Clinical Chemistry Trainee Council
Pearls of Laboratory Medicine
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TITLE: Genetic Testing for Hereditary Breast Cancer

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Slide 1:
Hello, my name is Melody Caramins. I am a Genetic Pathologist, also known as a Clinical Molecular Geneticist, at New South Wales Health Pathology. Welcome to this Pearl of Laboratory Medicine on "Genetic Testing for Hereditary Breast Cancer."

Slide 2:
Cancer is a one of the major causes of mortality and morbidity worldwide, with breast cancer being the most common cause of cancer death in women worldwide.

Cancer Research UK estimates that in 2008, breast cancer was responsible for ~458,500 female deaths. To put that into context, that would be equivalent to 1146 fatal 747 crashes in a year; which is a staggering statistic.

A small proportion of all breast cancer cases, about 5-10%, will have a strong hereditary component. This amounts to tens of thousands out of the previously quoted 458,000, and about 5% of these will be due to autosomally dominant inherited mutations in highly penetrant genes.

Slide 3:
Firstly, how does one go about in trying to identify the high-risk individuals who might benefit from genetic testing? Well, there are a number of national guidelines which can be consulted and provide some information regarding what criteria might constitute a high-risk individual. For example, in the UK, the National Institute for Health and Clinical Excellence (NICE) guidelines.

One of the most important components of assessment is a comprehensive family history, particularly from a specialist hereditary cancer clinical service. This might usually involve a multi-generation family history of cancer, including risk considerations such as: the age at diagnosis, family history of malignancy, bilateral disease, the presence of male breast cancer, 2 or more blood relatives being affected with cancer, or high-risk ethnicity (e.g. Ashkenazi Jewish).

Other risk factors which might be taken into account might include age, parity, or obesity.

There are some computer-based probability modeling programs that can be used in this sort of clinical setting, which can calculate a likelihood estimation that a BRCA1 or BRCA2 mutation might be found in a consultant. Two examples of such programs include BRCAPRO and BODICEA. Programs like these can be helpful when deciding whether to offer genetic testing in a clinical setting.

Slide 4:
Some national guidelines might suggest testing based on probability of mutation detection.

The UK guidelines by the National Institute for Health and Clinical Excellence (NICE) advocate gene testing if there is a high risk of breast cancer, which is defined as a 1 in 3 lifetime risk of breast cancer, or a greater than 1 in 12 risk of breast cancer prior to age 50.

The American Society of Clinical Oncologists (ASCO) doesn't set a numerical threshold value above which testing is recommended, but does suggest an individual risk assessment.

Slide 5:
Cancer Australia recommends testing only be carried out through specialist hereditary cancer clinics. Their website (http://canceraustralia.gov.au/affected-cancer/cancer-types/breast-cancer/your-risk/calculate) offers an online risk assessment questionnaire, which takes about 5-10 minutes to complete and can be undertaken by patients on their own or with a health professional.

The questionnaire is based on health and family history and provides patients with a risk categorization (low, medium, moderate) of breast cancer, and provides information on which aspects of their history contribute towards higher or lower risk of breast cancer development.

In individual clinic assessments in a hereditary cancer setting, the evaluation criteria for offering testing might also include a Bayesian calculation of the probability of mutation identification, as calculated by some of the programs discussed before.

Slide 6:
Penetrance refers to the proportion of individuals with a particular genetic variant who also express the associated phenotype.

In cancer, highly penetrant genes/variants confer a relative cancer risk of >5 compared with the baseline risk, moderately penetrant genes/variants confer relative risk of 1.5 – 5, and low-penetrant genes confer a relative risk of ~1.5.

Clearly deleterious mutations in highly penetrant genes such as BRCA1 and 2 increase lifetime risk of breast cancer to approximately 80% and the lifetime risk of ovarian cancer to approximately 40%, although this will be slightly different depending on exactly which gene and which mutation. Lifetime risk might also be affected by other variants in other genes, as well as environmental and lifestyle factors.

It is important to remember that even in some highly penetrant breast cancer susceptibility genes, such as BRCA1 and 2, variants might exist which show moderate penetrance - such as the BRCA1 arginine to glutamine change at amino acid position 1699 - or perhaps variants may have unknown penetrance; so for some patients and their families, the identification of a variant in a known hereditary cancer gene can sometimes be the be the beginning of a journey, rather than an end.

Slide 7:
Hereditary cancer is the ultimate complex genetic disease; cancer risk may be elevated by mutations in a number of genes, and each mutation may increase risk for more than one type of cancer.

Testing has traditionally involved serial testing of high penetrance genes such as BRCA1 and BRCA2. These tests have been shown over many years to have proven clinical utility, with available protocols and recommendations for management and clinical care of mutation positive patients. Testing is also generally delivered in a setting which limits the potential for harm and maximizes the benefits to patients.

Slide 8:
As mentioned, traditionally, testing has involved comprehensive BRCA1 and BRCA2 mutation analysis, usually comprising of sequencing of both genes, with simultaneous detection of large deletions. Testing has been established mostly around these two genes, as they are the most common genetic causes of hereditary breast/ovarian cancer, because deleterious mutations are generally highly penetrant and also because the clinical utility of testing has been comprehensively assessed and proven.

In some higher risk populations (e.g. Ashkenazi Jewish), genotyping for a limited number of targeted founder mutations in these two genes has also been undertaken as a cost-effective start to risk assessment.

Slide 9:
At the most fundamental level, cancer is a disease in which normal regulation of tissue growth is disturbed.

In hereditary cancers, this regulation is disturbed by the presence of a heritable constitutional change in a gene which regulates cell growth (oncogene), or a gene which inhibits cell division and survival (tumor suppressor gene); which then leads to a significant increase in cancer risk.

The hypothesis that cancer results from accumulated DNA mutations which subsequently led to the identification of oncogenes and tumor suppressor genes was first proposed by Carl Nordling in the British Journal of Cancer in 1953, and subsequently further developed by Alfred Knudson's statistical analyses in children with inherited retinoblastoma. Knudson suggested that multiple "hits" to DNA were necessary to cause cancer. In the children with inherited retinoblastoma, the first insult was inherited in the DNA, and any second insult would rapidly lead to cancer. In non-inherited retinoblastoma, two "hits" had to take place before a tumor could develop, explaining the earlier age of onset in inherited retinoblastoma.

Slide 10:
BRCA 1 and BRCA 2 are tumor suppressor genes; their protein products are essential for repairing damaged DNA and maintaining genomic integrity.

BRCA1 is part of a large complex of proteins, the BASC (BRCA1-associated genome surveillance complex), and has a central role in DNA repair by monitoring the genome for damage and signaling downstream effectors. BRCA1 may also function as a coordinator of multiple activities required for maintenance of genomic integrity during the process of DNA replication. BRCA1 has been implicated in two pathways of double strand DNA break repair: homologous recombination (HR) and non-homologous end joining (NHEJ). BRCA1-deficient cells are sensitive to ionizing radiation and DNA damaging drugs, such as mitomycin C.

BRCA2 belongs to a family of genes called FANC (Fanconi anaemia, complementation groups), the main purpose of which is in the cellular response to DNA damage. BRCA2 has been implicated in a number of functions including:

- Maintenance of genomic integrity by regulation of homologous recombination (HR)
- Maintenance of cell cycle checkpoints by affecting mitotic spindle assembly
- Transcription regulation
- Meiotic recombination

Slide 11:
The BRCA2 c.5946delT deleterious variant is a variant commonly found in the Ashkenazi Jewish population as a “founder mutation.” This variant in exon 11 leads to a frame-shift mutation and premature truncation of the protein at the 2003rd amino acid. The truncation of the BRCA2 protein prior to the carboxy-terminal nuclear localization signals results in exclusion of the mutant protein from the nucleus, and the inactivation of all its associated nuclear functions, including:

- Increased sensitivity to DNA cross-linking agents
- Homology directed repair
- Genomic instability (centrosome amplification)

Slide 12:
Genetic testing for hereditary breast and ovarian cancer is rapidly changing. This is mostly due to recent technological advances and the ability to offer rapid and cost-effective sequencing by massively parallel sequencing.

Some laboratories now offer simultaneous sequencing of “panels” of genes, where 12-40 genes are sequenced simultaneously, as opposed to sequential sequencing of single genes. This model of testing is now more widely available in a worldwide diagnostic context.

Each of the genes on the available multiplex panels may be uncommonly mutated; therefore, collaborative work will become critical to establish large cohorts of patients to adequately inform patient management in the future, given the lack of current established and comprehensive evidence on clinical utility for testing using this pathway.

Slide 13:
Gene testing panels for hereditary breast cancer may now offer up to 10-40 genes.

When designing such a panel, some considerations may be:

To group them according to penetrance, and offer tiered testing. So initially, one might screen for high penetrance genes for which there are already well-established clinical testing and management criteria (such as BRCA1/2, TP53, PTEN).

If negative, then other moderate or low penetrance genes, such as e.g. CHECK2, ATM could then potentially be offered but with the caveat that the clinical utility of this is not firmly established and may be limited.

Another way to offer testing would be to offer panels grouped according to function or cancer site.

Slide 14:
An important thing to bear in mind is that different cancer risks can be associated not only with specific genes but also specific mutation positions.

Different mutations can also be associated with specific histopathological features, so clinical phenotype, the laboratory phenotype, and the genotype together all provide important information.

For example, BRCA 1-related tumors are more likely than sporadic tumors to be “triple negative,” of higher histological grade, and derived from basal epithelial layers.

Families with ovarian cancer are more likely to have mutations in ovarian cluster region of BRCA 2 exon 11.

Odds ratios for prostate cancer can also vary significantly with position of BRCA2 mutation.

Slide 15:
The sequencing of any gene will almost invariably identify some missense variants. There exists a significant publication history on BRCA1 and 2 missense variants, and there are also well-curated locus specific databases for these genes containing information to help in the interpretation of the biological significance of missense variants. Despite the tremendous international efforts aimed at characterizing variants of unknown significance in these genes, there are still a significant number of these for BRCA1 and BRCA2. This issue is even more significant for genes which haven’t been the target of research efforts as comprehensive as BRCA1 and 2.

Functional assays to directly assess effects of specific missense variants are not available in many instances, and the application of these research methods is beyond what can be expected in a routine diagnostic laboratory practice.

Computational prediction tools which infer pathogenicity through evolutionary conservation or structural changes have uncertain clinical validity and it has been recommended that these not be used in isolation in the diagnostic setting to make a judgment about biological significance of a variant. The National Genetics Laboratory (Manchester) has produced a report on the use of missense prediction tools which is included in the list of references for this presentation. Clinicians should be particularly careful not to over-interpret the results from variants of unknown clinical significance to their patients.

Slide 16:

As I mentioned in the previous slide, ensuring appropriate interpretation of variants is a complex task, even when the variants are in well-known and studied genes. This becomes even more challenging when the test includes a gene panel with moderate-penetrance genes. The more genes one sequences, the more variants of unknown significance are likely to be identified.

Peak professional bodies such as the American College of Medical Genetics have produced publications on their recommendations for interpretation and reporting of sequence variants.

However, even for genes where there is an extensive evidence base (e.g. CHECK2) on variants, the appropriate clinical response and management may not be clear.

The absence of a mutation may not justify the relaxation of surveillance, depending on the family history, and conversely, the presence of a variant may not justify surgery or additional surveillance beyond that justified by family history.

Slide 17:

Current counseling models generally do not provide in-depth education for simultaneous testing of multiple genes/diverse syndromes, as this is likely to be quite time-consuming.

Individuals being tested need to understand the implications of clearly pathogenic variants that increase cancer risk in high penetrance genes both for themselves and their families. They will need to understand that this might include the risk of a spectrum of cancers which may not have been previously seen in a particular family; for example, deleterious CDH1 mutations in breast cancer families without history of gastric cancer. They also need to understand that the results might include a spectrum of risk for different kinds of cancers.

Unprepared individuals could experience much more distress by receiving unanticipated results, and the aim of testing should be to first do no harm; therefore, there should be an option to opt-out of receiving results that patients wish not to receive.

Slide 18:

Certainly it seems that multiplex testing for hereditary breast cancer as a standard approach is probably inevitable due to technological advances in sequencing, and a greater understanding of the genetic architecture of cancer. However, individuals that choose to undergo this kind of multiplex testing need to be aware of the complexity of implications which might ensue from the results.

Similarly, laboratories proposing to implement such multiplex testing need to be aware of the increasing complexity in the interpretation from such testing.

As clinical care standards are still incompletely developed, the most responsible approach would need to incorporate a framework for reviewing and monitoring the clinical utility as well as risk vs. benefit for multiplex genetic testing in this setting.

Slide 19: References

Slide 20: Disclosures

Slide 21: Thank You from www.TraineeCouncil.org

Thank you for joining me on this Pearl of Laboratory Medicine on "Genetic Testing for Hereditary Breast Cancer." I am Melody Caramins.