



Australian Government

Department of Health

MEDICAL SERVICES ADVISORY COMMITTEE

CLINICAL UTILITY CARD FOR HERITABLE MUTATIONS WHICH PREDISPOSE TO BREAST AND/OR OVARIAN CANCER

Introduction

The Medical Services Advisory Committee (MSAC) is piloting arrangements to assess the utility of germline genetic testing for predisposition to broad disease areas, such as cancer, cardiovascular or mental illness. This approach will be used to inform consideration of the circumstances under which testing for predisposition to disease 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 will be conducted in two stages. The first will assess the clinical utility of germline testing for disease predisposition; the second will evaluate the economics and budgetary implications of testing. This second stage will include assessment of the cost-effectiveness of testing index cases/probands (mutation screening) and the marginal cost effectiveness of testing family members (cascade testing). In contrast to previous assessments of germline testing, the pilot will be conducted from the perspective of disease predisposition 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 germline genetic testing 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>) 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 impact on 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.

1. DISEASE CHARACTERISTICS

1.1. List the names of the monogenic diseases proposed for predisposition testing within the disease area and provide the clinical rationale for this grouping

Familial breast and/or ovarian cancer.

1.2. OMIM# of the diseases

114480

1.3. List the names of the corresponding genes which are proposed for testing and, 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)

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.

1.4. OMIM# of the genes

BRCA1, 113705

BRCA2, 600185

PTEN, 601728

STK11, 602216

PALB2, 610355

CHEK2, 604373

TP53, 191170

CDH1, 192090

1.5. 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 would be $\geq 10\%$.

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 BOADICEA¹, BRCAPRO² or pathology-adjusted Manchester score (combined score of 16 or greater)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%.

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.

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.

³ Evans, D. G., F. Laloo, 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.

2.2.2. Would disease management of the affected person be influenced by the result of the genetic test? For guidance, compare how management differs for the incident cancer (only relevant for cancer-affected probands) and future cancers in a person who has a positive mutation test vs those who are mutation negative.

Summarize optimal treatment for mutation positive and negative individuals for the incident current cancer, with each management strategy for mutation-positive individuals and mutation-negative individuals being compared with clinical management in the absence of testing.

| <i>Incident cancer*</i> | <i>Mutation positive</i> | <i>Mutation negative</i> | <i>Incremental benefit of the differential approach</i> |
|-------------------------------|---|---|---|
| Early breast | | | |
| 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 |
| Ovarian/fallopian tube | | | |
| 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 individuals, with each management strategy for mutation-positive individuals and mutation-negative individuals being compared with clinical management in the absence of testing.

| Prevention of cancer | Mutation positive | Mutation negative | Incremental benefit of differential approach |
|-------------------------------|--|--|--|
| Breast | | | |
| Surgery | A. Bilateral mastectomy B. Bilateral salpingo-oophorectomy | Unchanged | A. Risk of breast cancer reduced by $\geq 90\%$ B. Risk of breast cancer reduced by $\sim 50\%$ if patient 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 | <ul style="list-style-type: none"> • Tamoxifen • Raloxifene | Unchanged | Proven 40% reduction of breast cancer incidence in those at higher baseline risk |
| Ovarian/fallopian tube | | | |
| Surgery | Bilateral salpingo-oophorectomy | Unchanged | Risk of ovarian/fallopian cancer reduced by $\geq 80\%$ and risk of breast cancer reduced by $\sim 50\%$ if patient 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.

Supporting evidence for the above is referenced in:

<https://www.eviq.org.au/Protocol/tabid/66/categoryid/66/id/170/Risk+Management+for+a+n+Unaffected+Female+BRCA1+Mutation+Carrier.aspx>

3. DESCRIPTION OF PREDISPOSITION TESTING FOR BREAST CANCER

3.1. Proposed description of testing for differential 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 BRCA2 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.

Genes other than BRCA1 and BRCA2 listed in section 3.2, whilst associated with significantly increased risk of developing breast cancer, mutations, 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 section 3.2 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 hereditary 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 person in a high risk family is unavailable, tissue samples from deceased individuals may be provided.

3.2. Scale of gene analysis?

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

- | | |
|--|-----|
| <i>A. monogenic testing – limited mutation testing or whole gene testing</i> | yes |
| <i>B. small gene panel – assaying 2 to ≤10 genes</i> | yes |
| <i>C. medium gene panel – assaying 11 to ≤200 genes</i> | no |
| <i>D. large gene panel – assaying >200 genes, but remaining sub-exome</i> | no |
| <i>E. non-targeted – whole exome sequencing or whole genome sequencing</i> | no |

Provide responses to sections 3.3 to 3.6 below consecutively for each category selected above (A to E).

A. Monogenic testing

3.3. 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 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.

3.4. 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.

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.

3.5. 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).

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.

3.6. Interpretive complexity

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

- | | |
|------------------------|-----|
| A. <i>low</i> | no |
| B. <i>intermediate</i> | yes |
| C. <i>high</i> | 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).

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

3.3. 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.

3.4. 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.

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.

3.5. 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).

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.

3.6. Interpretive complexity

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

- | | |
|------------------------|-----|
| A. <i>low</i> | no |
| B. <i>intermediate</i> | yes |
| C. <i>high</i> | 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).

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.

4. TEST CHARACTERISTICS OF BRCA1 AND BRCA2

4.1. Analytical performance

*Is there an analytical reference standard used to establish genotype: yes
[If yes, complete 4.1.1 below; if no, complete 4.1.2 below]*

4.1.1. Analytical validity possible (to be answered if 4.1 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.

| <i>Reference</i> | <i>Analytical sensitivity</i> | <i>Analytical specificity</i> | <i>Genes included</i> | <i>Additional comments</i> |
|--|-------------------------------|-------------------------------|--|--|
| Dacheva D. <i>et al.</i> Mol Diagn Ther. 2015 19(2):119-30. | 100% | 95.9% | BRCA1 BRCA2 | |
| Ruiz A. <i>et al.</i> Biomed Res Int. 2014:542541. doi: 10.1155/2014/542541. Epub 2014 Jun 26. | 100% | 97.35% | BRCA1 BRCA2 | |
| Guan Y. <i>et al.</i> Fam Cancer. 2015 14(1):9-18. | 93.66% | 99.98% | BRCA1 BRCA2 | Accuracy evaluated at 99.97% |
| Judkins T. <i>et al.</i> 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 |

4.1.2. Analytical validity not possible (to be answered if 4.1 was marked “no”)

Present analytical concordance across testing options (using proportions with their 95% CI and/or kappa statistics):

Present analytical reproducibility of each testing option (using proportions with their 95% CI and/or kappa statistics):

Present inter-rater or inter-laboratory reliability of each testing option (using kappa statistics):

Present the limit of detection of each testing option:

4.2. Clinical validity

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

[Possible events include disease progression, developing a new cancer, 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.

4.2.2. 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).]

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 ca ¹ Cumulative risk % | BRCA1 ² | | | BRCA2 ² | | |
|--------------|--|--------------------|-------------|------------|--------------------|-------------|------------|
| | | Cumulative risk % | 95%CI | RR | Cumulative risk % | 95%CI | 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).

4.2.3. 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).

4.2.4. 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.

4.2.5. Prevalence (or diagnostic yield) associated with this ratio:

[If the prevalence is likely to vary from the study population of affected patients 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 4.2.2 may be considered conservative.

5. IMPLEMENTATION ISSUES AND RATIONALE FOR ANY CASCADE TESTING

5.1. 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 worse breast cancer outcomes
- the individual is at increased risk of worse ovarian cancer outcomes
- family members of the individual are at increased risk of developing cancer.

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 first-degree family members:

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

If required, nature of counselling or information to be provided: [TBA].

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

5.2. Genetic risk assessment in first-degree family members of a diseased person

(to be answered if in 2.1, B was marked “yes”)

5.2.1. Would disease management of the family member be influenced by the result of the genetic test?

Summarize the differences between prevention strategies for mutation positive and negative individuals, with each management strategy for mutation-positive individuals and mutation-negative individuals being compared with 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.]

| Prevention of cancer* | Mutation positive | Mutation negative | Incremental benefit of differential approach |
|------------------------|--|---|---|
| Breast (female) | | | |
| Surgery | A. Bilateral mastectomy B. Bilateral salpingo-oophorectomy | Unchanged (but avoids unnecessary surgery) | A. Risk of breast cancer reduced by $\geq 90\%$ B. Risk of breast cancer reduced by $\sim 50\%$ if patient 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 | <ul style="list-style-type: none"> • Tamoxifen • Raloxifene | Unchanged (but avoids unnecessary medical prophylaxis) | Proven 40% reduction of breast cancer incidence in those at higher baseline risk |

| <i>Prevention of cancer*</i> | <i>Mutation positive</i> | <i>Mutation negative</i> | <i>Incremental benefit of differential approach</i> |
|-------------------------------|---|--|--|
| Ovarian/fallopian tube | | | |
| Surgery | Bilateral salpingo-oophorectomy | Unchanged (but avoids unnecessary surgery) | Risk of ovarian/fallopian cancer reduced by $\geq 80\%$ and risk of breast cancer reduced by $\sim 50\%$ if patient 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 | | | |
| Surgery | Unchanged | Unchanged | Not 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 cancer. For family members who already have a diagnosis of the disease, refer to the treatment table in Subsection 2.2.2.*

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

Supporting evidence for the above is referenced in:

<https://www.eviq.org.au/Protocol/tabid/66/categoryid/66/id/170/Risk+Management+for+a+n+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>

5.2.2. Clinical context of testing for a first-degree family member of an index patient

Clinical setting in which first-degree 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 for the first-degree family member: required.

Role of post-test genetic counselling for the first-degree family member:

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

If required, nature of counselling to be provided: [TBA].

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