Meeting the challenges of interpreting variants of unknown clinical significance in BRCA testing

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SUMMARY IMPORTANCE OF BRCA1 AND BRCA2 GENE SCREENING

- Many BRCA1 and BRCA2 genetic mutations are known to result in an elevated breast cancer risk.
- Routine BRCA1/2 gene screening is offered to patients thought to have an increased risk of carrying a deleterious mutation.
- 5–10% of genetic tests identify a variant of unknown clinical significance (VUCS), creating significant challenges to health care providers.
- Recent advances in sequencing technologies allow more genes to be screened in an increasing number of individuals and at an ever decreasing cost.
- Significantly more VUCS will be identified, adding to the uncertainty of how to manage these patients.
- The addition of splicing assays to current variant classification tools may be instrumental towards understanding the disease risk of these variants and improve the reliability of these assays.

Genetic mutations in the breast cancer susceptibility genes BRCA1 and BRCA2 are known to confer a highly elevated risk of breast cancer in at least 20% of multi-case families,1 ranging from 44% to 75% risk of cancer for BRCA1 mutation carriers and 41% to 70% risk for BRCA2 mutation carriers.2 Moreover, BRCA1 and BRCA2 mutations are estimated to account for up to 84% of families with four or more cases of breast cancer diagnosed younger than age 60 years.3

Routine diagnostic BRCA1 and BRCA2 gene screening for deleterious mutations is offered to affected individuals from high-risk breast-ovarian cancer families to identify the genetic cause for their disease. Currently, the pre-test probability of a genetic test identifying a pathogenic mutation in these high risk individuals is very low, while we are also yet to gain a thorough understanding of how to interpret many of the variants that are identified. This uncertainty may lead to possible adverse psychosocial consequences for patients and their families.4

While elevated stress levels are observed in women confirmed to carry pathogenic mutations, the intensive precautionary and preventative measures implemented as a result of this knowledge can significantly improve both disease-free survival and outcome if disease does develop.5,6 A negative genetic test result usually decreases anxiety in these patients, whereas it also reduces health costs as treatment, and preventative measures can be focused on those who are thought to have a significantly elevated risk of disease.

Currently, affected patients are offered BRCA1 and BRCA2 screening by Genetic Health Services New Zealand (GHSNZ) if they have one or more criteria outlined by evIQ Cancer Genetics—Breast and Ovarian Referral Guidelines (https://www.eviq.org.au/) (Box 1).

In New Zealand, approximately 300–500 of the 1,600 women referred to GHSNZ for genetic risk assessment are offered BRCA1 and BRCA2 gene screening each year. A further 350–400 individuals are
Variants of unknown clinical significance

An important practical issue associated with genetic testing is the identification of rare sequence variants that are not predicted to lead to obvious or easily detectable molecular aberrations, such as protein truncation or RNA splicing defects. These variants are identified in approximately 5–10% of BRCA1/BRCA2 clinical test results and are difficult to classify clinically as pathogenic (associated with disease risk) or neutral. Variants of unknown clinical significance (VUCS or unclassified variants) create a significant challenge for counselling and clinical decision making when identified in patients with a strong family history of breast and/or ovarian cancer. Over the last 10 years, up to 100 New Zealand individuals from a large number of families will have received a report from their BRCA genetic test indicating that they carry a VUCS. The associated uncertainty is known to leave many variant carriers with higher levels of anxiety, depression, and distress compared with those individuals receiving a negative result. Thus, interpreting unclassified sequence variants is not only necessary for the patient undergoing genetic testing but also for their relatives and future generations who may inherit these unclassified variants.

Seventy percent (1757/2474) of the entries in the commonly utilised genetic database, Breast Cancer Information Core database (http://research.nhgri.nih.gov/bic/ accessed 11th June 2015) remain unclassified. We predict that this number will rise substantially with the increased uptake of next-generation sequencing technologies in testing laboratories. Such technologies offer cheaper (per base), user-friendly, high-throughput sequencing, and this will enable more diagnostic laboratories to offer genetic testing across the entire gene of interest (exonic and intronic regions) on a greater number of individuals. Furthermore, advances in NGS technology have enabled the development of multiplex gene panels so that numerous genes can be assessed simultaneously for breast/ovarian cancer-risk sequence variants. Expanding the number of genes included in each test will inevitably lead to a rise in the number of unclassified variants being detected. Indeed, a recent study evaluating the coding regions and exon-intron boundaries (±10 base pairs) of a 42-cancer gene sequencing panel (including BRCA1 and BRCA2) identified unclassified

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**Box 1. Example of criteria used to prioritise individuals for germline BRCA1/BRCA2 testing**

- Triple negative (oestrogen, progesterone and HER2 receptor negative) breast cancer and <40 yrs at diagnosis
- Invasive non-mucinous ovarian, fallopian tube or primary peritoneal cancer at any age and a family history of breast or ovarian cancer
- Personal and/or family history of both breast cancer and epithelial ovarian cancer
- Ashkenazi Jewish ethnicity (for Jewish founder mutations)
- Member of family with confirmed BRCA1 or BRCA2 mutation
- Individual with a calculated BRCA1/2 mutation probability of 10–20% or more using a BRCA1/2 mutation probability risk calculator e.g. BRCAPRO or BOADICEA
- Woman with bilateral breast cancer and a family history of breast and/or epithelial ovarian cancer
- Male breast cancer
- Breast cancer diagnosed before age 30 years
- Bilateral breast cancer with first diagnosis under age 50 years

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offered predictive testing for a known family mutation per annum, returning around 200 positive results. Diagnostic screening is carried out across the exons and intron-exon boundaries of the relatively large BRCA1 and BRCA2 genes (22 and 26 protein coding exons, respectively), using next-generation sequencing technologies, in addition to multiplex ligation-dependent probe amplification (MLPA). Up to 80% of genetic tests for BRCA1 and BRCA2 mutations in New Zealand breast cancer patients do not identify a pathogenic mutation. Moreover, it is unclear how many BRCA1 and BRCA2 mutation carriers within New Zealand fail to be referred for a genetic test despite these tools and guidelines. Identification of cancer-causing mutations in the breast cancer susceptibility genes BRCA1 and BRCA2 has well-defined and actionable implications for disease prevention. The development of better methods for prioritising patients for mutation screening will no doubt improve clinical management of high-risk breast-ovarian cancer families and reduce health care costs.
variants in approximately 90% patients who had previously undergone BRCA1/2 testing. The trend towards sequencing deeper into the intronic regions will also undoubtedly increase the numbers of unclassified variants identified in BRCA1 and BRCA2 genetic tests. Managing high rates of unclassified variants will become increasingly difficult for oncologists and genetic counsellors as they try to explain the significance of these results to patients. Although surveillance of such patients will likely continue, based on personal and family history, health providers and/or diagnostic laboratories also need routine protocols for 1) re-examining the genetic results on a regular basis as international databases update; and 2) anonymising genetic data so that they too can contribute to international databases. In addition, existing variant classification tools need to be adopted and further developed by New Zealand laboratories, to keep abreast of the technological advances.

### Classifying BRCA1 and BRCA2 variants of unknown clinical significance

An important tool to evaluate the clinical significance of unclassified variants is the multifactorial likelihood model. This statistical tool integrates data from several sources, such as clinical, tumour pathology and molecular data, targeting characteristics associated with known BRCA1/BRC2 pathogenic mutations (Figure 1). This multifactorial approach can help gain an understanding of the role each variant is having on disease development.

The posterior probability calculated from the multifactorial model is assigned to one of five classes using a scheme developed by a panel of experts at the 2008 IARC (International Agency for Cancer Research) Unclassified Genetics Variants Working Group. This scheme defines class 1 low clinical significance variants as having a <0.01% probability of being pathogenic; class 2 variants—low clinical significance, probability 0.1%–4.9%; class 3 variants—uncertain probability 5%–94.9%; class 4 variants—likely pathogenic, probability 95%–99%; and class 5—pathogenic, probability >99%.

Research is currently underway to assess the potential of mRNA splicing assays for determining the molecular impact of unclas-
sified variants in patient samples. Aberrant mRNA isoforms may occur as a result of rare sequence variants and have the potential to disrupt the normal function of BRCA1 and BRCA2 proteins. The implementation of RNA splicing assays has previously been described as an indispensable tool in a clinical diagnostic setting, and the inclusion these assays into the above multifactorial analysis is only a matter of time. However, a number of guidelines need to be adopted to improve the reliability of these assays and ease of use for everyday clinical practice, including the standardisation of assay designs and reporting. As future assays will likely require both qualitative and quantitative assessment of mRNA splicing aberrations, it is easy to envisage another role for NGS, such as targeted RNA-seq.

Of note, New Zealand researchers are currently involved in several molecular and sequencing-based projects in collaboration with members of the international ENIGMA (Evidence-Based Network for the Interpretation of Germline Mutant Alleles) consortium (enigmaconsortium.org). ENIGMA was established to focus on developing and improving methods to classify variants in breast cancer genes, including BRCA1 and BRCA2, by pooling resources from international collaborative diagnostic and research laboratories. Their work includes an assessment of the IARC system, which highlighted several of the recommendations and challenges mentioned above. Furthermore, the New Zealand Familial Breast Cancer Study was established to recruit a cohort of women who have a personal or family history of breast cancer, and meet the Genetic Health Services New Zealand criteria for genetic testing. This study will allow local researchers to maintain collaborative links with ENIGMA and other international consortia to help understand the effects of BRCA1 and BRCA2 variants on cancer risk.

**Future impact of genetic testing for breast cancer families in New Zealand**

The recent evolution in sequencing technologies will enable more affordable genetic testing for New Zealand breast cancer families. However, it will also likely mean that an increased number of tests will return ambiguous results, thus providing a significant challenge to health care providers. Current efforts to improve variant classification systems, such as those by ENIGMA, will be crucial to understand the impact these variants are having on the risk of disease for an individual. It is imperative that oncologists, genetic counsellors and general practitioners are resourced sufficiently to take advantage of current and future classification tools. This may include routine collection of necessary clinical and pathological data along with bio-specimens to enable laboratory based analyses.

In the age of advancing genetic sequencing technologies patients are required to be fully informed of the implications and increasing likelihood of obtaining an unclassified variant result in their genetic test. Emphasising that many of these variants may not have an impact on disease risk is important, but also, such variants should not be included in patient management decisions until strong evidence is obtained that determines whether or not the variant is deleterious. Improvements to the current classification guidelines to help establish risk will be ongoing, as new information becomes available and interpretation processes advance. It is therefore recommended that all variants recorded in public databases are date-stamped to mark the time of classification. Furthermore, variant information should be submitted with donor consent to locus specific databases, such as the Breast Cancer Information Core, and the Leiden Open Variation Database 2.0, to facilitate accurate interpretation of genetic tests and contribute to the national and international health and research community. Use of quantitative tools to assess variants, such as the multifactorial likelihood model, is time consuming and therefore may not be feasible for routine application by genetic associates and clinical geneticists. An alternative approach is to develop a multidisciplinary collaboration of stakeholders (health care providers, patients and researchers) to curate DNA variants identified in New Zealand breast (or ovarian) cancer patients and assign clinical relevance. Analysis of genetic data would be carried...
out nationally by dedicated research and/or diagnostic laboratories and expertly curated in collaboration with the international community through consortia, such as ENIGMA.

Establishing better variant classification tools (eg, laboratory-based assays) and national practices is critical to meet the challenges associated with the increased sensitivity and specificity of genetic tests. If successful for BRCA1 and BRCA2 variants, then these methods will provide exemplars when striving to understand the risk behind variants in other cancer-associated genes.

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