

The Changing Landscape of Genetic Testing for Inherited Breast Cancer Predisposition

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Opinion statement

The advent of multiple-gene germline panel testing has led to significant advances in hereditary breast and ovarian cancer risk assessment. These include guideline-specific cancer risk management recommendations for patients and their families, such as screening with breast magnetic resonance imaging and risk-reducing surgeries, which have the potential to reduce substantially the morbidity and mortality associated with a hereditary cancer predisposition. However, controversy remains about the clinical validity and actionability of genetic testing in a broader patient population. We discuss events leading to the wider availability of commercialized multiple-gene germline panel testing, the recent data that support using this powerful tool to improve cancer risk assessment and reduction strategies, and remaining challenges to clinical optimization of this new genetic technology.

Introduction

Over the past decade, significant advances in clinical cancer genetics have strengthened personalized cancer medicine. The breast cancer susceptibility genes, *BRCA1* and *BRCA2* (*BRCA1/2*), were identified more than 20 years ago after extensive studies of families

that had several members with early-onset breast cancer [1]. Although only about 5 to 10% of women with breast cancer have an identifiable genetic predisposition, up to 20 to 30% of those with a family history of breast and ovarian cancer have a mutation in a breast

cancer susceptibility gene, primarily *BRCA1/2* [1, 2]. The discovery of these two genes and subsequent clinical availability of germline *BRCA1/2* gene testing enabled insights into the natural history of *BRCA1/2* mutation-associated cancers and development of evidence-based risk-reduction guidelines for mutation carriers. Furthermore, it advanced the field of targeted cancer therapeutics with the development

of agents such as poly (ADP-ribose) polymerase (PARP) inhibitors. In the current era of massively parallel next-generation sequencing, technologies that deliver more genetic information at a lower cost than ever before, the clinical implications of genetic testing will exponentially increase. We summarize recent progress in clinical cancer genetics and highlight research priorities.

The breast and ovarian cancer susceptibility genes: *BRCA1* and *BRCA2*

The breast and ovarian cancer susceptibility gene *BRCA1*, mapped to chromosome arm 17q, was identified in 1994 [3]. Soon thereafter, *BRCA2*, also implicated in breast and ovarian cancer development, was mapped to chromosome 13q [4]. Cancers arising in the setting of an inherited *BRCA1/2* mutation were found to have defects in the repair of DNA double-stranded breaks and in the recovery of stalled or broken DNA replication forks [5]. Initial studies of the cancer risks associated with a deleterious mutation in these genes (also known as mutation penetrance) were confined to families with very high cancer burden and young age at diagnosis. As a consequence, these studies produced very high penetrance estimates for *BRCA1/2* mutations, approaching a 90% lifetime risk of breast cancer development [6, 7]. Larger, more broadly representative population-based studies subsequently enrolled breast and ovarian cancer patients without a known family cancer history or early age at onset, and these analyses yielded lower and more generalizable penetrance estimates. Meta-analyses reported 57 and 49% risks of developing breast cancer, and 40 and 18% risks of developing ovarian cancer, among *BRCA1* and *BRCA2* mutation carriers, respectively [8–10]. Clinical practice guidelines for *BRCA1/2* carriers recommend risk-reduction strategies including annual breast magnetic resonance imaging (MRI) and prophylactic surgeries, some of which have a demonstrated survival benefit (Table 1) [11–13].

The era of multiple-gene germline panel testing for cancer predisposition

The first commercially available genetic test for breast cancer risk was launched and patented by Myriad Genetics, Inc., in 1996, and included sequencing of the *BRCA1/2* genes. In 2004, the European Patent Office limited the *BRCA1/2* patent claim as it did not meet the legal standards for inventiveness. Likewise, in the USA, there was debate as to whether the Patent Act applied to naturally occurring gene sequences [14]. This culminated in a landmark US Supreme Court ruling in 2013

Table 1. Estimated cancer risks and guidelines for breast cancer-associated genes on multigene germline panels

Gene	Breast cancer relative risk	Other cancer risks and syndromes	Clinical practice guidelines	References
<i>ATM</i>	Two to three-fold (c.7271T>G missense mutation with estimated 60% risk of breast cancer by age 80)	Ataxia telangiectasia syndrome in homozygotes; colon, pancreas, prostate (possibly family history dependent)	National Comprehensive Cancer Network (NCCN): Screening with breast MRI; consider RRM based on family history	[13, 35, 37]
<i>BRCA1</i>	10-fold	Ovarian	American Cancer Society (ACS) and NCCN: Screening with breast MRI, recommend RRBSO, discuss RRM	[8, 13, 18, 35, 38]
<i>BRCA2</i>	10-fold	Ovarian, pancreatic, prostate, melanoma	NCCN: Screening breast MRI, recommend RRBSO, discuss RRM	[8, 13, 18, 35, 38]
<i>CDH1</i>	Fivefold (particular association with lobular breast carcinoma)	Gastric	NCCN: Screening breast MRI; consider RRM based on family history	[13, 35, 39–41]
<i>CHEK2</i>	Two to threefold (threefold risk with truncating mutation 1100delC)	Possible link with colorectal, thyroid, lung (possibly family history dependent)	NCCN: Screening breast MRI, Patient with first-degree relative with colorectal cancer, consider colonoscopy every 5 years at 40, or 10 years prior to age of cancer diagnosis. Patient with no first-degree relative with colorectal cancer, consider colonoscopy every 5 years beginning at age 40	[13, 35, 42, 43]
<i>NBN</i>	Two to threefold	Nijmegen breakage syndrome in homozygotes; possibly ovarian	Consider screening breast MRI	[35, 44, 45]
<i>NF1</i>	Two to threefold	Central nervous system, peripheral nerve sheath	Consider screening breast MRI	[35, 46]
<i>PALB2</i>	Three to fivefold	Pancreas; possibly ovarian	NCCN: Screening breast MRI, discuss RRM	[13, 35, 47]
<i>PTEN</i>	At least fivefold	Thyroid, endometrial	NCCN: Screening breast MRI, discuss RRM	[13, 35, 48, 49]
<i>STK11</i>	At least fivefold	Pancreas, colon, ovarian sex cord-stromal	NCCN: Screening breast MRI	[13, 35, 50]
<i>TP53</i>	At least 10-fold	Multiple sites including adrenocortical, brain, leukemia, sarcoma	NCCN: Screening breast MRI, discuss RRM; whole-body MRI, colonoscopy, complete blood count, and other tests	[13, 35, 51]

RRBSO bilateral risk-reducing salpingo-oophorectomy, *RRM* risk-reducing mastectomy

determining that human genes in their natural form may not be patented [1]. The Supreme Court ruling in conjunction with rapid technological advances in next-generation gene sequencing opened doors to a competitive marketplace of commercialized multiple-gene germline panel testing.

Massively parallel, next-generation sequencing techniques allow analysis of many different genes simultaneously [15]. Clinical panels for cancer risk assessment have evolved from 6 to more than 100 genes. Although whole-genome approaches are also under study, to date, they have offered little additional yield and lower efficiency than smaller cancer-focused gene panels [16]. While commercially available panels differ in gene number and composition, there is substantial overlap of genes in which pathogenic mutations confer at least a two-fold elevation over the average breast cancer risk. This two-fold risk increase is the threshold value set by guidelines and payers for more intensive breast cancer screening that incorporates magnetic resonance imaging, and thus is a current threshold for clinical relevance [17, 18]. Genes associated with a risk in the two-fold range are often labeled as “moderate to high penetrance” cancer susceptibility genes. This distinguishes them from *BRCA1/2*, considered to be high-penetrance genes due to their five-fold or greater associated increase in breast cancer risk, and having well-established guidelines for intensive screening and prevention.

Immediately after the 2013 US Supreme Court ruling against gene patenting, multiple-gene germline sequencing panels incorporating *BRCA1/2* and other genes became available at a fraction of the previous cost for testing *BRCA1/2* only [1]. Initially, limited tests of four breast cancer-associated genes beyond *BRCA1/2* (*CDH1*, *PTEN*, *STK11*, *TP53*, which cause recognized syndromes with established clinical management guidelines) were adopted. Subsequent panels increased to contain an additional 15–20 candidate genes with DNA double-stranded repair functions similar to *BRCA1/2* (e.g., *ATM*, *BARD1*, *CHEK2*, *PALB2*) and an additional 25–40 genes encompassing cancer risk at multiple organ sites (e.g., *CDKN2A*, *MEN1*, *MLH1*, *MSH2*, *MSH6*, and *MUTYH*). Recently, panels have expanded to more than 100 genes that have potential cancer associations, although many lack sufficiently robust clinical data to guide patient care (e.g., *AXIN2*, *CYLD*, *SLX4*) [19].

With the widespread availability of such multigene panels, a simultaneous paradigm shift has occurred in hereditary cancer genetics clinics. In the past, gene testing was primarily phenotype-driven; by contrast, the current approach is increasingly panel-based. This means that patients are often tested for pathogenic mutations in several genes, even if they meet traditional criteria for genetic testing of only one or two. The primary reasons for seeking genetic consultation and testing for hereditary breast and ovarian cancer (HBOC) predisposition genes are as follows: (1) personal history of early-onset breast cancer (≤ 45 years of age), (2) a personal history of triple-negative breast cancer (≤ 60 years of age; this is the breast cancer subtype most associated with *BRCA1*), (3) family history of first- or second-degree relatives with breast or ovarian cancers, or other cancers associated with HBOC predisposition genes, and (4) personal history of male breast cancer.

The goal of such broadened, panel-based genetic testing is to define the optimal care of cancer patients and their families, specifically by assessing the risk of contralateral breast cancer and of developing other cancers (such as ovarian, colorectal, pancreatic cancers) and by enabling cancer prevention among unaffected family members [20]. Over the next decade, a major

priority is to define targeted chemoprevention strategies as well as selective agents for patients with curable and advanced cancers with a known genetic predisposition. The currently FDA-approved targeted agents in this setting are olaparib and rucaparib. Both of these drugs are PARP inhibitors and have been approved for the management of advanced *BRCA1* or *BRCA2* mutation-associated ovarian cancer [21, 22].

Prevalence and clinical features of moderate- and high-penetrance genes beyond *BRCA1/2*

The estimated prevalence of mutations in genes other than *BRCA1/2* is reported to be approximately 4–6% based on the currently published data using next-generation DNA sequencing for HBOC screening [20] (Table 2). These prevalence estimates stem from studies encompassing different cohorts, including African-Americans [23], patients with triple-

Table 2. Overview of multiplex germline gene panel testing results

Study	Study and patient population	Multigene panel	Results
Thompson et al. [31]	Case-control study; cases 2000 patients, primarily with breast cancer controls 1997 women without cancer diagnosis	Custom-targeted sequencing panel of 18 genes	3.9% of “cases” and 1.6% of “controls” were found to carry actionable mutations (excluding <i>BRCA1/2</i>)
Tung et al. [29]	Prospectively collected data; 488 stage I–III breast cancer patients enrolled (not based on HBOC family history)	Myriad Inc., MyRisk 25-gene panel	10.7% were found to have a germline mutation (6.1% in <i>BRCA1/2</i> , 4.6% in other HBOC genes)
Desmond et al. [28•]	Prospectively collected data; 1046 individuals referred for genetic counseling/testing for HBOC	Invitae Inc., 29-gene panel; Myriad Inc. MyRisk 25-gene panel	40 <i>BRCA1/2</i> -negative individuals (3.8%) harbored deleterious mutations in HBOC and Lynch syndrome genes
Tung et al. [27]	Cross-sectional study of 2158 individuals referred for <i>BRCA1/2</i> testing	Myriad Inc., 25-gene panel	9.3% of individuals had <i>BRCA1/2</i> mutation, 3.9% carried a mutation in other HBOC gene
Kurian et al. [26]	Cross-sectional study; 198 individuals referred for <i>BRCA1/2</i> testing	Custom 42-gene panel	57 (28.8%) patients carried <i>BRCA1/2</i> mutations; 16 germline mutation in other genes (11.4%)
Castera et al. [52]	708 patients seen in high-risk/cancer genetics clinic	Custom-targeted sequencing panel	3% of <i>BRCA1/2</i> -negative individuals were found to have deleterious mutations in other HBOC genes
Cancer Genomic Atlas [53]	Cross-sectional study of 507 breast cancer patients	Whole-exome sequencing	9.8% of germline mutations in HBOC genes; 5.5% in <i>BRCA1/2</i> , 4/3% in other HBOC genes

BRCA1/2 *BRCA1* and *BRCA2*, *HBOC* hereditary breast and ovarian cancer

negative breast cancer [24•], and patients seen in high-risk cancer genetics clinic settings [25–27, 28•].

A recent study by Tung and colleagues investigated the prevalence of deleterious germline mutations in 25 cancer susceptibility genes among patients with a personal history of breast cancer who were unselected for family history. The study cohort comprised of 488 breast cancer patients, with 49% of patients reported having a first- or second-degree relative with breast or ovarian cancer (mean age at time of breast cancer diagnosis was 50.3 years, 7.8% of the patients were of Ashkenazi Jewish descent, and 18% of the women had triple-negative breast cancer). Fifty-five deleterious mutations were identified in 52 (10.7%) patients. While 30 (6.1%) of the patients had a germline *BRCA1/2* mutation, 20 (4.1%) of patients had a total of 21 deleterious mutations in non-*BRCA1/2* breast cancer predisposition genes, including *CHEK2* ($n = 10$), *ATM* ($n = 4$), *BRIP1* ($n = 4$), and one each in *PALB2*, *PTEN*, and *NPN*. Notably, at least one variant of uncertain significance (VUS) was identified in 162 (32.2%) of women. Factors that significantly predicted *BRCA1/2* mutation carriage (including younger age at breast cancer diagnosis, Ashkenazi Jewish heritage, triple-negative breast cancer, and higher grade) did not predict carriage of a mutation in the other breast cancer predisposition genes, when these genes were analyzed as a single group [29]. Given the absence of predictive factors for carriage of non-*BRCA1/2* mutations, it may be that most or all breast cancer patients will ultimately benefit from multiple-gene germline panel testing [29].

As testing costs fall and access grows, a major question is the clinical utility of multiple-gene germline panel testing for cancer susceptibility. We prospectively enrolled and conducted multiple-gene germline panel testing of patients who were appropriate candidates for HBOC screening and who lacked deleterious *BRCA1/2* mutations [26, 28•]. Among the 1046 participants, 63 *BRCA1/2*-negative patients harbored deleterious mutations, most commonly in moderate-risk breast and ovarian cancer genes (*ATM*, *CHEK2*, and *PALB2*) and the Lynch syndrome genes. Nearly one third of mutation-positive patients (20 of 63) had mutations in genes for which guidelines recommended a change in cancer screening or prevention approaches (Table 1). Among patients with deleterious mutations in low- to moderate-risk breast cancer genes, a management change was recommended in one quarter of cases, with additional implications for unaffected relatives [28•]. Our work and that of others (Table 1) emphasize the clinical relevance of multiple-gene germline panel testing for hereditary cancer risk.

Similarly, Idos and colleagues presented on the interim analysis of a study assessing the yield of multigene panel testing versus expert genetic opinion among 1000 patients with a $\geq 2.5\%$ likelihood of carriage of a deleterious gene mutation at the 2016 American Society of Clinical Oncology Annual Meeting. A total of 11.6% patients tested positive for at least one pathogenic variant, and 36.5% of the individuals had at least one VUS. One quarter of patients with a pathogenic variant had a gene mutation that was “missed” by expert opinion, several in a clinically “actionable” gene with implications for these patients’ care [30]. Notably, patients with “missed” mutations lacked the classic phenotype:

for example, a 45-year-old woman with a family history of endometrial adenocarcinoma had a deleterious *BRCA2* mutation, which is not associated with this cancer type. Likewise, a 65-year survivor of two primary breast cancers had a deleterious mutation in *PMS2*, not a usual breast cancer predisposition gene. Significant questions remain as to whether mutation carriers without the classic family history should undergo the stringent cancer risk-reduction strategies (e.g., full-body MRI for patients with a *TP53* mutation) developed for more penetrant families.

Challenges in multiple-gene germline panel testing

Despite the advantages of multiple-gene germline panel testing, a number of questions remain about its clinical utility. Thompson and colleagues assessed the prevalence of deleterious mutations in 18 genes commonly included in hereditary breast cancer panels among 2000 predominantly breast cancer-affected women and 1997 cancer-free women. Seventy-eight cases (3.9%) and 33 controls (1.6%) had potentially actionable mutations. The authors concluded that the frequency of non-*BRCA1/2* mutations is relatively low and similar among breast cancer patients and cancer-free populations; and therefore that multiple-gene germline panels may provide clinical misinformation and harm at the individual patient level [31]. Such concerns may apply particularly to mutation carriers lacking the classic phenotype associated with a cancer syndrome, as their cancer risk may be lower than that of previously estimated.

Another major challenge is variants of uncertain significance (VUS). VUS are genetic alterations whose disease association is unknown. The large majority of VUS are expected to be re-interpreted as benign polymorphisms after sufficient data accumulate; however, uncertainty about the clinical relevance of such genetic findings can be frustrating and anxiety-provoking for patients and families. VUS are relatively rare (2–5%) when only *BRCA1/2* genes are tested, due primarily to increasingly widespread *BRCA1/2* testing in diverse populations [32]. However, the advent of multiple-gene germline panel testing has increased the prevalence of at least one VUS among all sequenced genes to 20–40% [27, 28, 33–35]. Further complications arise when two genetic laboratories provide conflicting interpretations of the same genetic variant—a conundrum that did not arise during the past two decades of patented *BRCA1/2* testing by a single laboratory. Balmana and colleagues recently reported on the discrepancy in VUS rates between different Clinical Laboratory Improvement Amendment (CLIA)-approved genetic testing laboratories. Interpretation varies among reporting laboratories from pathogenic or likely pathogenic to VUS for one quarter of variants, which raises major concerns about the appropriate clinical management strategy for a patient with such conflicting results [36]. Data sharing in a common and publicly available registry will be a step toward resolving the high VUS rate, which is a serious threat to successful implementation of next-generation sequencing.

Future directions

The landscape of clinical cancer genetics has dramatically changed in the recent years; these advances have yielded concrete and comprehensive guidelines with

great potential to benefit patients and families at risk of HBOC. Research must address gaps in the current landscape of clinical cancer genetics: one major priority is to reduce the prevalence of reported VUS by sequencing larger and more diverse populations. Population-based studies are essential to understand the true prevalence of breast cancer susceptibility gene mutations in the general population, outside of high-risk cancer genetics clinics. Furthermore, evidence-based clinical guidelines for patients carrying such pathogenic gene mutations can be implemented only if their prevalence and penetrance are better understood. Another key approach is biomarker-driven clinical trials of targeted agents such as PARP inhibitors for the treatment and prevention of hereditary breast, ovarian, and other cancers.

Compliance with Ethical Standards

Conflict of Interest

Anosheh Afghahi declares that she has no conflict of interest.

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Human and Animal Rights and Informed Consent

This article does not contain any studies with human or animal subjects performed by any of the authors.

References and Recommended Reading

Papers of particular interest, published recently, have been highlighted as:

- Of importance

1. Offit K, Bradbury A, Storm C, et al. Gene patents and personalized cancer care: impact of the myriad case on clinical oncology. *J Clin Oncol*. 2013;31:2743–8.
2. Couch FJ, Nathanson KL, Offit K. Two decades after BRCA: setting paradigms in personalized cancer care and prevention. *Science*. 2014;343:1466–70.
3. Miki Y, Swensen J, Shattuck-Eidens D, et al. A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. *Science*. 1994;266:66–71.
4. Wooster R, Neuhausen SL, Mangion J, et al. Localization of a breast cancer susceptibility gene, BRCA2, to chromosome 13q12-13. *Science*. 1994;265:2088–90.
5. Li X, Heyer WD. Homologous recombination in DNA repair and DNA damage tolerance. *Cell Res*. 2008;18:99–113.
6. Ford D, Easton DF, Stratton M, et al. Genetic heterogeneity and penetrance analysis of the BRCA1 and BRCA2 genes in breast cancer families. The breast cancer linkage consortium. *Am J Hum Genet*. 1998;62:676–89.
7. King MC, Marks JH, Mandell JB, et al. Breast and ovarian cancer risks due to inherited mutations in BRCA1 and BRCA2. *Science*. 2003;302:643–6.
8. Antoniou A, Pharoah PD, Narod S, et al. Average risks of breast and ovarian cancer associated with BRCA1 or BRCA2 mutations detected in case series unselected for family history: a combined analysis of 22 studies. *Am J Hum Genet*. 2003;72:1117–30.
9. Chen S, Parmigiani G. Meta-analysis of BRCA1 and BRCA2 penetrance. *J Clin Oncol*. 2007;25:1329–33.
10. Kurian AW, Kingham KE, Ford JM. Next-generation sequencing for hereditary breast and gynecologic cancer risk assessment. *Curr Opin Obstet Gynecol*. 2015;27:23–33.
11. Domchek SM, Friebel TM, Singer CF, et al. Association of risk-reducing surgery in BRCA1 or BRCA2 mutation carriers with cancer risk and mortality. *JAMA*. 2010;304:967–75.
12. Finch AP, Lubinski J, Moller P, et al. Impact of oophorectomy on cancer incidence and mortality in women with a BRCA1 or BRCA2 mutation. *J Clin Oncol*. 2014;32:1547–53.
13. Daly MB, Pilarski R, Berry M, et al. NCCN guidelines insights: genetic/familial high-risk assessment: breast and ovarian, version 2.2017. *J Natl Compr Cancer Netw*. 2017;15:9–20.

14. Kesselheim AS, Mello MM. Gene patenting—is the pendulum swinging back? *N Engl J Med*. 2010;362:1855–8.
 15. Shendure J, Ji H. Next-generation DNA sequencing. *Nat Biotechnol*. 2008;26:1135–45.
 16. Dewey FE, Grove ME, Pan C, et al. Clinical interpretation and implications of whole-genome sequencing. *JAMA*. 2014;311:1035–45.
 17. Mainiero MB, Lourenco A, Mahoney MC, et al. ACR appropriateness criteria breast cancer screening. *J Am Coll Radiol*. 2013;10:11–4.
 18. Saslow D, Boetes C, Burke W, et al. American Cancer Society guidelines for breast screening with MRI as an adjunct to mammography. *CA Cancer J Clin*. 2007;57:75–89.
 19. Kurian AW, Ford JM. Multigene panel testing in oncology practice: how should we respond? *JAMA Oncol*. 2015;1:277–8.
 20. Graffeo R, Livraghi L, Pagani O, et al. Time to incorporate germline multigene panel testing into breast and ovarian cancer patient care. *Breast Cancer Res Treat*. 2016;160:393–410.
 21. Ledermann J, Harter P, Gourley C, et al. Olaparib maintenance therapy in patients with platinum-sensitive relapsed serous ovarian cancer: a preplanned retrospective analysis of outcomes by BRCA status in a randomised phase 2 trial. *Lancet Oncol*. 2014;15:852–61.
 22. Swisher EM, Lin KK, Oza AM, et al. Rucaparib in relapsed, platinum-sensitive high-grade ovarian carcinoma (ARIEL2 part 1): an international, multicentre, open-label, phase 2 trial. *Lancet Oncol*. 2017;18:75–87.
 23. Churpek JE, Walsh T, Zheng Y, et al. Inherited predisposition to breast cancer among African American women. *Breast Cancer Res Treat*. 2015;149:31–9.
 24. • Couch FJ, Hart SN, Sharma P, et al. Inherited mutations in 17 breast cancer susceptibility genes among a large triple-negative breast cancer cohort unselected for family history of breast cancer. *Journal of Clinical Oncology* 2015;33.
- Characterized predisposition genes associated at higher frequency in triple-negative breast cancer beyond BRCA1/2.
25. Castera L, Krieger S, Rousselin A, et al. Next-generation sequencing for the diagnosis of hereditary breast and ovarian cancer using genomic capture targeting multiple candidate genes. *Eur J Hum Genet* 2014.
 26. Kurian AW, Hare EE, Mills MA, et al. Clinical evaluation of a multiple-gene sequencing panel for hereditary cancer risk assessment. *J Clin Oncol*. 2014;32:2001–9.
 27. Tung N, Battelli C, Allen B, et al. Frequency of mutations in individuals with breast cancer referred for BRCA1 and BRCA2 testing using next-generation sequencing with a 25-gene panel. *Cancer*. 2015;121:25–33.
 28. • Desmond A, Kurian AW, Gabree M, et al. Clinical actionability of multigene panel testing for hereditary breast and ovarian cancer risk assessment. *JAMA Oncol*. 2015;1:943–51.
- Assessed the potential clinical effect of multigene testing by determining the clinical actionability of such test results for individuals harboring deleterious gene mutations.
29. Tung N, Lin NU, Kidd J, et al. Frequency of germline mutations in 25 cancer susceptibility genes in a sequential series of patients with breast cancer. *J Clin Oncol*. 2016;34:1460–8.
 30. Idos G, Kurian AW, Ricker CN, et al. Yield of multiplex panel testing compared to expert opinion and validated prediction models. Chicago: Presented at the American Society of Oncology Annual Meeting; 2016.
 31. Thompson ER, Rowley SM, Li N, et al. Panel testing for familial breast cancer: calibrating the tension between research and clinical care. *J Clin Oncol*. 2016;34:1455–9.
 32. Hall MJ, Reid JE, Burbidge LA, et al. BRCA1 and BRCA2 mutations in women of different ethnicities undergoing testing for hereditary breast-ovarian cancer. *Cancer*. 2009;115:2222–33.
 33. LaDuca H, Stuenkel AJ, Dolinsky JS, et al. Utilization of multigene panels in hereditary cancer predisposition testing: analysis of more than 2,000 patients. *Genet Med*. 2014;16:830–7.
 34. Selkirk CG, Vogel KJ, Newlin AC, et al. Cancer genetic testing panels for inherited cancer susceptibility: the clinical experience of a large adult genetics practice. *Familial Cancer*. 2014;13:527–36.
 35. Kurian AW, Antoniou AC, Domchek SM. Refining breast cancer risk stratification: additional genes, additional information. *Am Soc Clin Oncol Educ Book*. 2016;35:44–56.
 36. • Balmana J, Digiovanni L, Gaddam P, et al. Conflicting interpretation of genetic variants and cancer risk by commercial laboratories as assessed by the prospective registry of multiplex testing. *J Clin Oncol*. 2016;34:4071–8.
- Assessed the discrepancies in variants of uncertain significance between genetic testing laboratories. Results show that a substantial number of conflicting interpretations exist between laboratories, and these may have clinical implications for patients.
37. Renwick A, Thompson D, Seal S, et al. ATM mutations that cause ataxia-telangiectasia are breast cancer susceptibility alleles. *Nat Genet*. 2006;38:873–5.
 38. Mavaddat N, Peock S, Frost D, et al. Cancer risks for BRCA1 and BRCA2 mutation carriers: results from prospective analysis of EMBRACE. *J Natl Cancer Inst*. 2013;105:812–22.
 39. Kaurah P, MacMillan A, Boyd N, et al. Founder and recurrent CDH1 mutations in families with hereditary diffuse gastric cancer. *JAMA*. 2007;297:2360–72.
 40. Pharoah PD, Guilford P, Caldas C, et al. Incidence of gastric cancer and breast cancer in CDH1 (E-cadherin) mutation carriers from hereditary diffuse gastric cancer families. *Gastroenterology*. 2001;121:1348–53.
 41. van der Post RS, Vogelaar IP, Carneiro F, et al. Hereditary diffuse gastric cancer: updated clinical guidelines with an emphasis on germline CDH1 mutation carriers. *J Med Genet*. 2015;52:361–74.

42. Meijers-Heijboer H, van den Ouweland A, Klijn J, et al. Low-penetrance susceptibility to breast cancer due to CHEK2* 1100delC in noncarriers of BRCA1 or BRCA2 mutations. *Nat Genet.* 2002;31:55–9.
43. Weischer M, Bojesen SE, Ellervik C, et al. CHEK2* 1100delC genotyping for clinical assessment of breast cancer risk: meta-analyses of 26,000 patient cases and 27,000 controls. *J Clin Oncol.* 2008;26:542–8.
44. Bartkova J, Tommiska J, Oplustilova L, et al. Aberrations of the MRE11-RAD50-NBS1 DNA damage sensor complex in human breast cancer: MRE11 as a candidate familial cancer-predisposing gene. *Mol Oncol.* 2008;2:296–316.
45. Zhang G, Zeng Y, Liu Z, et al. Significant association between Nijmegen breakage syndrome 1 657del5 polymorphism and breast cancer risk. *Tumour Biol.* 2013;34:2753–7.
46. Seminog OO, Goldacre MJ. Age-specific risk of breast cancer in women with neurofibromatosis type 1. *Br J Cancer.* 2015;112:1546–8.
47. Antoniou AC, Casadei S, Heikkinen T, et al. Breast-cancer risk in families with mutations in PALB2. *N Engl J Med.* 2014;371:497–506.
48. Tan MH, Mester JL, Ngeow J, et al. Lifetime cancer risks in individuals with germline PTEN mutations. *Clin Cancer Res.* 2012;18:400–7.
49. Pilarski R, Eng C. Will the real Cowden syndrome please stand up (again)? Expanding mutational and clinical spectra of the PTEN hamartoma tumour syndrome. *J Med Genet.* 2004;41:323–6.
50. Hearle N, Schumacher V, Menko FH, et al. Frequency and spectrum of cancers in the Peutz-Jeghers syndrome. *Clin Cancer Res.* 2006;12:3209–15.
51. Gonzalez KD, Noltner KA, Buzin CH, et al. Beyond Li Fraumeni syndrome: clinical characteristics of families with p53 germline mutations. *J Clin Oncol.* 2009;27:1250–6.
52. Castera L, Krieger S, Rousselin A, et al. Next-generation sequencing for the diagnosis of hereditary breast and ovarian cancer using genomic capture targeting multiple candidate genes. *Eur J Hum Genet.* 2014;22:1305–13.
53. Comprehensive molecular portraits of human breast tumours. *Nature* 2012;490:61–70.