Breast cancer in high-risk Afrikaner families: Is *BRCA* founder mutation testing sufficient?

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Background. Germline pathogenic mutations in cancer susceptibility genes result in inherited cancer syndromes. In the Afrikaner population of South Africa (SA), three founder mutations in the *BRCA* genes that lead to hereditary breast and ovarian cancer syndrome (HBOCS) have been identified.
Objectives. To investigate the uptake and type of molecular testing performed on patients for HBOCS, to determine the prevalence of the three Afrikaner founder *BRCA* mutations as well as non-founder *BRCA* mutations in the study population, and to analyse the utility of two mutation prediction models (Breast and Ovarian Analysis of Disease Incidence and Carrier Estimation Algorithm (BOADICEA) and Manchester scoring method) in assisting with the decision for the most cost-effective testing option.
Methods. A retrospective file review was performed on counsellees of self-reported Afrikaner ancestry from Johannesburg, SA (2001 - 2014), with a personal or family history of breast and/or ovarian cancer. Demographic and family history information was recorded and Manchester and BOADICEA scores were calculated for each patient.
Results. Of 86 unrelated counsellees whose files were reviewed, 54 (62.8%) underwent *BRCA* genetic testing; 18 (33.3%) tested positive for a mutation, and 14 of these (77.8%) for an Afrikaner founder mutation. Twelve counsellees had the *BRCA2* c.7934delG mutation. Four non-founder mutations were identified. BOADICEA scores were significantly higher in counsellees who tested positive for a mutation than in those who tested negative.
Conclusions. Founder mutation testing should be performed as a first-line option. BOADICEA is very useful in identifying counsellees at high risk for a *BRCA* mutation and also assists with the decision to pursue further testing following a negative founder mutation result. These findings assist in guiding an informed genetic counselling service for at-risk individuals with an Afrikaner background.

S Afr Med J 2016;106(3):264-267. DOI:10.7196/SAMJ.2016.v106i3.10285

Breast cancer is the most common form of cancer in women worldwide. Altogether 6 224 cases were reported in South Africa (SA) in 2009.[1] Up to 10% of breast cancer cases are attributable to germline mutations in cancer susceptibility genes, leading to hereditary syndromes.[2] The most well described of these cancer syndromes is hereditary breast and ovarian cancer syndrome (HBOCS), which is an autosomal dominant inherited syndrome predisposing to several cancers, particularly those of the breast and ovaries. This syndrome is caused by the presence of heterozygous, pathogenic germline mutations in either the *BRCA1* or *BRCA2* genes.

Founder mutations (those that occur more frequently or almost exclusively in a specific founder population group) account for a significant proportion of *BRCA* mutations. One such example of a founder population is the SA Afrikaner population group, which dates back approximately 330 years, when European settlers arrived in what is now the Western Cape Province of SA. This population (of individuals primarily of Dutch, German and French ancestry) grew rapidly in the first century after arriving in SA, and as a result the mutations in the initial population increased in frequency. The outcome of these events can be seen in the large number of genetic conditions in which Afrikaner founder mutations are documented.[3] The Afrikaner population group, comprising 12.2% of individuals living in the Gauteng Province of SA,[4] has had three founder *BRCA* mutations identified to date that result in HBOCS: *BRCA1* c.1374delC, *BRCA1* c.2641G>T and *BRCA2* c.7934delG.[5-6]

The Division of Human Genetics at the National Health Laboratory Services (NHLS) and the University of the Witwatersrand (Wits) in Johannesburg, SA, has offered genetic counselling at clinics in various hospitals in the Johannesburg area since the 1970s. Afrikaner individuals with a personal or family history of breast and/or ovarian cancer are seen at these clinics. The role of the genetic counsellor in this setting is to provide information regarding the genetics of HBOCS and the risks of carrying and passing on a mutation, and to assist the counsellee in making informed decisions about genetic testing. Genetic counsellors consider a variety of factors when analysing each individual case. The family history of cancers, tumour histological features and age of onset of the cancers can influence risk assessment and decisions regarding genetic testing. There are also a number of risk assessment tools available to aid in the analysis of cases. The two tools widely used at the NHLS/Wits are the online Breast and Ovarian Analysis of Disease Incidence and Carrier Estimation Algorithm (BOADICEA) risk calculation program[7] and the revised Manchester scoring method.[8] Both prediction models calculate the likelihood of carrying a deleterious mutation in one of the *BRCA* genes. Although these tools were initially designed for use in European populations, studies have been performed to validate their uses in ethnically diverse populations.[9-10] This type of analysis has not been undertaken in the Afrikaner population of SA.

Once the appropriate analyses and risk assessments have been undertaken, the counsellee may be offered a molecular genetic test, including testing for the Afrikaner founder mutations as a first-line testing option (prices range from approximately ZAR1 600 to ZAR3 500). Following a negative founder mutation result, further molecular testing may be offered should the clinical and family history warrant it. These further tests include sequencing

of the *BRCA1* and *BRCA2* genes and large rearrangement analysis to detect large deletions/duplications (prices range from approximately ZAR9 000 to ZAR25 000). The range in prices for *BRCA* testing is laboratory dependent.

No formal guidelines currently exist to assist genetic counsellors or their counsellees in determining whether founder mutation analysis is sufficient or whether additional testing should be pursued following a negative result for the founder mutations identified in this population group.

Objectives

To conduct a retrospective file review of a cohort of Afrikaner individuals presenting for genetic counselling for HBOCS. The objectives were to: (*i*) investigate the uptake and type of molecular testing carried out; (*ii*) determine the prevalence of the founder and non-founder mutations identified in this cohort; and (*iii*) analyse the utility of two prediction models used in genetic counselling for inherited cancers.

Methods

A retrospective file review of all counsellees of self-reported Afrikaner ancestry who received genetic counselling for HBOCS in the genetic counselling clinics offered by the NHLS/Wits Division of Human Genetics in Johannesburg from August 2001 to December 2014 was conducted. The files are maintained and archived at the Division of Human Genetics at the NHLS/Wits.

The specific counselling clinics were held at Charlotte Maxeke Johannesburg Academic Hospital, Chris Hani Baragwanath Academic Hospital, the Donald Gordon Medical Centre and Helen Joseph Hospital.

Subjects

Participants for the study were selected on the basis of the following criteria: at least one side of the family had to be of self-identified Afrikaner descent, and the family history of cancer had to be from the Afrikaner side of the family. All counsellees enrolled into the study were unrelated to one another.

The counsellee's family history and demographic information was recorded and analysed. During a genetic counselling session the genetic counsellor discussed HBOCS and testing options with the counsellees. Clinical judgement alone has been used historically to categorise counsellees as having an average, moderate or high risk of having HBOCS. In more recent years, various tools and prediction models have been used to assist genetic counsellors in determining HBOCS risks.

Genetic counsellors at the NHLS/Wits calculate counsellees' risks of carrying a deleterious *BRCA1* or *BRCA2* mutation using the online BOADICEA risk calculation program[7] and/or the Manchester scoring method.[8] The outputs of these tools were incorporated into the overall analysis of risk for each counsellee.

A data collection sheet was constructed to record the counsellees' demographic information, the specific tests undertaken, the cancers reported in the family and HBOCS risks given by the genetic counsellors, as well as risks calculated using BOADICEA and the revised Manchester scoring method for each individual. In cases where the BOADICEA or Manchester scores had not been calculated, these were computed from the pedigree data as part of the study for each counsellee (to obtain a pretesting risk output). These data allowed for standardised comparison among the different groups of counsellees.

Mann-Whitney tests were carried out to compare the BOADICEA and Manchester risk scores. The first was a comparison between counsellees who tested positive and negative for an Afrikaner founder mutation; this was done to evaluate whether the prediction models could be accurately applied to this population. The second comparison was carried out between those who tested positive and negative for a non-founder mutation after further sequencing was done. A median BOADICEA risk score, given as a percentage chance of having a mutation, was then calculated for each group. The Manchester scores, given as whole numbers, correspond to the following risks: a score of >16 corresponds to a ≥10%

chance of carrying a deleterious mutation and a score of ≥20 indicates a 20% chance of carrying a deleterious mutation.[11] As with the BOADICEA risk scores, the median Manchester scores for each group were calculated for comparison.

Once the counsellees were checked and found to be unrelated to one another, the data were anonymised in accordance with appropriate ethical protocols. Ethics clearance (reference: M101141) was obtained from the University of the Witwatersrand's Human Research Ethics Committee (Medical).

Results

A total of 122 self-reported Afrikaner counsellees were seen at the genetic counselling clinics for discussion around testing for HBOCS during the period 1 August 2001 - 31 December 2014, and their files were available for review. Twenty were found to be related to others already included in the study, 4 were reported to have a family history of cancer on the non-Afrikaner side of their family, and a further 12 were excluded because insufficient information was provided. A total of 86 counsellees (70.5%) therefore matched the inclusion criteria specified for the study.

Of the 86 counsellees whose files were reviewed, 54 (62.8%) underwent *BRCA* Afrikaner founder mutation testing, and 14/54 (25.9%) tested positive for one of the three founder mutations. Of the 40/54 (74.1%) who tested negative for a founder mutation, only 10/40 (25.0%) opted for further analysis. Four of these 10 (40.0%) were found to carry a non-founder mutation (Fig. 1).
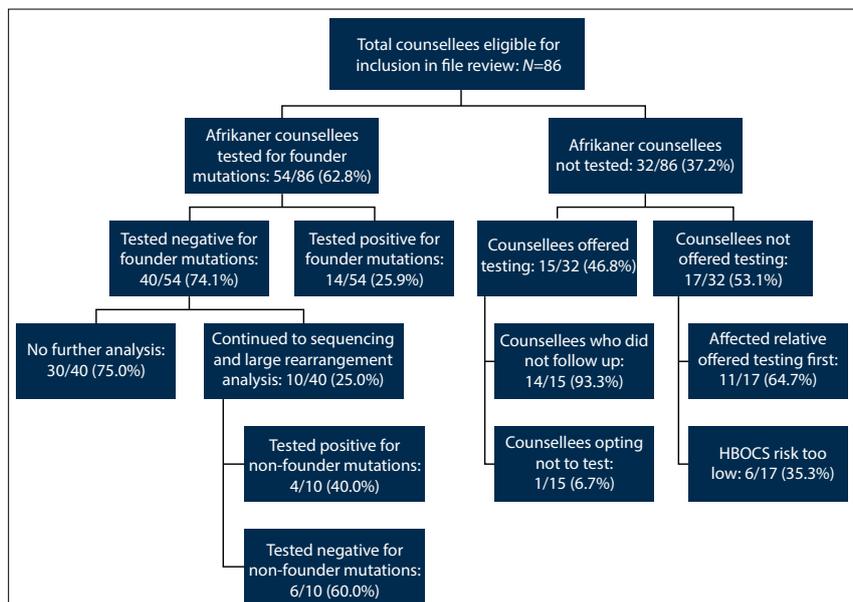
Fig. 1. Outline of testing options recommended and the outcomes for counsellees in this study (N=86).

Of the 86 counsellees, 32 (37.2%) did not undergo any testing, although 15/32 (46.9%) were offered founder mutation testing. One counsellee opted not to undergo molecular testing for personal reasons, and 14 did not follow through with providing a blood sample for testing. The remaining 17/32 (53.1%) were not offered testing, either because the risk for HBOCS was considered too low or because an affected relative who presented as a better candidate was offered testing.

Eighteen counsellees tested positive for a deleterious mutation in one of the *BRCA* genes, and 14 of these mutations (77.8%) were founder mutations in the Afrikaner population. The other four non-founder mutations identified were *BRCA1* c.45dupT, *BRCA1* c.181T>G, *BRCA2* c.6621delA and *BRCA2* c.6761_6762delTT. The Afrikaner *BRCA2* c.7943delG founder mutation was the most common mutation in this cohort, with 12 of the 18 counsellees (66.7%) testing positive for it. The mutations found are summarised in Table 1.

A BOADICEA risk score and a Manchester score were calculated for each counsellee, based on personal and family history alone; molecular results were excluded from these calculations so that a pretest prediction of identifying a pathogenic *BRCA* mutation could be performed. Median scores and ranges for counsellees tested for the Afrikaner founder mutations were calculated, since the data did not follow a normal distribution (Table 2). A Mann-Whitney test was then carried out to examine whether or not the BOADICEA and Manchester prediction models were useful in this founder population. In order to compare the scores effectively, counsellees who tested positive for a founder mutation (*n*=14) were compared with counsellees who tested negative for a founder mutation (*n*=40). Significance was assumed at a one-tailed *p*-value of <0.05. There was a highly significant difference in the BOADICEA risk scores for counsellees who tested positive and negative for an Afrikaner founder mutation ($p<10^{-3}$); however, there was no significant difference in Manchester scores between the two groups (*p*=0.06). An outlier with a Manchester score of 106 who tested negative for an Afrikaner founder mutation and did not undergo further testing was removed from the group of counsellees. As a result, a significant difference was observed between the two groups (*p*=0.04).

Another Mann-Whitney test was conducted on the 10 counsellees who opted for further testing after receiving a negative *BRCA* Afrikaner founder mutation result.

Table 1. Frequencies of *BRCA1* and *BRCA2* mutations identified in the Afrikaner cohort (*N*=18)

Gene	Mutation	Counsellees who tested positive, *n* (%)
BRCA1	c.45dupT	1 (5.5)
	c.181T>G	1 (5.5)
	c.1374delC*	1 (5.5)
	c.2641G>T*	1 (5.5)
BRCA2	c.6621delA	1 (5.5)
	c.6761_6762delTT	1 (5.5)
	c.7934delG*	12 (67.0)

*The three founder mutations previously identified in the Afrikaner population of SA.

Table 2. Median pretesting BOADICEA and Manchester risk scores for counsellees tested for Afrikaner founder mutations in the *BRCA* genes (*N*=54)

	Risk score, median (range)	
Risk assessment tool	Counsellees who tested positive for a founder mutation (*n*=14)	Counsellees who tested negative for a founder mutation (*n*=40)
BOADICEA, %	35.6 (8.2 - 91.6)	5.6 (0.5 - 93.2)
Manchester score	21 (16 - 40)	16.5 (6 - 42)*

*An outlier with a Manchester score of 106 was excluded.

Table 3. Median pretesting BOADICEA and Manchester risk scores for counsellees who underwent further *BRCA* analysis (*N*=10)

	Risk score, median (range)	
Risk assessment tool	Counsellees who tested positive for a non-founder mutation (*n*=4)	Counsellees who tested negative for a non-founder mutation (*n*=6)
BOADICEA, %	44.6 (2.7 - 74.3)	3.15 (0.9 - 19.0)
Manchester score	24.5 (16 - 32)	15.5 (8 - 28)

They were divided into those who tested positive (*n*=4) and negative (*n*=6) for a *BRCA* mutation (Table 3). A significant difference in BOADICEA scores was observed between the two groups (*p*=0.04), but there was no significant difference in the Manchester score (*p*=0.07).

Discussion

According to the study data, the majority of the counsellees in this Afrikaner cohort (54/86, 62.8%) decided to undergo molecular genetic testing to detect a deleterious founder mutation in either of the *BRCA* genes. Among the counsellees who opted to undergo testing, the total mutation detection rate was 33.3% (18/54); 14 of these mutations (77.8%) were founder mutations prevalent in the Afrikaner population. Considering that a few of the counsellees who did not have molecular testing were also considered to be at a high risk for HBOCS, this detection rate has the potential to be even higher.

The most common mutation detected in this cohort was the *BRCA2* c.7934delG Afrikaner founder mutation, which was identified in 12 counsellees (22.2% of those tested). The other two Afrikaner founder mutations (*BRCA1* c.1374delC and *BRCA1* c.2641G>T) occurred only once each, which is less than expected. The reason for this distribution is unclear, but may represent a geographical variation. In the original article describing these two mutations, five families with the c.2641G>T mutation and two families with the c.1374delC mutation were reported in a cohort made up of 90 individuals from various population groups.[5] The authors stated that after their article was completed, an additional eight families were identified as having the c.2641G>T mutation and a further two families were reported to have the c.1374delC mutation.

The four non-founder mutations were identified only once each. The *BRCA1* c.181T>G mutation is a European founder mutation.[11] The other three non-founder mutations (*BRCA1* c.45dupT, *BRCA2* c.6621delA and *BRCA2* c.6761_6762delTT) have not been reported in the literature before. *BRCA1* c.45dupT has been identified during *BRCA1/2* molecular testing in labora-

tories around SA (Dr Nico de Villiers, personal communication) and the *BRCA2* c.6761_6762delTT mutation has been reported in the National Centre for Biotechnology Information, ClinVar database.[12] Further studies, including haplotype analysis around the mutation region, are needed to characterise *BRCA1* c.45dupT in SA.

Fifteen of the counsellees who were offered testing did not pursue it (17.4%). This raises concern. Counsellees who could potentially be carrying deleterious mutations are not being tested and would therefore not benefit from prevention strategies.

A study by Petrucelli *et al.*[13] on the Ashkenazi Jewish population of Michigan found that only 1 out of 166 (0.6%) had a non-founder *BRCA1/2* mutation.[13] The authors observed that the National Comprehensive Cancer Network recommends further molecular analysis following a negative founder mutation result only when there is evidence of non-Ashkenazi Jewish ancestry. Even though only a small number of counsellees from the current study opted for further analysis when initial founder mutation testing was negative, 40% (4/10) had a non-founder mutation, which is a higher proportion than reflected in Petrucelli *et al.*'s research. This suggests that, in the Afrikaner population of SA, further analysis is required more frequently than suggested by Petrucelli *et al.*

Mutation prediction models have been useful in recent years to assist genetic counsellors in deciding whether or not to offer their clients further analysis. Prediction models of this kind have been validated in various ethnic groups,[9-10] but have not yet been modified to account for founder mutations in populations such as the SA Afrikaner population. Comparison of the two prediction models in the present study has revealed that the BOADICEA prediction model seems to discriminate high-risk individuals better than the Manchester model. Even though the numbers were small, there was a significant difference in the BOADICEA scores between counsellees who tested positive and negative upon further *BRCA* analysis. This indicates that, following a negative founder result, *BRCA* sequencing and deletion/duplication analysis should be pursued if the BOADICEA score is >10%. The application of this threshold could reduce unnecessary costs if further testing is not pursued. Further studies are necessary in larger samples to examine the role of these prediction models comprehensively in the Afrikaner founder population. However, in this study, the BOADICEA prediction model provided a good additional indication as to whether or not further testing was warranted.

Conclusions

The findings of this study have shown that 54/86 Afrikaner counsellees underwent *BRCA* testing in the Johannesburg area over the period 2001 - 2014. Insight is provided into the prevalence of *BRCA* founder mutations in the local Afrikaner population. The sample in the present study may not be representative of the wider Afrikaner population in the country, and further analysis of samples from at-risk families living elsewhere may indicate a need for regional-specific practice.

The presence of four non-founder mutations in the study cohort suggests that screening for founder mutations alone in high-risk counsellees may be insufficient. Clinical judgement and appropriately assessed prediction tools should be used to determine the most cost-effective course of testing and which counsellees would benefit from full sequencing and deletion/duplication analysis after a negative founder screen. A similar analysis to that described here, but performed on a larger sample, would provide more comprehensive results. Efforts in this regard are ongoing locally.

While the BOADICEA and Manchester risk prediction models were not designed to take into account founder mutations for the Afrikaner population, it is evident that these prediction models are very useful in this population. Genetic counsellors should be encouraged to utilise these models to aid in decision-making regarding testing for *BRCA* mutations. A high BOADICEA score would support further *BRCA* testing if the founder mutation screen is negative.

Genetic counsellors in SA need to be cognisant of the Afrikaner founder mutations and their possible presence in ethnic groups that may have Afrikaner admixture, and testing options should be considered accordingly.

The use of a retrospective, file-based approach limited this study to an analysis of the information contained in the counsellees' files. Further studies of a prospective and qualitative nature, could obtain insight into the attitudes and perceptions of Afrikaner counsellees regarding the process of genetic counselling and decision-making with regard to *BRCA* testing.

The findings from this study will be useful in the provision of an informed genetic counselling service to at-risk individuals with an Afrikaner background in an SA setting. This research illustrates the necessity of genetic counselling and testing of appropriate patients. Patients with a family history of breast and/or ovarian cancer, women <50 years of age with breast cancer, males with breast cancer and families with cancer from high-risk ethnic groups such as Afrikaners and Ashkenazi Jews should be referred for genetic counselling.

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