Clinical Chemistry
Trainee Council

PEARLS OF
LABORATORY MEDICINE

Genetic Testing for Hereditary Breast Cancer

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How big is the problem?

- Breast cancer is the most common cause of cancer death in women worldwide. Cancer research UK estimates breast cancer was responsible for ~458,500 female deaths in 2008. (Equivalent to 1146 fatal 747 crashes)
- 5-10% of cases have a strong hereditary component with 5% of these explained by AD inherited mutations in highly penetrant genes

Identification of high-risk individuals

- Consult national guidelines regarding criteria deeming high-risk. (E.g. UK NICE National Institute for Health and Clinical Excellence)
- Assessment by specialist hereditary cancer clinical service incl. multi-generation family history.
- Risk considerations include: Age at diagnosis, family history of malignancy, bilateral disease, male breast cancer, 2 or more blood relatives, high risk ethnicity (e.g. Ashkenazi Jewish).

Guidelines for offering testing

- American Society of Clinical Oncologists (ASCO) does not set numerical value but suggest individual risk assessment.
- UK guidelines by the National Institute for Health and Clinical Excellence (NICE) advocate gene testing if there is a high (1 in 3) lifetime risk of breast cancer, or greater than 1 in 12 risk of breast cancer prior to age 50.

Guidelines for offering testing

- Cancer Australia recommends testing only through specialist hereditary cancer clinics and offers online risk assessment based on health and family history questionnaires for patients and also as aids for health professionals.
- In individual clinic assessments, criteria for offering testing may include calculation of the probability of mutation identification, with testing offered at set criteria (e.g. a 10% chance of mutation detection)

Penetrance

- Penetrance refers to the proportion of individuals with a particular genetic variant who also express the associated phenotype
  - Highly penetrant genes confer a relative cancer risk of >5
  - Moderately penetrant genes confer relative risk of 1.5 – 5
  - Low-penetrant genes confer relative risk of ~1.5
- Important to remember some highly penetrant genes can have variants with moderate penetrance (e.g. BRCA1 p.Arg1699Gln, see Spurdle AB *et al.* 2012)

Complex genetic architecture

- Hereditary cancer is the ultimate complex genetic disease; 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, however massively parallel "panel" sequencing (12-40 genes) is now being offered in the USA and beyond *(as an example see: http://web.labmed.washington.edu/tests/genetics/BROCA)*

Which genes?

- Traditionally, comprehensive BRCA1 and BRCA2 mutation analysis, where clinical utility is proven
- These are the most common genetic causes of hereditary breast/ovarian cancer
- Deleterious mutations are generally highly penetrant
- In some populations (e.g. Ashkenazi Jewish) screening for a limited number of targeted founder mutations may be a cost-effective start

Pathophysiology

- Fundamentally, cancer is a disease in which normal regulation of tissue growth is disturbed.
- In hereditary cancers, a heritable constitutional change in a gene which regulates cell growth (oncogene), or a gene which inhibits cell division and survival (tumor suppressor gene), leads to a significant increase in cancer risk.

Pathophysiology – cont'd

- BRCA1 and BRCA2 are tumor suppressor genes; their protein products are essential for repairing damaged DNA and maintaining genomic integrity
- BRCA1 is part of a complex that repairs double-strand breaks in DNA
- BRCA2 belongs to a family of genes called FANC (Fanconi anaemia, complementation groups), the main purpose of which is to locate DNA damage and trigger its repair

Pathophysiology – example

- The BRCA2 c.5946delT deleterious variant:
  - Truncates BRCA2 product prior to the COOH-terminal nuclear localization signals, resulting in exclusion of the mutant proteins from the nucleus, inactivating all its associated nuclear functions, including:
    - Increased sensitivity to DNA cross-linking agents
    - Homology directed repair
    - Genomic instability (centrosome amplification)

Testing

- Genetic testing for hereditary breast and ovarian cancer is rapidly changing
- This is mostly due to the ability to offer rapid and cost-effective sequencing by MPS
- Many companies now offer simultaneous sequencing of “panels” of genes as opposed to sequential sequencing of single genes

Which genes (cont'd)

- Gene panels now offer 10-40 genes
- When designing a panel some considerations may be:
  - Grouping according to penetrance
    - High penetrance e.g. BRCA1/2, TP53, PTEN
    - Moderate/low penetrance e.g. CHECK2, ATM
  - Grouping according to function or cancer site
    - Genes functionally related to BRCA1/2 (e.g. ATM, BARD1, CHECK2, RAD50)
    - Other genes in Fanconi Anaemia pathway (e.g. PALB2, BRIP1)
    - Other cancer syndrome genes (e.g. TP53)

Genotype/Phenotype correlations

- Cancer risks can differ by gene and mutation position
  - E.g. 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

Appropriate interpretation (BRCA1/2)

- Sequencing any gene frequently identifies missense variants.
- Functional assays to directly assess effects of these variants are not available in many instances.
- Computational prediction tools which infer pathogenicity through evolutionary conservation or structural changes have uncertain clinical validity and should not be used in isolation in the diagnostic setting.

Ensuring appropriate interpretation

- Ensuring appropriate interpretation when the test includes a gene panel with moderate-penetrance genes is a complex task
  - Even for genes where there is an extensive evidence base (e.g. CHECK2), appropriate clinical response and management is not clear
  - Absence of mutation may not justify relaxation of surveillance
  - Presence of mutation my not justify surgery or additional surveillance beyond that justified by family history

The importance of counselling

- Current counselling models generally do not provide in-depth education for simultaneous testing of multiple genes/diverse syndromes
- Unprepared individuals could experience much more distress by receiving unanticipated results
  - E.g. Deleterious CDH1 mutations in breast cancer families without history of gastric cancer

Conclusions

- Advances in understanding of genetic architecture of cancer, coupled with technological advances in sequencing, are leading inevitably towards multiplex testing for hereditary breast cancer as a standard approach.
- Individuals should be made aware of the complexity of implications which may result from multiplex testing.
- A responsible approach would incorporate an appropriate framework to review and monitor clinical utility and risk vs. benefit for multiplex testing in this setting.

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Disclosures/Potential Conflicts of Interest

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