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**[MEDICAL SERVICES ADVISORY COMMITTEE](http://www.msac.gov.au/) CLINICAL UTILITY CARD FOR HERITABLE MUTATIONS WHICH INCREASE RISK IN  
BREAST AND/OR OVARIAN CANCER**

# Eligible investigative purposes of genetic testing for this clinical utility card (CUC)

*The investigative purposes of genetic testing of heritable mutations which are in scope for this CUC are:*

A. *clinically affected individuals, to make a genetic diagnosis and thus estimate their variation in (predisposition for) future risk of further disease – for these individuals, this is also called diagnostic testing;*

*and, when also appropriate*

B. *cascade testing of family members of those individuals who test positive for one or more relevant mutations, to make a genetic diagnosis and thus estimate each family member’s variation in (predisposition for) future risk of developing the clinical disease (and, less commonly, future risk of further disease if the disease has already been diagnosed) – for these individuals, this is also called predictive testing.*

*For each disease area, “star performer” gene(s) for testing are selected on the basis of having the strongest case for clinical utility, and the evidence provided in the CUC focusses on these genes. Other genes may be added to the panel of genes to be tested for the disease area on the basis of also having clinical utility, of not detracting from the clinical utility of the “star performer” genes, and of incurring negligible incremental costs for genetic testing. The evidence provided in the CUC for these other genes is commensurately reduced.*

*For each disease area, the characteristics of the clinically affected individuals who should be selected as eligible for this genetic testing are defined. This reflects an MSAC preference for a low probability of an actionable result over a high probability of an uninterpretable or unactionable result. Cascade testing is then only contemplated for family members of those individuals who test positive for a relevant mutation, and only when this mutation is also associated with having clinical utility for the family members.*

*MSAC is the target audience of the CUC. However, it should also be readily interpretable to non-experts in genetics, including the Evaluation Sub-Committee, contracted assessment groups, and those who will read the resulting Public Summary Documents from MSAC.*

# Background

*The Medical Services Advisory Committee (MSAC) is piloting arrangements to assess the utility of germline genetic testing for broad disease areas, such as cancer, cardiovascular or mental illness. This approach will be used to inform consideration of the circumstances under which germline genetic testing for these diseases should be publicly funded. Contributions to this pilot are being sought from pathologists with the support of the Royal College of Pathologists of Australasia (RCPA) and from the national reference group on cancer genetics (eviQ, www.eviq.org.au). Additional support for this pilot is being provided by the Australian Government Department of Health and the Monash University Assessment Group.*

*The pilot is being conducted in two stages. The first assesses the clinical utility of genetic testing of relevant heritable mutations grouped by disease area; the second evaluates the economics and budgetary implications of this testing. This second stage includes assessment of the cost-effectiveness of testing clinically affected individuals and the marginal cost effectiveness of also testing family members (cascade testing) where appropriate. In contrast to previous assessments of germline testing, the pilot will be conducted from a clinical perspective of disease management rather than a single gene by gene approach.*

*The clinical utility card (CUC) proforma is modelled on the clinical utility gene card format used by EuroGentest. When completed, a CUC provides relevant information regarding the clinical utility of genetic testing of relevant heritable mutations in particular circumstances. Clinical utility refers to the ability of a genetic test to significantly affect clinical management and patient outcomes. CUCs cover all elements relevant for assessing risks and benefits of a genetic test. Their clear and concise format will facilitate MSAC consideration across a large volume of tests.*

*The EuroGentest website (*[*http://www.eurogentest.org/index.php?id=668*](http://www.eurogentest.org/index.php?id=668)*) explains that the main components of a CUC are analytical validity, clinical validity, clinical utility and ethical, legal and social issues. A major challenge lies in balancing clinical validity, clinical utility and cost-effectiveness of testing. Some tests have excellent analytical validity, but are not viable from the clinical or economical point of view. On the other hand, some tests have poor analytical validity, but nevertheless affect patient and family management. Therefore it is important that the requirements for a test are defined in the clinical context and that the laboratory genetic test is only one of the components of an overall evaluation.*

# SUMMARY

## **Proposed disease**:

familial breast and/or ovarian cancer.

## **Proposed genes for testing**:

BRCA1, BRCA2, PTEN, STK11, PALB2, CHEK2, TP53, CDH1.

## Key analytical performance results:

* analytical sensitivity = 94%-100%
* analytical specificity = 96%-99.99%

## Key clinical validity results:

risk ratio of 15-year risk of contralateral breast cancer in non-familial breast cancer and affected mutation carriers (any age affected):

* BRCA1 = 28.7/8.4 = 3.4
* BRCA2 = 19/8.4 = 2.3

## Key clinical utility consequences:

* As treatment, contralateral mastectomy in affected mutation carriers reduces the risk of contralateral breast cancer to 1%
* As prevention, bilateral mastectomy reduces the risk of breast cancer in mutation carriers by ≥ 90%
* As prevention, bilateral salpingo-oophorectomy reduces the risk of breast cancer in mutation carriers by ~ 50% if the carrier is aged ~ 40 years, and reduces the risk of ovarian/ fallopian cancer in mutation carriers by ≥80%

## Proposed MBS item descriptor(s):

Affected individuals

*“Characterisation of germline gene variants, including at minimum BRCA1 and/or BRCA2 genes, in a patient with breast or ovarian cancer, in whom clinical and family history criteria have been determined by a treating specialist to be strongly suggestive of heritable breast/ovarian cancer predisposition based on the following criteria:*

* *A patient with breast and/or ovarian cancer whose personal or family history of cancer using a mutation prediction score predicts a combined mutation carrier probability of >10% according to either BOADICEA, BRCAPRO or pathology-adjusted Manchester score (combined score of 16 or greater) OR*
* *A patient who falls into one or more of the following specific categories:* 
  + *with a triple negative breast cancer and aged ≤40 years*
  + *with an isolated high grade (Grades 2 & 3) invasive non-mucinous ovarian, fallopian tube or primary peritoneal cancer aged ≤70 years*
  + *with invasive non-mucinous ovarian, fallopian tube or primary peritoneal cancer at any age and a family history of breast or ovarian cancer*
  + *with a personal and/or family history of breast and/or ovarian cancer, from a population where a common founder mutation exists.”*

Family members

*“Request by a specialist familial cancer physician for the detection of a previously identified single gene variant, in a relative of a patient with known breast or ovarian cancer where previous genetic testing has detected a variant causative of hereditary familial cancer predisposition."*

Proposed MBS fee(s):Affected individuals (initial) $1725.00

Positive affected individuals (confirmatory) $402.50

Post-test genetic counselling (positive only) $263.90

Family members $402.50

Post-test genetic counselling (positive only) $263.90

## **Key economic evaluation results:**

[TBA]

## Key financial implications:

[TBA]

# DISEASE CHARACTERISTICS

## List the names of the disease(s) proposed for genetic testing within the disease area

**and provide the clinical rationale for this grouping**

Familial breast and/or ovarian cancer.

## OMIM# of the disease(s)

114480

## List the names of the corresponding actionable genes which are proposed for genetic testing

**Of these, identify the “star performer(s)” in this list (that is, the actionable gene(s) for which the strongest clinical utility and/or cost-effectiveness argument is likely to apply for an affected individual)**

*[Included genes which are not the “star performer(s)” must have both sufficient penetrance and also have some evidence that the results would have clinical utility (eg inclusion in well-regarded clinical guidelines).]*

BRCA1, HGNC ID = 1100 = “star performer gene\* in breast and/or ovarian cancer”  
BRCA2, HGNC ID = 1100 = “star performer gene\* in breast and/or ovarian cancer”  
PTEN, HGNC ID = 9588  
STK11, HGNC ID = 11389  
PALB2, HGNC ID = 26144  
CHEK2, HGNC ID = 16627  
TP53, HGNC ID = 11998  
CDH1, HGNC ID = 1748

\* In practical terms, the “star performer gene” for this CUC means testing for (or detecting) any BRCA1 mutation or any BRCA2 mutation.

## OMIM# of the genes

BRCA1, 113705  
BRCA2, 600185  
PTEN, 601728  
STK11, 602216  
PALB2, 610355  
CHEK2, 604373  
TP53, 191170  
CDH1, 192090

## Target population for testing

**that is, what clinical/pathological or other diagnostic criteria should be used to determine the “phenome” which should be eligible for testing? Provide the evidence and/or clinical rationale for these criteria which would ensure that the pre-test probability of a pathogenic heritable mutation or combination of mutations for the “star performer(s)” would be ≥10%.**

*[Although accepted as being more influenced by clinical judgement than objective facts, the threshold of 10% for a pre-test probability of pathologic heritable mutation(s) for the “star performer(s) is influenced by MSAC preference for a low probability of an actionable result over a high probability of an uninterpretable or unactionable result.]*

Heritable BRCA1/BRCA2 mutation testing should be considered in an individual:

* with breast and/or ovarian cancer whose personal or family history of cancer using a mutation prediction score predicts a combined mutation carrier probability of >10% according to either BOADICEA[[1]](#footnote-1), BRCAPRO[[2]](#footnote-2) or pathology-adjusted Manchester score (combined score of 16 or greater)[[3]](#footnote-3) OR
* who falls into one or more of the following specific categories:
  + with a triple negative breast cancer and aged ≤40 years
  + with an isolated high grade (Grades 2 & 3) invasive non-mucinous ovarian, fallopian tube or primary peritoneal cancer aged ≤70 years
  + with invasive non-mucinous ovarian, fallopian tube or primary peritoneal cancer at any age and a family history of breast or ovarian cancer
  + with a personal and/or family history of breast and/or ovarian cancer, from a population where a common founder mutation exists.

## 1.6 Estimated prevalence of heritable mutations of BRCA1/BRCA2

*At birth:* 1:500 to 1:1000.

*In the target population for testing identified at 1.5:* ≥10%, with 15% as the base case estimate, and any sensitivity analyses to be 10% and 20%.

1.7 Estimated proportion of affected individuals **who fall within the target population for testing identified at 1.5**

10% as the base case, with any sensitivity analysis to be 5%.

# TEST CHARACTERISTICS OF BRCA1 AND BRCA2

## 2.1 Analytical performance

*Is there an analytical reference standard used to establish genotype:* yes

*[If yes, complete 2.1.1 below; if no, complete 2.1.2 below.]*

### ***2.1.1 Analytical validity possible*** (to be answered if 2.1 was marked “yes”)

*Define the analytical reference standard:*

The established analytical reference standard for detection of sequence variants in diagnostics has been Sanger sequencing; established reference methods for the detection of copy number variation due to large insertions or deletions include MLPA and DNA microarrays.

These reference methods have been in accepted use for over a decade with well-established quality assurance programs (QAP). The methods have a specificity and sensitivity of 96-100% for constitutional variants (expected to be present at proportions of 0%, 50%, or 100%). Sensitivity and specificity of Sanger sequencing is however much lower for mosaic variants, with limit of detection for mosaicism considered to be 20-30%. Low-level, multiple-tissue, constitutional mosaicism in BRCA1 has recently been reported and highlights the need to consider deep sequencing in affected individuals clinically suspected of having cancer predisposition (see Friedman E et al 2015).

The analytical performance and characteristics of other detection methods (e.g. High Resolution Melt-curve Analysis (HRMA) with Sanger sequencing confirmation, or alternatively, massively parallel sequencing with or without Sanger sequencing confirmation) are generally established by comparison with the reference method, and may be implemented if analytical performance is demonstrated to be equal or superior to the reference method.

For simplicity, the analytical reference standard will be considered as Sanger sequencing with massively parallel targeted sequencing considered as the comparator method.

*Present analytical sensitivity as the proportion of positive test results if the genotype is present according to the reference standard:*

*Present analytical specificity as the proportion of negative test results if the genotype is not present according to the reference standard:*

There are numerous publications in which the analytical sensitivity and specificity of targeted gene panels have been established as being equivalent to Sanger sequencing, both in the setting of the BRCA1 and BRCA2 analysis, or in other smaller targeted gene panels.

The table below provides a recent review of analytical validity of BRCA1 and BRCA2 massively parallel sequencing compared to established reference methods.

| *Reference* | *Analytical sensitivity* | *Analytical specificity* | *Genes included* | *Additional comments* |
| --- | --- | --- | --- | --- |
| Dacheva D. *et al.* Mol Diagn Ther. 2015 19(2):119-30. | 100% | 95.9% | BRCA1  BRCA2 |  |
| Ruiz A. *et al.* Biomed Res Int. 2014:542541. doi: 10.1155/2014/542541. Epub 2014 Jun 26. | 100% | 97.35% | BRCA1  BRCA2 |  |
| Guan Y. *et al.* Fam Cancer. 2015 14(1):9-18. | 93.66% | 99.98% | BRCA1  BRCA2 | Accuracy evaluated at 99.97% |
| Judkins T. *et al.* BMC Cancer. 2015; 15: 215. | 99.92% | 99.99% | 25 gene inherited cancer panel including BRCA1 and BRCA2, PALB2, ATM, TP53 | Comprehensive comparative analysis comparing NGS results for BRCA1 and BRCA2 with Sanger sequencing in 1864 patients who had undergone previous clinical testing |

### ***2.1.2 Analytical validity not possible***

*(to be answered if 2.1 was marked “no”)*

*Present* *analytical concordance across testing options (using proportions with their 95% CI and/or kappa statistics):* not applicable.

*Present analytical reproducibility of each testing option (using proportions with their 95% CI and/or kappa statistics):* not applicable.

*Present inter-rater or inter-laboratory reliability of each testing option (using kappa statistics):* not applicable.

*Present the limit of detection of each testing option:* not applicable.

* 1. **Clinical validity**

*[Clinical validity is assessed in terms of variation in risk of future events between a cohort of affected individuals who test positive for the “star performer” mutation(s) and a cohort of affected individuals who test negative for the “star performer” mutation(s).]*

* + 1. ***Definition of clinical event used to determine clinical validity of the test in an affected individual:***

*[Possible events include developing a new clinical event related to the disease, or death.]*

Women with a diagnosis of breast cancer represent the greatest proportion of affected individuals. For these individuals the key clinical event is the occurrence of a metachronous second primary cancer, most commonly contralateral breast cancer (CBC) or serous ovarian cancer. Available data on the incidence of ovarian cancer in mutation carriers frequently does not distinguish between the risk in unaffected women and the risk of ovarian cancer as a second primary. In contrast there is extensive published data on the rate of CBC and this will be used to evaluate clinic validity.

* + 1. ***Ratio of clinical events occurring in affected individuals who test positive to events occurring in affected individuals who test negative:***

*[Depending on the event and the type of cohort study or studies available, ratios can be presented as an odds ratios (OR), relative risk (RR), or hazard ratio (HR). Also report the rate of development of the clinical event in either mutation test-positive affected individuals or mutation test-negative affected individuals.]*

The risk of CBC is elevated for carriers of both BRCA1 and BRCA2. The risk is greatest for women who have a first diagnosis of breast cancer at an early age (< 50 years), but remains elevated at all ages. Representative data from the largest published cohort (Rhiem et al., Breast Cancer Res 2012 14:R156) is shown below:

**15-year risk of contralateral breast cancer in non-familial breast cancer and affected BRCA1 and BRCA2 mutation carriers**

| Age affected | Population breast ca1 Cumulative risk % | BRCA12  Cumulative risk % | BRCA12  95%CI | BRCA12  **RR** | BRCA22  Cumulative risk % | BRCA22  95%CI | BRCA22  **RR** |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Any | 8.4 | 28.7 | (24.4-32.9) | **3.4** | 19 | (13.5-24.4) | **2.3** |
| < 40 years | 8.5 | 40.8 | (33.2-48.3) | **4.8** | 20.9 | (9.7-32.1) | **2.5** |
| 40-49 years | 8.5 | 23.2 | (16.9-29.6) | **2.7** | 22 | (12.1-31.9) | **2.6** |
| ≥ 50 years | 8.4 | 18.7 | (11.0-26,3) | **2.2** | 15.5 | (7.8-23.3) | **1.8** |

1Breast cancer in women without a strong family history. Figures from: Narod et al., Clin Genet 2015 Apr 29. doi: 10.1111/cge.12604 2Rhiem et al., 2012 Breast Cancer Res 14(6) R156

The level of risk, variation with age of first breast cancer diagnosis, and variation between carriers of mutations in BRCA1 and BRCA2 have been confirmed in prospective studies and a meta-analysis of published data (Molina-Montes et al., The Breast 2014 23:721-742).

* + 1. ***Mean or median duration of follow-up across the cohort study associated with this ratio:***

*[Preferably, more than one ratio should be reported for more than one time point.]*

The relative risks are estimated from 6235 BRCA1/BRCA2 carriers with a mean follow up of 7.76 years (48,390 person-years of observation).

* + 1. ***Mean or median age across the cohort study associated with this ratio:***

*[If the ratio is expected to vary by age, present any available data which enables an assessment of the association between age and clinical validity.]*

The median age of first breast cancer (i.e. study entry) for individuals included in the estimate of CBC risk was 43.5 years (inter-quartile range 37.5-51.5) for BRCA1 carriers and 48.1 (40.4-58.5) for BRCA2 carriers. The median age of CBC was 47.7 years (40.1-55.5) for BRCA1 and 53.1 (44.7-62.6) for BRCA2.

* + 1. ***Prevalence (or diagnostic yield) associated with this ratio:***

*[If the prevalence is likely to vary from the study population of affected individuals and the target population for testing at 1.5, present the attributable fractions for the two populations.]*

The study cohort was ascertained on standard selection criteria consistent with those outlined at 1.5. The cumulative risk of CBC in this study was measured in non-index cases from families carrying a mutation to reduce the effect of ascertainment. The higher CBC rate in index cases means that the RRs provided at Subsection 2.2.2 above may be considered conservative.

# CLINICAL UTILITY OF BRCA1 AND BRCA2 FOR AFFECTED INDIVIDUALS

* 1. **Can a genetic diagnosis be made other than through a genetic test?** no
  2. **How would disease management of the affected individual be influenced by the result of the genetic test compared with not testing?**

*Summarize the differences in optimal treatment for mutation positive and negative affected individuals for the incident current manifestation of the disease, with each management strategy for mutation-positive affected individuals and mutation-negative affected individuals being compared with the comparator of clinical management in the absence of testing.*

**Early breast cancer**

| *Incident disease\** | *Mutation positive* | *Mutation negative* | *Incremental benefit of the differential approach* |
| --- | --- | --- | --- |
| Surgery | Bilateral mastectomy recommendation (especially if aged ≤ 50 years) | Breast conserving surgery or mastectomy as appropriate for the cancer-affected breast | Contralateral mastectomy in mutation carriers reduces the risk of contralateral breast cancer to 1% |
| Radiotherapy | Unchanged, but in some cases avoided | Unchanged | Expected better cosmetic outcome if bilateral mastectomy without radiotherapy is possible |
| Chemotherapy | Adjuvant chemotherapy unchanged at present but consideration is given to platinum agents in the relapse setting. May be involved in clinical trials of targeted therapy, eg PARP inhibitors | Unchanged | Expected future developments in targeted therapy |

*\* Of tested individuals, 5% are likely to receive the management identified for the mutation-positive column irrespective the testing outcome (due to other high risk factors).*

**Ovarian/fallopian tube cancer**

| *Incident disease\** | *Mutation positive* | *Mutation negative* | *Incremental benefit of the differential approach* |
| --- | --- | --- | --- |
| Surgery | Unchanged | Unchanged | Not applicable |
| Radiotherapy | Unchanged | Unchanged | Not applicable |
| Chemotherapy | Adjuvant chemotherapy unchanged at present but consideration is given to repeated use of platinum agents in the relapse setting. May be involved in clinical trials of targeted therapy, eg PARP inhibitors | Unchanged | Expected future developments in targeted therapy |

*\* Of tested individuals, 5% are likely to receive the management identified for the mutation-positive column irrespective the testing outcome (due to other high risk factors).*

*Provide supporting evidence for the claimed magnitudes of benefits for the selections above.*

Supporting evidence for the above is referenced in:  
<http://guidelines.canceraustralia.gov.au/guidelines/gene_mutation/index.php>

*Summarize the differences between prevention strategies for mutation positive and negative affected individuals, with each management strategy for mutation-positive affected individuals and mutation-negative affected individuals being compared with the comparator of clinical management in the absence of testing.*

**Breast cancer**

| *Prevention of disease* | *Mutation positive* | *Mutation negative* | *Incremental benefit of differential approach* |
| --- | --- | --- | --- |
| Surgery | 1. Bilateral mastectomy 2. Bilateral salpingo-oophorectomy | Unchanged | 1. Risk of breast cancer reduced by ≥ 90% 2. Risk of breast cancer reduced by ~ 50% if carrier is aged ~ 40 years |
| Screening | MRI is superior to mammogram/ultrasound for detection of breast cancer in mutation carriers aged ≤ 50 years because MRI detects tumours which are smaller and more likely to be node-negative | Unchanged | Early detection expected to reduce breast cancer mortality (no data yet available to estimate extent of reduction) |
| Medical prophylaxis | * Tamoxifen * Raloxifene | Unchanged | Proven 40% reduction of breast cancer incidence in those at higher baseline risk |

**Ovarian/fallopian tube cancer**

| Surgery | Bilateral salpingo-oophorectomy | Unchanged | Risk of ovarian/ fallopian cancer reduced by ≥80% and risk of breast cancer reduced by ~ 50% if carrier is aged ~ 40 years, with proven reduction of breast cancer related, ovarian cancer related, and all-cause mortality |
| --- | --- | --- | --- |
| Screening | Not applicable as no effective screening | Not applicable: no effective screening | Not applicable |
| Medical prophylaxis | Unchanged | Unchanged | Not applicable |

*Provide supporting evidence for the claimed magnitudes of benefit for the selections above.*

Supporting evidence for the above is referenced in:  
<https://www.eviq.org.au/Protocol/tabid/66/categoryid/66/id/170/Risk+Management+for+an+Unaffected+Female+BRCA1+Mutation+Carrier.aspx>

# IMPLEMENTATION ISSUES AND RATIONALE FOR ANY CASCADE TESTING

## 4.1 Clinical context of testing for an individual presenting with an eligible “phenome”

*Clinical setting in which testing can be ordered and the test results are interpreted for an individual presenting with an eligible “phenome”:* specialist physician/surgeon.

*Role of pre-test genetic counselling or information for this individual:* required.

*If required, nature of counselling or information to be provided:* a positive test result would mean:

* the individual is at increased risk of further breast cancer
* the individual is at increased risk of ovarian cancer
* there may be an increased risk of additional other cancers
* cancer management decisions may change
* there are options for early detection/cancer prevention
* family members of the individual may be at increased risk of developing cancer
* there may be psychosocial implications
* there may be insurance implications
* there may be family planning implications.

*If not required, brief explanation of why not:* not applicable.

*Role of post-test genetic counselling or information for this individual, including in relation to any referral to a hereditary cancer clinic or family cancer centre to manage family members (focussing on first-degree relatives for simplicity, noting that this is not intended to necessarily limit any public funding of cascade testing to first-degree relatives):*

A. *in event of a positive test result:* required.

*If required, nature of counselling or information to be provided:*

* a general explanation of cancer and hereditary cancer predisposition
* the individual is at increased risk of breast cancer or a further breast cancer
* the individual is at increased risk of ovarian cancer
* there may be an increased risk of additional other cancers
* cancer management decisions may change
* there are options for early detection/cancer prevention
* adult family members of the individual may be at increased risk of developing cancer and require referral to a family cancer clinic to consider predictive genetic testing
* there may be psychosocial implications
* there may be insurance implications
* there may be family planning implications.

*If not required, brief explanation of why not:* not applicable.

B. *in event of a negative test result:* required.

*If required, nature of counselling or information to be provided:*

If no definite genetic cause is identified by the testing the following issues may still need to be considered in post-test counselling on the basis of the clinical situation (personal and family history):

* the individual may be at increased risk of breast cancer or a further breast cancer
* the individual may be at increased risk of ovarian cancer
* there may be an increased risk of additional other cancers
* cancer management decisions may change
* there are options for early detection/cancer prevention
* family members of the individual may be at increased risk of developing cancer, but there is no genetic test available to them.

*If not required, brief explanation of why not:* not applicable.

## 4.2 Genetic risk assessment in family members of a proband

**(i.e. an affected individual who has tested positive for mutation)**

**4.2.1 Definition of clinical event used to determine clinical validity of the test in a family member of a proband:**

*[Possible events include diagnosis of the disease.]*

For family members of a proband, the primary clinical event of interest is the diagnosis of breast cancer.

* + 1. ***Ratio of clinical events occurring in mutation test-positive family members to clinical events occurring in mutation test-negative family members:***

*[Depending on the event and the type of cohort study or studies available, ratios can be presented as an odds ratios (OR), relative risk (RR), or hazard ratio (HR). Also report the rate of development of the clinical event in either mutation test-positive family members or mutation test-negative family members.]*

The relative risk of female breast and ovarian cancer has been established in multiple large scale international cohort studies in both the population setting (summarised in Antoniou et al., Am J Hum Genet 2002 72:1117-30) and clinic-based cohorts (summarised in Chen et al., JCO 2007 25(10):1329-33) that have been adapted to the Australian setting (Suthers. ANZ J Surg 2007 77:314-19). All studies have confirmed markedly elevated risks for female breast and ovarian cancer for both genes and more moderate increases in risk for male breast and prostate cancer in male BRCA2 carriers. The relative risks are greatest for early onset cancers (<50 years) but remain substantially increased at all ages. Representative data from a combined analysis of 8,139 individuals with breast or ovarian cancer and their families, including 280 BRCA1-mutation and 218 BRCA2-mutation families, is shown below (from Antoniou et al):

**Relative risk (95% CI) of cancer for female BRCA1 and BRCA2 mutation carriers**

| Age | BRCA1 | BRCA2 |
| --- | --- | --- |
| 20-29 | 17 (4.2-71) | 19 (4.5-81) |
| 30-39 | 33 (23-49) | 16 (9.3-29) |
| 40-49 | 32 (24-43) | 9.9 (6.1-16) |
| 50-59 | 18 (11-30) | 12 (7.4-19) |
| 60-69 | 14 (6.3-31) | 11 (6.3-20) |

These risks have been validated in a prospective series (EMBRACE) of 978 BRCA1-mutation carriers and 909 BRCA2-mutation carriers as presented in the table below.

**Cumulative lifetime risk (95% CI) of female breast cancer in Australian population and affected BRCA1 and BRCA2 mutation carriers**

|  | Australian population1 | BRCA12 | BRCA22 |
| --- | --- | --- | --- |
| Cumulative lifetime risk, % (95% CI)  Female, any age breast cancer | 9.3 | 60 (44-75) | 55 (41-70) |

1cumulative risk to age 75, AIHW cancer statistics 2011

2cumulative risk to age 70 in a UK series (Mavaddat et al., J Natl Cancer Inst 2013 105:812-822)

The relative risks over the Australian population are 60/9.3 = 6.4 for BRCA1 and 55/9.3 = 5.9 for BRCA2.

* + 1. ***Mean or median duration of follow-up across the cohort study associated with this ratio:***

*[Preferably, more than one ratio should be reported for more than one time point.]*

The Antoniou et al combined analysis did not report any duration of follow-up; Mavaddat et al reported a mean duration of follow-up of 3.3 years for women unaffected with breast cancer or ovarian cancer, 3.0 years for women without an ovarian cancer diagnosis and 3.0 years for women with unilateral breast cancer.

* + 1. ***Mean or median age across the cohort study associated with this ratio:***

*[If the ratio is expected to vary by age, present any available data which enables an assessment of the association between age and clinical validity.]*

The Antoniou et al combined analysis did not report any mean or median age; Mavaddat et al reported a mean age at start of follow-up of 41.2 years for women unaffected with breast cancer or ovarian cancer, 43.7 years for women without an ovarian cancer diagnosis and 50.2 years for women with unilateral breast cancer.

* + 1. ***Prevalence (or diagnostic yield) associated with this ratio:***

Cumulative lifetime risks are reported in Subsection 2.2.2 above.

* + 1. ***How would disease management of the family member be influenced by the result of the genetic test compared with not testing?***

*Summarize the differences between prevention strategies for mutation positive and negative family members, with each management strategy for mutation-positive individuals and mutation-negative individuals being compared with the comparator of clinical management in the absence of testing. [Where appropriate, split the following table into males and females or nominate the gender for which the clinical utility arguments are the strongest.]*

## Breast cancer (female)

| *Prevention of disease\** | *Mutation positive* | *Mutation negative* | *Incremental benefit of differential approach* |
| --- | --- | --- | --- |
| Surgery | 1. Bilateral mastectomy 2. Bilateral salpingo-oophorectomy | Unchanged  (but avoids unnecessary surgery) | 1. Risk of breast cancer reduced by ≥ 90% 2. Risk of breast cancer reduced by ~ 50% if carrier is aged ~ 40 years |
| Screening | MRI is superior to mammogram/ultrasound for detection of breast cancer in mutation carriers aged ≤ 50 years because MRI detects tumours which are smaller and more likely to be node-negative | Unchanged  (but avoids unnecessary additional screening) | Early detection expected to reduce breast cancer mortality (no data yet available to estimate extent of reduction) |
| Medical prophylaxis | * Tamoxifen * Raloxifene | Unchanged  (but avoids unnecessary medical prophylaxis) | Proven 40% reduction of breast cancer incidence in those at higher baseline risk |

**Ovarian/fallopian tube cancer**

| *Prevention of disease\** | *Mutation positive* | *Mutation negative* | *Incremental benefit of differential approach* |
| --- | --- | --- | --- |
| Surgery | Bilateral salpingo-oophorectomy | Unchanged  (but avoids unnecessary surgery) | Risk of ovarian/ fallopian cancer reduced by ≥80% and risk of breast cancer reduced by ~ 50% if carrier is aged ~ 40 years, with proven reduction of breast cancer related, ovarian cancer related, and all-cause mortality |
| Screening | Not applicable as no effective screening | Not applicable: no effective screening | Not applicable |
| Medical prophylaxis | Unchanged | Unchanged | Not applicable |

**Male breast/prostate cancer**

| *Prevention of disease\** | *Mutation positive* | *Mutation negative* | *Incremental benefit of differential approach* |
| --- | --- | --- | --- |
| Surgery | Unchanged | Unchanged | Nota applicable |
| Screening | Breast: advice on chest area awareness and pectoral area palpation on a regular basis  Prostate: consider annual PSA + Digital Rectal Exam (DRE) from early 40 years of age | Unchanged | Unknown.  Breast: no trials have evaluated whether manual palpation is effective.  Prostate: preliminary evidence for value in surveillance, as data suggests that BRCA-related prostate cancer is aggressive and occurs at a younger age than sporadic prostate cancer. |
| Medical prophylaxis | Not applicable | Not applicable | Not applicable |

*\* Note that the above generally refers to individuals unaffected by disease. If test results mean that further investigations detect signs of sub-clinical disease (for example an ECHO detecting hypertrophy of the ventricle in cardiac disease), extend the table to include any differences in clinical management of sub-clinical disease. For family members who already have a clinical diagnosis of the disease, refer to the treatment table in Subsection 3.2.*

*Provide supporting evidence for the claimed magnitudes of benefit for the selections above.*

Supporting evidence for the above is referenced in:  
<https://www.eviq.org.au/Protocol/tabid/66/categoryid/66/id/170/Risk+Management+for+an+Unaffected+Female+BRCA1+Mutation+Carrier.aspx>

and in:  
<https://www.eviq.org.au/Protocol/tabid/66/categoryid/66/id/656/Risk+Management+for+Unaffected+Male+BRCA1+or+BRCA+2+Mutation+Carrier.aspx>

*Does the previous table provide sufficient justification in terms of clinical utility for cascade testing when limited to first-degree family members of a proband?* yes

*If yes, there is no need to extend the justification further, noting that this is not intended to necessarily limit any public funding of cascade testing to first-degree relatives.*

*If no, briefly describe the significance of any variation in prevention strategies across first- to third-degree family members.* Not applicable.

*If cascade testing is undertaken on a relevant actionable gene other than the “star performer gene(s)”, indicate whether the expected benefits would be:*

A. *the same as* yes

B. *less than or* no

C. *greater than* no

*that observed with the “star performer gene(s)”.*

* + 1. ***Clinical context of testing for a family member of a proband***

*Clinical setting in which family members can be approached, testing can be ordered, and the test results are interpreted:* hereditary cancer clinic or family cancer centre.

*Role of pre-test genetic counselling or information for the family member:* required.

*If required, nature of counselling or information to be provided:*

* a general explanation of cancer and hereditary cancer predisposition
* the individual may be at increased risk of breast cancer or a further breast cancer
* the individual may be at increased risk of ovarian cancer
* there may be an increased risk of additional other cancers
* cancer management decisions may change
* there are options for early detection/cancer prevention
* adult family members of the individual may be at increased risk of developing cancer and require referral to a family cancer clinic to consider predictive genetic testing
* there may be psychosocial implications
* there may be insurance implications
* there may be family planning implications.

*If not required, brief explanation of why not:* not applicable.

*Role of post-test genetic counselling or information for the family member:*

A. *in event of a positive test result:* required.

*If required, nature of counselling or information to be provided:*

* the individual is at increased risk of breast cancer or a further breast cancer
* the individual is at increased risk of ovarian cancer
* there may be an increased risk of additional other cancers
* cancer management decisions may change
* there are options for early detection/cancer prevention
* adult family members of the individual may be at increased risk of developing cancer and require referral to a family cancer clinic to consider predictive genetic testing
* there may be psychosocial implications
* there may be insurance implications
* there may be family planning implications.

*If not required, brief explanation of why not:* not applicable.

B. *in event of a negative test result:* not required.

*If required, nature of counselling or information to be provided:* not applicable.

*If not required, brief explanation of why not:*

If an individual tests negative for a known cancer associated gene mutation, they and their offspring revert to the background population risk for the involved cancers unless there is further family history (not accounted for by the gene mutation), or unless there are other non-genetic risk factors.

# DESCRIPTION OF GENETIC TESTING FOR BREAST AND/OR OVARIAN CANCER

## 5.1 Proposed description of testing for differential genetic diagnosis

*Describe the mutational spectrum in terms of the frequency and nature of the aberrations (such as deletions and copy number variations) that occur within the target genes listed in 1.3 in order to justify the nature and range of the proposed testing needed both to detect any relevant mutations and also to validate their detection.*

### Genes with mutations frequently associated with a high relative risk for breast cancer

BRCA1 and BRCA2 (“star performers”)

BRCA1 and BRCA1 are the “star performers” in relation to determining the heritable risk of developing breast cancer with well over 2000 mutations distributed over the entire coding regions of the two genes having been identified. The vast majority (>40%) of class 5 (pathogenic) mutations reported in the Human Genome Mutations Database (HGMD) database are small deletions, insertions and indels that result in a shift in the reading frame and premature truncation of the BRCA1 or BRCA2 proteins followed by nonsense substitutions that again result in premature truncation of the BRCA1 or BRCA2 proteins. There is one reported example of a BRCA2 protein truncating mutation, p.Lys3326Ter that is considered a polymorphic variant with low relative risk (1.4) due likely, to its position at the 3’ end of the gene, for breast cancer however, the majority of BRCA1 and BRCA2 protein truncating mutations are considered to be associated with a high relative risk. Mutations that alter the splicing of BRCA1 or BRCA2 and which usually occur at the invariant splice donor or acceptor, or at highly conserved sequences within ~5 bases of an exon boundary constitute another 9% of mutations. A small number of mutations that affect splicing and are more remote to an exon boundary have also been associated with breast cancer development. Approximately 10% of reported mutations in BRCA1 (8%) and BRCA2 (1.8%) involve larger rearrangements such as deletions or insertions of exons or multiple exons which remain largely undetected by common sequencing strategies. Missense mutations in BRCA1 and BRCA2 account for approximately 30% of all detected variants and are the most challenging in terms of classifying their associated risk for the development of breast cancer.

Mutations in genes other than BRCA1 and BRCA2 listed in Subsection 1.3, whilst associated with significantly increased risk of developing breast cancer, occur at much lower frequency within high risk breast cancer families and are sometimes associated with other syndromic features suggestive of a particular gene involvement. The mutation spectrum across these genes in terms of breast cancer risk is variable therefore a number of key points will be made about selected specific genes.

### Genes with mutations infrequently associated with a moderate-high relative risk for breast cancer

PALB2 and CHEK2

Mutations in the **PALB2** gene have been reported to be associated with a relative risk of developing breast cancer of 5.3. While the relative risk for other PALB2 mutations is still under investigation, the PALB2:c.3113G>A truncating mutation has been associated with a high estimated risk of breast cancer risk in Australian women from high risk breast and/or ovarian cancer families. Large rearrangements of the PALB2 gene represent 9% of all PALB2 mutations reported in HGMD and splice site mutations account for approximately 8%.

The 1100delC mutation is relatively common in the **CHEK2** gene and has been associated with an increased relative risk of 3.0 for breast cancer however, the relative breast cancer risk associated with other mutations in the CHEK2 have not yet been established. Large rearrangements and splice site mutations represent 7% each of the CHEK2 mutations reported in HGMD.

### Genes associated with other heritable tumour syndromes that have breast cancer as a feature

Tp53, PTEN, STK11 and CDH1

Note that ascertainment bias makes estimating the frequency of mutations in these genes outside of their specific syndromes difficult.

Mutations in **Tp53** are typically associated with the Li Fraumeni syndrome which has breast cancer as a feature and HGMD indicates that 12% of reported Tp53 mutations are associated with a breast cancer phenotype. Large rearrangements and splice mutations comprise 4% and 11% of all reported Tp53 mutations in this database, respectively.

Mutations in the **PTEN** genes are associated with Cowden syndrome which includes breast cancer in its clinical phenotype and HGMD indicates that 4% of reported PTEN mutations are associated with a breast cancer phenotype. Large rearrangements and splice site mutations account for 9% and 8% of all reported PTEN mutations in this database, respectively.

Mutations in the **STK11** gene are typically associated with Peutz Jeghers syndrome which confers an increased risk of developing breast cancer. However, HGMD indicates only 0.3% of STK11 mutations being associated with a breast or breast/ovarian cancer phenotype. Large rearrangements of the STK11 gene comprise 21% of all the STK11 mutations reported in HGMD and would be largely undetectable by common sequencing strategies. Therefore, assays designed to detect CNV (copy number variations) are likely to detect a significant number of clinically actionable mutations in this gene. Splice site mutations represent 10% of the total STK11 mutations reported in HGMD.

Protein truncating mutations in the **CDH1** gene are typically associated with diffuse gastric cancer, however 11% of CDH1 mutations reported in HGMD are associated with a breast cancer phenotype. An increased relative risk of 6.6 for developing breast cancer, particularly the lobular subtype, due to harbouring a CDH1 truncating mutation, has been reported. Large deletions represent 6% of the CDH1 mutations reported in HGMD.

*What is the range of testing which therefore needs to be done, and what is the justification for this approach to testing? What samples are involved (eg cheek swabs, blood)?*

### Range of testing required to determine heritable breast cancer predisposition risk

The mutational spectrum described above for the genes listed in Subsection 1.3 requires both sequencing and copy number assays to identify the majority of mutations likely to be identified in these genes. DNA sequencing performed by either Next generation or Sanger methodologies should be designed to ensure that all coding regions of BRCA1 and BRCA2, including exon/intron boundaries are interrogated with high sensitivity and specificity (>95%). The minimum regions covered for the genes other than BRCA1 and BRCA2 comprise those regions of each gene that, if a mutation is identified, provides a moderate to high relative risk estimate for the development of hereditable breast cancer. Information currently available suggests that full gene screening for Tp53, PTEN, and STK11 should be performed however, as a minimum a targeted region in CHEK2 covering the 1100delC mutation may be appropriate. Presently, risk estimates for individual PALB2 mutations are not defined. However, data for specific truncating mutations such as PALB2:c.3113G>A indicate high risk estimates and it is likely that other protein truncating mutations will exhibit similar risk estimates, warranting full gene screening.

Copy number assessment can be achieved by techniques such as MLPA or exon resolution microarray analysis and must cover the relevant minimal regions of each gene.

### Samples

The samples analysed are most commonly blood samples from affected individuals except in the case of cascade testing where duplicate and independent blood samples from affected and/or unaffected family members are submitted for specific analysis. In situations where a blood sample from an affected individual in a high-risk family is unavailable, tissue samples from deceased individuals may be provided.

## Scale of gene analysis?

*What is the scale of gene analysis? Select one or more from the following five categories:*

A. *monogenic testing – limited mutation testing or whole gene testing* yes

B. *small gene panel – assaying 2 to ≤10 genes* yes

C. *medium gene panel – assaying 11 to ≤200 genes* no

D. *large gene panel – assaying >200 genes, but remaining sub-exome* no

E. *non-targeted – whole exome sequencing or whole genome sequencing* no

*Provide responses to Subsections 5.3 to 5.6 below consecutively for each category selected above (A to E).*

**A. Monogenic testing**

## Analytical validation of testing

*Is it possible?*  yes

*If yes, elaborate on what is required. If no, briefly explain why not.*

The scope of the test would need to include comprehensive analysis for all described/possible DNA variants in any *single gene* of the eight (8) listed genes. This would therefore necessarily include

1. Sequence level variants/changes and
2. DNA copy number variants/changes.

This would involve sequencing of all exons and intron/exon boundaries (two sequencing methodologies in potential current use – Sanger or massively parallel sequencing) for any single gene of the eight listed genes, in association with a method to detect copy number changes (two methods potentially in current use – MLPA and DNA microarrays). Future methods may incorporate sequencing and copy number change data into a single workflow/method.

## Need for any analytical confirmatory testing

*If a mutation is detected, is any further testing required to confirm its presence using an orthogonal method?*  yes

*If yes, identify the confirmatory assays required. If no, briefly explain why not.*

In some instances, additional analytical confirmatory testing would be required.

Details are provided below:

Single gene testing performed by Sanger sequencing **does not require** additional confirmatory testing of clinically relevant (actionable) mutations due to the degree of prior validation, and known performance characteristics of the Sanger sequencing methodology. However, confirmatory testing on a second sample from the tested individual prior to performing family cascade testing would constitute best laboratory practice in this situation.

However, analytical confirmation of single exon deletions detected by a single MLPA probe **is required** due to the possibility of the presence of a single nucleotide polymorphism (SNP) within the probe sequence and particularly near the MLPA ligation site that could provide a false positive result for an exon deletion. The possibility of a SNP should be excluded by comparison to sequence obtained from the probe site. If the full MLPA probe site is not covered by the routine sequencing strategy employed, this region should be sequenced in a separate assay. Alternatively, confirmation for both single and multiple exon deletions could be provided by MLPA analysis with alternative probe sites if available. The use of microarrays would be subject to similar considerations and consideration should be given to the number of probes interrogated for a single exon deletion.

## Need for any other supplementary testing

*Is there a need for any other supplementary testing (for example, gene expression studies, deletion screens or checking for copy number variations)?*  yes

*If yes, identify all supplementary assay(s), explain why each is needed, and estimate how often supplementary testing would be needed (as a % of all those for whom the primary test would be rendered). If no, briefly explain why not.*

The clinical expectation of comprehensive testing in this setting includes copy number analysis, and this is therefore included as mandatory in the scope of analysis, as above.

In ~ 2% of cases, supplementary RNA studies would be recommended to assess the significance of variants predicted by *in silico* algorithms to potentially affect normal splicing.

## Interpretive complexity

*What is the interpretive complexity? Select one from the following three categories:*

A. *low* no

B. *intermediate* yes

C. *high* no

*Considerations here include qualitative aspects (for example, level of expertise required, complexity of bioinformatics pipelines, software requirements), and quantitative aspects (for example, time component of labour required, cost of software licencing). This information should be sufficient to enable an estimate of the resources required to generate an adequate interpretation of the test results.*

Interpretive complexity for genetic testing results is dependent on:

1. The type of variants identified and
2. The number of variants identified (this is often proportional to the amount of sequence which is interrogated and analysed).

Published studies have reported the amount of time taken to effectively manually review, report and curate actionable variants is ~one hour per variant.

The analyses discussed here (single gene and small gene panels), interrogate and report on only a very small portion of the genome, and therefore a relatively small and manageable number of variants is expected to be identified. These variants would be expected to span a range of interpretive complexity, from well-recognised and annotated to complex variants of unknown significance. Thus, overall, the interpretive complexity would be expected to be intermediate and well within the capability of most laboratories already performing gene sequencing in a diagnostic setting for familial cancer predispositions.

**B. Small gene panel**

* 1. **Analytical validation of testing**

*Is it possible?*  yes

*If yes, elaborate on what is required.*

The scope of the test would need to include comprehensive analysis for all described/possible DNA variants in all genes tested. This would therefore necessarily include

1. Sequence level variants/changes and
2. DNA copy number variants/changes

for any combination of the eight (8) listed genes that are tested with a minimum requirement for BRCA1 and BRCA2 where neither of these genes has been previously tested.

This would involve sequencing of all exons and intron/exon boundaries (two methods potentially in current use – Sanger or massively parallel sequencing) for any combination of the eight listed genes with a minimum requirement for BRCA1 and BRCA2, in association with a method to detect copy number changes (two methods in potential current use – MLPA and DNA microarrays). Future methods may incorporate sequencing and copy number change data into a single workflow/method.

## Need for any analytical confirmatory testing

*If a mutation is detected, is any further testing required to confirm its presence using an orthogonal method?* yes

*If yes, identify the confirmatory assays required. If no, briefly explain why not.*

In some instances, additional analytical confirmatory testing would be required.

Details are provided below:

For small number gene panels, the requirement for confirmatory testing is dependent on the level of validation and experience of the individual laboratory with the technique in use.

In general, sequence variants detected by Sanger sequencing do not require confirmatory testing by a second method due to known and acceptable performance characteristics of the Sanger sequencing methodology in diagnostics. It is expected that, with growing experience and improvements in massively parallel sequencing technologies, samples testing with these methods for small gene panels may also not need additional confirmatory testing within the next 3-5 years.

Currently, requirements are as follows:

If the validation has been performed to NPAAC IVD and NPAAC nucleic acid standards for an individual gene, then variants identified in that gene **do not require** mandatory analytical confirmatory testing, although confirmatory testing on a second sample prior to performing family cascade testing would constitute best laboratory practice in this situation.

Where validation of an individual gene within a panel has not been performed to NPAAC IVD and NPAAC nucleic acid standards, then confirmatory testing **would be required** for all **actionable variants** identified in those genes. Ideally, this confirmatory testing should be performed by Sanger sequencing. Where Sanger sequencing is not possible due to the number of genes analysed and variants identified, this confirmatory testing could be performed by duplicate testing using NGS.

As per single gene testing (discussed above), analytical confirmation of single exon deletions detected by a single MLPA probe is required due to the possibility of the presence of a single nucleotide polymorphism (SNP) within the probe sequence and particularly near the MLPA ligation site that could provide a false positive result for an exon deletion. The possibility of a SNP should be excluded by comparison to sequence obtained from the probe site. If the full MLPA probe site is not covered by the routine sequencing strategy employed, then this region should be sequenced in a separate assay. Alternatively, confirmation for both single and multiple exon deletions could be provided by MLPA analysis with alternative probe sites where available. This is available for both BRCA1 and BRCA2. The use of microarrays would be subject to similar recommendations and consideration should be given to the number of probes interrogated for a single exon deletion.

## Need for any other supplementary testing

*Is there a need for any other supplementary testing (for example, gene expression studies, deletion screens or checking for copy number variations)?*  yes

*If yes, identify all supplementary assay(s), explain why each is needed, and estimate how often supplementary testing would be needed (as a % of all those for whom the primary test would be rendered). If no, briefly explain why not.*

The clinical expectation of comprehensive testing in this setting includes copy number analysis, and this is therefore included as mandatory in the scope of analysis, as above.

In ~ 2% of cases, supplementary RNA studies would be recommended to assess the significance of variants predicted by *in silico* algorithms to potentially affect normal splicing.

## Interpretive complexity

*What is the interpretive complexity? Select one from the following three categories:*

A. *low* no

B. *intermediate* yes

C. *high* no

*Considerations here include qualitative aspects (for example, level of expertise required, complexity of bioinformatics pipelines, software requirements), and quantitative aspects (for example, time component of labour required, cost of software licencing). This information should be sufficient to enable an estimate of the resources required to generate an adequate interpretation of the test results.*

Interpretive complexity for genetic testing results is dependent on:

1. The type of variants identified and
2. The number of variants identified (this is often proportional to the amount of sequence which is interrogated and analysed).

Published studies have reported the amount of time taken to effectively manually review, report and curate actionable variants is ~one hour per variant.

The analyses discussed here (single gene and small gene panels), interrogate and report on only a very small portion of the genome, and therefore a relatively small and manageable number of variants is expected to be identified. These variants would be expected to span a range of interpretive complexity, from well-recognised and annotated to complex variants of unknown significance. Thus, overall, the interpretive complexity would be expected to be intermediate and well within the capability of most laboratories already performing gene sequencing in a diagnostic setting for familial cancer predispositions.

# ECONOMIC EVALUATIONS OF TESTING AFFECTED INDIVIDUALS AND CASCADE TESTING

[TBA]

# FINANCIAL IMPLICATIONS OF TESTING AFFECTED INDIVIDUALS AND CASCADE TESTING

[TBA]

1. Antoniou, A. C., A. P. Cunningham, J. Peto, et al. 2008. "The BOADICEA model of genetic susceptibility to breast and ovarian cancers: updates and extensions." Br J Cancer 98(8):1457-1466. [↑](#footnote-ref-1)
2. James, P. A., R. Doherty, M. Harris, et al. 2006. "Optimal selection of individuals for BRCA mutation testing: a comparison of available methods." J Clin Oncol 24(4):707-715. [↑](#footnote-ref-2)
3. Evans, D. G., F. Lalloo, A. Cramer, et al. 2009. "Addition of pathology and biomarker information significantly improves the performance of the Manchester scoring system for BRCA1 and BRCA2 testing." J Med Genet 46(12):811-817. [↑](#footnote-ref-3)