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The use of panel testing in familial breast and ovarian cancer

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ABSTRACT

Advances in sequencing technology have led to the introduction of panel testing in hereditary breast and ovarian cancer. While direct-to-consumer testing services have become widely available, the clinical validity of many of the genes on panel tests remains contentious and risk management guidelines are often lacking. This article gives an overview of advantages with panel testing as well as important challenges, including clinical translation of test results.

Introduction

High-throughput sequencing of a number of genes (via targeted next-generation sequencing) at a declining cost is rapidly replacing sequential testing of single genes in both public and commercial service laboratories. The use of large multi-gene disease-targeted panels (see Table 1) is a cost-effective approach, also allowing incorporation of our improved understanding of the genetic architecture of disease. In this review, we focus on hereditary breast and ovarian cancer as a paradigm to illustrate the benefits and pitfalls of multi-gene panel testing in the clinic.

Susceptibility genes and panel testing

Germline mutations in the *BRCA1* and *BRCA2* genes remain the most important cause of familial forms of breast and ovarian cancer.[1] Identification of a deleterious *BRCA1/2* variant within a family has profound implications in risk management, including enhanced surveillance, prophylactic surgery and chemoprevention options.[2] However, in many families with a strong history of breast and/or ovarian cancer, *BRCA1/2* testing does not identify a causative mutation.

Since the discovery of the *BRCA1* and *BRCA2* genes in the mid-1990s, numerous other susceptibility genes for breast or ovarian cancer have been identified (Table 2). Rare mutations in *CDH1, PTEN, STK11* and *TP53* genes, associated with hereditary diffuse gastric cancer, Cowden syndrome, Peutz-Jeghers syndrome and Li Fraumeni syndrome, respectively, can confer a high risk of breast cancer.[1,3] Besides these, intermediate breast cancer risk genes, such as *ATM, CHEK2,* and *PALB2,* have been identified.[1,3] Large-scale genotyping studies have also led to the identification of numerous low risk variants, predominantly consisting of single nucleotide polymorphisms in non-coding sequences.[4,5] Inherited mutations in other genes, such as *RAD51C, RAD51D* and *BRIP1,* and mismatch-repair (MMR) genes (*MLH1, MSH2, MSH6, PMS2, EPCAM*) that cause Lynch syndrome when mutated, also influence the risk of ovarian cancer.[6]

These genetic discoveries have occurred alongside major advances in sequencing technology that has led to the introduction in the clinic of simultaneous testing of panels of selected multiple genes for hereditary breast and ovarian cancer predisposition.[1] In theory, panel testing for hereditary breast and ovarian cancer allows for a more comprehensive

Key points

Additional susceptibility genes for familial breast and ovarian cancer have been identified since the discovery of the *BRCA1/2* genes in the mid-1990s.

Deleterious mutations confer different levels of cancer risks, with lack of sufficient evidence for clinical actionability in many of these new genes.

Advances in sequencing technology led to the introduction of simultaneous multi-gene (panel) testing which is rapidly replacing sequential single-gene testing in public and commercial service laboratories.

Panel testing for familial breast/ovarian cancer has a slightly increased diagnostic yield compared to conventional *BRCA1/2* testing.

There is a lack of consensus guidelines on panel testing, including eligibility criteria and clinical translation of results.

KEYWORDS: gene panel, breast cancer, ovarian cancer, genetic testing and hereditary cancer.

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Table 1. Examples of disease-targeted gene panels available via UKGTN where testing criteria and a gene dossier on prevalence and sensitivity of mutation detection is also included.[10] Websites such as GeneReviews and OMIM (Online Mendelian Disorder in Man) provide clinicians with up-to-date information on genes associated with a Mendelian disorder in question. A comprehensive directory of available genetic testing services can then be accessed via webpages, such as UK Genetic Testing Network (UKGTN) and European Directory of DNA Diagnostic Laboratories (EDDNAL).

Disease category	Disease	Genes
Hereditary cancer	Familial breast/ovarian cancer	*ATM, BARD1, BRIP1, BRCA1, BRCA2, CDH1, CHEK2, PALB2, PTEN, PIK3CA, STK11, TP53*
	Familial bowel cancer including hereditary nonpolyposis colorectal cancer (HNPCC) phenotype or polyposis	*APC, BMPR1A, POLD1, POLE, KIT, MLH1, MSH2, MSH6, MUTYH, PDGFRA, PMS2, GALNT12, STK11, SMAD4*
Cardiac disorders	Familial hypertrophic cardiomyopathies	*ACTC1, CSRP3, FHL1, GLA, LAMP2, MYBPC3, MYL2, MYL3, MYLK2, MYH6, MYH7, NEXN, PLN, PRKAG2, SLC25A4, TTN*
	Aortopathy disorders	*ACTA2, COL3A1, FBN1, FBN2, MYH11, SMAD3, SLC2A10, TGFB2, TGFB3, TGFBR1, TGFBR2*
Neurological and neuromuscular disorders	Early infantile epileptic encephalopathy	*ALDH7A1, ARHGEF9, ARX, BTD, CHD2, CNTNAP2, CDKL5, FOXG1, GABRB3, GABRG2, GLUD1, GRIN2B, HCN1, POLG*
	Congenital muscular dystrophies	*B3GALNT2, CHKB, COL4A1, COL4A2, COL6A1, COL6A2, COL6A3, COL12A1, DOLK, DPM1, DPM2, DPM3, DAG1, FKTN, FKRP, GMPPB, ITGA7, ITGA9, ISPD, LAMA2*

risk assessment, incorporating up-to-date knowledge on risk variants. Moreover, it may allow risk stratification in individuals who would not otherwise meet traditional testing criteria based on prior syndrome-focused approaches. However, panel testing is not without its limitations, including interpretation of results and subsequent risk management, especially in the absence of consensus guidelines.

Availability and testing criteria

Since the US Supreme Court decision in 2013 against Myriad Genetics' patent claims for *BRCA1/2* testing, a number of gene panel testing services for hereditary breast and ovarian cancer, including *BRCA1/2* genes have become available.[1] These cover different combinations of genes with varying degrees of evidence of an association with breast and/or ovarian cancer which can be counterproductive where the evidence is limited. A case in point is *BRIP1*, which was first reported to be associated with breast cancer in 2006,[7] an observation that was not substantiated by a much larger study published ten years later.[8] Yet this gene is still found on many commercially available hereditary breast cancer panels.[1] In contrast, screening for single nucleotide polymorphisms is not typically included in panel tests, although it has been estimated that they can cumulatively confer a risk of 29% (by age 80 years for women in the top 1% for polygenic risk score[4]) which is comparable to moderate risk susceptibility genes (30% and 28% for *CHEK2* and *ATM*, respectively[9]).

While testing criteria for germline *BRCA1/2* gene mutations are well defined, there are no equivalent guidelines for multi-gene panel testing in familial breast–ovarian cancer. In a UK-based survey conducted in 2016, 8 out of 19 participating centres offered panel testing in selected individuals following discussion at the weekly departmental meeting (personal communication by MT and JS). In some centres, *BRCA1/2* testing was routinely run on a panel, with data on other genes reported upon the clinician's request. Eligibility criteria varied widely from centre to centre.

In the commercial setting, panel tests for breast cancer, such as the 17-gene BreastNext test by Ambry Genetics, are generally marketed to women who had uninformative *BRCA1/2* testing and either have a personal history of early onset breast cancer (diagnosed ≤ 45 years) or multiple close family members with breast and other cancers. Similarly, a 13-gene familial breast–ovarian cancer panel is available on the UKGTN with minimum requirements including the equivalent criteria for *BRCA1/2* testing.[10] These observations highlight the current inconsistencies in panel testing for breast and/or ovarian cancer susceptibility, in both clinical and commercial settings, and that access is largely dependent on availability and local guidelines.

Diagnostic yield and variant interpretation

Numerous studies have assessed the performance of targeted next-generation sequencing for the diagnosis of familial breast and ovarian cancer (Table 3). The analytical validity of panel testing was comparable to traditional sequencing methods (separate Sanger sequencing and deletion/duplication analyses of each gene).[11,12] Overall, 'high-risk' breast cancer susceptibility genes are thought to account for 20–25% of familial breast cancer, with 'intermediate' risk genes explaining a further 5% and 'low risk' gene variants cumulatively covering an additional 14% of the familial risk.[12]

In a recent study by Desmond *et al*,[13] panel testing in 1046 *BRCA1/2*-negative cases referred for hereditary breast–ovarian cancer predisposition led to identification of deleterious mutations in other hereditary cancer predisposition genes

Table 2. Breast and ovarian cancer susceptibility genes (excluding *BRCA1/2* genes).

Gene	OMIM gene ID	Associated syndrome and/or other cancers
Breast cancer susceptibility genes		
High risk		
CDH1	602118	Diffuse gastric cancer
PTEN	601728	Cowden syndrome (thyroid, endometrial cancer)
STK11	602216	Peutz-Jeghers syndrome (colon, pancreas, ovarian sex cord-stromal tumours)
TP53	191170	Li Fraumeni syndrome (sarcoma, adrenocortical carcinoma, brain tumours)
Intermediate risk		
ATM	607585	Ataxia telangiectasia (pancreas)
CHEK2	604373	
NF1	613113	Neurofibromatosis Type I (malignant tumours of peripheral nerve sheath, brain, central nervous system)
PALB2	610355	Pancreas
Unknown/disputed risk		
BRIP1	605882	Ovary (*moderate risk*)
NBN	602667	Unknown
RAD51C	602774	Ovary (*moderate risk*)
RAD51D	602954	Ovary (*moderate risk*)
Ovarian cancer susceptibility genes		
BRIP1	605882	
EPCAM	185535	Lynch syndrome (colon, endometrium, stomach, small intestine, hepatobiliary tract, urinary tract, brain, skin)
MLH1	120436	Lynch syndrome
MSH2	609309	Lynch syndrome
MSH6	600678	Lynch syndrome
RAD51C	602774	
RAD51D	602954	

in 3.8% of individuals, which was consistent with previous similar studies.[12,14–16] In the majority of these (26/40), the identified variant was in 'low-moderate risk' breast–ovarian cancer susceptibility genes, such as *CHEK2*, with another eight cases harbouring mutations in genes associated with Lynch syndrome (which are not proven to cause breast cancer), and only three variants detected in high-risk breast cancer genes (all in *CDH1*). Apart from the increased likelihood of identifying a disease-causing mutation, panel testing can also lead to unexpected (incidental) findings in 'atypical' cases. For example, panel testing in 360 women with primary ovarian, peritoneal, or fallopian tube carcinoma led to identification of two *MSH6* deleterious germline mutations in individuals with no family history of Lynch syndrome and three *TP53* mutation carriers without a family history of Li-Fraumeni syndrome.[16] Identifying such unexpected deleterious germline mutations could have implications for unaffected family members by way of cascading genetic tests, risk-assessment and future screening advice.

An important challenge for laboratories performing next-generation sequencing is the interpretation of identified variants, especially in genes other than *BRCA1/2* where available evidence is less well established. Although there is evidence of pathogenicity for protein-truncating variants (assumed to result in loss of function) for most genes included in hereditary breast–ovarian gene panels, the interpretation of missense variants is much more challenging. Missense variants in the coding region of genes that do not interfere with the reading frame are common and sometimes misinterpreted as pathogenic. Interpretation of these generally relies on use of *in silico* prediction tools, conservation data to assess if the variant is present in other species, familial segregation studies, and possibly tumour studies.[1] Another useful tool can be the assessment of a variant frequency in affected and non-affected cohorts from multi-ethnic groups but this is dependent on the establishment and maintenance of international databases.

As a result of the above, the likelihood of identifying so-called variants of uncertain significance (VUS) is high. In a study by Kurian *et al* assessing the performance of panel testing in 175 women meeting *BRCA1/2* gene testing criteria, sequencing of 39 genes led to identification of 2.1 VUS on average per participant, out of which the vast majority (88.8%) were novel.[17] Similarly, one third (168/488) of women with breast cancer undergoing sequencing of 25 breast/ovarian cancer susceptibility genes harboured VUS, with as many as three variants found per patient.[18] Most VUS will likely turn out to be non-pathogenic, but until this is known they will remain a source of considerable anxiety for the patient and their family.

Clinical translation of results

Most familial breast–ovarian cancer panels include moderate risk susceptibility genes for which consensus guidelines for risk management are not widely established and only beginning to be addressed in part.[19,20] For many of these genes, there is limited evidence on the degree of associated cancer risk. For example, most existing data on *CHEK2* gene derive from the 1100delC variant, which is the commonest truncating variant in northern European populations.[1] Mutations in three other genes, *BRIP1, RAD51C,* and *RAD51D,* have been clearly associated with ovarian cancer but there is conflicting and limited evidence regarding their association with breast cancer.[1]

Thus a mutation-positive result arising from panel testing may not necessarily be clinically actionable. In the aforementioned study by Desmond *et al*, the most common deleterious mutations in *BRCA*1/2-negative cases were identified in low to moderate breast cancer risk genes with a recommended management change in the minority of these by either increased screening or prophylactic breast surgery (in the context of significant family history) based on National Comprehensive Cancer Network (NCCN) guidelines.[13] Notably, there are no equivalent gene-specific NICE guidelines on the management

Table 3. Summary of panel testing studies in familial breast and/or ovarian cancer.

Study	Diseases	N subjects	N genes	N deleterious *BRCA1/2* mutations (%)	N deleterious mutations in all other genes (%)
Walsh *et al*, 2011[16]	Ovarian, fallopian tube, peritoneum	360	21	63 (17.5)	22 (6.1)
Castera *et al*, 2014[12]	Hereditary breast/ovarian cancer	708	27	66 (9.3)	44 (6.2)
Kurian *et al*, 2014[17]	Hereditary breast/ovarian cancer	198	42	57 (28.8)	16 (8.0)
LaDuca *et al*, 2014[14]	Ambry-tested, non *BRCA1/2*	874 (breast panel) 223 (ovarian panel)	14–22	0 (by definition)	65 (7.4) for breast panel 16 (7.2) for ovarian panel
Couch *et al*, 2014[22]	Triple-negative breast cancer	1824	17/122	204 (11.1)	67 (3.7)
Maxwell *et al*, 2014[15]	Breast cancer <40 years, non *BRCA1/2*	258	22	0 (by definition)	31 (11)
Tung *et al*, 2015[23]	Myriad tested	1781	25	165 (9.3)	79 (4.4)
Desmond *et al*, 2015[13]	Hereditary breast/ovarian cancer (Invitae or Myriad tested), non *BRCA1/2*	1046	25–29	0 (by definition)	40 (3.8)

of women who carry deleterious mutations in genes other than *BRCA1/2* and genes associated with rare conditions that carry a high risk of breast cancer (see Table 2).[21]

Another important aspect of panel testing is the value of a negative result in an unaffected relative for a familial deleterious variant in an intermediate cancer risk gene. Incorporation of truncating mutations in three intermediate breast cancer risk genes (*ATM, CHEK2*, and *PALB2*) to the BOADICEA risk-prediction algorithm, illustrated that the reduction in risk for women whose mother carried a *PALB2* mutation was comparable to a *BRCA2* mutation, whereas negative testing for an *ATM* or *CHEK2* familial mutation in the same scenario only led to a slight decrease in risk, even with a strong family history (Fig 1).[9] In line with the above, a counselling framework has been recently proposed for moderate penetrance cancer-susceptibility mutations, suggesting that relatives who test negative for the familial mutation should be managed on the basis of their family history and may still warrant some enhanced surveillance.[20]

However, one could argue that if a woman's mutation status for these genes does not change her clinical management, there is little to be gained for testing them in the first place.

Conclusion

In the era of next-generation sequencing with continuously falling prices, the need for complex eligibility criteria for panel testing may be eliminated. Revision of the NICE guidelines in 2013 lowered the threshold for *BRCA1/2* germline mutation testing, which is now offered to all individuals and their relatives who have a >10% (instead of 20%) likelihood of having a mutation based on risk algorithms, such as BOADICEA and the Manchester scoring system.[21] Panel tests for familial breast/ovarian cancer in the US typically cost around $1500–3000 and include 10–30 genes, although the price is coming down rapidly with some companies offering panel tests for as little as $300; in the UK, 13-gene panel testing costs around £600–800 versus *BRCA1/2* testing in the region of £400.[10]

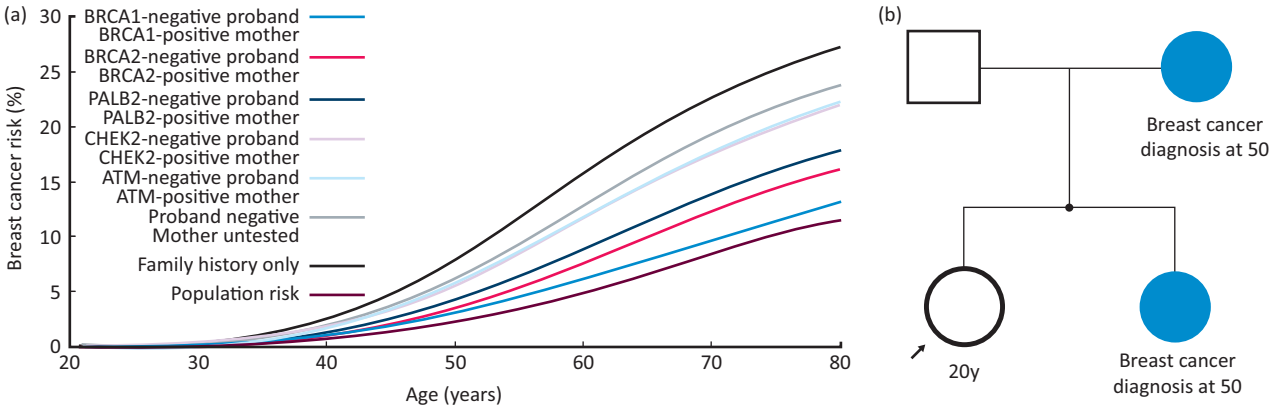

Fig 1. Value of negative testing for familial deleterious variants in moderate/high risk breast cancer genes. (a) shows the BOADICEA predicted breast cancer risk for negative testing (coloured curves) and based on family history alone (black curve). The proband, highlighted with an arrow in pedigree **(b)**, is assumed to be a 20 year old woman in the United Kingdom. As illustrated, the reduction in risk following negative testing for a *PALB2* familial mutation is comparable to a *BRCA2* mutation whereas negative testing for an *ATM* or *CHEK2* familial mutation is associated with a smaller decrease in risk. Image adjusted from Lee *et al.*, with permission.[9]

Despite the above, panel testing is unlikely to overtake clinical acumen in the medium term, considering its limitations and our current gap in knowledge. The complexity of human variation, such as missense variants and incomplete penetrance due to environmental and other gene modifier factors, means that genetic data can at most add predictive value and always need to be interpreted within a clinical context. In the future, clinical geneticists may devolve diagnostic panel testing to mainstream specialties but would support them through interpretation of results and cascading genetic tests to relatives. Importantly, the absence of a pathogenic variant does not necessarily exclude the presence of familial and/or other inherited susceptibility factors and therefore, genetic counselling should still be considered an integral part of the process. ■

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