Next-generation sequencing for hereditary breast and gynecologic cancer risk assessment

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Purpose of review
To summarize advances in next-generation sequencing and their application to breast and gynecologic cancer risk assessment.

Recent findings
Next-generation sequencing panels of 6–112 cancer-associated genes are increasingly used in patient care. Studies report a 4–16% prevalence of mutations other than *BRCA1/2* among patients who meet evidence-based practice guidelines for *BRCA1/2* testing, with a high rate (15–88%) of uninterpretable variants of uncertain significance. Despite uncertainty about results interpretation and communication, there is early evidence of a benefit from multiple-gene sequencing panels for appropriately selected patients.

Summary
Multiple-gene sequencing panels appear highly promising for the assessment of breast and gynecologic cancer risk, and they may usefully be administered in the context of cancer genetics expertise and/or clinical research protocols.

Keywords
breast cancer, genetic risk assessment, multiple-gene sequencing panels, next-generation sequencing, ovarian cancer

INTRODUCTION

Following the identification in 1994 of *BRCA1* and *BRCA2*, genetic assessment of breast and gynecologic cancer risk has become an increasingly important aspect of women's healthcare [1,2]. In the last 2 decades, incremental advances in genetic testing, clinical risk assessment, and risk reduction strategies have transformed our approach to patients who seek advice about their probability of developing cancer. Since the emergence of the high-throughput, relatively inexpensive next-generation sequencing (NGS) technology and the 2013 US Supreme Court ruling against gene patenting, our understanding and practice of cancer genetics have undergone particularly rapid change. This review will summarize recent advances and current research priorities in clinical risk assessment for breast and gynecologic cancers.

LESSONS FROM THE *BRCA1* AND *BRCA2* ERA, 1994–2013

The clinical application of genetic testing for mutations in *BRCA1* and *BRCA2* (*BRCA1/2*) has generated crucial observations and advances that shape our current approaches. Early studies [3,4] of the cancer risks associated with a *BRCA1/2* mutation were limited to families with an exceptionally high cancer burden, having multiple affected relatives, and very young ages at diagnosis; these analyses produced very high estimates of mutation-associated cancer risk (also known as 'penetrance'), approaching an 80–90% lifetime probability of developing breast cancer. Subsequent population-based studies [5,6] of breast and ovarian cancer patients with *BRCA1/2* mutations who were not selected for family history or early diagnosis have estimated far lower lifetime risks, with meta-analyses reporting risks for breast cancer of 57 and 49%, and for ovarian cancer of 40 and 18%, among

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KEY POINTS

- NGS produces large-volume genetic data quickly and cheaply, and multiple cancer-gene sequencing panels are rapidly emerging into clinical practice.
- For approximately 4–16% of women who meet relevant practice guidelines yet test negative for *BRCA1/2* mutations, NGS panels identify another cancer-associated gene mutation.
- The complexity of gene panel selection and interpretation, including high rates of VUS, means that such testing is most effectively and safely conducted by an expert in cancer genetics.
- Early clinical studies suggest that multiple-gene testing for breast and gynecologic cancer risk benefits appropriately selected patients.
- Larger, longer-term studies of the clinical utility of NGS panels for breast and gynecologic cancer risk assessment are a major priority.

BRCA1 and *BRCA2* mutation carriers, respectively. This experience emphasizes the importance of using widely inclusive, population-based study cohorts to estimate cancer risks for the average carrier of a genetic mutation, rather than generalizing from smaller, highly selected clinic-based samples. Another major advance was the design and evaluation of targeted cancer risk reduction strategies for *BRCA1/2* mutation carriers, including risk-reducing mastectomy and salpingo-oophorectomy and breast screening protocols that incorporate magnetic resonance imaging. Studies [7–9] have demonstrated a survival benefit due to these risk-targeted interventions, which have informed the development of evidence-based clinical practice guidelines and decision support tools [10,11▪▪,12,13], and now chart a pathway for other high-risk patient groups. The third noteworthy observation has been the economic and societal barriers that limit access to effective genetic testing. Due to the high costs of clinical *BRCA1/2* testing (rising above $3000 during 1994–2013) and variable insurance coverage, racial/ethnic minorities have been less widely tested than non-Hispanic Whites (NH Whites) in the United States. This access disparity has perpetuated a knowledge disparity about the normal sequence of *BRCA1/2* among racial/ethnic minorities: African Americans, Asians, and Hispanics are far more likely to receive uninterpretable results [defined as variants of uncertain significance (VUS)] when they do undergo *BRCA1/2* sequencing [14]. Although racial/ethnic disparities in clinical cancer genetics remain a significant problem, fears of genetic discrimination due to *BRCA1/2* mutation carriage have been more effectively addressed. The Genetic Information Non-Discrimination Act of 2008 prohibits discrimination in health insurance or hiring and stipulates that mutation carriage may not be used as a preexisting condition by insurers. The Genetic Information Non-Discrimination Act and the related laws offer a protective legal infrastructure, which will be crucial for patients' well-being as the scope of genetic testing expands [15–17].

NEXT-GENERATION SEQUENCING: AN ADVANCE IN TECHNOLOGY

NGS employs a massively parallel approach, enabling the rapid analysis of many genetic sequences at the same time. In comparison with traditional Sanger sequencing, NGS generates exponentially more genomic information at a dramatically lower cost. Whole-exome sequencing (WES) and whole-genome sequencing (WGS) are increasingly achievable at costs in the $1000 range (Fig. 1). Concerns about NGS have included its accuracy in sequence detection, which may fall short of the accuracy of older techniques and therefore require verification, and the sheer magnitude of data that it provides. Interpretation of NGS results, both in the genetics laboratory and in the clinic, currently lags behind the dramatic progress in sequencing methods [18,19▪▪]. In a departure from past decades when costs were limiting, challenges in results interpretation are now the primary barrier to widespread clinical dissemination of WGS.

MULTIPLE-GENE SEQUENCING PANELS FOR HEREDITARY CANCER RISK ASSESSMENT

Clinical trials of germline WES and WGS are underway in general medical and specialty settings, including oncology. As described above, however, routine clinical use of these comprehensive sequencing analyses is likely premature due to the volume and complexity of their results. In our present transition period from sequencing fewer to more genes, disease-focused multiple-gene sequencing panels may offer a clinically useful compromise. Gene selection for existing multiple-gene panels has followed the published literature, including genes implicated in known cancer syndromes with a breast or gynecologic component (e.g., the Lynch syndrome genes: *MLH1*, *MSH2*, *MSH6*, *PMS2*, and *EPCAM*), genes with a moderate (approximately two-fold) breast and/or gynecologic cancer risk association (e.g., *CHEK2* and *ATM*), and genes that function with or analogously to *BRCA1/2* in DNA repair pathways (e.g., *PALB2* and the Fanconi anemia-associated genes)

[18,20▪,21]. Although mutations in some panel genes have been convincingly demonstrated to confer high breast and/or gynecologic cancer risks (e.g., *PALB2* and the Lynch syndrome genes), other genotype–phenotype associations are less well substantiated (e.g., *MRE11A* and *NBN*) [22▪,23,24].

Until very recently, the prohibitive price of genetic sequencing limited clinical testing to those patients who met stringent diagnostic criteria for identified conditions such as hereditary breast/ovarian cancer syndrome, Cowden syndrome, and Li–Fraumeni syndrome (Fig. 1) [25]. In June 2013, however, a convergence of technological progress and legislative deregulation transformed the genetic testing landscape. The US Supreme Court's decision against gene patenting enabled numerous companies to offer clinical *BRCA1/2* testing, ending a 20-year period of testing by only one company; the resulting competition immediately reduced prices [26]. Economic incentives thus shifted quickly toward sequencing more genes instead of fewer, resulting in multiple-gene panels for clinical cancer risk assessment. Most of the commercially available multiple-gene panels sequence 25–30 cancer-associated genes, with a range of 6–112 genes (Table 1). Many experts urge caution in testing genes that have less established cancer risk associations, and guideline organizations have yet to issue specific recommendations for the clinical management of patients with mutations in moderate penetrance cancer-associated genes (e.g., *ATM* and *CHEK2*) [11▪▪,20▪]. However, a 2014 survey of North American genetic counselors demonstrated rapid uptake of multiple-gene panel testing over the preceding year, with marked variation in use by geographic region and healthcare setting [27].

CLINICAL STUDIES OF NEXT-GENERATION SEQUENCING

Several studies have reported on the yield of multiple-gene panels for the assessment of breast and gynecologic cancer risk in relevant patient populations. Study subjects include ovarian cancer patients unselected for age at diagnosis or family cancer history [21,28]; families with striking breast/ovarian cancer histories and/or young age at diagnosis [29–31]; clinical referral populations who meet standard *BRCA1/2* testing guidelines [11▪▪,32,33▪▪,34]; and patients recorded by the administrative databases of genetic testing companies [35▪,36,37▪▪]. With some exceptions, most studies have enrolled primarily NH Whites. Nearly 9000 patients have been included across nine published or presented studies as of September 2014; the evaluated panels have considerable overlap in the genes they include, ranging from a total of 13 to 42 sequenced genes (Table 2). The prevalence of

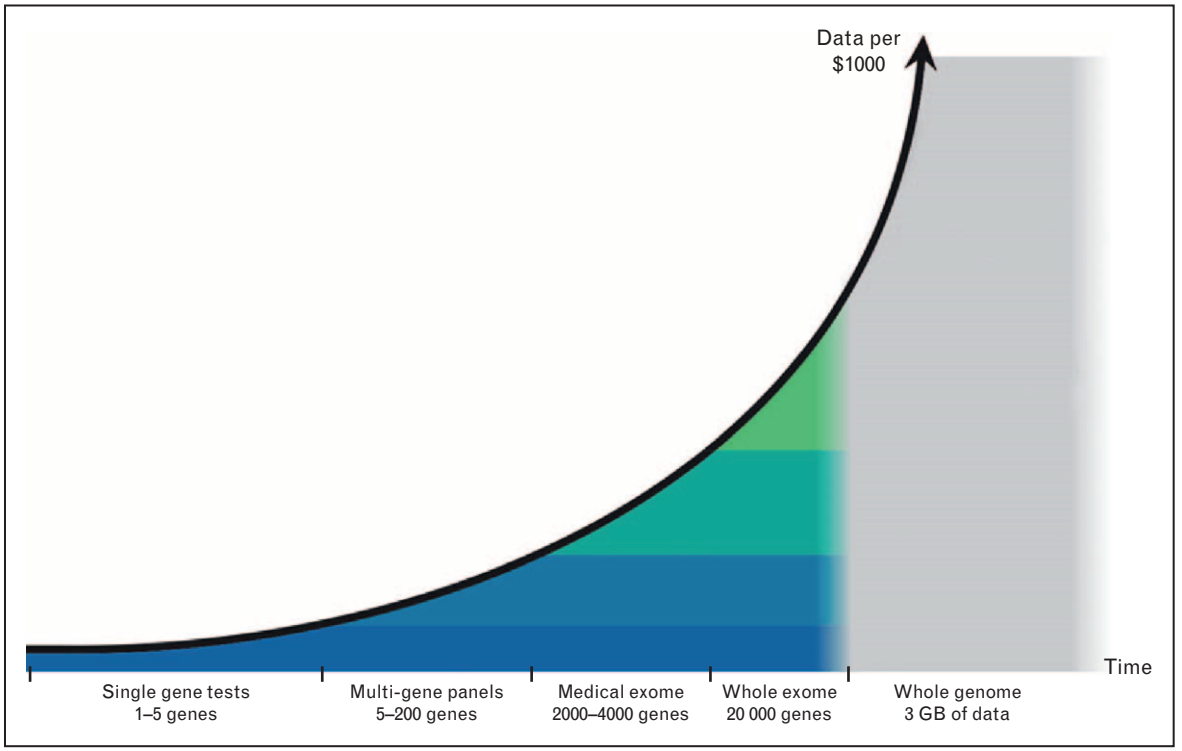

FIGURE 1. Evolution of next-generation sequencing technologies, demonstrating the exponential increase in genomic data available for a fixed cost in the last 2 decades (image provided courtesy of Steven Lincoln, PhD).

Table 1. Panels and genes available for clinical risk assessment of patients with personal and/or family history of breast and gynecologic cancers*

Genes (*N* = 122)	Ambry BRCAPlus (*N* = 6)	Ambry BreastNext (*N* = 16)	Ambry OvaNext (*N* = 19)	Ambry GYNPlus (*N* = 9)	Ambry CancerNext (*N* = 24)	Ambry CancerNext Expanded (*N* = 43)	Fulgent Breast-Ovarian Panel (*N* = 39)	Fulgent Hereditary Cancer Panel (*N* = 112)	GeneDx OncoGeneDx Panel (*N* = 29)	Invitae Women's Cancer Panel (*N* = 17)	Invitae Hereditary Cancer Panel (*N* = 29)	Myriad MyRisk (*N* = 25)	University of WA BROCA (*N* = 49)
AKT1													X
AIP								X					
*APC**					X	X	X	X	X		X	X	X
ATM		X	X		X	X	X	X	X	X	X	X	X
ATR							X	X					X
AXIN1									X				
AXIN2								X					
BAP1							X	X					X
BARD1		X	X		X	X	X	X	X			X	X
BLM							X	X					
*BMPR1A**					X	X	X	X	X		X	X	X
*BRCA1**	X	X	X	X	X	X	X	X	X	X	X	X	X
*BRCA2**	X	X	X	X	X	X	X	X	X	X	X	X	X
BRIP1		X	X		X	X	X	X	X	X	X	X	X
BUB1B								X					
*CDH1**	X	X	X		X	X	X	X	X	X	X	X	X
*CDK4**						X	X	X	X		X	X	X
CDKN1B								X					
*CDKN2A**						X	X	X	X		X	X	X
CHEK1													X
CHEK2		X	X		X	X	X	X	X	X	X	X	X
CTNNA1													X
CTNNB1							X	X					
CYLD								X					
DDB2								X					
DICER								X					
EGFR								X					
EGLN1								X					
*EPCAM**			X	X	X	X	X	X	X	X	X	X	X
ERCC2								X					
ERCC3								X					

Table 1 (*Continued*)

Genes (*N* = 122)	Ambry BRCAPlus (*N* = 6)	Ambry BreastNext (*N* = 16)	Ambry OvaNext (*N* = 19)	Ambry GYNPlus (*N* = 9)	Ambry CancerNext (*N* = 24)	Ambry CancerNext Expanded (*N* = 43)	Fulgent Breast-Ovarian Panel (*N* = 39)	Fulgent Hereditary Cancer Panel (*N* = 112)	GeneDx OncoGeneDx Panel (*N* = 29)	Invitae Women's Cancer Panel (*N* = 17)	Invitae Hereditary Cancer Panel (*N* = 29)	Myriad MyRisk (*N* = 25)	University of WA BROCA (*N* = 49)
ERCC4								X					
ERCC5								X					
EXO1								X					
EXT1								X					
EXT2								X					X
FAM175A							X						X
FANCA								X					
FANCB								X					
FANCC								X	X				
FANCD2								X					
FANCE								X					
FANCF								X					
FANCG								X					
FANCI								X					
FANCL								X					
FANCM								X					
FH						X		X					
FLCN						X		X					
GALNT12								X					X
GEN1													X
GPC3								X					
GREM1													X
HOXB13							X	X					X
HRAS								X					
KIF1B								X					
KIT								X					
MAX						X		X					
MC1R								X					
*MEN1**								X			X		
MET						X		X			X		
MITF						X		X					

Table 1 (*Continued*)

Genes (*N* = 122)	Ambry BRCAPlus (*N* = 6)	Ambry BreastNext (*N* = 16)	Ambry OvaNext (*N* = 19)	Ambry GYNPlus (*N* = 9)	Ambry CancerNext (*N* = 24)	Ambry CancerNext Expanded (*N* = 43)	Fulgent Breast-Ovarian Panel (*N* = 39)	Fulgent Hereditary Cancer Panel (*N* = 112)	GeneDx OncoGeneDx Panel (*N* = 29)	Invitae Women's Cancer Panel (*N* = 17)	Invitae Hereditary Cancer Panel (*N* = 29)	Myriad MyRisk (*N* = 25)	University of WA BROCA (*N* = 49)
*MLH1**			X	X	X	X	X	X	X	X	X	X	X
MPL								X					
MRE11A		X			X	X	X	X					X
*MSH2**			X	X	X	X	X	X	X	X	X	X	X
MSH3								X					
*MSH6**			X	X	X	X	X	X	X	X	X	X	X
*MUTYH**		X	X		X	X	X	X	X		X	X	X
NBN		X	X		X	X	X	X	X	X	X	X	X
*NF1**						X		X					
*NF2**								X					
PALB2		X	X		X	X	X	X	X	X	X	X	X
PALLD							X				X		
PDGFRA								X					
PICALM								X					
PIK3CA													X
PMS1								X					
*PMS2**			X	X	X	X	X	X	X	X	X	X	X
POLD1								X					X
POLE													X
PRKAR1A								X					
PRKDC								X					
PRSS1								X					X
PTCH1							X	X			X		
*PTEN**	X	X	X	X	X	X	X	X	X	X	X	X	X
PTPN11								X					
RAD50		X			X	X	X	X					X
RAD51			X				X	X					X
RAD51C		X			X	X	X	X	X	X	X	X	X
RAD51D						X	X	X	X			X	X
*RB1**								X					
RBBP8								X					

Table 1 (*Continued*)

Genes (*N* = 122)	Ambry BRCAPlus (*N* = 6)	Ambry BreastNext (*N* = 16)	Ambry OvaNext (*N* = 19)	Ambry GYNPlus (*N* = 9)	Ambry CancerNext (*N* = 24)	Ambry CancerNext Expanded (*N* = 43)	Fulgent Breast-Ovarian Panel (*N* = 39)	Fulgent Hereditary Cancer Panel (*N* = 112)	GeneDx OncoGeneDx Panel (*N* = 29)	Invitae Women's Cancer Panel (*N* = 17)	Invitae Hereditary Cancer Panel (*N* = 29)	Myriad MyRisk (*N* = 25)	University of WA BROCA (*N* = 49)
RBM15								X					
RECQL4								X					
*RET**						X		X			X		X
ROBO2								X					
SDBS								X					
SDHA						X		X					
SDHAF2						X		X					
*SDHB**						X		X					X
*SDHC**						X		X					X
*SDHD**						X		X					X
SLX4								X					
*SMAD4**			X		X	X	X	X	X		X	X	X
SMARCB1								X					
*STK11**	X	X	X		X	X	X	X	X	X	X	X	X
SUFU								X					
TERT								X					
TMEM127						X		X					
*TP53**	X	X		X	X	X	X	X	X	X	X	X	X
*TSC1**						X		X					
*TSC2**						X		X					
TSHR								X					
TYR								X					
TP53BP1													X
*VHL**						X	X	X	X		X		X
WRN								X					
*WT1**								X					
*XPA**								X					
*XPC**								X					
XRCC2							X	X	X				X
XRCC3							X	X					

*Genes with an asterisk are associated with a known hereditary cancer syndrome and/or clinical practice guidelines; others are not.

Table 2. Clinical studies of next-generation sequencing panels, reporting prevalence of deleterious mutations and variants of uncertain significance

Study	*N*	Population	Race/Ethnicity	Testing Panel	Deleterious Mutations	Variants of Uncertain Significance	Reference
Walsh *et al.* Proc Natl Acad Sci, 2011	360	Ovarian cancers, unselected	Not reported	BROCA (University of Washington, Seattle, WA; 21 genes)	6.1% (non-BRCA1/2)	Not reported	[21]
Harrell *et al.* American Society of Human Genetics Annual Meeting, 2013	1412	Ovarian cancers, unselected (extension of above study)	Not reported	BROCA (University of Washington; 41 genes)	5.5% (non-*BRCA1/2*)	Not reported	[28]
Walsh *et al.* American Society of Human Genetics Annual Meeting, 2013	800	*BRCA1/2*-negative, personal or family history of at least three breast or ovarian cancers	Not reported	BROCA (University of Washington; 41 genes)	15.8% (non-*BRCA1/2*)	Not reported	[30]
Olopade *et al.* American Society of Human Genetics Annual Meeting, 2013	395	Cancer genetics clinic testing sample	100% African American	BROCA (University of Washington; 41 genes)	4.1% (non-*BRCA1/2*)	Not reported	[34]
Tung *et al.* Cancer, 2014	2158	Testing laboratory database (Myriad Genetics, for *BRCA1/2*)	Mostly non-Hispanic White	MyRisk (Myriad, 25 genes)	4.3% (non-*BRCA1/2*)	42%	[37▪▪]
Castera *et al.* Eur J Hum Genet, 2014	708	Met practice guidelines criteria for Hereditary Breast/Ovarian Cancer Syndrome	Not reported	Custom designed (16 genes	5.6% (non-*BRCA1/2*)	Not reported	[29]
Kurian *et al.* J Clin Oncol, 2014	198	Met practice guidelines criteria for *BRCA1/2* testing (most *BRCA1/2*-negative)	70% non-Hispanic White, 20% Asian American	Custom designed (42 genes)	11.4% (non-*BRCA1/2*)	88%	[33▪▪]
Ford *et al.* Montreal Hereditary Breast and Ovarian Cancer Symposium, 2014	380	Met practice guidelines criteria for *BRCA1/2* testing (extension of above study)	70% non-Hispanic White, 20% Asian American	Hereditary Cancer Panel (Invitae, 29 genes)	9% (non-*BRCA1/2*)	35%	[32]
LaDuca *et al.* Genet Med, 2014	2079	Testing laboratory database (Ambry)	72% non-Hispanic White, 3% African American, 2% Hispanic	BreastNext, CancerNext, ColoNext, OvaNext (Ambry, 13–24 genes)	7–10% (non-*BRCA1/2*)	15–25%	[35▪]
Maxwell *et al.* American Society of Clinical Oncology Annual Meeting, 2014	278	Breast cancer at age<40, *BRCA1/2*-negative	69% non-Hispanic White, 24% African American	Custom designed (22 genes)	11% (non-*BRCA1/2*)	19%	[31]
Langer *et al.* American Society of Clinical Oncology Annual Meeting, 2014	648	Ovarian cancer patients in laboratory database (Myriad Genetics)	Not reported	MyRisk (Myriad, 25 genes)	6.2% (non-*BRCA1/2*)	40.6%	[36]
Summary	8858	Mostly high-risk, clinical referral samples	Primarily non-Hispanic White, with some exceptions	13–42 genes, overlapping across panels	4–16% (non-*BRCA1/2*)	15–88%	Cited above

pathogenic mutations in genes other than *BRCA1/2* ranges from 4 to 16%, and the prevalence of VUS ranges from 15 to 88%. Notably, VUS rates for multiple-gene panels have fallen quickly over a short period, dropping from 88–97% to 35–42% in subsequent updates of previously reported studies [32,33▪▪,37▪▪,38]. This finding reflects rapid progress in VUS classification algorithms as data accumulate regarding the sequences of less familiar genes. Nonetheless, even the lower end of multiple-gene panel VUS rates substantially exceeds VUS rates with testing *BRCA1/2* only [14]. As observed with *BRCA1/2* testing, multiple-gene panel VUS rates are significantly higher among racial/ethnic minorities than NH Whites, evincing a persistent health disparity that must be addressed [14,31].

Few studies to date have investigated the clinical outcomes of multiple-gene panel testing. We recently reported on the disclosure of multiple-gene sequencing results and the early outcomes of result-targeted screening and prevention strategies among 198 women referred for clinical *BRCA1/2* testing. All patients who were invited to receive their results of multiple-gene panel sequencing, which was conducted on a research protocol such that disclosure required recontacting and reconsenting of study participants, chose to do so. Initial risk-adapted interventions among 14 identified carriers of non-*BRCA1/2* mutations included a screening colonoscopy that detected and removed a tubular adenoma, and thus probably prevented a cancer [33▪▪]. These results suggest that multiple-gene panel testing may benefit appropriately selected patients, and larger, longer-term studies of clinical utility are a high priority.

GENETIC RESULTS COMMUNICATION AND COUNSELING

The emergence of NGS into clinical practice poses fundamental challenges to the existing process of results disclosure and genetic counseling. For breast and gynecologic risk assessment, the paradigm has shifted rapidly from a discussion of two genes (*BRCA1/2*) whose prevalence, penetrance, and management options have been extensively studied for 20 years, to panels of at least 15 genes whose prevalence, penetrance, and clinical implications are poorly understood [20▪,39,40,41▪,42▪]. Three degrees of uncertainty complicate multiple-gene panel testing that include the following: first, uncertainty about whether variants in clinically unfamiliar genes are deleterious or benign (given the high VUS rate); second, uncertainty about the magnitude of cancer risk conferred by a pathogenic variant in a clinically unfamiliar gene; and third, uncertainty about the scope of cancer risk (in terms of organ sites affected and histologic subtypes) conferred by a pathogenic variant in a clinically unfamiliar gene. A particularly thorny question is the residual cancer risk of a patient who tests 'true negative' for an identified familial mutation in a clinically unfamiliar gene. Large population-based studies have demonstrated that women who did not inherit their parents' *BRCA1/2* mutation have no increase over the average woman's breast cancer risk [43]; by contrast, we currently lack such reassuring data for noncarriers of familial mutations in other genes (e.g., *CHEK2*), and this uncertainty greatly limits the value of a 'true-negative' test result. Another challenging problem is how best to counsel patients who lack suggestive family cancer history, yet are found unexpectedly on multiple-gene panel testing to carry a high-risk gene mutation (e.g., *CDH1*). We do not know whether mutation carriers from such nonsyndromic families have the very high cancer risks that have previously been reported, nor whether they benefit from targeted risk-reducing procedures (e.g., prophylactic gastrectomy for *CDH1* mutation carriers) [33▪▪]. Furthermore, there is a major concern that patients, incorrectly believing a VUS to confer high cancer risk, will undertake invasive and irreversible prophylactic surgeries. This caveat is enhanced by evidence of rising bilateral mastectomy rates for breast cancer treatment in the last decade, in the absence of any survival benefit [44–46]. Moreover, a recent celebrity disclosure of *BRCA1* mutation carriage and prophylactic mastectomy has precipitated public demand for genetic testing [47–49], a phenomenon that compounds the danger of excessive intervention.

Given the increasing scope and complexity of NGS panels, it is strongly recommended that testing be conducted and interpreted by experts in cancer genetics [11▪▪,18,20▪,40]. Debate continues among insurers and professional societies as to the credentials and/or experience that practitioners must have to deliver high-quality care. Certified genetic counselors represent the gold standard for the practice of clinical cancer genetics, and training programs must expand to ensure a future workforce that is sufficient to meet patients' needs.

RESEARCH PRIORITIES

As discussed above, the clinical emergence of multiple-gene sequencing panels has generated more questions than answers to date. Major research priorities include the following:

(1) Which genes, and how many, should be included on breast and gynecologic cancer panels?

(2) Do WES/WGS add meaningfully to the results of cancer-focused gene panels?
(3) How can VUS be reclassified into pathogenic or benign status more rapidly?
(4) What is the penetrance of mutations in unfamiliar genes, and how much does penetrance vary?
(5) Do the prevalence and penetrance of mutations vary across race/ethnicity?
(6) What is the residual cancer risk for patients who test 'true negative' for identified familial mutations in clinically unfamiliar genes?
(7) How can results be most effectively communicated by practitioners to patients?
(8) What is the impact of multiple-gene panel testing on:
    (a) utilization of prevention and screening interventions?
    (b) clinical yield of prevention and screening interventions?
    (c) cost and cost-effectiveness of genetic testing and risk-targeted interventions?
    (d) patients' and their family members' quality of life?
    (e) cancer-specific and overall survival?

International consortia will be required to answer these pressing questions; the existing Evidence-based Network for the Interpretation of Germline Mutant Alleles (ENIGMA, http://enigmaconsortium.org/) and the new Prospective Registry for Outcomes of MultiPlex Testing (PROMPT, http://www.promptstudy.org) exemplify two such efforts.

CONCLUSION

Next-generation germline sequencing panels for breast and gynecologic cancer risk assessment are increasingly used in clinic practice. Studies report a 4–16% prevalence of non-*BRCA1/2* mutations in representative patient samples, along with early evidence of a clinical benefit from multiple-gene sequencing panels for appropriately selected patients. The uncertainty surrounding gene panel selection and results interpretation render cancer genetics expertise indispensable for good patient care. The many unknowns in this emerging field chart an exciting research agenda that will transform both our knowledge and our practice of clinical cancer risk assessment.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:
■ of special interest
■■ of outstanding interest

1. 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.
2. Wooster R, Neuhausen SL, Mangion J, *et al.* Localization of a breast cancer susceptibility gene, *BRCA2*, to chromosome *13q12-13*. Science 1994; 265:2088–2090.
3. 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; 62:676–689.
4. King MC, Marks JH, Mandell JB; New York Breast Cancer Study Group. Breast and ovarian cancer risks due to inherited mutations in *BRCA1* and *BRCA2*. Science 2003; 302:643–646.
5. 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; 72:1117–1130.
6. Chen S, Parmigiani G. Meta-analysis of *BRCA1* and *BRCA2* penetrance. J Clin Oncol 2007; 25:1329–1333.
7. Domchek SM, Freibel TM, Singer CF, *et al.* Association of risk-reducing surgery in *BRCA1* or *BRCA2* mutation carriers with cancer risk and mortality. JAMA 2010; 304:967–975.
8. Kurian AW, Sigal BM, Plevritis SK. Survival analysis of cancer risk reduction strategies for *BRCA1/2* mutation carriers. J Clin Oncol 2010; 28:222–231.
9. Rebbeck TR, Kauff ND, Domchek SM. Meta-analysis of risk reduction estimates associated with risk-reducing salpingo-oophorectomy in BRCA1 or BRCA2 mutation carriers. J Natl Cancer Inst 2009; 26:1331–1337.
10. Culver JO, MacDonald DJ, Thornton AA, *et al.* Development and evaluation of a decision aid for *BRCA* carriers with breast cancer. J Genet Couns 2011; 20:294–307.
11. ■■ Daly MB, Pilarski R, Axilbund JE, *et al.* Genetic/familial high-risk assessment: breast and ovarian, version 1.2014. J Natl Compr Canc Netw 2014; 12:1326–1338.
These are the current evidence-based practice guidelines of the National Comprehensive Cancer Network for breast and gynecologic cancer risk assessment.
12. Kurian AW, Munoz DF, Rust P, *et al.* Online tool to guide decisions for *BRCA1/2* mutation carriers. J Clin Oncol 2012; 30:497–506.
13. van Roosmalen MS, Stalmeier PF, Verhoef LC, *et al.* Randomized trial of a shared decision-making intervention consisting of trade-offs and individualized treatment information for BRCA1/2 mutation carriers. J Clin Oncol 2004; 22:3293–3301.
14. 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; 115:2222–2233.
15. Greely HT. Banning genetic discrimination. N Engl J Med 2005; 353:865–867.
16. Laedtke AL, O'Neill SM, Rubinstein WS, Vogel KJ. Family physicians' awareness and knowledge of the Genetic Information Non-Discrimination Act (GINA). J Genet Couns 2012; 21:345–352.
17. Gabai-Kapara E, Lahad A, Kaufman B, *et al.* Population-based screening for breast and ovarian cancer risk due to *BRCA1* and *BRCA2*. Proc Natl Acad Sci U S A 2014; 111:14205–14210.
18. Hall MJ, Forman AD, Pilarski R, *et al.* Gene panel testing for inherited cancer risk. J Natl Compr Canc Netw 2014; 12:1339–1346.
19. ■■ Stadler ZK, Schrader KA, Vijai J, *et al.* Cancer genomics and inherited risk. J Clin Oncol 2014; 32:687–698.
This is a comprehensive review of new genomic technologies and their role in cancer risk assessment.
20. ■ Domchek SM, Bradbury A, Garber JE, *et al.* Multiplex genetic testing for cancer susceptibility: out on the high wire without a net? J Clin Oncol 2013; 31:1267–1270.
This is an editorial by leaders in the field of clinical cancer genetics that clearly discusses the challenges of incorporating NGS into the clinic.

21. Walsh T, Casadei S, Lee MK, *et al.* Mutations in 12 genes for inherited ovarian, fallopian tube, and peritoneal carcinoma identified by massively parallel sequencing. Proc Natl Acad Sci U S A 2011; 108:18032–18037.

22. ■ 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.

This is an article that reports on the high breast cancer risks associated with mutations in the *PALB2* gene.

23. Bartkova J, Tommiksa 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.

24. Win AK, Lindor NM, Young JP, *et al.* Risks of primary extracolonic cancers following colorectal cancer in Lynch syndrome. J Natl Cancer Inst 2012; 104:1363–1372.

25. Daly MB, Axilbund JE, Buys S, *et al.* Genetic/familial high-risk assessment: breast and ovarian. J Natl Compr Canc Netw 2010; 8:562–594.

26. 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; 31:2743–2748.

27. Hooker GW, Leventhal KD, DeMarco T, *et al.* Cancer genetic counseling and testing in an era of rapid change [abstract]. Presented at the American College of Medical Genetics Annual Meeting; March 2014; Nashville, TN.

28. Harrell M, Norquist B, Walsh T, *et al.* Germline loss-of-function mutations in 15 different DNA repair genes are present in 22% of 1412 patients with ovarian, peritoneal or fallopian tube cancers not selected for age at diagnosis or family history [abstract]. Presented at the American Society of Human Genetics Annual Meeting; October 2013; Boston, MA.

29. 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; 22:1305–1313.

30. Walsh T, Casadei S, Lee MK, *et al.* More than 25% of breast cancer families with wild-type results from commercial genetic testing of BRCA1 and BRCA2 are resolved by BROCA sequencing of all known breast cancer genes [abstract]. Presented at the American Society of Human Genetics Annual Meeting; 2013; Boston, MA.

31. Maxwell K, Wubbenhorst B, Garman B, *et al.* Prevalence of mutations in a panel of breast cancer susceptibility genes in patients with early onset breast cancer [abstract]. Presented at the American Society of Clinical Oncology Annual Meeting; June 2014; Chicago, IL.

32. Ford JM, Kurian AW, Kobayashi Y, *et al.* Clinical impact of a multiple gene sequencing panel for hereditary breast cancer families [abstract]. Presented at the Montreal Hereditary, Breast and Ovarian Cancer Symposium; April 2014; Montreal, QC, Canada.

33. ■■ 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; 32:2001–2009.

This is the first study of the early clinical impact of NGS for breast and gynecologic cancer risk assessment.

34. Olopade O, Walsh T, Zheng Y, *et al.* Nine genes for inherited predisposition to breast cancer among African-American women [abstract]. Presented at the American Society of Human Genetics Annual Meeting; October 2013; Boston, MA.

35. ■ Laduca H, Stuenkel AJ, Dolinsky JS, *et al.* Utilization of multigene panels in hereditary cancer predisposition testing: analysis of more than 2000 patients. Genet Med 2014; 16:830–837.

This is a large study of the results of NGS panel testing, derived from the database of a genetic testing laboratory.

36. Langer L, McCoy H, Moyes K, *et al.* A study of ovarian cancer patients tested with a 25-gene panel of hereditary cancer genes [abstract]. Presented at the American Society of Clinical Oncology Annual Meeting; June 2014; Chicago, Illinois.

37. ■■ 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 2014; doi: 10.1002/cncr.29010. [Epub ahead of print]

This is a recent large study of multiple-gene sequencing panel testing for breast cancer risk assessment in a clinically relevant population.

38. Tung N, Batelli C, Allen B, *et al.* Prevalence of gene mutations among hereditary breast and ovarian cancer patients using a 25 gene panel [abstract]. Presented at the San Antonio Breast Cancer Symposium; December 2013; San Antonio, TX.

39. Bradbury AR, Patrick-Miller L, Domchek S. Multiplex genetic testing: reconsidering utility and informed consent in the era of next-generation sequencing. Genet Med 2014; doi: 10.1038/gim.2014.85. [Epub ahead of print]

40. Domchek SM, Nathanson KL. Panel testing for inherited susceptibility to breast, ovarian, and colorectal cancer. Genet Med 2014; 16:827–829.

41. ■ Fecteau H, Vogel KJ, Hanson K, Morrill-Cornelius S. The evolution of cancer risk assessment in the era of next generation sequencing. J Genet Couns 2014; 23:633–639.

This is an informative study that outlines the changes in genetic risk assessment required by new NGS approaches.

42. ■ Rainville IR, Rana HQ. Next-generation sequencing for inherited breast cancer risk: counseling through the complexity. Curr Oncol Rep 2014; 16:371.

This is a review and perspective article on the experience of genetic counseling in the era of clinical NGS.

43. Kurian AW, Gong GD, John EM, *et al.* Breast cancer risk for noncarriers of family-specific BRCA1 and BRCA2 mutations: findings from the Breast Cancer Family Registry. J Clin Oncol 2011; 29:4505–4509.

44. Agarwal S, Pappas L, Neumayer L, *et al.* Effect of breast conservation therapy vs mastectomy on disease-specific survival for early-stage breast cancer. JAMA Surg 2014; 149:267–274.

45. Kurian AW, Lichtensztajn DY, Keegan TH, *et al.* Use of and mortality after bilateral mastectomy compared with other surgical treatments for breast cancer in California, 1998–2011. JAMA 2014; 312:902–914.

46. Tuttle TM, Habermann EB, Grund EH, *et al.* Increasing use of contralateral prophylactic mastectomy for breast cancer patients: a trend toward more aggressive surgical treatment. J Clin Oncol 2007; 25:5203–5209.

47. Borzekowski DL, Guan Y, Smith KC, *et al.* The Angelina effect: immediate reach, grasp, and impact of going public. Genet Med 2014; 16:516–521.

48. James PA, Mitchell G, Bogwitz M, Lindeman GJ. The Angelina Jolie effect. Med J Aust 2013; 199:646.

49. Kamenova K, Reshef A, Caulfield T. Angelina Jolie's faulty gene: newspaper coverage of a celebrity's preventive bilateral mastectomy in Canada, the United States, and the United Kingdom. Genet Med 2014; 16:522–528.