded by breast screening, after which patients are discharged. Chemoprevention is not available for cancer-unaffected women in the South African state system and was therefore not assessed.

6.3.6 Data analysis

All statistical analysis was done using RStudio version 2024.4.0 (R Core Team, 2022), and $p<0.05$ was considered statistically significant. Univariate logistic regression was used to compare the distribution of both categorical and continuous variables for the uptake of genetic and family member testing. Factors with $p<0.2$ were then included in a multivariate logistic regression analysis for uptake of genetic testing in probands. Highly correlated factors, determined using variance inflation factors, were dropped from the model. As only one variable was found to have $p<0.2$ when factors impacting test uptake in family members was examined, multivariate analysis was not required. Results are reported as odds ratios (OR) with 95% confidence intervals (CI) and associated *p*-values.

Family pedigrees were used to determine the number of family members eligible for cascade testing. Family members were not considered eligible if <18 years unless the familial P/LP variant was associated with childhood cancers (i.e. *TP53*), or if unrelated to the side of the family with the P/LP variant.

6.4 RESULTS

6.4.1 Proband uptake of genetic counselling and testing

In the study period 534 women with a previous or current diagnosis of BC (probands) were seen for pre-test genetic counselling, of whom 488 (91.4%) were offered genetic testing, and 86.9% (424/488) accepted (Figure 6.1). Demographic and clinical data for all study participants are shown in Table 6.3.

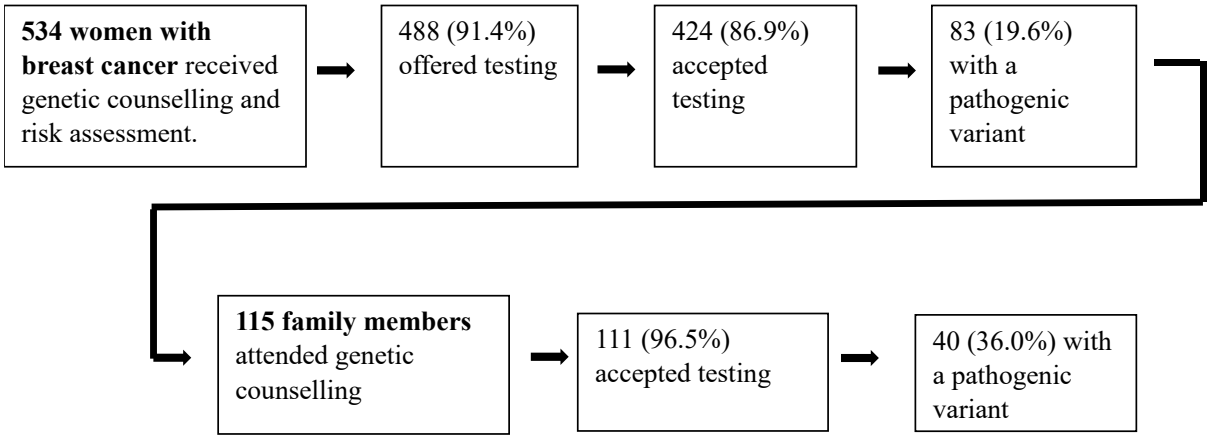


Figure 6.1: Flow chart of the genetic counselling and testing process for hereditary breast cancer in probands and family members

*Percentages in rows are as a proportion of the previous block

Table 6.3: Demographic and clinical data for study participants

		Probands offered testing (n=488)	Tested probands (n=424)	Tested family members (n=111)
Age at testing	median	41	41	35
	range	18-76	18-76	3-66
	unknown	0	0	0
Gender	Female	488	424	87 (78.4%)
	Male	0	0	24 (21.6%)
Distance from hospital	<50km	379 (77.7%)	322 (75.9%)	30 (27.0%)
	50-100km	57 (11.7%)	53 (12.5%)	2 (1.8%)
	100-200km	41 (8.4%)	39 (9.2%)	0
	>200km	10 (2.0%)	9 (2.1%)	2 (1.8%)
	unknown	1 (0.2%)	1 (0.2%)	77 (69.4%)
Ethnicity / Population group	Mixed Ancestry	254 (52.0%)	237 (55.9%)	38 (34.2%)
	Black African	100 (20.5%)	91 (21.5%)	25 (22.5%)
	White	70 (14.3%)	61 (14.4%)	24 (21.6%)
	unknown	64 (13.1%)	35 (8.3%)	24 (21.6%)
Financial subsidy[a] for GCT	Full subsidy of consult & test (H0)	382 (78.3%)	368 (86.8%)	21 (18.9%)
	Subsidised fee for consult & test (H1)	76 (15.6%)	41 (9.7%)	2 (1.8%)
	Subsidised consult & 20% test cost (H2)	11 (2.3%)	5 (1.2%)	1 (0.9%)
	No subsidy (H3 or Pvt)	18 (3.7%)	9 (2.1%)	5 (4.5%)
	Unknown	1 (0.2%)	1 (0.2%)	82 (73.9%)
Breast cancer diagnosis	yes	488 (100%)	424 (100%)	7 (6.3%)[f]
	no	0	0	102 (91.9%)
	unknown	0	0	2 (1.8%)
Breast cancer histology	DCIS[c]	7 (1.4%)	6 (1.4%)	
	Invasive ductal	418 (85.7%)	387 (91.3%)	
	Invasive lobular	17 (3.5%)	15 (3.5%)	
	unknown	46 (9.4%)	16 (3.8%)	7[e]
Cancer Stage[b]	*in situ*	7 (1.4%)	6 (1.4%)	
	Stage 1	28 (5.7%)	24 (5.7%)	
	Stage 2	158 (32.4%)	141 (33.3%)	
	Stage 3	194 (39.8%)	178 (42.0%)	
	Stage 4	70 (14.3%)	61 (14.4%)	
	Recurrence	5 (1.0%)	4 (0.9%)	
	unknown	26 (5.3%)	10 (2.4%)	7
Genetic tests done[c]	8 founder variants[d] (*BRCA1/2*)		50 (11.8%)	
	BRCA1/2 sequencing		22 (5.2%)	
	NHLS panel test		8 (1.9%)	
	Invitae panel test		368 (86.8%)	
	Family variant testing		4 (0.9%)	111 (100%)

Table footnotes: GCT – genetic counselling and testing, DCIS = ductal carcinoma *in situ,* [a] Subsidy levels are determined by the Department of Health based on income (Department of Health and Wellness, 2022), [b] Clinical breast cancer stage was determined by the tumour-node-metastasis (TNM) system, as indicated or inferred from the clinical notes. [c]Number of tests done (452) exceeds number of tested probands (424), because >1 test was done in some cases, [d]South African founder variants listed in the Supplementary Table 6.1, [e] 7 family members had breast cancer prior to testing.

Age at diagnosis (p<0.01), the presence of a cancer family history meeting NDOH testing criteria (p=0.05), living >50 km from the hospital (p=0.02) and subsidisation category (H0 and H1 vs others) (p<0.01) were included in the multivariate logistic regression analysis. Cancer stage (stages 0, 1, 2 vs stages 3, 4 and recurrence) (p=0.96) and age at consultation (p=0.02) were excluded from the model due to lack of significance or collinearity with other variables. Testing uptake was found to be associated with younger age at diagnosis, the presence of a family history and higher subsidisation level of test costs (Table 6.4). The impact of test cost on uptake was supported by the finding that 93.3% (42/45) of probands who gave a reason for declining testing, named financial concerns.

Table 6.4: Logistic regression showing factors impacting acceptance of genetic testing

Outcome: uptake of testing	Regression coefficient	Std. error	Z value	P value	Odds Ratio (95% CI)
Intercept	1.88	0.82	2.28	0.02	6.56 (1.33-34.02)
Age at diagnosis	-0.04	0.01	-2.63	0.01	0.96 (0.94-0.99)
Dist. to hospital	-0.59	0.43	-1.36	0.17	0.56 (0.22-1.22)
Family history	0.94	0.37	2.54	0.01	2.57 (1.28-5.55)
Financial subsidy	2.21	0.44	5.07	0.00	9.14 (3.88-21.83)

CI = confidence interval

6.4.2 Detection of pathogenic variants in probands

A heterozygous P/LP variant was detected in 19.6% (83/424) of tested probands, with variants in *BRCA1/2* accounting for 78.3% (65/83) (Supplementary Table 6.2). The prevalence of P/LP variants in *BRCA1* and *BRCA2* was 5.9% (25/424) and 9.4% (40/424) respectively, with fewer founder variants in *BRCA1* than *BRCA2* (6/424 vs 32/424, n=424, p<0.001). The detection rate of P/LP variants in other high-risk susceptibility genes tested was: *PALB2* (4/373, 1.1%), *TP53* (2/373, 0.5%); and in moderate-risk susceptibility genes: *ATM* (7/372, 1.9%), *CHEK2* (3/372, 0.8 %), *RAD51C* (1/321, 0.3%) and *RAD51D* (1/321, 0.3%).

6.4.3 Family member genetic counselling and testing

Of the 83 probands with a P/LP variant, result feedback was documented in all but one case. Almost half of the probands (45.8% (39/83)) had at least 1 family member receive genetic testing (median: 2, range: 1-11). Only 3.5% (4/115) of family members who attended genetic counselling declined testing. The median time between the first proband consultation and

family member consultation was 3.3 months (range: 0.4-20.8 months). Probands reporting more family members diagnosed with cancers (including melanoma, breast, ovarian, prostate, or pancreatic) were more likely to have family members test (OR 1,73, 95% CI 1.16-2.57, $p<0.01$). No relationship was found between having a family member attend and the age of the proband's cancer diagnosis ($p=0.26$), the proband's cancer stage ($p=0.91$), whether the proband lived <50km from the hospital ($p=0.47$), the number of eligible family members ($p=0.55$), whether the test provider offered free family variant testing ($p=0.31$), or the probands financial subsidy category ($p=0.75$).

Using the family pedigrees collected at the time of consultation, there were 1202 first-, second- and third-degree relatives eligible for testing (Table 6.5), of whom 111 (9.2%) were tested. Female family members (OR: 2.0, 95% CI: 1.16-3.43, $p=0.01$) and first-degree relatives (OR: 17.1, 95% CI: 5.27-60.48, $p<0.001$) were significantly more likely to have testing, with 25.4% of female first degree relatives receiving cascade testing.

The overall detection rate for P/LP variants in tested family members was 36.0% (40/111). Of the 40 family members found to carry a P/LP variant, 31 were female and 9 were male, with median ages of 43 (range 20-66), and 35 (range 20-56) years respectively. P/LP variants were found in the following genes for males: *BRCA1* (6), *BRCA2* (3); and in females: *ATM* (3), *BRCA1* (11), *BRCA2* (14), *PALB2* (3). There was evidence of result feedback in all family members. The identification of test-positive probands and cascade testing of their family members allowed detection of one test-positive family member for every 11 probands tested (40/424).

Table 6.5: Detection of P/LP variants in eligible family members

Degree of relationship to proband	Gender	Number of eligible family members	Family members tested (as % of eligible members)	Family members with P/LP variant as proportion of tested (%)
First-degree	F	228	58 (25.4%)	24/58 (41.4%)
	M	180	24 (13.3%)	9/24 (37.5%)
	Total	408	82 (20.1%)	33/82 (40.2%)
Second-degree	F	308	24 (7.8%)	6/24 (25%)
	M	271	0	0
	Total	579	24 (4.1 %)	6/24 (25%)
Third-degree	F	125	5 (4.0%)	1/5 (20%)
	M	90	0	0
	Total	215	5 (2.3%)	1/5 (20%)
All	F	661	87 (13.2%)	31/87 (35.6%)
	M	541	24 (4.4%)	9/24 (37.5%)
	Total	1202	111 (9.2%)	40/111 (36.0%)

6.4.4 Uptake of risk-reducing strategies

Most probands with a P/LP variant (64/81, 79.0%) had breast surgery as part of their treatment for BC. Reasons BC surgery was not performed included advanced cancer stage, inoperability, loss to follow-up or the proband declining the procedure. Among the probands who underwent BC surgery, 82.8% (53/64) also had surgery performed on the contralateral breast. However, the reason for surgery was frequently undocumented. Of the 50 probands who were eligible for BSO, considering guidelines for the relevant gene, their age, and prognosis, 17 (34.0%) had it performed and 8 (16.0%) said they would consider having it done in the future although they were already at the recommended age for the procedure.

Of the tested family members, seven had a prior diagnosis of BC, six of whom (85.7%) tested positive for a P/LP variant. As they had previously had surgical treatment, the uptake of RRM was not assessed. However, 4/6 (66.7%) were eligible for risk-reducing BSO considering their age and the applicable gene guidelines, and two proceeded.

Table 6.6 outlines risk reducing measures undertaken by cancer-unaffected family members who tested positive for a P/LP variant. As a proportion of those eligible for each intervention,

more had RRM (52.6%; 10/19) than mammographic surveillance (30.0%; 3/10) or BSO (15.4%; 2/13). Of those eligible for breast surveillance and/or RRM, 13/20 (65.0%) had one of these interventions. Only one eligible family member had both RRM and BSO procedures done.

Table 6.6: Risk-reducing measures used by female family members without breast cancer

Risk-reducing measure	No. eligible for intervention (n=25)[a]	Outcome	No. of participants	Median age and range
Breast surveillance in non-RRM participants by mammogram and/or ultrasound	10	Performed at least once	3 (30.0%)	37 (36-66)
		Participant reported no breast surveillance done	3 (30.0%)	37 (35-43)
		No record of surveillance[b]	4 (40.0%)	52 (34-63)
Risk-reducing mastectomy (RRM)	19	RRM	10 (52.6%)	43.5 (33-56)
		Not done	7 (36.8%)	37 (34-66)
		No record of surgery or associated histology[b]	2 (10.5%)	50 (37-63)
Bilateral salpingo-oophorectomy (BSO)	13	BSO	2 (15.4%)	51.5 (51-52)
		BSO not done	6 (46.2%)	47.5 (35-66)
		No record of surgery or associated histology[b]	5 (38.5%)	43 (36-63)

[a] [a]Indication for the risk-reducing measure was determined based on gene-guidelines and participant age according to NCCN guidelines (Table 6.1) , [b] When no record could be found for surgery or associated histology, it is highly likely that the procedure was not done in the state system, as electronic health records were thoroughly searched for each participant.

6.5 DISCUSSION

In our assessment of the GCT and management path for hereditary BC in a LMIC, we found a high uptake of genetic testing in women with BC, but test cost was a significant factor in determining uptake. Testing in eligible family members was most strongly associated with female gender, being a first-degree relative and having multiple family members affected by cancer. Although uptake of cascade testing was low overall, more than half of eligible test-positive relatives proceeded with RRM. BSO and mammographic surveillance were less frequently used.

6.5.1 Genetic counselling and testing of probands

The high uptake (86.9%) of genetic testing suggests that it is an acceptable option for most women with BC but may be unaffordable for some. This was indicated by the finding that probands with higher subsidisation of healthcare costs were nine times more likely to test. This probably relates to the fact that all the available genetic tests were expensive: approximately USD125 for founder mutation testing and over USD300 for sequencing of the *BRCA1/2* genes or broader gene panels. The individuals without subsidy, typically referred to as the "missing-middle", have sufficient household income to disqualify them from assistance, but lack the financial resources to pay for medical insurance for genetic testing. This issue is not unique to South Africa. A scoping review of 24 studies on BC testing, all conducted in the USA, found that women without private insurance were not only less likely to be referred for testing but, when referred, were also less likely to proceed with it (Grant et al., 2021). This likely results in a substantial segment of the population unable to access testing and therefore ineligible for cancer risk-reducing measures. Family history was also important as probands with a family history of *BRCA1/2* related cancers were found to be more than twice as likely to proceed with testing.

The high detection rate of P/LP variants (19.6%) in probands likely reflects the relatively strict criteria for offering genetic testing to women with BC in South Africa as we have discussed elsewhere (Osler et al., 2025).

6.5.2 Cascade Testing

Uptake of cascade testing in our study was generally low, and it dropped steeply from 20.1% in first-degree relatives to 4.1% and 2.3% in second- and third-degree relatives respectively. The uptake of cascade testing in our study was much lower than reported by a recent meta-analysis of 87 studies, where uptake of cascade testing was 41% in eligible first- and second-degree relatives (Frey et al., 2022). However, studies included in the meta-analysis were mainly from HIC, and often nested in research environments, settings in which better uptake is expected (Yip et al., 2019).

There are very few publications from LMIC on the uptake of cascade testing for hereditary BC, or hereditary cancer more broadly. Only two studies in the above-mentioned meta-analysis were conducted in LMIC as defined by the World Bank (2022) - one in Malaysia on hereditary

BC (Yoon et al., 2011) and one in South Africa on Lynch syndrome (Bruwer et al., 2013). The former reported a low uptake of cascade testing, with 78% of probands informing their families but only 11% of relatives coming forward, despite the availability of free testing within a research setting (Yoon et al., 2011). The authors noted that families expressed concerns regarding cultural taboos about cancer diagnoses, social marginalization and lack of regulatory control of genetic discrimination". Our study showed a similarly low uptake, but the reasons for this require further exploration. The study on Lynch syndrome showed a contrastingly high uptake of cascade testing , with 97% of siblings and 73.6% of children accepting predictive testing (Bruwer et al., 2013). Like our study, it was set in South Africa, but it described a bespoke research-based outreach program to rural areas with high rates of Lynch syndrome. Our study assessed a clinical practice environment covering a healthcare region and therefore appears more representative of the medical mainstream.

The predominance of testing by female family members (77.0%) aligns with findings from a meta-analysis, which reported that female relatives were almost twice as likely to undergo testing compared to males (Frey et al., 2022). This trend is likely attributable to their elevated risk for associated cancers and a greater propensity to utilize health services (Abera Abaerei et al., 2017). Similarly, the meta-analysis, consistent with our findings, demonstrated that first-degree relatives were significantly more likely to undergo testing than second-degree relatives (Frey et al., 2022). This may be due to their closer relationship with the proband, which places them at higher risk and facilitates better communication (Stoffel et al., 2008). Higher test uptake was associated with a family history of cancer in both probands and family members. Although not frequently reported in the literature, this finding is intuitive, as individuals from families with significant cancer histories often experience heightened cancer-related anxiety, which increases compliance with testing and screening (Vrinten et al., 2017).

Close to half of probands had at least one family member attend genetic counselling but the number of family members attending varied widely. The reasons for this require further assessment in our setting. Many specific family and contextual factors have also been reported to influence the uptake of testing in at-risk family members in Western and Asian countries. Described family factors include family support, communication and dynamics, while contextual factors include access to and cost of healthcare services (Srinivasan et al., 2020). Of the contextual factors, low income(Raspa et al., 2016), lack of health knowledge (Finlay et al.,

2008), and difficulty accessing genetic services (Srinivasan et al., 2020), are barriers that plausibly impacted many family members in our study.

6.5.3 The flow from proband genetic counselling to family member testing

The model developed by Offit et al. (2020), demonstrated that if all individuals with BC had testing, and there was a 50% to 70% uptake among first-, second- and third-degree relatives of those identified with a P/LP variant, the majority of carriers in the population could be detected (Offit et al., 2020). This is far removed from our study, where (1) we could offer genetic testing only to probands at high risk of hereditary cancer and (2) cascade testing uptake even in those at highest risk (female first-degree relatives) was only 25.7%. In our setting, public health-level as opposed to individual impact from cascade testing will be difficult to achieve.

Instead, we provide real-world data on the drop-offs at each stage of GCT in probands, and the detection of carrier status in their relatives. The high uptake and detection rate in probands, together with cascade testing uptake that was low but mainly among first-degree relatives, resulted in detection of 1 carrier relative being detected for every 13 probands receiving genetic counselling, and for every 11 probands receiving genetic testing. This information will facilitate future assessment of the cost-benefit of the GCT program.

6.5.4 Uptake of risk-reducing measures

Uptake of mammographic surveillance in family members (30%) was lower than that of RRM, and far lower than found by Metcalfe et al (2019), where uptake was above 96% in all countries offering mammographic surveillance (Metcalfe et al., 2019). In contrast, the uptake of RRM by 52.6% of eligible family members with a P/LP variant is above the upper end of the range (4.5-45%) observed by Metcalfe et al., (2019) across 10 mostly HIC (Metcalfe et al., 2019). The impact of RRM on BC-specific and overall mortality compared to recommended screening, is debated in HIC (Domchek, 2019). However, in our setting, where MRI is not widely available and access to advanced cancer therapies is limited, the higher uptake of RRM may be appropriate (Coetzee et al., 2018; Moukadem et al., 2022; O'Neil et al., 2019).

In contrast to RRM, the uptake of BSO (15.4 %) in eligible women without BC was considerably lower than that found in other studies, which had uptake rates varying from 36.7% to 83.3% (Makhnoon et al., 2021; Metcalfe et al., 2019; M. J. Smith et al., 2021). Likewise,

the uptake of BSO in probands and family members with BC (35.2%), although higher than in unaffected family members, was half that found in women with BC by Metcalfe et al., (2019) (70.7%) (Metcalfe et al., 2019). It is not clear if this relates to lack of access or awareness, or other reasons. The finding of Makhnoon et al., (2021) that higher parity was associated with BSO uptake independent of age, suggests that a desire to maintain fertility may be a factor (Makhnoon et al., 2021). The low uptake found in our study is concerning as BSO may be more effective in preventing morbidity and mortality in *BRCA1/2* and *PALB2* carriers than RRM (Li et al., 2016).

In probands the available information on risk-reducing measures was more difficult to interpret than in family members. Contralateral breast surgery in a person with unilateral BC does not necessarily imply that it was done for purposes of risk reduction, because of the common practice to offer contralateral breast reduction for cosmetic reasons. The reason for contralateral surgery was most often undocumented, and therefore we could make no inferences from the fact that over 80% of breast surgeries were bilateral. BSO is more likely to be performed to reduce the risk of future cancer, and half of eligible probands either had BSO performed or indicated they would do it in the future.

6.5.5 Strengths and limitations

There were several limitations in this study. Firstly, we relied on clinical records whose completeness was dependent on the consulting clinicians. This was evident in the pedigree data used where a higher number of female family members and first-degree relatives were counted as being eligible for genetic testing, suggesting that males and more distant relatives were undercounted. However, routine clinical and demographic data was nearly complete, indicating good data quality. This was expected because each electronic data source had proven reliable in clinical practice, and most data points were covered by two sources if required.

The uptake of cascade testing found is likely the "best-case scenario" because Invitae cascade testing was available in the country at the time which provided free family member testing if done within a 3-month period and therefore encouraged expeditious decision-making about family member testing. Conversely, the uptake of risk-reducing measures likely represents a minimum estimate. While we expect to have identified most participants who underwent mastectomies and BSOs in the regional state healthcare system given the multiple data sources

that we used, we may have missed some who had interventions in the private sector or elsewhere. In addition, the COVID pandemic may have negatively affected uptake; this was not directly assessed, but there was however no change in the frequency of cascade testing per year.

A comprehensive assessment of both cascade testing and uptake of risk-reducing measures would require longer prospective follow-up, as decisions may take time and eligibility for interventions is age dependent. Lastly, the small number of family members detected with P/LP variants meant we did not have sufficient power to investigate factors associated with uptake of risk-reducing measures.

6.5.6 Conclusion

To our knowledge, this is the first assessment of a BC genetic service from detection, through to cascade testing and uptake of risk-reducing strategies in a LMIC country, and it provides a useful framework for further investigations. The high proband test uptake and detection rate suggest that testing is acceptable and relevant. However, limited uptake of both cascade testing and some preventive interventions (BSO and mammography) reduced the effectiveness of the program. Reasons for the low uptake of cascade testing and variable uptake of risk-reducing measures should be explored. Comparative data from within and beyond South Africa, and a cost-benefit analysis, will help guide policy development in our and other LMIC settings.

6.6 DATA AVAILABILITY

The de-identified participant data used and analysed in this study is available from the corresponding author on request.

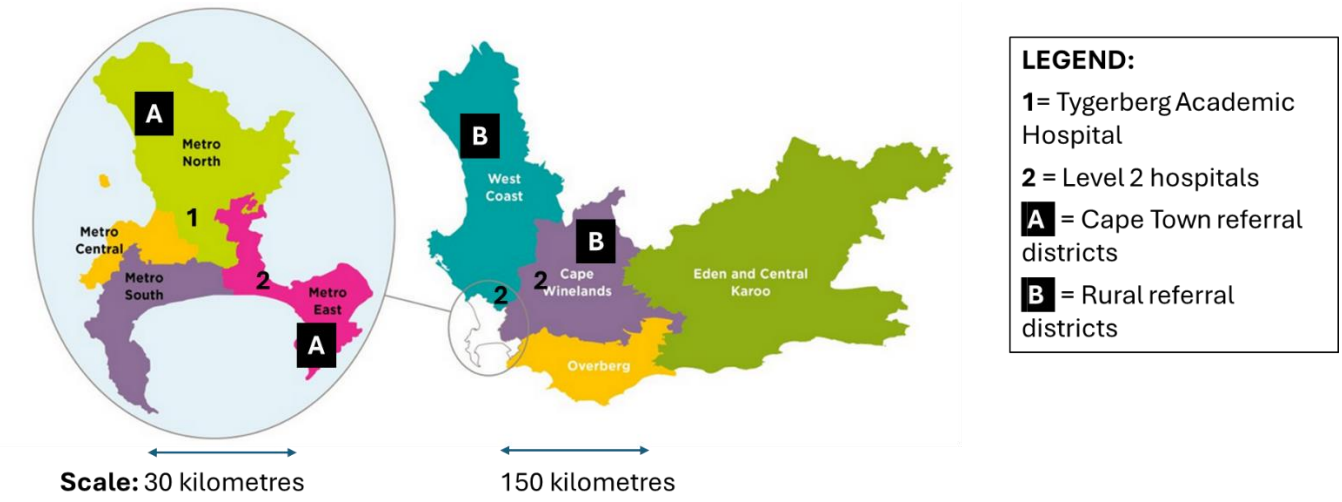
6.7 AUTHOR CONTRIBUTIONS

Conceptualization: M.U., T.O.; Data curation: T.O., W.P., J.E., F.E.; Formal analysis: T.O.; Funding acquisition: M.U., M.S., C.M.; Investigation: T.O., M.S., F.R., J.E., W.P.; Methodology: M.U., T.O.; Project administration: M.U., W.P.; Supervision: M.U., C.M.; Validation: T.O., M.U.; Visualization: T.O., M.U.; Writing-original draft: T.O.; Writing-review & editing: T.O., M.S., W.P., F.R., J.E., C.M., M.U.

6.8 ETHICS DECLARATION

The study was approved by the Human Research Ethics Committees of Stellenbosch University (N22/04/039) and the University of the Witwatersrand (M231195). As the study was a retrospective record review, participant consent was not required. All data were de-identified. The study adhered to the principles of Declaration of Helsinki.

6.9 SUPPLEMENTARY MATERIALS



Supplementary Figure 6.1: Tygerberg Hospital referral region

*Figure modified from https://wcedonline.westerncape.gov.za/contact/districts

Supplementary Table 6.1: Founder variants found in *BRCA1* and *BRCA2* in South African populations

Ancestry	Founder Variant
Afrikaner:	NM_007294.3 (*BRCA*1):c.1374del
	NM_007294.3 (*BRCA1*):c.2641G>T
	NM_000059.3 (*BRCA2*):c.7934del
Black African:	NM_000059.3 (*BRCA2*):c.5771_5774del
	NM_000059.3 (*BRCA2*):c.582G>A
Ashkenazi	NM_007294.3 (*BRCA1*): c.68_69del
Jewish:	NM_000059.3(*BRCA2*):c.5946del
	NM_007294.3 (*BRCA1*):c.5266dup

Supplementary Table 6.2: P/LP variants detected in probands

Gene	P/LP variant	Frequency in probands	Founder variant[a]
ATM	NM_000051.3:c.5228C>T	2	
	NM_000051.3:c.5279dup	1	
	NM_000051.3:c.6139_6146del	1	
	NM_000051.3:c.7271T>G	2	
	NM_000051.3:c.8307G>A	1	
BRCA1	NM_007294.3:c.1016dup	1	
	NM_007294.3:c.1360_1361del	1	
	NM_007294.3:c.1374del	1	Afrikaner
	NM_007294.3:c.1398del	1	
	NM_007294.3:c.181T>G	1	
	NM_007294.3:c.1953_1956del	2	
	NM_007294.3:c.2307_2313del	1	
	NM_007294.3:c.2641G>T	5	Afrikaner
	NM_007294.3:c.4484G>T	1	
	NM_007294.3:c.5096G>A	1	
	NM_007294.3:c.5153-1G>A	1	
	NM_007294.3:c.66dup	3	
	NC_000017.11:g.(?_43082404)_(43124096_?)del	2	
	NC_000017.11:g.(?_43070928)_(43124096_?)del	1	
	NM_007294.4:c.5278_5406del	2	
	NC_000017.11:g.(?_43104261)_(43106533_?)del	1	
BRCA2	NM_000059.3:c.3865_3868del	2	
	NM_000059.3:c.3881T>A	2	
	NM_000059.3:c.5771_5774del	13	Black African
	NM_000059.3:c.582G>A	3	Black African
	NM_000059.3:c.5946del	1	Ash. Jew
	NM_000059.3:c.6447_6448dup	4	
	NM_000059.3:c.7558C>T	1	
	NM_000059.3:c.7934del	12	Afrikaner
	NM_000059.3:c.8961_8964delGAGT	1	
	NM_000059.3:c.9105T>A	1	
CHEK2	NM_007194.3:c.1100del	1	
	NM_007194.3:c.283C>T	1	
	NM_007194.3:c.779del	1	
PALB2	NM_024675.4:c.2167_2168del	1	
	NM_024675.4:c.2835-1G>C	3	
RAD51C	NM_058216.3:c.491_492del	1	
RAD51D	NM_002878.4:c.619T>C	1	
TP53	NM_000546.6:c.742C>T	1	
	NM_000546.6:c.831_835del	1	

[a] Eight founder variants occur commonly in South Africa:.6 were found in one or more probands and are indicated according to the population in which they were first described. Two not found were Ashkenazi Jewish founders (BRCA1:c.5266dup and BRCA1: c.68_69del).

CHAPTER 7

INTEGRATED DISCUSSION

The overarching aim of this thesis was to assess the genetic counselling, testing and clinical management pathway in patients with BC and their family members in the South African context. This was achieved through four separate projects detailed in Chapters 3 to 6. Each chapter provides an in-depth discussion of its respective findings, which will not be repeated here. Instead, this chapter integrates the results from each project and discusses how they addressed the PhD study objectives. The novel contributions of this research are highlighted, along with potential implications for clinical practice, policy changes, and future research in South Africa. Additionally, the strengths and limitations of this study are addressed. For clarity and ease of reference, we refer to the study in Chapter 3 as the *Clinical Utility* project, the study in Chapter 4 as the *Population Prevalence* project, the study in Chapter 5 as the *Guideline* project, and the study in Chapter 6 as the *GCT* project.

7.1 OBJECTIVE 1: PREVALENCE OF P/LP VARIANTS

Objective 1, which aimed to determine the prevalence of P/LP variants in South African women of different ancestries with BC in both diagnostic and population-based settings, was addressed in the *Clinical Utility* (Chapters 3), *Population Prevalence* (Chapter 4) and *Guideline* (Chapter 5) projects. Chapter 4 examined the population-based prevalence of P/LP variants in BC susceptibility genes among Black South African women aged 50 and younger with BC. In Chapters 3 and 5, we compared the prevalence of P/LP variants in women who underwent diagnostic testing for hereditary BC across three South African ancestry groups. The results from these three studies are summarized for ease of reference in Table 7.1. The significantly higher prevalence of P/LP variants in the *Guideline* and *Clinical Utility* projects compared to the *Population Prevalence* project is expected, as participants in the former projects were selected based on meeting high-risk criteria. This is further reflected in their lower average age of diagnosis which is one of the selection criteria for diagnostic testing.

Table 7.1: Comparison of P/LP variant prevalence by ancestry found in three studies of South African women with BC.

		Chapter 4: *Population Prevalence* project (n=272)	**Chapter 3: *Clinical Utility* project (n=331)**	**Chapter 5: *Guideline* project (n=376)**
Participant recruitment		JCS study	Tygerberg Hospital Genetic Counselling Clinic	Tygerberg Hospital Genetic Counselling Clinic
Participant selection criteria		Black African with BC before ≤50 years.	Patients who had Invitae gene panel testing done	Met NDOH genetic test criteria (strictly applied)
No. of genes tested		12 BC genes	9 BC genes	2 genes (*BRCA1/2*)
Black African	No. participants	272	72	93
	Prevalence overall	10.3%	16.7%	
	BRCA1/2 prevalence	8.5%	15.3%	20.4%
	Average Age	41 years	38.5 years	38.2 years
Mixed Ancestry	No. participants		206	226
	Prevalence overall		16.5%	
	BRCA1/2 prevalence		9.2%	11.9%
	Average Age		44.3 years	42.7 years
White	No. participants		53	54
	Prevalence overall		22.6%	
	BRCA1/2 prevalence		17.0%	18.5%
	Average Age		50 years	45.5 years

[a] list of South African founder variants can be found in Supplementary Table 6.1.

Studies in West African and African American women with BC have generally reported a higher prevalence of *BRCA1/2* P/LP variants and lower prevalence of P/LP variants in other BC susceptibility genes compared to studies of women of European ancestry (Adedokun et al., 2020; Fackenthal et al., 2012; C. Hu et al., 2021; Purrington et al., 2020; Zheng et al., 2018). In our *Population Prevalence* project (Chapter 4), the first population-based study of P/LP variant prevalence in BC genes in a Southern African population, we found that the prevalence

in Black South African women with BC aged 50 or younger was consistent with the findings in other African populations (Table 7.1). As discussed in Chapter 4, our findings together with previous studies suggest that although there may appear to be a higher prevalence of *BRCA1/2* P/LP variants among African and African American women with BC, this may be accounted for by population demographic differences which influence the average age of diagnosis of participants. Similarly, since most P/LP variants in genes other than *BRCA1/2* are associated with BC at an older age, their lower prevalence in our study is expected, given that our participants are, on average, younger than those in studies from Western countries (Breast Cancer Association Consortium et al., 2021; C. Hu et al., 2021).

Studies that have directly compared the prevalence of P/LP variants between African American and non-Hispanic White women within the same study cohorts have yielded inconsistent results (Bishop et al., 2020; Caswell-Jin et al., 2017; Domchek, 2019; Hall et al., 2009; Yadav et al., 2021). These discrepancies may stem from differences in study designs, such as the use of multi-gene BC panels rather than focusing solely on *BRCA1/2* P/LP variants and/or different participant selection criteria, along with the relatively small numbers of African American participants included. More recently, Ahearn et al. (2022) found that the prevalence of *BRCA1/2* P/LP variants in Ghanaian women was double that found in European and Asian women in the BRIDGES study. However, this difference may be attributed to the fact that women in the BRIDGES study were on average 10-15 years older than in the Ghanaian study (Ahearn et al., 2022).

In our *Clinical Utility* and *Guideline* projects (Chapters 3 and 5), we compared the prevalence of P/LP variants in *BRCA1/2* in South African women at increased risk of a P/LP variant, across different South African ancestry groups (Table 7.1). We found no significant difference in the prevalence of P/LP variants across ancestry groups in our *Clinical Utility* project (Chapter 3). However, the multivariate regression analysis done in the *Guideline* project, showed that when controlling for other variables, Black African participants (20.4%) were more likely to carry a *BRCA1/2* P/LP variant than those of Mixed Ancestry (11.9%). No significant difference was observed between either of these groups and South African White probands (18.5%), whose prevalence of *BRCA1/2* P/LP variants fell between the two groups. These findings suggest that the prevalence of *BRCA1/2* P/LP variants is similar in Black and White South African participants, however the small number of White probands in the study limits the strength of the conclusions that can be drawn from comparisons involving this group. Since women in

both projects were selected based on high-risk criteria, the observed difference in prevalence may reflect a bias in the referral criteria met in one or other of the ancestry groups. Further population-based studies will be required to confirm that women with BC from different ancestry groups in South Africa have similar P/LP prevalences.

7.2 OBJECTIVE 2: ASSESSING THE CLINICAL UTILITY OF AN EXPANDED GENE PANEL

Our second objective was to assess the clinical utility of using an expanded gene panel for testing across different South African ancestry groups. This objective was addressed in Chapter 3 (*Clinical Utility* project), by comparing the prevalence of P/LP variants in known BC genes, as well as VUS, in different ancestry groups. We found that across all included ancestry groups, significantly more P/LP variants were detected in the 9-gene panel than in the additional 75 genes. Most P/LP variants in the 75 genes were identified in genes without a confirmed association with BC or established management guidelines and testing these genes resulted in the detection of significantly more VUS, particularly in Black African and Mixed Ancestry participants. Here, we discuss the implications of these findings for hereditary BC genetic testing in South Africa, focusing on optimizing the detection of P/LP variants in actionable genes, minimizing uncertain results, and reducing costs to the State.

In our GCT project (Chapter 6), we demonstrated the clinical utility of genetic testing for hereditary BC, identifying one family member with a P/LP variant for every 11 probands tested, which led to RRM in half of affected relatives, though BSO uptake was lower. Additionally, in Chapter 3, we showed that testing beyond *BRCA1/2* increases the detection of causative variants; however, the clinical utility and validity of genes included in a gene panel must be carefully considered. Without known risks and established guidelines, knowledge of P/LP variants is not clinically useful and can lead to higher levels of patient anxiety (Shickh et al., 2023). In addition, the higher incidence of VUS especially in Black African and Mixed Ancestry individuals, can create further distress in patients (Lumish et al., 2017). Testing additional genes also creates an increased workload for laboratory staff who must curate all detected variants (Tagliafico et al., 2018). At the time of the study, the highly automated and globalised Invitae system allowed for the testing of large gene panels at no additional cost (Invitae Corporation, 2023). Invitae testing is no longer available in the South African State system, and there is thus a need for the NHLS to provide testing, with the issue that local genomic resources and economies of scale are much smaller. The challenge is to provide effective testing and a rapid turnaround time.

A previous study done in South Africa recommended that *BRCA1/2* founder variant testing is performed before more costly gene panel testing (van der Merwe, Combrink, et al., 2022). Our finding in the *GCT* project (Chapter 6) that founder variants accounted for only ~46% of P/LP variants detected in BC genes, suggest that this testing approach is unlikely to be cost effective. In addition, this testing strategy will cause longer waiting times for results for the majority of individuals who will be negative on founder testing, increasing emotional distress and loss to follow-up (Bennett et al., 2007). Therefore, except in cases where there is a known family variant, we suggest that testing for hereditary breast cancer with a gene panel should be the first line test where resources permit.

Findings from this study underscore the limitations of broad gene panels demonstrating that while larger panels provide more extensive coverage, they also introduce greater complexity and uncertainty in result interpretation. A more cautious, targeted approach to genetic testing will reduce ambiguous results, particularly in African and Mixed Ancestry populations as well as reducing laboratory costs. We therefore concur with Kurian et al. (2021) who suggest that only actionable genes known to be associated with BC should be included in a gene panel test to maximise the detection of clinically relevant P/LP variants and to minimize the number of uncertain results.

7.3 OBJECTIVE 3: RECLASSIFICATION OF VUS WITH AFRICAN GENOMIC DATA

The third objective of our study was to establish the degree to which the use of African genomic data can contribute to the re-classification of VUS in African BC patients. This was assessed in our *Clinical Utility* (Chapter 3) and *Population Prevalence* (Chapter 4) projects. In the *Clinical Utility* project, over a third of the VUS identified in participants were present in the African genome data that we had collated. Using ClinGen gene-specific AF thresholds or an AF threshold of ≥ 0.01 when gene-specific guidelines were not available, resulted in additional evidence for variant classification in more than a quarter of VUS occurrences. However, we were unable to replicate this finding in the *Population Prevalence* project, possibly due to the way in which VUS classifications were made in this study (discussed further in Chapter 4).

It was however noted that the *PALB2* c.474G>C (p.Gln148His) variant which we recommended be reclassified as likely benign in Chapter 3 due to its high prevalence in our collated pan-African control data, was observed at a significantly higher frequency in both of

our BC cohorts compared to our African control dataset. This raised the question of whether these data could support a pathogenic classification through a case-control comparison. However, several important considerations must be taken into account. First, the ACMG guidelines specify that case-control comparisons are valid only when cases and controls are derived from the same population, as AF can vary significantly between different populations (Choudhury et al., 2020; Richards et al., 2015). While our control dataset included individuals from across SSA, both of our BC cohorts consisted exclusively of South African participants. When considering the datasets that included only South African participants (AWI-Gen and ARK), the variant prevalence was found to be significantly higher (6/1796 (0.33%)) than in the non-South African datasets (1/2012 (0.05%)). Therefore, comparing the prevalence of variants in a pan-African control datasets to South African cases for a case-control analysis is likely inappropriate. Secondly, the BC cases from both the Tygerberg and BROCA cohorts were sequenced in diagnostic laboratories using platforms with coverage depths of at least 50×. In contrast, the control datasets had much lower sequencing depths, ranging from 3.6× to 30×. This discrepancy in sequencing depth means that variants in the control datasets were substantially less likely to be detected, thus underestimating the frequency of the variants in controls (Meynert et al., 2013). For these two reasons, a case-control analysis was not used to inform the variant classification.

Findings from our *Clinical Utility* project highlight the potential of African genomic data to facilitate the reclassification of a significant number of VUS using population allele frequency. Reducing VUS in Black African and Mixed Ancestry participants is important, not only to decrease the number of uncertain results but also to enhance our understanding of variant effects, which could ultimately improve pathogenicity predictions globally. Expanding publicly available African genomic data resources should therefore be a priority for funding agencies and researchers.

7.4 OBJECTIVE 4: ASSESSMENT OF THE SOUTH AFRICAN NDOH TESTING GUIDELINES

Our fourth objective was to evaluate the South African NDOH genetic testing guidelines, which was addressed in Chapter 5 (*Guideline project*). We showed a substantial prevalence of P/LP variants detected in high-penetrance BC genes among individuals meeting these guidelines, and demonstrated their ability to stratify risk, which underscores their utility in our

setting. However, we identified inadequacies in how the NDOH criteria are integrated for risk stratification in our population, especially those of Black African ancestry.

Although the NDOH criteria, including family history of cancer, age at diagnosis, TNBC, and multiple primary BCs, were each associated with a similar prevalence of P/LP variants in *BRCA1/2,* the proportion of participants meeting each criterion varied significantly by ancestry. Participants with Black African ancestry were less likely to meet the family history criterion but more likely to have been referred based on their age at diagnosis. This has important implications for P/LP variant detection because a family history in keeping with the NDOH criterion, was associated with both the presence of a *BRCA1/2* P/LP variant (*Guideline* project) and testing uptake (*GCT* study) using multivariate analyses (see Figure 7.1).

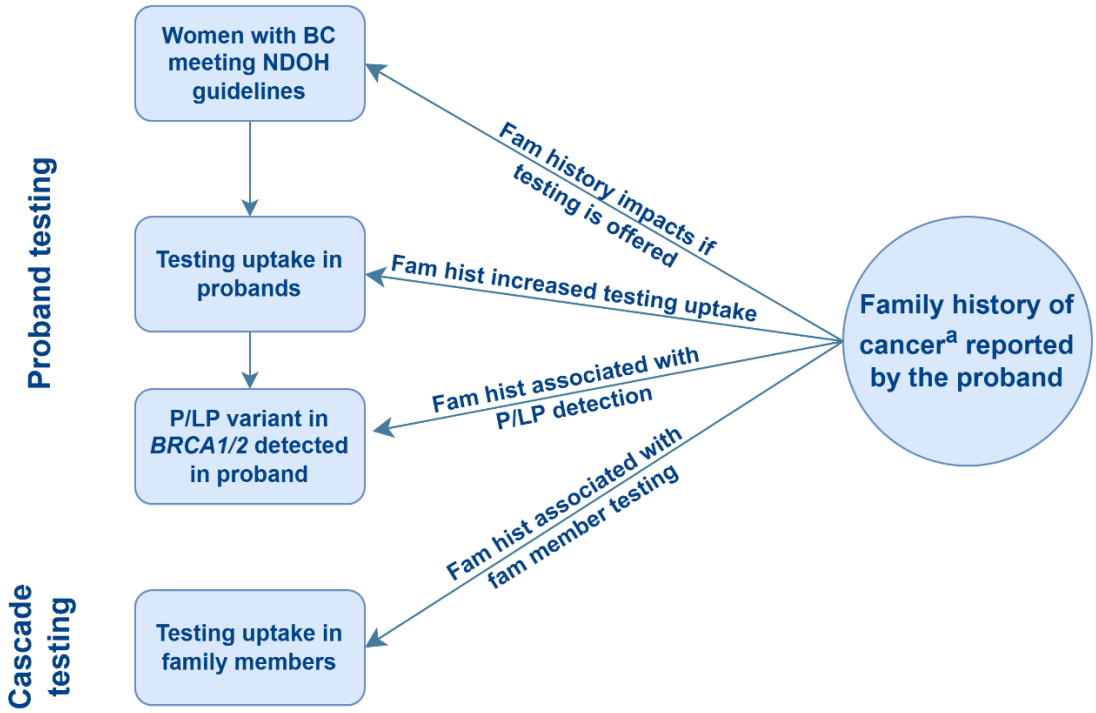


Figure 7.1: Steps in the GCT pathway associated with reporting a family history of cancers associated with hereditary breast cancer

[a] Family history of cancer refers to a history of cancers associated with hereditary BC as outlined in the NDOH guidelines.

Given that a smaller proportion of Black African probands were found to meet the NDOH family history criterion, reasons for which are discussed in Chapter 5, Black African women may be less likely to qualify for genetic testing than women of other ancestry groups (Figure

7.1). Similar concerns have been raised in the USA, where it has been argued that because African Americans are less likely to have/report a family history, testing opportunities for different ancestry groups may be discriminatory. The authors suggest that population-based testing would be an equitable solution (Jakuboski et al., 2022). South Africa, however, will need to come up with other solutions as population-based genetic screening is unlikely to be realised due to testing costs and implementation challenges (Ginsburg & Narod, 2018).

Since family history was found to influence testing uptake in our *GCT* project and Black African women were less likely to have/report a family history, they may also be less likely to accept testing when it is offered (Figure 7.1). While numerous U.S. studies have shown that African Americans are less likely to be referred for genetic counselling and/or testing (Armstrong et al., 2019; Childers et al., 2017; Liu et al., 2022; Reid et al., 2020), the few studies that have examined testing uptake after genetic counselling have found similar rates across ancestry groups (Butrick et al., 2015; Chapman-Davis et al., 2021). Unfortunately, missing data limited our ability to explore differences in testing uptake by ancestry. However, if lower uptake is confirmed, this, combined with fewer Black African women meeting the family history criteria, may result in proportionally fewer Black African women being tested for hereditary BC.

In the *GCT* project, we also showed that the higher the number of family members with *BRCA*-related cancers reported by the proband, the more likely their family members were to test (Figure 7.1). Again, because Black African probands were less likely to report a family history, cascade testing in this group may be less successful than in other ancestry groups. This hypothesis is consistent with non-significant trends in our data showing that test-positive Black African probands with a family history were more likely to have family members test (7/13, 53.8%) compared to those without a family history (3/10, 30%).

These findings have important implications for genetic counselling and until more data is available, ancestry should be considered when applying the NDOH guideline criteria (as detailed in Chapter 5) to enhance inclusivity and predictive accuracy for the Black African population. As it remains unclear whether the lower reported familial clustering of cancer in African women reflects a true difference or a lack of awareness and communication about cancer diagnoses within families, further studies are needed to explore this. Gaining insight into these factors could help refine future testing guidelines. Public health initiatives that

encourage the recording of medical family histories may also play a key role in addressing this gap in family history knowledge.

In addition to reviewing the family history criterion for Black African patients, the diagnostic age threshold in the NDOH guidelines should also be reconsidered. In Chapter 4 (*Population Prevalence* project), we showed that the number of P/LP variants in BC susceptibility genes did not differ significantly between Black South African women diagnosed at age 40 or younger and those diagnosed between 41 and 50 years. However, the NDOH guidelines set the diagnostic age threshold for testing at age ≤40 in the absence of other risk factors (National Department of Health, 2018). Based on our findings, consideration should be given to extending this threshold to include Black South African women diagnosed with BC at ≤50 years of age.

7.5 OBJECTIVES 5 & 6: GENETIC TESTING AND RISK MANAGEMENT UPTAKE

To meet objectives 5 & 6, we investigated the uptake of genetic testing in probands and their family members and determined the use of risk-reducing surgeries in those who had a P/LP variant. This study is described in detail in Chapter 6 (*GCT* project).

We found a high uptake of genetic testing among probands attending genetic counselling which indicates that testing was acceptable to most women with BC in our setting. However, we did not assess the proportion of eligible women with BC who were referred for genetic counselling or the number of referred women who did not attend. These two steps have been identified as major barriers to genetic testing uptake in the USA (Wehbe et al., 2022). While these barriers may be less significant at Tygerberg Hospital, where women with BC are recruited and seen by a genetic counsellor during the same visit as their BC Clinic appointment, they are likely to be substantial obstacles in other regions of the country, particularly where genetic services are scarce. Studies are required to determine the magnitude of barriers to proband referral and attendance at genetic counselling.

The low participation of at-risk relatives in GCT that we demonstrated in the *GCT* project is concerning, suggesting that cascade testing through family member contact may not be an effective method for identifying individuals at risk of hereditary BC in South Africa (Chapter 6). Consideration must be given to the fact there are two processes that need to occur for family members to benefit from GCT. First, the proband must share their result with the family

member and secondly the family member must take the initiative to instigate testing. It is likely that a combination of barriers to both of these processes results in the low uptake of cascade testing which is reported worldwide (Braley et al., 2022; Fehniger et al., 2013; Frey et al., 2022). Numerous barriers to cascade testing have been identified in the literature including individual level factors such as demographics, lack of knowledge, perceived lack of responsibility for informing relatives, as well as beliefs and attitudes towards genetic testing. Interpersonal barriers also play a role, such as the degree of familial relationship, communication difficulties, strained family relationships, and perceptions of relatives' response to the information. Additionally, contextual factors, like difficulty accessing services and low socio-economic status further impact testing uptake in family members (Levine et al., 2024; Srinivasan et al., 2020).

Studies have also shown that family members of African American probands are both less likely to have been informed of their at-risk status and are less likely to have cascade testing performed (Fehniger et al., 2013; Kassem et al., 2023). This may also be the case in Black African and Mixed Ancestry populations in South Africa as these groups are more likely to face the testing barriers already mentioned (Bredenkamp et al., 2021). In addition, other barriers may also be present. For example, a systematic review found that certain cultural beliefs such as superstitions and curses, which are more prevalent in non-Western societies, also likely impede testing uptake in LMIC (Zhong et al., 2021). We did not confirm a significant difference in testing uptake between family members of White, Black African or Mixed Ancestry probands.

Several methods aimed at improving uptake of testing in family members have been explored. Montgomery et al. (2013) investigated whether receiving a communication skills intervention increased the sharing of test results with family members. However, they found no difference between cases who received the intervention and controls who did not. Instead the study found that both cases and controls were more likely to share results based on demographic and relationship factors (Montgomery et al., 2013). In a meta-analysis, Frey et al. (2022) found that significantly higher number of relatives attended genetic counselling when contacted by a healthcare worker (63%) directly as opposed to when probands were left to inform family members (35%). Direct contact was via letters, emails or telephone calls with telephone calls resulting in the highest uptake (Frey et al., 2022). This strategy is however limited by privacy concerns (Levine et al., 2024) and the additional demand placed on already over-burdened

genetics professionals (Ormond et al., 2024). Other issues relevant to this strategy in our setting include language barriers between providers and patients, as well as communication instability due to unreliable contact methods. Prior to developing interventions, it will be necessary to explore the barriers to cascade testing in the country. Identifying these obstacles can then inform strategies for patient education, intervention design, and healthcare policies that support families in high-risk groups.

To meet objective 6, we assessed the uptake of risk-reducing interventions in probands and family members in the *GCT* project. Although assessing uptake of RRM in probands was not possible in this study, we found that half of the probands had BSO or intended to have BSO in the future. We found that less than a third of eligible family members had mammographic surveillance compared to over 95% in a systematic review of women in HIC (Metcalfe et al., 2019). In contrast, more than half of eligible family members proceeded to have RRM which was significantly more than reported in any of the studies in the previously mentioned systematic review. The higher uptake of RRM may be appropriate in our setting given limited MRI availability and restricted access to advanced cancer therapies (discussed in Chapter 6). On the other hand, we found that the uptake of BSO was much lower than reported in other studies. This may reflect barriers related to access, awareness, or possibly cultural issues as identified in other studies (Makhnoon et al., 2021; Metcalfe et al., 2019; M. J. Smith et al., 2021). Given evidence that BSO may be more effective than RRM in reducing morbidity and mortality in *BRCA1/2* and *PALB2* carriers (Li et al., 2016), its low uptake is concerning.

The reasons for the higher-than-expected uptake of RRM but low uptake of BSO in at-risk women need further study including assessment of the availability of surgical services as well as attitudes of both clinicians and women towards surgical risk-reducing interventions in South Africa. Barriers to breast screening for high-risk women should also be addressed. Availability of these services is an important consideration if genetic oncology services are extended.

7.6 STRENGTHS AND LIMITATIONS OF THE STUDY

This study offers novel insights into the GCT process within a sub-Saharan African context, offering a critical assessment of the strengths and weaknesses of the service, from guidelines to management, in a tertiary setting. Access to state-provided genetic services in South Africa is limited to 5 urban centres (South African Society of Human Genetics, n.d.), highlighting the

need for innovative solutions to extend access. The findings of this PhD study can assist in informing policies to support service expansion, guiding clinical practice and shaping future research. While the strengths and limitations of each of the four studies are detailed in their respective chapters, this section provides a broader overview of these aspects.

While this study provides valuable insights from a sub-Saharan African context, the findings may not be generalizable to other parts of South Africa, or the broader region given the significant genetic diversity of populations and differences in healthcare systems. We assessed services in the Western Cape, which has better health infrastructure (Stuckler et al., 2011) than other provinces, including breast cancer services, a small but active regional genetic counselling service, functional electronic records, and access to advanced genetic testing at the time of the study. These factors allow for more accurate assessment of patient and family interest, service uptake, and more effective assessment of testing indications and detection rates. However, the province is not fully representative of the national system. But, given its similar funding level to other tertiary hospitals in the country, it provides a benchmark for what is achievable nationwide.

Furthermore, the study findings may have been influenced by biases in referral and attendance at genetic counselling due to factors such as socio-economic status, geographic location, and cultural attitudes toward genetic testing. In addition, the evaluation of the GCT service largely reflects short-term outcomes, and the study did not assess long-term impacts on patient care or policy changes. Resource constraints, including limited access to genetic counsellors and diagnostic tools, will also likely impact the feasibility and scalability of these services' country wide. It is also important to note that the automated method used to call variants in the *Population Prevalence* project (Chapter 4) may have resulted in an underestimate of the number of P/LP variants compared to the number reported by diagnostic laboratories in Chapters 3 and 5.

Our *Clinical Utility* project (Chapter 3) was novel in accessing a large set of African genomic data (1855 individuals) from the international research community to highlight the importance of this data for variant interpretation. However, the availability of comprehensive datasets that fully capture the genetic diversity across African populations remains a challenge. Legal, ethical, and technical barriers to accessing genomic data also restricted the number of data sets we were able to collect, reducing the impact on variant curation.

These limitations underscore the need for further studies, including population-based research, to address these gaps and improve the overall effectiveness and equity of genetic testing and management in South Africa and sub-Saharan Africa.

7.7 CONCLUSIONS

This PhD study has generated new insights into the GCT pathway, from establishing genetic testing criteria to the cascade testing and management of at-risk individuals. These findings have the potential to contribute to much-needed policy reforms, shape future research agendas, and transform clinical practice in South Africa and beyond. By highlighting gaps in the current approach, this research underscores the urgency of reviewing policies to better serve diverse populations and ensure equitable access to effective genetic testing and management for hereditary cancer. Moreover, this study provides evidence that incorporating additional African genomic data can play a pivotal role in improving the classification of genetic variants. The inclusion of this data can significantly reduce the uncertainty of genetic test results, offering clearer, more actionable insights for patients with BC and their families. This advance not only enhances the clinical utility of genetic testing but may also foster greater accuracy in diagnosing hereditary conditions. It also illustrates the importance of detailed genomic analysis in understudied populations as an important driver of global progress in genetic research and patient care.

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APPENDICES

9.1 APPENDIX A: AUTHORS AGREEMENT

Declaration: Student's contribution to article(s) and agreement of co-author(s)

I, Tabitha Osler, student number 9807768f, declare that this thesis is my own work and that I contributed adequately towards research findings published in the articles stated below which are included in my thesis.

Signature of Student TOsler **Date**……29/01/2025

Name of Primary Supervisor…Prof Christopher Mathew

Signature of Primary Supervisor ……Chris Mathew **Date**……29/01/2025……..

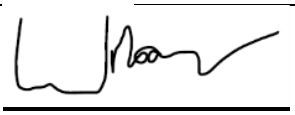
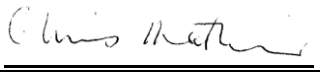
Agreement by co-authors: By signing this declaration, the co-authors listed below agree to the use of the article(s) by the student as part of his/her Thesis/Dissertation/Research Report. In cases where the student is not the 1st author of a published article, the primary supervisor must explain (under comments) why the student is entitled to use the paper for his/her degree purposes.

Article 1:

Title: Prevalence and reclassification of genetic variants in South African populations with breast cancer

Journal name, year, volume and page numbers: Genes, Chromosomes and Cancer (2024) 63: e23275 https://doi.org/10.1002/gcc.23275

Authors	Name	Signature	Date
1st author	Tabitha Osler	TOsler	19/11/2024
2nd author	Jean-Tristan Brandenburg	JBrandenburg	30/01/2025
3rd author	Mardelle Schoeman	MSchoeman	30/01/2025

4th author	Wenlong Carl Chen		30/01/2025
5th author	Michael F. Urban		11/02/2025
6th author	Christopher G. Mathew		29/01/2025

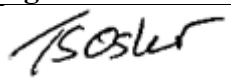
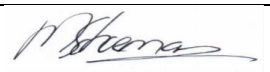
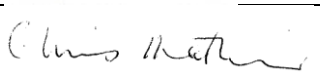
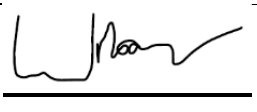
Comments by primary supervisor:

Tabitha Osler carried out the analysis for this paper and wrote and submitted the paper. The paper is an integral part of the research for her PhD.

Article 2:

Title: Application of genetic testing criteria for hereditary breast cancer in South Africa

Journal name, year, volume and page numbers: Breast Cancer Research and Treatment (2025) https://doi.org/10.1007/s10549-024-07585-3

Authors	Name	Signature	Date
1st author	Tabitha Osler		7/03/2025
2nd author	Mardelle Schoeman		30/01/2025
3rd author	Willem JS Pretorius		04/02/2025
4th author	Christopher G. Mathew		29/01/2025
5th author	Jenny Edge		6/2/25
6th author	Michael F. Urban		11/02/2025

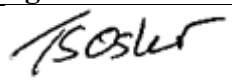
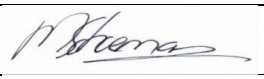
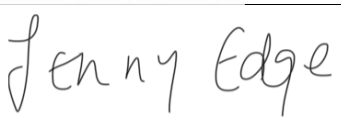
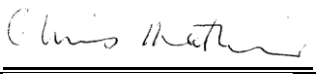
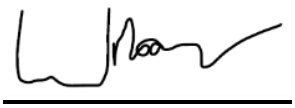
Comments by primary supervisor:

Tabitha Osler carried out the analysis for this paper and wrote and submitted the paper. The paper is an integral part of the research for her PhD.

Article 3:

Title: Breast Cancer Genetic Services in a South African Setting: Proband Testing, Cascading and Management

Journal name, year, volume and page numbers: Cancer Medicine Cancer Medicine (2025) 14: e70743. https://doi.org/10.1002/cam4.70743

Authors	Name	Signature	Date
1st author	Tabitha Osler		07/03//2025
2nd author	Mardelle Schoeman		30/01/2025
3rd author	Jenny Edge		6/2/25
4th author	Willem JS Pretorius		04/02/2025
5th author	Felix H. Rabe		03/01/25
6th author	Christopher G. Mathew		29/01/2025
7th author	Michael F. Urban		11/02/2025

Comments by primary supervisor:

Tabitha Osler carried out the analysis for this paper and wrote and submitted the paper. The paper is an integral part of the research for her PhD.

9.2 APPENDIX B: ETHICS CLEARANCE CERTIFICATE

UNIVERSITY OF THE WITWATERSRAND JOHANNESBURG

R49 Ms T Osler

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
CLEARANCE CERTIFICATE NO. M231195

NAME: (Principal Investigator)	Ms T Osler **DEGREE:** PhD
SCHOOL:	Sydney Brenner Institute for Molecular Bioscience Faculty of Health Sciences
PROJECT TITLE:	*Genetic counselling, testing and clinical management of hereditary breast cancer in South Africa* Change of study title noted on 2025/02/03
DATE CONSIDERED:	Ad hoc
DECISION:	Approved unconditionally
CONDITIONS:	Sub-study under M23/09/77 and N22/04/039 (University of Stellenbosch)
NOTE:	For permission to use or contact student study participants, please contact the Registrar's office - <Nicoleen.Potgieter@wits.ac.za>
SUPERVISOR:	Profs C Mathew & M Urban; Drs WC Chen & J-T Brandenburg
APPROVED BY:	Professor P Ruff, Chairperson, HREC (Medical)
DATE OF APPROVAL:	2024/01/26 **EXPIRY DATE:** 2029/01/25

This Clearance Certificate is valid for 5 years from the date of approval. An extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office secretariat on the 3rd floor, Phillip Tobias Building, Parktown, University of the Witwatersrand, Johannesburg.

I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated from the research protocol as approved, I/we undertake to submit details to the Committee. **I agree to submit a yearly progress report** in the format available at https://wits.ac.za/research/researcher-support/research-ethics/ethics-committees/. This report is due annually, for the duration of the Clearance Certificate, beginning on the first anniversary of Date of Approval above. Unreported changes to the study may invalidate the clearance given by the HREC (Medical).

Signature of Principal Investigator _____ Date _____

9.3 APPENDIX C: PUBLISHED PAPERS

Check for updates

Genes, Chromosomes and Cancer

WILEY

RESEARCH ARTICLE OPEN ACCESS

Prevalence and Reclassification of Genetic Variants in South African Populations with Breast Cancer

Tabitha S. Osler[1,2] | Jean-Tristan Brandenburg[1,3] | Mardelle Schoeman[4] | Wenlong Carl Chen[1,3,5] | Michael F. Urban[2,6] | Christopher G. Mathew[1,2,7]

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Keywords: African ancestry | African genomic data | allele frequency | *BRCA1/2* | gene panel testing | hereditary breast cancer | variant classification | variant of uncertain significance (VUS)

ABSTRACT

Concurrent testing of numerous genes for hereditary breast cancer (BC) is available but can result in management difficulties. We evaluated use of an expanded BC gene panel in women of diverse South African ancestries and assessed use of African genomic data to reclassify variants of uncertain significance (VUS). A total of 331 women of White, Black African, or Mixed Ancestry with BC had a 9-gene panel test, with an additional 75 genes tested in those without a pathogenic/likely pathogenic (P/LP) variant. The proportion of VUS reclassified using ClinGen gene-specific allele frequency (AF) thresholds or an AF > 0.001 in nonguidelines genes in African genomic data was determined. The 9-gene panel identified 58 P/LP variants, but only two of the P/LP variants detected using the 75-gene panel were in confirmed BC genes, resulting in a total of 60 (18.1%) in all participants. P/LP variant prevalence was similar across ancestry groups, but VUS prevalence was higher in Black African and Mixed Ancestry than in White participants. In total, 611 VUS were detected, representing 324 distinct variants. 10.8% (9/83) of VUS met ClinGen AF thresholds in genomic data while 10.8% (26/240) in nonguideline genes had an AF > 0.001. Overall, 27.0% of VUS occurrences could potentially be reclassified using African genomic data. Thus, expanding the gene panel yielded few clinically actionable variants but many VUS, particularly in participants of Black African and Mixed Ancestry. However, use of African genomic data has the potential to reclassify a significant proportion of VUS.

1 | Introduction

It is estimated that 5%–15% of women with breast cancer (BC) have a clinically actionable variant, defined as a pathogenic or likely pathogenic (P/LP) variant in a moderate to high-risk BC susceptibility gene [1–4]. Since the advent of high-throughput sequencing (HTS), concurrent testing of hundreds of genes for BC susceptibility is now possible and available. Given the option

to test more genes, clinicians and patients frequently opt for a "the more the better" approach, especially when there is no additional cost [5]. However, it is important to consider the clinical utility of testing, which is a balance between achieving improved outcomes due to testing and the risks associated with the test [6]. Negative consequences of testing using expanded panels include risk and management uncertainties for genes lacking guidelines [7, 8], and an increase in the number of variants of uncertain significance (VUS) detected [9].

VUS are genetic variants for which the clinical impact is unclear. They can cause considerable uncertainty and anxiety to both patients and their clinicians, and may even lead to unnecessary interventions [10, 11]. Individuals of African ancestry have been found to be significantly more likely to have a VUS identified on genetic testing [12–14]. This is due both to the increased genetic diversity found in African populations and the under-representation of African genomic data necessary for variant classification. The increased level of genetic diversity in continental African populations has been confirmed in multiple studies [15–19]. One of these studies showed that five populations from sub-Saharan Africa had, on average, 20% more single nucleotide polymorphisms/variants than either European or East Asian populations [15]. More recently Choudhury et al. discovered over three million undescribed variants in their study of genomic diversity in Africa using whole genome sequencing (WGS) [17]. However, the number of African genomes contained in global genomic databases is not representative of this diversity [20]. This is evidenced by the fact that only 6.5% of the exome/genome data in v3.1.1. of the largest publicly available database of population variation, the Genome Aggregation Database (gnomAD), are derived from continental African or African–American populations [21, 22].

The lack of population variation data significantly impacts variant classification in individuals of African ancestry. According to the American College of Medical Genetics and Genomics and the Association of Molecular Pathology (ACMG-AMP) guidelines, the most compelling evidence type for variant classification is an allele frequency (AF) in a control population that is higher than the prevalence of the disease. The ACMG-AMP guidelines recommend that a variant with an AF of ≥ 0.05 in gnomAD can be designated as benign without the addition of other evidence (evidence code BA1 in the guidelines). An AF ≥ 0.01 is considered strong evidence that a variant is benign (code BS1), but additional evidence types are needed before a benign classification can be made [22, 23].

More recently, the ClinGen Sequence Variant Interpretation (SVI) working group refined the guidelines to state that any population database of at least 2000 alleles from unrelated individuals can be used to establish control population AF in a gene without a gene- or variant-specific modification. The SVI did not specify the read depth or quality filtering that should be applied to the population database used [24]. Since then, several ClinGen Variant Curation Expert Panels (VCEPs) under the auspices of the NIH-funded ClinGen Consortium have released modifications to the ACMG-AMP guidelines for specific genes [25, 26]. These modifications include lower AF thresholds for both BA1 and BS1 (Table S1) determined using the Whiffin calculator, which takes into account disorder prevalence, allelic and genetic heterogeneity, and penetrance of the specific gene [27].

The South African population consists of people with diverse origins, languages, and cultures. The National Census describes South Africa's three largest ancestry groups as Black African (80.2%), Colored (8.8%), and White (7.7%) [28]. These categories were inherited from the Apartheid era but are now used to identify and address inequalities and discrimination. The Black African populations indigenous to South Africa are an ethnically diverse group of Bantu-speaking peoples [16], and in the post-Apartheid period include an increasing diversity of people originating elsewhere in Africa. White South Africans are predominantly descendants of European settlers, although their ancestry includes some degree of nonEuropean admixture with those of white Afrikaner descent having about 5% nonEuropean admixture [29]. The term "Colored" (referred to as Mixed Ancestry in this study) refers to peoples with a degree of shared cultural identity who have genetic ancestry from Khoisan, Black, European, and Asian populations [16, 30].

Given the diversity of the South African population, we aimed to determine if an expanded gene panel for hereditary BC had similar utility in different ancestry groups by comparing the P/LP and VUS prevalences. In addition, we aimed to determine the proportion of VUS detected in our participants that could be reclassified with the use of additional African genomic data.

2 | Methods

2.1 | Participants

Participants included South African females with invasive or in situ BC who attended Tygerberg Hospital in the Western Cape, South Africa for genetic counseling and proceeded with gene panel testing between January 2018 and June 2022. Participants were offered diagnostic genetic testing if they had been diagnosed with BC ≤ 40 years; had two primary BCs ≤ 60 years of age; had triple negative BC; had one or more close relatives with invasive ovarian cancer, male BC, or BC ≤ 50 years; or two or more close relatives with breast or pancreatic cancer with one diagnosed ≤ 60 years old. The genetic counselor recorded the proband's ancestry group, which was required by the testing laboratory to assist with variant interpretation, according to the categories of the South African Census: White, Black African, or Colored (Mixed Ancestry).

2.2 | Gene Panel Testing

Saliva or buccal swabs were collected from participants and sent to Invitae Laboratory in the USA for gene panel testing [31]. Invitae performed gene sequencing and deletion/duplication analysis using HTS, which covered all coding exons and 10–20 base pairs of intronic sequence on either side of the coding exon [32]. To classify variants, Invitae used Sherloc, a variant classification framework developed to refine the rules within the ACMG-AMP guidelines [33]. Population AF data is incorporated

into the Sherloc framework using a machine-learning model that estimates variant pathogenicity based on AF data from large population databases [34].

All study participants underwent testing of nine genes known to be associated with BC. If no P/LP variant was found in these genes, participants were then tested for an additional 75 genes (see gene lists in Data S1). The prevalence of P/LP variants and VUS in the initial nine genes tested was then compared to the prevalence found after testing the additional 75 genes. This comparison aimed to assess the utility of testing a large gene panel in demographically diverse, understudied African populations.

In view of the wide range in the number of VUS identified in different genes, we also investigated whether a relationship existed between the number of VUS and the size of the gene's coding region. The length of the coding region was determined using the number of amino acids in the gene's corresponding protein as a proxy.

2.3 | Re-Curating VUS With African Genomic Data

The use of African genomic data for the reclassification of VUS was assessed using five data sources, which provided WGS data from a total of 1855 individuals of African ancestry (Table 1). After application to the relevant data access committees, these five data sources were shared with us in either VCF or CRAM file format. Variant calling was performed on the CRAM files from the MalariaGen dataset using GATKv4.4.0.0 best practice guidelines [35]. VCFtools v0.1.17 was used to search each of the five data sources individually using the appropriate genomic position (GRCh37 or GRCh38) of the VUS [36]. Subsequently, an overall AF using the information from each data source was calculated for every VUS. The online Whiffen calculator [37] was then used to find the filtering AF (the lower bound of the 95% confidence interval [CI]), for each VUS [27]. Two VUS, one identified in two participants, were excluded from this analysis because they were duplications with unknown breakpoints.

Using the filtering AF, the VUS were curated using the original population frequency thresholds from the ACMG-AMP guidelines and the VCEP gene-specific specifications, when available (Table S1). Three of the data sources used did not meet the sequencing depth specified in some of the gene-specific guidelines, but all other criteria were observed during curation. The GroupMax AF (lower bound 95% CI AF from the genetic ancestry group with the highest AF) of the detected VUS was also determined in gnomAD v2.1.1 and v3.1.2 using the Ensembl Variant Effect Predictor [38].

2.4 | Data Analysis

All statistical tests were done using RStudio Version 2023.12.0 [42]. Statistical differences were calculated using a Chi-square test or Fisher Exact test for categorical variables and Kruskal–Wallis for continuous variables. Bonferroni correction was used to correct for multiple testing. Spearman's correlation was used to examine relationships between continuous variables. Odds ratios with 95% CI were calculated

TABLE 1 | African genomic data used in the analysis of VUS identified in the study.

Database and Reference	Country of origin (no. of participants in brackets)	Total no. alleles	Reference genome	Sequencing depth	Data type received	No. VUS in dataset ($n = 323$)
African Genome Variation Project [18]	Ethiopia (120), South Africa (100), and Uganda (100)	640 alldes	37	4–8×	WGS (VCF)	39
Awi-Gen SA [39]	South Africa (100)	200 alldes	38	30×	WGS (VCF)	64
ARK [40]	South Africa (798)	1596 alleles	38	3.6×	WGS (VCF)	75
MalariaGen [41]	Burkina Faso (57), Cameroon (38), and Tanzania (247)	494–504 alldes[a]	37	10×	WGS (CRAM)	20
H3Africa Consortium WGS VCF [17]	Benin (41), Nigeria (49), Botswana (48), Zambia (29), and Burkina Faso (31), Ghana (26), Cameroon (50), Uganda (51), Guinea (27), Ivory Coast (22), and DRC (12)	772 alleles	37	30×	WGS (VCF)	46

Abbreviations: CRAM, compressed reference-oriented alignment Map; VCF, variant call format; WGS, whole genome sequencing.
[a]Due to areas without good coverage, the number of alleles at some sites varied.

using the median-unbiased estimate method for contingency data [43]. A p-value less than 0.05 was considered statistically significant.

3 | Results

There were 344 female BC patients who had gene panel testing in the study period but 13 were excluded because they had previously tested negative for a founder disease-causing variant in *BRCA1/2* or had not had testing of all included nine genes. Data from the remaining 331 participants were used in the analysis of the 9-gene panel results. Of these participants, a P/LP variant was detected in 58 and further testing was not done. A total of 273 participants had additional testing of the same set of 75 genes and these results were compared to those from the 9-gene panel.

3.1 | P/LP Variants Detected With 9 and 75 Gene Panels

No significant difference was found between the prevalence of P/LP variants in the three ancestries for either the 9-gene ($p = 0.56$) or the 75-gene panel ($p = 0.43$) (Table 2). All ancestry groups had significantly more P/LP variants detected with the 9-gene panel compared to the 75-gene panel ($p < 0.01$ for all groups). P/LP variants in *BRCA1* (5.7%) and *BRCA2* (7.6%) were the most common and there was no significant difference in their prevalence between the three ancestries. Other genes from the 9-gene panel found to have a P/LP variant in one or more of the participants were *PALB2*, *CHEK2*, *TP53*, and *ATM* (Table S2). A P/LP variant was detected in 4.4% (12/273) of the participants tested with the 75-gene panel (). Of the nine genes in which a P/LP variant was detected using this larger panel, two were in genes (*RAD51C* and *RAD51D*) conclusively linked to BC [3, 44] and six had management guidelines available (Table 3) [45]. No difference in the frequencies of P/LP variants between the three ancestry groups was found for any of the 84 genes tested (Table S3). Including the results from both panels, 60 participants had a P/LP variant in a gene with an established association with BC (18.1%).

3.2 | Prevalence of VUS

When comparing the three ancestry groups, no significant difference in the average number of VUS per participant was found in the 9-gene panel ($p = 0.16$). However, Black African and Mixed Ancestry participants had significantly more VUS per participant detected with the 75-gene panel than White participants ($p = 3.8 \times 10^{-6}$). In addition, the ratio of P/LP variants to VUS detected was significantly lower using the 75-gene panel compared to the 9-gene panel for all ancestries, Black African ($p < 10^{-5}$), Mixed Ancestry ($p < 10^{-5}$), and White ($p < 10^{-5}$) (Figure 1).

3.3 | Analysis and Reclassification of VUS

A total of 71.9% (238/331) of the participants carried one or more of the 611 VUS occurrences, 323 of which were distinct variants. Nearly half (43.3%; 140/323) of the distinct VUS were found in only 10 of the 84 genes tested: *ATM* (25, 7.7%),

TABLE 2 | Number of P/LP variants and VUS detected in each ancestry group.

Gene panel size	Ancestry	Total no. participant	No. participant with P/LP variant	No. participant with ≥ 1 VUS	Total no. VUS detected[a]	Ratio of P/LP variants to VUS
9 genes ($n = 331$)	Black African	72	12 (16.7%)	17 (23.6%)	17	1:1.4
	White	53	12 (22.6%)	8 (15.1%)	10	1:0.8
	Mixed Ancestry	206	34 (16.5%)	57 (27.7%)	63	1:1.9
	All participants	331	58 (17.5%)	82 (24.8%)	90	1:1.6
75 genes ($n = 273$)	Black African	60	1 (1.7%)	49 (81.7%)	102	1:102
	White	41	1 (2.4%)	20 (48.8%)	39	1:39
	Mixed Ancestry	172	10 (5.8%)	149 (86.6%)	383	1:38
	All participants	273	12 (4.4%)	218 (79.9%)	524	1:44

Abbreviations: P/LP, pathogenic/likely pathogenic; VUS, variant of uncertain significance.
[a]Multiple participants had > 1 VUS.

TABLE 3 | Information available for genes with P/LP variants detected with the 75-gene panel.

Gene	No. P/LP variants detected	Established link to BC[a]	NCCN guidelines available [45]
BAP1	1	No	Yes
BRIP1	1	No	Yes
FH	2	No	No
MSH6	1	No	Yes
MUTYH	2	No	Yes[b]
RAD50	1	No	No
RAD51C	1	Yes	Yes
RAD51D	1	Yes	Yes
RECQL4	2	No	No

Abbreviations: P/LP, pathogenic/likely pathogenic; NCCN, National Comprehensive Cancer Network.
[a]Link to BC as per standardized curations [46].
[b]Both individuals were heterozygous and guidelines for MUTYH do not recommend additional cancer screening for heterozygotes.

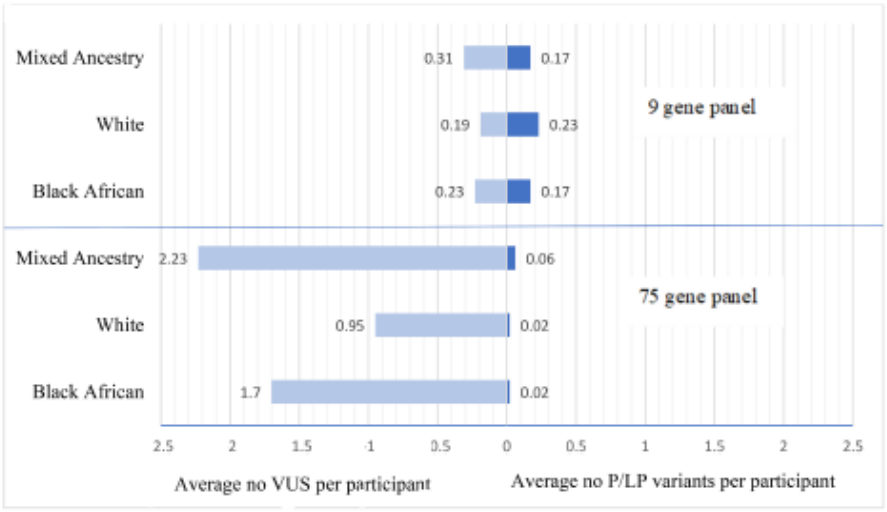

FIGURE 1 | Comparison of the average number P/LP variants and VUS after initial 9 gene and then further 75 gene testing.

POLE (25, 7.7%), *APC* (16, 5.0%), *WRN* (12, 3.7%), *PALB2* (11, 3.4%), *RECQL4* (11, 3.4%), *AXIN2* (10, 3.1%), *BARD1* (10, 3.1%), *DIS3L2* (10, 3.1%), and *MET* (10, 3.1%). A significant positive relationship was found between the length of the gene's coding region and the number of distinct VUS detected in the gene ($p = 3.4 \times 10^{-9}$).

A total of 35.3% (114/323) of the VUS were found to be present in the African genomes but none had a filter AF ≥0.01 and therefore did not meet the AF threshold in the original ACMG-AMP guidelines. Gene-specific AF thresholds were available for 12 of the 73 genes in which VUS were found (see Figure 2). Of the 83 distinct VUS in genes with guidelines, two met the gene-specific frequency thresholds for BA1 and could be reclassified as benign (Table 4). There were 15 additional VUS that met the gene-specific AF threshold for BS1, but of these, eight had less than five alleles, and BS1 could therefore not be applied as specified in the guidelines. Of the seven VUS that met BS1, five had additional evidence available and were reclassified as likely benign. Therefore, 7 (8.4%) of the 83 distinct VUS in guideline genes could be reclassified, and because many of these VUS occurred in more than one participant, this resulted in a total of 19.1% (26/136) of VUS occurrences in guideline genes being reclassified.

However, 61 genes in our panel did not have guidelines specifying gene-specific AF thresholds, resulting in most of the VUS in our study remaining classified as VUS. We determined the number of VUS with a filter AF ≥0.001, which was the most stringent threshold in any of the gene-specific guidelines used. We found that 10.8% (26/240) of distinct VUS in nonguideline genes met this threshold, accounting for 22.7% (139/611) VUS occurrences. Therefore, in total, 27.0% (165/611) of the VUS occurrences had

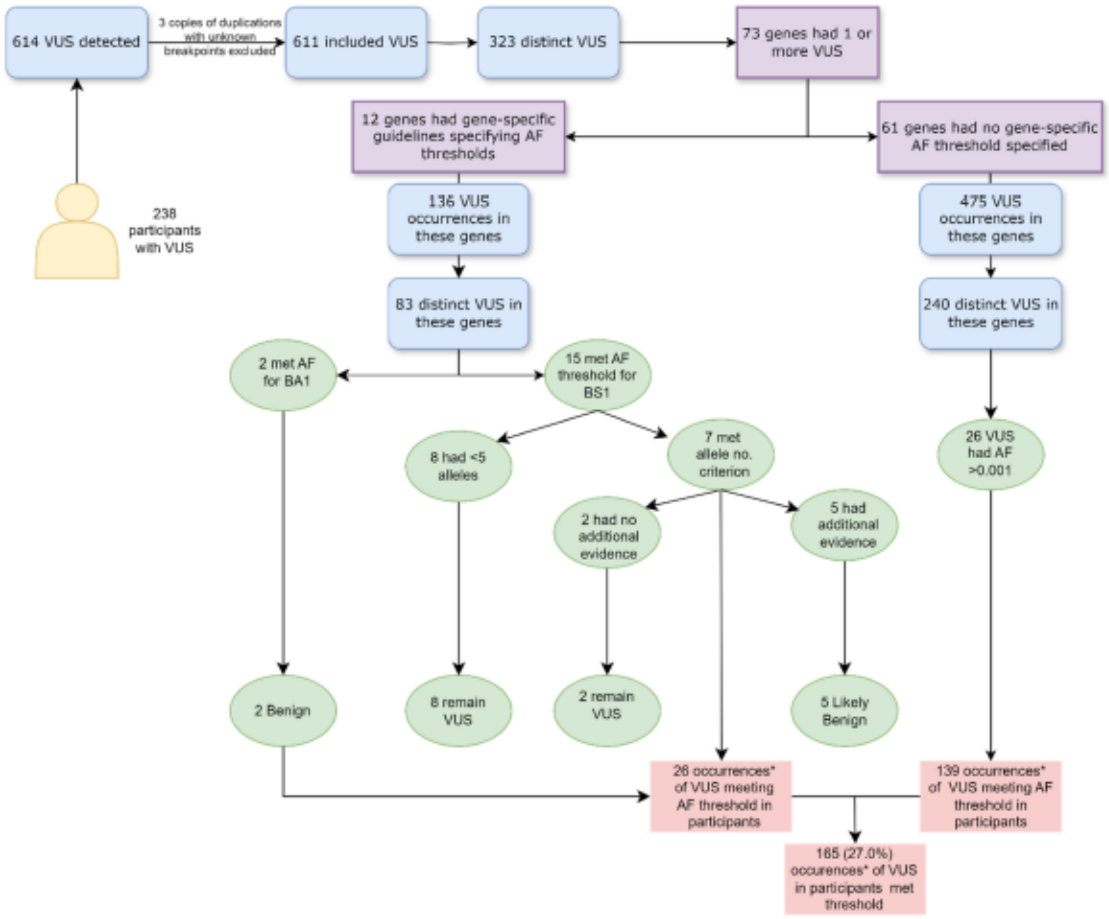

FIGURE 2 | Reclassification of VUS using allele frequencies from African genomic data *Some of the VUS occurred in multiple individuals (occurrences).

a filter AF above either 0.001 or the gene-specific threshold in the African genomes.

On searching gnomAD, we found that 4.8% (4/83) of the distinct VUS in our participants had an AF above the stipulated gene-specific threshold, and 1.7% (4/240) had an AF > 0.001 in nonguideline genes. This resulted in a total of 3.4% (21/611) of VUS occurrences having an AF ≥ 0.001 or above the gene-specific threshold in gnomAD. The odds of a VUS from our sample being present at an AF above the gene-specific threshold or > 0.001 was 10.1 times higher (95% CI: 6.4–16.6, $p < 0.0001$) in the African genomes than in gnomAD.

4 | Discussion

Here, we report on the prevalence of P/LP variants and VUS in BC susceptibility genes in three South African ancestries and assess the utility of expanded genetic testing. We also show the potential benefit of increasing available African genomic data for the re-curation of VUS.

We found a similar prevalence of P/LP variants in participants from all three South African population groups. This is in line with studies comparing the frequency of P/LP variants in African–American and nonHispanic White Americans [12, 47, 48]. However, the admixture in the African–American population and their predominantly West African origins makes it challenging to draw conclusions for resident African-ancestry populations [49]. In addition, the diversity of African populations means that genetic research findings cannot be generalized across the continent [17, 50]. Studies of unselected women with BC in Nigeria [51], Uganda and Cameroon [52], and Ghana [53] have found P/LP variant prevalence in BC genes of 14.7% (11.1% in *BRCA1/2*), 15.8% (11.2% in *BRCA1/2*), and 11.9% (6.1% in *BRCA1/2*), respectively. The higher prevalence of P/LP variants associated with BC risk in our study (18.1%) is likely due to our selection criteria, which included family history, young age of diagnosis, or triple negative BC. Further studies are needed to determine whether the prevalence of P/LP variants is similar between African ancestry groups in both BC patients and in the general population.

TABLE 4 | VUS meeting gene-specific AF thresholds in African genomes.

Gene	VUS (HGVS Nomenclature)	Protein prediction	No. alleles in study participants ($n = 331$)	No. alleles in African genomes ($n = 1854$)	Filtering AF[a] in African genomes	Groupmax filtering AF in gnomAD[b]	ACMG-AMP code[c] met with African genome AF	Other gene-specific ACMG-AMP criteria met	New classification
APC	NM_000038:c.-30530G>C	Intronic	2	3	0.0002205	0	Not met	—	—
	NM_000038:c.-30582T>C	Intronic	7	3	0.0002205	0	Not met	—	—
	NM_000038:c.3139G>A	p.Glu1047Lys	2	3	0.0002205	0	Not met	—	—
ATM	NM_000051:c.131A>G	p.Asp44Gly	1	18	0.0031376	0.00046	BS1	None	—
	NM_000051:c.4279G>A	p.Ala1427Thr	1	14	0.0031376	0.0002042	BS1	BP4	Likely benign
	NM_000051:c.4082A>G	p.Gln1361Arg	1	12	0.0018673	0.000728	BS1	BP4	Likely benign
	NM_000051:c.3746+18A>G	Intronic	1	9	0.0012662	0	BS1	BP7	Likely benign
	NM_000051:c.2813C>T	p.Pro938Leu	1	5	0.0005313	0	BS1	BP4	Likely benign
	NM_000051:c.6537T>G	p.Ile2179Met	2	5	0.0005313	0.000479	BS1	None	—
BRCA1	NM_007294:c.3076A>G	p.Ile1026Val	1	3	0.0002205	0	Not met	—	—
BRCA2	NM_000059:c.4502A>G	p.Asn1501Ser	4	4	0.0003684	0	Not met	—	—
	NM_000059:c.3448A>T	p.Thr1150Ser	1	3	0.0002205	0	Not met	—	—
DICER1	NM_177438:c.4852T>A	p.Ser1618Thr	2	4	0.0003684	0	Not met	—	—
PALB2	NM_024675:c.474G>C	p.Gln158His	7	7	0.000886	0	BS1	BP1, BP4	Likely benign
RUNX1	NM_001754:c.82-84del	p.Ser28del	1	11	0.0016637	0.000149	BA1	N/A	Benign
VHL	NM_000551:c.631A>C	p.Met211Leu	11	13	0.00207	0.0000789	BA1	N/A	Benign
	NM_000551:c.18G>T	p.Glu6Asp	2	4	0.0003684	0	Not Met	—	—

Abbreviations: ACMG/AMP, American College of Medical Genetics and Genomics and the Association of Molecular Pathology; AF, allele frequency; VUS, variant of uncertain significance.
[a]Filtering AF refers to the lower 95% confidence interval of the allele frequency calculated using the Whiffin calculator [27].
[b]The lower bound 95% confidence interval allele frequency in gnomAD from the genetic ancestry group with the highest AF.
[c]AF meets BS1 gene-specific threshold.

There has been debate regarding the appropriate number of genes to include in a panel to test for inherited BC susceptibility [54, 55]. It is widely held that testing beyond *BRCA1/2* is appropriate and increases the detection rate of P/LP variants substantially [1, 56–58]. All genes included in the 9-gene panel are known to be associated with BC and have established management guidelines [45]. After excluding participants in our study with P/LP variants in *BRCA1/2*, we found that an additional 4.8% (14/287) had P/LP variants detected with the 9-gene panel. This detection rate is consistent with findings from other studies [58, 59].

Significantly more participants had a P/LP variant detected with the 9-gene panel (17.5%) than with the 75-gene panel (4.4%). Using the 75-gene panel, only 0.7% (2/273) participants had a P/LP variant in a gene associated with BC, which was the primary indication for testing. 83.3% (10/12) of P/LP variants detected with the 75-gene panel were in genes with disputed or no evidence of an association with BC (referred to here as secondary findings [SF]), and of these, 5 (41.7%) had no current clinical utility because management guidelines for the gene were not available (Table 3) [6]. Although detecting these variants leads to uncertainty [8], multiple studies have found no evidence of psychological harm caused to individuals receiving them [60–63]. It has however been shown that a considerable number of individuals choose not to receive SF, especially when the SF has no clinical utility [61, 62].

SF may be of much benefit to individuals when they occur in genes with guidelines on risk-reducing measures. There were three participants in this study with SF in genes (*BAP1*, *BRIP1*, and *MSH6*) with guidelines recommending interventions to mitigate other cancer types. Although detecting these P/LP variants has the potential to reduce mortality and morbidity, studies conducted in high-income settings (HIC) have found that participants with SF and their family members often do not pursue cascade testing or adopt recommended risk-reducing measures [60, 61]. This may be even more likely to be the case in our setting, where uptake of risk-reducing measures in women with hereditary BC have been shown to be lower than in HIC [64].

A further issue related to expanded gene panels is the associated increase in the number of VUS detected. The detection of VUS can create uncertainty for both patients and clinicians, potentially resulting in unnecessary medical interventions [65–67]. As reported by several other studies, we confirmed that the frequency of VUS in populations not of European ancestry is significantly higher than those of European ancestry when testing a large number of genes [12–14]. This may ultimately increase the risk of a negative consequence from genetic testing in populations of African ancestry compared with European populations, but this should be balanced against the limited additional detection of actionable variants. Also, such data is scarce in under-resourced settings and may have a role in clinical research.

Allele frequencies in aggregated African genomes enabled the reclassification of 8.4% of distinct VUS in genes with ClinGen gene-specific guidelines [25]. When an AF threshold of 0.001 was applied for VUS in genes without guidelines, 10.8% of distinct VUS met this threshold in the African genomes and could potentially be reclassified if additional information was available. This was significantly more than met the same threshold in gnomAD and highlights the value of including genomes from diverse populations for variant classification. However, to realize the full potential of African genomic data, lower AF thresholds may be required, which will necessitate gene-specific thresholds to be determined and implemented for more genes. In addition, gene-specific guidelines need to be modified to allow the use of datasets other than gnomAD. Also, the sequencing depth criteria specified should be reviewed when AF is used to support a benign interpretation, as over-counting of variants is unlikely and the observed AF can be considered the minimum AF [68]. Removing this sequencing depth requirement would make multiple other data sources available for curation.

Current limitations to using the African genome AF data are the absence of gene-specific guidelines for most genes and the relatively small number of genomes in our database. Several VUS met the AF threshold in the data, but due to the insufficient number of African genomes, they did not have the specified five alleles, which limited VUS reclassification. Also, future work would benefit from availability of genome data specifically from the populations being studied, such as Southern African Black and Mixed Ancestry populations. An additional limitation of this study was the limited sample size of participants ($N = 331$); consequently, small differences in the prevalence of variants in individual genes between ancestries may not have been detected due to insufficient power.

5 | Conclusions

We have shown that the prevalence of P/LP variants in commonly tested BC susceptibility genes is similar in women with BC from different ancestral populations in South Africa. Also, no apparent differences in the prevalence of P/LP variants in the genes tested were found, and thus there is no indication that gene panels need to be adjusted for different ancestral groups. Expansion of testing with the 75-gene panel produced only a small increase in the number of P/LP variants detected in BC susceptibility genes, and a significant increase in the number of VUS, especially for participants from Black African and Mixed Ancestry populations. Although secondary findings are of potential clinical relevance, further studies are needed to investigate whether feedback of such results would be welcomed by participants, and to what extent they would have clinical utility. We showed that using African genomic data for variant curation resulted in the potential reclassification of a considerable number of VUS. This indicates that increasing the number of African genomes while decreasing the AF threshold through gene-specific guidelines for more cancer susceptibility genes would be of significant benefit. In conclusion, our study indicates that expanding gene panels for hereditary BC testing in South Africa offers limited clinical benefits and increases the number of VUS substantially. However, increasing the representation of African genomes and refining classification guidelines could substantially improve the reclassification of these variants.

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Ethics Statement

The study was approved by the Human Research Ethics Committees of Stellenbosch University (N22/04/039) and the University of the Witwatersrand (M231195). As the study was a retrospective record review, participant consent was not required. All data were de-identified.

Consent

The authors have nothing to report.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The de-identified participant data used and analyzed in this study is available from the corresponding author on reasonable request. The African genomic data were used under license for the current study. Restrictions apply to the availability of these data but applications for use can be made via the European Genome Archive at the following links: African Genome Variation Project: https://ega-archive.org/datasets/EGAD00001001663, Awi-Gen SA: https://ega-archive.org/datasets/EGAD00001006418, MalariaGen: https://ega-archive.org/studies/EGAS00001003648, H3Africa Consortium WGS VCF: https://ega-archive.org/datasets/EGAD00001008577, and ARK—the Principal Investigator can be contacted for data access at june.fabian@mweb.co.za.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.

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RESEARCH

Application of genetic testing criteria for hereditary breast cancer in South Africa

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Abstract

Purpose Breast cancer (BC) is the commonest cancer in South African women. A proportion are associated with a pathogenic or likely pathogenic (P/LP) variant in a BC susceptibility gene. Clinical guidelines for genetic testing are used to optimise variant detection while containing costs. We assessed the detection rate in women of diverse ancestries who met the South African National Department of Health (NDOH) testing guidelines, and analysed relationships between testing criteria, participant characteristics and presence of a *BRCA1/2* P/LP variant.

Methods Records from 376 women with BC who met NDOH criteria and had genetic testing were included. Demographic, clinical and test result data were collated to describe detection rates according to criteria met, and a multivariate analysis conducted to find variables most frequently associated with a P/LP variant.

Results P/LP variant prevalence in women meeting NDOH testing criteria was 19.9% (75/376). Women meeting ≥ 2 guideline criteria were over twice as likely to have a P/LP variant (OR 2.27, 95%CI 1.27–4.07, $p=0.006$), highlighting the guidelines' capacity to stratify risk. Family history (OR 1.97; 95%CI 1.05–3.70, $p=0.03$) and Black African ancestry (OR 2.58; 95%CI 1.28–5.18, $p<0.01$) were independently associated with having a *BRCA1/2* P/LP variant when controlling for other variables. Notably, although Black African participants were less likely to report a family history, those that did had higher odds of a P/LP variant in *BRCA1/2*.

Conclusion These results demonstrate the usefulness of the NDOH guidelines in women of diverse ancestries and provide insight into the factors associated with P/LP variants in understudied African populations.

Keywords Hereditary breast cancer · *BRCA1/2* · Testing guidelines · Family history · LMIC

Introduction

There were 11,020 women diagnosed with breast cancer (BC) in South Africa in 2022, making it the commonest cancer in females [1]. A proportion are expected to be attributable to pathogenic or likely pathogenic (P/LP) variants in a germline BC susceptibility gene, such as *BRCA1/2*. Population-based studies from the USA put this proportion at 5–10% [2, 3]. Similar data from Africa is scarce, but West African studies give estimates of 8–14% [4–6]. P/LP variants in these genes are associated with lifetime risks of BC of 40%–85% for high risk genes and 20%–40% for moderate risk genes, causing significant morbidity and mortality [7]. In addition, the risks of other cancers, such as ovarian and pancreatic cancers, are often also increased in gene carriers. Identifying individuals with these P/LP variants allows intensified surveillance, risk-reducing surgical interventions and modifications to cancer treatment to be made [8].

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Given the modest proportion of BC associated with Mendelian susceptibility genes, and the historically high cost of genetic testing, clinical guidelines were developed to direct testing [9, 10]. Guidelines initially aimed to identify individuals with a 10%–20% risk of carrying a germline P/LP variant in a high risk BC susceptibility gene, such as *BRCA1/2*, *TP53* and *PALB2* [7]. Following the introduction of extended gene panel testing it has been noted that these guidelines are less successful at identifying individuals with P/LP variants in moderate penetrance genes, such as *ATM* and *CHEK2* [11]. As a result of this, the increasing availability of targeted therapies and the decreasing cost of genetic testing over time, many high-income countries (HIC) have adjusted their policy guidelines to reduce the stringency of genetic testing criteria, with some calling for universal testing of women with BC [10, 12]. In low- and middle-income countries (LMIC) the successful implementation of genetic testing for hereditary cancer faces technological, health system, and patient-level barriers [13]. Clinical practice guidelines are necessary for ensuring accessible genetic testing and are recognised as a key need in some LMICs [14]. Although guidelines have been published in a few LMICs [15, 16] there is limited data available on their usefulness [13].

In 2018, the South African National Department of Health (NDOH) published guidelines to establish standards for BC prevention and control. This included eligibility criteria for genetic testing, which remain in effect [17]. These criteria were adapted from the widely used National Comprehensive Cancer Network (NCCN) guidelines from the USA namely, "Genetic/Familial High-Risk Assessment: Breast and Ovarian, version 2.2016", which have since been updated [18]. Criteria used in the NDOH and NCCN guidelines are summarised in Table 1 and show that the NDOH criteria were made more stringent by lowering the eligible age at diagnosis. Minimal local data was available to guide this process.

The suitability of these guidelines in our setting is uncertain for several reasons. The NCCN guidelines were developed using data from high-resource settings with predominantly European ancestry populations [19]. In contrast, South Africa is an upper middle income country with a majority Black African population [20]. Incidence rates of BC vary considerably in different countries and populations. In South Africa, the age standardised incidence rate per 100 000 of BC in 2022 was 94.6 in White females (similar to European populations), 47.5 in Mixed Ancestry females and 21.2 in Black African females [1, 21]. These differences raise uncertainty about whether similar genetic factors contribute to BC risk across the populations. In addition, the predictiveness of individual criteria is uncertain. For instance, one criterion is the presence of a breast tumour before the age of 60 that is estrogen and progesterone receptor negative with no overexpression of human epidermal growth factor receptor 2 (HER2), referred to as triple negative breast cancer (TNBC). Notably, populations with African ancestry may have a higher prevalence of TNBC [22, 23]. Although TNBC has been shown to be associated with *BRCA1* P/LP variants in Nigerian patients [5], African American women with TNBC are less likely to have a P/LP variant in *BRCA1* compared to women from other ancestry groups [24]. Consequently, the positive predictive value of

Table 1 A comparison of NDOH and NCCN v2.2016 criteria used to select women with breast cancer for genetic testing

Genetic testing criteria for women with breast cancer	NDOH[a] criteria [36]	NCCN[b] v2.2016 criteria [18]
Known P/LP variant in a cancer predisposition gene in the family	Yes	Yes
Age of breast cancer diagnosis	≤ 40 years	Early-age onset BC (suggested ≤ 50y)
Triple negative breast cancer[c] < 60 years	Yes	Yes
Two primary breast cancers at any age	Yes	Yes
≥ 1 close relatives with breast cancer < 50 years	Yes	Yes
≥ 1 close relative/s with invasive ovarian cancer	Yes	Yes
≥ 1 close relative/s with male BC	Yes	Yes
≥ 2 close relatives with breast/pancreatic cancer with at least one < 60 years old	Yes	Yes
From a population at increased risk	Absent	Yes

[a]NDOH – National Department of Health (South Africa)

[b]NCCN – National Comprehensive Cancer Network (USA)

[c]Triple negative breast cancer—cancers that are estrogen and progesterone hormone receptor negative and human epidermal growth factor receptor 2 (HER2) amplification negative

TNBC for identifying P/LP variants in African populations remains unclear. Furthermore, Black African women in South Africa have on average a younger age of diagnosis than White women, [25] which suggests that the age of BC diagnosis criterion may be less predictive of a P/LP variant in Black African women. Whether the NDOH guidelines are an effective tool for detecting P/LP variants in BC genes in South African women is unknown.

In South Africa, there is tension between optimising the detection rate of BC susceptibility genes and the cost of testing. The objectives of this study were to determine the P/LP variant prevalence in BC susceptibility genes in women meeting the NDOH guidelines in a South African setting and to compare the detection rates of individual criteria. In addition, we conducted multivariate analysis to assess the association of various demographic, clinical and tumour histological features with *BRCA1/2* detection rates.

Methods

Participants

Participants included females with invasive or in situ BC who attended genetic counselling and testing for hereditary BC between 1 January 2018 and 1 October 2022 at Tygerberg Academic Hospital (TAH) in the Western Cape, South Africa. Participants were included if they met the NDOH testing criteria as shown in Table 1.

Of the 424 women who had testing during this period, 26 individuals were excluded from this study because they did not meet NDOH criteria. Additionally, 13 participants were excluded because they tested negative for common founder variants in *BRCA1/2* but were not tested for the presence of other potential P/LP variants in *BRCA1/2*. There were 9 participants with leiomyosarcoma, ovarian or pancreatic cancer who were excluded, as although these cancers are associated with P/LP variants in BC susceptibility genes, they are not included in the NDOH criteria. The remaining 376 participants were included in the study to determine P/LP variant prevalence. Three participants with a known familial P/LP variant were excluded from the multivariate analyses, as this indication is associated with a substantially higher likelihood of having a P/LP variant compared to other NDOH criteria.

Data collection

Demographic, clinical, and test result data were collected for participants from TAH genetic counselling and histology records. Information on proband ancestry, required by the genetic testing laboratory to assist in variant interpretation, was inferred by the genetic counsellor in keeping with the population group categories of the South African National Census of 2022; White, Black African or Coloured. The latter descriptor is referred to as Mixed Ancestry in this study as the population has various ancestral roots, notably Khoe-San, European, Black African, and South and South-East Asian [26]. For all variables described in the study, data was available for > 97% of patients.

Genetic testing

During the study period, genetic testing for hereditary BC was done at Invitae Corporation [27] in the USA in 90.4% (340/376) of participants, with 12.8% (48/376) of participants tested locally at the National Health Laboratory Service (NHLS) [28]. Although participants had testing of between 2 and 102 genes, we included only the results for 13 genes (*ATM, BARD1, BRCA1, BRCA2, CDH1, CHEK2, NF1, PALB2, PTEN, RAD51C, RAD51D, STK11, TP53)* known to be associated with BC and having clinical guidelines for risk-reduction. Because not all probands had testing of the same genes, with the exception of *BRCA1/2*, denominators used to calculate the prevalence of P/LP variants differed. In addition, it meant we could not include the detection of P/LP variants in genes other than *BRCA1/2* in the multivariate analysis of NDOH criteria.

The Invitae gene panel protocol used next-generation sequencing (NGS) to detect copy number and single nucleotide variants in coding exons and 10–20 flanking base pairs for most genes [27, 29]. The Sherloc modifications of the American College of Genetics and Genomics and the Association for Molecular Pathology (ACMG-AMP) guidelines were used by Invitae for variant interpretation [30]. NHLS genetic testing was done using various methods: targeted analysis of 8 well-documented founder pathogenic variants in *BRCA1/2*; single nucleotide and copy number variant (CNV) detection within the exons and splice-site junctions of *BRCA1/2*; family variant testing; and a next generation sequencing (NGS) panel of 15 genes [31]. NHLS testing was conducted in a diagnostic laboratory accredited by the South African National Accreditation System (SANAS) [32], with variants interpreted using the ACMG-AMP variant interpretation guidelines [33].

Data analysis

Study data were stored in a RedCap Database [34] and statistical analysis was done using RStudio [35]. A P value < 0.05 was considered statistically significant. Chi-square or Fischer's exact test were used to find group differences when variables were categorical and the Kruskal–Wallis test when data was continuous. When significant differences were found, odds ratios, 95% confidence intervals and associated p values were calculated.

Logistic regression was used to examine the relationship between detecting a P/LP variant in *BRCA1/2* and demographic and clinical variables, as well as NDOH criteria. Initially, each variable was included in a univariate model to determine its predictive value (Table 2). Variables with $p < 0.2$ were included in the multivariate analysis. The absence of multicollinearity between the predictor variables was confirmed using variance inflation factors. As ancestry was comprised of three categories, the Mixed Ancestry group was used as the reference/index category with White and Black African groups compared to it. Results are reported as odds ratios (OR) with 95% confidence intervals (CI) and associated p-values.

Results

The prevalence of P/LP variants found in the 376 study participants was 19.9% (75/376), with 17.0% in high-penetrance (*BRCA1/2, PALB2, TP53*) and 2.9% in moderate penetrance (*ATM, CHEK2, RAD51C, RAD51D*) genes. 15.4% (58/376) of the participants had a P/LP variant in *BRCA1/2*, with more in *BRCA2* (35/376, 9.3%) than in *BRCA1* (23/375, 6.1%) (see Supplementary Table 1 for a list of P/LP variants detected).

To assess the NDOH criteria, we first compared the prevalence of P/LP variants for each criterion (Table 2). Of participants who satisfied only one of the NDOH criteria (either family history, triple negative breast cancer (TNBC), young age of cancer diagnosis or > 1 primary BC), 11.7% (31/265) had a P/LP variant in *BRCA1/2* and 5.1% (12/235) in one of the other BC susceptibility genes. Individually, each of the four criteria was associated with a similar prevalence of P/LP variants in *BRCA1/2* ($p = 0.88$) and other BC susceptibility genes ($p = 0.35$). However, participants who met two or three of the criteria were twice as likely to have a P/LP variant detected in *BRCA1/2* (OR 2.27, 95% CI 1.27–4.07, $p = 0.006$) than those meeting only one. This increased likelihood did not apply to other BC susceptibility genes, where meeting one or more criteria resulted in similar odds of having a P/LP variant (OR 1.02, 95% CI 0.35–2.98, $p = 0.82$).

Table 3 presents the univariate analysis of test indications, demographic data and clinical variables of 373 participants (three participants with known family variant were excluded) by their *BRCA1/2* P/LP variant status. Overall, age at diagnosis was the most frequently met NDOH test criterion (55.0%), followed by TNBC (35.4%) and family history (34.3%), with > 1 primary BC (9.4%) being the least frequent. We assessed the influence of each of these four

Table 2 Pathogenic/Likely Pathogenic variant detection rates by NDOH testing criteria

No. NDOH criteria met ($n = 376$)	Testing criteria	No. proband	Gene with P/LP variant detected[a]						Total detection rate in all genes[f]
			BRCA1/2	*ATM*	*CHEK2*	*PALB2*	*TP53*	*RAD51C*	
	Known variant in the family	3	2/3 (66.7%)						66.7%
One criterion	Family history	58	8/58 (13.8%)	4/56 (7.1%)	1/56 (1.8%)				22.9%
	TNBC[b] at < 60 years	73	8/73 (11.0%)					1/57 (1.8%)	12.7%
	Age of diagnosis ≤ 40	119	14/119 (11.8%)	2/109 (1.8%)	2/109 (1.8%)	1/110 (0.9%)			16.3%
	> 1 primary BC	15	1/15 (6.7%)			1/12 (8.3%)			15.0%
Two criteria[c]		90	19/89 (21.3%)			2/85 (2.4%)	2/85 (2.4%)		26.1%
Three criteria[c]		18	6/18 (33.3%)					1/15 (6.7%)	40.0%

[a]Denominators reflect the number of participants who had testing for the gene in question,

[b]TNBC refers to triple negative breast cancer which is estrogen and progesterone receptor negative and HER2 amplification negative,

[c]Indications include any combination of the following criteria: family history, triple negative breast cancer, young age of diagnosis and > 1 primary BC. Known variant in the family was not included in this analysis

[d]Comparison of *BRCA1/2* P/LP variant prevalence in participants meeting only one of the four criteria (family history, triple negative breast cancer, age at diagnosis, > 1 primary BC)

[e]Comparison of prevalence of P/LP variants in other BC genes in participants meeting one of the four criteria (family history, triple negative breast cancer, age at diagnosis, > 1 primary BC)

[f]Calculated by summing percentages in the preceding columns

Table 3 Demographic and clinical data for study participants by *BRCA1/2* status

		Participants ($n=373$)	*BRCA1/2* negative ($n=317$)	*BRCA1/2* positive ($n=56$)	*P* value
Demographic and clinical variables					
Ancestry/population group	Mixed Ancestry	226 (60.6%)	199/226 (88.1%)	27/226 (11.9%)	0.05*
	Black African	93 (24.9%)	74/93 (79.6%)	19/93 (20.4%)	
	White	54 (14.5%)	44/54 (81.5%)	10/54 (18.5%)	
Age at diagnosis	Median	39	39	38	0.45
	Range	18–76	18–76	24–73	
Breast cancer type[a]	Ductal carcinoma in situ	6/364 (1.6%)	5/6 (83.3%)	1/6 (16.7%)	0.36
	Invasive ductal carcinoma	323/364 (88.7%)	274/323 (84.8%)	49/323 (15.2%)	
	Invasive lobular carcinoma	14/364 (3.8%)	12/14 (85.7%)	2/14 (14.3%)	
	Other	21/364 (5.8%)	20/21 (95.2%)	1/21 (4.8%)	
Breast tumour receptors	Triple negative breast cancer	133/370 (35.9%)	113/133 (85.0%)	20/133 (15.0%)	0.94
	Other	237/370 (64.1%)	202/237 (85.2%)	35/237 (14.8%)	
Cancer stage[b]	in situ	6/368 (1.6%)	5/6 (83.3%)	1/6 (16.7%)	0.10*
	Stage 1	21/368 (5.7%)	18/21 (85.7%)	3/21 (14.3%)	
	Stage 2	127/368 (34.5%)	102/127 (80.3%)	25/127 (19.7%)	
	Stage 3	158/368 (42.9%)	140/158 (88.6%)	18/158 (11.4%)	
	Stage 4	53/368 (14.4%)	44/53 (83.0%)	9/53 (17.0%)	
	Recurrence	3/368 (0.8%)	3/3 (100%)	0	
NDOH indications					
Family history	Meets NDOH family history criterion	128/373 (34.3%)	103/128 (80.5%)	25/128 (19.5%)	0.09*
	Does not meet NDOH family history criterion	245/373 (65.7%)	214/245 (87.3%)	31/245 (12.7%)	
Triple negative breast cancer < 60 years	Triple negative breast cancer	132/373 (35.4%)	112/132 (84.8%)	20/132 (15.2%)	1.0
	Other	241/373 (64.6%)	205/241 (85.1%)	36/241 (14.9%)	
Age at diagnosis	≤ 40 years	205/373 (55.0%)	172/205 (83.90%)	33/205 (16.1%)	0.56
	> 40 years	168/373 (45.0%)	145/168 (86.3%)	23/168 (13.7%)	
> 1 primary BC	Yes	35/373 (9.4%)	26/35 (74.3%)	9/35 (25.7%)	0.08*
	No	338/373 (90.6%)	291/338 (86.1%)	47/338 (13.9%)	

*Variables with $p<0.2$ which were included in the multivariate analysis

[a]P value calculated by comparing those with invasive ductal carcinoma to other categories combined

[b]P value calculated by comparing in situ and stages 1/2 vs stages 3, 4 and recurrence

criteria, along with participant demographic and clinical variables, on the odds of detecting a P/LP variant in *BRCA1/2* using multivariate logistic regression. Based on the initial univariate analysis, we included ancestry, family history, > 1 primary BC and cancer stage at diagnosis in the logistic regression model (Table 4).

The model showed that family history and ancestry were independently associated with the presence of a P/LP variant in *BRCA1/2*. Participants with a family history were almost twice as likely to have a P/LP variant in *BRCA1/2* (OR 1.97; 95% CI 1.05–3.70, $p=0.03$), while participants of Black African ancestry had 2.58 (95% CI 1.28–5.18, $p<0.01$) times higher odds of having a P/LP variant than the reference group (those of Mixed Ancestry). From the regression analysis, we calculated that participant of Black African ancestry reporting a family history had a 5.01 times higher chance of having a P/LP variant in *BRCA1/2* compared to the reference (Mixed Ancestry participants without a family history).

Given that ancestry was a significant variable in the logistic regression analysis, we compared the frequency of the four testing indications by ancestry (Fig. 1). The indications TNBC ($p=0.77$) and > 1 primary BC ($p=0.08$) were found to occur at similar frequencies across the three ancestry groups. However, participants of Black African ancestry were significantly less likely to meet the NDOH family

Table 4 Logistic regression model predicting *BRCA1/2* P/LP variant from demographic, clinical and test indication data in study participants

Outcome: *BRCA1/2* P/LP variant	Regression coefficient	Std Error	Z value	*P* value	OR (95% CI)
Intercept	− 2.17	0.32	− 6.772	0*	0.11 (0.06–0.21)
Family history reported	0.68	0.32	2.13	0.033*	1.97 (1.05–3.70)
> 1 primary BC	0.82	0.43	1.88	0.060	2.26 (0.92–5.15)
Late cancer stage	− 0.45	0.30	− 1.48	0.138	0.64 (0.35–1.17)
Black African	0.95	0.35	2.67	0.008*	2.58 (1.28–5.18)
White	0.38	0.42	0.92	0.357	1.47 (0.62–3.25)

*Indicates significant values

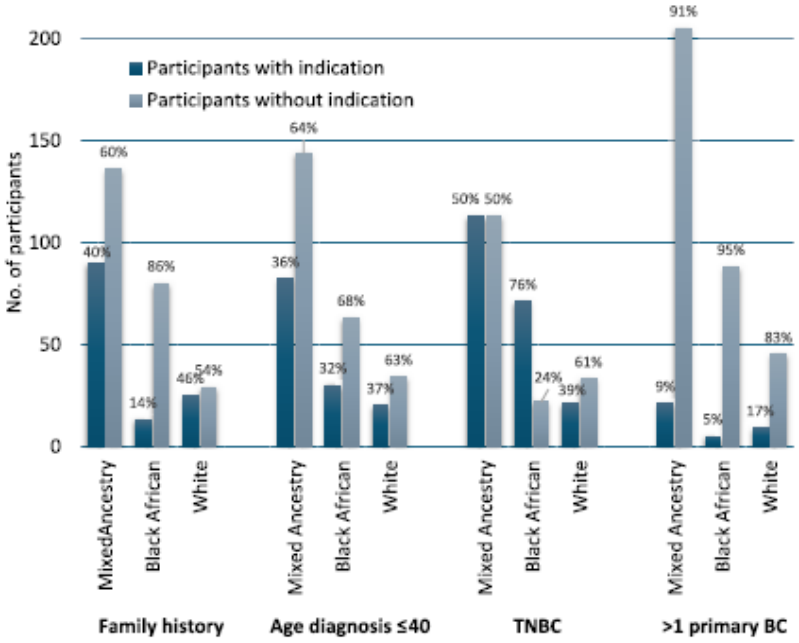

Fig. 1 Frequency of NDOH genetic testing criteria met in South African Mixed Ancestry, Black African and White ancestry groups. Testing criteria included family history, age of diagnosis ≤40 years, triple negative breast cancer (TNBC) and >1 primary breast cancer (BC)

history criterion ($p < 0.001$), and more likely to meet the age at diagnosis criterion ($p < 0.001$). Participants from all three ancestry groups were equally likely to meet one ($p = 0.62$), two ($p = 0.73$) or three test indications ($p = 0.34$).

Discussion

The existence of a guideline for genetic testing demonstrates that the South African Department of Health acknowledges the benefit of identifying women with P/LP variants in BC susceptibility genes [36]. Our finding that almost 20% of participants meeting the recommended criteria had a P/LP variant detected indicates a high detection rate and suggests that these guidelines are useful in our setting. The higher prevalence of P/LP variants in *BRCA1/2* found in our study (15.4%) compared to other studies (9.2% and 6.0%) which investigated prevalence in women meeting different versions of the NCCN guidelines, may be due to the more stringent criteria specified in the NDOH guideline [37, 38]. Although, expanding genetic testing to the entire BC population would be ideal to optimise detection, limited resources in the country make this unachievable [39]. These resources include not only genetic testing, but also genetic counselling and changes to clinical management for those testing positive. Therefore, we recommend prioritizing the expansion of genetic counselling and testing services and the development of specific guidelines tailored to local needs as an equitable way to increase identification of women with P/LP variants.

Family history of cancer, age at diagnosis, TNBC and > 1 primary BC on their own were associated with a similar prevalence of P/LP variants in *BRCA1/2*. However, as has

been demonstrated in other studies, participants with two or three indications were significantly more likely to have a P/LP variant in *BRCA1/2* detected [37, 38, 40]. This confirms that the criteria have a cumulative effect, each contributing to the likelihood of a P/LP variant, thereby making risk stratification possible. Although the NDOH guidelines were developed to detect *BRCA1/2* P/LP variants, clinically actionable variants in other BC susceptibility genes were found in an additional 5% of participants, supporting the utility of extending testing beyond *BRCA1/2* in our setting. However, the similar prevalence of P/LP variants among participants meeting one versus two or three of the criteria suggests that these criteria are ineffective at differentiating gradations of risk for variants in BC genes other than *BRCA1/2*. This finding is consistent with reports that the NCCN guidelines are less effective at identifying P/LP variants in moderate-risk BC susceptibility genes [11, 13, 41]. Further evaluation of the guidelines is needed to determine the proportion of all P/LP variants in women with BCs that are detected by applying the NDOH criteria.

We found that when controlling for other factors, reporting a family history and being of Black African ancestry were independently associated with having a P/LP variant in *BRCA1/2*. Family history is a well-established risk factor for a *BRCA1/2* P/LP variant. The reason proportionally more participants of Black African ancestry had a *BRCA1/2* P/LP variant is uncertain. One possibility is that *BRCA1/2*-related BC is more common in Black African women in South Africa, as found in case–control studies in West African and African American populations [4–6], although population studies have not been conducted locally. Alternatively, but not mutually exclusive, the proportion of *BRCA1/2*-related BC may be higher because environmental factors contribute less risk. This is compatible with South African registry data indicating that the prevalence of BC in the Black African population (lifetime risk 1 in 43) is lower than that of both Mixed Ancestry (1 in 18) and White (1 in 10) populations [42]. It is also consistent with evidence of increasing BC incidence in African countries, that has been attributed to "westernization" of lifestyles [43]. Population-based studies would be required to disaggregate these possibilities. A further possible cause of the disparity in *BRCA1/2* prevalence may be differences in the NDOH criteria met by Black African participants; they were less likely to meet the family history and far more likely to meet the age of diagnosis criterion. However, as age of diagnosis was not found to be associated with a P/LP variant any more strongly than the other criteria, but family history was, this seems an unlikely explanation.

The low proportion of participants of Black African ancestry meeting the family history criterion may be attributed to the above-mentioned lower incidence of BC or possibly to a lower penetrance of *BRCA1/2* P/LP variants, as was found in a case–control study in Ghana [4]. Additionally, health-related disparities, which disproportionately affect Black South Africans [44], may contribute to shorter life expectancy, reducing the likelihood of a cancer family history, given the positive association of cancer with age [45]. The disparity in reported family history may also be the result of a lower awareness of medical family history in Black African participants. This supposition is supported by findings from a study in the USA which showed that medically under-served groups (primarily African Americans and non-English speakers) had less knowledge of their family cancer history [46]. Likewise, a South African study found that 30% of their predominantly Black African participants with idiopathic dilated cardiomyopathy did not have knowledge of their family history. Reasons included having little recent contact with relatives living elsewhere, poor medical literacy and difficulty accessing relatives' death certificates [47]. The low frequency of family history being reported by Black African women requires exploration. However, our finding suggests that ancestry should be considered when offering genetic testing for hereditary breast cancer. We therefore recommend that in our setting Black African women with BC who have any family history of hereditary BC-associated cancers should be offered genetic testing even if they do not meet the defined NDOH criteria. In addition, awareness of the medical family history should be promoted.

Our assessment of the NDOH guidelines was limited by the relatively small numbers of P/LP variants in demographic sub-groups and in genes other than *BRCA1/2*. Consequently, we could not determine the predictive value of the different indications for each ancestry group or gene. The NDOH criteria themselves are premised on prevalence and penetrance of P/LP variants being relatively similar to those in Western populations. Our study adds to evidence from studies elsewhere in Africa that this may not be the case. It is important to note that we assessed a clinical setting in which various genetic tests were performed depending on availability, cost and other factors. Targeted mutation analysis alone was performed in a minority of cases, and 13 participants were excluded because this was negative. Also, in those receiving sequencing, the number of genes sequenced varied. The quoted prevalence of hereditary breast cancer (19.9%) is therefore an estimate, but if all 13 excluded cases were included but negative, this would give a lower bound of prevalence of 19.3% (75/389).

The high prevalence of P/LP variants detected in participants as well as evidence that P/LP variant risk can be stratified by the number of criteria met, provides evidence of the value of the NDOH guidelines in a South African public health service setting. The increased detection rate of *BRCA1/2* P/LP variants in women with either a relevant family history or Black African ancestry, and especially both together is important to guide genetic testing and thereby is

relevant to genetic counselling. Further research and possible refinement of the NDOH guidelines should be considered to address the fact that Black African women were less likely than other women to meet the family history criterion and more likely to meet the age of onset criterion.

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Data availability The datasets generated and analysed during the current study are not publicly available but are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors declare no competing interests.

Ethical approval This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Human Research Ethics Committees of Stellenbosch University (06/02/2023, N22/04/039) and the University of the Witwatersrand (26/01/2024, M231195).

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Breast Cancer Genetic Services in a South African Setting: Proband Testing, Cascading and Clinical Management

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Keywords: *BRCA1/2* | cancer prevention | cascade testing | genetic testing

ABSTRACT

Purpose: Limited data exist on managing hereditary breast cancer in low- middle-income countries (LMICs). We assessed proband and cascade genetic testing, and risk-reducing measures in a South African regional breast cancer service.

Methods: We analysed records from 534 consecutive female probands receiving genetic counselling for breast cancer and their 115 relatives who attended genetic counselling. Demographic and clinical data, family pedigrees and genetic test data were collated from hospital clinical records, regional laboratory data, screening appointments and radiological records.

Results: Test uptake in probands was high (86.9%), although cost was a deterrent in some. There were 83 (19.6%) probands who tested positive, and 45.0% of them had one or more family members have testing. This resulted in 9.2% of relatives (first- to third-degree) having cascade testing. Family testing was associated with a stronger family history of cancer, female gender and being a first-degree relative (uptake was 25.6% in female first-degree relatives). Risk-reducing mastectomy was accepted by 52.6% of eligible female family members, while mammographic surveillance (30%) and bilateral salpingo-oophorectomy (15.4%) were less frequent.

Conclusion: Genetic testing was well accepted by probands, but uptake was low in family members. Overall, one family member carrying a pathogenic variant was identified for every 13 probands receiving genetic counselling and for every 11 probands tested. Risk-reducing measures were taken up by over half of those eligible. Limited uptake of cascade testing and variable uptake of risk-reducing options impacted the programme. To our knowledge, this is the first study in Africa of the real-world effectiveness of a breast cancer genetic service.

1 | Introduction

Breast cancer (BC) is the commonest cancer in South African women, with prevalence estimates from the National Cancer Registry of 1 in 43 for Black African women, 1 in 18 for women of Mixed Ancestry, and 1 in 10 for White women [1]. Internationally, 5%–10% of unselected cases of BC are attributable to a pathogenic or likely pathogenic (P/LP) variant in a BC susceptibility gene [2, 3]. Identifying these individuals through genetic counselling and testing (GCT) allows the implementation of recommended treatment modifications and risk-reducing strategies [4].

Although GCT is the standard of care in high-income countries (HIC), it is not widely available in low-to-middle-income countries (LMIC) like South Africa [5]. Barriers to these services in LMIC include underfunded and fragmented health care services, as well as financial, geographical and logistical barriers [6, 7]. South Africa's healthcare system comprises a private sector, which primarily serves individuals with medical insurance, and a state-provided sector, on which approximately 80% of the population relies [8]. GCT services are available within the state healthcare system at tertiary facilities in only three of the country's nine provinces. Medical costs, including those for GCT, are subsidised in state facilities on an income-based sliding scale, ranging from full subsidies to none [9]. In South Africa, cancer genetic services are provided mainly by genetic counsellors - nationally there are 28 practicing genetic counsellors, the majority of whom work in private practice. This number is estimated to represent just 5% of the genetic counselling workforce needed to meet the country's demands. In addition to the workforce shortage, there is a significant disparity in geographic distribution, with most genetic counsellors concentrated in urban centres [10]. Qualitative studies have shown that while many South Africans are unfamiliar with genetic counselling, they generally respond positively to the process after experiencing it [11, 12].

Identifying individuals with a P/LP variant in a BC susceptibility gene enables the use of risk-reducing and surveillance measures for cancer recurrence and/or occurrence [4]. It is well established that risk-reducing mastectomy (RRM) reduces the risk of BC by at least 90% in asymptomatic *BRCA1/2* carriers, while bilateral salpingo-oophorectomy (BSO) significantly reduces morbidity and all-cause mortality [13–15]. In addition, the National Comprehensive Cancer Network (NCCN) guidelines developed in the USA recommend surveillance for BC with MRI and/or mammogram starting from between 25 and 30 years of age. Similar evidence-based guidelines are available for other BC susceptibility genes [4]. The uptake of risk-reducing strategies has been shown to vary widely between countries [16], but little data is available from LMIC.

When an individual is identified to have a P/LP variant in a cancer susceptibility gene, cascade testing, the process of extending GCT to family members, can proceed. Offit et al. [17] calculated that if 70% of first, second- and third-degree family members were to have testing, most individuals with a P/LP variant in the population would be identified within a decade. In practice, this has been difficult to realize even in HIC, with various barriers identified including contextual factors [18], poor family communication and/or relationships [19, 20], and others [21–23]. Currently, there is also little data on the uptake of cascade testing for hereditary cancer in LMIC, with two studies, both in research cohorts, showing divergent outcomes [24, 25].

Considering the dearth of data on GCT for inherited BC in South Africa and other LMICs, we aimed to assess its effectiveness in facilitating the pathway from proband to cascade genetic testing and risk-reducing measures among family members in a regional BC service.

2 | Methods

2.1 | Study Site

This study was conducted at Tygerberg Academic Hospital (TAH), a tertiary hospital that serves as the regional cancer genetic referral centre for ~3.5 million people, 60% living in metropolitan Cape Town and the remainder in towns and farms up to 300 km away (see Figure S1). Within the region and during the study period, most aspects of BC care were provided at TAH, although breast surgery or cancer surveillance was also available at three associated secondary hospitals. Care was coordinated by a multidisciplinary team that included the breast surgery, oncology and anatomical pathology services, as well as a genetic counsellor based at TAH. Pre-test genetic counselling was provided at TAH in person by a genetic counsellor or medical geneticist. Post-test counselling, including the discussion of management options for clinically relevant genetic variants, was provided in person or telephonically.

2.2 | Participants

Records were reviewed for female probands and their family members. Initial contact with the genetic service was almost always by a proband (a woman with invasive or in situ BC), following referral from the BC service. They were offered testing if the genetic health provider considered them eligible according to the South African National Department of Health (NDOH) guidelines [26]. Based on these guidelines, a woman with BC was eligible if she met any of the following criteria: diagnosis with BC at ≤ 40 years; two primary BCs or triple-negative BC at ≤ 60 years; or a family history of either ≥ 1 close relative with invasive ovarian cancer, male BC, or BC at ≤ 50 years, or ≥ 2 close relatives with breast or pancreatic cancer, with one diagnosed ≤ 60 years. Probands were excluded if they had genetic testing prior to attending genetic counselling. Probands identified to have a P/LP variant in a BC susceptibility gene were asked to invite family members to attend GCT.

2.3 | Study Period

Probands were included if they were seen for pre-test GCT between 1 January 2018 and 31 August 2022. This timeframe was chosen because it was the period for which next generation sequencing (NGS) gene panel testing was available through the global testing platform of Invitae Corporation (San Francisco, USA) [27]. Family members were included if they attended GCT

TABLE 1 | Genes assessed—number of probands tested and guidelines used to assess risk-reducing interventions.

Gene	Included on NHLS panel [30]	Included on invitae panel [27]	Number of probands tested[a]	NCCN guideline starting age recommendations for risk-reducing interventions		
				Mammo-gram[b]	RRM	BSO
ATM	Yes	Yes	372	40 years	No	No
BARD1	Yes	Yes	313	40 years	No	No
BRCA1	Yes	Yes	393	25 years	25 years	35 years
BRCA2	Yes	Yes	393	25 years	25 years	40 years
CDH1	—	Yes	313	30 years	30 years	No
CHEK2	Yes	Yes	372	40 years	No	No
NF1	—	Yes	321	30 years	No	No
PALB2	Yes	Yes	372	30 years	30 years	45 years
PTEN	—	Yes	372	30 years	30 years	No
RAD51C	—	Yes	321	40 years	No	45 years
RAD51D	—	Yes	321	40 years	No	45 years
STK11	—	Yes	343	30 years	30 years	No
TP53	Yes	Yes	372	20 years	20 years	No

Abbreviations: BSO, bilateral salpingo-oophorectomy; NCCN, National Comprehensive Cancer Network (USA); NHLS, National Health Laboratory Service (South Africa); RRM, risk-reducing mastectomy.
[a]Does not include probands who only had *BRCA1/2* founder variant ($n = 29$) or known family variant testing ($n = 2$).
[b]Due to lack of access to MRI at TAH Hospital, only mammogram surveillance was analyzed.

at TAH by 31 December 2022. To allow a minimum of a full year for implementation of risk-reducing measures, medical records of both probands and family members were reviewed until 31 December 2023.

2.4 | Genetic Testing

Of the probands tested, 86.8% had NGS gene panel testing through Invitae and 19.8% received testing through the South African National Health Laboratory Service (NHLS) (Table 3) [27, 28]. Testing by the NHLS was done using various methods: targeted analysis of 8 well-documented founder mutations in *BRCA1/2* (see Table S1); single nucleotide and copy number variant (CNV) detection within the exons and splice-site junctions of *BRCA1/2*; family variant testing; and an NGS panel of 15 genes. NHLS testing is conducted in an accredited diagnostic laboratory, with variants interpreted using standard guidelines [29–31].

The Invitae NGS gene panel protocol included assessment of single nucleotide variants and, for most genes, also copy number variants in the coding and flanking DNA, with variants interpreted using their Sherloc guideline [27, 32]. Family variant analysis performed by Invitae was available free of charge for 3 months via the TAH genetic counselling service to family members. By comparison, the cost of family variant analysis through the NHLS depended on patient eligibility for a financial subsidy; this also applied to both Invitae and NHLS proband tests.

In this study, we included only the results for 13 genes known to be associated with BC and having clinical guidelines for risk reduction (Table 1). Because not all probands had testing of the same genes, the denominators used to calculate the prevalence of P/LP variants differed.

2.5 | Data Sources and Collection

Demographic, clinical, genetic counselling and testing data were collected for probands, and more limited data for family members. Five electronic data sources were used: NHLS and Invitae online portals for laboratory data, the TAH Enterprise Content Management system (ECM) (the hospital electronic archive of scanned clinical notes) for clinical data, the Picture Archiving and Communication System (PACS) for imaging results, and Clinicom for clinic appointments. Both Clinicom and PACS provided information on appointments and imaging results not only for TAH but also for secondary hospitals in the region, enabling a comprehensive assessment of screening performed across the area. Two supplementary sources of clinical information were hard copy records held by the Genetic Counselling service and the BC service. The variables collected from each data source are summarised in Table 2.

Ethnicity was inferred by the genetic health professional and recorded on request forms, as required by testing laboratories, and categorised according to South African National Census groups: Black African, White, South African Coloured (Mixed Ancestry in this study) and Indian/Asian [34]. The Black African population is Bantu-speaking peoples indigenous to Southern Africa, accounting for 38.8% of the Western Cape (WC) population [35, 36]. White South Africans, accounting for 16.4% of the WC population, are

TABLE 2 | Types and sources of data collected for probands and their family members at Tygerberg Academic Hospital (TAH).

Data source	Access (coverage)	Data type	Collected for probands	Collected for family members
TAH Enterprise Content Management (ECM system) [33]	Electronic (TAH)	Demographic data	Date of birth, gender, ethnicity, distance from TAH, financial subsidy level	Date of birth, gender
		Clinical data	BC histology, BC stage, age at BC diagnosis, any surgeries done	History of previous cancers, notes on RRM and/or BSO
		Family pedigree data	Number of FDR, SDR and TDR with cancer, family member age, family member age at cancer diagnosis	Relationship to proband
TAH Genetic Counselling Clinic records	Hard copy (Departmental)	Demographic data Genetic test data Family pedigree data	To supplement data from electronic and online sources	To supplement data from electronic and online sources
TAH Breast Surgery departmental records[a]	Hard copy (Departmental)	Clinical data	BC stage, breast surgery done, notes on BSO (if done)	RRM and/or BSO (if done)
Invitae online portal	Internet	Genetic test data	Result of Invitae genetic testing, ethnicity	Invitae genetic test result
NHLS reporting system (Trakcare)[b]	Internet (national)	Clinical data (histology records)	BC histology, BC stage, age at BC diagnosis, surgeries done	RRM and/or BSO (if done)
		Genetic test data	Results of genetic testing done at NHLS	Results of genetic testing done at NHLS
Picture Archiving and Communication (PACS) System	Electronic (regional reports)	Radiological procedures	Mammogram, breast ultrasound records	Mammogram, breast ultrasound records
Clinicom	Electronic (regional)	Appointment data	Attendance at screening mammogram and/or breast ultrasound	Attendance at mammogram and/or breast ultrasound

Abbreviations: BC, breast cancer; BSO, bilateral salpingo-oophorectomy; FDR, first degree relative; NHLS, National Health Laboratory Service; RRM, risk reducing mastectomy; SDR, second degree relative; TDR, third degree relative.

[a]Available from January 2021.

[b]Laboratory reports from tests done in the state system countrywide.

TABLE 3 | Demographic and clinical data for study participants.

	Probands offered testing ($n=488$)	Tested probands ($n=424$)	Tested family members ($n=111$)
Age at testing			
Median	41	41	35
Range	18–76	18–76	3–66
Unknown	0	0	0
Gender			
Female	488	424	87 (78.4%)
Male	0	0	24 (21.6%)
Distance from hospital			
< 50 km	379 (77.7%)	322 (75.9%)	30 (27.0%)
50–100 km	57 (11.7%)	53 (12.5%)	2 (1.8%)
100–200 km	41 (8.4%)	39 (9.2%)	0
> 200 km	10 (2.0%)	9 (2.1%)	2 (1.8%)
Unknown	1 (0.2%)	1 (0.2%)	77 (69.4%)
Ethnicity/Population group			
Mixed Ancestry	254 (52.0%)	237 (55.9%)	38 (34.2%)
Black African	100 (20.5%)	91 (21.5%)	25 (22.5%)
White	70 (14.3%)	61 (14.4%)	24 (21.6%)
Unknown	64 (13.1%)	35 (8.3%)	24 (21.6%)
Financial subsidy[a] for GCT			
Full subsidy of consult and testing (H0)	382 (78.3%)	368 (86.8%)	21 (18.9%)
Subsidised fee covering consult and testing (H1)	76 (15.6%)	41 (9.7%)	2 (1.8%)
Subsidised consult fee and 20% of test cost (H2)	11 (2.3%)	5 (1.2%)	1 (0.9%)
No subsidy–pay consult and test cost (H3 or Pvt)	18 (3.7%)	9 (2.1%)	5 (4.5%)
Unknown	1 (0.2%)	1 (0.2%)	82 (73.9%)
Breast cancer diagnosis			
Yes	488 (100%)	424 (100%)	7 (6.3%)
No	0	0	102 (91.9%)
Unknown	0	0	2 (1.8%)
Breast cancer histology			
DCIS[c]	7 (1.4%)	6 (1.4%)	
Invasive ductal	418 (85.7%)	387 (91.3%)	
Invasive lobular	17 (3.5%)	15 (3.5%)	
Unknown	46 (9.4%)	16 (3.8%)	7[c]
Cancer stage[b]			
In situ	7 (1.4%)	6 (1.4%)	

(Continues)

TABLE 3 | (Continued)

	Probands offered testing ($n=488$)	Tested probands ($n=424$)	Tested family members ($n=111$)
Stage 1	28 (5.7%)	24 (5.7%)	
Stage 2	158 (32.4%)	141 (33.3%)	
Stage 3	194 (39.8%)	178 (42.0%)	
Stage 4	70 (14.3%)	61 (14.4%)	
Recurrence	5 (1.0%)	4 (0.9%)	
Unknown	26 (5.3%)	10 (2.4%)	7
Genetic tests done[c]			
8 founder variants[d] (*BRCA1/2*)		50 (11.8%)	
BRCA1/2 sequencing		22 (5.2%)	
NHLS panel test		8 (1.9%)	
Invitae panel test		368 (86.8%)	
Family variant testing		4 (0.9%)	111 (100%)

Abbreviations: DCIS, ductal carcinoma in situ; GCT, genetic counselling and testing.
[a]Subsidy levels are determined by the Department of Health based on income [9].
[b]Clinical breast cancer stage was determined by the tumour-node-metastasis (TNM) system, as indicated or inferred from the clinical notes.
[c]The number of tests done (452) exceeds the number of tested probands (424), because >1 test was done in some cases.
[d]South African founder variants are listed in the Table S1.
[e]7 family members had breast cancer prior to testing.

predominantly descendants of European settlers [36, 37]. People of Mixed Ancestry have genetically admixed ancestry, including Khoisan, Black African, European and Asian population roots [35, 38]. They form the largest population group in the WC, accounting for 42.1%. The Indian/Asian population represents a small minority (1.1%) of the WC population [36].

The financial subsidy provided to individuals for state healthcare, including genetic testing, is determined through a means-tested system [9]. Patients with very limited financial means receive almost full subsidies for the direct costs of healthcare, including genetic testing and cancer management, while those with greater financial means receive only partial subsidies or none at all. There are four levels of financial subsidy (H0, H1, H2, H3), as detailed in Table 3. However, due to the smaller number of patients in the H1, H2 and H3 categories, we combined H0 with H1 and H2 with H3 for the purposes of comparison.

Probands and family members were considered eligible for interventions based on the NCCN guidelines (Table 1). Surgeries such as mastectomies and BSOs performed in the state healthcare system should result in both a clinical note and a NHLS histology report. Mammogram and breast ultrasound screening required an electronic appointment on the Clinicom system and were reported in the Picture Archiving and Communication System (PACS) database. Therefore, for both surgical interventions and screening procedures, two sources of evidence were assessed. In addition, screening was reviewed for all probands and family members, except in cases where an RRM had already been performed. This is because RRM is routinely preceded by breast screening, after which patients are discharged. Chemoprevention is not available for cancer-unaffected women in the South African state system and was therefore not assessed.

2.6 | Data Analysis

All statistical analysis was done using RStudio version 2024.4.0 [39]. and $p<0.05$ was considered statistically significant. Univariate logistic regression was used to compare the distribution of both categorical and continuous variables for the uptake of genetic and family member testing. Factors with $p<0.2$ were then included in a multivariate logistic regression analysis for uptake of genetic testing in probands. Highly correlated factors, determined using variance inflation factors, were dropped from the model. As only one variable was found to have $p<0.2$ when factors impacting test uptake in family members were examined, multivariate analysis was not required. Results are reported as odds ratios (OR) with 95% confidence intervals (CI) and associated *p*-values.

Family pedigrees were used to determine the number of family members eligible for cascade testing. Family members were not considered eligible if <18 years unless the familial P/LP variant was associated with childhood cancers (i.e., *TP53*), or if unrelated to the side of the family with the P/LP variant.

3 | Results

3.1 | Proband Uptake of Genetic Counselling and Testing

In the study period, 534 women with a previous or current diagnosis of BC (probands) were seen for pre-test genetic counselling, of whom 488 (91.4%) were offered genetic testing, and 86.9% (424/488) accepted (Figure 1). Demographic and clinical data for all study participants are shown in Table 3.

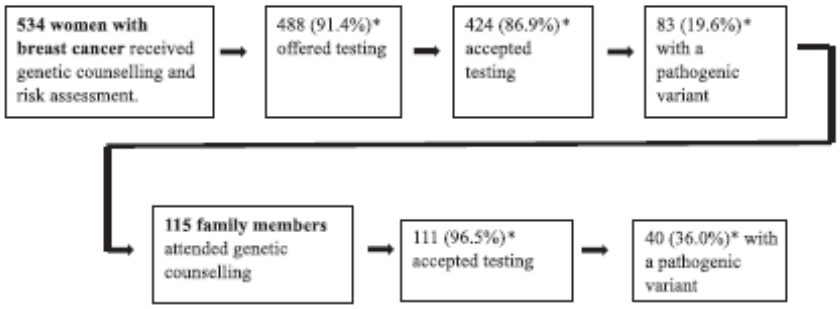

FIGURE 1 | Flow chart of the genetic counseling and testing process for hereditary breast cancer in probands and family members. *Percentages in rows are as a proportion of the previous block.

TABLE 4 | Logistic regression showing factors impacting acceptance of genetic testing.

Outcome: uptake of testing	Regression coefficient	Std. error	*Z*	*p*	Odds ratio (95% CI)
Intercept	1.88	0.82	2.28	0.02	6.56 (1.33–34.02)
Age at diagnosis	−0.04	0.01	−2.63	0.01	0.96 (0.94–0.99)
Dist. to hospital	−0.59	0.43	−1.36	0.17	0.56 (0.22–1.22)
Family history	0.94	0.37	2.54	0.01	2.57 (1.28–5.55)
Financial subsidy	2.21	0.44	5.07	0.00	9.14 (3.88–21.83)

Abbreviation: CI, confidence interval.

Age at diagnosis ($p<0.01$), the presence of a cancer family history meeting NDOH testing criteria ($p=0.05$), living >50 km from the hospital ($p=0.02$) and subsidisation category (H0 and H1 vs. others) ($p<0.01$) were included in the multivariate logistic regression analysis. Cancer stage (Stages 0, 1, 2 vs. Stages 3, 4 and recurrence) ($p=0.96$) and age at consultation ($p=0.02$) were excluded from the model due to a lack of significance or collinearity with other variables. Testing uptake was found to be associated with younger age at diagnosis, the presence of a family history, and a higher subsidisation level of test costs (Table 4). The impact of test cost on uptake was supported by the finding that 93.3% (42/45) of probands who gave a reason for declining testing named financial concerns.

3.2 | Detection of Pathogenic Variants in Probands

A heterozygous P/LP variant was detected in 19.6% (83/424) of tested probands, with variants in *BRCA1/2* accounting for 78.3% (65/83) (Table S2). The prevalence of P/LP variants in *BRCA1* and *BRCA2* was 5.9% (25/424) and 9.4% (40/424) respectively, with fewer founder variants in *BRCA1* than *BRCA2* (6/424 vs. 32/424, $n=424$, $p<0.001$). The detection rate of P/LP variants in other high-risk susceptibility genes tested was: *PALB2* (4/373, 1.1%), *TP53* (2/373, 0.5%); and in moderate-risk susceptibility genes: *ATM* (7/372, 1.9%), *CHEK2* (3/372, 0.8%), *RAD51C* (1/321, 0.3%) and *RAD51D* (1/321, 0.3%).

3.3 | Family Member Genetic Counselling and Testing

Of the 83 probands with a P/LP variant, result feedback was documented in all but one case. Almost half of the probands (45.8% [39/83]) had at least 1 family member receive genetic testing (median: 2, range: 1–11). Only 3.5% (4/115) of family members who attended genetic counselling declined testing. The median time between the first proband consultation and family member consultation was 3.3 months (range: 0.4–20.8 months). Probands reporting more family members diagnosed with cancers (including melanoma, breast, ovarian, prostate, or pancreatic) were more likely to have family members test (OR 1.73, 95% CI 1.16–2.57, $p<0.01$). No relationship was found between having a family member attend and the age of the proband's cancer diagnosis ($p=0.26$), the proband's cancer stage ($p=0.91$), whether the proband lived <50 km from the hospital ($p=0.47$), the number of eligible family members ($p=0.55$), whether the test provider offered free family variant testing ($p=0.31$), or the proband's financial subsidy category ($p=0.75$).

Using the family pedigrees collected at the time of consultation, there were 1202 first-, second- and third-degree relatives eligible for testing (Table 5), of whom 111 (9.2%) were tested. Female family members (OR: 2.0, 95% CI: 1.16–3.43, $p=0.01$) and first-degree relatives (OR: 17.1, 95% CI: 5.27–60.48, $p<0.001$) were significantly more likely to have testing, with 25.4% of female first-degree relatives receiving cascade testing.

The overall detection rate for P/LP variants in tested family members was 36.0% (40/111). Of the 40 family members found to carry a P/LP variant, 31 were female and 9 were male, with median ages of 43 (range 20–66) and 35 (range 20–56) years, respectively. P/LP variants were found in the following genes for males: *BRCA1* (6), *BRCA2* (3); and in females: *ATM* (3), *BRCA1* (11), *BRCA2* (14), *PALB2* (3). There was evidence of result feedback in all family members. The identification of test-positive probands and cascade testing of their family members allowed

TABLE 5 | Detection of P/LP variants in eligible family members.

Degree of relationship to proband	Gender	Number of eligible family members	Family members tested (as % of eligible members)	Family members with P/LP variant as proportion of tested (%)
First-degree	F	228	58 (25.4%)	24/58 (41.4%)
	M	180	24 (13.3%)	9/24 (37.5%)
	Total	408	82 (20.1%)	33/82 (40.2%)
Second-degree	F	308	24 (7.8%)	6/24 (25%)
	M	271	0	0
	Total	579	24 (4.1%)	6/24 (25%)
Third-degree	F	125	5 (4.0%)	1/5 (20%)
	M	90	0	0
	Total	215	5 (2.3%)	1/5 (20%)
All	F	661	87 (13.2%)	31/87 (35.6%)
	M	541	24 (4.4%)	9/24 (37.5%)
	Total	1202	111 (9.2%)	40/111 (36.0%)

detection of one test-positive family member for every 11 probands tested (40/424).

3.4 | Uptake of Risk-Reducing Strategies

Most probands with a P/LP variant (64/81, 79.0%) had breast surgery as part of their treatment for BC. Reasons BC surgery was not performed included advanced cancer stage, inoperability, loss to follow-up, or the proband declining the procedure. Among the probands who underwent BC surgery, 82.8% (53/64) also had surgery performed on the contralateral breast. However, the reason for surgery frequently was undocumented. Of the 50 probands who were eligible for BSO, considering guidelines for the relevant gene, their age and prognosis, 17 (34.0%) had it performed, and 8 (16.0%) said they would consider having it done in the future, although they were already at the recommended age for the procedure.

Of the tested family members, seven had a prior diagnosis of BC, six of whom (85.7%) tested positive for a P/LP variant. As they had previously had surgical treatment, the uptake of RRM was not assessed. However, 4/6 (66.7%) were eligible for risk-reducing BSO considering their age and the applicable gene guidelines, and two proceeded.

Table 6 outlines risk-reducing measures undertaken by cancer-unaffected family members who tested positive for a P/LP variant. As a proportion of those eligible for each intervention, more had RRM (52.6%; 10/19) than mammographic surveillance (30.0%; 3/10) or BSO (15.4%; 2/13). Of those eligible for breast surveillance and/or RRM, 13/20 (65.0%) had one of these interventions. Only one eligible family member had both RRM and BSO procedures done.

4 | Discussion

In our assessment of the GCT and management path for hereditary BC in a LMIC, we found a high uptake of genetic testing in women with BC, but test cost was a significant factor in determining uptake. Testing in eligible family members was most strongly associated with female gender, being a first-degree relative, and having multiple family members affected by cancer. Although the uptake of cascade testing was low overall, more than half of eligible test-positive relatives proceeded with RRM. BSO and mammographic surveillance were less frequently used.

4.1 | Genetic Counselling and Testing of Probands

The high uptake (86.9%) of genetic testing suggests that it is an acceptable option for most women with BC but may be unaffordable for some. This was indicated by the finding that probands with higher subsidisation of healthcare costs were nine times more likely to test. This probably relates to the fact that all the available genetic tests were expensive: approximately USD125 for founder mutation testing and over USD300 for sequencing of the *BRCA1/2* genes or broader gene panels. The individuals without subsidy, typically referred to as the 'missing-middle' have sufficient household income to disqualify them from assistance but lack the financial resources to pay for medical insurance for genetic testing. This issue is not unique to South Africa. A scoping review of 24 studies on BC testing, all conducted in the USA, found that women without private insurance were not only less likely to be referred for testing but, when referred, were also less likely to proceed with it [40]. This likely results in a substantial segment of the population unable to access testing and therefore ineligible for cancer risk-reducing measures. Family history was also important as probands with a family history of

TABLE 6 | Risk-reducing measures used by female family members without breast cancer.

Risk-reducing measure	No. eligible for intervention ($n = 25$)[a]	Outcome	No. of participants	Median age and range
Breast surveillance in non-RRM participants by mammogram and/or ultrasound	10	Performed at least once	3 (30.0%)	37 (36–66)
		Participant reported no breast surveillance done	3 (30.0%)	37 (35–43)
		No record of surveillance[b]	4 (40.0%)	52 (34–63)
Risk-reducing mastectomy (RRM)	19	RRM	10 (52.6%)	43.5 (33–56)
		Not done	7 (36.8%)	37 (34–66)
		No record of surgery or associated histology[b]	2 (10.5%)	50 (37–63)
Bilateral salpingo-oophorectomy (BSO)	13	BSO	2 (15.4%)	51.5 (51–52)
		BSO not done	6 (46.2%)	47.5 (35–66)
		No record of surgery or associated histology[b]	5 (38.5%)	43 (36–63)

[a]Indication for the risk-reducing measure was determined based on gene guidelines and participant age according to NCCN guidelines (Table 1).
[b]When no record could be found for surgery or associated histology, it is highly likely that the procedure was not done in the state system, as electronic health records were thoroughly searched for each participant.

BRCA1/2 related cancers were found to be more than twice as likely to proceed with testing.

The high detection rate of P/LP variants (19.6%) in probands likely reflects the relatively strict criteria for offering genetic testing to women with BC in South Africa, as we have discussed elsewhere [41].

4.2 | Cascade Testing

Uptake of cascade testing in our study was generally low, and it dropped steeply from 20.1% in first-degree relatives to 4.1% and 2.3% in second- and third-degree relatives, respectively. The uptake of cascade testing in our study was much lower than reported by a recent meta-analysis of 87 studies, where the uptake of cascade testing was 41% in eligible first- and second-degree relatives [42]. However, studies included in the meta-analysis were mainly from HIC and often nested in research environments, settings in which better uptake is expected [7].

There are very few publications from LMIC on the uptake of cascade testing for hereditary BC, or hereditary cancer more broadly. Only two studies in the above-mentioned meta-analysis were conducted in LMIC as defined by the World Bank (2022)—one in Malaysia on hereditary BC [25] and one in South Africa on Lynch syndrome [24]. The former reported a low uptake of cascade testing, with 78% of probands informing their families but only 11% of relatives coming forward, despite the availability of free testing within a research setting [25]. The authors noted that families expressed concerns regarding 'cultural taboos about cancer diagnoses, social marginalization and lack of regulatory control of genetic discrimination'. Our study showed a similarly low uptake, but the reasons for this require further exploration. The study on Lynch syndrome showed a contrastingly high uptake of cascade testing, with 97% of siblings and 73.6% of children accepting predictive testing [24]. Like our study, it was set in South Africa, but it described a bespoke research-based outreach programme to rural areas with high rates of Lynch syndrome. Our study assessed a clinical practice environment covering a healthcare region and therefore appears more representative of the medical mainstream.

The predominance of testing by female family members (77.0%) aligns with findings from a meta-analysis, which reported that female relatives were almost twice as likely to undergo testing compared to males [42]. This trend is likely attributable to their elevated risk for associated cancers and a greater propensity to utilize health services [43]. Similarly, the meta-analysis, consistent with our findings, demonstrated that first-degree relatives were significantly more likely to undergo testing than second-degree relatives [42]. This may be due to their closer relationship with the proband, which places them at higher risk and facilitates better communication [44]. Higher test uptake was associated with a family history of cancer in both probands and family members. Although not frequently reported in the literature, this finding is intuitive, as individuals from families with significant cancer histories often experience heightened cancer-related anxiety, which increases compliance with testing and screening [45].

Close to half of test-positive probands had at least one family member attend genetic counselling, but the number of family members attending varied widely. The reasons for this require further assessment in our setting. Many specific family and contextual factors have also been reported to influence the uptake of testing in at-risk family members in Western and Asian countries. Described family factors include family support, communication and dynamics, while contextual factors include access to and cost of healthcare services [18]. Of the contextual factors, low income [20], lack of health knowledge [23] and difficulty accessing genetic services [18] are barriers that plausibly impacted many family members in our study.

4.3 | The Flow From Proband Genetic Counselling to Family Member Testing

The model developed by Offit et al. [17] demonstrated that if all individuals with BC had testing, and there was a 50% to 70% uptake among first-, second- and third-degree relatives of those identified with a P/LP variant, the majority of carriers in the population could be detected. This is far removed from our study, where (1) we could offer genetic testing only to probands at high risk of a hereditary cancer and (2) cascade testing uptake, even in those at highest risk (female first-degree relatives) was only 25.7%. In our setting, public health-level, as opposed to individual-level, impact from cascade testing will be difficult to achieve.

Instead, we provide real-world data on the drop-offs at each stage of GCT in probands and the detection of carrier status in their relatives. The high uptake and detection rate in probands, together with cascade testing uptake that was low but mainly among first-degree relatives, resulted in the detection of 1 carrier relative being detected for every 13 probands receiving genetic counselling and for every 11 probands receiving genetic testing. This information will facilitate future assessment of the cost–benefit of the GCT programme.

4.4 | Uptake of Risk-Reducing Measures

Uptake of mammographic surveillance in family members (30%) was lower than that of RRM, and far lower than found by Metcalfe et al. [16], where uptake was above 96% in all countries offering mammographic surveillance. In contrast, the uptake of RRM by 52.6% of eligible family members with a P/LP variant is above the upper end of the range (4.5%–45%) observed by Metcalfe et al. (2019) across 10 mostly HIC [16]. The impact of RRM on BC-specific and overall mortality compared to recommended screening is debated in HIC [46]. However, in our setting, where MRI is not widely available and access to advanced cancer therapies is limited, the higher uptake of RRM may be appropriate [47–49].

In contrast to RRM, the uptake of BSO (15.4%) in eligible women without BC was considerably lower than that found in other studies, which had uptake rates varying from 36.7% to 83.3% [16, 50, 51]. Likewise, the uptake of BSO in probands and family members with BC (35.2%), although higher than in unaffected family members, was half that found in women with BC by Metcalfe et al. [16] (70.7%). It is not clear if this relates to a lack of access or awareness or other reasons. The finding of Makhnoon et al. [50] that higher parity was associated with BSO uptake independent of age suggests that a desire to maintain fertility may be a factor. The low uptake found in our study is concerning as BSO may be more effective in preventing morbidity and mortality in *BRCA1/2* and *PALB2* carriers than RRM [13].

In probands, the available information on risk-reducing measures was more difficult to interpret than in family members. Contralateral breast surgery in a person with unilateral BC does not necessarily imply that it was done for purposes of risk reduction because of the common practice to offer contralateral breast reduction for cosmetic reasons. The reason for contralateral surgery was most often undocumented, and therefore we could make no inferences from the fact that over 80% of breast surgeries were bilateral. BSO is more likely to be performed to reduce the risk of future cancer, and half of eligible probands either had BSO performed or indicated they would do it in thefuture.

4.5 | Strengths and Limitations

There were several limitations in this study. Firstly, we relied on clinical records whose completeness was dependent on the consulting clinicians. This was evident in the pedigree data used where a higher number of female family members and first-degree relatives were counted as being eligible for genetic testing, suggesting that males and more distant relatives were undercounted. However, routine clinical and demographic data was nearly complete, indicating good data quality. This was expected because each electronic data source had proven reliable in clinical practice, and most data points were covered by two sources if required.

The uptake of cascade testing found is likely the 'best-case scenario' because Invitae cascade testing was available in the country at the time, which provided free family member testing if done within a 3-month period and therefore encouraged expeditious decision-making about family member testing. Conversely, the uptake of risk-reducing measures likely represents a minimum estimate. While we expect to have identified most participants who underwent mastectomies and BSOs in the regional state healthcare system given the multiple data sources that we used, we may have missed some who had interventions in the private sector or elsewhere. In addition, the COVID pandemic may have negatively affected uptake; this was not directly assessed, but there was, however, no change in the frequency of cascade testing per year.

A comprehensive assessment of both cascade testing and uptake of risk-reducing measures would require longer prospective follow-up, as decisions may take time and eligibility for interventions is age-dependent. Lastly, the small number of family members detected with P/LP variants meant we did not have sufficient power to investigate factors associated with the uptake of risk-reducing measures.

5 | Conclusion

To our knowledge, this is the first assessment of a BC genetic service from detection through to cascade testing and uptake of risk-reducing strategies in an LMIC country, and it provides a useful framework for further investigations. The high proband test uptake and detection rate suggests that testing is acceptable and relevant. However, limited uptake of both cascade testing and some preventive interventions (BSO and mammography) reduced the effectiveness of the programme. Reasons for the low uptake of cascade testing and variable uptake of risk-reducing measures should be explored. Comparative data from within and beyond South Africa and a cost–benefit analysis will help guide policy development in our and other LMIC settings.

Author Contributions

T. S. Osler: conceptualization (equal), data curation (equal), formal analysis (lead), investigation (lead), methodology (equal), validation (lead), visualization (lead), writing – original draft (lead), writing – review and editing (lead). **M. Schoeman:** funding acquisition (supporting), investigation (lead), writing – review and editing (equal). **J. Edge:** data curation (supporting), investigation (supporting), writing – review and editing (supporting). **W. J. S. Pretorius:** data curation (equal), investigation (equal), project administration (supporting), writing – review and editing (supporting). **F. H. Rabe:** data curation (supporting), writing – review and editing (supporting). **C. G. Mathew:** funding acquisition (equal), supervision (equal), writing – review and editing (equal). **M. F. Urban:** conceptualization (lead), funding acquisition (lead), methodology (lead), project administration (lead), supervision (lead), validation (equal), visualization (equal), writing – review and editing (lead).

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Ethics Statement

The study was approved by the Human Research Ethics Committees of Stellenbosch University (N22/04/039) and the University of the Witwatersrand (M231195). As the study was a retrospective record review, participant consent was not required. All data were de-identified. The study adhered to the principles of the Declaration of Helsinki.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The de-identified participant data used and analyzed in this study are available from the corresponding author on request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.

9.4 APPENDIX D: PLAGIARISM DECLARATION AND TURNITIN REPORT

PLAGIARISM DECLARATION TO BE SIGNED BY ALL HIGHER DEGREE STUDENTS

SENATE PLAGIARISM POLICY: APPENDIX ONE

I Tabitha Osler (Student number: 9807768f) am a student registered for the degree of PhD in the academic year 2024.

I hereby declare the following:

- I am aware that plagiarism (the use of someone else's work without their permission and/or without acknowledging the original source) is wrong.
- I confirm that the work submitted for assessment for the above degree is my own unaided work except where I have explicitly indicated otherwise.
- I have followed the required conventions in referencing the thoughts and ideas of others.
- I understand that the University of the Witwatersrand may take disciplinary action against me if there is a belief that this is not my own unaided work or that I have failed to acknowledge the source of the ideas or words in my writing.
- I have included as an appendix a report from "Turnitin" (or other approved plagiarism detection) software indicating the level of plagiarism in my research document.

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