

Gene panel testing for hereditary breast cancer

It is estimated that less than 5% of breast cancer occurs as a result of single gene predispositions; some 2% is explained by mutations in the well known *BRCA1* or *BRCA2* genes.¹ The Australian Institute of Health and Welfare estimated that 15 740 people would be diagnosed with breast cancer in 2015,² suggesting about 780 of these cancers would be due to genetic predisposition. While this number seems small, the people affected have many more family members (most of whom are unaffected) who can have their potentially high risk of breast cancer managed once it is identified.

Recognition of a familial predisposition to breast cancer led to a race for the “responsible” genes in the late 20th century. The *BRCA1* gene was the first gene found to have very rare mutations associated with high penetrance of breast and ovarian cancers.^{3,4} These rare mutations were often identified in young women or in members of families with multiple cases of breast or ovarian cancer. In 1994, the *BRCA2* gene was identified,^{5,6} and mutations in this gene were found to be associated with increased risk of several cancer types; breast and ovarian cancers were seen in families who also had a higher incidence of melanoma, pancreatic cancer and prostate cancer. A characteristic feature of mutations in *BRCA2* is the increased risk of breast cancer in men.⁶

Twenty years later, the “missing heritability” of breast cancer remains elusive. A network of genetic services and familial cancer centres across Australia investigate families when there is an evident inherited predisposition to breast cancer. However, most of these families do not have mutations in either the *BRCA1* or *BRCA2* gene. Rare mutations in other genes have subsequently been recognised as playing a role, especially in cases where breast cancer is diagnosed at an early age.⁷⁻¹⁰

A new approach – multiple gene testing

To date, tests have been conducted as single gene tests in an iterative manner, one gene at a time, at very high cost. Although some information could be used to direct this testing (eg, specific histopathological features of the breast cancer, such as mitotic index and trabecular growth pattern),¹¹ the testing process is slow and laborious, and most test results remain uninformative. However, the revolution in our capacity to conduct genetic analyses in very recent years has had a profound impact on how genetic testing services can be applied. Now, a panel of multiple genes can be screened for mutations in a single test, at considerably reduced cost, via the application of massively parallel sequencing technology. This approach applies across many disciplines, most notably cardiology; gene panel testing is now the standard of care in the cardiac genetic clinic at the Royal Melbourne Hospital.¹²

Summary

- Inherited predisposition to breast cancer is explained only in part by mutations in the *BRCA1* and *BRCA2* genes. Most families with an apparent familial clustering of breast cancer who are investigated through Australia’s network of genetic services and familial cancer centres do not have mutations in either of these genes.
- More recently, additional breast cancer predisposition genes, such as *PALB2*, have been identified.
- New genetic technology allows a panel of multiple genes to be tested for mutations in a single test. This enables more women and their families to have risk assessment and risk management, in a preventive approach to predictable breast cancer.
- Predictive testing for a known family-specific mutation in a breast cancer predisposition gene provides personalised risk assessment and evidence-based risk management.
- Breast cancer predisposition gene panel tests have a greater diagnostic yield than conventional testing of only the *BRCA1* and *BRCA2* genes.
- The clinical validity and utility of some of the putative breast cancer predisposition genes is not yet clear.
- Ethical issues warrant consideration, as multiple gene panel testing has the potential to identify secondary findings not originally sought by the test requested.
- Multiple gene panel tests may provide an affordable and effective way to investigate the heritability of breast cancer.

Increases in genetic technology capacity have, in parallel, enabled work that has identified additional breast cancer predisposition genes, and many other candidate breast cancer predisposition genes have been reported (however, these require further evidence). Today, gene panel tests for breast cancer predisposition are offered by numerous providers and service laboratories.^{10,13} However, few of these tested genes are bona fide breast cancer predisposition genes. Indeed, most of the genetic variation identified by these gene panel tests cannot be interpreted in terms of breast or ovarian cancer risk.

The *PALB2* gene is an example of an important new breast cancer predisposition gene, as it has rare mutations that are associated with high penetrance for breast cancer.^{14,15} The *PALB2* protein plays an essential role in interacting with the *BRCA1* and *BRCA2* proteins and enabling DNA repair. The risk of breast cancer for women with heterozygous germline mutations in the *PALB2* gene is as much as eight to nine times as high as in the general population, depending on the age of the woman.¹⁵ Mutations in

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Hypothetical case illustrating the utility of gene panel testing

A 37-year-old woman had been diagnosed with high-grade breast cancer at 35 years of age. She had a positive family history of breast cancer, with her paternal aunt being diagnosed at the age of 41 years and her paternal grandmother at 49 years. In keeping with usual practice in Australia, she was offered genetic testing on the basis that she had a greater than 10% likelihood of carrying a mutation in either the *BRCA1* or *BRCA2* gene, calculated by the BOADICEA risk prediction algorithm.¹⁷

No mutation was found in either the *BRCA1* or *BRCA2* gene. This result was regarded as inconclusive, but the family was still deemed to be at high risk of an inherited breast cancer predisposition (as three relatives on the same side of the family had been diagnosed with breast cancer before the age of 50 years). As a result, all women on the paternal side of her family underwent high-risk surveillance, as no individualised (ie, genetically informed) risk assessment could be offered.

During a review appointment, it was disclosed that the woman's paternal cousin, aged 42 years, had also recently been diagnosed with breast cancer. In view of the high likelihood of a mutation, further testing was undertaken using a gene panel approach.

This testing identified a *PALB2* c.3113G>A mutation. As there are recommendations for the risk management of unaffected women who have this *PALB2* gene mutation, the woman's extended family were offered predictive testing.¹⁸ Two affected relatives who were still living and four of the seven unaffected women tested were also found to carry the *PALB2* mutation and were referred to breast surgeons and gynaecological oncologists for management of their high breast cancer risk.

BOADICEA = Breast and Ovarian Analysis of Disease Incidence and Carrier Estimation Algorithm. ♦

PALB2 account for about 2.4%^{14,16} of the familial aggregation of breast cancer in the Australian population, but occur at extremely low rates in the general population, making single gene testing for *PALB2* cost-ineffective at a population health level.

The low yield of single gene testing and the potential for inclusion of other genes with low frequency mutations (such as *PALB2*) make a gene panel test approach ideal in the investigation of breast cancer predisposition. Genes that are regularly included in breast cancer susceptibility gene panels that are currently available through academic or commercial testing laboratories include *BRCA1*, *BRCA2*, *PALB2*, *PTEN*, *STK11*, *TP53*, *ATM*, *BARD1*, *BRIP1*, *CDH1*, *CHEK2*, *MRE11A*, *MUTYH*, *NBN*, *NF1*, *RAD50* and *RAD51C*.^{10,13}

The utility of gene panel testing is illustrated by the hypothetical case outlined in the [Box](#).

Implications for breast cancer risk management

There is an evidence-based strategy for managing women who harbour high penetrance mutations in genes such as *BRCA1* and *BRCA2*, and emerging evidence for the risk management of women with mutations in several other genes, including *PALB2*. For *BRCA1* and *BRCA2* mutation carriers, it is now known that bilateral salpingo-oophorectomy around the age of 40 years or before the natural menopause will halve the risk of breast cancer, while substantially lowering the risk of ovarian cancer.¹⁹ Likewise, chemoprevention and risk-reducing mastectomy are effective in reducing the risk of breast cancer in women with pathogenic mutations in these genes.²⁰ In the

absence of a mutation, where there is a theoretical 50% risk that each woman in such families will not have inherited the genetic predisposition, this irreversible and invasive approach is hard to justify.

Genetic testing is thus the only way to provide personalised risk assessment, through predictive testing of at-risk unaffected family members, although this approach depends on identifying a pathogenic mutation in the index case. Effective risk management is thus predicated on accurate risk estimation; as is release from high-risk cancer surveillance for those found not to be at high risk of cancer. The initial inability to find a pathogenic mutation in our illustrative case ([Box](#)) meant that this risk management strategy was not possible, whereas gene panel testing and the resulting detection of the *PALB2* gene mutation enabled personalised management of the extended family.

Considerations in gene panel testing

The choice of genes to include in panel testing needs careful consideration. The ACCE framework, named for the acronym derived from its descriptors, calls for genetic tests to be underpinned by analytical validity, clinical validity, clinical utility and ethical issues.²¹

Clinical validity and utility

Clinical validity and consequent clinical utility are currently the most complex areas — is the genetic variation detectable in these genes associated with clear and quantifiable risk of disease? Initially, genetic testing entered clinical practice without much process, nor an evidence base, but spurred by an enthusiasm to reduce the risk of potentially predictable cancers. In contrast, adoption by clinical services of the newly recognised breast cancer predisposition genes, such as *PALB2*, has been slow, due to an increased awareness of the complexity of interpretation of genetic test results.²² In Australia, protocols for genetic testing and subsequent management based on genetic knowledge have been built over time and are now consolidated in the eviQ guidelines,¹⁸ used by clinical genetic and familial cancer services nationally.

It was important initially to ensure that panel testing was as good as the standard of care in finding mutations in *BRCA1* and *BRCA2*, before considering potential additional benefits. In a recent study of 966 patients undergoing breast cancer genetic testing,²³ deleterious *BRCA1* or *BRCA2* mutations were identified at equivalent rates between single gene testing and a multiple gene panel test that included *BRCA1* and *BRCA2*. An additional 3.9% of patients tested by the multiple gene panel had pathogenic mutations identified in other breast cancer predisposition genes, most commonly *PALB2*, *CHEK2* and *ATM*. In a retrospective review of 90 consecutive patients undergoing genetic testing by a multiple gene panel test,²⁴ pathogenic mutations were identified in nine individuals: five in *BRCA1* or *BRCA2*, and four in other breast cancer predisposition genes. As single gene testing has only

identified mutations in *BRCA1* and *BRCA2* genes, multiple gene panel testing finds more clinically significant mutations.

However, gene panel tests also identify many variants of uncertain significance. Classification of variants is important. In short, there are sequence variants that are clearly disease-associated and classed as pathogenic mutations, while at the other end of the spectrum, much variation in DNA is clearly not associated with disease predisposition and can be discounted. A large grey zone exists in between, the so-called variants of uncertain significance, where classification is not possible, and this information cannot be used clinically. For this reason, interpretation of results, whether derived from gene panels or single gene testing, remains highly specialised and requires a team of skilled scientists, informaticians and genetic practitioners, along with research-derived evidence.

Ethical issues

Technology that allows analysis of many genes at once has a raft of associated ethical issues when applied in a diagnostic setting.²⁵ It is prudent to consider the ability of such tests to produce secondary findings (previously also referred to as incidental findings), wherein genetic changes may be found that were not anticipated.

The recent confirmation in the High Court of Australia that the *BRCA1* gene is not patentable has been welcomed, and represents a moral victory for the women and families concerned.²⁶ Indeed, in 2013, following the

ruling by the Supreme Court in the United States to invalidate patent claims made by Myriad Genetics, the *BRCA1* and *BRCA2* genes were included in existing panels that had hitherto excluded them and thus had reduced the comprehensiveness of the analyses being conducted.¹⁰

Conclusions

The use of new genetic technology can provide an affordable, effective way of investigating the heritability of breast cancer. Early studies suggest that the gene panel testing approach will detect all mutations found by conventional testing and increase the diagnostic yield through analysis of more genes. This gives more women and their families the opportunity to have personalised risk assessment and risk management, in a preventive approach to predictable breast cancer.

On a cautionary note, the consequences — that is, the clinical validity and utility of many of the genes listed — are not yet clear. Large scale studies through international collaborations into the genes included in these gene panels are essential to yield the information that will allow the safe and effective use of newer technologies and new genetic information in clinical practice.

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