

A new paradigm of genetic testing for hereditary breast/ovarian cancers

Ava Kwong *, JW Chen, Vivian Y Shin

ABSTRACT

Introduction: Genetic risk factors and family history play an important role in breast cancer development. This review aimed to summarise the current genetic testing approach to hereditary breast/ovarian cancer.

Methods: A systematic literature review was performed by searching the PubMed database. Publications available online until January 2015 that addressed issues related to hereditary breast/ovarian cancer genetic counselling/testing were selected. The search terms used were “familial breast/ovarian cancer”, “susceptibility genes”, “genetic counselling”, and “genetic testing”. The data extracted for this review were analysed by the authors, with a focus on genetic testing for hereditary breast/ovarian cancer.

Results: Although a greater proportion of inherited breast/ovarian cancers are due to the *BRCA1* and *BRCA2* mutations, a number of new genes have emerged as susceptibility candidates, including rare germline mutations in high penetrance genes, such as *TP53* and *PTEN*, and more frequent mutations in moderate/low penetrance genes, such as *PALB2*, *CHEK2* and *ATM*. Multi-gene testing, if used appropriately, is generally a more cost- and time-

effective method than single-gene testing, and may increase the number of patients who can be offered personal surveillance, risk-reduction options, and testing of high-risk family members.

Conclusions: Recent advances in molecular genetics testing have identified a number of susceptibility genes related to hereditary breast and/or ovarian cancers other than *BRCA1* and *BRCA2*. The introduction of multi-gene testing for hereditary cancer has revolutionised the clinical management of high-risk patients and their families. Individuals with hereditary breast/ovarian cancer will benefit from genetic counselling/testing.

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A Kwong *, FRCS (Edin), PhD

JW Chen, PhD

VY Shin, PhD

Breast Surgery Division, The University of Hong Kong, Pokfulam, Hong Kong

* Corresponding author: akwong@asiabreastregistry.com

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Introduction

Breast cancer is one of the most common cancers and the second most common leading cause of cancer-related death among women with 1.67 million new cases diagnosed in 2012 (25% of all cancers).¹ About 39% of these new cases are found in Asia.¹ In the US, women have a 12% lifetime risk of developing breast cancer including women of young age. In addition, approximately 1 in 250 women in their 30s will develop breast cancer in the next 10 years.² Assessment of an individual's risk for breast cancer is complex, and based on different aspects such as personal lifestyle, environmental exposure, reproductive influences, and drug use. Genetic risk factors and family history, however, also play important roles in breast cancer development. Only 5% to 10% of breast cancer cases are characterised as hereditary and follow the autosomal dominant pattern of transmission.³ On the other hand, 15% to 20% of breast cancer cases are familial, referring to women who have two or more first- or second-degree relatives with the disease.^{4–6} Hereditary cancers follow a Mendelian inheritance pattern and

tend to have an earlier age of onset. Familial cancers do not follow a specific inheritance pattern. Defects in the *BRCA1* and *BRCA2* genes are the most well-known high-risk factors among inherited breast cancers. Results from genome-wide association studies have broadened our knowledge over the last few years about the specific genes that contribute to familial breast cancer. Other genes such as *TP53* and *PTEN* have also been identified to be associated with an increased risk of breast cancer.⁷ High-risk women are likely to benefit from genetic testing as there are now emerging targeted therapies and interventions that have been shown to improve outcome in mutation carriers.

Methods

A search of the medical literature was performed to identify the relevant studies and reviews on genetic testing for hereditary breast/ovarian cancer. The PubMed database was searched for publications available online until January 2015 that address the related issues; “familial breast/ovarian cancer”, “susceptibility genes”, “genetic counselling”, and

遺傳性乳腺癌/卵巢癌的新一代基因檢測

鄺靄慧、陳嘉偉、冼念慈

引言：遺傳風險因素和家族史是乳癌發病的關鍵因素。本文概括目前針對遺傳性乳腺癌/卵巢癌的基因檢測方法。

方法：通過PubMed數據庫搜索直至2015年1月為止所有有關遺傳性乳腺癌/卵巢癌和基因諮詢/檢測的文章。用以搜索文獻的關鍵詞為「家族性乳腺癌/卵巢癌」(familial breast/ovarian cancer)、「易感基因」(susceptibility genes)、「遺傳諮詢」(genetic counselling)和「基因檢測」(genetic testing)。針對遺傳性乳腺癌/卵巢癌的基因檢測，筆者把搜索到的文章作重點分析。

結果：縱使大多數遺傳性乳腺癌/卵巢癌均由BRCA1和BRCA2基因突變引起，另一些新的易感基因也逐漸被認為是這種癌症的關聯基因，包括具有高外顯率如TP53和PTEN的罕見突變基因，以及具有中/低外顯率如PALB2、CHEK2和ATM的常見突變基因。一般來說，如使用得當，多基因檢測組合較單基因檢測省時及更具成本效益，令更多基因檢測呈陽性的患者得到個人監測、更多降低風險的選擇，以及替高風險的家族成員作檢測。

結論：分子遺傳學測試的最新發展讓我們確定除了BRCA1和BRCA2基因突變，還有其他引致遺傳性乳腺癌/卵巢癌的易感基因。引入多基因檢測組合對於高風險患者和其家族成員的臨床管理來說起了革命性的作用。遺傳性乳腺癌/卵巢癌患者將受惠於遺傳諮詢/檢測。

“genetic testing” were used as the search terms.

High-penetrance genes

BRCA1 and BRCA2

Hereditary breast and ovarian cancer syndrome (HBOC) refers to a germline mutation in either the *BRCA1* or *BRCA2* gene, and individuals who carry a mutation have an increased risk of developing cancers. *BRCA1* and *BRCA2* are tumour-suppressor genes that code for proteins that help repair damaged DNA and therefore play vital roles in securing the stability of the cell's genetic material. Defects in these two genes may result in protein with malfunction, thus DNA damage may not be repaired properly. As a result, cells are prone to develop genetic mutations leading to cancer development. Scientists discovered in the 1990s that *BRCA1* and *BRCA2* are breast cancer susceptibility genes.^{8,9} Women have a 57% to 60% and 49% to 55% lifetime risk of developing breast cancer if they carry a *BRCA1* or *BRCA2* mutation, respectively.^{10,11} Women with mutations in the *BRCA1* cancer susceptibility gene associated with HBOC have a 39% to 46% risk of developing ovarian cancer by the age of 70 years while approximately 10% to 27% *BRCA2*-positive women are at risk.¹²⁻¹⁴ The result of genetic testing for the *BRCA* mutation is important to decisions made about management of breast cancer. For example, a woman diagnosed with breast cancer and who harbours the *BRCA1*

or *BRCA2* mutation has a greater risk of developing a second breast cancer in the contralateral breast, and this risk is age-related. Women diagnosed with breast cancer at a younger age have a higher risk of developing contralateral malignancy compared with those diagnosed at an older age.¹⁵ *BRCA1* mutation carriers tend to have more triple-negative breast cancer (TNBC), medullary histopathology, somatic *TP53* mutations, higher histological grade, and present at a younger age compared with women with sporadic breast cancers. Basal markers such as cytokeratin (*CK14*, *CK5/6*, *CK17*), osteonectin, and *EGFR* are more commonly expressed in *BRCA1*-positive tumours than in control tumours unselected for mutation status.¹⁶⁻¹⁸ The National Comprehensive Cancer Network (NCCN) annually updates guidelines with respect to genetic counselling and testing (www.nccn.org) and the most updated guidelines recommend it for individuals who meet the HBOC testing criteria. Guidelines are based on young age of onset, family history of breast cancer, specific histological types of breast cancer (TNBC), ovarian (epithelial and peritoneal), and prostate cancer (Gleason score ≥ 7). For details refer to NCCN guidelines (Genetic/Familial High-Risk Assessment: Breast and Ovarian), version 1.2016.¹⁹

Knowing the mutation status of germline *BRCA1* and *BRCA2*, patients may be offered alternative screening and/or therapeutic interventions (Table 1¹⁹), including intensive breast surveillance (magnetic resonance imaging [MRI] of the breasts in addition to standard breast imaging such as mammography and ultrasonography), mastectomy instead of breast conservation surgery, prophylactic mastectomy and salpingo-oophorectomy, or the prescription of chemopreventive drugs and more recently the choice of chemotherapy as primary treatment, for example, carboplatin. A recent study has shown that treatment with carboplatin produces no advantage over docetaxel in patients with TNBC, although those with *BRCA1* or *BRCA2* mutation benefited from either drug.²⁰ A number of targeted therapies, such as poly(ADP-ribose) polymerase inhibitors, have been shown to be effective in *BRCA* mutation carriers.^{21,22} The evolution of sequencing technologies enables parallel testing of multiple genes, leading to simultaneous analysis of breast cancer predisposition genes with either high or intermediate penetration. Multi-gene panel testing, however, has raised new issues regarding patient eligibility for gene testing other than *BRCA1* and *BRCA2*, and more importantly, interpretation of genetic results.

TP53

One of the high penetrance genes is *TP53*, which is a tumour-suppressor gene that encodes the transcription factor protein *p53*. It is a ubiquitous

TABLE 1. NCCN management of the hereditary breast and/or ovarian cancer syndrome¹⁹
(Adapted from the NCCN guidelines, version 1.2016)

Management of hereditary breast and/or ovarian cancer syndrome	Details
Breast screening	Annual breast MRI screening or mammogram for age 25-29 years. Breast MRI screening and mammogram for age ≥35-70 years. Individual consideration for age >70 years
Risk-reducing mastectomy	Counselling may include a discussion regarding degree of protection, reconstruction options, and risks
Risk-reducing salpingo-oophorectomy is recommended for those aged 35-40 years and upon completion of child bearing	Counselling may include a discussion of reproductive wishes, extent of cancer risk, degree of protection for breast and ovarian cancer, and management of menopausal symptoms
Address the psychosocial, social, and quality-of-life aspects	For those who have not selected risk-reducing salpingo-oophorectomy, consider concurrent transvaginal ultrasound and CA-125 monitoring starting at 30 years or 5-10 years before the earliest age of first diagnosis of ovarian cancer in the family
Consider chemoprevention for breast cancer and ovarian cancer	-
Consider investigational imaging and screening studies	-

Abbreviations: MRI = magnetic resonance imaging; NCCN = National Comprehensive Cancer Network

protein implicated in preservation of an intact genome. It regulates cell cycle, DNA repair, apoptosis, cellular senescence, and metabolism. It has been shown to be involved in various kinds of cancer progression such as osteosarcomas, colon cancer, and lung cancer.²³⁻²⁸ Li-Fraumeni syndrome (LFS) is a rare but highly penetrance familial cancer syndrome that is characterised by germline *TP53* mutations inherited in an autosomal dominant manner, in which 60% to 80% of LFS families carry a mutant *TP53*.²⁹ In addition to soft-tissue sarcomas and osteosarcomas, LFS families are likely to exhibit a pattern of early-onset and multiple primary cancers including breast, brain, and adrenocortical tumours^{29,30}; LFS is thought to account for approximately 1% of all breast cancers.^{31,32} Approximately 1% of women diagnosed with breast cancer before the age of 40 years carry a *TP53* mutation.³² Breast cancer is the most frequent malignancy among female *TP53* mutation carriers and accounts for up to one third of all cancers in LFS families.³³ Although LFS is only responsible for a tiny fraction of breast cancers, women with LFS have a breast cancer risk of 56% by the age of 45 years and greater than 90% by the age of 60 years, and LFS accounts for a 60-fold increased risk for early-onset breast cancer compared with the general population.^{34,35} Women with LFS-related breast cancer are reported to have very early disease onset (20s or 30s) and a relatively advanced disease staging.³⁶⁻³⁸ Studies have shown that 3% to 8% of women who are diagnosed with breast cancer younger than 30 years without a significant family history of cancer have *TP53* mutation.^{31,39,40} The NCCN has included early-onset breast cancer as one of the criteria for offering *TP53* genetic testing, regardless of the family history of cancer. *TP53* mutations can be tested either

through sequencing the entire encoding region that identifies approximately 95% of *TP53* mutations or just selected regions. Analysis of hot-spot regions located in exons 4-9 can detect approximately 90% of all *TP53* mutations.^{19,41,42} When the *TP53* mutation is present in an individual, breast screening and preventive guidelines are similar to those for *BRCA* mutation carriers. In addition, a full-body MRI scan is an option as a screening tool. Individuals with the following should be included for genetic testing of *TP53*¹⁹: early-onset breast cancer (≤35 years), a combination of diagnosis of a sarcoma at the age of <45 years, AND a first-degree relative diagnosed at the age of <45 years with cancer, multiple cancers (brain tumours, sarcomas, and leukaemia).

PTEN

PTEN is a phosphatase tensin homologue located on chromosome 10q23.3 that plays a tumour-suppressive role due to its *PI3K* (phosphatidylinositol-3-kinase) phosphatase activity. Abnormal *PTEN* cannot activate cell cycle arrest and apoptosis and leads to uncontrolled cell survival.⁴³ Germline *PTEN* mutations have been identified in a variety of disorders such as Cowden syndrome (CS) or *PTEN* hamartoma tumour syndrome. Affected individuals have multiple hamartomas in a variety of tissues with an increased risk of malignant transformation.⁴⁴ Breast cancer is the most common tumour associated with CS. Although CS is responsible for <1% of all breast cancers, women with this syndrome have a 25% to 50% risk of developing breast cancer in a lifetime and are prone to early onset.^{45,46} The frequency of multifocal and bilateral disease is increased in CS-associated breast cancers compared with sporadic cases.^{47,48}

Women with CS also have an increased risk (67%) of benign breast disease characterised by mammary hamartomas that can be multiple and bilateral.⁴⁹ Similar to *TP53* mutation carriers, *PTEN* mutation carriers are advised to have breast surveillance and interventions as recommended for *BRCA* mutation carriers. The testing criteria for CS are those who present with breast cancer, endometrial cancer, follicular thyroid cancer, multiple gastrointestinal hamartomas, ganglioneuromas, or other diseases including macrocephaly, macular pigmentation of glans penis, and mucocutaneous lesions.¹⁹

Moderate- and low-penetrance genes

PALB2

PALB2 (partner and localiser of *BRCA2*) is involved in homologous recombination and double-strand break repair along with *BRCA2*.^{50,51} Loss-of-function mutations are associated with a 2 to 4 times higher risk than non-mutation carriers for familial breast cancer.⁵²⁻⁵⁴ A study analysed the risk of breast cancer among 362 members of 154 families who had deleterious *PALB2* mutations.⁵⁵ The results revealed that the risk of having breast cancer for female *PALB2* mutation carriers was 8 to 9 times higher among those younger than 40 years, 6 to 8 times higher among those 40 to 60 years, and 5 times higher among those >60 years when compared with the general population. The estimated cumulative risk of breast cancer among female mutation carriers increased from 14% to 35% from the age of 50 to 70 years. In addition, the risk of breast cancer for *PALB2* mutation carriers was significantly increased by familial factor.⁵⁶ Thus, it has been advised that *PALB2* mutation testing should be performed routinely to identify mutations in HBOC families since it may be of clinical relevance. This is increasingly being tested.

Other hereditary breast cancer susceptibility genes

There are other low-penetrance genes that are associated with hereditary breast cancer such as *STK11*, *CDH1*, and *MMR* genes, and that are responsible for Peutz-Jeghers syndrome, hereditary diffuse gastric cancer syndrome, and Lynch syndrome, respectively.⁵⁷⁻⁵⁹ Some moderate-penetrance genes such as *CHEK2*, *ATM*, *BRIP1*, *RAD51C*, *RAD51D*, *BARD1*, *MRE11*, *RAD50*, *NBS1*, and *FANCM* have been recognised as breast cancer susceptibility genes.⁶⁰

The recent development of multi-gene testing for hereditary cancer has had a great impact on the clinical management and genetic counselling of high-risk patients and their families. The decision to use multi-gene testing should be no different

than the rationale for testing a single gene. Multi-gene testing is more cost-effective than sequentially testing multiple genes associated with a phenotype. For example, young women diagnosed with breast cancer can be tested for mutations in *BRCA1*, *BRCA2*, and *TP53*. Detailed testing criteria for genes can be found in NCCN guidelines version 1.2016.¹⁹ Next-generation sequencing enables simultaneous analysis of a specific panel of genes, but there are limited outcome data on clinical interventions, particularly in lower-penetrance-gene-mutation-related breast cancers. Results of a multi-gene panel may pose difficulty in interpretation and clinical decisions. At present, multi-gene testing is largely performed for research purposes. There are limited data regarding the degree of cancer risk associated with some of the genes on the recurrent multi-gene test. There is a lack of well-established guidelines for risk management for carriers of mutations in some of the genes, which may lead to extra surveillance and surgeries.

Nonetheless multi-gene testing is more cost-effective and time-effective than single-gene testing, and provides a higher mutation detection rate. It may reduce the number of high-risk families with negative results of finding a gene mutation due to the increased coverage. The lifetime breast cancer risk estimates associated with gene mutations are listed in Table 2.^{10,13,36,56,58,61-74}

In Hong Kong, breast cancer is the most common cancer in the female population. The Hong Kong Hereditary Breast Cancer Family Registry was established in 2007. It functions as a data registry of hereditary breast, ovarian and prostate cancer families and is also an established charitable organisation that subsidises the cost of genetic testing for underprivileged individuals. More than 1900 patients with breast and/or ovarian cancer who satisfied the selection criteria have received genetic testing in Hong Kong. Each individual underwent thorough genetic counselling to ensure the implications of genetic testing were understood.

TABLE 2. Estimated lifetime risk of breast cancer associated with selected susceptibility genes^{10,13,36,56,58,61-74}

Gene	Estimated lifetime risk of breast cancer
<i>BRCA1</i>	55-65% ¹⁰
<i>BRCA2</i>	45-47% ¹³
<i>TP53</i>	49-60% ³⁶
<i>PTEN</i>	25-50% ^{61,62}
<i>PALB2</i>	33-58% ⁵⁶
<i>STK11</i>	30-50% ^{58,63,64}
<i>CDH1</i>	39-52% ^{65,66}
<i>ATM</i>	15-52% ⁶⁷⁻⁷⁰
<i>CHEK2</i>	20-44% ⁷¹⁻⁷⁴

Around 600 probands were screened for *BRCA1* and *BRCA2* mutations by bi-directional Sanger sequencing of all coding exons and multiplex ligation-dependent probe amplification.⁷⁵ The sensitivity of identifying mutations is comparable with the gold-standard method with good bioinformatics support. Next-generation sequencing meets rigorous quality standards and can provide clinical sequencing results that are equivalent to those obtained from Sanger DNA sequencing analysis.⁷⁶ We started employing next-generation DNA sequencing to expedite analysis workflow and expand the gene panel in 2011 to include *TP53* and *PTEN* for sequencing. Cases with a negative result after screening with our in-house developed gene panel are further sequenced using 454 GS Junior System (Roche Life Sciences) or MiSeq (Illumina). Sequencing data are analysed by an in-house fully developed automatic bioinformatics pipeline. The mutation screening result of a 4-gene panel *BRCA1*, *BRCA2*, *TP53*, and *PTEN* in our recruited patients revealed that 9% carried such mutations. Nonetheless a number of clinically high-risk patients have tested negative for the above genes. This indicates that there is further potential in expanding the coverage to different lower-penetrance genes such as *PALB2*, which has recently been reported to be important to cause hereditary breast cancer in our testing strategy.⁵⁶

Conclusions

Clinical assessment of an individual's risk of hereditary cancer is based on the evaluation of family history, age of onset, and type of cancer. Advances in molecular genetics testing have identified a number of genes associated with inherited susceptibility to breast and/or ovarian cancers such as *BRCA1*, *BRCA2*, *PTEN*, and *TP53*. The recent introduction of next-generation sequencing technology and multi-gene panel testing for hereditary cancer has rapidly altered the clinical approach to high-risk patients and their families. Although there are still limitations, individuals with hereditary or familial breast/ovarian cancer are likely to benefit from strategies including prevention, screening, and targeted treatment. Suitable patients and families should be offered genetic counselling and testing.

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