

PATHCHAT

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BRCA gene testing: A general overview

BRCA1 and BRCA2 gene testing is anything but straightforward. This article attempts to discuss the salient points to consider when faced with testing requests.

BRCA1 and BRCA2 mutations: An overview

- These mutations account for 5% of all cases of breast cancer and 10 to 15% of all cases of ovarian cancer. Women with BRCA1 and BRCA2 mutations have markedly elevated risks of developing breast and ovarian cancer. Their lifetime risk of developing breast cancer is 50 to 85%, and they have a 15 to 40% chance of developing ovarian cancer. There is also an increased risk of a second breast cancer diagnosis.
- Over 1 000 mutations in BRCA1 and BRCA2 have been reported. Not all of them are high-risk; some appear to be harmless variations. Mutations occur throughout the entire coding regions of the BRCA genes, but the most clinically adverse mutations result in a truncated protein when translated. The cancer risk associated with any given mutation varies significantly and depends on the exact type and location of the mutation, and possibly other individual factors.
- An undefined increased lifetime risk of developing breast cancer and prostate cancer exists in men with BRCA1 mutations, while those with BRCA2 mutations have a lifetime risk greater than 10% of developing breast cancer, while their lifetime risk of developing prostate cancer is elevated five- to sevenfold. Prostate cancer patients who are BRCA1 and BRCA2 mutation carriers experience a more aggressive clinical course.
- Men aged between 40 and 69 years with a positive family history of prostate cancer, a family

history of known BRCA1 or BRCA2 mutations, or a family history of females with breast or ovarian cancer should be advised to undergo annual prostate-specific antigen (PSA) testing. Those with PSA levels higher than 3 ng/ml should be offered BRCA1 and BRCA2 mutation testing and a prostate biopsy. Screening mammography should also be offered to male BRCA mutation carriers, especially those of BRCA2.

How to determine risk and the appropriateness of referral (Table 1)

- Risk assessment requires an accurate three-generation pedigree, using both paternal and maternal histories. The paternal history is equally important considering that men are more likely to be non-penetrant gene carriers.
- The best approach for minimising non-informative negative gene tests is to test the individual in the family who is most likely to carry a mutation, even if this is not the individual initially presenting for genetic risk assessment.
- DNA storage (after extraction from an ethylenediaminetetraacetic acid (EDTA) blood specimen/tumour tissue) from an individual with advanced breast, ovarian or prostate cancer and a family history of these malignancies also provides an invaluable future asset for baseline information when other family members have to be assessed for their personal risk.
- A diagnosis of triple-negative breast cancer (ER-, PR-, HER2-) in women diagnosed before the age of 60 is an indication for BRCA mutation testing regardless of family history. Triple-negative breast cancer represents 10 to 20% of invasive breast cancer, and is reported to be three times more common in women of African descent.

Table I: The National Comprehensive Cancer Network (NCCN) Referral Criteria for Further Genetic Risk Evaluation for Hereditary Breast and Ovarian Cancer (HBOC) – www.nccn.org

An affected individual with one or more of the following:

- Early-age-onset breast cancer
- Triple-negative (ER-, PR-, HER2-) breast cancer
- Two breast cancer primaries in a single individual
- Breast cancer at any age, and more than one close blood relative with breast cancer under 50 years old, or more than one close blood relative with epithelial ovarian/fallopian tube/primary peritoneal cancer at any age, or more than two close blood relatives with breast cancer and/or pancreatic cancer at any age from a population at increased risk
- A combination of breast cancer with one or more of the following: thyroid cancer, sarcoma, adrenocortical carcinoma, endometrial cancer, pancreatic cancer, brain tumours, differentiated use gastric cancer, dermatologic manifestations or leukemia/lymphoma on the same side of the family (especially if early onset)
- Ovarian, fallopian tube or primary peritoneal cancer
- Male breast cancer

An unaffected individual with a family history of one or more of the following: more than two breast primaries, either in one individual or two different individuals from the same side of the family (maternal or paternal); more than one ovarian primary from the same side of the family (maternal or paternal); first- or second-degree relative with breast cancer under 45 years.

- A combination of breast cancer with one or more of the following: thyroid cancer, sarcoma, adrenocortical carcinoma, endometrial cancer, pancreatic cancer, brain tumours, differentiated use gastric cancer, dermatologic manifestations or leukemia/lymphoma on the same side of the family (especially if early onset)
- A known mutation in a breast cancer susceptibility gene within the family

- Population-specific effects can also determine the initial testing strategy in some families:

- a) Founder effects have led to some specific mutations being very prevalent in certain populations of defined geographical or ethnic background, e.g., Ashkenazi Jewish descent or Afrikaners. Since these founder mutations account for about 90% of BRCA gene mutations in Afrikaner and Jewish populations, cost-effective genetic testing is made possible by using restricted gene panels.
- b) A frequent founder mutation in the Western Cape has been identified in coloured and Xhosa women, possibly due to gene flow from the indigenous tribes, such as the Xhosa, to the coloured population.

Regarding BRCA test requests

- BRCA1 and BRCA2 screening is not a diagnostic procedure. It determines lifetime risk in mutation carriers. BRCA tests are expensive and are currently not covered by most healthcare funders; are not relevant in all cases with a family history of breast cancer; and cannot detect all forms of genetic susceptibility to familial breast cancer. Substantial risk may still

be present when BRCA results are interpreted as “inconclusive”, and even when “BRCA – negative”. All usual surveillance approaches (regular self examination, mammograms, etc.) remain mandatory.

- A diagnosis of breast cancer requires histopathological confirmation. Genetic testing at the time of diagnosis can incorporate test results in management decisions. This may obviate the need for a second breast surgery for risk reduction, as some women found to have deleterious mutations choose to simultaneously undergo therapeutic surgery for the affected breast, and risk reducing surgery for the contra-lateral breast.
- When confronted with a request for BRCA1 or BRCA2 testing, there may be the perception that it only requires a blood sample to obtain a simple “yes” or “no” result, indicating the presence or absence of an inherited risk for breast or ovarian cancer. Further points to consider are discussed below.
- Aspects related to BRCA testing can be obtained from <http://www.myriad.com/treating-diseases/hereditary-breast-cancer/>.

Pretest genetic counselling: A genetic counsellor can assist with the following aspects:

- Ascertainment of risks in the context of a particular family history where tests in the available test repertoires may be applicable in particular cases (for example, related to specific population groups, gender considerations such as males with breast and/or prostate cancer, etc.).
- Communicating the test limitations, costs and expected test turnaround times. Several tiers of testing may have to be considered in some instances and the potential associated financial outlay that is required has to be revealed to the patient.

Testing: points to consider

a) Having other relatives tested/considering other cancers in the family pedigree:

- Mutations can be inherited from either parent and may be passed on to both sons and daughters. Each child of a carrier has a 50% chance of inheriting the mutated gene from the carrier parent. Half of those with BRCA gene mutations are male. Both men and women with hereditary breast and ovarian cancer, have increased risks for other cancers (see Table 1 and Table 2).
- Homozygosity for mutations in BRCA2 has been shown to cause Fanconi anaemia (FANCD1).

b) Testing of minors:

Consensus guidelines state that genetic testing for adult onset conditions should be deferred until after legal adulthood (age 18), when individuals can decide for themselves and are emotionally mature enough to deal with results that potentially confer a cancer risk. The age at which screening strategies should ideally be initiated is in the mid-20s, as many clinicians recommend that this is the best timing for the initiation of testing for at-risk women.

c) When to consider full BRCA sequencing:

Jewish or Afrikaner individuals with mixed ancestry and/or those individuals from other population groups who are at the highest risk of testing positive (for example, male breast cancer or breast or ovarian cancer in the family) may get the most informative results from full gene sequencing done at the outset.

d) Targeted testing of relatives when the mutation in a family has already been identified:

Once a mutation is identified in a family, further targeted analysis for the specific mutation can be performed in relatives, and is much less expensive than the original investigation.

Post-test genetic counselling: This is essential regardless of the outcome of the results.

In **BRCA-positive** cases, an explanation of the nature of the inherited risk, the clinical implications and a review of risk management options is required.

In **BRCA-negative** cases, 70% of cases with negative/inconclusive results require a thoroughly considered follow-up approach for patients with strong family histories.

- Maternal and paternal lineage needs to be considered separately in the interpretation of a negative test. If the cancer predisposition is evident in only one lineage, and this predisposition has been adequately explained by the presence of a mutation in one or more relatives of that lineage, then a negative gene test is highly informative. The client can be reassured that his or her cancer risk is likely to be no greater than that of the general population. When none of these criteria are met, the test result is classified as non-informative negative.
- A negative test should be carefully interpreted with all the available information. Despite uninformative results, clinical recommendations should be made for the patient and family, based on empirical evidence from the family history, and the presence of certain associated clinical features (see Table 2). The clinically relevant point to remember is that gene tests are rarely capable of identifying every possible deleterious mutation in a gene.
- Each of the following issues should be addressed for every patient with a non-informative negative test result:
 - 1) Could the patient have inherited an identifiable gene mutation from the other side of the family?
 - 2) Could the gene test have missed a deleterious mutation (is more extensive testing indicated even after full BRCA sequencing). In patients who have breast cancer and family histories of cancer and who also test negative (wild type) for BRCA1 and BRCA2, approximately 12% can be expected to carry a large genomic deletion or duplication in one of these genes.
 - 3) Should a different gene be tested? The rationale and costs of any further testing strategies need to be ascertained in each individual situation. A genetic counsellor can assist. For example, 5% of patients with negative BRCA1 or BRCA2 results carry a mutation in CHEK2 or TP53 (see Table 2); and germline mutations in breast and ovarian cancer pedigrees recently established RAD51C as a susceptibility gene.
 - 4) If a non-informative negative gene test cannot be resolved, and additional clinical features have been identified in the family pedigree, the decision to test other risk genes can be guided by the family history and phenotype clues listed in Table 2.

Table 2: Other major breast cancer predisposition genes and syndromes

Gene	Lifetime risk for breast cancer (%)	Gene frequency (%)	Family history and phenotype clues
BRCA1	65–81	0.06–1.5	Hereditary breast and ovarian cancer syndrome: early-onset breast cancer, ovarian cancer, modest increase in male breast cancer risk
BRCA2	45–85	0.06–1.5	Hereditary breast and ovarian cancer syndrome: early-onset/late-onset breast cancer, ovarian cancer, melanoma, pancreatic cancer, male breast cancer
TP53	50–80	<0.0005	Li-Fraumeni syndrome: very-early-onset breast cancer, sarcoma, adrenocortical carcinoma, brain tumours, phyllodes tumour, others (many), ER-positive, PR-positive, human epidermal growth factor receptor 2–positive breast cancer
PTEN	50–85	0.0005	Cowden syndrome: breast cancer, benign and malignant thyroid disease, endometrial cancer, colorectal cancer, macro-cephaly, trichilemmomas, palmar-plantar keratoses, oral mucosal papillomatosis, benign breast disease; de novo mutations 11–48%
CDH1	39–52	Unknown	Infiltrating lobular cancer, diffuse gastric cancer with signet ring cells
STK11/LKB1	35–50	0.004–0.0003	Peutz-Jeghers syndrome: very-early-onset breast cancer, gastro-intestinal cancer, pancreatic cancer, ovarian cancer, hamartomatous polyps of the gastrointestinal tract, oral-labial pigmentation; de novo mutation rate may be as high as 50%
PALB	20–30	0.2	Later-onset breast cancer, male breast cancer, pancreatic cancer
CHEK2	15–25	0.3–1.7	Similar cancer spectrum as Li-Fraumeni syndrome but lower penetrance; male breast cancer
NF1	15–25	0.02	Neurofibromatosis: early-onset breast cancer, gliomas, malignant peripheral nerve sheath tumours, cafe' au lait spots; de novo mutations 50%
p16	15–25	Unknown	Familial atypical multiple mole melanoma syndrome: melanoma, pancreatic cancer, dysplastic nevi, breast cancer

References and recommended reading:

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