Annals of Oncology 26: 2057–2065, 2015
doi:10.1093/annonc/mdv278
Published online 7 July 2015

BRCA1 and *BRCA2* genetic testing—pitfalls and recommendations for managing variants of uncertain clinical significance

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Received 8 December 2014; revised 5 February 2015 and 21 April 2015; accepted 8 June 2015

Background: Increasing use of *BRCA1/2* testing for tailoring cancer treatment and extension of testing to tumour tissue for somatic mutation is moving *BRCA1/2* mutation screening from a primarily prevention arena delivered by specialist genetic services into mainstream oncology practice. A considerable number of gene tests will identify rare variants where clinical significance cannot be inferred from sequence information alone. The proportion of variants of uncertain clinical significance (VUS) is likely to grow with lower thresholds for testing and laboratory providers with less experience of *BRCA*. Most VUS will not be associated with a high risk of cancer but a misinterpreted VUS has the potential to lead to mismanagement of both the patient and their relatives.

Design: Members of the Clinical Working Group of ENIGMA (Evidence-based Network for the Interpretation of Germline Mutant Alleles) global consortium (www.enigmaconsortium.org) observed wide variation in practices in reporting, disclosure and clinical management of patients with a VUS. Examples from current clinical practice are presented and discussed to illustrate potential pitfalls, explore factors contributing to misinterpretation, and propose approaches to improving clarity.

Results and conclusion: Clinicians, patients and their relatives would all benefit from an improved level of genetic literacy. Genetic laboratories working with clinical geneticists need to agree on a clinically clear and uniform format for reporting *BRCA* test results to non-geneticists. An international consortium of experts, collecting and integrating all available lines of evidence and classifying variants according to an internationally recognized system, will facilitate reclassification of variants for clinical use.

Key words: variants of uncertain significance, VUS, *BRCA*, clinical utility, classification

introduction

Germline inactivating variants in the tumour suppressor genes *BRCA*1 and *BRCA*2 confer high lifetime risks of breast cancer, ovarian cancer and less frequently also other cancers [1]. These pathogenic variants are conventionally called 'mutations' or 'deleterious variants' in *BRCA* genetic testing parlance and the term pathogenic variant is used here for the sake of precision. Pathogenic variants in either gene confer a high lifetime risk of developing ovarian or (another) primary breast cancer in female carriers but they explain only ~20% of familial breast cancer [2]. The more cancers, the younger the onset and the admixture of ovarian with breast cancer among relatives all increase the chance that a familial cluster is due to a *BRCA* gene mutation but, nonetheless, most familial clusters of breast cancer are not due to an inherited mutation in *BRCA*1 or *BRCA*2. In addition to familial clusters, *BRCA* mutations account for over 10% of patients with early onset triple-negative breast cancer and over 10% of women with non-mucinous ovarian cancer unselected

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for family history [3, 4]. Female carriers with pathogenic variants can make informed decisions about prophylactic surgery or intensified screening programmes. High-profile media coverage increases patient expectations from genetic testing [5, 6]. The indications for germline genetic testing for *BRCA1* and *BRCA2* are further increasing to include directing cancer chemotherapy, novel targeted treatments and informing choices about the extent of therapeutic surgery [7–9]. Germline genetic testing for *BRCA1* and *BRCA2* for cancer risk prediction and management is routinely delivered by clinical genetics professionals but increasing demand is overwhelming the current delivery model with insufficient capacity among trained geneticists and genetic counsellor. Safe integration of genetic counselling and testing to identify Hereditary Breast and Ovarian Cancer in mainstream oncology is an ongoing challenge. Challenges exist at many levels but key are genetic literacy and genetic test outcome interpretation and reporting.

Variability between individuals' genetic code is common within the general population and between individuals of different ethnic background, and this intrinsic variability can lead to difficulties in interpreting some types of sequence change (see Figure 1 for more information). Variants of uncertain clinical significance (VUS) represent a particular challenge since the clinical significance cannot be inferred from sequence information alone. Misinterpretation of VUS can lead to real clinical harms for both patients and families [10–12]. Furthermore, terms used in genetic test clinical reports vary (see Table 1). Up to 20% of *BRCA1/2* tests will report genetic VUS but, in a well-characterized ethnic population, the proportion may drop to 5% or less [13–15]. The Evidence-based Network for the Interpretation of Germline Mutant Alleles (ENIGMA) consortium has received over 6000 submissions of unique VUS, identified in over 13 000 families from over 17 countries [16]. Laboratories following generic reporting rules and with limited *BRCA*-specific experience may result in reports with more categoric conclusions about variants in *BRCA* genes than is supported when multiple lines of evidence are taken into account [17]. The percentage of gene tests resulting in a VUS is expected to increase when the extent of sequencing increases to include untranslated and deeper intronic regions and when tumour testing is offered. We restrict examples in this paper to the *BRCA* genes tested in blood samples.

Members of the ENIGMA consortium's Clinical Working Group have collated real case scenarios from clinical practice to illustrate the pitfalls that can arise after a VUS test result and suggest some strategies to mitigate this risk. Clinicians who are requesting *BRCA* tests need to consider these issues when using *BRCA* test results in the management of patients and their families.

the VUS report: why are VUS difficult to classify?

pitfalls associated with variability in the information content of genetic test reports. Based on the experience of representatives from 17 countries, it is clear that there is currently no internationally accepted standard for *BRCA* testing reporting (Table 1), and no agreed consistent classification system: some laboratories report variants without interpretation, some use a narrative approach and some use locally developed guidelines or published schemes [18, 19]. For some types of *BRCA* gene variants, additional evidence may be essential before a variant can be clearly classified. Different lines of evidence may appear to conflict, so an integrated estimation of probability taking all available evidence into account is essential to reach a final classification for many variants [20, 21].

multiple lines of evidence may be required to establish pathogenicity. Software packages are available to help interpret genetic variants, each with strengths and weaknesses (Table 2). Understanding of how the multiple functions of the *BRCA*1 and *BRCA*2 proteins relate to cancer predisposition is limited and few validated functional assays are available [22]. The interpretation of functional studies is often technically complex, and results may not be calibrated against clinical parameters in order to give measures of sensitivity and specificity. A major breakthrough in the field of *BRCA* variant classification has been the development of a multifactorial likelihood classification model [20, 23]. It is anticipated that functional data from *BRCA*1 and *BRCA*2 will soon be incorporated in this model [22, 24, 25].

The multifactorial likelihood method uses a number of different independent features in order to establish a combined likelihood estimate that a *BRCA* variant has the characteristics of known pathogenic variants [15]. The model currently combines the prior probability of variant pathogenicity (based on evolutionary conservation and amino acid physicochemical properties) with additional estimates of pathogenicity from likelihoods based on clinical information, including variant co-occurrence with a known pathogenic mutation in the same gene, tracking of the variant with cancer-affected family members (segregation) and *BRCA* tumour features. The concept underlying multiparametric methods is that empiric probabilities that a person 'does' have or 'does not' have a pathogenic *BRCA* variant can be established for each available line of evidence [20]. Multiple lines of evidence can be factored together as they become available in an iterative manner to produce an increasingly accurate probability of pathogenicity estimates for an individual variant. These estimates can be used to classify variants into clinically relevant strata (Table 3) [18].

several classification systems have been proposed and there is no international consensus on which to adopt. A universal system for classification of variants common across all countries would facilitate education among new users of genetic tests and minimize the risk of misinterpretation. Unfortunately, multiple systems have been proposed and are currently in use with many reporting laboratories not offering a clinically relevant classification. A World Health Organization-funded expert workshop at the International Agency for Research on Cancer (IARC) in 2008 recommended that the score from a multifactorial likelihood model is used to categorize high-risk cancer gene variants [26] based on the multifactorial likelihood estimates of variant pathogenicity [18]. This is the only published classification system that links clinical recommendations to each class (Table 3). The American College of Medical Genetics are recommending ClinVar as a common repository for genetic variants to help standardise reporting in the United States (Table 4).

databases reporting genetic variants. A number of web-based resources catalogue variants reported in the BRCA1 and 2 genes (Table 4). For all variant databases, ongoing curation of deposited information, both at the time of deposition and reclassification,

Variation in DNA is common	
Facts and terms	Further information
When a gene is tested for mutations, the sequence of that gene in the individual is compared to an accepted reference sequence for the gene and variation from the reference sequence is often observed.	The human genome contains ~ 6 billion bases of genetic sequence in any diploid cell and on average there is a change in the reference sequence approximately every 500 bases, much of this variation does not obviously impact on function but occasionally it does.
BRCA1 and *BRCA2* are very large genes	Random variation in the reference sequence occurs frequently across the population, not associated with any clinical effect. This is true whether the individual has had cancer or not.
Non-pathogenic variation in sequence is as frequent in the general population as in the cancer affected patient	It is tempting to believe that because a person with cancer has been tested for a mutation in the BRCA1/2 gene, that any deviation from the expected code is causative but in reality, in the absence of clear evidence of a loss of function in the mutated copy of the gene, most of the genetic variation is of little or no significance in relation to disease causation
Different ethnic groups have different frequencies of variants	For ethnic populations where relatively little genetic testing has been undertaken, common polymorphisms in that ethnic minority group may be unrecognised in the testing laboratory and are therefore more likely to be reported as of uncertain significance (VUS).
Terms used in describing genetic variants	
Polymorphisms (meaning "many forms") are common variations observed in more than 1% of the normal population.	When observed frequently in population controls (unaffected by cancer), this type of sequence change is generally not associated with a high cancer risk.
A change in a single base (T,G,A or C) is a single nucleotide variant and if the particular variant is relatively frequently observed in a particular population, it is called a single nucleotide polymorphism (SNP)	Single nucleotide variants usually have no major clinical consequence especially if frequent (i.e. SNPs) but rarely they may affect the function of the protein being coded. Synonymous: a single base change in the exon which results in no change to the expected amino acid at that position so is usually of no consequence but can create or destroy a native donor or acceptor splice site. Non-synonymous: also known as missense mutations lead to a change in one amino acid, these may affect a critical functional domain, although most do not. Variants within an intron (ie non coding part of the gene) can also rarely destroy or create false donor or acceptor splice sites.
In-frame deletions or insertions	An insertion or deletion of three bases starting with the first base of an amino acid code is an in frame deletion or insertion and leads to the insertion or deletion of one or sometimes more amino acids but the full length protein is otherwise predicted to be complete. These may have no effect on protein function since most of the protein is translated correctly.
Variants of uncertain significance (VUS)	Where a change to the expected sequence is observed that has not been observed with any frequency in the testing laboratory and has not been classed as non-pathogenic in literature or databases, genetic testing laboratories will usually report these as a VUS. Gathering multiple lines of additional information may help to clarify pathogenicity [15]. In reality most of these are unlikely to be pathogenic mutations but a few will be.

Figure 1. Genetic variability information.

is a significant challenge. Some databases are actively curated while others rely heavily on the classification by the submitting laboratory with varying levels of supporting data provided. Also important is the use of standardized HGVS nomenclature (http://www.hgvs.org/) allowing unambiguous comparison of all data on the same variant in the database and across the literature. Apart from occasional national consortia, reporting of variants is not mandated so databases cannot usually be used to derive population frequencies of variants to aid classification (e.g. laboratories tend to under-report variants that have been found several times) and many do not catalogue the supporting evidence used to determine pathogenicity. A new attempt to collate BRCA VUS for the purpose of classification is the PROMPT registry (www.promptstudy.org). Patients receiving a

Table 1. Types of sequence variants reported in *BRCA* mutation detection tests according to risk relevant for clinical management

Clinical risk	Descriptors observed in clinical reports	Interpretation
High	Functionally deleterious mutation Pathogenic mutation High-risk mutation Deleterious variant	Variants that result in a high lifetime risk of breast and ovarian cancer. Deleterious variants disrupt normal protein function. They include nonsense changes, out-of-frame insertions or deletions, large gene rearrangements or splicing variants altering the canonical splicing acceptor and donor sites disrupting regulatory regions, as well as some missense changes. Supportive evidence from multiple sources may be required to call a variant deleterious where predictions of functional consequences are unclear[a].
Uncertain	Missense mutation Rare variant Variant of uncertain clinical significance (VUS, VOUS) Uncertain variant Variant of uncertain Variant of unknown clinical significance	Variants that differ from the published reference DNA sequence (RefSeq BRCA1: U14680; BRCA2: NM00059) and are not classifiable as either deleterious or as neutral based on available evidence. The association with 'clinical phenotype' at this time is 'unknown' and cannot be used to inform clinical decisions.
None	Frequent mutation Common variant Polymorphism Variants of low clinical significance Variants of no clinical significance Neutral variants Benign variants	Changes in the DNA sequence that do not disrupt the normal function of the encoded protein and are not associated with a clinically important increased risk of disease. If present in the population at a frequency of >1%, these may be referred to as polymorphisms.

[a]For example assays showing partial loss of normal transcript from aberrant splicing or partially reduced function from an amino acid substitution may require additional evidence to classify as class 4 or 5.

BRCA VUS result are informed about the registry directly by the collaborating testing laboratory (including Myriad and Ambry Genetics) and/or their clinician. The patient can submit a range of medical information, genetic test report and tumour pathology reports and opt to participate in a variety of variant-specific research studies.

Case 1: Two families were identified in separate countries with the same missense variant in *BRCA1* designated c.5212G > A, NM_007294.3 (p.Gly1738Arg). A number of women in both families were seeking risk-reducing surgery because of a strong family history of early age of onset of breast cancer in their families. The variant was a class 3 (uncertain) variant (Table 3) but it occurred at a highly conserved residue, and it was recognized that more evidence may successfully confirm it was pathogenic. Predictive testing could then be offered to at-risk family members to facilitate their preventive choices. Each family provided insufficient power for an informative segregation analysis but collaboration across centres in two countries with the same variant, allowed a segregation analysis to be completed and the VUS could then be re-classified as class 5 (definitely pathogenic) [27]. It took a fortuitous academic collaboration and over 3 years to re-classify this VUS. Unless the phenotypes and genotypes are deposited in a freely accessible database, then such a variant will remain class 3. The ENIGMA consortium [16] provides a mechanism to rapidly link clinical teams looking after families with the same VUS to pool evidence. Since *BRCA* germline mutations explain only a minority of all cases presenting with breast or ovarian cancer, two unrelated patients with these relatively common phenotypes and the same rare *BRCA*1 variant provide insufficient evidence of pathogenicity. In this scenario, additional evidence from segregation analyses is essential to allow a multifactorial estimation of the likelihood of pathogenicity before confidently using the variant in clinical predictive testing.

pitfalls derived from frequency and population of origin of the VUS. Rare variants (allele frequency <0.01) are usually not classifiable by an individual laboratory due to paucity of information and lack of statistical power. When the ethnicity of the patient being tested differs from the patient groups where most testing has been done, a sequence variant may have little publically available data and be rare in that laboratory so is more likely to be considered a VUS.

Case 2: A 35-year-old woman of African ancestry developed two primary breast tumours presenting at age 25 and 33. Both tumours were estrogen receptor negative (ER-), progesterone receptor negative (PR-) and human epidermal growth factor receptor 2 negative (HER2-). She had no family history of cancer, but testing was initiated on the basis of her personal cancer history. She was found to have two VUS in *BRCA1;* variant 1, c.5154G > T, NM_007294.3 (p.Trp1718Cys) and variant 2 in the 3′ UTR region, c.*36C > G, NM_007294.3. DNA samples from relatives indicated that both variants are likely located on the same allele. Variant 2 is frequent (6%–11%) in the African-American population [28] and could be assigned to class 1 (non-pathogenic). Variant 1 involves amino acid substitution at a highly conserved residue predicted *in silico* to have a functional impact but could not be classified unambiguously due to insufficient data. The clinical dilemma for this patient thus

Table 2. Examples of software tools available for clinical variant evaluation online

Resource	Description	Website
Polyphen2	POLYmorphism PHENotypes Polyphen2 is freely available, web-based program from Harvard University that predicts whether an amino acid substitution affects protein structure and function.	http://genetics.bwh.harvard.edu/pph2/
SIFT	Sorting Intolerant From Tolerant SIFT software is freely available and predicts whether an amino acid substitution affects protein function.	http://sift.jcvi.org/
Align-GVGD	Align Grantham Variation and Grantham Deviation. Align-GVGD is a freely available, web-based program from International Agency for Cancer Research (IARC) that combines the known chemical nature of amino acids and the alignments of DNA sequences across species to predict the likely pathogenicity of missense substitutions.	http://agvgd.iarc.fr/about.php
HCI database of prior probabilities	The Huntsman Cancer Institute Database of Prior Probabilities of Pathogenicity for single-nucleotide substitutions provides the prior probability of pathogenicity estimate which is the starting point for the multifactorial likelihood estimate for a novel SNP identified through genetic testing for a BRCA gene.	http://priors.hci.utah.edu/PRIORS/
GeneSplicer	GeneSplicer is a freely available flexible system for detecting splice sites in the genomic DNA of various eukaryotes.	http://www.ncbi.nlm.nih.gov/pmc/articles/PMC29713/
Human Splicing Finder	This tool is freely available and aimed at studying pre-mRNA splicing and identifying variants that may disrupt normal splicing.	http://www.umd.be/HSF3/
Commercial software, e.g. Alamut	Alamut is commercially available software that provides a single interface to bring together multiple data sources including many of those listed above in a simplified and streamlined tool for rapid assembly of data about any one variant aimed at busy diagnostic laboratories.	http://www.interactivebiosoftware.com/software/alamut/overview
Entrez-Pubmed Google Scholar	Web-based search engines to look for research publications including a specific mutation.	http://www.ncbi.nlm.nih.gov/pubmed http://scholar.google.com
UMD-*BRCA1* UMD-*BRCA2*	The UMD-BRCA1/BRCA2 databases have been set up in a joined national effort through the network of the 16 French diagnostic laboratories. A classification is proposed based on the classical parameters including splicing algorithms. A strong effort is done to perform co-segregation studies.	http://www.umd.be/BRCA1/ http://www.umd.be/BRCA2/

Table 3. 5-tier classification *BRCA1/2* VUS modified from ref. [15]

Class	Description	Probability of being pathogenic	Clinical predictive testing of at risk relatives	Management recommendations if at-risk relative has the variant	Research testing of family members
5	Definitely pathogenic	>0.99	Yes	Full high-risk guidelines	Not indicated
4	Likely pathogenic	0.95–0.99	Yes	Full high-risk guidelines	May be helpful to further classify variant
3	Uncertain	0.05–0.949	No	Presence of variant is irrelevant to risk assessment, manage risk based on family history only	May be helpful to further classify variant
2	Likely not pathogenic or of no clinical significance	0.001–0.049	No	Manage risk based on family history only	May be helpful to further classify variant
1	Not pathogenic or of no clinical significance	<0.001	No	Manage risk based on family history only	Not indicated

becomes whether the available information about variant 1 is sufficient to predict a high risk of ovarian cancer and recommend risk-reducing bilateral salpingo-oophorectomy (BSO).

A literature search identified two reports of variant 1. The first report was of two families from Asturias, Northern Spain [29]. Contact with the authors clarified the following details, some directly applicable for variant interpretation: Asturian proband 1 was diagnosed with bilateral invasive ductal breast cancer at age 28 (grade III, ER−) and 30 (grade II, ER−, PR−, HER2−); Asturian proband 2 developed breast cancer at age 40 (ER and grade unknown), and probably also ovarian cancer at age 63. The second report of the variant was in an African-American patient and family members where the variant was present in 4/4 women with cancer and absent in 3/3 at-risk women without cancer. Pathogenicity was supported by the functional assay showing loss of transcriptional activity [30].

Table 4. Commonly used online database resources that provide some interpretation of *BRCA* sequence variants although

Resource	Description	Website
IARC/LOVD [Leiden Open (source) Variation Database]	Separate *BRCA1* and *BRCA2* variant databases curated by experts at the University of Leiden and IARC. Includes only those VUS with literature references and records the published literature associated which each variant. Each variant is given an IARC class [15] and links to published source data. Submissions of variants are from registered submitters largely from the research community. Curation of submissions for each gene is undertaken by a named expert curator on a voluntary basis.	http://brca.iarc.fr/LOVD/home.php soon to be replaced by http://hci-exlovd.hci.utah.edu/home.php
The Breast Cancer Information Core (BIC)	A database that acts as a central repository for BRCA1 and BRCA2 variants (deleterious, neutral or VUS) deposited by submitters from research and clinical sites internationally. Recently, a central curation process working to classify all variants according to the IARC 5-tier classification [15] scheme has been introduced.	https://research.nhgri.nih.gov/projects/bic
ClinVar	ClinVar is a freely accessible, public archive of reports of the relationships between human variations and phenotypes presented with supporting evidence and an indication of likely clinical significance. Submissions are from research and some diagnostic laboratories and submitters include a classification for submitted variants, submissions are not currently curated.	http://www.ncbi.nlm.nih.gov/clinvar/
Human Variome Project	The Human Variome Project, under the auspices of UNESCO, has been created as a unified reporting portal and lists four separate databases for *BRCA1* and four for *BRCA2*. As yet it offers no formal curation or attempt to classify variants.	http://www.humanvariomeproject.org

Using multifactorial likelihood analysis [23] combining all currently available evidence: a prior probability of 0.81 based on Align-GVGD prediction [31], and likelihood ratios based on additional information gained about breast tumour pathology [32], the posterior probability of pathogenicity for the variant is 0.98 which places it in class 4. Thus, sufficient evidence is now available to estimate a substantially raised ovarian cancer risk and permit a clinically sound recommendation of BSO to reduce ovarian cancer risk.

why is genetic counselling around VUS complex?

Although it is expected that most class 3 variants will represent non-pathogenic variants, it is critical that we improve and accelerate clinical annotation and classification.

limited genetic literacy among clinical professionals. Disclosure of a VUS result can test the skills of even highly experienced genetic counselling teams. There can be considerable variation in experience with molecular genetics and with clinical *BRCA* gene testing leading to diverse clinical management recommendations. This situation is compounded by complex and variable presentation of diagnostic reports from laboratories and limited genetic literacy among treating clinicians, and exacerbated further by the patient's perception, prior experiences and beliefs.

Case 3: A *BRCA2* missense variant p.Thr3033Ile (c.9098C > T, NM_000059.3) was identified in a 53-year-old woman with ovarian cancer and no family history of breast or ovarian cancer. A class 3 report was issued as little supporting evidence was available. The managing clinician treated the variant as clinically significant and offered genetic testing to the patient's daughter and sister. The patient's sister did not carry the variant, but the daughter did. The daughter was given the same cancer risk management advice as a pathogenic variant carrier including risk-reducing surgery. Additional information from a *BRCA2* functional assay was subsequently used to reclassify this variant to class 2 (likely of no clinical significance) [33]. Cancer risk estimates for the daughter and sister should have been based on family history alone. Since there was no family history, testing in relatives could not even provide useful additional information about segregation. It is worth noting here that the majority of breast and ovarian cancer patients who will be offered germline *BRCA* testing to guide cancer treatment will not have a family history of cancer amenable to segregation studies.

This case illustrates the need to ensure improved genetic literacy among non-genetics professionals ordering DNA tests, particularly the uncertainty around VUS results, the implications of testing in other family members and the need for a clear pathway for referral to colleagues experienced in the interpretation and follow-up of variant results [34]. Many patients will readily assume that any reported variant must be pathogenic unless the potential for a VUS has been clearly flagged as part of the pre-test discussion.

perceptions of cancer risks associated with VUS. A genetic test for a disease like breast cancer that uncovers a rare variant may lead to both patients and clinicians to think, 'what is the chance this rare variant has nothing to do with this clinical presentation?' and convince themselves it cannot be coincidental. The decision to undertake preventive surgery is complex and patients are likely to have had personal and family experiences which may have strongly contributed to their risk management decision. Class 3 VUS reports discussed with counselees are too frequently inaccurately perceived typically leading to overestimation of cancer risks, adverse psychological outcomes and more radical medical decisions [35, 36]. Furthermore, 'almost the same large number of counselees with an unclassified variant decided to have preventive surgery as pathogenic mutation carriers' [37].

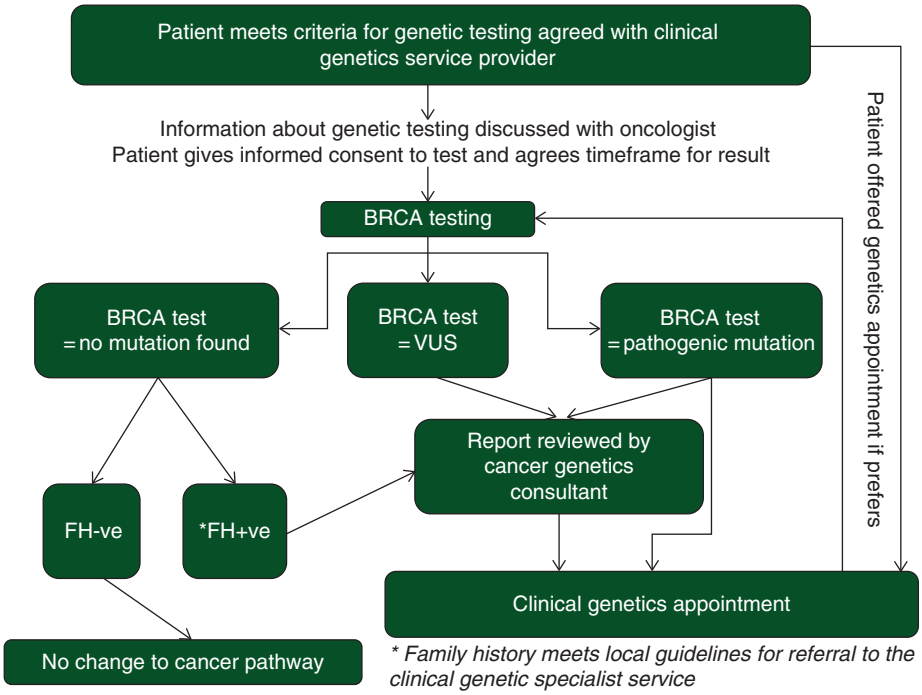

Figure 2. Suggested model of a clinical pathway for breast and ovarian cancer patients. Initial genetic testing through an oncologist with basic training, expertise routinely contributed by the clinical genetics consultant. This model works where there is a close working relationship between oncologist and geneticist through a regular multidisciplinary team meeting.

Case 4: A *BRCA1* VUS was identified some years previously in a woman who developed breast cancer at the age of 45 in the setting of a strong family history of early onset breast and ovarian cancer. The family understood that the variant must be the answer to the strong family history despite the inconclusive evidence. Several family members came forward for predictive genetic testing which they incorrectly believed was available. Genetic testing was repeated some years later and a clearly pathogenic large exonic deletion in *BRCA1* was then identified on the opposite allele confirming irrefutably that the original VUS could not be pathogenic and demonstrating that new or optimized mutation screening approaches will uncover missed mutations in some families.

This case illustrates the importance of understanding a 'non-informative' or inconclusive test aimed at detecting a genetic predisposition to cancer. It is important to be aware that mutation testing is <100% sensitive and a negative *BRCA* test result does not exclude an underlying hereditary cause. *BRCA1* or *BRCA2* gene mutations only account for an estimated 20%–30% of familial clustering of breast cancer, so cancer patients negative for a *BRCA* test, but with a strong family history, should be referred for specialist assessment and advice about risk management in the cancer genetics clinic.

The testing clinician has a responsibility to ensure that they have received appropriate training about the genetic testing process and understand the possible outcomes and have appropriate expert support from a genetic specialist. A helpful starting point is to be able to estimate the probability of a deleterious *BRCA* mutation given the family history and tumour characteristic [38]. Testing clinicians should be able to explain the likely outcomes of the test to their patients; in patients with a low a priori probability of finding a pathogenic variant, the most likely outcome from testing would be no pathogenic variant found, the second most likely outcome would be a VUS, and the least likely outcome a pathogenic variant. It is good practice before embarking on a *BRCA* gene test to discuss with the patient the likelihood of these possible outcomes. The test result must be interpreted in the context of the family history and clear guidelines should be agreed for referral of cases to a specialist genetic clinic (Figure 2). Finally, the experience gathered with VUS in *BRCA*1/2 is relevant to developing ethical norms and policy issues, including duty to re-contact patients, as cancer gene panel, exome and genome sequencing become increasingly commonplace [38].

recommendations

We believe that some essential elements are necessary for the optimal clinical use and interpretation of VUS in mainstream medical practice.

variant reporting and classification

- An internationally accepted terminology and a clinically relevant classification to report and discuss *BRCA* test results.
- A framework for clinicians, clinical scientists and research groups to work together towards classification of VUS.

Reporting of sequence variants to a single, transparently and expertly curated database providing clinically relevant classification for each reported *BRCA* variant based on clearly stated lines of evidence and freely available to all providers of genetic testing. To submit variants to ENIGMA, go to contacts page http://www.enigmaconsortium.org/

risk communication

- Improve the genetics literacy of medical providers by structured training and integrating genomics into undergraduate, basic and specialist medical training curriculae.
- Access and clear referral guidelines to specialist clinical genetics services for patients with a pathogenic variant, a VUS or a strong family history and no detectable genetic cause.
- A close working relationship between genetic diagnostic laboratories, genetic specialists and cancer clinicians delivering an integrated care pathway for patients and their families.

data review

- A standard operating procedure within reporting laboratories to review variant classification each time new evidence emerges.
- A clearly agreed process for reporting updated classifications (laboratories) and re-contacting patients (clinicians) if a VUS becomes classified into clinically actionable or definitively non-pathogenic.

concluding remarks

VUS identified in *BRCA* genes represent a major clinical challenge. Individuals with significant family history, a pathogenic variant or a VUS should be referred to a genetic specialist service. Patients with no pathogenic variant or a VUS should be managed based on the family history only. Standardized reporting and better genetic literacy must be implemented to safely introduce genetics into mainstream oncology. In conclusion, concerted action between the clinical and research communities is the best approach to optimally managing *BRCA* variants for maximum patient benefit. The international ENIGMA consortium is focussed on improving the interpretation of *BRCA* VUS and incorporates both clinical and research expertise. Through a collaborative approach and a global and unified source of data and variant classification, important advances in this complex field will continue to benefit patients.

disclosure

The authors have declared no conflicts of interest.

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appendix

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