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A review of genetic testing for females referred to genetic counselling for a personal or family history of gynaecological cancer

by

Sebastian Barnard

A research report (in the format of a "submissible" paper) submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in partial fulfilment of the requirements for the degree of Master of Science in Medicine (Genetic Counselling)

Johannesburg, 2023

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Declaration

I, Sebastian Barnard, declare this research report (in the format of a "submissible" paper) is my own, unaided work. It is being submitted for the Degree of Master of Science in Medicine at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.

I, Sebastian Barnard, principal investigator, have full access to the original data generated through this study and take responsibility for the integrity of the data. All authors contributed to the interpretation of the data and take responsibility for the accuracy of the data analysis.

Sebastian Barnard

26 February 2024, in Johannesburg

Contribution of the candidate to this paper

Declaration: Student's contribution to article and agreement of co-authors

I, Sebastian Barnard, student number: 708672, declare that this Research Report is my own work and that I contributed significantly towards research findings presented in the paper intended for publication below.

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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 Research Project.

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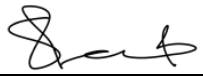
1st Author	Sebastian Barnard		26 February 2024
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Dedication

This work is dedicated to those who are curious, who seek, and are brave enough to write their findings down.

Abstract

Hereditary cancer syndromes, caused by pathogenic variants in specific genes, substantially increase an individual's risk for cancer and are estimated to cause 10% of all uterine cancers and 20% of all ovarian cancers. However, these data are primarily based on high-income countries and to date there is no published data on the known variants and testing of cancer predisposition genes associated with gynaecological cancers in South Africa. In this study, patient records for those with either a confirmed diagnosis or family history of gynaecological cancer that were seen by the Division of Human Genetics (NHLS/Wits) were retrospectively analysed (n = 104). Associations between patient characteristics, genetic testing availability and the detection of pathogenic variants as well as the utility of risk assessment tools were investigated using statistical analysis. The majority of patients underwent diagnostic genetic testing (78/104, 75.0%), 25 (32.1%) were positive, 41 (52.6%) were negative, and 12 (15.4%) returned a variant of unknown significance. Test results were significantly different between European and non-European patients ($p << 0.05$) with non-European patients being 30% less likely to have a pathogenic variant detected (OR 0.7, 95% CI 0 0.22, 2.21). Patients who met genetic testing criteria according to online risk assessments were more likely to have a positive genetic test result than those who did not ($p < 0.05$). A disparity exists not only in genetic testing availability but also clinic attendance between public and private healthcare which is likely limiting the ability to diagnose hereditary cancer syndromes associated with gynaecological cancers in public healthcare hospitals.

Acknowledgements

As with any human endeavour worth pursuing, this body of work is a testament to the indirect efforts of several important individuals whom I would like to acknowledge.

Firstly, my supervisors, Bianca and Elzette, for their continued support and vision throughout this process. The work you do for our profession and the invaluable knowledge and patience that you have shared with me have been strong sources of inspiration for me this year.

To my dearest friends, Sam, Chad and Daniël, and cherished siblings, Deyna and Nicolas, thank you for always believing in me. Your collective love and support form the foundations upon which my achievements stand.

To my loving mother, this achievement is as much yours as it is mine. Thank you for the incalculable sacrifices you have made out of love and for only ever wishing for my happiness. And to my father, thank you for providing me with the opportunities that have made this degree possible.

Finally, I would like to acknowledge my peers who have stood by me on my journey through Academia. To Sarah and Luke, my Honours compatriots, and Megan, my MSc brother-in-arms, thank you for walking this difficult journey alongside me and for all the support along the way.

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List of abbreviations

ACMG	American College of Medical Genetics and Genomics
B	Benign
BSO	Bilateral salpingo-oophorectomy
BRCA1	Breast Cancer gene 1
BRCA2	Breast Cancer gene 2
BRIP1	BRCA1-interacting Protein 1 gene
CI	Confidence interval
df	Degrees of freedom
EPCAM	Epithelial Cellular Adhesion Molecule gene
FDR	First degree relative
HBOC	Hereditary Breast and Ovarian Cancer
HCS	Hereditary cancer syndrome
HREC	Human Research Committee
LB	Likely benign
LP	Likely pathogenic
MLH1	MutL Homolog 1 gene
MSH2	MutS Homolog 2 gene
MSH6	MutS Homolog 6 gene
NHLS	National Health Laboratory Service
OOP	Out-of-pocket
OR	Odds ratio
P	Pathogenic
PALB2	Partner and Localizer of BRCA2 gene
PARP	poly (ADP-ribose) polymerase
PMS2	Postmeiotic Segregation Increased 2 gene
PPV	Positive predictive value
$PREMM_5$	Prediction Model for gene Mutations version
PTEN	Phosphatase and Tensin Homolog gene
PV	Pathogenic variants

RAD51C	RAD51 Paralog C gene
RAD51D	RAD51 Paralog D gene
REDCap	Research Electronic Data Capture
SA	South Africa
SD	Standard deviation
STK11	Serine/Threonine Kinase 11 gene
VUS	Variant of unknown significance
Wits	University of the Witwatersrand

Research report in the format of a “submissible” paper

This work has been written up as a paper for submission to Frontiers Oncology. The author guidelines for submission have been adhered to and can be found in Appendix G. Tables and Figures have been included in the text for ease of reference and do not exceed the author guidelines.

A review of genetic testing for females referred to genetic counselling for a personal or family history of gynaecological cancer

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Abstract for paper submission

Background: Hereditary cancer syndromes, caused by pathogenic variants in specific genes, substantially increase an individual's risk for cancer and are estimated to cause 10% of all uterine cancers and 20% of all ovarian cancers. However, these data are primarily based on high-income countries and to date there is no published data on the known pathogenic variants or testing of cancer predisposition genes associated with gynaecological cancers in South Africa. Thus, this study aimed to retrospectively analyse records of patients seen by the Division of Human Genetics (NHLS/Wits), South Africa with a confirmed diagnosis or family history of gynaecological cancer.

Methods: Patient records dated between 2003 and 2023 were evaluated for inclusion criteria. Associations between patient characteristics, genetic testing availability and the detection of pathogenic variants as well as the utility of risk assessment tools were investigated using statistical analysis.

Results: A total of 104 records were included in analysis. The majority of patients underwent diagnostic genetic testing (78/104, 75.0%), 25 (32.1%) were positive, 41 (52.6%) were negative, and 12 (15.4%) returned a variant of unknown significance. Test results were significantly different between European and non-European patients ($p << 0.05$) with non-European patients being 30% less likely to have a pathogenic variant detected (OR 0.7, 95% CI 0 0.22, 2.21). Patients who met genetic testing criteria according to online risk assessments were more likely to have a positive genetic test result than those who did not ($p < 0.05$).

Conclusion: A disparity exists not only in genetic testing availability but also clinic attendance between public and private healthcare which is likely limiting the ability to diagnose hereditary cancer syndromes associated with gynaecological cancers in public healthcare hospitals.

Keywords: gynaecological oncology, hereditary cancers, genetic counselling, hereditary breast and ovarian cancer (HBOC), ovarian cancer, uterine cancer, Lynch syndrome

1. Introduction

Gynaecological cancers were the fourth most frequently diagnosed cancer worldwide in 2020, accounting for 15.3% of cancer deaths in women (1). In South Africa (SA) cervical cancer is the most common gynaecological cancer, followed by uterine and ovarian cancers (2). Where cancer of the cervix is nearly entirely caused by human papilloma virus infection (3), approximately 10% of uterine cancers and more than 20% of ovarian cancers are caused by hereditary cancer syndromes (HCSs) (4,5). The majority of hereditary cancers are caused by two HCSs, Hereditary Breast and Ovarian Cancer (HBOC) syndrome and Lynch syndrome (6). HBOC syndrome is caused by pathogenic variants (PVs) in the tumour suppressor genes *BRCA1* and *BRCA2* and Lynch syndrome by PVs in DNA mismatch repair genes *MLH1*, *MSH2*, *MSH6* and *PMS2*. Pathogenic variants in *BRCA1* increases the lifetime risk for developing ovarian cancer up to 44% compared to the general population risk of 1–2% while *BRCA2* raises the risk to 17% (6,7) (Table 1). In addition, PVs in several other genes are responsible for hereditary ovarian cancers such as *PALB2*, *RAD51C*, *RAD51D*, and *BRIP1* (7) (Table 1). Lynch syndrome is associated with a substantial risk (31–49%) for uterine cancer compared to the general population risk of 3%, in addition to an elevated risk for ovarian cancer (6) (Table 1). Two less common genes known to cause gynaecological cancers are *STK11* (Peutz-Jeghers syndrome) and *PTEN* (Cowden syndrome) (6).

Table 1. Lifetime risk of gynaecological cancers for those with Hereditary Breast and Ovarian Cancer syndrome, Hereditary ovarian cancers, Lynch syndrome, Peutz-Jeghers syndrome and Cowden syndrome

Cancer type	**General population risk (%)**	**Heritable cancer syndrome risk (%)***											
		BRCA1	*BRCA2*	*PALB2*	*RAD51C*	*RAD51D*	*BRIP1*	*MLH1*	*MSH2*	*MSH6*	*PMS2*	*STK11*	*PTEN*
Ovarian	1.2	44	17	5	11	13	6	11	17	11	3	32.4	-
Uterine	3.1	-	-	-	-	-	-	37	49	41	31	49.6	28.2
Cervical	0.6	-	-	-	-	-	-	-	-	-	-	0.9	-

*Data sourced from references 14–16

Before genetic testing for a HCS can be considered, a risk assessment is carried out to estimate how likely a patient is to be a carrier for a germline PV in one of the causative genes (8). A manual risk assessment is often done in clinic and takes into consideration factors such as age of onset and cancer type (ovarian cancer under 60 years of age; uterine cancer under 50 years of age), histology of cancer (high-grade serous carcinoma), personal history of cancer (single or multiple primary tumours; bilateral disease), as well as family history and ancestry. Additional tools are available online which can account for additional factors such as tumour histology and health factors (e.g., age of menarche), these include the Manchester Scoring System and CANRisk for HBOC syndrome and Prediction Model for gene Mutations version 5 ($PREMM_5$) for Lynch syndrome (9–11). These tools are used to quantify the risk that an individual is carrying a pathogenic variant as a percentage, each having a threshold beyond which genetic testing is indicated. For Manchester Scoring this threshold is greater than 14 points, equivalent to a 10% risk of having a PV in either *BRCA1* or *BRCA2* (9). For CANRisk the threshold is 10% or greater, representing the risk of having a PV in *BRCA1*, *BRCA2*, *PALB2,* CHEK2, ATM, BARD1, *RAD51D*, *RAD51C* or *BRIP1* (10). The threshold for testing based on PREMM5 is set as 2.5% or greater, representing the risk of having a PV in *MLH1*, *MSH2*, *MSH6*, *PMS2*, or *EPCAM* (11). Manchester scoring and CANRisk are set at a 10% threshold in accordance with the criteria for genetic testing offered in the public healthcare of the United Kingdom, while $PREMM_5$ is set at 2.5% as a means of improving its positive predictive value (9–11). Despite being originally validated for a European population, these tools have since been adopted for South African Afrikaner and Israeli Jewish ethnic populations (12,13).

In developed countries, multi-gene sequencing panels have become routine in diagnosing HCSs, with the advantage of detecting PVs in multiple genes simultaneously (17). This type of testing is particularly useful for HCSs where multiple genes cause the same cancer predisposition. Genetic testing for an individual affected with cancer is referred to as diagnostic testing, with variants detected classified according to American College of Medical Genetics and Genomics (ACMG) guidelines as either pathogenic (P), likely pathogenic (LP), variant of unknown significance (VUS), likely benign (LB) or benign (B) (18). Pathogenic and LP variants are known to be disease causing, thus clinically actionable, B and LB variants are considered part of normal genetic diversity while VUSs have insufficient evidence to classify as either pathogenic or benign and cannot be used in clinical decision-making (18). A

disadvantage of broad genetic testing is the high yield of VUSs which can be confusing and anxiety inducing for patients (19).

The molecular diagnosis of a HCS, achieved by a positive (P or LP variant) genetic test result, enables potentially life-saving opportunities in the treatment and prevention for affected individuals. Knowing the causative gene clarifies which types of cancer an individual is at elevated risk for as well as the degree of risk for each (Table 1) (16). In certain instances, prophylactic risk-reducing surgeries can be offered even before cancer develops. A prophylactic bilateral salpingo-oophorectomy (BSO) reduces the risk of ovarian cancer by 80% in females with hereditary ovarian cancers and a prophylactic hysterectomy can reduce the uterine cancer risk in patients with Lynch syndrome by up to 18% (20,21). Personalized and more extensive cancer screening can be implemented for cancers associated with the HCS to improve the likelihood of early cancer detection and prognosis (22). Furthermore, genetic testing can inform patient care and management, an example being poly (ADP-ribose) polymerase (PARP) inhibitors which are a well-established targeted therapy for ovarian cancer caused by PVs in *BRCA1* or *BRCA2* (23). Lastly, by knowing their genetic status, women have the opportunity to make more informed lifestyle, contraceptive and reproductive choices (16).

These benefits are not limited to the affected individual. HCSs exhibit autosomal dominant inheritance with first degree relatives (FDRs) having a 50% risk of carrying the familial PV (6). At-risk relatives can be offered expedited and less costly genetic testing by testing for the single family variant, termed predictive testing. Unlike diagnostic testing, predictive testing either confirms or excludes the presence of the familial variant and so does not generate VUSs. For all who test positive (i.e., carrying the same PV) the same aforementioned preventative measures are available, even in the absence of cancer (8). Predictive testing for HCSs thus could serve as an effective means of reducing cancer incidence and mortality, making this especially useful in the resource-limited context of SA (24). Those who test negative would be considered population risk for developing cancer negating the need to undergo frequent and invasive screening, such as that for Lynch syndrome, they are also then not at-risk of having children with the familial HCS.

The South African healthcare system is polarized with the lower socio-economic classes almost totally reliant on public healthcare, while the wealthy, many of whom pay separately for health insurance, utilize private healthcare (25). Historically, this segregation has reflected a racial divide with the majority of indigenous South Africans relying on public healthcare whereas

those of European descent are commonly able to afford private healthcare. Since 1996, access to public healthcare is free for citizens subject to a means-test (26). If fully subsidized by the government, a patient eligible for genetic testing is not required to pay for this testing. If partially subsidized the patient may be required to pay a portion of the genetic testing cost out-of-pocket (OOP). All public healthcare testing is offered through a single National Pathology Service. Individuals accessing private healthcare do so either by OOP payments or by claiming from their health insurance and even then, may still incur a co-payment. This testing is offered through either local or international laboratories. International genetic testing is not routinely covered by health insurance in South Africa requiring private patients to pay OOP but offers several advantages including more comprehensive and cost-effective testing as well as free family variant testing. Private healthcare services only 16% of the population while public healthcare services the remaining 84% (25).

Genetic testing for HCSs in SA began in 2005 but was limited to *BRCA1* and *BRCA2* in public healthcare (27). Only recently has genetic testing for cancers been expanded to gene panel testing, including *BRCA1* and *BRCA2* as well as *ATM, BARD, BRIP1, CDK12, CHEK2* and *PALB2*, which has led to an increased detection rate of actionable PVs (28). The limitation of this panel is that only half of these genes are associated with hereditary ovarian cancers (*BRCA1, BRCA2, BRIP1, PALB2*) and none of the genes causing Lynch syndrome are included. For private healthcare patients more comprehensive testing for HCSs has been available for much longer through private laboratories both locally and internationally. South African studies on HCSs have mostly focused on breast cancer, producing valuable contributions to the discovery of genetic differences in various South African populations, as well as screening and testing procedures (13,27,29). However, there is limited research into genetic testing for gynaecological cancers, detection rates and causative variants (30).

Therefore, the aims of this study were: 1) To conduct a file audit of patients seen by the Division of Human Genetics, National Health Laboratory Service (NHLS) and the University of the Witwatersrand (Wits) with a confirmed diagnosis or family history of gynaecological cancer and 2) To assess the utility of risk assessment tools in our sample. The outcomes of which were to assess whether adequate genetic testing is available to patients, whether patient factors (age of cancer diagnosis, family history, ancestry etc.) correlate with a positive genetic test result and evaluate the predictive value of risk assessments.

2. Materials and methods

2.1. Study population

The study included all individuals who received genetic counselling from a qualified genetic health practitioner (i.e., genetic counsellor, medical geneticist etc.) for a confirmed personal or family history of gynaecological cancer between January 2003 and May 2023. The patients were all seen through the Division of Human Genetics (NHLS/Wits) at one of the affiliated hospitals in Johannesburg, namely: Chris Hani Baragwanath Hospital, Rahima Moosa Mother and Child Hospital, Helen Joseph Hospital, Charlotte Maxeke Johannesburg Academic Hospital, and the Wits Donald Gordon Medical Centre.

2.2. Clinical and genetic data collection

All data were obtained from either electronic or hardcopy patient records held by the Division of Human Genetics (NHLS/Wits) after ethics approval was obtained from the Human Research Committee (HREC) at the University of the Witwatersrand (clearance number: M230313, Appendix C). Records were excluded where 1) neither the Patient nor their family history included a gynaecological cancer, 2) family history was non-specific (e.g., unconfirmed "womb cancer"), or 3) records were incomplete or unattainable. Patient records were reviewed to obtain relevant data (demographics, self-reported ancestry, relevant medical history including cancer diagnosis and histology, genetic testing, and family history) and organized using Research Electronic Data Capture (REDCap) software (Appendix B) (31). In addition, various risk assessments were performed where appropriate for each patient (Manual, Manchester Scoring System (9), CANRisk [https://www.canrisk.org/canrisk_tool/] and/or PREMM$_5$ [https://premm.dfci.harvard.edu/]). Records were anonymized and subsequently given unique record identity to maintain confidentiality.

2.3. Statistical analysis

Categorical variables were summarized as frequencies and percentages while the normally distributed continuous variables, such as age, were summarized using mean and standard deviation. The association of positive test results with various patient factors, including risk assessment, were assessed using the chi-squared test for group comparison. Logistic regression analysis was applied to evaluate the predictive power of online risk assessment tools in our sample. Independent t-test was used to determine significant differences between mean ages for different groups (patients w/ gynaecological cancer versus patients with family history of gynaecological cancer; age of diagnosis for ovarian versus uterine cancer). This test was

performed after data was determined to be normally distributed with equal variance using Shapiro-Wilk test and Levene's mean test respectively. The Unequal Variance t-test was performed as an alternative when data was not normally distributed, and the assumption of equal variance could not be made. Statistical significance was set at a p-value of < 0.05. Statistical analysis was done using the Real Statistics Resource Pack software (Release 8.9.1) for Excel® Microsoft 365 (32).

3. Results

3.1. Patient characteristics

A total of 104 patients with either a personal or family history of gynaecological cancer were included in this study after files during the allocated time periods were evaluated for inclusion criteria (Figure 1).

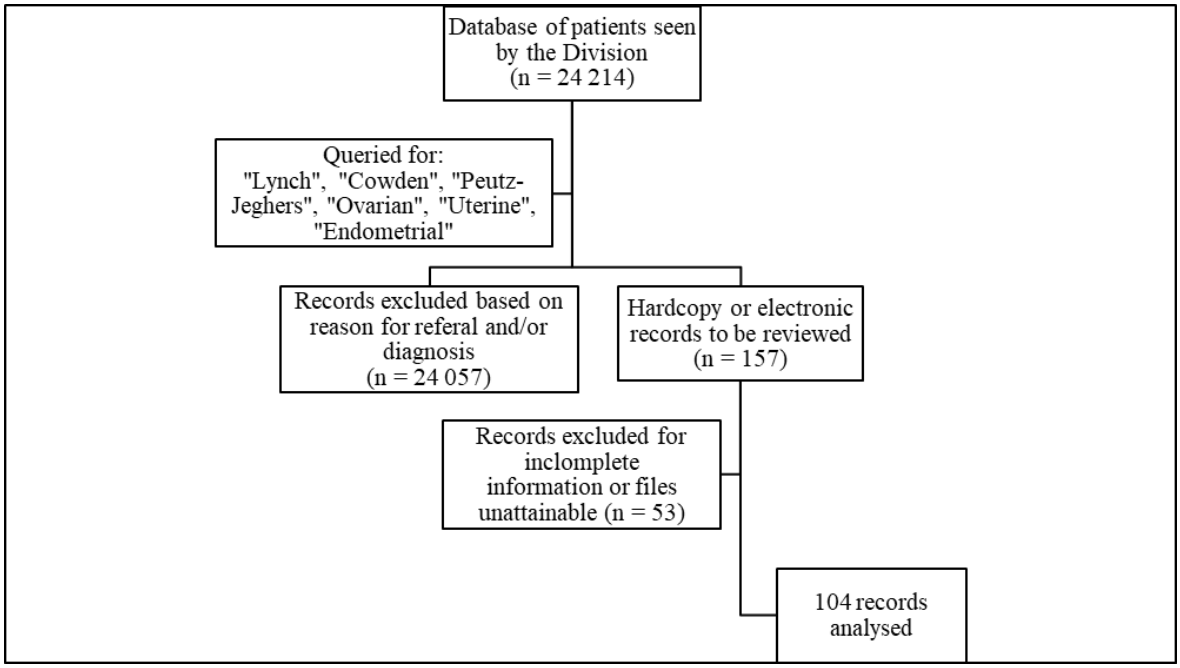

Figure 1. A flowchart of patient record selection for retrospective analysis

The majority of patients included in this study had a diagnosis of a gynaecological cancer (45/104, 43.3%), in addition a proportion of these also had a family history of gynaecological cancer (10/45, 22.2%). A smaller proportion (40/104, 38.5%) had a family history of a gynaecological cancer only. Nineteen (18.3%) patients presented for predictive testing. The mean age of patients presenting with a gynaecological cancer was 54.3 years (SD 2.2, 15.3 – 78.7) which was not significantly different from the mean age of patients presenting with a

family history of gynaecological cancer, 49.3 years (SD 2.0, 23.8 – 74,5) ($p > 0.05$). The mean age of diagnosis of uterine cancer was 47.1 years while the mean age of diagnosis of ovarian cancer was 49.5 years. The majority of patients in the study, 73 (70.2%), were seen via private healthcare and the remaining 31 (29.8%) patients via public healthcare. There were differences in the distribution of ancestry among patients seen in private and public healthcare, highlighted by Figure 2. Notably, Indigenous African patients comprised the majority of those in public healthcare but the minority of those in private.

Table 2. Patient characteristics

Sex of patients	
Female	98 (94.2%)
Male	6 (5.8%)
Age of patients at time of genetics consultation (years)	
Mean (standard deviation), range	49.6 (SD = 14.7), 15.3 – 78.7
<18	1 (0.1%)
18≤ x <30	9 (8.7%)
30≤ x <40	19 (18.3%)
40≤ x <50	20 (20.2%)
50≤ x <60	31 (29.8%)
>60	24 (23.1%)
Reason for referral	
Patient with a gynaecological cancer	35 (33.7%)
Patient with a family history of gynaecological cancer	40 (38.5%)
Patient with personal and family history of gynaecological cancer	10 (9.6%)
Family variant testing for a hereditary cancer syndrome associated with gynaecological cancers	19 (18.3%)
Population ancestry	
Indigenous African	16 (15.4%)
Afrikaner	24 (23.1%)
European	35 (33.7%)
Ashkenazi Jewish	13 (12.5%)
Other	16 (15.4%)
Healthcare sector patient seen in	
Public healthcare	31 (29.8%)
Private healthcare	73 (70.2%)

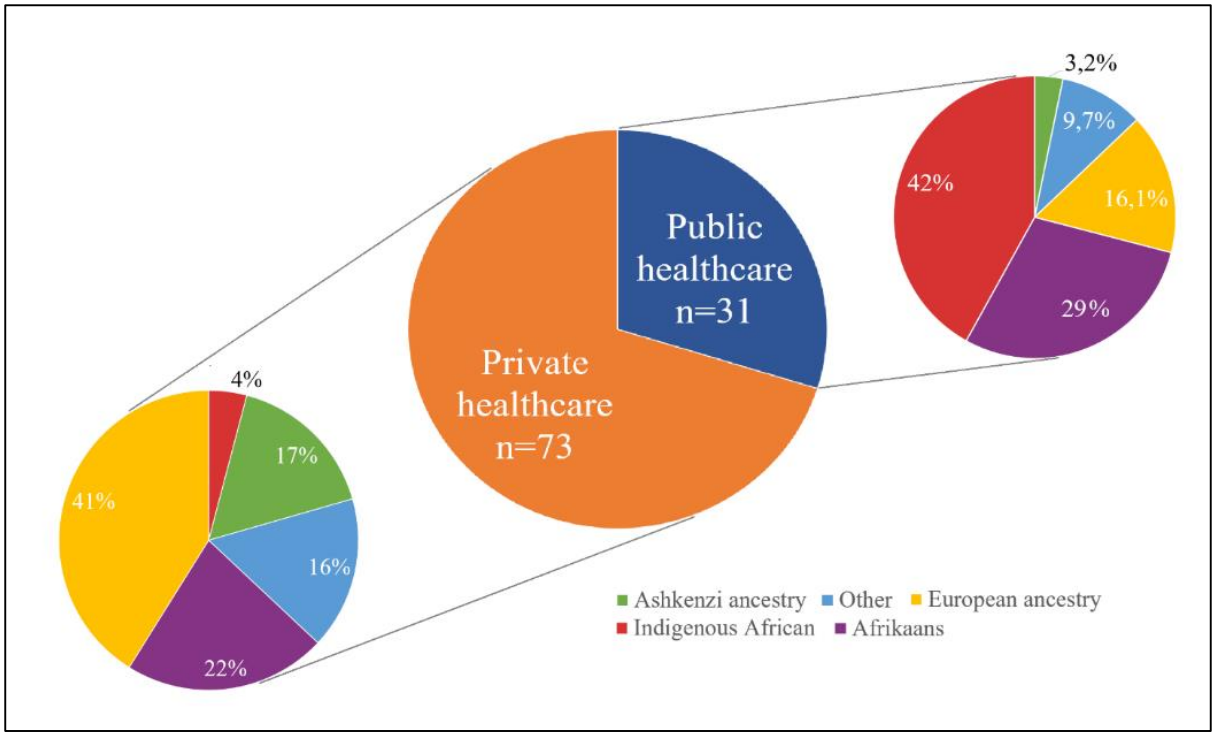

Figure 2. Pie chart showing ancestry distribution among study participants in public and private healthcare settings

The mean age of diagnosis for non-European patients with a gynaecological cancer was 43.3 years (SD = 4.3), 8.7 years earlier than European patients (51.9 years), which was statistically significant ($p << 0.05$). Patients with gynaecological cancer in public healthcare took on average 7.4 years after their diagnosis to attend Genetics clinic while those in private healthcare took on average 3.5 years. The delay between public and private was not statistically significant ($p > 0.05$).

3.2. Genetic testing

Across all 104 patients, eight (7.7%) did not receive any genetic testing. The most common reason for this was that the patient was not an appropriate candidate for testing (i.e., unaffected). Of the 96 (92.3%) who received genetic testing the majority, 67 (69.8%), were patients from private healthcare (Table 3). A total of 100 tests were ordered with four (4.2%) patients receiving additional or follow-up testing after a negative result. Access to genetic testing in public and private healthcare was notably different (Table 3). Nearly all family variant testing was done in private (17/18, 94.4%) and the majority of private patients received testing by broader gene panels through international laboratories (30/67, 44.8%). In public the

most routine testing done was *BCRA1/2* sequencing (13/33, 39.4%), with only four (12.1%) having testing done internationally.

Table 3. Genetic testing that was performed for patients in our cohort

Genetic Testing	**Count**	**Positive results**
No. of patients who underwent testing	96 (92.3%)	
Diagnostic testing	78 (81.3%)	25 (32.1%)
Family variant testing	18 (18.8%)	11 (61.1%)
No. of genetic tests conducted	100[*]	36 (36.0%)
Types of Genetic Testing		
Public healthcare	33	9 (27.2%)
Founder mutation testing[†]	4 (4.0%)	1 (25.0%)
BRCA1/2 sequencing	13 (13.0%)	3 (23.1%)
Local gene panel [‡]	9 (9.0%)	1 (12.5%)
Research testing[§]	2 (2.0%)	2 (100%)
International testing[#]	4 (4.0%)	1 (25.0%)
Family variant testing	1 (1.0%)	1 (100%)
Private healthcare	67	27 (40.3%)
Founder mutation testing	7 (7.0%)	1 (14.3)
BRCA1/2 sequencing	3 (3.0%)	1 (33.3%)
Local private testing[¶]	10 (10.0%)	5 (50.0%)
International testing	30 (30.0%)	10 (33.3%)
Family variant testing	17 (17.0%)	10 (58.8%)

[*]Four patients each had one additional genetic test done after initial testing was negative.
[†]Testing for a limited number of specific mutations with a high prevalence in a population of distinct ancestry (e.g., *BRCA1* c.68_69delAG, *BRCA1* c.5266_5267insC, and *BRCA2* c.5946delT).
[‡]Genes included on local panel: *ATM*, *BARD1*, BRCA1, *BRCA2*, *BRIP1*, *CHEK2*, *PALB2*, *TP53*.
[§]More extensive/appropriate gene panel testing provided through research recruitment which includes genes not available on the local gene panel.
[#]Gene panels for: Breast and gynaecological cancers; Breast cancer; Colorectal cancer; Common hereditary cancers; Lynch syndrome; Paediatric solid tumour (on average gene panels included 22 genes).
[¶]Four tests (40.0%) were *BRCA1/2* sequencing, five (50.0%) were nine-gene panels (*BRCA1*, *BRCA2*, *CDH1*, *PALB2*, *PTEN*, *RAD51C*, *RAD51D*, *STK11*, and *TP53*), and one (10.0%) was a Lynch syndrome gene panel (*EPCAM*, *MLH1*, *MSH2*, *MSH6*, and *PMS2*).

In all four instances (all in public) where additional testing was done no PVs were detected. Private patients had a higher PV detection (27/67, 40.3%) than those in public (9/33, 27.2%). This difference was determined to be statistically significant ($p << 0.05$). The odds that a patient who underwent genetic testing had a PV detected was higher in those who had private testing, compared to those who had public testing (OR 1.5, 95% CI = 0.59, 3.78).

3.2.1. Diagnostic yield

Of the 96 patients who underwent testing, 78 (81.3%) underwent diagnostic testing trying to establish a cause for their cancer and 18 (18.8%) had family variant testing to determine whether they had inherited a family variant. Diagnostic testing yielded 25 PVs across eight different genes (Figure 3). No patient received multiple PVs and so 25 (32.1%) patients received a PV, most often found in *BRCA1* and *BRCA2*, with seven (28%) PVs detected in each. Fourteen (56%) patients were diagnosed with HBOC syndrome and eight (32.0%) with Lynch syndrome.

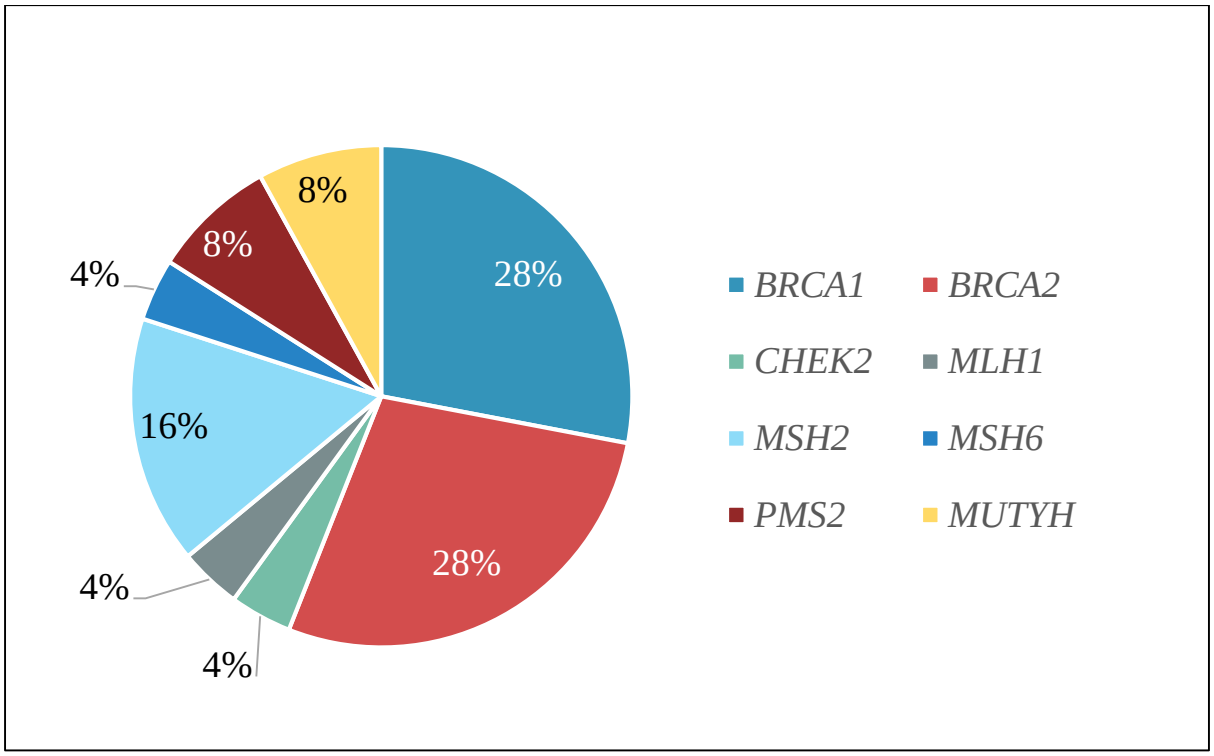

Figure 3. Pie chart of pathogenic variants (n = 25) found across different genes

In addition, diagnostic testing detected 16 VUSs. One (1.3%) patient received both a PV and VUS, and two (2.6%) patients received multiple VUSs. In total, 12 (15.4%) patients received VUSs which could not clarify the cause of their cancer. Only two (2/16, 12.5%) VUSs have been reclassified since testing was done, a *BRCA2* variant which was reclassified as benign and a *WT1* variant reclassified as likely benign. Of the remaining 14 VUSs, four (28.6%) were listed on ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) as "Conflicting interpretations of pathogenicity" having been reported differently by multiple submitters (i.e. laboratories) (33).

Table F1 (appendix F) summarizes all variants found, their classification and the cancer history (personal or familial) of the patient.

In terms of family variant testing, 18 relatives of patients were seen in our clinic while an additional 22 (totalling 40) were tested through other laboratories. Of these, 24 (24/40, 60%) tested positive for the familial PV and 17 (17/24, 70.8%) were asymptomatic at the time of testing.

3.2.2. Population differences in detection rate

Of the 104 patients included in the study, 78 (75%) patients had diagnostic testing done, with 25 (32.1%) having a positive result (detecting a PV) and 53 (67.9%) did not receive a positive result (either negative or a VUS). These patients fell into one of five ancestral categories: Afrikaner (24, 30.8%), European (24, 30.8%), Ashkenazi Jewish (11, 14.1%) Indigenous African (13, 16.7%), and 'Other' (6, 7.7%). A statistically significant association was found between a patient's population ancestry and whether they are in public or private healthcare ($p << 0.05$). The Cramer's V value was calculated to be 1.14 which is considered a large effect size ($df = 1$; $p >> 0.5$), thus the observed statistical difference is unlikely due to chance. Furthermore, fewer PVs were detected in non-Europeans than European patients, 5 (20%) and 20 (80%) respectively. Similarly, only four (4/16, 25%) VUSs were detected in non-European patients. The difference between genetic testing outcomes for non-European and European patients was determined to be statistically significant ($p << 0.05$). The odds that a patient who underwent genetic testing had a PV detected was significantly lower in non-European patients (OR 0.7, 95% CI = 0.22, 2.21).

3.3. Risk assessment

Risk assessments were retrospectively performed for patients who had received diagnostic genetic testing, this included a manual assessment and additionally one or more of the following online risk assessment tools: CANRisk, Manchester Scoring, and $PREMM_5$. A manual risk assessment could be performed for all 78 (100%) patients. Whereas CANRisk, Manchester Scoring and $PREMM_5$ were performed where appropriate, based on cancer history, for 64, 66 and 35 patients respectively. The summary of how many patients were determined to be at high risk of a HCS by each risk assessment is summarised by Figure 4.

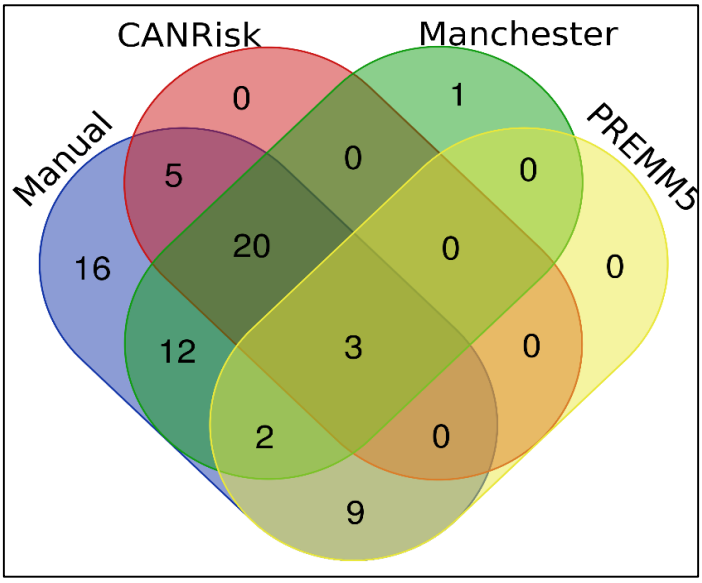

Figure 4. Overlap of patients predicted to be high risk for a hereditary cancer syndrome based on the different risk assessment tools used

This Venn diagram depicts where there was a consensus to warrant genetic testing among the four key risk assessment tools—CANRisk, Manchester Scoring, Manual, and PREMM5. Notably, only 3 (4.4%) patients were evaluated as high risk by all four methods and Manual, CANRisk, and Manchester Scoring had the highest concordance (20/59, 33.9%).

3.3.1. Manual risk assessment

A manual risk assessment was performed by the lead author for all patients who received diagnostic testing, these assessments were reviewed by two registered genetic counsellors. Of the 78 patients, 66 (84.6%) were assessed as being high risk for having a genetic (heritable) cause for their cancer and 22 (33.3%) patients went on to have a PV detected by genetic testing. The positive predictive value (PPV) for the manual risk assessment in this cohort is 32.8%. Ten (13.0%) patients did not meet testing criteria, being evaluated as either low or moderate risk, but were able to access testing through private laboratories. Despite not being indicated, this testing identified PVs in two (20.0%) patients. The association between an individual's manual risk assessment and whether a PV was detected was determined to be statistically significant ($p << 0.05$). The odds that a patient who underwent genetic testing had a PV detected was higher in those considered high risk through manual risk assessment, compared to those considered low risk on manual risk assessment (OR 1.95, 95% CI = 0.38, 9.99).

3.3.2. CANRisk risk assessment

CANRisk was used to quantitatively assess the likelihood that a patient was carrying a PV in one of nine genes associated with breast and ovarian cancer (*BRCA1*, *BRCA2*, *PALB2*, *CHEK2*, *ATM*, *BARD1*, *RAD51D*, *RAD51C* or *BRIP1*). This was done for all patients with a personal or

family history of breast and/or ovarian cancer, excluding those presenting for predictive testing (n=64). Twelve patients were excluded due to a personal or family history of uterine and/or colon cancer, and an additional two patients were excluded from analysis due to insufficient information to perform the assessment. Of the 64 patients, 28 (43.8%) were calculated as being high risk (10% or greater) of carrying a PV in one of the nine genes previously mentioned, 12 (42.9%) of which went on to have a PV detected. Only 4 (11.1%) patients who were calculated as low risk had a PV detected. Two of the PVs detected in these low-risk patients were in *MUYTH* and did not explain the cancer history. The PPV for the CANRisk risk assessment in this cohort was 42.9%. The association between an individual's CANRisk risk assessment and whether a PV was detected was determined to be statistically significant ($p < 0.05$). The odds that a patient who underwent genetic testing had a PV detected was 6-fold higher in those who met the CANRisk threshold for testing, compared to those with a low risk assessment (OR 6, 95% CI = 1.67, 21.6).

Additionally logistic regression was used to analyse the relationship between the CANRisk score an individual received based on personal factors and family history and whether they received a positive genetic test result. A Likelihood Ratio Test determined using CANRisk score as a predictor statistically improved the model's fit (Chi squared = 14.5; *df* 1; $p << 0.05$). The model accounted for 30% of the variation in the test result (Nagelkerke's R-squared = 0.3), indicating a reasonable fit to the data given the binary nature of the dependent variable. The odd of a positive test result occurring increased by 6.7% (95% CI = 2.6%, 10.9%) for the one-

unit increase in CANRisk score ($p << 0.05$). In the logistic regression analysis, the model achieved a high predictive performance (Figure 5).

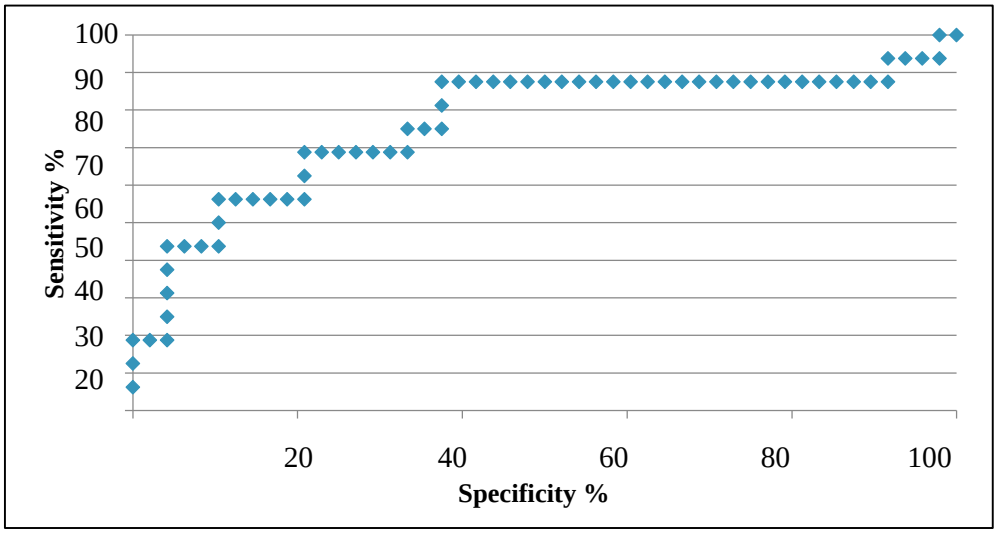

Figure 5. Receiver Operating Characteristic Analysis for predicting pathogenic variants in patients with a personal or family history of gynaecological cancer using CANRisk

The Receiver Operating Characteristic (ROC) curve illustrates the performance of the logistic regression model in predicting positive genetic test outcomes based on CANRisk score. The area under the ROC curve (AUC) is 0.76, indicating a robust ability to discriminate between individuals with positive and negative test results. The curve represents the trade-off between sensitivity and specificity, offering insights into the model's predictive accuracy in our study population.

3.3.3. Manchester scoring risk assessment

The Manchester Scoring System was used to quantitatively assess the likelihood that a patient was carrying a PV in either the *BRCA1* or *BRCA2* genes. This was done for all patients with a personal or family history of breast and/or ovarian cancer, excluding those presenting for predictive testing (n = 66). These were the same patients for whom a CANRisk assessment was done (n = 64), two additional patients could be included as less information is required to perform the assessment. Of the 66 patients, 38 (57.6%) were assessed as being high risk (10% or greater) of carrying a PV in *BRCA1/2*, 13 (34.2%) of whom went on to have a PV detected. Again, only 4 (14.3%) patients who received a risk assessment less than 10% had a PV detected. Three of whom were the same patients identified as low risk by CANRisk. The PPV for the Manchester Scoring risk assessment in this cohort was 34.2%. The association between an individual's Manchester Score risk assessment and whether a PV was detected was determined to be statistically significant ($p << 0.05$). The odds that a patient who underwent genetic testing had a PV detected was much higher in those who met the Manchester Score threshold for testing, compared to those with a low risk assessment (OR 3.12, 95% CI = 0.89, 10.92).

3.3.4. PREMM$_5$ risk assessment

The PREMM$_5$ risk assessment was used to quantitatively assess the likelihood that a patient was carrying a PV in either the *MLH1, MSH2, MSH6, PMS2* or *EPCAM* genes. This was done for all patients with a family history suggestive of Lynch syndrome (n=35), excluding those presenting for predictive testing. Of the 35 patients, 14 (40%) were assessed as being high risk (≥2.5%) of carrying a PV in the genes previously mentioned, of whom 8 (57.1%) went on the have a PV detected. Of those who received a low risk assessment (21 patients, 60%), eight (38.1%) had a PV detected. The PPV for the PREMM$_5$ risk assessment in this cohort was 57.1%. The association between an individual's PREMM$_5$ risk assessment and their genetic testing outcome was not determined to be statistically significant ($p > 0.05$). However, the odds that a patient who underwent genetic testing had a PV detected was higher in those who met the PREMM$_5$ threshold for testing, compared to those with a low risk assessment (OR 2.17, 95% CI = 0.55, 8.57).

4. Discussion

4.1. Patient characteristics and demographics

This is the first study to assess the genetic testing provided to patients with a personal or family history of gynaecological cancer in South African. Almost all the patients included were female, which was to be expected given the selection criteria. The cohort consisted of individuals from various ethnic backgrounds, however, indigenous African patients were the minority (15.4%) and so the cohort is not representative of the country's population distribution where 81.0% are indigenous African (34). This disparity was unexpected because the Division of Human Genetics (NHLS/Wits) operates more clinics in public healthcare which services 84% of the population, 81% of whom are Indigenous African (26, 34). The disparity is likely due to multiple contributing factors that may be clinician-related, patient-related and/or healthcare-related.

Clinicians may be unaware when to refer patients and risk factors pertinent to gynaecological cancers, or that genetic testing has clinical utility (35,36). This knowledge gap seems to persist particularly for Lynch syndrome when compared to HBOC syndrome (37). Furthermore, clinicians who are familiar with the risk factors of heritable cancers may not know how to refer the patient to genetic services, or simply not follow-up to ensure the patient attends the referral

(38). The health-seeking behaviours of patients accessing public healthcare is also known to reduce or delay presenting to tertiary hospitals, reasons include lack of cancer knowledge and importance of screening, superstitions providing alternative explanations for disease, and limited household income which hinders transport to seek healthcare (39–41). in public healthcare the lack of genetic testing for Lynch syndrome is a significant limiting factor for diagnosing HCSs for patients, especially in the case of uterine cancers. In addition, the strain placed on public healthcare leads to resource scarcity which negatively impacts patient care, this is further exacerbated by lack of trained genetic counsellors (42).

4.2. Genetic testing

The diagnostic yield of genetic testing for our cohort was 30.1%, with 24 PVs detected which is higher than previous studies investigating hereditary cancers that have reported a yield of between 10% and 20% (43, 44). In addition, 16 VUSs were detected which is relatively low given that gene sequencing technology often results in an increased detection of VUSs, especially when multiple genes are analysed simultaneously or when patients belong to underrepresented population groups such as those of African ancestry (45). Despite many patients undergoing multi-gene panel testing, the combination of high PV and low VUS detection indicates that for our cohort the referral to genetics was generally appropriate, with many patients receiving accurate risk assessments and subsequently, appropriate testing. However, another contributor may be the population bias towards European ancestry. Two individuals received VUSs in multiple genes and in both instances no PVs were found. One was of Afrikaner ancestry who had 36 genes analysed while the other was of indigenous African ancestry who had 47 genes analysed. Roughly 90% of VUSs are reclassified as benign, and attributed to normal variation, although the few which are later determined to be pathogenic have clinical utility (46). In our cohort only two VUSs (12.5%) had been reclassified as benign/likely benign. Ten (62.5%) have remained unchanged for, on average, three years and four (25%) variants are currently reported to have conflicting interpretations of pathogenicity. With gene panels becoming the standard of care it is important for routine re-analysis of VUSs and variants with conflicting interpretations (47).

Nearly all PVs detected were either associated with HBOC syndrome and Lynch syndrome which was expected given that these are two of the most common HCSs (48). The high proportion of *BRCA1/2* pathogenic variants may also be due to our cohort having a higher

incidence of ovarian than endometrial cancer, with PVs in *BRCA1* being more prevalent due to its higher penetrance (up to 44%) for ovarian cancer (49). Similarly, a high *MSH2* PV detection can be explained by the fact that it confers not only the highest overall cancer risk but the highest risk for endometrial and ovarian cancers (46% and 17% respectively) (50).

Non-European ancestry individuals had a 30% lower likelihood of receiving positive genetic testing results, potentially due to factors such as limited access to testing and population-specific considerations. In public healthcare, patients, who are more often non-European, face constraints in comprehensive genetic testing, limited to an eight-gene panel, focused on breast and ovarian cancer genes (28), placing a significant limitation on PV detection in the context of gynaecological cancers. Furthermore, with genetic research predominantly conducted on European populations it is possible the cancers seen in indigenous African populations are caused by genes yet to be identified which are not included in current testing (51). Population factors related to disease penetrance may also be reducing PV detection rates in non-Europeans. Numerous studies looking at inherited cancers have shown that indigenous African patients diagnosed with cancer are unlikely (<10%) to have a family history of cancer (52–54). This demonstration of reduced disease penetrance may in turn limit the referrals and thus testing of cancer patients of African ancestry.

After reviewing the testing each patient received, 36 (37.5%) were deemed insufficient due to the limitations of testing (e.g., Founder mutation testing, or *BRCA1/2* sequencing only) and would benefit from more comprehensive testing. Instances of insufficient testing occur when, based on personal and familial cancer history, the initial testing conducted did not include all the appropriate genes which could be responsible for the cancer history. Of these, 17 (47.2%) were public patients and 19 (52.8%) private patients. In public healthcare, families presenting with a history of uterine and colon cancer suggestive of Lynch syndrome do not receive sufficient testing due to the lack of a panel of genes associated with colorectal cancers.

Across the 20-year period, it was noted that the number of patients seen by our genetic services increased substantially. From the year 2000 in intervals of 5 years (e.g., 2000 to 2004 and then 2005 to 2009 etc.) the number of patients seen were 1, 4, 11, 23, and 46 respectively. This increase in patients could be attributed to the expansion of genetic services since its inception in the late 90's (42). Equally important is the increase in visibility of genetic services amongst healthcare providers through the establishment of these clinics (35). Expansion has also followed the trend of improved genetic testing in the public healthcare sector.

4.3. Family variant testing

Nearly all family variant testing was done in private, with only one patient in our cohort having family variant testing in public healthcare. Internationally, the uptake of family variant testing for HCSs by at-risk relatives is considered to be low. Investigations have shown possible reasons include family communication, family history and experience related to cancer, fear of a positive result and accessibility of testing (55–57). Of particular relevance to our cohort is that the testing done in private was most often done through an international laboratory which offered free family variant testing in the even a pathogenic variant was found which is known to significantly improve uptake (55). Although other factors such as family communication cannot be excluded as a cause for the uptake discrepancy between public and private and are beyond the scope of this study.

The diagnostic testing that was done diagnosed 21 patients with a HCS, as a result 40 additional individuals presented for family variant testing, illustrating the attractive cost-effective and large impact diagnostic testing can have. The majority of individuals who tested positive for a familial variant were asymptomatic at the time of testing. This means that in addition to the 21 patients, a further 24 relatives became eligible for increased screening. For those asymptomatic women diagnosed with HBOC syndrome, increased mammography screening has been shown to reduce overall breast cancer mortality by up to 19% (58). In addition, a prophylactic mastectomy could reduce breast cancer mortality by between 81% and 100%; while prophylactic BSO is associated with a decreased incidence of both breast and ovarian cancer together with a reduction of all-cause mortality by between 55% and 100% (59). These surgeries can be considered in asymptomatic women diagnosed with Lynch syndrome but should be tailored based on the patient's causative gene, family cancer history, comorbidities, whether childbearing is complete and in the case of BSO, menopause status (60). Due to their prevalence and treatability, population-based screening to identify asymptomatic carriers by genetic testing has been proposed for Lynch syndrome and HBOC syndrome (44). The challenges that would need to be overcome in the resource limited context of South Africa, including cost, obtaining informed consent, and variant interpretation, make this far from feasible. Predictive testing of at-risk family members, however, stands as an attractive, cost-effective alternative which should be considered (24).

4.4. Risk assessment tools

Of the four risk assessment methods utilized in this study, manual, CANRisk and Manchester scoring assessments all significantly increased the chances of detecting a PV. Manual risk assessments were the least specific of the three, labelling almost 90% of patients as being 'high risk' and eligible for genetic testing. The advantage, however, is this method can be readily applied in a genetic counselling session and allows the counsellor to adjust risk criteria. In particular, Indigenous African patients are held to a less stringent criteria due to the observation of a higher degree of incomplete penetrance (52–54). The Manchester score is similarly convenient in terms of ease of use in clinic, performing similarly to a manual assessment in the detection of PVs in high-risk groups (34.2% versus 32.8%) but only calculates the risk of a PV in *BRCA1* and *BRCA2* and was validated in a European population (9). The authors of the Manchester score note, however, adjustments could be made to accommodate populations of lower penetrance by decreasing the score necessary to reach the 10% risk threshold for testing.

CANRisk was the most specific risk assessment tool and produced the highest proportion of positive test results, with the risk of finding a PV being 6-fold higher in those who were assessed as high risk, increasing the diagnostic yield of testing from 32.8% using manual risk assessment to 43.8%. This improved accuracy is likely be due to the assessment making use of extensive personal medical information (age of menarche and menopause, age at the birth of first child, exposure to contraception and/or hormone replacement therapy etc.) in addition to family history (10). This does, however, make it time-consuming. Another possibility is the lack of population diversity in our sample, with most patients being of European ancestry and includes a marked proportion of patients from high-risk populations for which the online tool has already been validated (10,12,13).

A specific challenge encountered when using CANRisk was the requirement of dates of birth in addition to age of diagnosis for those affected with cancer. In many cases, especially in grandparental generations, birth dates were not listed which meant a 25-year generation gap was assumed to perform the calculation. If a sibling did not have a date or birth noted, a 5-year age difference was assumed. Furthermore, the program did not allow for half-siblings (the same genetic similarity as a second degree relative) and so affected half-siblings were incorporated as full siblings according to the general principle of a false positive screen being less detrimental than a false negative (59). Using logistic regression, we determined that for our cohort, an increase of 1% for the CANRisk risk value led to a 6% increase in the odds of finding a PV. An explanation for this overestimation could be 1) the small sample size, with only 64

patients (83.1% of those who were tested) eligible for a CANRisk assessment; 2) that the cohort was primarily European ancestry; 3) the sample was skewed by the nature that all patients had received testing thus was enriched for high-risk individuals.

The $PREMM_5$ assessment tool follows a similar simplicity and ease of use to that of the Manchester score, making it convenient and accessible in clinic, though it requires internet access. Unlike Manchester score and CANRisk, this tool assesses the risk of having a PV in the mismatch repair genes and *EPCAM* associated with Lynch syndrome. In our cohort the effect of $PREMM_5$ risk assessment was determined not to have a statistically significant effect on testing outcomes, however, the power of analysis was limited as only 35 risk assessments were performed. Another confounding factor is that 20% of these patients did not receive sufficient testing (i.e., not tested for mismatch repair genes and *EPCAM*) and so additional positives may have been missed.

4.5. Limitations

This is a retrospective study performed at a single institution with inherent selection bias, including the error that may be associated with relying on patient recall in a counselling session. Patient records that were incomplete or unobtainable limited the number of patients that could be included. The relatively small patient cohort and the large data collection period may have reduced the power to detect some of the associations between patient factors and genetic testing outcomes, in particular genetic testing availability was not consistent across the collection period. The small patient cohort also limited the extent to which the effect patient characteristics had on testing could be investigate. Furthermore, our sample included predominantly Caucasian patients of either European or Afrikaner ancestry and so is unlikely to be representative of the genetic testing outcomes for indigenous African patients with a personal or family history of gynaecological cancer.

5. Conclusion

With the advancement of genetic testing in the era of sequencing and improved understanding of cancer heritability, an increasing number of cancer patients are referred to Genetic services. Genetic testing can identify those with an inherited cancer syndrome that puts them at risk for various cancers depending on the syndrome which then aids in patient care and management. Effective genetic testing in a resource-limited setting requires suitable referral of patients with

risk factors for hereditary cancer syndromes, accurate risk evaluation using available online assessment tools and for the type of testing to be guided by these assessments. From this study we found Indigenous African patients with gynaecological cancer are less frequently attending Genetic services. Furthermore, the genetic testing in public healthcare is inadequate for detecting hereditary cancer syndromes associated with gynaecological cancers pointing to the need of implementing a colon cancer gene panel. Highlighting the need for comprehensive genetic testing in Indigenous African patients with gynaecological cancers as well as the need to investigate reasons for poor referral so that they may be addressed.

6. Data availability public statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

7. Ethics public statement

This study involved the use of patient data which were reviewed and approved by the Human Research Ethics Committee (HREC) at the University of the Witwatersrand (clearance number: M230313). Written informed consent for participation was not required for this study in accordance with the institutional requirements.

8. Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

9. Author contributions

Conceptualization, BR and EG. Data curation, formal analysis and investigation, SB. Writing – original draft preparation, SB. Writing – review and editing, BR and EG. Supervision, BR and EG. All authors contributed to the article and approved the submitted version.

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Appendices

Appendix A: Approved research protocol

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

FACULTY OF HEALTH SCIENCES

CANDIDATE'S SURNAME: **Barnard**	FIRST NAME/S: **Sebastian**	STUDENT NUMBER: **708672**	
CURRENT QUALIFICATIONS: **BSc (Hons) Genetics**			
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DEGREE FOR WHICH PROTOCOL IS BEING SUBMITTED: **MSc (Med) Genetic Counselling**			
PART-TIME OR FULL-TIME: **Full-time**			
FIRST REGISTERED FOR THIS DEGREE:	TERM : **1**	YEAR: **2022**	
DEPARTMENT: **Human Genetics**			
TITLE OF PROPOSED RESEARCH: **A review of genetic testing for females referred to genetic counselling for a personal or family history of gynaecological cancer**			
CANDIDATE'S SIGNATURE:		DATE: **29/01/2023**	
SUPERVISOR 1 (NAME & SURNAME): **Bianca Rossouw**		% Supervision: **50%**	
SUPERVISOR'S QUALIFICATIONS: **MSc (Med) Genetic Counselling**			
SUPERVISOR'S DEPARTMENT: **Human Genetics**			
SUPERVISOR'S ADDRESS / TEL / E-MAIL: **Room 1, First floor, Jack Metz Building, Cnr Hospital & De Korte st, Braamfontein [T]: 011 489 9151/9223 | [E]: elzette.nienaber@nhls.ac.za**			
SUPERVISOR 2 (NAME & SURNAME): **Elzette Gilfillan**		% Supervision: **50%**	
SUPERVISOR'S QUALIFICATIONS: **MSc (Med) Genetic Counselling**			
SUPERVISOR'S DEPARTMENT: **Human Genetics**			
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SUPERVISOR 3 (NAME & SURNAME):		% Supervision	
SUPERVISOR'S QUALIFICATIONS			
SUPERVISOR'S ADDRESS / TEL / E-MAIL:			

SYNOPSIS OF RESEARCH:(Brief summary of proposed research project; between 200-300 words only; with subheadings: an introduction and justification for study, aim/s, proposed methodology and expected outcome/s)

Introduction & Justification:

The burden of cancer is rising with gynaecological cancers, those affecting the female reproductive system, ranking as the fourth most frequently diagnosed cancer worldwide. An important proportion of these cancers are hereditary, caused by pathogenic variants in specific genes. Three common hereditary cancer syndromes (HCSs), including Hereditary Breast and Ovarian Cancer syndrome, Hereditary Ovarian Cancer syndrome, and Lynch syndrome, result in a significantly increased risk of developing gynaecological cancers. Diagnosing a HCS through genetic testing can enable risk reducing prophylactic surgery, chemoprevention and more appropriate cancer screening for both the affected individual and all relatives who test positive for the same pathogenic variant. To date there is no published data on the prevalence of known mutations in cancer causing genes associated with gynaecological cancers in South Africa.

Aim:

The aim of this study is to conduct an audit of genetic testing and the associated clinical presentation of patients with either a diagnosis or family history of gynaecological cancer(s).

Methodology:

The study population will include at least 80 patients seen by the Division of Human Genetics at affiliated state and private hospitals during the last 20 years (2002-2022). Pertinent patient information including basic demographics, medical and family history, and genetic testing results will be gathered, in addition multiple risk assessments for each patient will be performed. Statistical analysis will be performed to evaluate whether patient factors (e.g., family history or risk assessments) correlate with a genetic diagnosis of a HCS causing gynaecological cancer. We will also assess whether adequate genetic testing is available to patients based on risk assessments and the impact of comprehensive genetic testing.

Expected outcomes:

We expect genetic testing provided to state patients who present with gynaecological cancer to be insufficient, highlighting a need to include additional genes to the routine genetic testing offered as a means of reducing the cancer burden in South Africa.

WITS ETHICS NOT REQUIRED: Yes (No) WITS ETHICS PENDING: Yes (No) WITS ETHICS APPROVED: Yes No (circle appropriate symbol)* ETHICS NUMBER HERE:	IF Y SUPPLY ETHICS: CLEARANCE CERTIFICATE AS ATTACHMENT AND INCLUDE

***Please note the final human ethics clearance certificate or animal ethics certificate must be available prior to starting research**

As supervisor/s, I/we confirm that I have read the protocol which has been submitted for assessment.

SIGNATURE OF SUPERVISOR/S:	B. Rossouw	Gilfillan
SIGNATURE PG OFFICE STAFF ……..	REGISTERED YES….. NO…..	STAMP

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PLAGIARISM DECLARATION TO BE SIGNED BY ALL HIGHER DEGREE STUDENTS

SENATE PLAGIARISM POLICY: APPENDIX ONE

I **Sebastian Barnard** (Student number: **708672**) am a student registered for the degree of

MSc (Med) Genetic Counselling in the academic year 2023.

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.

Signature:  Date: 31/01/2023

A review of genetic testing for females referred to genetic counselling for a personal or family history of gynaecological cancer

Sebastian Barnard

708672

MSc (Med) Genetic Counselling

Supervisor 1:

Bianca Rossouw

MSc (Med) Genetic Counselling, Genetic Counsellor and Lecturer, Clinical and Counselling Section, Division of Human Genetics, School of Pathology, Faculty of Health Sciences, University of the Witwatersrand

Supervisor 2:

Elzette Gilfillan

MSc (Med) Genetic Counselling, Genetic Counsellor and Lecturer, Clinical and Counselling Section, Division of Human Genetics, School of Pathology, Faculty of Health Sciences, University of the Witwatersrand

1. Background literature analysis and critique

1.1. Introduction

With the improvement of healthcare and quality of life increasing the average lifespan the global burden of non-communicable diseases, a predominant component being cancer, is on the rise. GLOBOCAN 2020 reported an estimated 19.3 million new cancer cases and almost 10 million cancer deaths to have occurred worldwide in 2020. New cases of uterine body, cervix, ovary, vagina, and vulva cancers together constituted 1.3 million cases making gynaecological cancers the fourth most frequently diagnosed cancer worldwide, accounting for 15.3% of cancer deaths in women (Sung et al., 2021). A proportion of gynaecological cancers are caused by hereditary cancer syndromes (HCSs) (Walsh et al., 2020). South African studies on HCSs have mostly focused on breast cancer and produced valuable contributions to the discovery of genetic differences in various native populations, as well as screening and testing procedures (Gardiner et al., 2019; Schoeman et al., 2013; Seymour et al., 2016). However, there is a dearth of research into genetic testing for gynaecological cancers, detection rates and causative mutations. With gynaecological cancers, particularly ovarian cancer, mostly being diagnosed in advanced stages, genetic testing may have a role in early detection and reducing the overall burden of these cancers (Walsh et al., 2020).

1.2. Gynaecological cancers

In total there are six gynaecological cancers: cervical, endometrial, ovarian, vaginal, vulva, and fallopian tube cancer. Of these cervical, endometrial, and ovarian cancers are the most commonly diagnosed gynaecological cancers in South Africa (SA) (Figure 1) (Ferlay et al., 2020). Despite a lower risk of females from SA to develop cancer than for those from Northern America or Europe, the risk of dying from cancer is significantly higher (Ferlay et al., 2020). These high rates of cancer mortality in SA can be partly attributed to poor access to

cancer prevention, screening, diagnosis, and treatment which in turn produce poorer health outcomes (Department of Health Republic of South Africa, 2017).

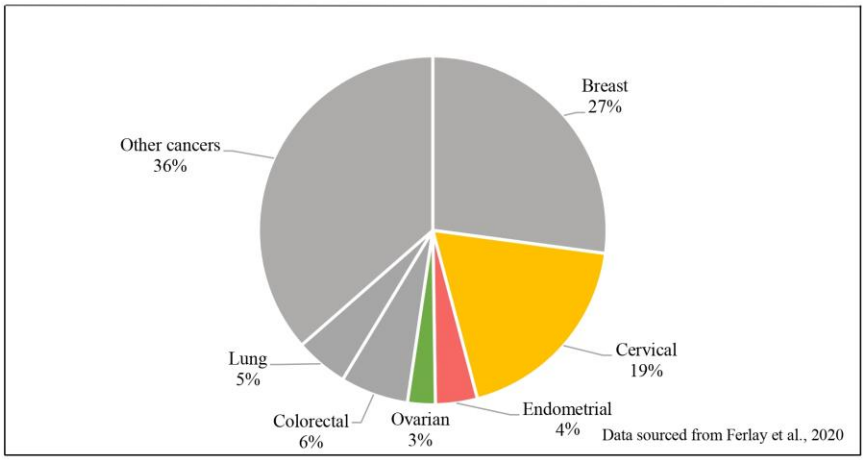

Figure 1. Number of new female cancer cases reported in South Africa for 2020, gynaecological cancers highlighted.

1.3. Hereditary gynaecological cancer syndromes

Although all cancers are genetic, in that they are the result of mutations (now referred to as pathogenic variants) in genes that regulate the cell cycle or are involved in DNA repair; some cancers are heritable, being caused by a germline pathogenic variant present in the gamete which goes on to reside in every daughter cell of the body, subsequently predisposing an individual to cancer of multiple tissues/organs; this is the basis of HCSs (Tinley & Lynch, 2000). These HCSs exhibit autosomal dominant inheritance with affected individuals having a 50% risk of passing on the pathogenic variant to offspring (Walsh et al., 2020). Several genes are known to cause HCSs associated with gynaecological cancers, most notably: Hereditary breast and ovarian cancer (HBOC) syndrome (Dark blue circle, figure 2), Hereditary ovarian cancer (HOC) syndrome (Red circle, figure 2), and Lynch syndrome (LS) (Brown circle, figure 2). Importantly, each gene is not only associated with certain cancers but also confers a distinct risk for developing them which sits well above population risk. Pathogenic variants in *BRAC1*

and *BRCA2* for example are found in up to 55% of cases of hereditary breast and ovarian cancers, where *BRCA1* increases the lifetime risk for developing ovarian cancer to 44% as opposed to the general population risk of 1,2% while *BRCA2* raises the risk to 17% (Figure 2) (Toss et al., 2015; Walsh et al., 2020). Up to 70% of Lynch syndrome is caused by pathogenic variants in one of four mismatch repair genes, each conferring a substantial (31% - 49%) risk for endometrial cancer where the general population risk is 3,1% in addition to an elevated risk for ovarian cancer (Figure 2) (Walsh et al., 2020). Two less common HCSs also known to cause gynaecological cancers are Peutz-Jeghers syndrome caused by *STK11* pathogenic variants (Pink circle, figure 2) and Cowden syndrome caused by *PTEN* pathogenic variants (Light blue circle, figure 2).

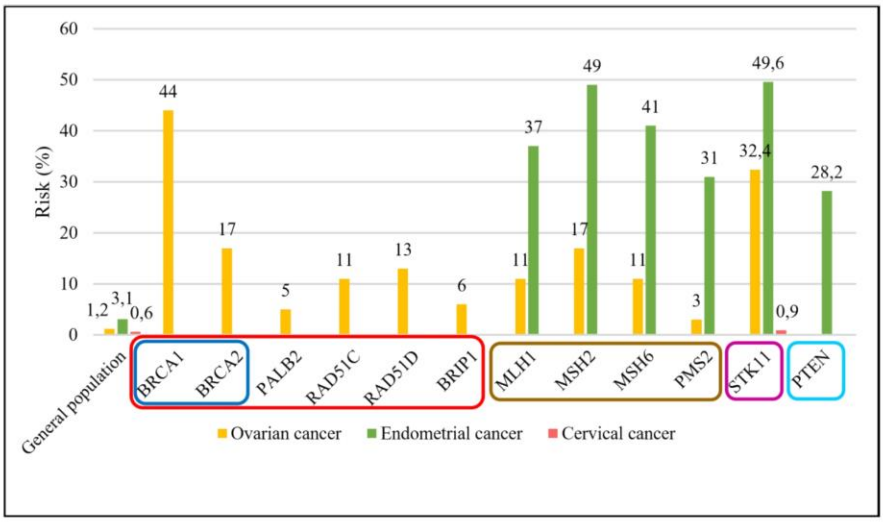

Figure 2. Lifetime risk of gynaecological cancers for those with Hereditary Breast and Ovarian Cancer syndrome, Hereditary
Ovarian Cancer syndrome, Lynch, Peutz-Jeghers and Cowden syndrome.
This figure illustrates the risk of developing endometrial, ovarian, and/or cervical cancer associated with specific genes known to cause hereditary cancer syndromes compared to that in the general population. The genes are grouped by the hereditary cancer syndrome they cause: Hereditary Breast and Ovarian Cancer syndrome (dark blue circle), Hereditary Ovarian Cancer syndrome (red circle), Lynch syndrome (brown circle), Peutz-Jeghers syndrome (pink circle) and Cowden syndrome (light blue circle). Data sourced from Beggs et al., 2010; Kalra et al., 2022; Lifetime Risk of Developing and Dying from Cancer, 2016-2018, 2022.

1.4. Risk assessment

Before genetic testing for a HCS can be considered a risk assessment is carried out to estimate how likely a patient is to be a carrier for a germline pathogenic variant in one of the causative genes (Daly et al., 2020). A risk assessment can be carried out based on a patient's personal and family history of cancer alone, termed a manual risk assessment, although additional tools are available online which quantify the risk of a germline pathogenic as a percentage, these include the Manchester Scoring System, CanRisk and PREMM5. For a manual risk assessment, factors that increase the risk include: young age of onset for a particular cancer (e.g. < 60 years for ovarian cancer); multiple related cancers in the family and/or the patient (e.g., breast, ovarian and pancreatic cancer in the case of HBOC); bilateral cancer of paired organs; multiple primary cancers in one individual; and/or high-risk ancestry (Afrikaner, Xhosa or Ashkenazi Jewish). The Manchester Scoring System uses cancer histology in addition to family history to calculate the risk for germline pathogenic variants in *BRCA1* or *BRCA2*, patients with a 10% or higher risk are eligible for genetic testing (Evans et al., 2017). CanRisk uses family history, cancer histology and personal risk factors to calculate both cumulative and individual risks for *BRCA1*, *BRCA2, PALB2, CHEK2, ATM, RAD51C, RAD51D* and *BRIP1* (Lee et al., 2019). PREMM®5 uses personal and family history to calculate the cumulative risk of carrying a pathogenic variant in one of the four Lynch syndrome genes (Kastrinos et al., 2017).

1.5. Genetic testing in South Africa

Genetic testing for HCSs began in 2005 but was limited to *BRCA1* and *BRCA2* in state healthcare (Schoeman et al., 2013). Only recently has genetic testing expanded to include eight genes on a panel, allowing for more time- and cost-effective sequencing of these genes in parallel for pathogenic variant detection, which is offered through the National Health

Laboratory Service (NHLS) in Bloemfontein. This panel includes *BRCA1* and *BRCA2* as well as *ATM, BARD, BRIP1, CDK12, CHEK2* and *PALB2*. The limitation of this panel is that only half of these genes are associated with hereditary gynaecological cancers (*BRCA1, BRCA2, BRIP1, PALB2*) and none of the genes causing Lynch syndrome are included. Nevertheless, the introduction of a broader panel has increased the detection rate of actionable pathogenic variants (van der Merwe et al., 2022). For private patients more comprehensive testing for HCSs has been available for much longer through private laboratories both locally (e.g., AMPATH and Lancet) and internationally (e.g., Invitae).

1.6. Clinical implications of hereditary cancer syndromes

When a HCS is diagnosed additional preventative measures can be offered which extend beyond what is required in the general population. These include prophylactic surgery, chemoprevention, and more appropriate screening for additional cancers yet to develop. Prophylactic salpingo-oophorectomy reduces the risk of ovarian cancer by 80% in females with hereditary ovarian cancers and prophylactic hysterectomy can reduce the endometrial cancer risk in patients with Lynch syndrome by up to 18% (Dominguez-Valentin et al., 2021; Hartmann & Lindor, 2016). Personalized and more extensive screening can begin at earlier ages to improve the likelihood of cancer detection (Walsh et al., 2020). Genetic testing also enables women to make lifestyle, contraceptive and reproductive choices that reflect their genetic status (Kalra et al., 2022). Lastly, genetic testing can direct management and care for patients, an example being poly(ADP-ribose) polymerase (PARP) inhibitors which are a wellestablished therapy for ovarian cancer caused by *BRCA1* or *BRCA2* (Fong et al., 2010).

The benefit of genetic testing can also extend to family members. Once a germline pathogenic variant is identified, relatives who are at risk of having inherited the variant can be offered expedited and less costly genetic testing to determine their genetic status. This would be

considered predictive in those who do not have cancer. For all who test positive (i.e., carrying the same pathogenic variant) the same preventative measures mentioned above could be offered even in the absence of cancer. Enhanced screening and preventative prophylaxis in relatives yet to develop cancer serves an effective means of reducing cancer incidence and mortality.

1.7. Rationale

To date there is no published data on the prevalence of known mutations in cancer causing genes associated with ovarian cancer in SA despite evidence of its incidence increasing (Gizaw et al., 2022). The same sentiment can be extended to all hereditary gynaecological cancers in SA. Given that gynaecological cancers often do not present with symptoms or present with vague symptoms that are dismissed by women and generally have ineffective screening, genetic testing is a potential answer to the growing burden of these cancers. To stimulate genetic research into gynaecological cancers first data is required on uptake of patients with gynaecological cancers for genetic testing and their testing results. Therefore, the aim of this study is to conduct an audit of genetic testing and associated clinical presentation in patients seen by the Division of Human Genetics at the and the University of the Witwatersrand (Wits) with a confirmed diagnosis or family history of gynaecological cancer and assess whether adequate genetic testing is available to patients, how testing has changed over time and whether patient factors (histology, family history, risk assessment, ect.) correlate with a positive genetic test result.

2. Aims and objectives

2.1. Aim

To conduct an audit of genetic testing and associated clinical presentation in patients seen by the Division of Human Genetics (NHLS/Wits) with either a confirmed diagnosis or family history of gynaecological cancer.

2.2. Objectives

1. To review the files of patients who meet inclusion criteria that were seen for genetic counselling.
2. To assess the genetic testing that was available for each patient and evaluate the results for patients who consented for testing.
3. To evaluate the uptake of cascade testing in families diagnosed with a hereditary gynaecological cancer.
4. To assess the predictive value of risk assessments tools and whether patients received sufficient testing as indicated by their risk assessment/s.
5. To evaluate associations between patient factors (such as age, ethnicity, cancer histology, and risk assessment score) and genetic test results.

3. Methods

3.1. Study population

The study sample will include all females who received genetic counselling, either by a registered medical geneticist, genetic counsellor, or genetic nurse, for a confirmed diagnosis or family history of gynaecological cancer between 2000 and 2022 through the Division of Human Genetics (NHLS/Wits) at one of the affiliated hospitals, namely: Chris Hani Baragwanath Hospital, Rahima Moosa Mother and Child Hospital, Helen Joseph Hospital, Charlotte Maxeke Johannesburg Academic Hospital, and Donald Gordon Medical Centre. We expect a sample size of approximately 80-100.

3.1.1. Inclusion criteria

1. State and private patients with either:
    a. A confirmed diagnosis of a gynaecological cancer.
    b. Family history of gynaecological cancer/s.

2. Adult and paediatric patients.
3. Patients seen between January 2000 and December 2022.

3.1.2. Exclusion criteria

1. Cases where family history is non-specific e.g., unspecified gynaecological cancer type.

3.2. Study design

This study is a retrospective case review, requiring an audit of patients' genetic counselling records. The principal investigator will create a database of pertinent information from patients meeting inclusion criteria. Relevant records will be reviewed for patient demographics, family history, relevant medical history including diagnosis and histology, genetic testing, and uptake of cascade testing (see appendix 1). The information will be accessed from the Division of Human Genetics Research Electronic Data Capture (REDCap) clinical database, or physical patient files located at the Division of Human Genetics, NHLS Braamfontein. This study is reliant on patient recollection for family history which is self-reported and documented on family pedigrees. Records with vague cancer histories such as "womb cancer" or insufficient information will be excluded. Appropriate cases for inclusion identified by the principle investigator will be reviewed by senior supervisors before data analysis. Family history information will not be confirmed by other means. In addition, the principal investigator will perform both qualitative and quantitative risk assessments for each patient, the former by manual assessment of family history while the latter by the Manchester Scoring System, CanRisk and/or PREMM5 online tools. A detailed outline of the data to be collected can be found in Appendix 1. The data collected will be anonymised and subsequently coded to maintain patient confidentiality.

3.3. Data analysis

Data collected for this study will be analysed quantitatively. Continuous data (e.g., age at consultation) will be tested for normality using the Shapiro-Wilk test and if the null hypothesis is met, described using means and standard deviations, otherwise continuous data will be reported using median and interquartile range. Categorical data (e.g., type of genetic testing performed) will be described using either percentages or frequencies. Chi-square tests will be applied to investigate potential associations between age groups, tumour histopathology (if available, family history, cancer type, and genetic test results. Logistic regression analysis will be applied to evaluate the predictive power of online risk assessment tools in our sample. Statistical significance to be set at the P value < 0.05. Statistical analysis will be done using R© v 4.2.2 using the *Basic Statistics and Data Analysis* package and Excel® Microsoft 365.

4. Ethics

Ethics approval is required in order to make use of data recorded within patient records. The data will be anonymised and subsequently coded with only the researcher and their supervisors able to access identifying information. Submission to the Human Research Ethics Committee (Medical) of the University of Witwatersrand is planned for February 2023. Data collection is to commence once ethical clearance is granted.

5. Timing

	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov
Literature Review													
Preparing protocol													
Protocol assessment													
Ethics application													
Collecting data													
Data analysis													
Writing up – Thesis													
Writing up - Paper													

6. Funding

6.1. Predicted budget

Activity	Cost
REDCap software and Excel® for Microsoft 365	R0.00
No transport costs, files available on site (NHLS)	R0.00
Printing and binding of research report (~80 pages) at 62c/page (2 copies)	R99.20
	R99.20

7. Study limitations

By nature of study design the analysis of this study is dependent on the review of patient records. This study may be limited by missing patient records and/or omitted patient data due to data collection during a genetic counselling session not being completely standardized. By choosing to include patients who themselves did not present with a gynaecological cancer but instead had a family history thereof increases the sample size but introduces the possibility for confounding data. A larger sample size is desirable for robust statistical analysis, however, family history criteria for inclusion will need to be strict to not skew genetic testing data away from gynaecological cancers. Lastly, given how genetic testing has evolved, the testing offered to patients has changed with time leaving broad comparisons across time misleading, however, by factoring in this as a consideration we hope to gain valuable insights into whether comprehensive testing has utility in our resource-limited setting.

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UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

FACULTY OF HEALTH SCIENCES

PROTOCOL ASSESSORS MEETING

Candidate Full Name: . . . . . Sebastian Barnard . . . . . .

Student Number: . . . . . 708672 . . . . . Date: . . . . 22nd February 2023 . . . . . .

School / Department / Division: . . . . . Pathology / Human Genetics . . . . . .

1. Type of study *(tick all that apply)*:

X Quantitative
- o Qualitative
- o Mixed Methods
- o Laboratory
- o Clinical
- o Other, please specify……

2. Is title of the study appropriate (*preferably fewer than 20 words*)**?** Yes

Comments: _____

3. Are the study objectives clear and linked to the research aim and title? Yes

Comments: _____

4. Is the design of the study appropriate to meet the study objectives? Yes

Comments: _____

5. **Are the proposed methods and tools appropriate to meet the research objectives?** Yes, but...

Comments (the following changes should be made to the 'Methods' section of the protocol unless otherwise stated):

- In the 'Data analysis' section it states that 'The analysis of variance (ANOVA) method will be used to assess correlations between genetic test results (positive/negative) and patient factors (age group; ethnicity; cancer type; risk assessment score).' ANOVA is not the correct test for these analyses. Data will be compared between subjects with a positive or negative genetic test result and the stats test used will depend on the data type. Thus, for a continuous variable like age it would be a Student t test or a Mann Whitney U test depending on data distribution. For ethnicity it will be a chi squared test, possibly using contingency tables if there are more than 2 ethnic groups. Will logistic regression be performed? Please clarify.
- Data will be used for subjects with a family history of cancer. How will this family history be verified?

6. **Is the study feasible within the resources of:**
   a) The applicant? Yes
   b) The department? Yes
   c) The time frame? Yes

7. **If this is a PhD protocol assessment:** **Not applicable**
   a) Is the content original? Yes No
   b) Does the content show the scope and depth of a PhD? Yes No

Comments: _____

_____

_____

_____

Do you recommend:

i. Additional revision/amendment of the protocol? Please be specific on the recommendations being made:

_____

_____

_____

ii. The appointment of the proposed Supervisor? Yes

Nominee/s: _____

_____

_____

11 March 2019/MP

iii. The appointment of the proposed Co-Supervisor/s and/or additional co-supervisors? Yes

Nominee/s: _____

_____

_____

iv. Has the Chair of the Assessor Group signed the RECOMMENDATION FOR APPOINTMENT OF SUPERVISOR(S) OF RESEARCH REPORT, DISSERTATION OR THESIS form? Please attach. Yes

v. Has the Chair informed the student and supervisor about the Wits ethics requirements, and that if required, they must have either a Wits Human Research Ethics clearance certificate or a Wits Animal Research Ethics clearance certificate? Yes

vi. Based on the protocol provided (including any proposed changes by the protocol assessor group), does the student require:

1.	Human Research Ethics clearance certificate	Yes
2.	Animal Research Ethics clearance certificate	No
3.	No human or animal ethics certificate is required	No
4.	Unclear, will seek appropriate guidance from the HREC/AREC committees	No

vii. Has the Postgraduate student and supervisor/s signed the ethics declaration form Yes

Overall recommendation regarding the protocol:

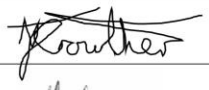
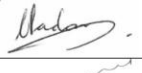
i. Revision of the protocol to the satisfaction of the Supervisor (**NB: if HoD approval is also required, please specify**): **Yes**
*(**Candidate**: one copy, list of corrections with page numbers and Supervisor approval letter – submit to PG Office).*

ii. Revision of the protocol to the satisfaction of the Assessor Group/Chair: No
*(**Candidate**: one copy, list of corrections with page numbers, Supervisor approval letter – submit to PG Office and PG Office to forward to the Assessor Group Chair).*

iii. Revision of the protocol and resubmission of the revised protocol to the next Assessor Group Meeting: No
*(**Candidate**: six copies, list of corrections with page numbers, Supervisor approval letter – submit one copy to PG Office / 5 to school assessor group administrator / for PhD, all six copies to be submitted to the PG Office).*

iv. Candidate goes ahead (*no revision required*): No

Details of Assessors:

Name:	Email:	Sign:
Nigel Crowther	nigel.crowther@nhls.ac.za	
Carolyn Padoa	carolyn.padoa@nhls.ac.za	
Nitien Naran	nitien.naran@nhls.ac.za	

Details of Assessor Group Chair:

Name:	Email:	Sign:
Nigel Crowther	nigel.crowther@nhls.ac.za	

Date: 22nd February 2023

Faculty of Health Sciences, Postgraduate Office
Phillip V Tobias Building, 2nd Floor
Cnr York & Princess of Wales Terrace, Parktown 2193
Tel: (011) 717 2745 | Fax: (011) 717 2119
Email: Kgomotso.Tlala@wits.ac.za

Dear Kgomotso Tlala,

RE: Protocol amendments approved by Supervisors

This letter serves to formally approve the proposed amendments Sebastian Barnard, student no. 708672, has made to the protocol for his Masters project titled *A review of genetic testing for females referred to genetic counselling for a personal or family history of gynaecological cancer*. These revisions are in accordance with the feedback he had received from the assessors of his protocol defence on the 22nd of February 2023. Kindly find attached the amended protocol to be submitted to the Postgraduate Office.

Bianca Rossouw
MSc (Med) Genetic Counselling, Genetic Counsellor and Lecturer, Clinical and Counselling Section, Division of Human Genetics, School of Pathology, Faculty of Health Sciences, University of the Witwatersrand

Elzette Gilfillan
MSc (Med) Genetic Counselling, Genetic Counsellor and Lecturer, Clinical and Counselling Section, Division of Human Genetics, School of Pathology, Faculty of Health Sciences, University of the Witwatersrand

List of amendments:

Section heading	Page number	Paragraph	Line	Amendment
3.2 Study design	8	1	7	This study is reliant on patient recollection for family history which is self-reported and documented on family pedigrees. Records with vague cancer histories such as "womb cancer" or insufficient information will be excluded. Appropriate cases for inclusion identified by the principle investigator will be reviewed by senior supervisors before data analysis. Family history information will not be confirmed by other means.
3.3 Data analysis	9	1	5	Chi-square tests will be applied to investigate potential associations between age groups, tumour histopathology (if available), family history, cancer type, and genetic test results. Logistic regression analysis will be applied to evaluate the predictive power of online risk assessment tools in our sample.

Appendix B: REDCap data collection

Return to methods.

Demographics

Hospital	_____
Age at Consultation	_____
Has the patient demised?	◯ Yes ◯ No
Population group	◯ Asian ◯ Black ◯ Indian ◯ Mixed Ancestry ◯ Other
Specify Population_Group	_____
Ethnolinguistic Group	☐ Afrikaner ☐ Ashkenazi Jewish ☐ European ☐ Hindi ☐ Muslim ☐ Ndebele ☐ Sepedi ☐ Sotho ☐ Swazi ☐ Tsonga ☐ Tswana ☐ Venda ☐ Zulu ☐ Xhosa ☐ Other ☐ Unknown
Specify Ethnolinguistic Group	_____
State or Private Patient	◯ State ◯ Private

Medical & Family History

What has the proband presented with?	◯ Proband has a gynaecological cancer ◯ Proband has a family history of gynaecological cancer only ◯ Both the proband and their relatives have gynaecological cancers
What type of gynaecological cancer?	◯ Cervical cancer ◯ Endometrial cancer ◯ Ovarian cancer ◯ Fallopian tube cancer ◯ Peritoneal cancer ◯ Other
Histology of cervical cancer	◯ Adenoma malignum ◯ Other ◯ Unknown
Specify type of cervical cancer:	_____
Histology of endometrial cancer	◯ Endometrioid adenocarcinoma ◯ Mucinous carcinoma ◯ Serous carcinoma ◯ Clear cell carcinoma ◯ Squamous cell carcinoma ◯ Undifferentiated carcinoma ◯ Mixed carcinoma ◯ Metastatic carcinoma ◯ Other ◯ Unknown
Specify type of endometrial cancer:	_____
Histology of ovarian cancer	◯ Serous carcinoma ◯ Mucinous carcinoma ◯ Endometrioid carcinoma ◯ Clear cell carcinoma ◯ Transitional cell carcinoma (Brenner tumors) ◯ Carcinosarcoma ◯ Epithelial carcinoma ◯ Undifferentiated carcinoma ◯ Other ◯ Unknown

How many maternal relatives have a history of gynaecological cancer?	◯ 1 ◯ 2 ◯ 3 ◯ 4 ◯ 5 ◯ 6 ◯ 7 ◯ 8 ◯ 9 ◯ 10 ◯ >10
How many first degree relatives?	◯ 1 ◯ 2 ◯ 3 ◯ 4 ◯ 5 ◯ 6
How many second degree relatives?	◯ 1 ◯ 2 ◯ 3 ◯ 4 ◯ 5 ◯ 6
How many third degree relatives?	◯ 1 ◯ 2 ◯ 3 ◯ 4 ◯ 5 ◯ 6
How many maternal relatives had a history of cervical cancer?	
How many maternal relatives had a history of endometrial cancer?	◯ 1 ◯ 2 ◯ 3 ◯ 4 ◯ 5 ◯ 6 ◯ 7 ◯ 8 ◯ 9 ◯ 10
How many maternal relatives had a history of ovarian cancer?	◯ 1 ◯ 2 ◯ 3 ◯ 4 ◯ 5 ◯ 6 ◯ 7 ◯ 8 ◯ 9 ◯ 10

How many paternal relatives have a history of gynaecological cancer?	◯ 1 ◯ 2 ◯ 3 ◯ 4 ◯ 5 ◯ 6 ◯ 7 ◯ 8 ◯ 9 ◯ 10 ◯ >10
How many first degree relatives?	◯ 1 ◯ 2 ◯ 3 ◯ 4 ◯ 5 ◯ 6
How many second degree relatives?	◯ 1 ◯ 2 ◯ 3 ◯ 4 ◯ 5 ◯ 6
How many third degree relatives?	◯ 1 ◯ 2 ◯ 3 ◯ 4 ◯ 5 ◯ 6
How many maternal relatives had a history of cervical cancer?	
How many maternal relatives had a history of endometrial cancer?	◯ 1 ◯ 2 ◯ 3 ◯ 4 ◯ 5 ◯ 6 ◯ 7 ◯ 8 ◯ 9 ◯ 10
How many maternal relatives had a history of ovarian cancer?	◯ 1 ◯ 2 ◯ 3 ◯ 4 ◯ 5 ◯ 6 ◯ 7 ◯ 8 ◯ 9 ◯ 10
Family Pedigree	

Genetic Testing

Was genetic testing accepted?	◯ Yes ◯ No
What genetic testing was performed first?	◯ Founder mutation testing ◯ Sequencing BRCA1/2 with MLPA ◯ Bloem panel testing (8 gene) ◯ Bloem panel testing (15 gene) ◯ Invitae panel ◯ Inherited diseases panel ◯ BROKA study testing ◯ HERCAN study testing ◯ Family variant testing ◯ Local private testing (AMPATH, Lancet ect.) ◯ Other
Please specify the testing performed:	_____
Please specify which genes were included in testing:	☐ ATM ☐ BARD1 ☐ BRCA1 ☐ BRCA2 ☐ BRIP1 ☐ CDH1 ☐ CHEK2 ☐ DICER1 ☐ EPCAM ☐ MLH1 ☐ MSH2 ☐ MSH6 ☐ NF1 ☐ PALB2 ☐ PMS2 ☐ PTEN ☐ RAD51C ☐ RAD51D ☐ SMARCA4 ☐ STK11 ☐ TP53 ☐ Other
What additional genes were included?	_____
Were pathogenic variants identified by testing?	◯ Yes ◯ No

In what gene/s was a mutation found?	☐ ATM ☐ BARD1 ☐ BRCA1 ☐ BRCA2 ☐ BRIP1 ☐ CDH1 ☐ CHEK2 ☐ DICER1 ☐ EPCAM ☐ MLH1 ☐ MSH2 ☐ MSH6 ☐ NF1 ☐ PALB2 ☐ PMS2 ☐ PTEN ☐ RAD51C ☐ RAD51D ☐ SMARCA4 ☐ STK11 ☐ TP53 ☐ Other
In what additional genes were pathogenic variants found?	_____
Specify the pathogentic variant(s) found along with rsID numbers:	_____ (e.g., "BRCA1 rs12345678; ATM rs87654321")
Did at-risk relatives present for testing?	◯ Yes ◯ No
How many at-risk relatives presented for testing?	◯ 1 ◯ 2 ◯ 3 ◯ 4 ◯ 5 ◯ 6 ◯ 7 ◯ 8 ◯ 9 ◯ >9
How many at-risk relatives tested positive for the same pathogenic variant(s)?	◯ 1 ◯ 2 ◯ 3 ◯ 4 ◯ 5 ◯ 6 ◯ 7 ◯ 8 ◯ 9 ◯ >9

Of these, how many were affected with cancer at the time of testing?	◯ 1 ◯ 2 ◯ 3 ◯ 4 ◯ 5 ◯ 6 ◯ 7 ◯ 8 ◯ 9 ◯ 10 ◯ >10
Reason for declining testing:	☐ Financial constraints ☐ Would prefer not to know ☐ No perceived benefit ☐ Not specified ☐ Out of the country ☐ Other
Please specify reason for declining testing:	_____
If initial test results were negative, were additional tests performed?	◯ Yes ◯ No
Would this family have benefited from additional testing?	◯ Yes ◯ No
What follow- up genetic testing was performed?	◯ Founder mutation testing ◯ Sequencing BRCA1/2 with MLPA ◯ Bloem panel testing (8 gene) ◯ Bloem panel testing (15 gene) ◯ Invitae breast & gyne panel (21 genes)
Were the results from the second test positive?	◯ Yes ◯ No
In what gene/s was a mutation found?	☐ BRCA1 ☐ BRCA2 ☐ PALB2 ☐ RAD51C ☐ RAD51D ☐ BRIP1 ☐ MHL1 ☐ MSH2 ☐ MSH6 ☐ PMS2 ☐ STK113 ☐ PTEN4
Specify the mutation found:	_____

Appendix C: Ethics clearance certificate

Return to Methods

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG	HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

Office of the Deputy Vice-Chancellor (Research and Innovation)

TO: Mr S Barnard
School of Pathology
Division of Human Genetics
Medical School
University

E-mail: Sebastian.Barnard@nhls.ac.za

CC: Supervisor: Mesdames B Rossouw and E Gilfillan
<Bianca.Rossouw@nicd.ac.za>
and <HREC-Medical Research Office@wits.ac.za>

FROM: Mr Iain Burns
Human Research Ethics Committee (Medical)
Tel: 011 717 1252

E-mail: Iain.Burns@wits.ac.za

DATE: 2023/05/18

REF: R14/49

PROTOCOL NO: **M230313** (This is your ethics application reference number. Please quote it in all enquiries, oral or written, relating to this study.)

PROJECT TITLE: *A review of genetic testing for females referred to genetic counselling for a personal or family history of gynaecological cancer*

Please find attached the Clearance Certificate for the above project. I hope it goes well and that an article in a recognized publication comes out of it. This will reflect well on your professional standing and contribute to Government funding of the University.

MSWorks2000/Iain0007/Clearscan.wps

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

R49 Mr S Barnard

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
CLEARANCE CERTIFICATE NO. M230313

NAME: (Principal Investigator)	Mr S Barnard
DEPARTMENT:	School of Pathology Division of Human Genetics Medical School University
PROJECT TITLE:	*A review of genetic testing for females referred to genetic counselling for a personal or family history of gynaecological cancer*
DATE CONSIDERED:	2023/03/31
DECISION:	Approved unconditionally
CONDITIONS:	
NOTE:	If contact information regarding student study participants is required, please contact the Registrar's office - <Nicoleen.Potgieter@wits.ac.za>
SUPERVISOR:	Mesdames B Rossouw and E Gilfillan
APPROVED BY:	Dr CB Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	2023/05/18

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**. When a funder requires annual re-certification, the application date will be one year after the date when the study was initially reviewed. In this case, the study was initially reviewed in **March** and therefore reports and re-certification will be due in the month of **March** each year. Unreported changes to the study may invalidate the clearance given by the HREC (Medical).

Signature of Principal Investigator

20/05/2023
Date

Appendix D: Plagiarism declaration

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

FACULTY OF HEALTH SCIENCES

PLAGIARISM DECLARATION TO BE SIGNED BY ALL HIGHER DEGREE STUDENTS

SENATE PLAGIARISM POLICY: APPENDIX ONE

I Sebastian Barnard (Student number: 708672) am a student registered for the degree of MSc (Med) Genetic Counselling in the academic year 2023.

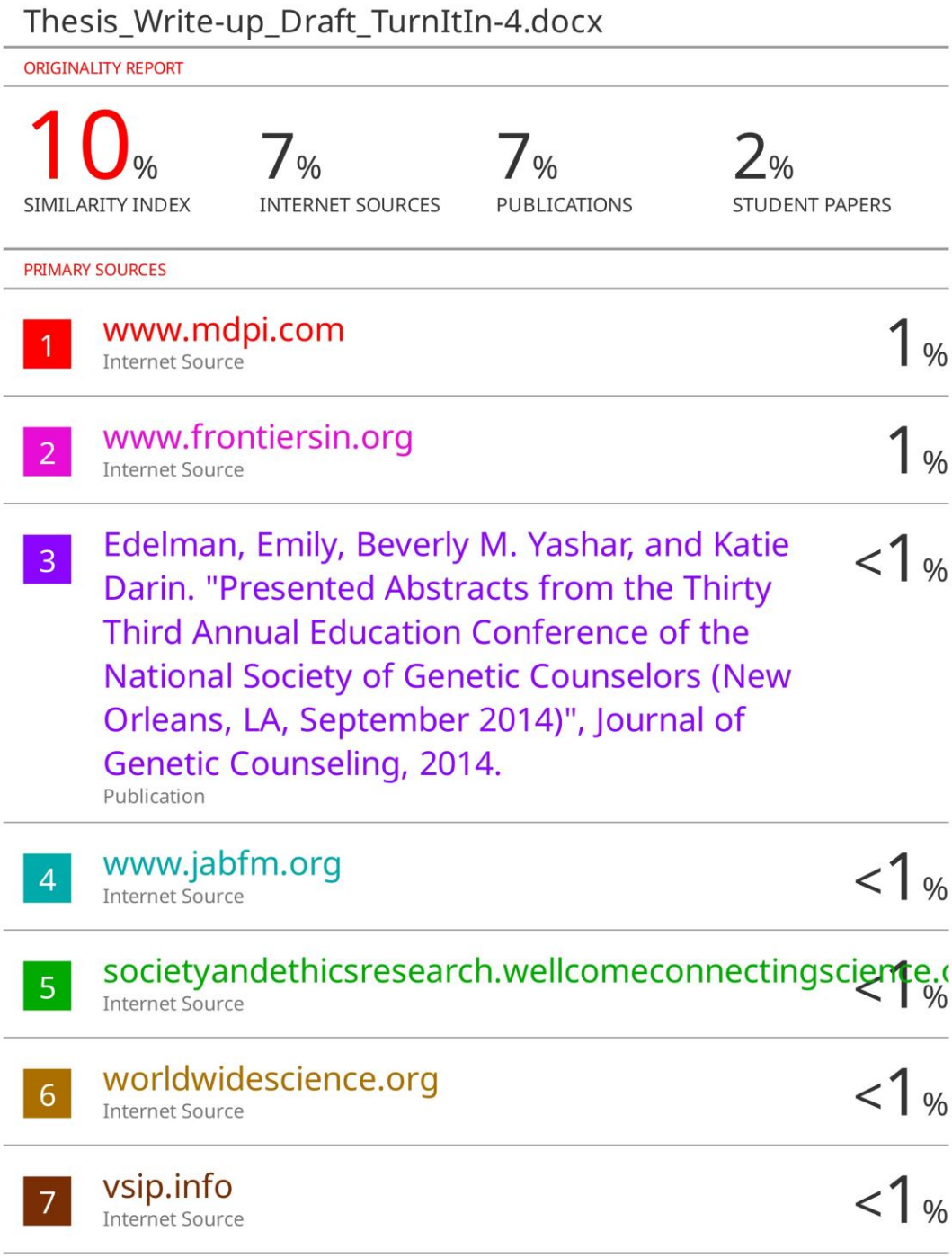
I hereby declare the following:

- 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.

Signature:  Date: 01/12/2023

1

Appendix E: Turn-it-in-report



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Appendix F: Variant detection and classification

Table 4. Genetic variants and classification summary

Gene	Variant	Variant classification	Personal or family history of gynaecological cancer
ATM	c.946T>A (p.Tyr316Asn)	VUS	SDR ovarian CA
BRCA1	c.1A>G (p.Met1Val)	Pathogenic	Patient & FDR ovarian CA
	c.2008G>T (p.Glu670Ter)	Pathogenic	Patient ovarian CA
	c.2641G>T (p.Glu881Ter)	Pathogenic	Patient & FDR ovarian CA
	c.3263T>A (p.Val1088Asp)	VUS	Patient ovarian CA
	c.45dup (p.Asn16Ter)	Pathogenic	FDR ovarian CA
	c.5266dup (p.Gln1756fs)	Pathogenic	Patient ovarian CA
	c.5533dup (p.Tyr1845fs)	Pathogenic	Patient uterine CA
	c.68_69del (p.Glu23fs)	Pathogenic	1 FDR, 1 TDR ovarian CA
BRCA2	c.3599_3600del (p.Asp1199_Cys1200insTer)	Pathogenic	TDR uterine CA
	c.5763del (p.Phe1921fs)	Pathogenic	Patient ovarian CA
	c.5946del (p.Ser1982fs)	Pathogenic	SDR ovarian CA; FDR ovarian CA
	c.6761_6762del (p.Phe2254fs)	Pathogenic	Patient & FDR ovarian CA
	c.7934del (p.Arg2645fs)	Pathogenic	Patient & FDR ovarian CA
	c.8954-2A>G	Pathogenic/Likely pathogenic	Patient uterine CA
	c.9976A>T (p.Lys3326Ter)	VUS → Benign	Patient ovarian CA
CHEK2	c.1263del (p.Ser422fs)	Pathogenic	FDR uterine CA
	c.538C>T (p.Arg180Cys)	Conflicting interpretations of pathogenicity	2 SDR uterine CA
FANCM	c.3998A>C (p.Gln1333Pro)	VUS	FDR ovarian CA
GPC3	c.49A>T (p.Ser17Cys)	VUS	Patient ovarian CA
HOXB13	c.153G>T (p.Leu51Phe)	VUS	Patient ovarian CA, SDR uterine CA
MLH1	c.2108_2117del (p.Glu703fs)	Pathogenic	SDR uterine CA
MSH2	c.1046C>G (p.Pro349Arg)	Pathogenic	Patient uterine CA
	c.1340_1341insGG (p.Phe447fs)	Pathogenic	Patient ovarian CA
	c.2502_2508del (p.Asn835fs)	Pathogenic	2 FDR ovarian CA
	Deletion (Exons 12-16)	Pathogenic	Patient uterine CA
MSH3	c.2659G>A (p.Asp887Asn)	VUS	Patient ovarian CA
MSH6	c.1871del (p.Gly624fs)	Pathogenic	Two FDR uterine CA
MUTYH	c.1103G>A (p.Gly368Asp)	Pathogenic	FDR ovarian CA

	c.1187G>A (p.Gly396Asp)	Pathogenic	Patient ovarian CA
	c.14_16dup (p.Arg5_Ala6insGly)	VUS	Patient ovarian CA
NBN	c.628G>T (p.Val219Phe)	Conflicting interpretations of pathogenicity	Patient ovarian CA
PMS2	c.137G>A (p.Ser46Asn)	Pathogenic	FDR uterine CA
	c.2012C>T (p.Thr671Met)	Conflicting interpretations of pathogenicity	Patient ovarian CA
	c.2478G>C (p.Glu826Asp)	VUS	Patient ovarian CA, SDR uterine CA
	c.736_741delCCCCCTins11 (p.?)	Pathogenic	FDR ovarian CA
POLE	c.3230G>A (p.Arg1077His)	Conflicting interpretations of pathogenicity	Patient ovarian CA
TSC2	c.3578G>T (p.Gly1193Val)	VUS	Patient ovarian CA, SDR uterine CA
WT1	c.203A>T (p.Gln68Leu)	VUS → Likely Benign	Patient ovarian CA
XRCC2	c.514A>G (p.Thr172Ala)	VUS	FDR ovarian CA

This table provides a summary of the genetic variants detected by diagnostic testing, their classification, and the cancer history for the individual that was tested. CA, cancer; FDR, first degree relative; SDR, second degree relative; TDR, third degree relative; VUS, variant of unknown significance; Reclassification is indicated by an arrow.

Appendix G: Frontiers Oncology author guidelines

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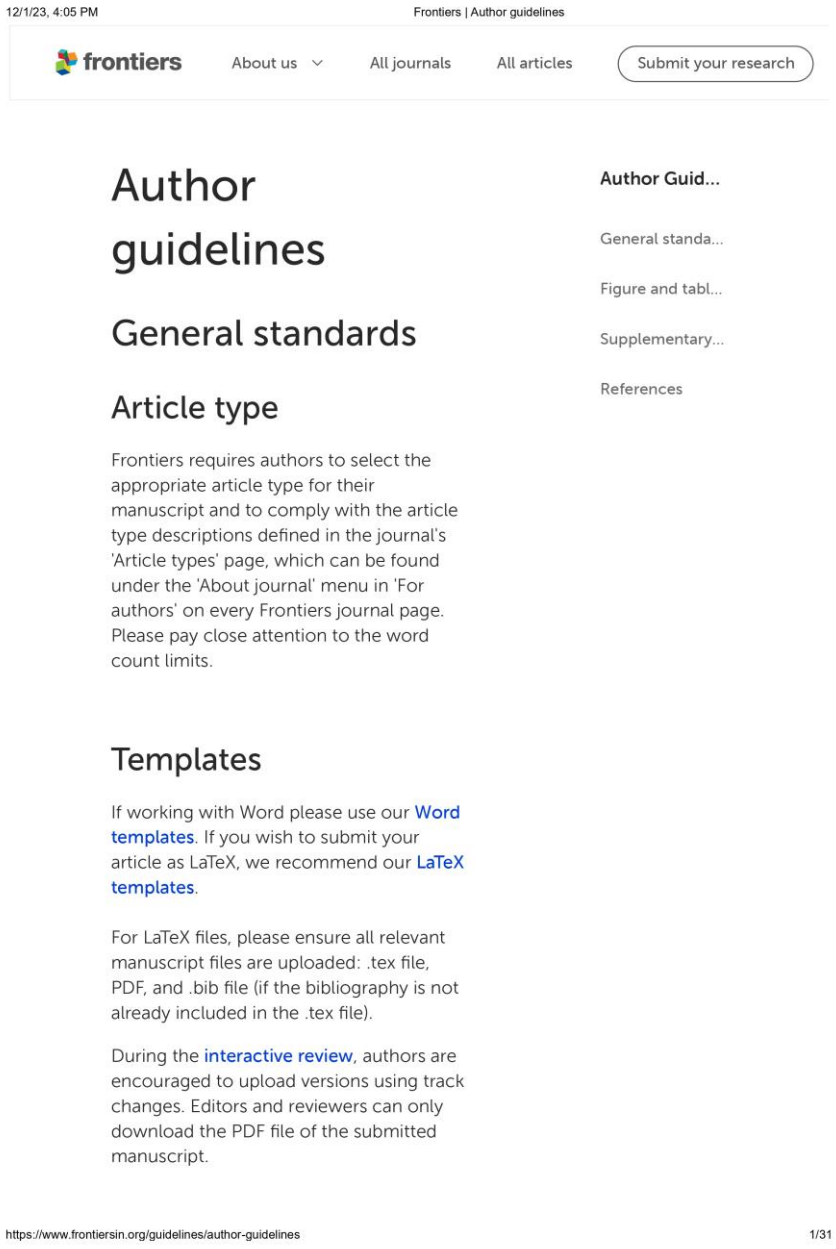

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- The citation of non-academic and non-peer-reviewed sources (e.g. blog posts, website content), as well as anonymous sources or commercial websites should be avoided or kept to a minimum
- Authors should avoid citing content that is not directly relevant to the scope of the article and the journal

proportion of citations to the same authors or sources, school of thought, etc.

- The length of the reference list should be appropriate depending on the article type, covering the relevant literature through sufficient referencing
- Authors should ensure that references are accurate, that all links are accessible, and that the citations/references adhere to the reference styles outlined below

Frontiers' journals use one of two reference styles, either Harvard (author-date) or Vancouver (numbered). These formats should be adhered to for the in-text citations and the reference lists. Please check our help center to find the correct style for the journal to which you're submitting.

- All citations of published works in the text, figures, or tables must be in the reference list and vice-versa.
- The names of the first six authors followed by et al. and the DOI (when available) should be provided.
- Given names of authors should be abbreviated to initials (e.g. Smith, J., Lewis, C.S., etc.).
- The reference list should only include articles that are published or accepted.
- Unpublished data, submitted manuscripts, or personal communications should be cited within the text only, for article types that allow such inclusions. Where additional details are available, these will be included as footnotes.
- For accepted but unpublished works use 'in press' instead of page numbers.

the version and unique identifier when available.

- Personal communications should be documented by a letter of permission.
- Website URLs should be included as footnotes.
- Any inclusion of verbatim text must be contained in quotation marks and should clearly reference the original source.
- Preprints can be cited provided that a DOI or archive URL is available, and the citation clearly mentions that the contribution is a preprint. If a peer-reviewed journal publication for the same preprint exists, the official journal publication is the preferred source. See the preprints section for each reference style below for more information.

Harvard reference style (author-date)

Reference examples for Frontiers' journals using the Harvard referencing system can be found below. For examples of other sources, and for general questions regarding the Harvard reference style, please refer to the Chicago Manual of Style.

- References should include the full last name and first name initials of the first six authors, followed by et al. and the year of publication in brackets.
- Alphabetical order is followed for the reference list.

style (numbered)

Vancouver reference style (numbered)

Reference examples for Frontiers' journals using the Vancouver referencing system can be found below. For more examples of citing other documents and general questions regarding the Vancouver reference style, please refer to Citing Medicine.

- In-text citations in the Vancouver reference style should be numbered consecutively in order of appearance in the text and identified by Arabic numerals in parenthesis.
- Use square brackets for physics and mathematics articles.
- The abbreviation 'Ref' should not be used, e.g.: [e.g., (1)] should NOT read [e.g. Ref. (1)].
- Style for comparing a citation should follow the number format, e.g. [cf. (1)]. The same applies when using 'see', e.g. [see (46)].
- References should be numbered and listed chronologically, according to the order they appear in the text.

Reference guidelines - Vancouver

Source	Reference list entry
Article in a print journal	1. Sondheimer N, Lindquis an epigenetic modifier of function in yeast. Mol Cell 5:163-172.