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

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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 alleles	37	4–8×	WGS (VCF)	39
Awi-Gen SA [39]	South Africa (100)	200 alleles	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 alleles[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.

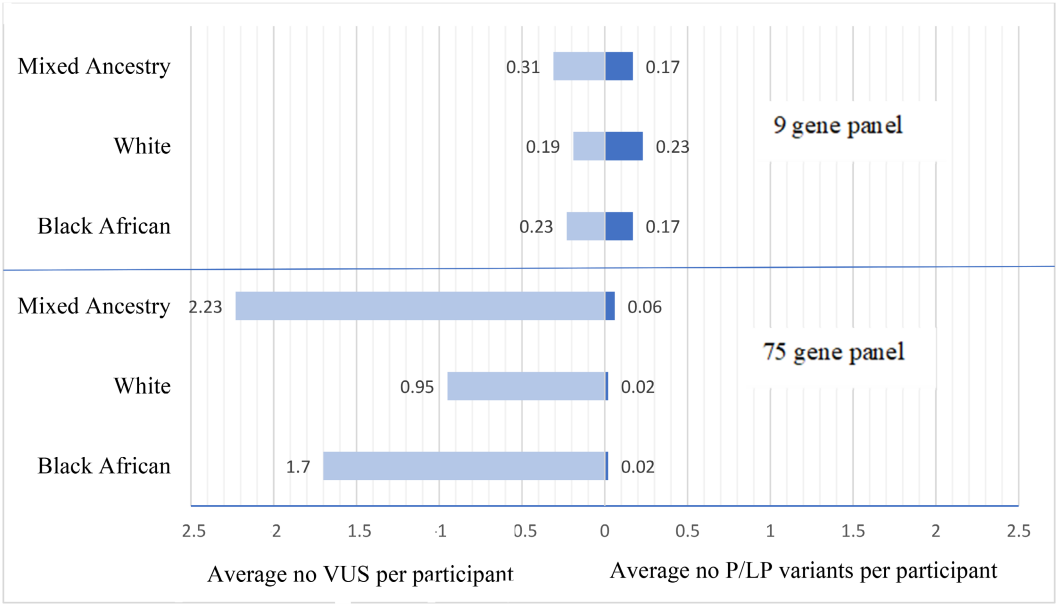

FIGURE 1 | Comparison of the average number P/LP variants and VUS after initial 9 gene and then further 75 gene testing.

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

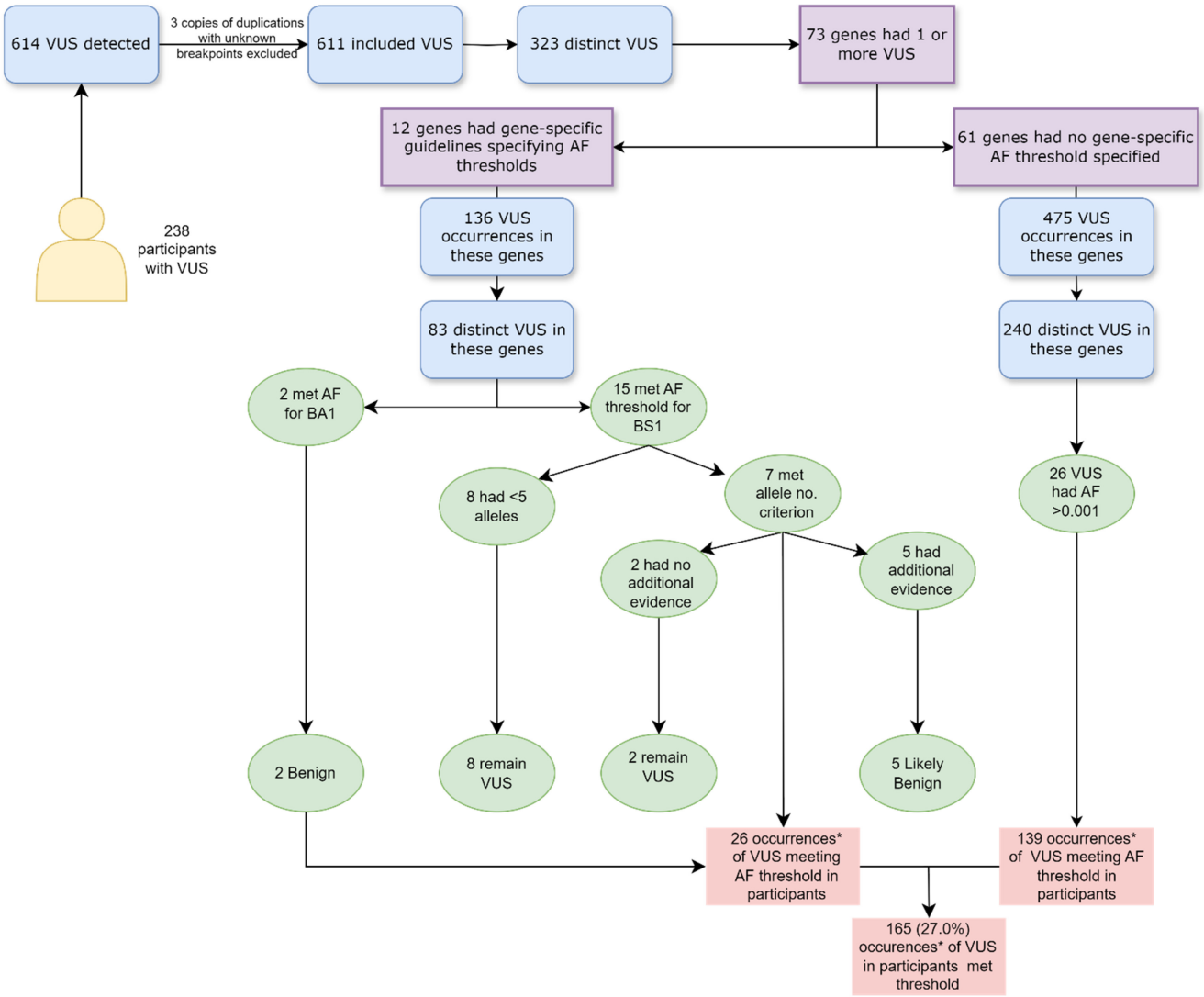

FIGURE 2 | Reclassification of VUS using allele frequencies from African genomic data *Some of the VUS occurred in multiple individuals (occurrences).

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.-30 582T > 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.