special article

Genetic Testing and Clinical Management Practices for Variants in Non-*BRCA1/2* Breast (and Breast/Ovarian) Cancer Susceptibility Genes: An International Survey by the Evidence-Based Network for the Interpretation of Germline Mutant Alleles (ENIGMA) Clinical Working Group

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executive summary

Purpose To describe a snapshot of international genetic testing practices, specifically regarding the use of multigene panels, for hereditary breast/ovarian cancers. We conducted a survey through the Evidence-Based Network for the Interpretation of Germline Mutant Alleles (ENIGMA) consortium, covering questions about 16 non-*BRCA1/2* genes.

Methods Data were collected via in-person and paper/electronic surveys. ENIGMA members from around the world were invited to participate. Additional information was collected via country networks in the United Kingdom and in Italy.

Results Responses from 61 cancer genetics practices across 20 countries showed that 16 genes were tested by > 50% of the centers, but only six (*PALB2*, *TP53*, *PTEN*, *CHEK2*, *ATM*, and *BRIP1*) were tested regularly. US centers tested the genes most often, whereas United Kingdom and Italian centers with no direct ENIGMA affiliation at the time of the survey were the least likely to regularly test them. Most centers tested the 16 genes through multigene panels; some centers tested *TP53*, *PTEN*, and other cancer syndrome–associated genes individually. Most centers reported (likely) pathogenic variants to patients and would test family members for such variants. Gene-specific guidelines for breast and ovarian cancer risk management were limited and differed among countries, especially with regard to starting age and type of imaging and risk-reducing surgery recommendations.

Conclusion Currently, a small number of genes beyond *BRCA1/2* are routinely analyzed worldwide, and management guidelines are limited and largely based on expert opinion. To attain clinical implementation of multigene panel testing through evidence-based management practices, it is paramount that clinicians (and patients) participate in international initiatives that share panel testing data, interpret sequence variants, and collect prospective data to underpin risk estimates and evaluate the outcome of risk intervention strategies.

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INTRODUCTION

Massively parallel sequencing technologies have transformed testing practices for hereditary breast cancer (BC) and breast and ovarian cancer (BOC) predisposition. Currently, several multigene panels are available that include from < 10 to > 100 known or candidate cancer susceptibility genes, which are tested for diagnostic or research purposes. Some panels are targeted at diverse cancers (pan-cancer panels), whereas others target specific cancers only (disease-specific panels).

The ability to run multigene panels at affordable prices has expanded the eligibility criteria and increased the demand for testing.[1-5] However, the rapid pace at which candidate risk genes are moving from research based to clinical diagnostic testing has its drawbacks. Consequently, diagnostic laboratories are making inferences and clinicians are making decisions based on limited data. The rate of variants of uncertain significance (VUS) has increased proportionally to the extent of the sequenced genome.[5-7] Moreover, many genes currently included on multigene panels have imprecise cancer risk estimates, and there is no consensus on when to test for a given gene or how to manage a reported (likely) pathogenic variant.[8,9]

The aim of this study was to describe a snapshot of the landscape of international genetic testing practices and risk management approaches for BC and BOC susceptibility genes beyond *BRCA1* and *BRCA2*. A survey was conducted among members of the Evidence-Based Network for the Interpretation of Germline Mutant Alleles (ENIGMA), an international consortium focused on determining the clinical significance of variants in *BRCA1*, *BRCA2*, and other (ascertained or suspected) BC and BOC susceptibility genes, providing expertise to global database and classification initiatives, and exploring optimal avenues of communication of such information at the provider and patient levels. Additional information was collected via country networks in the United Kingdom and in Italy, from centers that were not directly involved in ENIGMA research at the time of study initiation.

In total, respondents represented cancer genetic experts from 61 centers across 20 countries. To our knowledge, this is the first study to describe international testing practices and risk management guidelines for non-*BRCA1/2* genes implicated in BC and BOC susceptibility.

METHODS

This study was submitted for approval to the ethics committees of the two coordinating sites, the University of Chicago and Maastricht University. Both concluded that review by the institutional review board/official committee approval was not required, because the study was determined to be nonhuman subject research. A survey about genetic testing practices for non-*BRCA1/2* BC and BOC susceptibility genes was developed by ENIGMA Clinical Working Group (CWG) leaders during 2016 (Appendix Table A1). ENIGMA members were invited to complete the survey if they had a clinical genetic testing or diagnostic laboratory affiliation and were involved in ordering, performing, or interpreting DNA tests for inherited susceptibility to BC/BOC at their center. An ENIGMA member is currently defined as a researcher or research group (consortium) who is willing to work collaboratively toward classification of variants by contributing data from families and/or conducting statistical analysis or laboratory-based assays within a working group framework. There is no requirement for ENIGMA members to state their primary role (clinician, genetic counselor, laboratory scientist, basic researcher), but all members by definition have a research interest in the topic of gene/variant classification.

Individuals from the same center could work on the survey together or choose a designated representative to complete it, so only one survey per center was counted. Specific questions were asked about 16 BC/BOC genes with published evidence of risk association commonly included on commercial BC panels at the time of the survey: *ATM*, *BARD1*, *BRIP1*, *CDH1*, *CHEK2*, *MRE11A*, *NBN*, *NF1*, *PALB2*, *PTEN*, *RAD50*, *RAD51C*, *RAD51D*, *STK11*, *TP53*, and *MEN1* (which is considered a [candidate] BC susceptibility gene in the Netherlands[10]).

Information about testing and management approaches at individual sites, formulated as multiple-choice questions with a discrete number of options, was obtained through both in-person surveys (during conference session) and paper/electronic surveys, which included additional open-ended questions (Appendix Table A1).

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The survey process is outlined in Figure 1. In brief, an in-person survey of members of the CWG, consisting mainly of laboratory and clinical scientists from academic centers, was conducted during the ENIGMA consortium meeting in Limassol, Cyprus, in January 2017. A total of 30 centers from 17 countries participated.

A more detailed version of the survey was then distributed by e-mail (paper/electronic survey) to the same 30 centers that participated in the in-person survey and to additional ENIGMA-affiliated centers worldwide. This allowed collection of information from an additional eight centers and three countries.

Both in-person and paper/electronic survey data were reviewed for consistency and completeness. Participants were sent a copy of their answers and asked to verify them or to clarify any discrepancies.

Notably, in Italy and in the United Kingdom, the paper/electronic version of the survey was also distributed, via country networks, to centers that were not actively involved in ENIGMA research. This provided the opportunity to carry out additional subanalyses (ENIGMA *v* non-ENIGMA; described in Results). In Italy, all submissions were coordinated by A.D.N., as a liaison for the Network of Italian Collaborators to ENIGMA Studies and Trials. The effort comprised both the ENIGMA-affiliated Fondazione Istituto di Ricovero e Cura a Carattere Scientifico Istituto Nazionale dei Tumori (Milan) and the Santa Chiara University Hospital (Pisa), which were counted among the 38 participating ENIGMA centers, and 14 additional centers, which were not directly affiliated with ENIGMA at the time of the survey (henceforth referred to as non-ENIGMA; Fig 1, lower right). Of the 14 Italian non-ENIGMA centers, five were dedicated to diagnostic testing only, and nine were dedicated to both diagnostics and research; moreover, half of them were university affiliated, and half were not.

Similarly, in the United Kingdom, D.M.E. completed the survey for her own ENIGMA-affiliated center (one of the 38 participating ENIGMA centers) and also coordinated, with the assistance of Y.W., the distribution of the survey through SurveyMonkey via the Association for Clinical Genetic Science mailing list to cancer genetic leads from diagnostic laboratories providing genetic testing for the publicly funded National Health Service (NHS). The original ENIGMA survey was modified to encompass questions that were considered most relevant to NHS laboratories (Appendix Table A1, far-right column). Nine laboratories responded (anonymously), representing approximately half of the active NHS laboratories in the United Kingdom (also henceforth referred to as non-ENIGMA; Fig 1, lower left).

Comparisons were made between individual centers, US and non-US ENIGMA centers, and ENIGMA and non-ENIGMA centers.

RESULTS

In total, 61 centers from 20 countries participated in the survey. The recruitment flowchart and the global distribution of participants are illustrated in Figure 1.

Clinical Utility

To get a preliminary idea of the participants' opinions about the clinical utility of the 16 genes on which the survey focused, the CWG members present at the 2017 ENIGMA meeting in Cyprus were asked to answer the following questions relating to each of them: Should every patient with BC/OC who qualifies for (*BRCA1/2)* genetic testing (by criteria that we recognize may differ by country/center) be tested for the gene? and Do you agree that the cancer risk associated with (pathogenic variants in) the gene is high enough to inform clinical management? All participants (n = 23 at this specific session) stated that they would test every qualifying patient with BC (as defined in the question) for *PALB2* and every qualifying patient with OC (as defined) for *BRIP1*, *RAD51C*, and *RAD51D*. No participant stated that he or she would test every qualifying patient with BC for *NBN*, *MRE11A*, or *RAD50*. Results for the other nine genes were variable (Appendix Fig A1A).

With regard to clinical management, all participants agreed that *PALB2*, *TP53*, *CDH1*, *PTEN*, and *STK11* along with *BRIP1*, *RAD51C*, and *RAD51D* were associated with high enough (BC or OC) risk to alter clinical management. Many participants felt that the risk associated with *CHEK2* and *ATM* pathogenic variants could also alter clinical management. *NF1*, *BARD1*, *MEN1*, *MRE11A*, *NBN*, and *RAD50* were deemed by

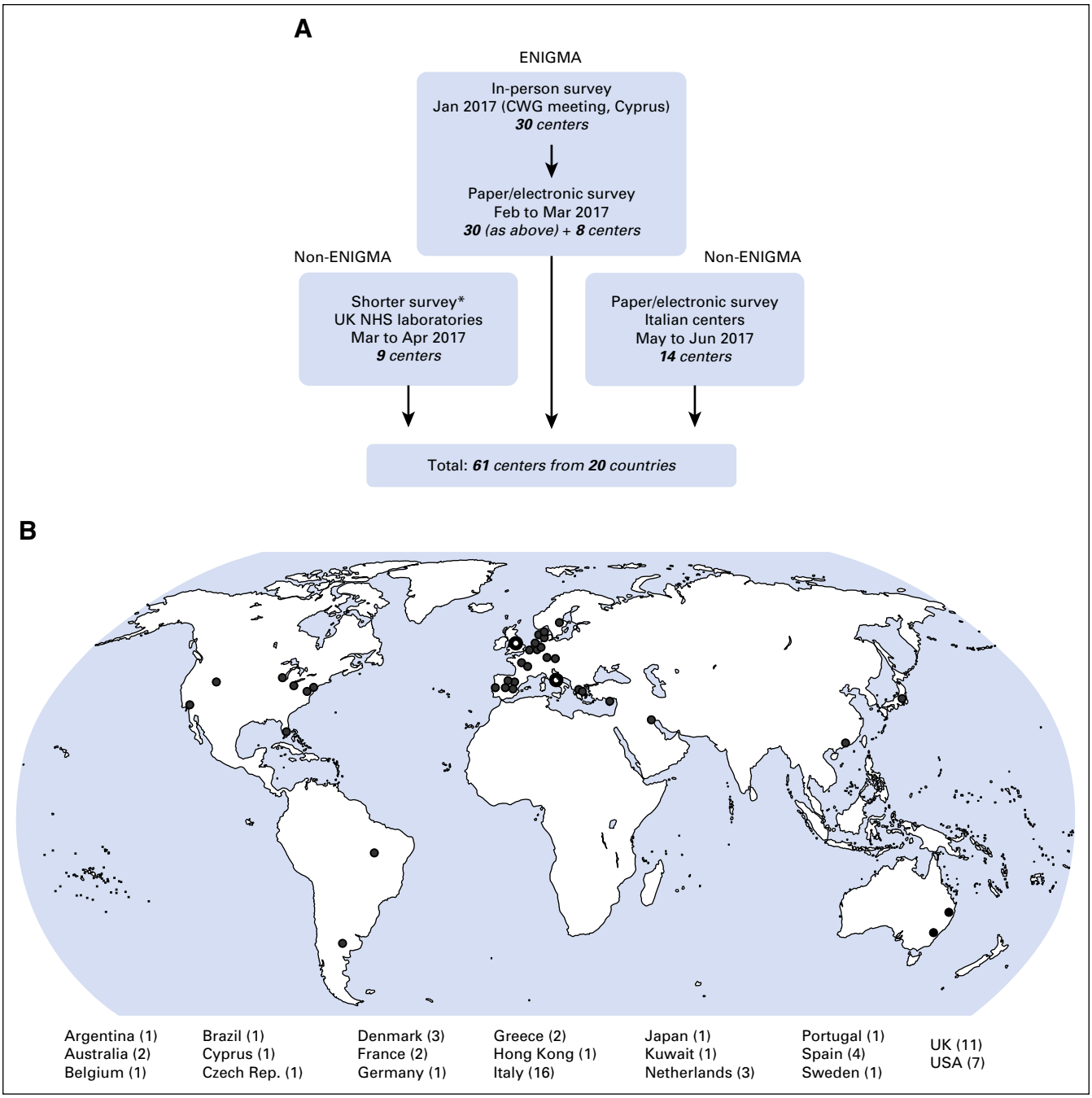

Fig 1. Survey distribution flow and global representation of participating centers. CWG, Clinical Working Group; ENIGMA, Evidence-Based Network for the Interpretation of Germline Mutant Alleles; NHS, National Health Service. (*) Via SurveyMonkey.

most of the participants as genes that currently do not affect clinical management of BC risk (Appendix Fig A1B). Please note that 95% CIs for this figure and for all the following figures are provided in Appendix (Tables A2-A10).

Testing Practices

Participants were also asked (via in-person and/or paper/electronic surveys) if and how frequently they tested each gene, the method (single gene *v* gene panel) and purpose of testing (clinical *v* research), and the practices of reporting (likely) pathogenic variants and VUS to patients. The aggregate of the responses is presented here.

Purpose and setting. Figure 2 shows the absolute number and proportion of the ENIGMA centers that tested for a specified gene (for clinical or research purposes) and that tested the gene regularly (ie, ordered the test for > 50% of patients who qualified for genetic testing, by criteria that we recognize may differ by center/

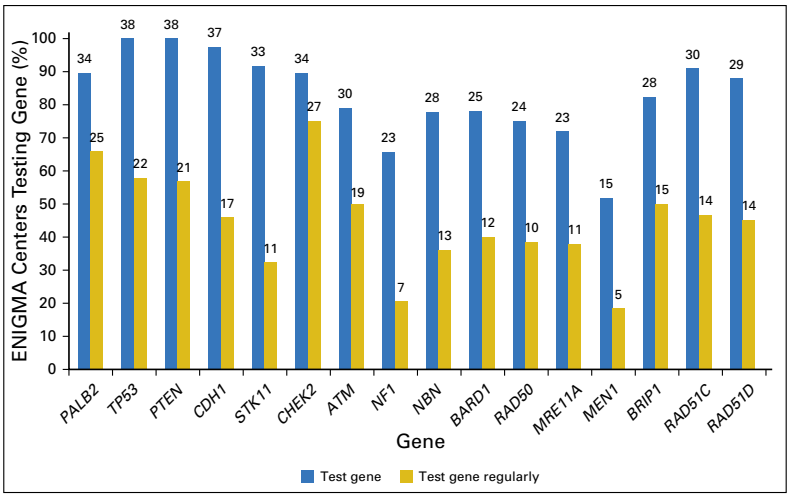

Fig 2. Frequency of testing. Absolute No. of centers testing given gene is shown above each bar. In total, there were 38 participating centers; however, the No. of centers that responded to the question varied by gene (range, 29 to 38 centers).Regularly was defined as ordered for > 50% of eligible patients (ie, those who qualified for genetic testing, by criteria that we recognize may differ by center/country). ENIGMA, Evidence-Based Network for the Interpretation of Germline Mutant Alleles.

country). Even though each gene was tested by > 50% of the centers (range, 52% to 100%), only *PALB2*, *TP53*, *PTEN*, *CHEK2*, *ATM*, and *BRIP1* were tested regularly by > 50% of centers.

Testing in a research setting in addition to the clinical setting was common for ENIGMA centers (Appendix Fig A2). The genes that were most frequently tested (ie, tested by at least > 30% of centers) for research purposes only were: *NBN*, *BARD1*, *RAD50*, and *MRE11A*. All the other genes were tested clinically by at least two thirds of the ENIGMA centers. No center tested *TP53* solely for research purposes.

Focusing only on clinical testing, a majority of ENIGMA centers used multigene panels (Fig 3). Single-gene testing was performed by a number of centers, varying from one to 21, for: *TP53*, *PTEN*, *CDH1*, *STK11*, *PALB2*, *CHEK2*, *NF1*, *ATM*, *MEN1*, and *NBN* (in decreasing order of frequency), often based on a specific phenotype (eg, *PTEN* hamartoma syndrome or neurofibromatosis type 1), or these genes were tested as a reflex only when *BRCA1/2* testing was noninformative. Notably, these methods were not mutually exclusive. Seven centers from four countries (Belgium, Brazil, the Netherlands, and Spain) testing *CHEK2* only tested for the 1100delC variant.

Regarding the types of gene panels used, US respondents typically ordered broad cancer panels from commercial laboratories, although the specific panels varied depending on patient preferences, insurance considerations, and clinical scenarios. The non-US ENIGMA centers used a combination of commercial and custom in-house panels.

The main issues that emerged regarding barriers to panel testing, among ENIGMA centers and non-ENIGMA Italian centers, were lack of knowledge of cancer risk/penetrance and of management guidelines (hence, lack of actionability); concerns about VUS; validation of testing method; and need for "robust, carefully curated, and constantly updated international databases" and for "global data sharing." Separately, the nine United Kingdom NHS laboratories were asked, "If you currently only report *BRCA* genes but might report broader panels in the future, what issues are major barriers/problems to overcome?" Responses were chosen from a menu of nine options plus "other," and the four main reasons selected (by half or more of respondents) were no request by the oncologists (of note, NHS oncologists can ask directly for *BRCA1* and *BRCA2* testing but not for multigene panels), lengthy and laborious process of variant interpretation, lack of standardization of reporting, and lack of demand for testing.

Reporting practices and cascade testing. For genes analyzed through clinical testing, > 90% of ENIGMA centers reported (likely) pathogenic variants to patients (for *CHEK2* and *NBN*, the percentages were slightly lower, at 88% and 71%, respectively; Fig 4). Some centers reported these variants only if the patient met criteria for the associated syndrome (eg, hereditary diffuse gastric cancer for *CDH1*, neurofibromatosis type 1 for *NF1*). Almost all centers (67% to 81% for *NBN*, *RAD50*, *MRE11A*, and *BARD1* and > 90% for the other genes) offered cascade testing to family members if a (likely) pathogenic variant was identified (data not shown). Notably, participants from the Netherlands reported that they only tested first-degree relatives for *CHEK2* 1100delC variant when the estimated risk based on family history was lower than the risk conferred by having the variant, so testing for the variant had clinical utility because it would change surveillance recommendations.[11]

A high percentage (50% to 82%) of ENIGMA centers reported VUS to patients (Fig 4). Most of these centers reported that they would not offer cascade testing for VUS unless it was in a research setting for cosegregation purposes to aid variant (re)classification (data not shown).

Variant Classification Systems

All respondents reported using the International Agency for Research on Cancer five-tier classification system,[12] and many also used American College of Medical Genetics and Genomics[13] classification criteria. Sources cited for (qualitative) variant classification were literature and public databases including ClinVar,[14] the Breast Cancer Information Core database,[15] and the Leiden Open Variant Database.[16] Respondents were also asked, "Who takes responsibility for interpreting the clinical significance of the variants identified?" This question was answered by 39 centers (including ENIGMA and non-ENIGMA centers) with the following responses: the clinical team (ie, a medical geneticist or oncologist specialized in genetics; n = 16), the laboratory team (n = 11), a combination of the two (n = 10), and a bioinformatic pipeline (n = 2).

Clinical Management Practices and Guidelines

Most ENIGMA centers (≥ 80%) had risk management guidelines for a majority of non-*BRCA1/2* genes considered reportable to patients (Fig 5). Exceptions were *BARD1*, *RAD50*, and *MRE11A*, for which ≤ 30% of centers had guidelines.

Although most ENIGMA centers reported having some type of management guidelines for all genes except *BARD1*, *RAD50*, and *MRE11A*, after review, only 10 of 20 countries had national guidelines for (some of) these genes (Table 1). Furthermore, in some countries (Denmark and Germany), the national guidelines were not gene specific (ie, they were broken down by high- and moderate-risk categories rather than by specific gene). Other guidelines were local (center or region specific) or international (meaning national guidelines from another country were used). Review of management guidelines disclosed both similarities and substantial differences in country-specific guidelines available for BC risk management according to gene (Table 1). Ten countries had national guidelines for high-risk cancer syndrome–associated genes such as *TP53*, *CDH1*, and *PTEN* (with the exception of Belgium not having guidelines for *CDH1*). National guidelines were limited for other BC genes considered clinically actionable, including *PALB2*. The primary differences between countries were the starting age and type of diagnostic imaging (mammography *v* magnetic resonance imaging [MRI] *v* sonography) and the policy on risk-reducing mastectomy. For instance, there was no consensus on the age to begin mammograms/MRI for carriers of pathogenic variants in *NF1*, *MEN1*, *PALB2* (age 25 *v* 30 years), or *TP53* (age 20 *v* 25 years). The United Kingdom guidelines differed from all others in that breast MRI was not the standard imaging technique for carriers of pathogenic variants in other gene carriers (except for *TP53*). Guidelines for risk-reducing mastectomy in carriers of *PALB2* pathogenic variants ranged among accepted (n = 1), consider depending on personal/family history (n = 5), and not enough evidence to recommend (n = 1). For *PTEN* and *CDH1*, the guidelines that commented on preventive surgery (four of the seven and five of the eight national guidelines, respectively) mentioned risk-reducing mastectomy as a possible option. There were no national management guidelines for *BARD1*, *RAD50*, or *MRE11A* pathogenic variant carriers, which is consistent with the indeterminate evidence for BC or OC risk associated with these genes.

For the OC susceptibility genes *BRIP1*, *RAD51C*, and *RAD51D*, the US-based National Comprehensive Cancer Network and the Dutch guidelines recommended risk-reducing salpingo-oophorectomy (RRSO) from age 45 to 50 years; RRSO was recommended only for *RAD51C* and *RAD51D* by the German Hereditary Breast and Ovarian Cancer Consortium. Before RRSO, the Czech Republic guidelines also advised sonography starting from age 30 years.

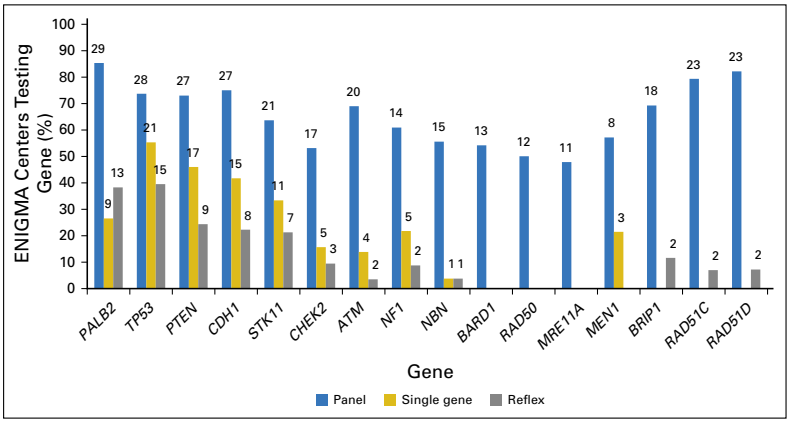

Fig 3. Clinical testing methods. Absolute No. of centers testing given gene through each method is shown above each bar. Only responses from those centers that reported they tested each gene were counted in the total, and the No. of centers that responded varied by gene (range, 14 to 38 centers). The three methods are not mutually exclusive; notably, the center in Kuwait performs whole-genome sequencing for all cases, which is not represented in the figure. ENIGMA, Evidence-Based Network for the Interpretation of Germline Mutant Alleles.

Subanalyses: ENIGMA-US Versus ENIGMA-Other Centers and Versus Non-ENIGMA Centers

Responses from the seven ENIGMA centers in the United States (ENIGMA-US) were compared with those of the other 31 ENIGMA centers (ENIGMA-other). In addition, responses from 14 non-ENIGMA centers in Italy and nine non-ENIGMA laboratories in the United Kingdom were compared with those from 38 ENIGMA centers across all countries.

Results of these comparisons are summarized in Appendix Figs A3 and A4. Briefly, the ENIGMA-US centers were more likely to regularly test all genes, particularly through multigene panels, compared with ENIGMA-other centers (Appendix Figs A3 and A4). A much smaller proportion of non-ENIGMA centers from Italy and the United Kingdom tested each gene compared with ENIGMA-affiliated centers (Appendix Fig A3).

Management guidelines were more likely to be available in the US-based ENIGMA centers compared with the other ENIGMA centers for all genes except *BARD1*, *RAD50*, *MRE11A*, and *MEN1*. Only a small proportion of the Italian and United Kingdom non-ENIGMA centers had management guidelines for the 16 genes. Non-ENIGMA United Kingdom centers reported guidelines to be available for *TP53* (71% of centers) and *CHEK2* (14%), whereas the non-ENIGMA Italian centers reported available guidelines for *PALB2* (19% of centers), *TP53* (50%), *PTEN* (19%), *CDH1* (38%), *STK11* (19%), *CHEK2* (13%), and *ATM* (6%).

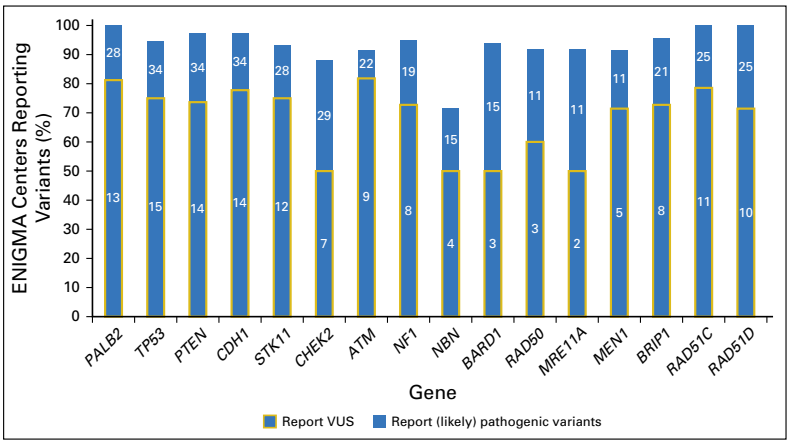

Fig 4. Reporting practices of (likely) pathogenic variants and variants of unknown significance (VUS; to patients). Absolute No. of centers reporting variants to patients is shown within each bar. Only responses from those centers that reported they clinically tested the given gene were counted in the total, and the No. of centers that responded varied by gene (range, 12 to 36 centers responding about reporting pathogenic variants; range, four to 20 centers responding about reporting VUS). ENIGMA, Evidence-Based Network for the Interpretation of Germline Mutant Alleles.

DISCUSSION

We surveyed a total of 61 cancer genetic centers across 20 countries asking about their genetic testing and management practices relating to 16 BC and BOC predisposition genes. Our global survey demonstrated that only a few genes are routinely analyzed beyond *BRCA1/2*; most centers clinically test them through multigene panels and report (likely) pathogenic variants (and VUS, to a slightly lesser extent) to patients; and gene-specific guidelines for BC and OC risk management are limited and differ between countries, especially in regard to starting age and type of imaging and risk-reducing surgery recommendations.

With falling costs of sequencing and more genes being identified that are associated with increased BC and BOC risk, multigene (panel) testing is becoming the norm. The results of our survey confirm this trend, showing that genes that are commonly offered on commercial panels were tested by > 50% of the surveyed centers.

Nevertheless, the value of multigene panel testing continues to be debated in the context of three main areas: limited additional yield of pathogenic variants in genes other than *BRCA1/2* coupled with significantly increased interpretation workload, reliability of penetrance estimates for moderate- or uncertain-risk genes (clinical validity), and evidence for informing management recommendations to improve patient outcomes (clinical utility).[9] Our international survey demonstrates that the use of panel testing varies widely among countries. US centers were early adopters of multigene testing, which is generally ordered more liberally (if insurance criteria are met), with broader gene panels. Moreover, differences were observed when comparing ENIGMA-affiliated centers with non-ENIGMA Italian and United Kingdom centers (with the latter testing non-*BRCA1/2* genes less than one third of the time). Conceivably, because ENIGMA is a research consortium, centers that are ENIGMA members are more involved in research and might become aware of, and hence implement, novel technologies before they become mainstream. Conversely, national/universal health service providers may require a higher threshold of benefit before adopting new tests.

The insufficient evidence in support of clinical validity and/or utility (hence, actionability) of

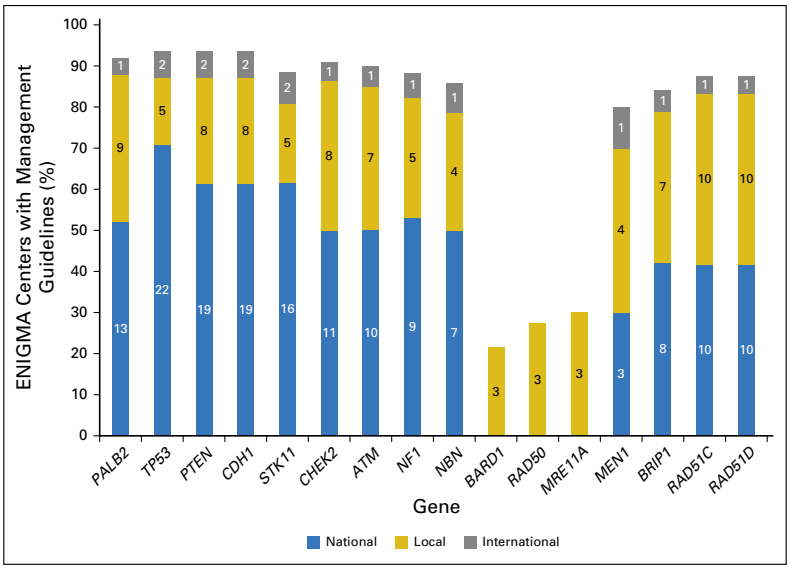

Fig 5. Sources of the management guidelines used by the Evidence-Based Network for the Interpretation of Germline Mutant Alleles (ENIGMA) centers. Absolute No. of centers reporting existing management guidelines for each gene is shown within each bar. Only responses from centers that reported they performed clinical testing and reported (likely) pathogenic variants to patients were counted in the total, and the No. of centers that responded varied by gene (range, 10 to 31 centers). If management guidelines were available, centers were asked to specify the source of such guidelines (local, national, or international, such as National Comprehensive Cancer Network or National Institute for Health and Care Excellence).

the genes included on panels was the most common concern raised by the participating centers. Easton et al[8] asserted that "a genomic test should not be offered until its clinical validity is established"[8(p2)]; however, the utility of a gene needs to be continuously reconsidered as more data become available, and this can only be done by analyzing results from large cohorts of individuals who have been tested. Concerns about the rates of VUS were frequently expressed by the study participants, but just as variant rates have significantly decreased over the years for *BRCA1/2* as a result of concerted classification efforts, the same trend will likely occur for other susceptibility genes, arguably at a faster pace as (and provided that) more laboratories worldwide contribute their testing data to population and peer-reviewed databases.[5,28,29] Despite the establishment of such databases, survey participants felt that "robust, constantly updated international databases" and "global data sharing" are still lacking. They also expressed the need for robust software that could help with annotation and real-time classification of each variant. This is a worthy goal, but expert judgment in variant classification methods is still required, because fully automated approaches to variant classification that apply guidelines are not ready for clinical practice.[6]

At a basic level, some centers reported validation of the testing method as a barrier. Therefore, it is important to recognize the technologic barriers in certain countries, although the transition to massively parallel sequencing is ultimately expected to increase throughput and optimize diagnosis without significantly elevating costs.[30]

There were also nonmedical barriers to implementing routine testing of many of these surveyed genes. Insurance can be a major barrier in the United States, where, for example, Medicare (a US federal health insurance program for people who are age ≥ 65 years and for certain younger people with disabilities) will only cover testing for individuals with a BC or OC diagnosis, and many insurers will not cover multigene panel testing if the patient has already had prior genetic testing. Confounding matters, direct-to-consumer testing is becoming increasingly common in the United States. In many other countries, particularly those with national (ie, universal) health care, testing is approved on a gene-by-gene basis or as a package if research-derived evidence is considered robust enough to change clinical management.

In terms of risk magnitudes, *PALB2* and *TP53* are the only BC genes, in addition to *BRCA1/2*, that consistently fall into the high-risk category across studies (ie, confer levels of risk greater than four times that in the general population)[8]; the remainder have conflicting evidence regarding the risk category into which they fit.[8,9,31-33] Our survey confirmed that ENIGMA centers test *PALB2* and *TP53* relatively frequently and regard them as clinically actionable genes. These two genes were tested much less consistently by non-ENIGMA centers, evidencing the lack of consensus, even for genes that are generally regarded as high risk. These differences in testing approaches may be, however, more directly linked to how health care is paid (ie, if certain genes have been approved or not for testing through the national/universal health care system).

Large-scale studies have become recently available that address the penetrance of moderate-risk (ie, two to four times the risk compared with the general population) BC/BOC genes and the risk magnitudes of the genes included in multigene panels.[8,9,31,32] These studies are providing a broader perspective of risk, particularly for genes like *CHEK2* or *NBN*, for which previous risk estimates were based primarily on studies of founder variants only.[8] However, most of these studies are based on predominantly white European populations, and therefore, the evidence may not be generalizable.

Table 1. National Guidelines for BC Management

Gene/ Management	United States[17]	Czech Republic[18]	The Netherlands[11]	Australia[19]	France[20]	Spain[21]	Belgium[22,23]	United Kingdom[24]	Germany[25-27]	Denmark*
PALB2										
Surveillance	Annual mammogram and consider breast MRI with contrast from age 30 years	SBE every month from age 20-25 years	Age 30-60 years: annual breast MRI	Age 30-50 years: annual breast MRI and mammogram ± US	Age 30-65 years: annual MRI, mammogram, and US	Annual mammogram and MRI from age 30 years			Age 30-70 years†: annual breast MRI + US every 6 months	High-risk (≥ 30% LR of BC) guidelines:
	Breast MRI with contrast and US, alternating every 6 months from age 25-29 years	Age 30-75 years: annual mammogram‡	Age > 50 years: annual mammogram ± US + CBE	Age > 65 years: mammogram and US					Mammogram age < 40 years only in conspicuous cases	Age < 50 years: annual mammogram from age of 30 years
	Mammogram and breast MRI with contrast, alternating every 6 months from age 30-65 years	Age 30-75 years: annual CBE by specialist	In families with BC diagnosed < 35 years, individualized surveillance recommendations may apply; otherwise, surveillance should start at age 30 years						Mammogram age > 40 years at least every 2 years, or more often depending on accessibility of other examination procedures, gland tissue density, and mammographic findings	Age 50-69 years: yearly clinical mammogram
	Mammogram and US, alternating every 6 months from age > 65 years									Age > 69 years: screening mammogram every 2 years
										General recommendations: Breast self-examination is not recommended as screening method; MR scanning can be used as part of clinical breast examination imaging but is not recommended as only screening method outside experimental protocol

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Table 1. National Guidelines for BC Management (Continued)

Gene/ Management	United States[17]	Czech Republic[18]	The Netherlands[11]	Australia[19]	France[20]	Spain[21]	Belgium[22,23]	United Kingdom[24]	Germany[25-27]	Denmark*
Surgical	Consider RRM based on FH	Consider RRM based on FH	Not enough evidence to recommend RRM	Offer RRM followed by self-surveillance of breast area if there is strong FH of BC in women diagnosed age < 50 years	RRM accepted	No statement made			RRM: individual case decision (consideration of pedigree and birth cohort)	RRM is not recommended, but request for it is granted to women with high lifetime risk (≥ 30%) who insist on it after receiving genetic counseling
TP53										
Surveillance	Age 20-25 years: CBE every 6-12 months	Age 20-25 years: SBE every month	Same as for *BRCA1/2* pathogenic variant carriers, from age 20-25 years	Breast awareness from age of breast development	Annual MRI and US from age 20 years	From age 20 years, annual breast MRI and add annual mammogram from age 30 years	Annual MRI recommended from age 25 years	Do not offer mammogram	Age 20-70 years†: annual breast MRI + US every 6 months	Same as *PALB2*
	Age 20-29 years: annual breast MRI with contrast (preferred) or mammography	Age 20-29 years: annual breast MRI with contrast (preferred) or mammography	No consensus about use of mammography in combination with MRI or only MRI	From age 20 years, annual breast MRI			Mammogram not recommended because of higher susceptibility to radiation	Age 20-49 years: annual MRI	Mammogram age < 40 years only in conspicuous cases	
	Age 30-75 years: annual mammogram and breast MRI with contrast	Age 30-75 years: annual mammogram and breast MRI with contrast		Other forms of imaging: mammogram ± US only if unable to access MRI			US useful to reduce No. of false positives when MRI is difficult to interpret	Age 50-60 years: consider annual MRI	Mammogram age > 40 years at least every 2 years, or more often depending on accessibility of other examination procedures, gland tissue density, and mammographic findings	
	Age > 75 years: management should be considered on individual basis	Age > 75 years: management should be considered on individual basis								

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Table 1. National Guidelines for BC Management (Continued)

Gene/ Management	United States[17]	Czech Republic[18]	The Netherlands[11]	Australia[19]	France[20]	Spain[21]	Belgium[22,23]	United Kingdom[24]	Germany[25-27]	Denmark*
Surgical	Discuss option of RRM	Discuss option of RRM	Same as for *BRCA1/2* pathogenic variant carriers	Offer RRM especially in women age < 50 years followed by self-surveillance of breast area	RRM accepted	RRM option should be discussed	Discuss with patient possibility to perform RRM	No statement made	RRM: individual case decision (consideration of pedigree and birth cohort)	Same as *PALB2*
PTEN										
Surveillance	From age 25 years or 5-10 years earlier before earliest BC in family: CBE every 6-12 months starting at age 25 years	From age 20 years: SBE every month	Age 25-60 years: annual physical examination and breast MRI	In families with BC diagnosed age < 35 years, individualized surveillance recommendations may apply; otherwise, surveillance should start at age 30 years	Annual MRI, mammogram, and US from age 30-65 years; then mammogram and US	Annual mammogram and breast MRI from age 30 years	Annual MRI from age 25 years onward	Age 30-39 years: consider annual mammogram	Age 30-70 years†: annual breast MRI + US	Annual mammogram and breast MRI from age (25 to) 30 years

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Table 1. National Guidelines for BC Management (Continued)

Gene/ Management	United States[17]	Czech Republic[18]	The Netherlands[11]	Australia[19]	France[20]	Spain[21]	Belgium[22,23]	United Kingdom[24]	Germany[25-27]	Denmark*
	Age 30-35 years or 5-10 years before earliest BC in family: annual mammogram and breast MRI with contrast	Age 30-35 years or 5-10 years before earliest BC in family: annual mammogram and breast MRI with contrast	Age 30-60 years: annual mammography	Age 30-50 years: annual MRI + mammogram (± US)	Anticipated surveillance if mastopathy with MRI and US		From age 40 years onward, annual MRI and annual mammography with interval of 6 months between both examinations can be used	Age 40-59 years: annual mammogram	Mammogram age < 40 years only in conspicuous cases	Rest as for *PALB2*
	Age > 75 years: management should be considered on individual basis	Age > 75 years: management should be considered on individual basis	From age 60 years and depending on difficulty to evaluate mammogram, can be chosen individually between annual or biannual mammogram as part of population surveillance program	Age > 50 years: annual mammogram ± US			Mammogram should be used with prudence between age 30 and 40 years but should not be used before age 30 years US is useful to reduce No. of false positives when MRI is difficult to interpret	From age 60 years, mammogram as part of population surveillance program	Mammogram age > 40 years at least every 2 years, or more often depending on accessibility of other examination procedures, gland tissue density, and mammographic findings	

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Table 1. National Guidelines for BC Management (Continued)

Gene/ Management	United States[17]	Czech Republic[18]	The Netherlands[11]	Australia[19]	France[20]	Spain[21]	Belgium[22,23]	United Kingdom[24]	Germany[25-27]	Denmark*
Surgical	RRM: discuss option	No statement made	No statement made	Discuss RRM followed by self-surveillance of breast area (consider individual residual risk for BC and comorbidities)	RRM accepted and discussed at age 25 years if mastopathy	Discuss option of RRM	No studies have assessed efficacy of prophylactic mastectomy in Cowden syndrome; discuss with patient balance of benefits/ harms of RRM and counsel regarding degree of protection, extent of cancer risk, and reconstruction options	No statement made	No statement made	

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Table 1. National Guidelines for BC Management (Continued)

Gene/ Management	United States[17]	Czech Republic[18]	The Netherlands[11]	Australia[19]	France[20]	Spain[21]	Belgium[22,23]	United Kingdom[24]	Germany[25-27]	Denmark*
CDH1										
Surveillance	Annual mammogram and consider breast MRI with contrast from age 30 years	SBE every month from age 18 years CBE every 6 months from age 18 years US and breast MRI with contrast, alternating every 6 months from age 35 years or 5-10 years before earliest BC in family	From age 30 years, annual MRI, mammography, and CBE performed by specialist	Age 30-50 years: annual MRI + mammogram (± US) Age > 50 years: annual mammogram ± annual US + CBE (consider also continuing MRI as may be superior for detection of lobular cancer)	Annual MRI, mammogram, and US from age 30-65 years, then mammogram and US	Annual mammography and breast MRI from age 35 years		Age 30-39 years: consider annual mammogram Age 40-59 years: annual mammogram From age 60 years, mammogram as part of population surveillance program	Same as *PTEN*	Same as *PALB2*
Surgical	Consider RRM based on FH	No statement made	Individual case decision	RRM may be considered	RRM accepted	No statement made		No statement made	RRM: individual case decision (consideration of pedigree and birth cohort)	Same as *PALB2*

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Table 1. National Guidelines for BC Management (Continued)

Gene/ Management	United States[17]	Czech Republic[18]	The Netherlands[11]	Australia[19]	France[20]	Spain[21]	Belgium[22,23]	United Kingdom[24]	Germany[25-27]	Denmark*
STK11										
Surveillance	Mammogram and breast MRI annually beginning at approximately age 25 years	SBE every month starting at age 20 years	Annual breast MRI from age 25 years	In families with BC age < 35 years, individualized surveillance recommendations may apply; otherwise screening should start at age 30 years				Age 30-39 years: consider annual mammography		Same as *PALB2*
		Annual mammogram and breast MRI with contrast at age 30-35 years or 5-10 years before earliest BC in family	Mammogram and breast MRI from age 30 years, rotating every 6 months	Age 30-50 years: annual MRI + mammogram (± US)				Age 40-59 years: annual mammogram		
		Age > 75 years: management should be considered on an individual basis		Age > 50 years: annual mammogram (± annual US) + CBE				From age 60 years, mammogram as part of population surveillance program		
Surgical	RRM: evidence insufficient; manage based on FH		No statement made	Consider RRM followed by self-surveillance of chest wall				No statement made		Same as *PALB2*

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Table 1. National Guidelines for BC Management (Continued)

Gene/ Management	United States[17]	Czech Republic[18]	The Netherlands[11]	Australia[19]	France[20]	Spain[21]	Belgium[22,23]	United Kingdom[24]	Germany[25-27]	Denmark*
CHEK2										
Surveillance	Annual mammogram and consider breast MRI with contrast at age 40 years	Women with BC heterozygous for *CHEK2* c.1100delC pathogenic variant: because of increased risk of contralateral BC, annual CBE and mammography until age 60 years, or up to 10 years after diagnosis of BC (if first BC occurred age > 50 years)							Same as *PTEN*	Moderate risk (20%-29% LR of BC) guidelines:
		Healthy heterozygotes: annual CBE and mammography from age 35-60 years								Age < 50 years: annual mammogram from age 40 years
	Risk data are based only on frameshift variants; risks for most missense variants are unclear	Healthy women not carrying familial *CHEK2* c.11100delC pathogenic variant: advise depending on FH								Age 50-69 years: screening mammogram every 2 years

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Table 1. National Guidelines for BC Management (Continued)

Gene/ Management	United States[17]	Czech Republic[18]	The Netherlands[11]	Australia[19]	France[20]	Spain[21]	Belgium[22,23]	United Kingdom[24]	Germany[25-27]	Denmark*
		Women homozygotes for *CHEK2* c.11100delC: same advice as BRCA1/2 carriers								Age > 69 years: none
										General recommendations: Breast self-examination is not recommended as screening method; MR scanning can be used as part of clinical breast examination imaging but is not recommended as only screening method outside experimental protocol
Surgical	RRM: evidence insufficient; manage based on FH		Consider for women homozygotes for *CHEK2* c.11100delC pathogenic variant						RRM: individual case decision; consideration of pedigree and birth cohort	RRM is not recommended
ATM										
Surveillance	Annual mammogram and consider breast MRI with contrast starting at age 40 years		Draft guidelines:	Guidelines for 7271T>G pathogenic variant only:					Same as *PTEN*	Same as *CHEK2*

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Table 1. National Guidelines for BC Management (Continued)

Gene/ Management	United States[17]	Czech Republic[18]	The Netherlands[11]	Australia[19]	France[20]	Spain[21]	Belgium[22,23]	United Kingdom[24]	Germany[25-27]	Denmark*
	Insufficient evidence to recommend against radiation therapy		Female ATM carriers (all pathogenic variants except for C.7271T>G):	Age 30-50 years: annual MRI + mammogram (± US)					Avoid radiation of contralateral breast	Guidelines for postoperative radiation therapy will not be modified as result of pathogenic variants in *ATM*
	7271T>G mutation has higher LR of BC (up to 60%) than truncating variants		Age 40-50 years: annual mammography Age 50-75 years: population surveillance	Age > 50 years: annual mammogram (± US) + CBE						
			Female carriers of c.7271T>G: Age 25-60 years: annual breast MRI							
			Age 30-75 years: annual mammography; exception is by heterogeneous density or high density of fibroglandular tissue (ACR 3 or 4); then advice is annual MRI alternating with mammography from age 60-75 years							

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Table 1. National Guidelines for BC Management (Continued)

Gene/ Management	United States[17]	Czech Republic[18]	The Netherlands[11]	Australia[19]	France[20]	Spain[21]	Belgium[22,23]	United Kingdom[24]	Germany[25-27]	Denmark*
Surgical	Consider RRM based on FH		No statement made	No statement made					RRM: currently not recommended	Same as *CHEK2*
NF1										
Surveillance	Annual mammogram starting at age 30 years and consider breast MRI with contrast from age 30-50 years	SBE examination every months starting at age 20 years	Age 35-50 years: annual mammogram and physical examination by specialist	All ages: breast awareness with prompt reporting to general practitioner of persistent or unusual changes				Annual BC screening should be done from age 40 years onward		Same as *CHEK2*
		Annual mammogram starting at age 30 years and consider breast MRI with contrast	Age > 50 years: population breast surveillance program	From age 40 years, annual mammography						
				From age 50 years, biannual mammogram						
Surgical	RRM: evidence insufficient; manage based on FH	No statement made	No statement made	No statement made						Same as *CHEK2*
NBN										
Surveillance	Annual mammogram and consider breast MRI with contrast from age 40 years									
	Recommendations based on data from c.657del5 Slavic truncating variant									
Surgical	RRM: evidence insufficient; manage based on FH									

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Table 1. National Guidelines for BC Management (Continued)

Gene/ Management	United States[17]	Czech Republic[18]	The Netherlands[11]	Australia[19]	France[20]	Spain[21]	Belgium[22,23]	United Kingdom[24]	Germany[25-27]	Denmark*
MEN1										
Surveillance		SBE every month starting at age 20 years	Age 35-50 years: annual mammogram and CBE by specialist							
		Biannual mammogram starting at age 40 years	Age > 50 years: population breast surveillance program							
Surgical		No statement made	No statement made							

Abbreviations: BC, breast cancer; CBE, clinical breast examination; FH, family history; LR, lifetime risk; MRI, magnetic resonance imaging; RRM, risk-reducing mastectomy; SBE, self-breast examination; US, ultrasound/ sonography.

*Danish Breast Cancer Group Secretariat and Statistical Office recommendations. M. Rossing, personal communication, February 2016.

†Or at least 5 years before earliest age of diagnosis in family.

‡Between ages 60 and 75 years and when mammogram is not easy to evaluate, alternate annual breast MRI with mammogram.

BRIP1, *RAD51C*, and *RAD51D* are ever more accepted as OC but not BC risk predisposition genes (two to five times the risk compared with the general population).[15,32] Notably, many respondents agreed that every patient with OC should be tested for these three genes (in addition to *BRCA1/2*). Although there is currently no indication that OC treatment for a carrier of a pathogenic variant in one of these three genes would differ from that for a noncarrier, carriers may benefit from RRSO at menopause.

The uncertainties and inconsistencies regarding risk and testing practices are magnified when it comes to syndromic cancer genes like *PTEN*, *CDH1*, *STK11*, *NF1*, *NBN*, and *MEN1*, as well as genes conferring an uncertain risk such as *BARD1*, *RAD50*, and *MRE11A*. Although there is significant evidence for elevated BC risk and lobular BC risk in carriers of pathogenic variants in *PTEN* and in *CDH1*, respectively,[34-36] it is likely that these BC risks (and those from the other syndromic genes) are overestimated and therefore unreliable, because they were derived from patients whose histories were consistent with these rare syndromes rather than from unselected patients.[8]

More robust and replicable penetrance estimates from large-cohort and population studies are certainly needed to further define risks. In addition, better understanding of gene-gene and gene-environment interactions that affect risk is required. However, on the basis of both the evidence available from the literature and the results of our survey, which incorporate an international clinical perspective, the 16 genes can be grouped into five categories: high BC risk: *PALB2*, *TP53*, *PTEN*, and *CDH1*; moderate BC risk: *ATM* and *CHEK2*; BC risk of unclear magnitude (but established risk for other cancer types): *STK11*, *NF1*, *NBN*, and *MEN1*; moderate OC risk: *BRIP1*, *RAD51C*, and *RAD51D*; and insufficient evidence for BC or OC risk: *BARD1*, *RAD50*, and *MRE11A*.

The clinical utility of multigene panel testing is assessed based on the improved outcomes of those managed by evidence-based surveillance or prevention approaches. Management guidelines are largely based on expert opinion. Easton et al[8] reviewed guidelines across various countries, but they were specific to women with a family history of BC or with *BRCA1/2* mutations. A framework for management of moderate-risk BC/BOC genes has been extensively reviewed by Tung et al[9] and includes a comparison of surveillance guidelines among the United States, United Kingdom, and Germany. Our survey offers a more extensive comparison of management guidelines among several countries for non-*BRCA1/2* risk genes. Results from the survey show that many countries do not yet have their own guidelines, and/or they use National Comprehensive Cancer Network guidance. There are limited national guidelines available even for genes such as *PALB2*, *BRIP1*, *RAD51C*, and *RAD51D*, which most participants felt should always be tested because they are clinically actionable. Most importantly, when management guidelines are available, they are largely based on expert opinion rather than being evidence based. This explains why the guidelines often differ in important aspects such as indication for risk-reducing surgery and type of diagnostic imaging recommendations.

Our study was initiated to provide a snapshot of ENIGMA clinical practice for non-*BRCA1/2* genes. It included countries and centers with ENIGMA affiliation and also a small subset of centers with no direct link to the ENIGMA consortium at the time of the survey. It provides a global, yet incomplete, picture of testing practices in the world. Indeed, countries like Poland and Israel, with founder pathogenic variants in some of these genes, did not participate in the survey. Because panel testing is currently being implemented in large regions of the world like Asia, Africa, and South America, similar surveys will need to be redistributed once more countries have established testing protocols. Even at the time of the survey, testing protocols and surveillance recommendations were in flux in some countries, and broader gene panels were expected to be offered within a short time. We acknowledge that our sampling of non-ENIGMA centers was limited, and we aim to survey a more diverse collection of US, Canadian, and other worldwide regional or community practices in future studies.

Massively parallel sequencing represents a transformational technology that we must learn to apply appropriately in health care. Although the number of genes, other than *BRCA1/2*, associated with BC/BOC risk is growing, only a small subset of them have clinical utility at the moment. Our survey reveals lack of consensus

among most countries regarding which genes to test, how to test them, how to most efficiently interpret variants, and how to manage patients carrying pathogenic variants. The goal of this study was to highlight the differences across countries and to determine what additional information and infrastructure are still needed to move toward more uniform testing practices and management guidelines internationally.

Our collected evidence suggests that the clinical usefulness of multigene panel testing for BC/BOC predisposition can be improved by a better definition of the cancer risks associated with genetic variation in cancer susceptibility genes and by the availability of evidence-based management guidelines. To this end, it is key that clinicians share clinical and genetic data, through ENIGMA and/or other international consortia focused on the clarification of the BC and OC risk associated with genetic variation, and that tested individuals are encouraged to participate in initiatives that collate genetic testing data and in long-term follow-up studies that evaluate intervention strategies. As ENIGMA CWG, we aim at promoting the use of internationally accepted, standard guidelines at the country level through sharing and discussion of all available management guidelines, and we will continue to evaluate testing practices and risk management recommendations periodically.

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REFERENCES

1. Walsh T, Lee MK, Casadei S, et al: Detection of inherited mutations for breast and ovarian cancer using genomic capture and massively parallel sequencing. Proc Natl Acad Sci USA 107:12629-12633, 2010
2. Kurian AW, Hare EE, Mills MA, et al: Clinical evaluation of a multiple-gene sequencing panel for hereditary cancer risk assessment. J Clin Oncol 32:2001-2009, 2014
3. Kurian AW, Kingham KE, Ford JM: Next-generation sequencing for hereditary breast and gynecologic cancer risk assessment. Curr Opin Obstet Gynecol 27:23-33, 2015
4. 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. J Clin Oncol 33:304-311, 2015
5. 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 16:830-837, 2014
6. Maxwell KN, Hart SN, Vijai J, et al: Evaluation of ACMG-guideline-based variant classification of cancer susceptibility and non-cancer-associated genes in families affected by breast cancer. Am J Hum Genet 98:801-817, 2016
7. 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 31:1267-1270, 2013
8. Easton DF, Pharoah PD, Antoniou AC, et al: Gene-panel sequencing and the prediction of breast-cancer risk. N Engl J Med 372:2243-2257, 2015
9. Tung N, Domchek SM, Stadler Z, et al: Counselling framework for moderate-penetrance cancer-susceptibility mutations. Nat Rev Clin Oncol 13:581-588, 2016
10. Dreijerink KM, Goudet P, Burgess JR, et al: Breast-cancer predisposition in multiple endocrine neoplasia type 1. N Engl J Med 371:583-584, 2014
11. Vereniging Klinische Genetica Nederland: Erfelijke tumoren. http://www.vkgn.org/vakinformatie/richtlijnen-en-protocollen/erfelijke-tumoren/

12. Plon SE, Eccles DM, Easton D, et al: Sequence variant classification and reporting: Recommendations for improving the interpretation of cancer susceptibility genetic test results. Hum Mutat 29:1282-1291, 2008

13. Richards S, Aziz N, Bale S, et al: ACMG Laboratory Quality Assurance Committee: Standards and guidelines for the interpretation of sequence variants: A joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 17:405-424, 2015

14. Landrum MJ, Lee JM, Benson M, et al: ClinVar: Public archive of interpretations of clinically relevant variants. Nucleic Acids Res 44:D862-D868, 2016

15. National Human Genome Research Institute: Breast cancer information core: An open access on-line breast cancer mutation. https://research.nhgri.nih.gov/bic/

16. Fokkema IF, Taschner PE, Schaafsma GC, et al: LOVD v.2.0: The next generation in gene variant databases. Hum Mutat 32:557-563, 2011

17. 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 Canc Netw 15:9-20, 2017

18. Janatová M, Borecká M, Soukupová J, et al: PALB2 as another candidate gene for genetic testing in patients with hereditary breast cancer in Czech Republic [in Czech]. Klin Onkol 29:S31-S34, 2016 (suppl 1)

19. Cancer Institute NSW, eviQ: Cancer genetics: Risk management. https://www.eviq.org.au/cancer-genetics/risk-management

20. Unicancer: Le Groupe génétique et cancer. http://www.unicancer.fr/recherche/les-groupes-recherche/groupe-genetique-et-cancer-ggc

21. Llort G, Chirivella I, Morales R, et al: SEOM clinical guidelines in hereditary breast and ovarian cancer. Clin Transl Oncol 17:956-961, 2015

22. Robays J, Stordeur S, Hulstaert F, et al: Oncogenetic testing and follow-up for women with hereditary breast/ovarian cancer, Li Fraumeni syndrome and Cowden syndrome. https://kce.fgov.be/sites/default/files/page_documents/KCE_236Cs_Oncogenetic_testing_Abstract.pdf

23. Robays J, Stordeur S, Hulstaert F, et al: Oncogenetic testing, diagnosis and follow-up in Birt-Hogg-Dubé syndrome, familial atypical multiple mole melanoma syndrome and neurofibromatosis 1 and 2. https://kce.fgov.be/sites/default/files/atoms/files/KCE_243_oncogenetic_testing_Neurofibromatosis_Report_0.pdf

24. National Institute for Health and Care Excellence: Familial breast cancer: Classification, care and managing breast cancer and related risks in people with a family history of breast cancer. https://www.nice.org.uk/guidance/cg164/chapter/recommendations#surveillance-and-strategies-for-early-detection-of-breast-cancer

25. German Consortium of Hereditary Breast and Ovarian Cancer: Früherkennung, Diagnostik, Therapie und Nachsorge des Mammakarzinoms. http://www.awmf.org/leitlinien/detail/ll/032-045OL.html

26. Rhiem K, Schmutzler RK: Risk-adapted surveillance: Focus on familial breast and ovarian cancer [in German]. Bundesgesundheitsblatt Gesundheitsforschung Gesundheitsschutz 57:307-311, 2014

27. Schmutzler RK: Konsensusempfehlung des Deutschen Konsortiums Familiärer Brust- und Eierstockkrebs zum Umgang mit Ergebnissen der Multigenanalyse. Geburtshilfe Frauenheilkd 77:733-739, 2017

28. Lincoln SE, Kobayashi Y, Anderson MJ, et al: A systematic comparison of traditional and multigene panel testing for hereditary breast and ovarian cancer genes in more than 1000 patients. J Mol Diagn 17:533-544, 2015

29. Kurian AW, Ford JM: Multigene panel testing in oncology practice: How should we respond? JAMA Oncol 1:277-278, 2015

30. Castéra 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 22:1305-1313, 2014
31. Couch FJ, Shimelis H, Hu C, et al: Associations between cancer predisposition testing panel genes and breast cancer. JAMA Oncol 3:1190-1196, 2017
32. Kurian AW, Hughes E, Handorf EA, et al: Breast and ovarian cancer penetrance estimates derived from germline multiple-gene sequencing results in women. JCO Precis Oncol
33. Antoniou AC, Casadei S, Heikkinen T, et al: Breast-cancer risk in families with mutations in PALB2. N Engl J Med 371:497-506, 2014
34. Tan MH, Mester JL, Ngeow J, et al: Lifetime cancer risks in individuals with germline PTEN mutations. Clin Cancer Res 18:400-407, 2012
35. Hearle N, Schumacher V, Menko FH, et al: Frequency and spectrum of cancers in the Peutz-Jeghers syndrome. Clin Cancer Res 12:3209-3215, 2006
36. Pharoah PD, Guilford P, Caldas C: Incidence of gastric cancer and breast cancer in CDH1 (E-cadherin) mutation carriers from hereditary diffuse gastric cancer families. Gastroenterology 121:1348-1353, 2001

Appendix

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Table A1. Questions Included in the Surveys (by mode of distribution)

Question	In-Person Survey Only	In-Person and Paper Surveys	Paper Survey Only	SurveyMonkey*
I Testing practices				
I-1				
Is DNA testing for inherited susceptibility to BC and/or OC I-2 carried out at your clinical practice?		Yes/no		Yes/no
I-2				
Which of the following BC/BOC II-2 susceptibility genes are tested?		*ATM*		*BRCA1*
		BARD1		*BRCA2*
		BRIP1		*CHEK2*
		CDH1		*ATM*
		CHEK2		*CDH1*
		MEN1		*NBN*
		MRE11A		*NF1*
		NBN		*PALB2*
		NF1		*PTEN*
		PALB2		*STK11*
		PTEN		*TP53*
		RAD50		Other genes (specify)
		RAD51C		
		RAD51D		
		STK11		
		TP53		
I-3				
Frequency of testing		Does your center test for gene X?		Which of the following genes are routinely reported for all BC susceptibility requests? (same list as above)
		Yes, regularly		
		Yes, occasionally		
		No, it does not		

(Continued on following page)

Table A1. Questions Included in the Surveys (by mode of distribution) (Continued)

Question	In-Person Survey Only	In-Person and Paper Surveys	Paper Survey Only	SurveyMonkey*
I-4				
Testing methods and setting	Which genes do you agree should be tested for every BC or OC patient eligible for genetic testing?	Which method is used to test for gene X?	Describe the gene panels currently used (if any) and if they are used in the diagnostic or research setting	
		Clinical	If you are not currently using gene panels but may in the future, what do you think is required before starting to use them?	
		i. Single gene		
		ii. Part of gene panel		
		iii. Reflex test (ie, tested only if other specified genes are wild type)		
		Research		
		i. Single gene		
		ii. Part of gene panel		
II Variant classification				
II-1				
Classification system		Which scheme/criteria are used for variant classification?		
		Specify the No. of tiers used for class definition		
II-2				
Reporting and cascade testing of variants		(Likely) pathogenic variants:	Do you (or your colleagues) request genetic testing directly and discuss results?	Do you routinely discuss results of uncertain significance with the referring clinician before reporting?
		Are they reported to patients?		If you currently only report *BRCA* genes but might report broader panels in the future, what are the major issues/problems that should be overcome?
		Is cascade testing performed?		
		VUS:		
		Are they reported to patients?		
		Is cascade testing performed?		

(Continued on following page)

Table A1. Questions Included in the Surveys (by mode of distribution) (Continued)

Question	In-Person Survey Only	In-Person and Paper Surveys	Paper Survey Only	SurveyMonkey*
II-3				
Variant interpretation			Who takes responsibility for interpreting the clinical significance of the identified variants?	For cancer susceptibility genes: Who takes responsibility for variant interpretation and reporting? Clinical scientist Clinical geneticist Genetic counselor Oncologist (medical/surgical) Other (specify) Who takes responsibility for discussing the clinical significance/utility of an identified variant? (same choices as above)
III Risk management guidelines	For which genes do you agree that the cancer-associated risks are high enough to alter clinical practice/management?	Are management guidelines available at your center for patients with (likely) pathogenic variants in these genes? Yes, national guidelines Yes, local guidelines or local adaptations of national guidelines No, guidelines are not currently available	If clinical management guidelines are available at your center for the specified genes, please provide digital copy, reference, or Web site link	Are there clinical guidelines for managing patients who carry a pathogenic or likely pathogenic variant in a BC susceptibility gene?

NOTE. Questions I-4 and III of the in-person survey were asked at a different time compared with the remainder of the survey; therefore, answers were collected from only 23 centers. Open questions were only part of the paper survey. The far right column shows the items included in the United Kingdom-specific survey conducted through SurveyMonkey.

Abbreviations: BC, breast cancer; OC, ovarian cancer; VUS, variant of unknown significance.

*United Kingdom National Health Service laboratories only.

Table A2. Frequency of Testing

Gene	Testing for Gene				Testing Regularly			
	No. of Informative Responses	No.	%	95% CI	No. of Informative Responses	No.	%	95% CI
PALB2	38	34	89	0.7587 to 0.9583	38	25	66	0.4989 to 0.7879
TP53	38	38	100	0.9082 to 1.0000	38	22	58	0.4219 to 0.7215
PTEN	38	38	100	0.9082 to 1.0000	37	21	57	0.4091 to 0.7133
CDH1	38	37	97	0.8651 to 0.9953	37	17	46	0.3104 to 0.6162
STK11	36	33	92	0.7817 to 0.9713	34	11	32	0.1913 to 0.4916
CHEK2	38	34	89	0.7587 to 0.9583	36	27	75	0.5893 to 0.8625
ATM	38	30	79	0.6365 to 0.8893	38	19	50	0.3485 to 0.6515
NF1	35	23	66	0.4915 to 0.7917	34	7	21	0.1035 to 0.3680
NBN	36	28	78	0.6192 to 0.8828	36	13	38	0.2246 to 0.5242
BARD1	32	25	78	0.6192 to 0.8828	30	12	40	0.2459 to 0.5768
RAD50	32	24	75	0.5789 to 0.8675	26	10	38	0.2243 to 0.5747
MRE11A	32	23	72	0.5463 to 0.8444	29	11	38	0.2269 to 0.5600
MEN1	29	15	52	0.3443 to 0.6861	27	5	19	0.0818 to 0.3670
BRIP1	34	28	82	0.6649 to 0.9165	30	15	50	0.3315 to 0.6685
RAD51C	33	30	91	0.7643 to 0.9686	30	14	47	0.3023 to 0.6386
RAD51D	33	29	88	0.7267 to 0.9518	31	14	4	0.2916 to 0.6223

Table A3. Clinical Testing Methods

Gene	No. of Informative Responses	Single Gene			Panel			Reflex		
		No.	%	95% CI	No.	%	95% CI	No.	%	95% CI
PALB2	34	9	26	0.1460 to 0.4312	29	85	0.6987 to 0.9355	13	38	0.2390 to 0.5496
TP53	38	21	55	0.3971 to 0.6985	28	76	0.5989 to 0.8664	15	41	0.2635 to 0.5651
PTEN	37	17	46	0.3104 to 0.6162	27	75	0.5893 to 0.8625	9	25	0.1375 to 0.4107
CDH1	36	15	42	0.2714 to 0.5780	27	77	0.6098 to 0.8793	8	23	0.1207 to 0.3902
STK11	33	11	33	0.1975 to 0.5039	21	64	0.4662 to 0.7782	7	21	0.1067 to 0.3775
CHEK2	32	5	16	0.0687 to 0.3176	17	53	0.3645 to 0.6913	3	9	0.0324 to 0.2422
ATM	29	4	14	0.0550 to 0.3056	20	69	0.5077 to 0.8273	1	3	0.0061 to 0.1718
NF1	23	5	22	0.0966 to 0.4190	14	61	0.4079 to 0.7784	2	9	0.0242 to 0.2680
NBN	27	1	4	0.0066 to 0.1828	15	56	0.3732 to 0.7242	1	4	0.0066 to 0.1828
BARD1	24	0	0	0.0000 to 0.1717	13	54	0.3508 to 0.7211	0	0	0.0000 to 0.1380
RAD50	24	0	0	0.0000 to 0.1717	12	50	0.3143 to 0.6857	0	0	0.0000 to 0.1380
MRE11A	23	0	0	0.0000 to 0.1431	11	48	0.2924 to 0.6704	0	0	0.0000 to 0.1431
MEN1	14	3	21	0.0757 to 0.4759	8	57	0.3259 to 0.7862	0	0	0.0000 to 0.2153
BRIP1	26	0	0	0.0000 to 0.1287	18	69	0.5001 to 0.8350	3	12	0.0400 to 0.2898
RAD51C	29	0	0	0.0000 to 0.1170	23	79	0.6161 to 0.9015	2	7	0.0191 to 0.2197
RAD51D	28	0	0	0.0000 to 0.1206	23	82	0.6441 to 0.9212	2	7	0.0198 to 0.2264

Table A4. Reporting Practices of (likely) Pathogenic Variants and VUS to Patients

	Pathogenic Variants				VUS			
Gene	No. of Informative Responses	No.	%	95% CI	No. of Informative Responses	No.	%	95% CI
PALB2	28	28	100	0.8794 to 1.0000	16	13	81	0.5699 to 0.9341
TP53	36	34	94	0.8186 to 0.9846	20	15	75	0.5313 to 0.8881
PTEN	35	34	97	0.8547 to 0.9949	19	14	74	0.5121 to 0.8819
CDH1	35	34	97	0.8547 to 0.9949	18	14	78	0.5479 to 0.9100
STK11	30	28	93	0.7868 to 0.9815	16	12	75	0.5050 to 0.8982
CHEK2	33	29	88	0.8788 to 0.7267	14	7	50	0.2680 to 0.7320
ATM	24	22	92	0.7415 to 0.9768	11	9	82	0.5230 to 0.9486
NF1	20	19	95	0.7639 to 0.9911	11	8	73	0.4344 to 0.9025
NBN	21	15	71	0.5004 to 0.8619	8	4	50	0.2152 to 0.7848
BARD1	16	15	94	0.7167 to 0.9889	6	3	50	0.1876 to 0.8124
RAD50	12	11	92	0.6461 to 0.9851	5	3	60	0.2307 to 0.8824
MRE11A	12	11	92	0.6461 to 0.9851	4	2	50	0.1500 to 0.8500
MEN1	12	11	92	0.6461 to 0.9851	7	5	71	0.3589 to 0.9178
BRIP1	22	21	95	0.8454 to 1.0000	11	8	73	0.4344 to 0.9025
RAD51C	25	25	100	0.8668 to 1.0000	14	11	79	0.5241 to 0.9243
RAD51D	25	25	100	0.8668 to 1.0000	14	10	71	0.4535 to 0.8828

Abbreviation: VUS, variant of unknown significance.

Table A5. Sources of Management Guidelines From ENIGMA Centers

Gene	No. of Informative Responses	Have Guidelines			National			Local			International		
		No.	%	95% CI	No.	%	95% CI	No.	%	95% CI	No.	%	95% CI
PALB2	25	23	92	0.7503 to 0.9778	13	52	0.335 to 0.6997	9	36	0.2025 to 0.5548	1	4	0.0071 to 0.1954
TP53	31	29	94	0.7928 to 0.9821	22	71	0.5341 to 0.839	5	16	0.0709 to 0.3263	2	6	0.0179 to 0.2072
PTEN	31	29	94	0.7928 to 0.9821	19	61	0.4382 to 0.7627	8	26	0.137 to 0.4	2	6	0.0179 to 0.2072
CDH1	31	29	94	0.7928 to 0.9821	19	61	0.4382 to 0.7627	8	26	0.137 to 0.4	2	6	0.0179 to 0.2072
STK11	26	23	88	0.7102 to 0.96	16	62	0.4253 to 0.7757	5	19	0.0851 to 0.3788	2	8	0.0214 to 0.2414
CHEK2	22	20	91	0.7219 to 0.9747	11	50	0.3072 to 0.6928	8	36	0.1973 to 0.5705	1	5	0.0081 to 0.218
ATM	20	18	90	0.699 to 0.9721	10	50	0.2993 to 0.7007	7	35	0.1812 to 0.5671	1	5	0.0089 to 0.2361
NF1	17	15	88	0.6566 to 0.9671	9	53	0.3096 to 0.7383	5	29	0.1328 to 0.5313	1	6	0.0105 to 0.2698
NBN	14	12	86	0.6006 to 0.9599	7	50	0.268 to 0.732	4	29	0.1172 to 0.5465	1	7	0.0127 to 0.3147
BARD1	14	3	21	0.0757 to 0.4759	0	0	0 to 0.2153	3	21	0.0757 to 0.4759	0	0	0 to 0.2153
RAD50	11	3	27	0.0975 to 0.5656	0	0	0 to 0.2588	3	27	0.0975 to 0.5656	0	0	0 to 0.2588
MRE11A	10	3	30	0.1078 to 0.6032	0	0	0 to 0.2775	3	30	0.1078 to 0.6032	0	0	0 to 0.2775
MEN1	10	8	80	0.4902 to 0.9433	3	30	0.1078 to 0.6032	4	40	0.1682 to 0.6873	1	10	0.0179 to 0.4042
BRIP1	19	16	84	0.6243 to 0.9448	8	42	0.2314 to 0.6372	7	37	0.1915 to 0.5896	1	5	0.0094 to 0.2464
RAD51C	24	21	88	0.69 to 0.9566	10	42	0.2447 to 0.6117	10	42	0.2447 to 0.6117	1	4	0.0074 to 0.2024
RAD51D	24	21	88	0.69 to 0.9566	10	42	0.2447 to 0.6117	10	42	0.2447 to 0.6117	1	4	0.0074 to 0.2024

Abbreviation: ENIGMA, Evidence-Based Network for the Interpretation of Germline Mutant Alleles.

Table A6. Clinical Utility: Every Patient With BC (or OC) Who Meets Criteria for Genetic Testing Should Be Tested for This Gene

Gene	No. of Informative Responses	No.	%	95% CI
PALB2	23	23	100	0.8569 to 1.0000
TP53	23	9	39	0.2216 to 0.5921
PTEN	23	6	30	0.1255 to 0.4647
CDH1	23	7	26	0.1560 to 0.5087
STK11	23	4	17	0.0698 to 0.3714
CHEK2	23	15	65	0.4489 to 0.8119
ATM	23	12	52	0.3296 to 0.7076
NF1	23	1	4	0.0077 to 0.2099
BARD1	23	6	26	0.1255 to 0.4647
MEN1	23	2	9	0.0242 to 0.2680
MRE11A	23	0	0	0.0000 to 0.1431
NBN	23	0	0	0.0000 to 0.1431
RAD50	23	0	0	0.0000 to 0.1431
BRIP1	23	23	100	0.8569 to 1.0000
RAD51C	23	23	100	0.8569 to 1.0000
RAD51D	23	23	100	0.8569 to 1.0000

Abbreviations: BC, breast cancer; OC, ovarian cancer.

Table A7. Clinical Utility: Cancer Risks Associated With This Gene Are High Enough to Affect Clinical Management

Gene	No. of Informative Responses	No.	%	95% CI
PALB2	23	23	100	0.8569 to 1.0000
TP53	23	23	100	0.8569 to 1.0000
PTEN	23	23	100	0.8569 to 1.0000
CDH1	23	23	100	0.8569 to 1.0000
STK11	23	23	100	0.8569 to 1.0000
CHEK2	23	20	87	0.6787 to 0.9546
ATM	23	18	78	0.5810 to 0.9034
NF1	23	8	35	0.1881 to 0.5511
BARD1	23	6	26	0.1255 to 0.4647
MEN1	23	3	13	0.0454 to 0.3213
MRE11A	23	0	0	0.0000 to 0.1431
NBN	23	0	0	0.0000 to 0.1431
RAD50	23	0	0	0.0000 to 0.1431
BRIP1	23	23	100	0.8569 to 1.0000
RAD51C	23	23	100	0.8569 to 1.0000
RAD51D	23	23	100	0.8569 to 1.0000

Table A8. Testing Setting: Clinical Versus Research

Gene	No. of Informative Responses	Clinical Testing Only			Clinical and Research			Research Testing Only		
		No.	%	95% CI	No.	%	95% CI	No.	%	95% CI
PALB2	34	20	59	0.4222 to 0.7363	11	32	0.1913 to 0.4916	3	9	0.0305 to 0.2296
TP53	37	23	62	0.4610 to 0.7594	14	38	0.2406 to 0.539	0	0	0.0000 to 0.0000
PTEN	36	21	58	0.4220 to 0.7286	14	39	0.2478 to 0.5514	1	3	0.0049 to 0.1417
CDH1	35	22	63	0.4634 to 0.7683	12	34	0.2083 to 0.5085	1	3	0.0051 to 0.1453
STK11	33	16	48	0.3250 to 0.6478	14	42	0.2724 to 0.5919	3	9	0.0314 to 0.2357
CHEK2	32	14	44	0.2817 to 0.6067	13	41	0.2552 to 0.5774	5	16	0.0686 to 0.3175
ATM	29	11	38	0.2269 to 0.5600	12	41	0.2551 to 0.5926	6	21	0.0985 to 0.3839
NF1	23	12	52	0.3296 to 0.7076	7	30	0.156 to 0.5087	4	17	0.0698 to 0.3714
NBN	27	6	22	0.1061 to 0.4076	10	37	0.2153 to 0.5577	11	41	0.2451 to 0.5927
BARD1	23	5	22	0.0966 to 0.4190	9	39	0.2216 to 0.5921	9	39	0.2216 to 0.5921
RAD50	23	6	26	0.1255 to 0.4647	7	30	0.156 to 0.5087	10	43	0.2563 to 0.6319
MRE11A	23	4	17	0.0698 to 0.3714	8	35	0.1881 to 0.5511	11	48	0.2924 to 0.6704
MEN1	14	5	36	0.1634 to 0.6124	6	43	0.2138 to 0.6741	3	21	0.0757 to 0.4759
BRIP1	27	10	37	0.2153 to 0.5577	10	37	0.2153 to 0.5577	7	26	0.1317 to 0.4468
RAD51C	29	12	41	0.2551 to 0.5926	12	41	0.2551 to 0.5926	5	17	0.0760 to 0.3455
RAD51D	28	12	43	0.2651 to 0.6093	12	43	0.2651 to 0.6093	4	14	0.0570 to 0.3149

Table A9. Genes Regularly Tested by ENIGMA-US Versus ENIGMA-Other Versus Italian and United Kingdom Non-ENIGMA Centers

	ENIGMA Overall			Italy				United Kingdom				ENIGMA-US				ENIGMA-Other			
Gene	No. of Informative Responses	No.	%	No. of Informative Responses	No.	%	95% CI	No. of Informative Responses	No.*	%	95% CI	No. of Informative Responses	No.	%	95% CI	No. of Informative Responses	No.	%	95% CI
PALB2	37	25	68	14	2	14	0.0401 to 0.3994	9	2	22	0.0632 to 0.5474	7	7	100	0.6457 to 1.0000	30	18	60	0.4232 to 0.7541
TP53	37	22	59	14	3	21	0.0757 to 0.4759	9	5	56	0.2667 to 0.8112	7	6	86	0.4869 to 0.9743	30	16	53	0.3614 to 0.6977
PTEN	36	21	58	14	2	14	0.0401 to 0.3994	9	3	33	0.1206 to 0.6458	7	7	100	0.6457 to 1.0000	29	14	48	0.3139 to 0.6557
CDH1	36	17	47	14	2	14	0.0401 to 0.3994	9	1	11	0.0199 to 0.4350	7	6	86	0.4869 to 0.9743	29	11	38	0.2269 to 0.5600
STK11	33	11	33	14	1	7	0.0127 to 0.3147	9	1	11	0.0199 to 0.4350	7	2	29	0.0822 to 0.6411	26	9	35	0.1941 to 0.5378
CHEK2	35	26	74	14	4	29	0.1172 to 0.5465	9	1	11	0.0199 to 0.4350	7	6	86	0.4869 to 0.9743	28	20	71	0.5294 to 0.8475
ATM	37	19	51	14	2	14	0.0401 to 0.3994	9	1	11	0.0199 to 0.4350	7	6	86	0.4869 to 0.9743	30	13	43	0.2738 to 0.6080
NF1	33	7	21	14	2	14	0.0401 to 0.3994	9	1	11	0.0199 to 0.4350	7	2	29	0.0822 to 0.6411	26	5	19	0.0851 to 0.3788
NBN	35	13	38	14	2	14	0.0401 to 0.3994	9	0	0	0.0000 to 0.0000	7	5	71	0.3589 to 0.9178	28	8	29	0.1525 to 0.4706
BARD1	29	12	1	14	0	0	0.0000 to 0.0000	9	0	0	0.0000 to 0.0000	6	4	67	0.3000 to 0.9032	23	8	35	0.1881 to 0.5511
RAD50	26	10	38	14	0	0	0.0000 to 0.0000	9	0	0	0.0000 to 0.0000	4	3	75	0.3006 to 0.9544	22	7	32	0.1636 to 0.5268
MRE11A	29	11	38	14	0	0	0.0000 to 0.0000	9	0	0	0.0000 to 0.0000	6	3	50	0.1876 to 0.8124	23	8	35	0.1881 to 0.5511

(Continued on following page)

Table A9. Genes Regularly Tested by ENIGMA-US Versus ENIGMA-Other Versus Italian and United Kingdom Non-ENIGMA Centers (Continued)

	ENIGMA Overall			Italy				United Kingdom				ENIGMA-US				ENIGMA-Other			
Gene	No. of Informative Responses	No.	%	No. of Informative Responses	No.	%	95% CI	No. of Informative Responses	No.*	%	95% CI	No. of Informative Responses	No.	%	95% CI	No. of Informative Responses	No.	%	95% CI
MEN1	26	5	19	14	1	7	0.0127 to 0.3147	9	0	0	0.0000 to 0.0000	4	1	25	0.0456 to 0.6994	22	4	18	0.0731 to 0.3852
BRIP1	30	15	50	14	1	7	0.0127 to 0.3147	9	1	11	0.0199 to 0.4350	7	5	71	0.3589 to 0.9178	23	10	43	0.2563 to 0.6319
RAD51C	30	14	47	14	1	7	0.0127 to 0.3147	9	3	33	0.1206 to 0.6458	7	5	71	0.3589 to 0.9178	23	9	39	0.2216 to 0.5921
RAD51D	30	14	47	14	1	7	0.0127 to 0.3147	9	3	33	0.1206 to 0.6458	7	5	71	0.3589 to 0.9178	23	9	39	0.2216 to 0.5921

Abbreviation: ENIGMA, Evidence-Based Network for the Interpretation of Germline Mutant Alleles.
*The United Kingdom version of the survey did not give "test regularly" as an option.

Table A10. Genes Tested Through Panel Testing by ENIGMA-US Versus ENIGMA-Other Centers

	ENIGMA-Other				ENIGMA-US			
Gene	No. of Informative Responses	No.	%	95% CI	No. of Informative Responses	No.	%	95% CI
PALB2	27	22	81	0.6330 to 0.9182	7	7	100	0.6457 to 1.0000
TP53	30	21	70	0.5212 to 0.8334	7	7	100	0.6457 to 1.0000
PTEN	29	20	69	0.5077 to 0.8272	7	7	100	0.6457 to 1.0000
CDH1	28	20	71	0.5294 to 0.8475	7	7	100	0.6457 to 1.0000
STK11	26	15	58	0.3895 to 0.7446	7	6	86	0.4869 to 0.9743
CHEK2	25	11	44	0.2667 to 0.6293	7	6	86	0.4869 to 0.9743
ATM	22	13	59	0.3873 to 0.7674	7	7	100	0.6457 to 1.0000
NF1	16	8	50	0.2800 to 0.7200	7	6	86	0.4869 to 0.9743
NBN	21	9	43	0.2447 to 0.6345	6	6	100	0.6097 to 1.0000
BARD1	19	7	37	0.1915 to 0.5896	6	6	100	0.6097 to 1.0000
RAD50	18	7	39	0.2031 to 0.6138	6	5	83	0.4365 to 0.9699
MRE11A	17	6	35	0.1731 to 0.5870	6	5	83	0.4365 to 0.9699
MEN1	10	7	70	0.3968 to 0.8922	4	1	25	0.0456 to 0.6994
BRIP1	20	12	60	0.3866 to 0.7812	6	6	100	0.6097 to 1.0000
RAD51C	22	16	73	0.5185 to 0.8685	7	7	100	0.6457 to 1.0000
RAD51D	21	16	76	0.5491 to 0.8937	7	7	100	0.6457 to 1.0000

Abbreviation: ENIGMA, Evidence-Based Network for the Interpretation of Germline Mutant Alleles.

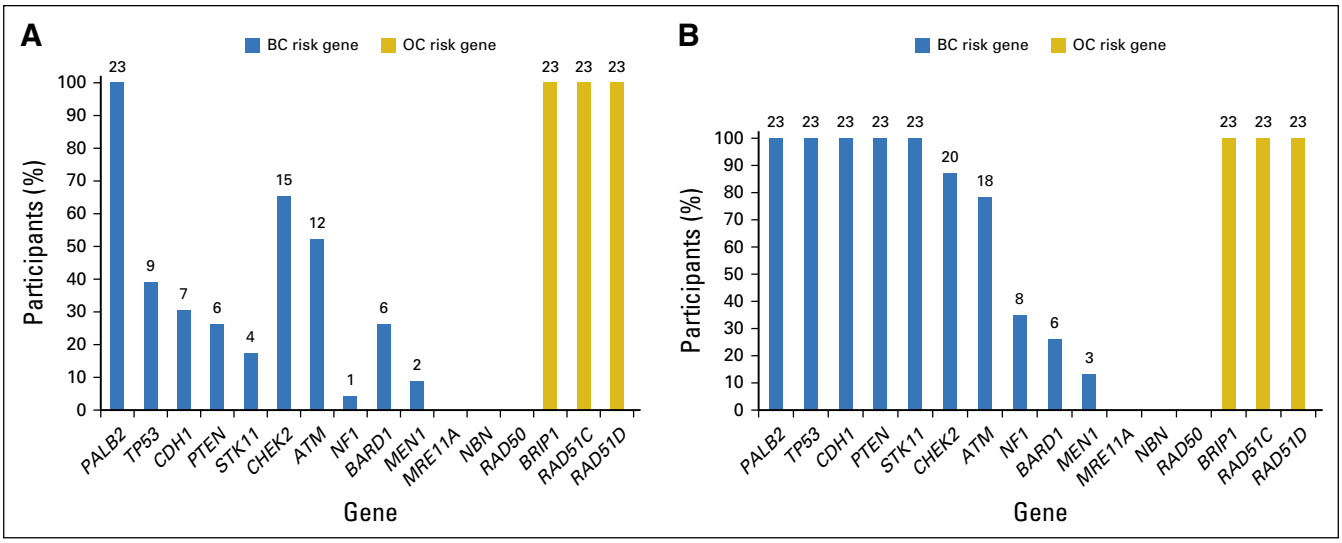

Fig A1. Opinions on clinical utility of non-*BRCA1/2* breast (BC) and ovarian cancer (OC) risk genes. Participants who agree with the following statements (No. shown above each bar): (A) every patient with BC (or OC) who meets criteria for (*BRCA1/2*) genetic testing should be tested for this gene, and (B) cancer risks associated with this gene are high enough to affect clinical management. *MRE11A*, *NBN*, and *RAD50* are candidate BC risk genes. These two questions were asked at a different time (during Evidence-Based Network for the Interpretation of Germline Mutant Alleles meeting in Cyprus in January 2017 compared with survey questionnaire). Therefore, only 23 centers answered these questions.

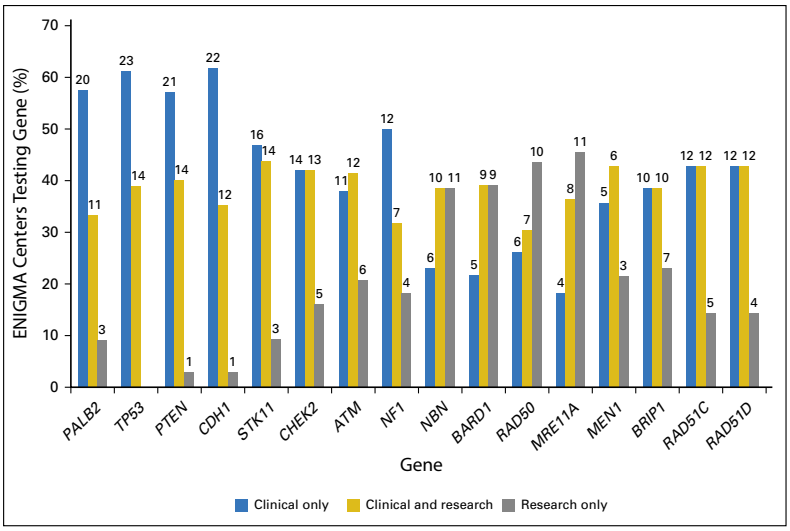

Fig A2. Testing setting: clinical versus research. Absolute No. of centers testing given gene through each method is shown above each bar. Only responses from those centers that reported they tested the gene were counted in the total, and the No. of centers that responded varied by gene (range, 14 to 37 centers). The centers that tested each gene through research only were compared with the proportion of centers that tested the gene only clinically and proportion of those that tested the gene for both clinical and research purposes. ENIGMA, Evidence-Based Network for the Interpretation of Germline Mutant Alleles.

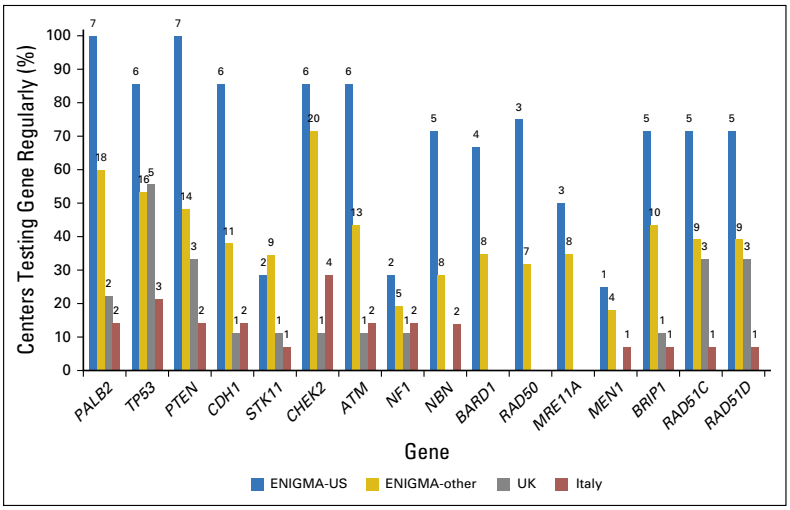

Fig A3. Genes tested regularly by Evidence-Based Network for the Interpretation of Germline Mutant Alleles (ENIGMA) centers in the United States (ENIGMA-US) versus other ENIGMA centers (ENIGMA-other) versus Italian and United Kingdom non-ENIGMA centers. Absolute No. of centers testing given gene regularly (defined as ordered for > 50% of patients eligible for genetic testing, by criteria that we recognize may differ by center/country) is shown above each bar. Of the seven total ENIGMA-US centers, the No. of centers that answered this question was four to seven, depending on the gene; of the 31 ENIGMA-other centers, a range of 22 to 30 centers answered this question. All 14 non-ENIGMA Italian centers answered this question; all nine non-ENIGMA United Kingdom centers answered this question. The United Kingdom version of the survey did not give "test regularly" as an option.

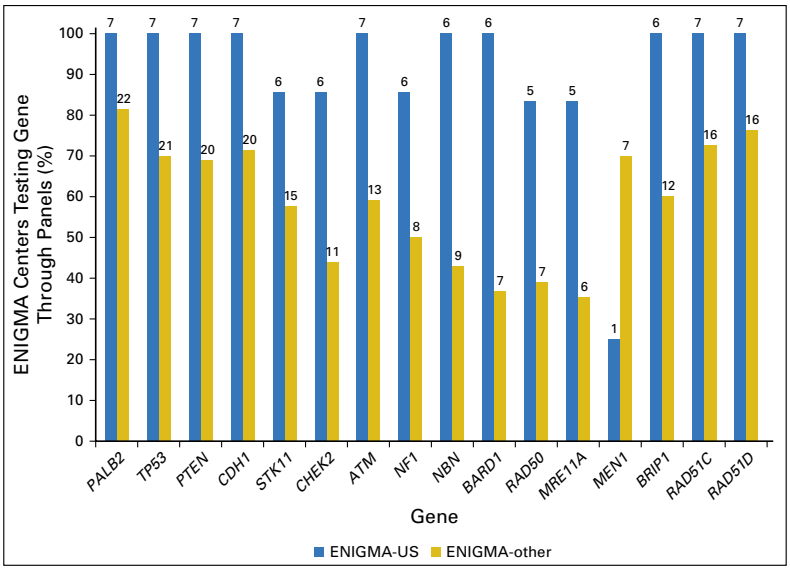

Fig A4. Genes tested through panel testing by Evidence-Based Network for the Interpretation of Germline Mutant Alleles (ENIGMA) centers in the United States (ENIGMA-US) versus other ENIGMA centers (ENIGMA-other). Absolute No. of centers testing given gene through panel testing is shown above each bar. Only responses from those centers that reported they tested the gene were counted in the total, and the No. of centers that responded varied by gene (of the seven total ENIGMA-US centers, four to seven centers responded depending on the gene; of the remaining 31 ENIGMA-other centers, a range of 10 to 30 centers responded).