

CLINICAL UPDATE

Genetic testing approaches for hereditary breast cancer: Perspectives from a private diagnostic laboratory

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Breast cancer is highly prevalent in South Africa, and up to 10% of breast cancer cases may be hereditary. The landscape of genetic testing options for hereditary breast cancer (HBC) has changed significantly over the past decade, and healthcare providers are faced with multiple options when referring breast cancer patients for genetic testing. We have performed a retrospective study of 3 years' worth of breast cancer genetic testing referrals to our laboratory. While Afrikaner and Ashkenazi Jewish founder screens may be appropriate as first-line tests in a limited subset of patients, we have shown that in the majority of cases it is more effective to adopt a multigene panel approach. While variants in the *BRCA1* and *BRCA2* genes still account for a significant proportion of cases, close to 40% of pathogenic variants were found in genes other than *BRCA1* or *BRCA2*. There are many factors that healthcare providers should consider when requesting genetic testing for breast cancer patients and families, including family history, ancestral background, cost, medical aid scheme reimbursement and scope of testing. We summarise our findings and provide advantages and disadvantages of each approach, with the aim of assisting clinicians and genetic counsellors to make appropriate testing decisions.

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Breast cancer is the most common malignancy in South African (SA) women, accounting for ~22% of local cancer cases,^[1] and 5 - 10% of these cases are considered to be hereditary.^[2] Historically, hereditary breast cancer (HBC) was associated primarily with pathogenic variants in the *BRCA1* and *BRCA2* genes, but in the post-genomic era there is increasing evidence of the contributions of other genes to inherited breast cancer risk.^[3,4] This means that clinicians and genetic counsellors are faced with an ever-broadening menu of genetic testing options for patients. Our colleagues have published on strategies for patients in the public healthcare sector,^[5-7] but no data are available on the detection rates of the various testing options available to SA patients in the private sector. Information regarding the detection rates of these tests may assist managing healthcare providers to select the most appropriate and economical approach to genetic testing for individuals and families with HBC.

Indications for genetic testing

Various international groups have proposed guidelines and criteria for identifying candidates for genetic testing.^[8-10] However, other groups have raised concerns about these criteria being too stringent, resulting in more than half of patients harbouring disease-causing variants being missed.^[11] In line with these findings, the American Society of Breast Surgeons recommends that genetic testing be made available to all patients with a personal history of breast cancer,^[12] although this approach in patients diagnosed at >65 years without a significant family history of relevant cancers has a very low probability (<2%) of yielding results with clinical utility.^[3,8]

Currently no national SA consensus guidelines exist for genetic testing for persons at risk of HBC. Individual academic units and genetic practitioners have adapted international guidelines^[8-10] to our local context, taking into consideration founder effects among certain population groups and the availability of tests in different healthcare sectors.^[5-7]

It is internationally recognised and recommended that any genetic testing be performed in the context of appropriate genetic counselling. This process aims to ensure informed consent by providing the patient with information regarding testing, including the benefits, risks and limitations of different testing options. The potential complexities of results, including the chance of identifying variants of uncertain clinical significance (VUSs), and the implications of results for the family are also discussed.^[3,7,8]

Overview of current genetic testing strategies in the private sector

Founder screening

In the local context, founder variant testing may be requested as a first-line genetic test for individuals of Ashkenazi Jewish or Afrikaner ancestry.^[5-7,13] It is important to note that there are >2 000 and >3 000 known 'likely pathogenic' and 'pathogenic' variants in the *BRCA1* and *BRCA2* genes, respectively (as listed in ClinVar), and each founder screen tests for only three of these variants (Table 1). It is therefore not appropriate to utilise these tests as first-line screening for individuals who are not of known Afrikaner or Jewish ancestry. Additionally, individuals who have negative results from these limited founder screens should be offered more extensive testing options, such as full sequencing of the *BRCA1* and *BRCA2* genes.

BRCA1 and *BRCA2* full gene sequencing

For patients who are not of known Afrikaner or Ashkenazi Jewish ancestry, clinicians may request comprehensive screening of the full *BRCA1* and *BRCA2* genes, or utilise multigene panels that include additional high- or moderate-risk genes such as *PALB2*, *CHEK2* and *ATM*. Germline disease-causing variants in *BRCA1* and *BRCA2* are estimated to account for ~24% of families with inherited breast and/or ovarian cancer in European populations.^[8] Comprehensive screening of the full coding regions of genes is usually achieved via a next-

Table 1. Summary of genetic testing options

	Afrikaner founder screen	Ashkenazi Jewish founder screen	BRCA1/2 sequencing (local)	Multigene panel (international)
Details	3 variants <ul style="list-style-type: none"> BRCA1 c.1374delC (p.Asp458Glufs) BRCA1 c.2641G>T (p.Glu881Ter) BRCA2 c.7934delG (p.Arg2645Asnfs) 	• 3 variants <ul style="list-style-type: none"> BRCA1 c.68_69delAG (p.Glu23Valfs) BRCA1 c.5266dupC (p.Gln1756Profs) BRCA2 c.5946delT (p.Ser1982Argfs) 	Sequencing of BRCA1 and BRCA2 genes (>5 000 variants)	Sequencing of multiple genes, including BRCA1, BRCA2, CHEK2, ATM
Cost*	~ZAR2 200	~ZAR2 200	~ZAR13 500 (varies between laboratories)	~ZAR4 600 (USD250 + ZAR750 courier/handling fee)
Turnaround time	1 - 2 weeks	1 - 2 weeks	6 - 8 weeks	2 - 3 weeks (1 week for STAT Panel [†])
Disadvantages	Only 3 variants tested Not appropriate for individuals of other ancestries	Only 3 variants tested Not appropriate for individuals of other ancestries	Risk of missing 40 - 50% of pathogenic variants in other HBC-associated genes Long turnaround times Patient may need to pay a significant proportion of cost	High VUS rates Rarely covered by medical aid
Advantages	Cost-effective first-line test for individuals with Afrikaner ancestry May be covered (in full or partially) by medical aid scheme	Cost-effective first-line test for individuals with Ashkenazi Jewish ancestry May be covered (in full or partially) by medical aid scheme	Higher detection rates than founder screens May be covered (in full or partially) by medical aid scheme	High detection rates Short turnaround times Free testing available for family members

HBC = hereditary breast cancer; VUS = variant of uncertain significance.
 *Approximate costs (private rates) correct as at March 2020.
[†]BRCA1, BRCA2, CDH1, PALB2, PTEN, STK11, TP53, ATM and CHEK2.

generation sequencing (NGS) approach. It is important to select an approach that includes deletion/duplication analysis (either through NGS analysis or complementary methods such as multiplex ligation-dependent probe amplification (MLPA)) since ~10% of pathogenic variants in BRCA1 and BRCA2 can only be detected in this manner.^[14] Although the cost of NGS-based testing has decreased substantially in the past decade, local NGS-based testing still comes with a significant cost and turnaround times of more than 6 weeks (Table 1).

Multigene panels

More recently, research has shown that more than half of the disease-causing variants underlying HBC can be found in genes other than BRCA1 and BRCA2.^[15] These non-BRCA genes include ATM, CHEK2 and PALB2, among others, and are also tested using an NGS-based approach. Multigene panel tests including these genes are available locally, although many clinicians and genetic counsellors opt to refer to the international laboratory Invitae (USA) because of the lower costs, quicker turnaround time, and additional benefits such as testing of family members at no additional cost if a clinically relevant variant is identified (Table 1). No data are available on the uptake of local multigene panel tests.

The laboratory where the study was conducted does not offer in-house multigene panel testing for HBC, but sends these requests to Invitae laboratory. Multigene panels offered by other local laboratories are not included in this article.

When referring for multigene panel testing, it is important to consider the likelihood of detecting VUSs. This risk increases the more genes the panel selected contains. Rather than falling into pathogenic or benign categories, these are sequence variants for

which the association with disease risk is unclear based on currently available evidence. Laboratories routinely report on VUS results to allow for periodic review and potential reclassification based on new evidence. However, until a VUS is reclassified, these variants should not be used in clinical decision-making.^[16]

Medical aid scheme considerations

The selection of tests in the private sector is often influenced by cost and medical aid scheme reimbursement. Medical aid schemes do not currently cover tests sent to international referral laboratories. Patients may apply to their medical scheme for reimbursement of local genetic testing, but this is often subject to internal risk assessment criteria, which differs per scheme. In our experience, the full cost of local BRCA1 and BRCA2 sequencing has rarely been covered by medical schemes, and patients often had out-of-pocket expenses of a significant proportion. In recent months it has become evident that more medical schemes are reimbursing these local tests in full; this option may therefore become more attractive to patients and healthcare providers. It is advisable for patients and their healthcare team to discuss the economic impact of all testing options, both local and international, and apply to medical schemes for approval prior to any testing.

Methods

An internal database was reviewed to determine the results obtained from HBC patient referrals to our laboratory over a period of 3 years (2016 - 2018). This period was selected since it represented the most comprehensive data set for the various testing options. All referrals were included in the data set, except for referrals for family variant

testing (testing of at-risk individuals in a family where a known pathogenic variant has been detected) which were excluded. Referrals came from private clinicians ranging from general practitioners to specialist physicians and genetic specialists throughout SA. The results from founder screens ($n=704$), in-house *BRCA1/2* sequencing ($n=260$) and internal multigene panel send-aways ($n=723$) were de-identified and reviewed to determine the positive and negative pick-up rates for each test. The VUS rate was also calculated for multigene panel tests. Clinical and demographic information rarely accompanies these referrals. It was therefore not possible to determine how appropriate requests were, based on the patients' ancestral backgrounds.

Ethics approval

Ethics approval was granted by the University of the Free State Health Sciences Research Ethics Committee (ref. no. UFS-HSD2019/0484/2805).

Results

Founder screening

Based on 655 requests for Afrikaner variant testing, 15% of results were positive for one of the three variants tested (Fig. 1). This is significantly lower than the detection rates which can be achieved in well-defined Afrikaner cohorts.^[5,6] The most commonly detected variant was *BRCA2* c.7934del (p.Arg2645Asnfs, historically referred to as *BRCA2* 8162delG) which was detected in 11% of referrals. This is in line with variant distributions reported in other local studies.^[5,6]

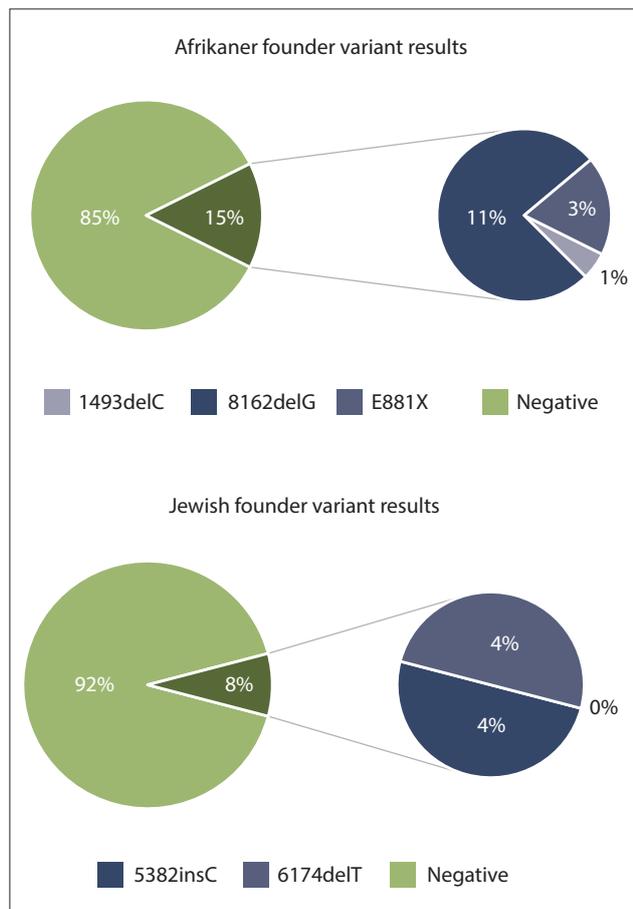


Fig. 1. Results of in-house founder variant tests over a period of 3 years (2016 - 2018).

Fewer requests were received for Ashkenazi Jewish founder variant screening ($n=49$). Eight percent of these requests were positive for either the *BRCA1* c.5266dupC (p.Gln1756Profs) or *BRCA2* c.5946delT (p.Ser1982Argfs) variants (Fig. 1). No individuals were positive for the *BRCA1* c.68_69delAG (p.Glu23Valfs) variant.

Sequencing of the *BRCA1* and *BRCA2* genes

Based on 260 referrals for *BRCA1* and *BRCA2* NGS-based sequencing over a 3-year period, we noted a 7% pathogenic variant pick-up rate. Sixty-five percent of disease-causing variants were found in *BRCA1* (Fig. 2), which is in line with internationally published distributions of *BRCA1* v. *BRCA2* disease-causing variants (66% v. 34%).^[14]

Multigene panels

From a dataset of >700 referrals to Invitae for multigene panel testing, we noted a positive pick-up rate ranging between 17% and 21%, depending on the panel selected. The 9-gene Breast Cancer STAT Panel (consisting of *BRCA1*, *BRCA2*, *CDH1*, *PALB2*, *PTEN*, *STK11*, *TP53*, *ATM* and *CHEK2*) showed the highest pick-up rate of 21% (Fig. 3). The 20-gene Breast and Gyn Cancers Guidelines-Based Panel and the 47-gene Common Hereditary Cancers Panel showed positive pick-up rates of 17% and 19%, respectively. We acknowledge that referrals for the STAT Panel may be biased towards affected patients in terms of needing an urgent result for clinical decision-making, compared with other panels that may include unaffected patients where no affected family member is available to test.

The risk of a higher VUS rate when larger panels are used is demonstrated in Fig. 3, with the largest 47-gene panel having a VUS pick-up rate of 56%. When a VUS is reported, the result is neither actionable nor completely reassuring; the interpretation is therefore complex and should take the family history as well as other clinical factors into account. The higher the VUS rate, the more difficult the result becomes to interpret, and these results become more challenging to convey and explain to the patient.^[3]

Multigenes and multidisciplinary teams

As mentioned previously, it is now possible to test for genetic contributions to HBC other than *BRCA1* and *BRCA2*. Based on our referrals for multigene panel testing, close to 40% of disease-causing variants were found in genes other than *BRCA1* or *BRCA2* (Fig. 4). This highlights the importance of comprehensive genetic testing, since the clinical management and associated risks of breast, ovarian and other cancers (such as colon, gastric or prostate cancer) vary greatly, depending on the causative gene.^[8] Larger panels could also include genes that are relatively newly identified, with the implication

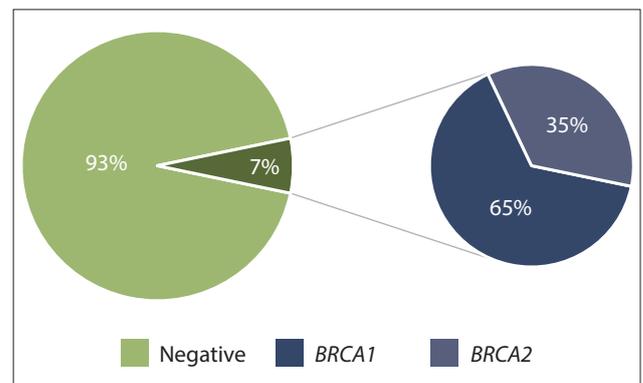


Fig. 2. Results of in-house *BRCA1* and *BRCA2* next-generation sequencing-based screening.

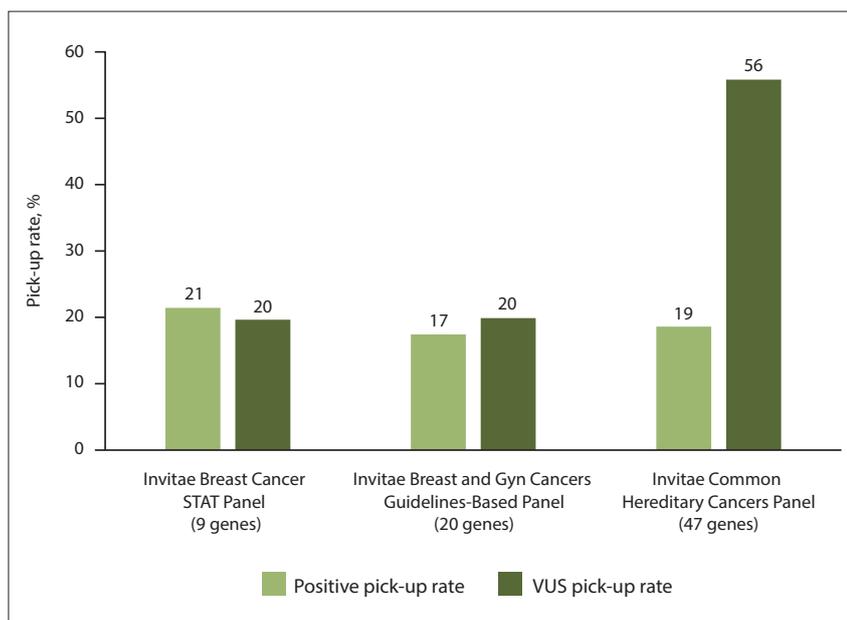


Fig. 3. Results of referrals to Invitae for multigene panel testing. (STAT Panel = BRCA1, BRCA2, CDH1, PALB2, PTEN, STK11, TP53, ATM and CHEK2; VUS = variant of uncertain significance.)

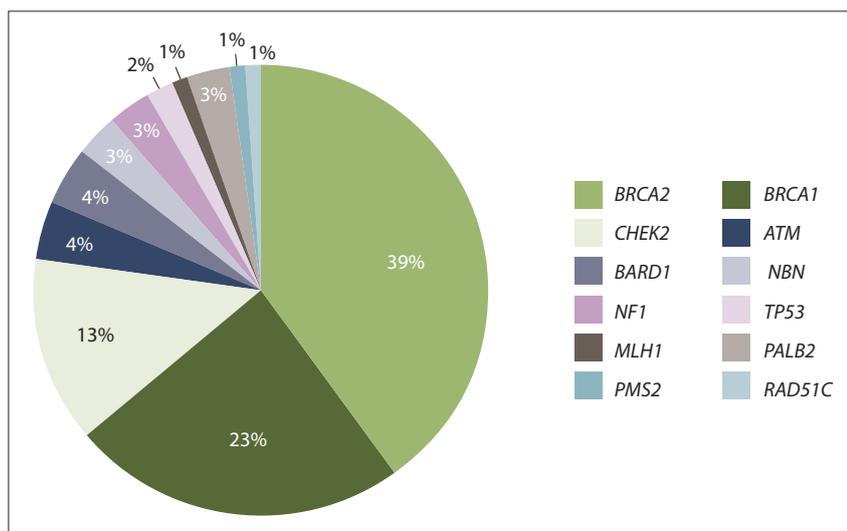


Fig. 4. Multigene panel results.

that cancer risks are not well delineated and no guidelines exist for medical management or the value of family predictive testing.^[3] Caution should be exercised when selecting a panel in which these genes are included.

Discussion

This retrospective study is the first to evaluate and compare the results obtained from different genetic testing options for HBC in the SA private sector. A summary of these testing options is given in Table 1. A summary of the test details and positive pick-up rates is provided in Table 2.

The results of this study are in line with findings from other international publications in terms of positive pick-up rates for the multigene panels and VUS

rates that increase with the larger multigene panels.^[17,18]

Our data set has shown positive pick-up rates of 15% and 8% for the Afrikaner and Jewish founder screens. Previous studies have indicated that these rates can be significantly higher in well-defined and appropriate patient populations.^[5,6,19] There is a concern that these tests are being requested inappropriately, and are often selected because of lower cost, quicker turnaround times and medical aid scheme reimbursement, rather than being based on clinical utility and family history/ancestry. The benefit of medical scheme coverage and lower cost will have to be weighed against the need for further testing if results are negative. The multigene panels will pick up

these six founder variants, in addition to other variants in *BRCA1* and *BRCA2* and the other HBC-associated genes in the panel selected.

Our data suggest that in the private sector in SA, targeted multigene panels (such as the Invitae Breast Cancer STAT or the Invitae Hereditary Breast and Gyn Cancers Guidelines-Based Panel in this data set) are appropriate testing options for patients with suspected HBC. While positive pick-up rates are high (17 - 21%), the VUS rate is lower than with larger panels such as the Invitae Common Hereditary Cancer Panel (20% v. 56%), making result interpretation less complex. While the pick-up rate for multigene panels is much higher than with *BRCA1* and *BRCA2* sequencing alone (17 - 21% v. 7%), the cost is also lower with the international multigene panels than local full gene sequencing of *BRCA1* and *BRCA2* (Table 1). Therefore, considering both cost and pick-up rate, the international send-away multigene panels may be a more suitable option for patients than local *BRCA1* and *BRCA2* sequencing. However, it is essential that patients and healthcare providers engage with medical schemes prior to any genetic testing, since schemes are covering local NGS-based testing more frequently.

We have alluded to some of the complexities involved in choosing the most appropriate genetic testing approach for breast/ovarian cancer patients, as well as the intricacies that may arise with the interpretation of genetic testing results. For these reasons it is recommended that multigene testing is ideally offered in the context of professional genetic expertise for pre- and post-test genetic counselling.^[3,8] Genetic counselling services are still under-utilised throughout SA and only available in some of the larger centres,^[20] although the majority of genetic counsellors in private practice offer telephonic or video-call consultations. Patients may also be referred to genetic services within the major academic hospitals.

Conclusions

In the SA private sector, multigene panel tests for HBC have good clinical utility in terms of pick-up rate for pathogenic variants. Founder mutation screens should only be requested in very specific contexts. Decisions regarding test options and interpretation of results can be complex and genetic counselling by genetic specialists is advised.

While national capacity building is important in order to expand the scope of local genetic services, the significantly lower costs of the international send-away panels

Table 2. Summary of test details and positive pick-up rates

	Afrikaner founder screen	Ashkenazi Jewish founder screen	BRCA1/2 sequencing (local)	Multigene panel (international)
Details	3 variants • <i>BRCA1</i> c.1374delC (p.Asp458Glufs) • <i>BRCA1</i> c.2641G>T (p.Glu881Ter) • <i>BRCA2</i> c.7934delG (p.Arg2645Asnfs)	3 variants • <i>BRCA1</i> c.68_69delAG (p.Glu23Valfs) • <i>BRCA1</i> c.5266dupC (p.Gln1756Profs) • <i>BRCA2</i> c.5946delT (p.Ser1982Argfs)	Sequencing of <i>BRCA1</i> and <i>BRCA2</i> genes (>5 000 variants)	Sequencing of multiple genes, including <i>BRCA1</i> , <i>BRCA2</i> , <i>CHEK2</i> , <i>ATM</i>
Number of referrals	655	49	260	723
Positive pick-up rate	15%	8%	7%	17 - 21%

make this option an attractive alternative. Further discussion is needed among product distributors, testing laboratories, healthcare providers, medical schemes, patients and their families, in order to increase the availability and accessibility of necessary genetic testing services in SA.

Study limitations

This study was conducted at a single laboratory in private practice in SA and results are therefore biased towards a population group having access to private medical schemes or an income level that would support self-payment for tests. Only data and testing options available for this laboratory were included in the article.

A correlation was not made between the pick-up rate of tests and the patient clinical information, as these data were not available.

Declaration. None.

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